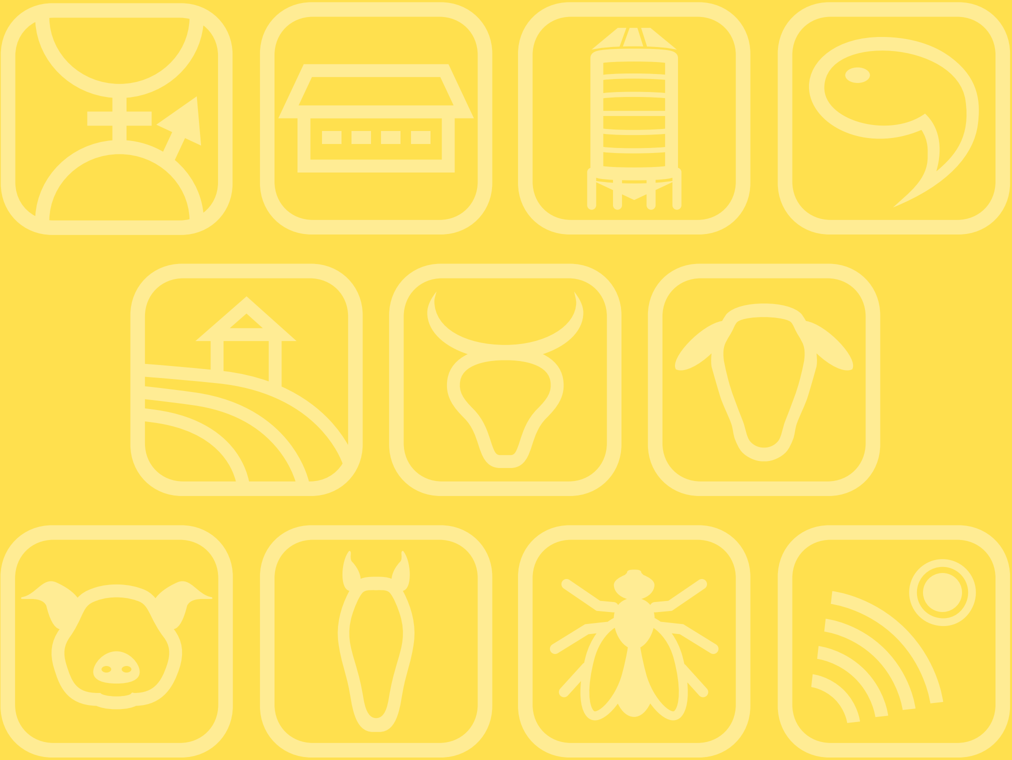
Book of Abstracts of the 72nd Annual Meeting of the European Federation of Animal Science



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Book of abstracts No. 27 (2021)
Davos, Switzerland
30 August – 3 September 2021

Book of Abstracts of the 72nd Annual Meeting of the European Federation of Animal Science

EAAP

European Federation of Animal Science

The European Federation of Animal Science wishes to express its appreciation to the
Ministero delle Politiche Agricole Alimentari e Forestali (Italy) and the
Associazione Italiana Allevatori (Italy)
for their valuable support of its activities.

Book of Abstracts of the 72nd Annual Meeting of the European Federation of Animal Science

Davos, Switzerland, 30th August – 3rd September, 2021

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OASES
Online Academic Submission and Evaluation System

EAN: 9789086863662
e-EAN: 9789086869183
ISBN: 978-90-8686-366-2
e-ISBN: 978-90-8686-918-3
DOI: 10.3920/978-90-8686-918-3

ISSN 1382-6077

First published, 2021

Wageningen Academic
Publishers

Wageningen Academic Publishers
P.O. Box 220
6700 AE Wageningen
The Netherlands
www.WageningenAcademic.com
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Welcome to the EAAP 2021 in Davos

On behalf of the Swiss Organizing Committee, I'm very delighted to welcome you to the 72nd EAAP Congress in Davos from the 29th of August to the 3rd of September 2021. Davos is a world-famous place due to Thomas Mann's Magic Mountain, the World Economic Forum and numerous sporting events.

To say it in advance: Our choice of the location in 2016 for the 2021 congress could not have been better. Davos is an uncluttered, airy little place, but it is used to dealing with extraordinary situations. Therefore, we can guarantee our participants that your health is our top priority. We are conducting the congress onsite with the best conscience, because Davos is ideal for this.

The main theme of the 2021 meeting is 'Scientific solutions to different demands on the livestock sector'. The conference will cover all aspects of animal science. In addition, challenge sessions will offer the opportunity to debate societal demands on the animal sector.

The EAAP commissions have designed a very attractive program with 74 scientific sessions. As in the previous year, the poster-session will be held exclusively virtually and will cover numerous interesting topics. The Plenary Session program is dedicated to the topic 'The multiple roles of livestock in sustainable development'. The scientific program will be presented in 11 parallel sessions.

Enjoy this EAAP platform for scientists, policy makers and industry representatives to acquire new knowledge and to exchange experiences about the latest research results from many areas of animal science.

This annual meeting is a unique opportunity to meet again on an international level face to face after a long break due to the pandemic, to exchange ideas, to feel comfortable among friends, to admire a unique mountain landscape, to exchange new ideas, and to develop professionally.

We are very happy that, despite the many uncertainties, our efforts in organizing EAAP 2021 were rewarded with enough registrations for on-site participation and additional 400 registrations for virtual participation.

We have planned a social program with a Swiss evening, a farewell dinner and technical tours. Due to possible restrictions because of the pandemic, we have to remain flexible until the congress starts. However, we will do our best to be able to offer you attractive social events as well.

Finally, we thank the numerous sponsors. They have remained loyal to us despite the pandemic. Without their solidarity and financial support, the event would not have been possible.

On behalf of the Swiss Organizing Committee, I wish you a good stay in Davos. We look forward to seeing you again in Davos!

Lucas Casanova
Chairman of the Swiss Organizing Committee

National Organisers of the 72nd EAAP Annual Meeting

Swiss Association for Animal Sciences

The 72nd EAAP annual meeting is organized by the Swiss Association for Animal Sciences (SAAS) in partnership with the Federal Office for Agriculture (FOAG) and Global Agenda for Sustainable Livestock (GASL).

Members:

Lucas Casanova	President, Finance, Sponsorships – Braunvieh Schweiz, Zug
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Andreas Hofer	Infrastructure, Congress venue – SUISAG, Sempach
Veronika Maurer	Scientific program and contact with the Scientific Committee of EAAP; Volunteers – Research Institute of Organic Agriculture FiBL, Frick
Stefan Probst	Social Events, Technical Tours – Bern University of Applied Sciences, School of Agricultural, Forest and Food Sciences BFH-HAFL, Zollikofen
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The Swiss Scientific Committee was led by **Veronika Maurer**, **Andreas Hofer** and **Beat Bapst** and supported by members of

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- Centre for Proper Housing of Ruminants and Pigs ZTHT, Tänikon
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- Qualitas AG, Zug
- Research Institute of Organic Agriculture FiBL, Frick
- SUISAG, Sempach
- Swissgenetics, Zollikofen
- Swiss Goat Breeders Association SZZV, Zollikofen

Conference website: www.eaap2021.org

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European Federation of Animal Science has close established links with the sister organisations American Dairy Science Association, American Society of Animal Science, Canadian Society of Animal Science and Asociación Latinoamericana de Producción Animal.

Friends of EAAP

By creating the 'Friends of EAAP', EAAP offers the opportunity to industries to receive services from EAAP in change of a fixed sponsoring amount of support every year.

- The group of supporting industries are layered in three categories: 'silver', 'gold' and 'diamond' level.
- It is offered an important discount (one year free of charge) if the sponsoring industry will agree for a four years period.
- EAAP will offer the service to create a scientific network (with Research Institutes and Scientists) around Europe.
- Creation of a permanent Board of Industries within EAAP with the objective to inform, influence the scientific and organizational actions of EAAP, like proposing choices of the scientific sessions and invited speakers and to propose industry representatives for the Study Commissions.
- Organization of targeted workshops, proposed by industries.
- EAAP can represent and facilitate activities of the supporting industries toward international legislative and regulatory organizations.
- EAAP can facilitate the supporting industries to enter in consortia dealing with internationally supported research projects.

Furthermore EAAP offers, depending to the level of support (details on our website: www.eaap.org):

- Free entrances to the EAAP annual meeting and Gala dinner invitation.
- Free registration to journal *animal*.
- Inclusion of industry advertisement in the EAAP Newsletter, in the banner of the EAAP website, in the Book of Abstract and in the Programme Booklet of the EAAP annual meeting.
- Inclusion of industry leaflets in the annual meeting package.
- Presence of industry advertisements on the slides between presentations at selected standard sessions.
- Presence of industry logos and advertisements on the slides between presentations at the Plenary Sessions.
- Public Recognition by the EAAP President at the Plenary Opening Session of the annual meeting.
- Discounted stands at the EAAP annual meeting.
- Invitation to meetings (at every annual meeting) to discuss joint strategy EAAP/Industries with the EAAP President, Vice-President for Scientific affair, Secretary General and other selected members of the Council and of the Scientific Committee.

Contact and further information

If the industry you represent is interested to become 'Friend of EAAP' or want to have further information please contact jean-marc.perez0000@orange.fr or EAAP secretariat (eaap@eaap.org, phone : +39 06 44202639).

The Association

EAAP (The European Federation of Animal Science) organises every year an international meeting which attracts between 900 and 1,500 people. The main aims of EAAP are to promote, by means of active co-operation between its members and other relevant international and national organisations, the advancement of scientific research, sustainable development and systems of production; experimentation, application and extension; to improve the technical and economic conditions of the livestock sector; to promote the welfare of farm animals and the conservation of the rural environment; to control and optimise the use of natural resources in general and animal genetic resources in particular; to encourage the involvement of young scientists and technicians. More information on the organisation and its activities can be found at www.eaap.org

What is the YoungEAAP?

YoungEAAP is a group of young scientists organized under the EAAP umbrella. It aims to create a platform where scientists during their early career get the opportunity to meet and share their experiences, expectations and aspirations. This is done through activities at the Annual EAAP Meetings and social media. The large constituency and diversity of the EAAP member countries, commissions and delegates create a very important platform to stay up-to-date, close the gap between our training and the future employer expectations, while fine-tuning our skills and providing young scientists applied and industry-relevant research ideas.

Committee Members at a glance

- Torun Wallgren (President)
- Ines Adriaens (Secretary)
- Marcin Pszczola (secretary)

YoungEAAP promotes Young and Early Career Scientists to:
- Stay up-to-date (i.e. EAAP activities, social media);
- Close the gap between our training and the future employer expectations;
- Fine-tune our skills through EAAP meetings, expand the special young scientists' sessions, and/or start online webinars/trainings with industry and academic leaders;
- Meet to network and share our graduate school or early employment experiences;
- Develop research ideas, projects and proposals.

Who can be a Member of YoungEAAP?

All individual members of EAAP can join the YoungEAAP if they meet one of the following criteria: Researchers under 35 years of age OR within 10 years after PhD-graduation

Just request your membership form (torun.wallgren@slu.se) and become member of this network!!!

73rd Annual Meeting of the European Federation of Animal Science

Porto, Portugal,
September 5th to 8th 2022

Organizing Committees

The 73rd EAAP annual meeting is organized by the Portuguese Association of Animal Science (APEZ) and the National Institute of agrarian research (INIAV), under the patronage of the Ministry of agriculture and rural development.

The organization of this event is also in close collaboration with other Portuguese institutions, including, FeedInov CoLab, University of Trás-os-Montes and Alto Douro (UTAD), University of Lisbon (ISA and FMV), University of Évora (UE), University of Porto (UP-ICBAS), University of Minho (UM), and the National Association of Politechnych institutes (ANP).

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Conference website: www.eaap2022.org

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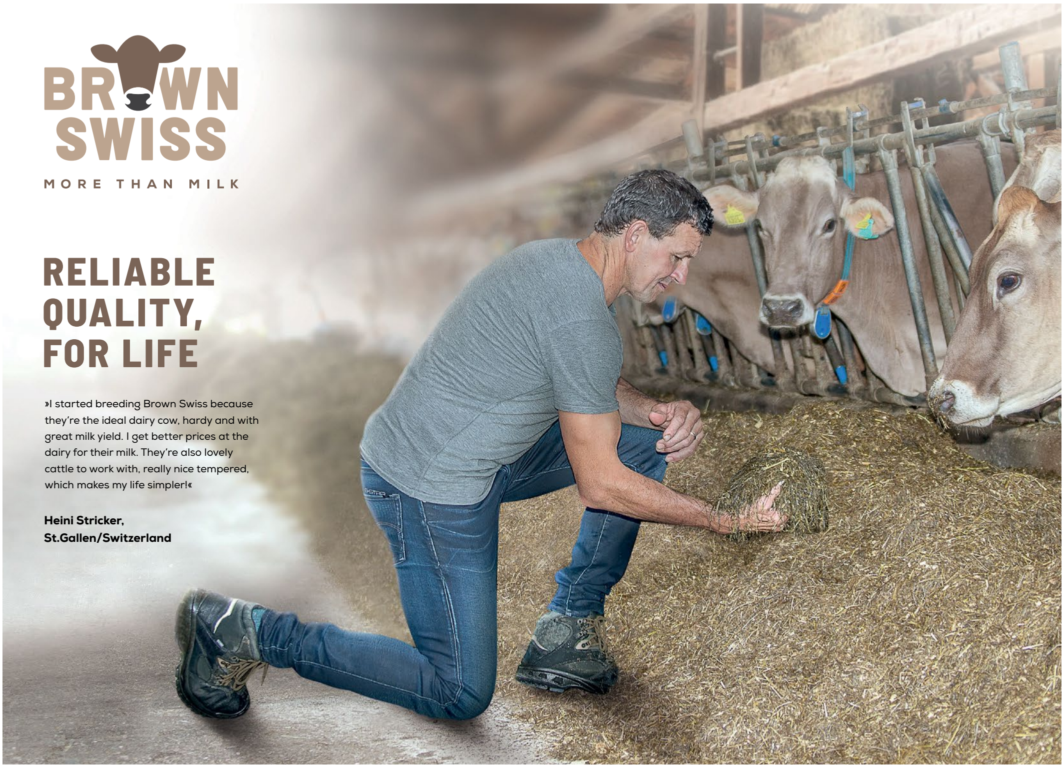

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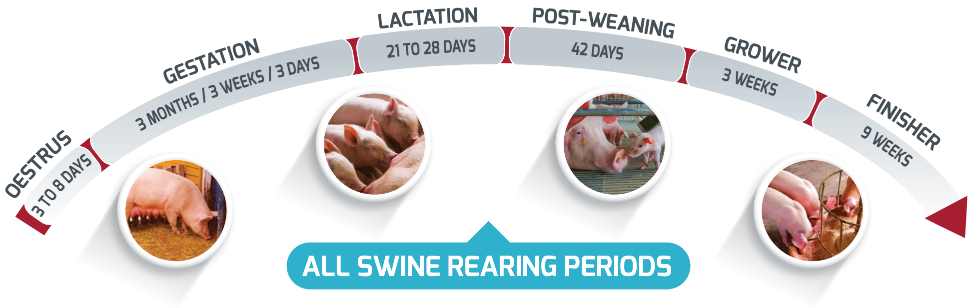

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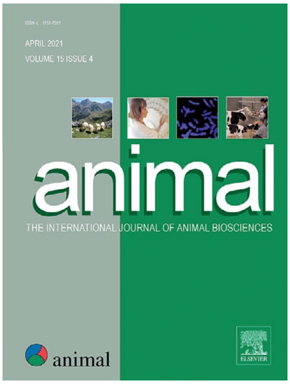

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Editor-in-Chief: Cledwyn Thomas

More information about the animal family of journals can be found on www.animal-journal.eu.

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[1] The Journal Citation Indicator (JCI) is a new metric of Clarivate. The JCI of 1.60 means that articles published in animal have 60% more citation impact than articles published in the average journal in this category.

Scientific programme EAAP 2021

Monday 30 August 8.00 – 12.00	Monday 30 August 13.30 – 17.30
Session 01 Phenotypes and strategies for breeding for climate friendly and efficient animals Chair: Pszczola **Session 02** Environmental impact on cattle genetics and breeding strategies Chair: König **Session 03** Use of genomic data for conservation activities Chair: Oldenbroek **Session 04** Effects of heat stress and other environmental challenges on the physiology of livestock Chair: Kenez / Montanholi **Session 05** Insects as feed and bioremediation tool Chair: Gasco / Ottoboni / Murta	**Session 11** Low carbon animal nutrition: methane emission and climate Chair: De Campeneere / Bruggeman **Session 12** Single-step genomic evaluation Chair: Wang **Session 13** (A) Joining forces in the use and conservation of genetic resources Chair: Hiemstra (B) Use of functional genome annotation in farmed animal breeding Chair: Charlier / Calus **Session 14** The Sm@RT and TechCare projects Chair: Morgan-Davies / Halachmi **Session 15** Methods and practices to assess and improve animal welfare Chair: Ammer / Dantas de Brito Almeida

Monday 30 August 9.00 – 13.00	Monday 30 August 14.30 – 18.30
Session 06 Sensing dynamics in livestock physiology disruption through images and other non-invasive alternatives Chair: Montanholi / Gondret **Session 07** Mixed crop/livestock systems – do they deliver more resilient food systems Chair: Boland **Session 08** Development of genetic and genomic systems in equid breeding Chair: Stock **Session 09** Feeding the gut: from microbiome to models Chair: Abdennebi-Najar / Goselink **Session 10** Early career competition: stressing innovation in pig and poultry industry Chair: Vigors / Nilsson / Trevisi	**Session 16** Recent advances in omics technologies applied to small ruminants Chair: Hernández-Castellano **Session 17** Going beyond the Feed vs Food competition: crops and animals together to address food and nutrition security Chair: O'Mara / Lee / Boland **Session 18** Alpine, organic and grassland based cattle production Chair: Berard **Session 19** Young train – Innovative research and extension Chair: Kuipers / Mielczarek **Session 20** Management of the sow and her litter Chair: Nilsson / Knol

Tuesday 31 August 8.30 – 12.30	Tuesday 31 August 13.30 – 17.30
Session 21 **Plenary session and Leroy Lecture** Chair: Casasús	**Session 22** Breeding for improved animal health, welfare and longevity I Chair: Minozzi / Gardan **Session 23** The tortoise and the hare: finding the best balance between resilience and efficiency Chair: Friggens **Session 24** From PLF sensor development to application in the field Chair: Bloch / Druyan **Session 25** Sustainable livestock farming in mountain areas and harsh environments - challenge or opportunity? Chair: Poulopoulou **Session 26** Regulatory framework and advances in genetics and genomics of farmed insects Chair: Sandrock / Lefebvre
	Tuesday 31 August 14.30 – 18.30
	Session 27 Sustainability and human health aspects of dairy and meat products and substitutes Chair: De Vries / Hocquette **Session 28** Evolutions and latest developments in equine production and management Chair: Auclair-Ronzaud / Cox / Mantovani / Vial / De Palo **Session 29** So you think your science is so great Chair: Keatinge **Session 30** Young EAAP – opportunities for early career scientists Chair: Wallgren / Adriaens / Pszczola **Session 31** ANIMAL NUTRTION 4.0 Chair: Pinotti / Ranilla García **Session 32** Meat and fat quality in broilers and finishing pigs Chair: Bee / Stadnicka

Wednesday 1 September **8.00 – 12.00**	**Wednesday 1 September** **13.30 – 17.30**
Session 33 Breeding for improved animal health, welfare and longevity: cattle II Chair: Poppe / Minozzi **Session 34** Breeding for the future: technology finds application in new phenotypes Chair: Granados Chapatte **Session 35** Good health of pigs and poultry: increasing robustness through feeding and management - part 1 Chair: Daş / Vigors **Session 36** Advancing sustainable livestock systems for resilient sustainable food systems Chair: Lee / Bourgeois Lüthi **Session 37** Sustainability assessments of insects as food and feed in a circular economy Chair: Smetana / Veldkamp	**Session 44** PLF methods for measuring health, welfare and caring for individual animals Chair: Adriaens / Bloch **Session 45** Free communications in animal genetics Chair: Cecchinato **Session 46** Use of genomic information Chair: Sell-Kubiak **Session 47** What levers can be activated by stakeholders to promote the livestock transition? Chair: Thenard / Siqueira **Session 48** Insects: standardisation and terminology Chair: Deruytter /Shumo
Wednesday 1 September **9.00 – 13.00**	**Wednesday 1 September** **14.30 – 18.30**
Session 38 Computing in PLF: cloud and edge computing and smart algorithms Chair: Pastell **Session 39** Nutrient competition between production and animal health – interactions of nutrition, immunity and microbiota Chair: Gross **Session 40** Heifer growth strategies for increased productivity, longevity and reduced environmental impact Chair: Foskolos	**Session 49** Transgenerational effect of nutrition (feeding of mother, effect on offspring) Chair: Kreuzer-Redmer / Alvarez Rodriguez **Session 50** Novel developments of milk removal in conventional, automated and cow-calf contact systems Chair: Bruckmaier **Session 51** Organic production of milk and meat Chair: De Marchi / Maurer
Joint EAAP/ADSA session	>>>
Session 41 'Future of housing for cattle' – part 1 Chair: Costa / Klopčič	**Session 52** 'Future of housing for cattle' – part 2 Chair: Costa / Klopčič
Session 42 Inclusive livestock nutrition: where we have a trade-off between performance, environmental sustainability and animal welfare Chair: Bruggeman / De Campeneere **Session 43** Optimisation of sheep and goats production systems: strategies for sustainable and resilient agroecosystems Chair: Cadavez	**Session 53** Good health of pigs and poultry: increasing robustness through feeding and management - part 2 Chair: Trevisi / Minozzi **Session 54** Sheep and goats management and reproduction Chair: Hadjipavlou
11.00 – 12.00 **Commission meeting** • Insects	

Thursday 2 September
8.00 – 12.00

Session 55
Sustainable Insect Chain (H2020 SUSINCHAIN)
Chair: Veldkamp / Gasco

Session 56
Free communications: Crossbreeding
- Conservation of local breeds
Chair: Hinrichs

Session 57
Exploiting sequence data
Chair: Berg

Session 58
Animal behaviour: from horses to hens
Chair: Boyle / Dantas de Brito Almeida / Mantovani

Session 59
CYBELE project on high performance
computing for precision livestock farming
Chair: Maselyne

Thursday 2 September
13.30 – 17.30

Session 64
Sustainable Insect Chain (H2020 SUSINCHAIN)
Chair: Veldkamp / Gasco

Session 65
Contribution of animal breeding to
solving societal challenges
Chair: Rydhmer

Session 66
Interaction between host genetics and gastro-intestinal tract microbiota genetics
Chair: Abdennebi-Najar

Session 67
Challenges and novel metrics of multi
objective tools at farm/regional level
Chair: Zehetmeier / Puillet

Session 68
PLF MIR tools to monitor cows› welfare in particular
the freedom from disease, hunger and stress
(in collaboration with HappyMoo project)
Chair: Gengler

Thursday 2 September
9.00 – 13.00

Session 60
European collaboration in research
towards sustainable pig production
Chair: Millet

Session 61
Workshop: Meta-analysis applied to animal science
Chair: Tzamaloukas

<<< **Joint EAAP/ADSA session**

Session 62
'Future of housing for cattle' – part 3
Chair: Costa / Klopčič

Session 63
Metabolism, oxidative stress and immune function
Chair: Sauerwein / Louveau

Thursday 2 September
14.30 – 18.30

Session 69
Improving animal welfare: do we need more
regulations, or should the society decide?
Chair: De Briyne / Boyle

Session 70
Improving sustainability of pig production
by participatory research
Chair: Aluwé / Bee

Session 71
Good practices and strategies in dairy farming
Chair: Mayer / Klopčič

Session 72
Methods and technologies for research and smart
nutrition management in dairy and beef cattle
Chair: Baumont

Session 73
Next generation in animal nutritional
science (Young Scientists Session)
Chair: Kreuzer-Redmer / Tretola

Session 74
Omics in animal nutrition and physiology
(the European joint doctorate program
Molecular Animal Nutrition "MANNA")
Chair: Eckersall / Sauerwein

11.00 – 12.00

Commission meetings

- Genetics
- PLF
- Health and Welfare
- LFS

12.00 – 13.00

Commission meetings

- Physiology
- Cattle
- Pig
- Nutrition
- Horse
- Sheep and Goats

Scientific programme

Session 01. Phenotypes and strategies for breeding for climate friendly and efficient animals

Date: Monday 30 August 2021; 8.00 – 12.00
Chair: Pszczola

Theatre Session 01

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Session 02. Environmental impact on cattle genetics and breeding strategies

Date: Monday 30 August 2021; 8.00 – 12.00
Chair: König

Theatre Session 02

Session 03. Use of genomic data for conservation activities

Date: Monday 30 August 2021; 8.00 – 12.00
Chair: Oldenbroek

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Session 04. Effects of heat stress and other environmental challenges on the physiology of livestock

Date: Monday 30 August 2021; 8.00 – 12.00
Chair: Kenez / Montanholi

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Session 05. Insects as feed and bioremediation tool

Date: Monday 30 August 2021; 8.00 – 12.00
Chair: Gasco / Ottoboni / Murta

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Poster Session 05

Session 06. Sensing dynamics in livestock physiology disruption through images and other non-invasive alternatives

Date: Monday 30 August 2021; 9.00 –13.00
Chair: Montanholi / Gondret

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Session 07. Mixed crop/livestock systems – do they deliver more resilient food systems

Date: Monday 30 August 2021; 9.00 –13.00
Chair: Boland

Theatre Session 07

Session 08. Development of genetic and genomic systems in equid breeding

Date: Monday 30 August 2021; 9.00 –13.00
Chair: Stock

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Session 09. Feeding the gut: from microbiome to models

Date: Monday 30 August 2021; 9.00 –13.00
Chair: Abdennebi-Najar / Goselink

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Poster Session 09

Session 10. Early career competition: stressing innovation in pig and poultry industry

Date: Monday 30 August 2021; 9.00 –13.00
Chair: Vigors / Nilsson / Trevisi

Theatre Session 10

Poster Session 10

Session 11. Low carbon animal nutrition: methane emission and climate

Date: Monday 30 August 2021; 13.30 – 17.30
Chair: De Campeneere / Bruggeman

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Poster Session 11

Session 12. Single-step genomic evaluation

Date: Monday 30 August 2021; 13.30 – 17.30
Chair: Wang

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Session 13. (A) Joining forces in the use and conservation of genetic resources

Date: Monday 30 August 2021; 13.30 – 17.30
Chair: Hiemstra

Theatre Session 13

Session 14. The Sm@RT and TechCare projects

Date: Monday 30 August 2021; 13.30 – 17.30
Chair: Morgan-Davies / Halachmi

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Session 15. Methods and practices to assess and improve animal welfare

Date: Monday 30 August 2021; 13.30 – 17.30
Chair: Ammer / Dantas de Brito Almeida

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Session 16. Recent advances in omics technologies applied to small ruminants

Date: Monday 30 August 2021; 14.30 – 18.30
Chair: Hernández-Castellano

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Session 17. Going beyond the Feed vs Food competition: crops and animals together to address food and nutrition security

Date: Monday 30 August 2021; 14.30 – 18.30
Chair: O'Mara / Lee / Boland

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Session 18. Alpine, organic and grassland based cattle production

Date: Monday 30 August 2021; 14.30 – 18.30
Chair: Berard

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Session 19. Young train – Innovative research and extension

Date: Monday 30 August 2021; 14.30 – 18.30
Chair: Kuipers / Mielczarek

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Session 20. Management of the sow and her litter

Date: Monday 30 August 2021; 14.30 – 18.30
Chair: Nilsson / Knol

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Session 21. Plenary session and Leroy Lecture

Date: Tuesday 31 August 2021; 8.30 – 12.30
Chair: Casasús

Theatre Session 21

Session 22. Breeding for improved animal health, welfare and longevity I

Date: Tuesday 31 August 2021; 13.30 – 17.30
Chair: Minozzi / Gardan

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Session 23. The tortoise and the hare: finding the best balance between resilience and efficiency

Date: Tuesday 31 August 2021; 13.30 – 17.30
Chair: Friggens

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Poster Session 23

Session 24. From PLF sensor development to application in the field

Date: Tuesday 31 August 2021; 13.30 – 17.30
Chair: Bloch / Druyan

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Session 25. Sustainable livestock farming in mountain areas and harsh environments - challenge or opportunity?

Date: Tuesday 31 August 2021; 13.30 – 17.30
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Session 26. Regulatory framework and advances in genetics and genomics of farmed insects

Date: Tuesday 31 August 2021; 13.30 – 17.30
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Session 27. Sustainability and human health aspects of dairy and meat products and substitutes

Date: Tuesday 31 August 2021; 14.30 – 18.30
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Session 28. Evolutions and latest developments in equine production and management

Date: Tuesday 31 August 2021; 14.30 – 18.30
Chair: Auclair-Ronzaud / Cox / Mantovani / Vial / De Palo

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Session 29. So you think your science is so great

Date: Tuesday 31 August 2021; 14.30 – 18.30
Chair: Keatinge

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Session 31. ANIMAL NUTRITION 4.0

Date: Tuesday 31 August 2021; 14.30 – 18.30
Chair: Pinotti / Ranilla García

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Session 32. Meat and fat quality in broilers and finishing pigs

Date: Tuesday 31 August 2021; 14.30 – 18.30
Chair: Bee / Stadnicka

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Session 33. Breeding for improved animal health, welfare and longevity: cattle II

Date: Wednesday 1 September 2021; 8.00 – 12.00
Chair: Poppe / Minozzi

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Session 34. Breeding for the future: technology finds application in new phenotypes

Date: Wednesday 1 September 2021; 8.00 – 12.00
Chair: Granados Chapatte / Schibler

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Session 35. Good health of pigs and poultry: increasing robustness through feeding and management - part 1

Date: Wednesday 1 September 2021; 8.00 – 12.00
Chair: Daş / Vigors

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Session 36. Advancing sustainable livestock systems for resilient sustainable food systems

Date: Wednesday 1 September 2021; 8.00 – 12.00
Chair: Lee / Bourgeois Lüthi

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Session 37. Sustainability assessments of insects as food and feed in a circular economy

Date: Wednesday 1 September 2021; 8.00 – 12.00
Chair: Smetana / Veldkamp

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Session 38. Computing in PLF: cloud and edge computing and smart algorithms

Date: Wednesday 1 September 2021; 9.00 – 13.00
Chair: Pastell

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Session 39. Nutrient competition between production and animal health – interactions of nutrition, immunity and microbiota

Date: Wednesday 1 September 2021; 9.00 – 13.00
Chair: Gross

Theatre Session 39

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Session 40. Heifer growth strategies for increased productivity, longevity and reduced environmental impact

Date: Wednesday 1 September 2021; 9.00 – 13.00
Chair: Foskolos

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Session 41. Joint EAAP/ADSA session 'Future of housing for cattle' – part 1

Date: Wednesday 1 September 2021; 9.00 – 13.00
Chair: Costa / Klopčič

Theatre Session 41

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Session 42. Inclusive livestock nutrition: where we have a trade-off between performance, environmental sustainability and animal welfare

Date: Wednesday 1 September 2021; 9.00 – 13.00
Chair: Bruggeman / De Campeneere

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Session 43. Optimisation of sheep and goats production systems: strategies for sustainable and resilient agroecosystems

Date: Wednesday 1 September 2021; 9.00 – 13.00
Chair: Cadavez

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Session 44. PLF methods for measuring health, welfare and caring for individual animals

Date: Wednesday 1 September 2021; 13.30 – 17.30
Chair: Adriaens / Bloch

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Session 45. Free communications in animal genetics

Date: Wednesday 1 September 2021; 13.30 – 17.30
Chair: Cecchinato

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Session 46. Use of genomic information

Date: Wednesday 1 September 2021; 13.30 – 17.30
Chair: Sell-Kubiak

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Session 47. What levers can be activated by stakeholders to promote the livestock transition?

Date: Wednesday 1 September 2021; 13.30 – 17.30
Chair: Thenard / Siqueira

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Session 48. Insects: standardisation and terminology

Date: Wednesday 1 September 2021; 13.30 – 17.30
Chair: Deruytter /Shumo

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Session 49. Transgenerational effect of nutrition (feeding of mother, effect on offspring)

Date: Wednesday 1 September 2021; 14.30 – 18.30
Chair: Kreuzer-Redmer / Alvarez Rodriguez

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Session 50. Novel developments of milk removal in conventional, automated and cow-calf contact systems

Date: Wednesday 1 September 2021; 14.30 – 18.30
Chair: Bruckmaier

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Poster Session 50

Session 51. Organic production of milk and meat

Date: Wednesday 1 September 2021; 14.30 – 18.30
Chair: De Marchi / Maurer

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Session 52. Joint EAAP/ADSA session 'Future of housing for cattle' – part 2

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Chair: Costa / Klopčič

Theatre Session 52

Session 53. Good health of pigs and poultry: increasing robustness through feeding and management - part 2

Date: Wednesday 1 September 2021; 14.30 – 18.30
Chair: Trevisi / Minozzi

Theatre Session 53

Poster Session 53

Session 54. Sheep and goats management and reproduction

Date: Wednesday 1 September 2021; 14.30 – 18.30
Chair: Hadjipavlou

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Session 55. Sustainable Insect Chain (H2020 SUSINCHAIN)

Date: Thursday 2 September 2021; 8.00 – 12.15
Chair: Veldkamp / Gasco

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Session 56. Free communications: Crossbreeding - Conservation of local breeds

Date: Thursday 2 September 2021; 8.00 – 12.00
Chair: Hinrichs

Theatre Session 56

Poster Session 56

Session 57. Exploiting sequence data

Date: Thursday 2 September 2021; 8.00 – 12.00
Chair: Berg

Theatre Session 57

Poster Session 57

Session 58. Animal behaviour: from horses to hens

Date: Thursday 2 September 2021; 8.00 – 12.00
Chair: Boyle / Dantas de Brito Almeida / Mantovani

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Session 59. CYBELE project on high performance computing for precision livestock farming

Date: Thursday 2 September 2021; 8.00 – 12.00
Chair: Maselyne

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Session 60. European collaboration in research towards sustainable pig production

Date: Thursday 2 September 2021; 9.00 – 13.00
Chair: Millet

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Session 61. Workshop: Meta-analysis applied to animal science

Date: Thursday 2 September 2021; 9.00 – 13.00
Chair: Tzamaloukas

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Session 62. Joint EAAP/ADSA session 'Future of housing for cattle' – part 3

Date: Thursday 2 September 2021; 9.00 – 13.00
Chair: Costa / Klopčič

Theatre Session 62

Session 63. Metabolism, oxidative stress and immune function

Date: Thursday 2 September 2021; 9.00 – 13.00
Chair: Sauerwein / Louveau

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Session 64. Sustainable Insect Chain (H2020 SUSINCHAIN)

Date: Thursday 2 September 2021; 13.30 – 17.30
Chair: Veldkamp / Gasco

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Session 65. Contribution of animal breeding to solving societal challenges

Date: Thursday 2 September 2021; 13.30 – 17.30
Chair: Rydhmer

Theatre Session 65

Session 66. Interaction between host genetics and gastro-intestinal tract microbiota genetics

Date: Thursday 2 September 2021; 13.30 – 17.30
Chair: Abdennebi-Najar

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Session 67. Challenges and novel metrics of multi objective tools at farm/ regional level

Date: Thursday 2 September 2021; 13.30 – 17.30
Chair: Zehetmeier / Puillet

Theatre Session 67

Poster Session 67

Session 68. PLF MIR tools to monitor cows' welfare in particular the freedom from disease, hunger and stress (in collaboration with HappyMoo project)

Date: Thursday 2 September 2021; 13.30 – 17.30
Chair: Gengler

Theatre Session 68

Poster Session 68

Session 70. Improving sustainability of pig production by participatory research

Date: Thursday 2 September 2021; 14.30 – 18.30
Chair: Aluwé / Bee

Theatre Session 70

Session 71. Good practices and strategies in dairy farming

Date: Thursday 2 September 2021; 14.30 – 18.30
Chair: Mayer / Klopčič

Theatre Session 71

Poster Session 71

Session 72. Methods and technologies for research and smart nutrition management in dairy and beef cattle

Date: Thursday 2 September 2021; 14.30 – 18.30
Chair: Baumont

Theatre Session 72

Poster Session 72

Session 73. Next generation in animal nutritional science (Young Scientists Session)

Date: Thursday 2 September 2021; 14.30 – 18.30
Chair: Kreuzer-Redmer / Tretola

Theatre Session 73

Session 74. Omics in animal nutrition and physiology (the European joint doctorate program Molecular Animal Nutrition "MANNA")

Date: Thursday 2 September 2021; 14.30 – 18.30
Chair: Eckersall / Sauerwein

Theatre Session 74

Poster Session 74

Session 01 Theatre 1

Phenotypes and strategies to reduce methane emissions of dairy cattle

Y. De Haas[1], A.E. Van Breukelen[1], R.F. Veerkamp[1], G. De Jong[2] and M.N. Aldridge[1]
[1]Wageningen University and Research, Animal Breeding and Genomics, P.O. Box 338, 6700 AH Wageningen, the Netherlands, [2]CRV, Animal Evaluation Unit, P.O. Box 454, 6800 AL Arnhem, the Netherlands; yvette.dehaas@wur.nl

The global livestock sector, particularly ruminants, contribute substantially to the total anthropogenic greenhouse gases. Management and dietary solutions to reduce enteric methane (CH_4) emissions are extensively researched. Animal breeding that exploits natural variation in CH_4 emissions is an additional mitigation solution that is cost-effective, permanent, and cumulative. We quantified the effect of including CH_4 production in the Dutch breeding goal using selection index theory. The current Dutch national index contains 15 traits, related to milk yield, longevity, health, fertility, conformation and feed efficiency. From literature we obtained a heritability of 0.21 for enteric CH_4 production, and genetic correlations of 0.4 with milk lactose, protein, fat and dry matter intake. Correlations between CH_4 production and other traits in the breeding goal were set to zero. When including CH_4 production in the current breeding goal with a zero economic value, CH_4 production increases yearly by 1.5 g/d as a correlated response. When extrapolating this, the average daily CH_4 production of 392 g/d in 2018 will increase to 442 g/d in 2050 (+13%). However, expressing the CH_4 production as CH_4 intensity in the same period shows a reduction of 13%. By putting economic weight on CH_4 production in the breeding goal, selective breeding can reduce the CH_4 intensity even by 24% in 2050. This shows that breeding is a valuable contribution to the whole set of mitigation strategies that could be applied in order to achieve the goals for 2050 set by the EU. If the decision is made to implement animal breeding strategies to reduce enteric CH_4 production, and to achieve the expected breeding impact, there needs to be a sufficient reliability of prediction. The only way to achieve that is to have enough animals phenotyped and genotyped. The power calculations offer insights into the difficulties that will be faced in trying to record enough data. Recording CH_4 data on 100 farms (with on average 150 cows each) for at least 2 years is required to achieve the desired reliability of 0.40 for the genomic prediction.

Session 01 Theatre 2

The usefulness of methane production predicted from cows' heat production

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Sniffers can be used to measure concentrations of gases in the air exhaled or eructated by cows. One way to convert concentration into production is to use daily CO_2 production estimated based on information on cows' characteristics. This conversion uses several coefficients that are assumed to be constant for all the cows. The effect of possible variation in these coefficients is unknown. The objective of this paper was to assess the effect of departure from assumptions of the equation used to estimate the daily CO_2 production on the estimated CH_4 production and the possible impact of such a variation on the genetic evaluation. To answer that question I tested the impact of departure from the assumed values and variation in the coefficients used to predict daily CH_4 production. The magnitude of departure and variation was 1, 10 and 20% of the initial values. The biggest differences from the baseline CH_4 production of 413 l were observed when all the coefficients were adjusted at the same time, the highest departure was due to changes in the c_2 constant. In most of the cases, the average production and SD were similar to the baseline scenario, despite the introduced variation in the coefficients The significant difference was observed only in the case of scenarios with modification of c_2, and c_5 with 10 and 20% of the variation, and when all the coefficients varied at the same time. The predicted methane production level is sensitive to the values of coefficients used in the estimation posing a question on the validity of this approach for the emission inventory. Most of the scenarios showed no substantial changes in the ranking of the animals, and therefore, the impact of variation in predicted methane production on the effectiveness of the selective breeding for lowering the CH_4 production should be limited.

Methane output versus production traits: estimates of genetic associations in Nordic Red cattle

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Understanding the genetic, nutritional, and microbial basis of methane (CH_4) emission in dairy cattle is an important step in developing tools for its mitigation. However, CH_4 is a difficult trait to measure and so far, routine measurement of the trait does not exist in commercial dairy production systems. Although lately concerted efforts have been made to develop CH_4 measurement systems, there is still lack of large-scale data and estimates of genetic parameters and particularly genetic associations between CH_4 output and production and functional traits. The objective was to estimate genetic parameters for the different CH_4 output traits and assess their genetic and phenotypic associations with some of the dairy cattle production traits. Methane was measured from cows using a non-invasive photoacoustic spectroscopy technique using F10 multigas analyser (Gasera Ltd, Turku, Finland). Data was from 309 Nordic Red cows from Finnish dairy research farm including 13,573 weekly records. Production traits were energy corrected milk (ECM), metabolic body weight (mBW), residual feed intake (RFI) and dry matter intake (DMI). Whereas CH_4 phenotypes included: CH_4 production (MeP=CH_4 g/day), CH_4 yield (MeY = g CH_4/kg DMI), CH_4 intensity (MeI = g CH_4/kg ECM) and residual CH_4 production (RMP). Univariate and bivariate repeatability animal models were used for the estimation of heritabilities and genetic correlations (r_g) between traits, respectively. The models included fixed effects of age, kiosk number, lactation week, measurement year-month and random permanent environment and animal effect. Mean MeP, MeY and MeI were 400.1±32.7 g/d, 20.6±4.3 g/kg, 13.9±3.5 g/kg and 6.07±2.1% of GE intake as CH_4. Heritabilities for the CH_4 traits: MeP, MeY, MeI and RMP were 0.04, 0.04, 0.08, and 0.16, respectively. Genetic correlations between MeP and production traits: ECM, mBW, RFI and DMI were moderate to high positive with 0.52, 0.67, 0.48 and 0.49, respectively. Especially, the moderate r_g between MeP with RFI suggest that improving dairy cattle feed utilization efficiency could be one sustainable option for mitigation of CH_4 emission from dairy production systems.

Genetic parameters for long term recording of enteric methane emissions of dairy cows

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Enteric methane emissions of dairy cows can be recorded long term and on a large scale with multi-gas analysers or 'sniffers', installed in the feed bin of milking robots. Sniffer data offers the potential to be applied in breeding programs, with the aim to reduce enteric methane emissions and limit the contribution that the dairy industry has to greenhouse gases. Currently we are investigating how to define methane traits for continuously recorded sniffer data. Methane concentration (ppm) was measured in milking robots in 14 herds from March 2019 to September 2020. In total, data from 181,597 robot visits of 1,698 Holstein Friesian cows were recorded. A robot visit, where methane was recorded, was defined to last at least two and a half minutes to capture both breathing and belching. The visit was defined to start one minute after a cow had entered the robot, to ensure that the cow has reached the feed bin and to account for a delay in the air sample entering the air intake and reaching the sensor. Furthermore, records after five minutes of milking were discarded, because when the cow has finished her pellets she will remove her head from the feed bin. The methane traits were defined as the mean concentration of a visit, daily means of visits, or weekly means of visits, and treated as repeated records. Genetic parameters were estimated with a univariate animal model with repeated measurements in ASReml 4.1. Fixed effects included herd×year×week, parity, and 2nd order Legendre polynomials for days in milk. Preliminary results show that the heritability ranged between 0.08 to 0.23, and is highest for weekly means (0.23±0.02). The genetic standard deviation for weekly means was 73 ppm, indicating that the genetic difference between the 1% highest and lowest emitting cows is 366 ppm. Currently the analyses are ongoing, with a focus on refining the model and trait definitions. The preliminary results indicate that there is genetic variation between cows, and decreasing methane emissions by selection is a possibility in the near future.

Enteric methane emissions are positively associated to residual feed intake in growing bulls

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With the current economic and environmental context, it is interesting to select livestock phenotypes combining high feed efficiency and low greenhouse gas emissions. The aim of this study was to relate the individual variability of enteric methane (CH_4) emissions to that of feed efficiency in growing bulls. One hundred young Charolais bulls were fattened for 6 months during two consecutive years in two independent groups (50/year). In each group, half of the animals received *ad libitum* a total mixed ration either based on maize silage (62% of dietary DM) and high-starch concentrate (MS-S), or based on grass silage (59% of dietary DM) and high-fibre concentrate (GS-F). The CH_4 emissions were individually measured with 2 GreenFeed (GF) systems during 88 days for group 1 and 64 days for group 2. Individual feed efficiency was measured as residual feed intake (RFI) during the same period. The daily number of visits to the GF systems averaged 2.2±0.9 for group 1 and 2.5±1 for group 2. Data were averaged for the whole period. Mean CH_4 emissions were higher (P=0.01) for MS-S diet than for GS-F diet (270±30 vs 254±29 g/d). The RFI values ranged between -0.98 and 1.08 (sd=0.59) and between -0.93 and 0.82 (sd=0.42) with MS-S and GS-F diet, respectively. Data were corrected first for group effect. The relationship between CH_4 emissions (Y) and RFI (X) was tested by linear regression with the diet and its interaction with RFI values included as fixed effects. Regression analysis revealed that daily CH_4 emissions (g/d) were positively linked with RFI (r=0.50, $P \leq 0.01$) with a slope of 26 g/kg DMI irrespective of the diet. With both diets, animals that ingested food in excess of their maintenance and growth requirements emitted more enteric CH_4. The relationship observed in this study should be deepened by considering other CH_4 units (e.g. g/kg DMI), other feed efficiency indices and by studying the mechanisms involved.

Re-thinking modelling of residual feed intake for genomic evaluation in dairy cattle

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Efficiency in utilizing feed has a multiplicative effect and therefore ratio traits are intriguing. Nevertheless, the use of residual feed intake (RFI) has been proposed by many studies. However, the usual modelling of RFI, where dry matter intake (DMI) is regressed on energy sinks, cannot account for the multiplicativity of efficiency. In this study we explored a different modelling of RFI. We posed a new measure (nRFI) where DMI is regressed on expected DMI (eDMI) calculated using feeding norms and realized production. The nRFI model was tested on feed efficiency data that included 15,940 weekly records from 679 primiparous Nordic Red cows from four Finnish research farms. The nRFI model comprised of a fixed regression on eDMI nested within herd effect and three random regressions on eDMI nested within herd-test-month, permanent environmental, and additive genetic effects. The model was compared to a standard RFI model where DMI was regressed on the energy sinks: energy corrected milk (ECM), metabolic body weight (BW), BW loss and BW gain. The standard RFI model included also fixed herd-test-month and lactation stage effects as well as random permanent environmental and additive genetic effects. The estimated heritability was 0.20 for the nRFI and 0.19 for the RFI model. Single-step genomic breeding values (GEBV) from both models were compared to GEBVs for energy conversion efficiency (ECE) and ECM that were estimated from the same data. The weekly ECE ratio observations were formed by dividing ECM observations by the corresponding metabolizable energy intake. Fixed regression coefficients from the nRFI model varied between 0.92 and 0.99 and agreed with the differences in metabolizable energy content of the diets fed. Regression coefficients from the RFI model were biologically not meaningful. The nRFI GEBVs were highly favourably (-0.71) correlated with ECE GEBVs and moderately favourably (-0.26) correlated with ECM GEBVs. In contrast, the RFI GEBVs were favourably (-0.49) correlated with ECE GEBVs but moderately unfavourably (0.21) correlated with ECM GEBVs. The correlation between nRFI and RFI GEBVs was only 0.64.

A new genomic evaluation for feed efficiency in Holsteins

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The large economic impact of feeding costs in dairy cattle production has motivated research and development of feed efficiency evaluations worldwide. Breeding animals that utilize feed more efficiently has the potential to reduce costs considerably, minimize environmental footprints and improve dairy industry sustainability. However, the cost of recording individual feed intake has been the most critical obstacle for selecting for feed efficiency in dairy cattle. Genomics has had a profound impact on the ability to enhance traits of economic importance, some of which have historically been too difficult or costly to include in industry-wide recording schemes. Therefore, genomics provides an excellent opportunity for selecting for feed efficiency. Effective April 2021, Lactanet Canada will launch official genomic evaluations for feed efficiency in Holsteins. This new genetic evaluation for feed efficiency was achieved as the main deliverable of a large collaborative research project involving the University of Guelph, the University of Alberta, and Lactanet Canada partnering with other Canadian and international organizations. The data originates from multiple herds in Canada, United States, Denmark, Australia, and Switzerland. The single-step genomic evaluation includes three traits within each of two periods during first lactation: 5-60 DIM and 61-305 DIM: Metabolic Body Weight, Energy Corrected Milk and Dry Matter Intake. All traits are weekly averages expressed in kg/day (ECM and DMI) or kg (MBW). Re-parametrization of GEBV for DMI gives a measure of feed efficiency that is genetically independent of ECM and MBW and is expressed as relative breeding value (RBV) for Feed Efficiency, with mean of 100 and SD of 5 as all functional traits in Canada.

Residual feed intake of genomic tested young Holstein bulls

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The Intergovernmental Panel on Climate Change has reported that anthropogenic greenhouse gases (GHG) have been responsible for most of the observed temperature increase since the middle of the twentieth century. Agriculture and livestock are major contributors to the rise in global temperature due to GHG emissions. Methane (CH_4) is associated with global warming, therefore the mitigation of emissions has become a hot research topic. The objective of this study was to calculate the residual feed intake (RFI) of young genomic tested Holstein bulls. The RFI is an indicator of feed efficiency which can indirectly quantify the reduction of GHG emissions. Data were collected on 111 young Holstein bulls between May 2018 and July 2020 at the genetic centre of the Associazione Nazionale Allevatori della Razza Frisona e Jersey Italiana (ANAFIJ, Cremona, Italy). Pens of the genetic centre were equipped with Roughage Intake Control system (RIC; Hokofarm Group, Voorsterweg, The Netherlands) to record dry matter intake (DMI, kg/d), and with the Automated System (GreenFeed C-Lock Inc., Rapid City, SD, USA) to record CH_4 emissions (g/d). Average daily gain was estimated as the regression of body weight on test days. Expected DMI was obtained from a linear regression of DMI on metabolic body weight (BW0.75) and average daily gain (ADG). Residual feed intake was calculated as the difference between measured DMI and expected DMI. Age, body weight, BW0.75, ADG, actual DMI, expected DMI, CH_4 emissions and RFI averaged 276±42 d, 298±63 kg, 71.91±11.71 kg, 1.12±0.29 kg/d, 8.24±2.20 kg/d, 8.10±1.47 kg/d, 220±41 g/d and 0.07±1.56, respectively. Pearson correlations between RFI and CH_4 was weak (0.16; $P<0.05$). Negative RFI values identify animals with lower feed intake and greater efficiency. Improving individual feed efficiency using RFI would lead to a reduction of GHG emissions in the long-term.

Evaluation of different models to define a more suitable residual feed intake estimation in sheep

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Residual feed intake (RFI) is the difference between an animal's actual feed intake (FI) and its expected feed intake based on its size and growth or level of production. There is a lack of studies investigating the incidence of wool growth in the RFI estimation. This study aimed to compare different models for the estimation of RFI in Merino lambs, based on data of 577 animals, born in 2018 and 2019, sired by 16 rams. For both generations, after 14 days of acclimatization, the one-year-old lambs (296±48 days) were allotted to one of six RFI tests based on sex, birth type and age. In each test, animals were allocated to one of five outdoor pens with five automated feeding systems each pen in accordance with body weight (BW), sex and sire, during a 42-day test period. Lambs were fed *ad libitum* with Lucerne haylage (CP 22.0%, FDA 27.4%, FDN 35.1%, EE 2.2%). Models were compared using the Akaike Information Criterion (AIC). A first comparison considered the basic model including as fixed effects sex-pen-trial, average daily gain (ADG), mean metabolic weight (MW), and then we evaluated the inclusion of the following effects: birth type, lambing batch-year, age, rib-eye area, fat thickness, and two estimates of wool growth during the test, Trial Clean Fleece Growth and Trial Staple Length Growth, calculated using the Wool Production Potential principles. Another analysis was conducted using only 2019 progeny when Staple Length Growth (SLG) was recorded during the test. The model was as described above but, in this case, estimates of wool growth were SLG, estimated greasy fleece growth based on SLG and an ADG that did not consider the weight of the wool were included. The results indicated that the basic model (sex-pen-trial, ADG, MW) is the most parsimonious for both analyses, the others fixed effects, body composition and fleece growth traits were not significant (P>0.05) (AIC difference >2). Furthermore, RFI values estimated with the basic and alternative models were highly correlated (r=0.99). In conclusion, it might be not necessary to include estimations of wool growth during 42-day tests in RFI models when evaluating Merino sheep.

Multitrait analysis of RFI: heterogeneity of residual variances and modelling of body weight change

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In the usual definition of RFI dry matter intake (DMI) is regressed on energy sinks. This implicitly assumes that energy sink covariates are measured with little or no measurement error and a missing covariate for an animal results in deletion of its records for RFI. An alternative is to apply a multitrait (MT) model which can address these deficiencies. In this approach Cholesky decomposition is applied on MT variance covariance matrix (VCV) of energy sinks to derive MT RFI. Here DMI is expressed in a sequential relationship that is conditional on the different energy sinks (energy corrected milk (ECM), metabolic body weight (mBW), body weight loss (BW_{LOSS}), body weight gain (BW_{GAIN})). Because accuracy of production and intake measurements vary across lactation stages the heterogeneity of residual variances have not been reported for MT approach. Also, it is hypothesized that some issues in deriving RFI from production traits may be rooted in the difficulty to accurately model BW changes during lactation. The objective was to model BW change during lactation, as BW_{LOSS} and BW_{GAIN} traits and test effects of heterogeneity of residual variances on estimates of genetic parameters for RFI. Data was from 731 Nordic Red cows from four Finnish research farms including 20,533 weekly records. Traits were ECM, mBW, BW_{LOSS}, BW_{GAIN} and DMI. Lactation period was divided into five residual classes: <5, 5-8, 9-12, 13-32 and >33 lactation weeks. A MT repeatability animal model with age, lactation week, year-season, herd-test-month, random permanent environment, and animal effects was used to estimate MT VCV matrix. A square-root free modified Cholesky decomposition was then applied on VCV matrix to derive the MT RFI. During lactation, heritabilities for sink traits ranged from 0.15-0.25, 0.65-0.80, 0.04-0.11, 0.08-0.27 and 0.30-0.36 for ECM, mBW, BW_{LOSS} and BW_{GAIN} and DMI, respectively. Heritabilities for MT RFI were 0.06, 0.08, 0.09, 0.09, 0.07, for the five residual classes, respectively. Accounting for heterogeneity of residuals and modelling body weight change gave a reasonable description of RFI across lactation.

Longitudinal residual feed intake criterion for selection

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Residual feed intake (RFI) corresponds to the difference between actual feed intake (FI) and that predicted on the basis for production and maintenance (P&M) requirements. It is most often obtained by a multiple regression (genetic or phenotypic) of FI on P&M indicators (growth rate, body composition, metabolic body weight). Estimating the genetic covariance matrix between components of RFI is therefore necessary to perform selection on RFI without detrimental effects on other traits, by directly using RFI obtained by genetic regression or by computing a selection index that combines RFI obtained by phenotypic regression and P&M traits with appropriate weights. This matrix can easily be derived from the result of a multiple trait model. However, this approach is difficult to apply in the case of multiple repeated measurements of FI and P&M that are now available thanks to the development of automatic self-feeders in different species. To overcome this difficulty, we used a structured antedependence approach (multi-SAD regression model) to account for the longitudinality of the data. The multi-SAD regression model has the advantage to be applicable even when some phenotypes for production traits are missing, to directly provide genetic regression coefficients of FI on P&M from its outputs, to easily compute genetic and phenotypic covariance matrix between time points and traits, and ultimately to provide RFI that are genetically independent of P&M traits at all time points. We applied the multi-SAD regression model to FI and P&M traits recorded for 2,435 French Large White pigs over a 10-week test period. Heritabilities of RFI obtained with the multi-SAD regression model remained quite stable over time, ranging from 0.14±0.04 to 0.16±0.05. Using a clustering approach, we identified 3 patterns of EBV trajectories that can be characterized using a limited number (2 to 3) of summarized breeding values (SBV) derived from the eigendecomposition of the genetic covariance matrix of RFI. Selection to the desired trajectory pattern is possible using SBV as selection criteria.

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Infrared thermography as indicator trait for feed efficiency in dairy cattle

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Breeding for feed efficient cows has become more important over the past years. Genetic selection on feed efficiency will lead to reduced feed costs. To improve feed efficiency, individual feed intake has to be collected. Unfortunately, individual feed intake records are sparse, because recording individual feed intake is expensive and limited numbers of animals can be measured at the same time. To breed for feed efficiency in dairy cattle, an indicator trait (e.g. infrared thermography) for feed efficiency could be an interesting alternative. Infrared thermography (IRT) is fast, cheap and non-invasive. For a large group of animals, IRT phenotypes can be collected in an automated system. The aim of this study was to investigate the relationship between IRT traits and feed efficiency in dairy cattle. In this study, 30 dairy cows (HF) in late lactation with milk production, body weight and feed intake records were used. Individual roughage intake was measured using Roughage Intake Control-bunks. Residual feed intake (RFI) was used as a measure for feed efficiency. IRT measurements were taken on Tuesday afternoon and Wednesday morning for two consecutive weeks. The IRT camera (FLIR A655sc, Flir Systems) was placed at the exit of the milking parlour. The camera made consecutive videos of two minutes each for the entire duration of milking. IRT traits investigated were the ocular area, forehead, cheek, snout, left and right side of the back and left and right flank. Temperature means were significantly ($P<0.05$) different between evening measurements but not between morning measurements. Pearson correlations were better for morning (0.41-0.85) compared to evening (0.35-0.79) measurements. The estimate for RFI was 0.20 ($P=0.04$) in a linear mixed model explaining average temperature measured on the right side of the back. Estimates for dry matter intake in relation to different IRT traits on the back were between 0.12-0.18 ($P<0.03$). Altogether, IRT traits forehead and back show potential to use as indicator trait for feed efficiency in dairy cattle which can be used in breeding. Further research will focus on the prediction of feed efficiency using IRT traits.

A hologenomic approach for genomic selection on methane emissions and feed efficiency

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Methane emission and feed efficiency are influenced by the cow and rumen microbial genes. Genetic improvement selects the most favourable genes for future generations and can modulate their microbiota composition and consequently the microbial gene functions involved in the digestion of feed. This study used 4,624 weekly averaged records of methane (MET) from 1,501 cows obtained with a nondispersive infrared methane detector, metagenome information obtained with nanopore sequencing from 437 cows, 11,042 dry matter intake (DMI) observations from 551 cows, as well as production and conformation information from the milk recording scheme in 19 farms. Eukaryote microorganisms have an important role in methane production, 192 KEGGs showed association with MET ($P_{adj}<0.05$; $\log_2FC>0.5$), many of them carried by the SAR (Stramenopila, Alveolata, and Rhizaria) group. The heritability of the relative abundance for these and other microorganisms ranged between 0.08 and 0.46. Large genetic correlations (>0.50) were estimated between Ciliophora, Euryarcheota and Chytridiomycota with methane production. We inferred that a larger relative abundance of fungi and protozoa has a causal effect on increased MET. The complexity of the rumen metagenome was aggregated in principal component variables that were used for downwards analyses. These aggregated metagenome variables showed heritabilities between 0.15 and 0.34, and strong positive genetic correlation with methane traits (0.50-0.80), and positive with DMI (0.32). DMI showed a positive (0.20-0.27) genetic correlation with MET traits. Furthermore, the analyses showed positive genetic correlation between MET and DMI with production traits and body capacity type traits. Our results showed that it is possible to use these data as a reference population for genomic selection on feed efficiency and methane emissions. DMI and MET must be properly accounted for in the Spanish total merit indices.

Strategies for deriving auxiliary traits for lameness prediction and breeding value estimation

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Lameness in dairy cattle presents a widespread animal welfare and economic issue. Early detection and prediction of lameness may help to reduce negative effects for farms. However, lameness is caused by a variety of factors such as housing, feeding or management and may be associated with changes in milk performance or behaviour. One aim of the D4Dairy project is the development of a decision-support tool for early lameness detection based on already existing data from national performance recordings, farm records, veterinary records, claw trimmings and different kinds of milking and sensor systems measuring e.g. activity, temperature or rumination (smaXtec, SCR by allflex). Additionally, these data should serve as basis for deriving auxiliary traits for breeding value estimation for claw health. However, integrating various types of farm data within and across farms presents a huge challenge in terms of data quality and model validity. The aim of this work is to present strategies for formulating prediction and genetic models for lameness when using various types of data but also different systems and input parameters. First of all, data from different sources, which are recorded at different scales, at different times and by different people have to be checked for completeness, reliability and plausibility and combined in a common format. To enhance prediction and breeding value estimation, the unknown variation due to farm and system differences has to be minimised using the most promising explanatory variables. For example, the type of milking system (automatic milking system vs conventional milking parlour with fixed milking times) may influence the occurrence of lameness itself, but at the same time affects cow behaviour and thus the validity of activity sensor data for lameness detection. Furthermore, farms use different types of sensors, which may vary in terms of suitability for lameness detection. Thus, predictive models as well as those for breeding value estimation have to fit various data while still being able to use large amounts of information to ensure validity.

Estimation of genetic parameters for dairy cattle behaviour traits generated by AMS

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The use of automatic milking systems (AMS) in dairy cattle housing steadily is increasing. Hence, apart from temperament, also the ability of the cow to cope with a technical environment is a new trait of interest. This new trait is largely determined by learning behaviour. For a dairy cow, an AMS may be viewed as a test system that automatically is documenting behaviour in general and specifically learning behaviour. Aim of the present study was the estimation of genetic parameters for traits defined as average daily visits and average daily milkings. Visits and milkings differ as the AMS rejects milking of cows when they visit the unit too frequently. As behaviour and specifically learning behaviour is highly influenced by adaptation, attention was given to the time period of familiarity or 'experience' with the AMS. Data from a large dairy farm equipped with 27 AMS units was analysed with the start of the observation period being the switch from the old milking system to AMS. Data comprised 2,245 Holstein cows with 4,190 lactations documented from May 2017 to June 2019 and was aggregated on a per-day basis to form 949,990 daily means for the traits no. of visits and no. of milkings. Time periods for 'experience' were defined as 0-75, 76-150, 151-251 and 251+ days. Stage of lactation was defined as three time periods for days in milk (DIM) 0-100, 101-200 and 201+. For the entire time covered by the study, 5 time periods were defined as seasons. Estimation of variance components via REML under a bivariate animal model was conducted with the fixed effects of parity (1, 2, 3, 4+), season, and the combined effect of stage of lactation × 'experience', nested within parity. Daily milk yield was included as a covariate. Random effects were a permanent environmental effect associated with the cow and the additive genetic effect of the animal. Estimates of heritability were 0.15 and 0.14 for visits and milkings, respectively, and a genetic correlation of 0.84 between the two traits. Average no. of visits and milkings decreased with increasing 'experience' and was more pronounced for cows in first lactation as compared to older cows.

Genotype by environment interaction for persistency of production in multiparous SA Holsteins

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Producers choose a pasture (PST) or total mixed ration (TMR) production system (PS) based on the average rainfall of the region in which SA Holstein herds are located. To avoid reranking of sires based on their daughters' performance requires appropriate estimated breeding values (EBV) in the presence of a genotype by environment interaction (GxE) between varying feeding approaches. Different heritabilities of separate analyses of the PS showed evidence of a GxE. It was shown using a test-day (TD), first-3-lactations (3L), alternative random regression model (aRRM) and was proposed for genetic evaluations (GE) of milk (MI), butterfat (BF) and protein (PR) production. The aRRM allow random effects to constantly change over the lactation and estimates an additive-genetic-average persistency (GPe) and -production effect (GPr). The GPe was shown to cause GxE between PS in primiparous cows in a joint aRRM analysis. In this study, we did 2 types of analyses using TD records from 3L using the aRRM. In the first analysis each of the 3L were defined as a TMR or PST lactation, resulting in a 6-separate-traits analysis, each trait having a GPe and GPr (12 principal components, PC) in the aRRM (aRRM12). In the second analysis (same dataset) each of the 3L were undefined as a TMR or PST lactation, resulting in a collective-PS, 3-trait analysis (6 PC) in the aRRM (aRRM6). The aRRM6 model represents a national GE model. These 2 types of analyses were done for MI, BF and PR each using REML. The GPe correlations between TMR and PST in the aRRM12 analysis were 0.21, 0.63 and 0.60 for the 3L of MI, respectively. Pearson correlations of the collective-PS persistency EBV (EBVpe, aRRM6) with the PST PS EBVpe (aRRM12) were 0.23, 0.67 and 0.67 for the 3L of MI, respectively. Corresponding Pearson correlations of the collective-PS EBVpe with the TMR PS EBVpe were (0.87, 0.91 and 0.89). Results for BF and PR were in line with MI. Reranking of sires between TMR and PST will take place in the 3L due to the GPe. Selecting on EBVpe from the aRRM6 will lead to consistent genetic response for persistency in TMR, but not in PST.

Preliminary evidence of genes associated with milk fatty acid profile in different cow breeds

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Bovine milk is an important source in human nutrition, however the fat content is often criticized as a risk factor in cardiovascular diseases. To improve dairy products benefit, 'precision breeding', using marker-assisted selection, could be used to modify the fatty acid composition. Genome-wide association study (GWAS) is the means to identify genes and variants associated with these molecules. Here a preliminary investigation of milk fatty acid profile was conducted in milk collected at around 60 and 120 days after calving from different cow breeds (Holstein; HO, Simmenthal; SM, Simmenthal × Holstein crossbred; SM×HO, and Podolica; POD). A total of 113 animals (26 HO, 30 SM, 27 SM×HO and 30 POD) were genotyped with different SNP chip platforms, and, after quality control, 37,000 common markers were analysed. GCTA software was used for the single-SNP GWAS and gene-based approach (fastBAT). Genes were functionally prioritized using PANEV R package. Among the obtained results, two genes (*STAT1* and *GLS*) were found suggestively associated with EPA (C20:5) fatty acid in HO. Both genes were previously associated with unsaturated fatty acid in HO cows. We also identified signals in common among all the breeds. *PPARD* and *AP2A2* genes were associated with total PUFA and total MUFA, respectively. In a previous study, in cattle, *PPAR* gene family was identified as regulator of *AP2A2* expression and both associated with lipid metabolism. *GAL3ST2* and *NEU4* genes were here associated with caprylic acid (C8:0). Finally, significant SNPs mapping nearby *SCD*, a well-known gene associated with lipid metabolism, were associated with C18 desaturation index. The results here obtained are preliminary, however identify new genes useful to improve milk healthiness through breeding. Acknowledgement: this research was funded by MIPAAF in the national research project MIQUALAT (D.M. 16844/7100/2019), and partly supported by CEF Highlander project (INEA/CEF/ICT/A2018/1815462).

Influence of pedigree on effective population size in European Red Dairy Cattle

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The effective population size and inbreeding per generation are among the most important measures to maintain genetic diversity. In our study, we calculated the effective population size of the simulated population, using the inbreeding coefficients estimated based on actual pedigree information. We simulated four different populations. Pedigrees were simulated on the basis of a real pedigree comprising more than four million animals from the European red dairy breeds. Each of the populations has a different number of individuals (from 20 to 19,610). Effective numbers of founders in each population vary from 3.8 to 77.7 individuals. The average and maximum inbreeding coefficients are different for each population. Inbreeding coefficients were estimated using Meuwissen and VanRaden approach. Meuwissen method is based on decomposition of the additive genetic relationship matrix, while VanRaden approach is based on the tabular method taking into account missing relatives. In this method, for animals with missing relatives, the inbreeding coefficient is assumed to be equal to the mean value of this coefficients calculated for animals with known relatives. We used calculated coefficients of inbreeding to estimate the effective population size. To obtain these estimates for each population, we chose the method that takes into account individual increase of inbreeding for each animal. Apart from calculating the effective population size, we estimated the effect of different levels of pedigree completeness for inbreeding coefficients. In that case, we applied a linear mixed model with the levels of pedigree depth incorporated as a random effect.

Session 02 Theatre 6

Benefits and perils of import in small cattle populations

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Small cattle populations can import genetic material to increase genetic gain and possibly genetic variability. Despite the benefits, import diminishes the importance of domestic selection. The aim of this paper was to determine in which settings small cattle populations benefit from importing. We simulated two populations that mimicked a larger foreign and a smaller domestic population. Foreign population implemented more accurate and intense selection to achieve a higher initial and annual genetic gain. We compared the genetic gain of 20 scenarios, in which the domestic population used foreign sires, that differed in the percentage of domestic females mated with foreign sires and the genetic correlation between the populations. Scenarios were tested in a setting in which the domestic population implemented the genomic selection either simultaneously with the foreign population or with a 10-year delay. We next partitioned genetic trends to quantify the contributions of domestic and foreign populations to the domestic genetic gain. When genetic correlation between the populations was 0.9, the use of foreign sires increased the domestic genetic gain regardless of the time of implementing domestic genomic selection. Here, the domestic genetic gain increased with increasing use of foreign sires but with a diminishing return. When the genetic correlation between the populations dropped to 0.8, the use of foreign sires only increased domestic genetic gain when we implemented the domestic genomic selection 10-years after the foreign population. The partitioning analysis revealed that the contribution of foreign population to domestic genetic gain increases with increasing use of foreign sires irrespective of the benefit to the domestic genetic gain. The results show that for small populations to benefit from importing, they should have a high genetic correlation with the foreign population. They should also adjust the use of foreign sires by weighing the benefit for genetic gain against the diminished contribution of domestic selection.

Session 02 Theatre 7

Spatial modelling improves genetic evaluation in smallholder breeding programs

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Breeders and geneticists use statistical models to separate genetic and environmental effects on phenotype. A common way to separate these effects is to model a descriptor of an environment, a contemporary group or herd, and account for genetic relationship between animals across environments. However, separating the genetic and environmental effects in smallholder systems is challenging due to small herd sizes and weak genetic connectedness across herds. We hypothesised that accounting for spatial relationships between nearby herds can improve genetic evaluation in smallholder systems. Furthermore, geographically referenced environmental covariates are increasingly available and could model underlying sources of spatial relationships. The objective of this study was therefore, to evaluate the potential of spatial modelling to improve genetic evaluation in dairy cattle smallholder systems. We performed simulations and real dairy cattle data analysis to test our hypothesis. We modelled environmental variation by estimating herd and spatial effects. Herd effects were considered independent, whereas spatial effects had distance-based covariance between herds. We compared these models using pedigree or genomic data. Results: The results show that in smallholder systems: (1) standard models do not separate genetic and environmental effects accurately; (2) spatial modelling increases the accuracy of genetic evaluation for phenotyped and non- phenotyped animals; (3) environmental covariates do not substantially improve the accuracy of genetic evaluation beyond simple distance-based relationships between herds; (4) the benefit of spatial modelling was largest when separating the genetic and environmental effects was challenging; and (5) spatial modelling was beneficial when using either pedigree or genomic data. We have demonstrated the potential of spatial modelling to improve genetic evaluation in smallholder systems. This improvement is driven by establishing environmental connectedness between herds, which enhances the separation of genetic and environmental effects. We suggest routine spatial modelling in genetic evaluations, particularly for smallholder systems.

Session 02 Theatre 8

Heat waves effect on Brown Swiss milk yield and quality

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The temperature humidity index (THI) is effective in estimating effects of the environment on dairy cows' efficiency and welfare. Heat waves (HW) have been entered as concept of long duration of warm environmental climatic conditions, able to affect dairy cows. The present study aims to estimate the effect of HW on the Italian Brown Swiss (IBS) population production efficiency. The definition of HW was adapted to the study's aims considering it as a period lasting from 2 to 5 consecutive days with a THI (minimum, mean and maximum) over the known threshold for the IBS population. The aim of this study was to estimate the effects of HW of different duration (from 2 to 5 days) in IBS, considering different production traits.10 years data from test day record system at national level were considered and merged with data from 76 weather stations. A mixed effects model for repeated measures was applied to evaluate the effects of HW on each production trait. Month, parity class and DIM class were considered as fixed effects, while herd and year were considered as random effects. When recorded THI exceeded the maximum THI threshold, cows yielded from 32 g (2 days) to 40 g (5 days) of daily protein less ($P<0.0001$). When THI exceeded the minimum THI threshold, cows produced from 30 g (2 days) to 32 g (5 days) less ($P<0.0001$) of daily protein yield. The FCM yield was lower in cows subjected to HW over the maximum THI, worsening in relation to the HW duration (from 554 g for a duration of 2 days to 850 g at 5 days lasting HW; $P<0.0001$). It is clear how HW affect efficiency in this breed, although it is considered more tolerant than others. Moreover, although from a physiological perspective minimum and maximum THI values have a different meaning, both showed to be equally effective in estimating how HW affect milk production traits in IBS.

Session 02 Theatre 9

Predicting lifetime production and longevity of organic dairy cows from 1st or 2nd lactation data

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Longevity of dairy cows is a key trait combining all functional traits and is decisive for sustainability of dairy production at economic, environmental and ethical level. We used herdbook data of culled Swiss dairy cows to predict longevity (days) and average lifetime daily milk yield (LT_DMY, kg milk) of dairy cows in low-input dairy farms from data easily available for the farmer. First, we assessed the suitability of 1st vs 2nd lactation data to predict longevity and LT_DMY without (n=10,031 cows, 384 farms) and with information on number of inseminations (n=6,011 cows, 372 farms). Second, we tested if lactation curve parameter estimates (LCPs) derived from test day records can be successfully used to predict LT_DMY and longevity (n=1,632 cows, 321 farms). Finally, we investigated breed differences between local dual-purpose breeds and pronounced dairy type breeds in a subset of mixed herds (n=1,796 cows, 72 farms). Models based on 2nd lactation data were consistently better across all traits investigated. Although estimation of LCPs was only possible with sufficient reliability for about 16% of the cows, models including LCPs performed best in predicting LT_DMY with a mean predictability of 73.3%. By contrast, longevity models performed best when using insemination data, but mean predictability only reached 4.6%. Somatic cell count, breed, calving interval, age at first calving, lactation curve persistency, fat protein ratio and information on alpine pasturing were additional traits improving predictions of both traits. Investigation of breed differences in mixed herds revealed lower LT_DMY in the local breeds Simmental and Original Braunvieh compared to Swiss Fleckvieh and Holstein cows. Original Braunvieh lived longer than Holstein (1,949±70 SE vs 1,709±54 SE days, P=0.046), while the local Simmental cows (1,681±61 SE days) did not. We conclude that it seems possible to develop models for TL_DMY, while reliable prediction of longevity remains challenging using information at farmer's hands.

Session 02 Theatre 10

Transgenerational heat stress effect on genetic parameters for weight traits in dual-purpose cattle

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The aim of the study was to analyse the transgenerational and direct impact of mean daily temperature-humidity index (mTHI) and number of heat stress days (nHS) at different recording periods (7 d and 56 d) during late pregnancy (a.p.) and postpartum (p.p.) on genetic parameter estimates for birth weight (BW) and weight gain traits (200 day- and 365 day-weight gain (200dg, 365dg)) in the offspring generation of the local dual-purpose cattle breed 'Rotes Hoehenvieh' (RHV). The dataset included 5,434 observations for BW, 3,679 observations for 200dg and 2,998 observations for 365dg. To calculate the mTHI, climate data from public weather stations with minimal distance to the farms was used. Within the two recording periods, the number of HS days (mTHI≥60) was counted. Both heat stress (HS) indicators mTHI and nHS were classified. Bivariate animal models were applied, considering same traits from the mTHI- and nHS-classes as different traits. The AI-REML algorithm was used to estimate (co)variance components, genetic correlations and estimated breeding values (EBV). Genetic correlations <0.80 between same traits from different mTHI- or nHS-classes as well as substantial alterations of EBV in different climatic conditions were taken as indicators for possible GxE. Additive genetic variances and heritabilities increased for all traits when calves and dams were kept under HS conditions, which was observed for all climate recording periods. A very low mTHI (indicating cold stress) during the 56 d p.p. period caused similar results for 365dg. Results indicate a pronounced genetic differentiation due to climatic stress with possible positive effect on selection response, also from a transgenerational aspect. Considering same traits from mTHI- and nHS-classes in greater distance, genetic correlations were <0.80, indicating GxE. Fluctuations of sire EBV across the mTHI- or nHS-classes support the impact of time-lagged THI impact on genetic (co)variance components. Some sires displayed stable EBV across the climatic range, indicating the possibility to improve robustness in the RHV outdoor population genetically.

Session 02 Theatre 11

Physiological determinants underlying differences in feed efficiency between crossbreed beef cattle

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The part of meat offer from crossbreed growing cattle originating from a dairy breed mother and a beef breed father increase steadily. Aim of the present study was to compare feed efficiency and prioritize its physiological and behavioural determinants among the most widespread crossbreeds used in Switzerland. 88 fattening bulls (169±13 kg BW) from Brown Swiss mothers and sires of Angus (AN), Limousin (LI) or Simmental (SI) breeds received one of two corn silage based TMR (A with alfalfa, B with beet pulp and grass silage), until slaughter either at 477 or 530 kg BW. Individual feed efficiency was computed over 85 days initiating at 230±18 kg BW as either residual feed intake (crossbreed not included in RFI model) or feed conversion ratio (FCR). Concomitantly, digestibility, feeding behaviour, physical activity, gaseous production and consumption, the isotopic N discrimination of plasma proteins ($\Delta^{15}N$) as a proxy of N use efficiency, and carcass composition were measured. All data were analysed by ANOVA with diet, breed type and their interaction as fixed effects. The relationships between RFI or FCR and the measured determinants were explored by regressions using GLM procedures. AN differed from LI and SI by higher DMI, RFI, FCR, frequency of ruminating chews, daily CH_4 production, $\Delta^{15}N$ and carcass fatness ($P<0.05$). LI differed from AN and SI by higher ingestion time (%) and carcass muscle percentage, and from SI by lower DMI and higher CH_4 yield ($P<0.05$). The best single variables explaining most of the variation were carcass fatness for RFI ($R^2=0.29$) and $\Delta^{15}N$ for FCR ($R^2=0.34$). Relationships were improved when CO_2 yield for RFI or daily O_2 consumption for FCR were combined with physical activity (%), carcass fatness and $\Delta^{15}N$ in multiple regressions ($R^2=0.41$ and 0.42 for RFI and FCR, respectively; every slopes >0, $P\leq0.05$). The inclusion of the crossbreed effect did not improved such relationships ($P>0.20$). In conclusion, when receiving high-energy diets, LI and SI crossbreed bulls showed higher feed efficiency than AN ones, which is at least partly linked to their higher lean mass and a more efficient use dietary N.

Session 02 Theatre 12

Genetic evaluation of carcass traits considering different fattening systems in Switzerland

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Genetic evaluations for carcass conformation (CC), carcass fat (CF) and carcass weight (CW) for calves (c) and adults (a) have been published for the six major beef cattle breeds in Switzerland. Since 2018, Mutterkuh Schweiz – the Swiss Beef Cattle Association – is verifying parentage based on SNP-chip technology. The genomic data from the parentage verification was used to develop a genomic selection framework for the Limousine breed. At the same time, the prediction of 'genomic-free' breeding values has been optimized. The previously used multivariate evaluation using six traits combining two fattening systems (c and a) and three different phenotypes (CC, CF and CW) was transformed to three separate evaluations for every fattening system (c, a and Natura-Beef (n) – referring to ten-month-old weaned and after weaning slaughtered animals raised in suckler cow husbandry). Phenotypic observations (CC, CF and CW) and pedigree data have been processed for estimating genetic parameters applying tri-variate (CC, CF and CW) animal models. Animal sex, abattoir, year×season at slaughter, breed combination sire×dam and grader (except for CW) were modelled as fixed effects. Herd×year at slaughter and animal were treated as random effects in the evaluations of the production systems c and a, while herd at slaughter and animal were treated as random effects for n. In order to correct for age at slaughter, linear and quadratic regression coefficients on age were fitted as covariates. In these multi-breed evaluations, genetic groups are defined by breed and birth year. Estimated heritabilities were moderate to high (CWc 0.18, CWa 0.20, CFc 0.22, CFa 0.25, CCc 0.38, CCa and CFn 0.39, CCn 0.42, CWn 0.55). Genetic correlations were moderate between CW and CC (c 0.46, n 0.45, a 0.40), CW and CF (c 0.30, n 0.42, a 0.21), and close to zero between CC and CF (c 0.19, n -0.06, a 0.01). The de-regressed predicted breeding values were used as pseudo-phenotypes to estimate direct genomic breeding values. The use of genomic information resulted in promising increases of reliabilities of genomic breeding values over those originating from parental averages.

Session 02 Theatre 13

Methane emission from dairy cows measured using laser methane detectors and respiration chambers

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Aim of the present study was to compare methane (CH_4) emissions from lactating cows assessed in a free-ranging barn with a handheld laser methane detector (LMD) and measured in open-circuit respiration chambers (RC, n=4). The study was carried out at FBN Dummerstorf using 20 lactating dual-purpose Fleckvieh cows. At the start of the experiment, cows were in average 283.3 DIM in 3.7 lactation. Cows were fed two total mixed rations (TMR) either based on maize silage or grass silage in a cross-over design with a 4-week adaptation period on each TMR. The 20 cows were studied in 5 blocks from November 2018 until August 2019. On each ration, CH_4 concentration measurements using the LMD were performed in the free-stall barn on 3 consecutive days. One week later, individual CH_4 production was measured in RCs on 2 consecutive days. Cows in the free stall barn as well as in RCs were milked and fed twice a day. Body weight, feed intake, milk yield and ambient climate parameters were recorded daily and milk composition was analysed weekly. Data analysis was carried out using SAS 9.4. Cows fed with the maize silage vs grass silage based TMR showed 11.4-23.0 g/cow higher daily CH_4-production. This difference was observed with both measurement methods. In RC, significantly higher CH_4 emissions (544.7 g/cow/day) were measured as compared to those assessed by LMD (337.3 g/cow/day). Interestingly, highly positive phenotypic correlations (r_p=0.752-0.916) between CH_4 production determined by RCs and LMD were observed when values were expressed as daily g CH_4/kg milk/cow. This underlines that measurements taken by LMD could serve as a proxy to assess CH_4 production measured in RC.

Time-lagged genotype by heat stress interaction analyses

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The aim of this study was to estimate genetic parameters for production, female fertility and health traits in the offspring generation in dependency of the temperature-humidity-index (THI) from the last week of gestation (i.e. an across-generation perspective). The dataset for production and female fertility traits considered 162,492 first parity Holstein Friesian cows from calving years 2003 to 2012. The health dataset included 69,986 first parity cows from calving years 2008 to 2016. The production traits were milk yield (MKG), fat percentage (FAT%) and somatic cell score (SCS) from the first official test-day in first lactation. Female fertility traits were the interval from calving to first insemination (ICFI) in first parity cows and the non-return-rate after 56 days (NRR56) in heifers. The health traits were clinical mastitis (MAST), digital dermatitis (DD) and endometritis (EM) in the early lactation period. For MKG, heritabilities and additive genetic variances decreased with increasing THI. Especially for THI>58, the decrease was very obvious with a minimal heritability of 0.19. For FAT% and SCS, heritabilities increased slightly in dependency of prenatal heat stress (HS) conditions at THI>60. For ICFI, heritabilities differed marginally across THI (h^2=0.05-0.08). For NRR56, MAST and DD, the heritability curves were U-shaped, with largest estimates at the extreme ends of the THI scale. For EM, heritabilities and genetic variances increased with increasing THI, from 0.12 in cold climates up to 0.34 for prenatal HS. The trait-specific alterations of genetic parameters along the THI gradient indicate pronounced genetic differentiation due to intra-uterine HS for NRR56, MAST, DD and EM, but decreasing genetic variation for MKG and ICFI. Genetic correlations smaller than 0.80 for NRR56, MAST, DD and EM between THI 65 with corresponding traits at remaining THI indicate genotype by HS interactions. Lowest genetic correlations were identified when considering the most distant THI. For MKG, FAT%, SCS and ICFI, genetic correlations were throughout larger than 0.80. As a possible explanation for the intergenerational climate effects on genetics, we assume epigenetic mechanisms, with long-lasting impact on gene activities.

Genomic tools to support decisions on conservation of animal genetic diversity

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Conservation of genetic diversity, both among and within breeds, is a costly process. Therefore, choices among breeds and animals within breeds are unavoidable, either for conservation *in vitro* (gene banks) or *in vivo* (maintaining small populations alive). Nowadays, genomic information on breeds and individual animals is the standard for the choices to be made in conservation. Genomics may measure accurately the genetic distances among breeds and the relationships among animals within breeds. In addition, genomics can be used to detect potentially valuable rare alleles and haplotypes, their carriers in these breeds and promote a specific management of these genomic regions. Genomic tools to characterize animal genetic diversity will be briefly introduced.

Combining whole-population genotyping and stochastic simulation to improve conservation management

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As the livestock sectors in many countries focus on highly productive breeds, locally adapted breeds decline in numbers, resulting in an elevated risk to lose genetic variability due to genetic drift and selection. Most conservation approaches employ pedigree-based measures to limit the increase in inbreeding. With the advent of genomic array data, one prominent approach to determine inbreeding became the estimation of Runs-of-Homozygosity (ROH) and ROH islands were taken as the result of lost or fixed haplotypes due to selection or drift. In this study we combine the analysis of HD array data of all existing breeding animals of the Göttingen Minipig (GMP) in the year 2019 and stochastic simulation, first to determine the genomic properties of our population, and second, to simulate comparable datasets as a proxy to understand the possible nature of loss of variation in this small, managed breed. In the GMP, around 40% per individual genome are covered by ROH. These regions overlap in only 38 islands, which account for 4% of the genome, indicating that most parts of the genome are still variable. Anyway, many regions were close to fixation. Two scenarios (S1 and S2) were simulated by simplified mimicking the GMP breed history. Mating 20 boars and 40 sows, selected out of 60 boars and 120 sows, respectively, for 60 generations resulted in populations that resembled the structure of ROH in the GMP. S1 assumed 50 QTL underlying the selection trait and S2 5 QTL, respectively. As the assumed QTL and original SNPs were known, we determined that about 50% of all SNPs were fixed over time, among them 35 QTL in S1 and all 5 in S2. In S1 roughly 25 QTL and in S2, 3 QTL were visibly associated with an ROH island. Hence, we conclude that in the GMP the main reason for ROH islands is drift, rather than selection. With this we show, that genomic data help to identify genomic regions under threat and stochastic simulation can be an appropriate tool to assess the effect of the known breed history on the genome thus helping to evaluate future conservation approaches.

Runs of homozygosity islands reveal highly inbred genomic regions across 124 chicken populations

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Runs of homozygosity (ROH) are homozygous chromosomal stretches often caused by inbreeding. By computing ROH islands, these ROH can be used to detect selection signatures. In a previous study, we analysed medium density SNP datasets available online from 8 animal species (cat, cattle, dog, goat, horse, pig, sheep and water buffalo) and 442 populations to construct an ROH island repository. In this study, we expanded our analyses with high density SNP data from the SYNBREED chicken diversity panel (3,235 individuals from 174 populations and 580,961 SNPs). PLINK v1.9 was used to perform quality control and ROH analyses based on Gorssen *et al*. Quality control was performed population specific and only populations with minimal 15 individuals were retained for analyses. Consequently, 124 out of the 174 populations were subjected to further quality control. For ROH detection, multiple settings on the number of allowed heterozygous (--homozyg-window-het and --homozyg-het) and missing (--homozyg-window-missing) SNPs were tested and a consensus was found to update our script for high density SNP analysis. ROH islands were defined per population as SNPs with a population incidence of minimum 30% and a P-value for ROH incidence larger than 0.999 using standard normal z-scores. Of all 124 analysed populations, fifteen genomic regions were found with ≥5 populations showing an ROH island. Some known ROH islands in chicken were confirmed. Moreover, we found two interesting regions were >25% of all studied populations showed an ROH island. Between 10-12 Mb on chromosome GGA8, 40 chicken populations had a ROH island (32% of all studied populations), whereas on chromosome GGA2, 32 chicken populations showed an ROH island between 51-54 Mb (26% of all studied populations). Furthermore, ROH patterns could also be linked to known history of joint populations. By examining these high density SNP data for ROH islands, we updated our script for ROH analysis. Our findings can serve as a reference for results in other (chicken) populations or other investigations and we believe these results can be valuable for future genetic research.

Session 03 Theatre 4

Effects of recent and ancient inbreeding on growth in American Angus cattle

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The accumulation of inbreeding can lead to an unfavourable change in the phenotypic value of individuals for traits related to fitness, also known as inbreeding depression. However, inbreeding accumulated at a more distant past (ancient inbreeding) is expected to have a smaller depressive effect than that accumulated more recently due to the loss of detrimental alleles caused by purifying selection. Therefore, the aim of this study was to quantify the inbreeding depression caused by recent and ancient inbreeding for birth weight, weaning weight, and post-weaning gain. Pedigree and genomic information were obtained from Angus Genetics, Inc. (St. Joseph, MO) for 569,364 individuals from the American Angus breed. Pedigree inbreeding and genomic inbreeding based on runs of homozygosity (ROH) were estimated using the SNP1101 software. Model-based genomic inbreeding based on the probability a marker is part of a homozygous-by-descent segment (HBD) was estimated using the RZooROH in R. The generational cut-offs for designating inbreeding as recent was that acquired 5 generations ago or sooner for pedigree, 6.25 generations ago or sooner for ROH, and 8 generations ago or sooner for HBD inbreeding. The effect of a 1% increase in inbreeding was modelled in males and females using a linear mixed model approach. Recent pedigree inbreeding was found to decrease birth weight by 0.04 and 0.03 kg, decrease weaning weight by 0.50 and 0.48 kg, and decrease post-weaning gain by 0.62 and 0.32 kg, in males and females respectively. Ancient pedigree inbreeding was generally found to have no effect on growth. For genomic inbreeding, when both recent and ancient inbreeding had a detrimental effect on growth, recent inbreeding generally had a larger effect. The results of this study demonstrate that inbreeding accumulated recently should be quantified and managed in beef cattle populations to avoid economic losses.

Session 03 Theatre 5

Body and reproduction traits are negatively affected by inbreeding depression in Belgian Milksheep

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The Belgian Milksheep is a dairy type sheep breed with a distinctive thin tail ('rat tail') and high milk production. The breed is closest related to the Friesian sheep breeds. Due to the small population size (approx. 350 registered lambs/year) and the heavy use of some rams as sires, inbreeding of the breed was investigated. The average inbreeding coefficient of 12% based on pedigree information and 14.5% based on runs of homozygosity. Moreover, effective population size was only around 24 so the question arose if inbreeding depression is of concern in the breed. In the present study, we evaluate the effect of inbreeding on body dimensions and reproductive traits. The evaluated body measurements are height at withers, length, chest width, chest circumference and chest dept. For reproductive traits, we evaluated the number of litters, average litter size, number of lambs born dead and number of lambs born alive. Individual inbreeding coefficients were calculated based on >9,000 pedigree records. Body dimension data were available for more than 1,300 animals, and reproductive traits for almost 1000 ewes. Animal models were fitted with the individual inbreeding estimates included as fixed effect (linear covariable or categorical). The degree of inbreeding had a unfavourable effect on the number of litters per ewe, and litter sizes (total, born alive and born dead). An increase of 10% inbreeding resulted in a reduction of 0.12 litters/ewe and an increase of 0.05 more dead lambs per litter. For body dimensions, we estimated negative regression coefficients, for example for height at withers 0.29 cm per 10% inbreeding. Although these effects are small in absolute terms, the combined effect may affect the overall (re) production levels of the breed negatively and this may further compromise the successful conservation of the breed.

Session 03 Theatre 6

Genomic and pedigree estimates of effective population size in the Old Kladruber and Noriker horse

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Effective population size (N_e) is a key parameter in population genetics because it is functionally related to genetic drift or stochastic variation in gene frequency, inbreeding, and linkage disequilibrium. Recently, a number of new estimators have been developed to estimate N_e from genomic information. The main objective of this study was to compare genomic estimates (N_{eLD}, N_{eGD}, N_{eROH}, $N_{eROHreg}$) with pedigree estimates ($N_{ePED\Delta F}$, $N_{e\Delta COAN}$, and $N_{ePEDreg}$) most frequently used in livestock populations. Our study was conducted on the Old Kladrub (OKH) and Silesian Noriker (SNH) horse breeds because these two populations are endangered and have deep well-documented pedigrees. In OKH, the lowest estimates were obtained with N_{eLD} (30) and N_{eGD} (46), were in the middle range with $N_{ePED\Delta F}$ (50), $N_{e\Delta COAN}$ (62), $N_{ePEDreg}$ (67) and $N_{eROH>2reg}$ (66), while the highest estimate was obtained with $N_{eROH>2\Delta F}$ (99). In SNH, the lowest estimates were obtained with N_{eLD} (45), were intermediate with $N_{e\Delta COAN}$ (73), $N_{ePEDreg}$ (74) and N_{eGD} (76), while the highest estimates were obtained with $N_{ePED\Delta F}$ (105) and $N_{eROH>2\Delta F}$ (201). Estimated $N_{eROH>2reg}$ was not defined in SLH because the estimated ΔF was negative. Our results show that the most similar (‘stable’) Ne estimates were obtained with $N_{ePED\Delta F}$, $N_{e\Delta COAN}$, $N_{ePEDreg}$, and N_{eGD}. For both breeds, the estimates with N_{eLD} and $N_{eROH>2\Delta F}$ were outliers that respectively most likely under- and overestimated the true N_e value. While $N_{eROH>2reg}$ was close to the other estimates in OKH, this estimate was not defined in SNH. Although genomic data occasionally provide good estimates, with the exception of NeGD, which appears to be ‘stable’, they are not reliable and need to be improved or better understood. As an alternative, we propose to apply all estimators used in this study and use the median across all estimates as a reasonable choice. The study was supported by the projects QK1910156, MZe-RO0718 and ANAGRAMS-IP-2018-01-8708.

Session 03 Theatre 7

Do standardised genotypes in Hardy-Weinberg equilibrium enhance local breeds assignment accuracy?

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According to article 19 of the EU Regulation 2016/1012 on Animal breeding, endangered breeds can benefit from a derogation to enable animals without origin to enter the main section of the herdbook. The development of a genotype-based model able to correctly assign animals to an endangered breed has already been proven to ease the application of this derogation. However, the impact of standardisation of genotypes (i.e. SNP column mean centred and divided by the standard deviation), Hardy-Weinberg (HW) equilibrium and their combination on the accuracy of a breed assignment model has not been studied yet. The objective of this study was to optimize the accuracy of a breed assignment model dedicated to three local cattle breeds: Dual-Purpose Belgian Blue (DPBB), East Belgian Red and White (EBRW) and Red-Pied of Ösling (RPO) through four pre-treatments modalities: no pre-treatment (1), standardisation of genotypes (2), HW equilibrium filter (3) and standardisation of genotypes in HW equilibrium (4). Most informative SNPs were selected using a partial least squares-discriminant analysis, making four panels of (1) 2,084; (2) 2,005; (3) 1,930 and (4) 1,843 SNPs. The method of nearest shrunken centroids was then parameterized and validated for each of these four panels. For DPBB, pre-treatment (4) provided the best validation accuracy (97.24%). For EBRW and RPO, pre-treatment (2) provided the best validation accuracy (94.42 and 53.24%, respectively). The low level of accuracy found in RPO was expected due to the genetic closeness of RPO to EBRW. If a priori RPO animals identified as RPO or EBRW are grouped, validation accuracy reached 83.45%. However, when comparing confidence intervals of each model for each breed, none of the pre-treatments was significantly better than others. According to the objective, different strategies can therefore be chosen when developing a breed assignment model. Pre-treatment (2) can be used to detect more animals to be included to the herdbook. Pre-treatment (1) can be applied to develop quickly a performant model. To limit the number of SNPs to use, pre-treatment (4) should be preferred.

Session 03 Theatre 8

Genomic diversity and population structure of Holstein Friesian: Czech population perspective

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With over 227 million individuals, situated in more than 150 countries, Holstein-Friesian (Holstein) is the most important dairy breed in the world. However, the population effective size and genetic diversity of this cattle breed is rapidly declining in recent decades due to heavy selection and overuse of a small number of elite bulls. The aim of this study was to analyse genomic diversity, ROH inbreeding (FROH>4 Mb) and gametic disequilibrium effective population size (NeGD) of the Czech Holstein population (CZE). We also estimated the population structure of the Holstein metapopulation, 1,831-1,530 bulls from Belgium (BLG), Canada (CAN), France (FRA), Germany (DEU), Ireland (IRL), the Netherlands (NED), the United Kingdom (UK), Switzerland (CHE), and the United States, with particular interest in determining their genomic relatedness to Czech Holstein (301 CZE bulls). The lowest observed (0.299) and close to the lowest expected (0.299) heterozygosity was observed in CZE. The lowest $F_{ROH>4Mb}$ was estimated in IRL (0.052), while the highest value was estimated in CZE (0.102), indicating the need for better mating management. In contrast, we estimated unexpectedly large N_{eGD} (144) in CZE, which was higher only in NLD (195), DEU (185) and USA (149) populations, with the median of the metapopulation equal to 141. The mean pairwise Wright's F_{ST} distance from CZE to all other Holstein populations was very low (0.008), slightly below the overall mean differentiation observed (overall mean FST=0.009). The very low differentiation within the Holstein metapopulation is caused by numerous migrations, which was evident in the admixture analysis. With an F_{ST} of 0.003, DEU and USA were the closest populations to CZE, while the most distant populations were IRL (F_{ST}=0.021) and CHE (F_{ST}=0.020). The results presented will help to improve the mating and breeding strategy of the CZE. The study was supported by projects QK1810253 and ANAGRAMS-IP-2018-01-8708.

Session 03 Theatre 9

Analysis of Y-chromosome sequences in twelve cattle breeds reveals low within-breed diversity

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In this study, we analysed pedigree information on males from 12 bovine breeds born in France between 2015 and 2019 (39,927≤n≤8,259,344). We report an overall small number of paternal lineages with, for example, a minimal number of ancestors accounting for 95% of the Y-chromosome pool of their breed ranging from only two to 15 individuals. We observed significant differences between dairy versus dual purpose and beef breeds for all the metrics calculated. Then, we analysed data from run 8 of the 1000 Bull Genomes Project and mined whole-genome sequence data from 811 sires (2≤n≤453 per breed) and built a median-joining network using 1,411 SNPs. In total, 1,266 SNPs (89.7%) supported the existence of the two main North-East (Y1) and South-West (Y2) European haplogroups as they were previously described in the literature. Most branches were breed-specific and in agreement with the geographic and genetic relatedness of these populations. The within-breed haplotype diversity was lower than expected based on genealogical information, which supports the existence of major male founder effects predating pedigree recording. We observed very rare carriers of the Holstein Y1 haplotype within the Simmental (1/70) and Montbéliarde (1/45) breeds, which result from planned introgressions during the 1970s and 1980s to improve milk production. We also identified six additional examples of Y1-haplotype carriers within breeds that are normally fixed for the Y2 haplotype and three of them are attributed to errors in correspondence between DNA samples and animal ID. In addition, we observed de novo mutation events among the descendants of the same ancestors, which are of interest to define paternal sub-lineages. Our results pave the way to future studies on the estimation of the effects of Y-chromosome haplotypes on male reproductive performances and on the conservation of Y-chromosome diversity.

Session 03 Theatre 10

Selection signatures in candidate genes affecting exterior traits in several rabbit breeds

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The European rabbit (Oryctolagus cuniculus) is a multi-purpose species globally kept and raised mainly for meat and fur production. Since its domestication, several breeds have been developed. Alongside a few highly-selected and production-oriented rabbit breeds, several other fancy breeds have been constituted. These breeds present animals having specific exterior traits, such as coat colours and patterns, fur structure and several morphometric traits (body size, shape and position of the ears, and structure of the skull). Here, we took advantage of this phenotypic variability to identify signatures of selection in the rabbit genome that could highlight candidate genes whose variability might contribute to explain the diversity of exterior traits that distinguish some commercial and fancy rabbit breeds. A total of 660 animals were genotyped with the Affymetrix Axiom OrcunSNP Array which interrogates 199,692 SNPs. Animals were from fancy rabbit breeds (Belgian Hare, n.24; Burgundy Fawn, 6; Champagne d'Argent, 19; Checkered Giant, 79; Dwarf Colored, 20; Dwarf Loop, 20; Ermine, 20; Giant Grey, 27; Giant White, 20; Rex, 19; Rhinelander, 28; and Thuringian, 9) and three Italian commercial rabbit breeds (Italian d'Argent, 20; Italian Spotted, 93; and Italian White, 256). Signatures of selection were investigated via window-based Fixation Index (FST) analyses. A first approach compared one breed against all others whereas a second approach compared two groups of breeds (groups were based on pigmentation or morphometric features). FST analyses highlighted many genome regions harbouring coat colour related genes already described to affect pigmentation in rabbits (ASIP, MC1R and TYR) and coat structure (LIPH). In addition, several other regions contain genes involved in body structure/stature, growth and reproduction performances. Overall, the present study added information that contributed to clarify the genetic architecture and mechanisms determining phenotypic differences among rabbit breeds.

Session 03 Theatre 11

Deciphering the genomic architecture and patterns of selection in Swiss cattle breeds

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Domestication, adaptation and human-mediated selection for different traits have created several cattle breeds with distinctive characteristics. To understand the genetic architecture and detect footprints of selection in the genome of the most prevalent dairy and dual-purpose breeds of Switzerland, we analysed whole-genome sequence data of the Original Braunvieh (OB), Brown Swiss (BSW), Grauvieh (GV), Holstein (HOL) and Fleckvieh (FV) cattle breeds. Reference-guided variant discovery in 124 unrelated cattle yielded 21,637,709 segregating biallelic SNPs on the autosomes. The nucleotide diversity was highest (1.5×10^{-3}) in GV and lowest (1.3×10^{-3}) in BSW. Principal components and admixture analyses clearly separated the animals by breeds and suggested only marginal admixture among the breeds. Phylogenetic analysis revealed an ancestral population which bifurcated into GV and a common node for BSW and OB. Genetic drift was strongest in BSW cattle which agrees with the low nucleotide diversity and likely results from a recent sharp reduction in effective population size. Signatures of selection that differed between the Swiss breeds (BSW, GV and OB) and HOL and FV encompassed genes associated with stature, pigmentation, dairy and beef traits (e.g. *KIT*, *CAPN13*, *CAPN14*, *NCAPG*, *ABCG2*). We overlapped the signatures of selection detected in OB and BSW with multi-trait meta-analyses of sequence-based association studies for 37 complex traits (confirmation, fertility, milk and beef production). Two QTL overlapped with a BSW-specific signature of selection and three QTL overlapped with a signature of selection detected between OB and BSW. These QTL were associated with dairy traits and mammary gland morphology. In conclusion, we provide insights into the history of Swiss cattle breeds and reveal evidence for strong genetic drift in BSW. The signature of selection and multi-trait meta-analyses suggest that selection for dairy and type traits may have contributed to the decline in effective population size in BSW cattle.

Session 03 Theatre 12

Effect of SNP related to reproduction traits on the genetic composition of local cattle

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The systematic differences in allele frequencies among dairy, beef and dual-purpose breeds resulting from selective breeding for traits of interest were tested by the screening of particular SNPs located inside genes responsible for genetic control of reproduction traits in cattle. Genome-wide data of 356 animals representing the gene pool of dairy (Jersey), beef (Charolais, Limousin) and dual-purpose (Slovak Spotted, Slovak Pinzgau) populations kept in Slovakia were used to compare their genetic composition and find particular allelic variants unique for each of them. The hypothesis assumed that the artificial selection during grading-up of breeds led to the specific changes in their genome manifested in the formation of specific haplotypes. From the 34,384 autosomal SNP markers, only loci located inside genomic sequences of 15 protein-coding genes (BTA 1, 2, 5, 6, 7, 11, 14, 15, and 19) previously associated with reproduction traits were left for analysis. The inter-population genetic differentiation was tested by principal component analysis. The effect of specific allelic variants depending on particular genes on intra-population genetic composition was then evaluated by factorial analysis. The individual differences in allelic frequencies were evident mainly in Jersey and Slovak Pinzgau cattle. Jersey population were separated from others due to polymorphism of GDF9, CWC15, LHCGR and NCAPG genes, whereas the Slovak Pinzgau population showed significant differences in genetic variants inside STAT3, IGF1 and PGR genes. The subsequent gene ontology term enrichment analysis showed that these genes are important for several biological processes related to reproduction, including ovulation cycle process (GO:0022602), regulation of gonad development (GO:1905939), response to growth hormone (GO:0060416) and regulation of generation of precursor metabolites and energy (GO:0043467).

Session 03 Theatre 13

Study of the variability of Csd alleles based on NGS data in Italian honeybees

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Sex determination in *Apis mellifera* is regulated by the complementary sex determiner (csd) gene. Drones are hemizygous at this locus and females are heterozygous; diploid drones, containing homozygous genotypes for csd, are eaten by worker bees during the larval stage. Therefore, the prevention of allelic variability in this locus is essential to prevent colony losses and for conservation strategies. Focus of this study is to investigate the genomic variability of the csd gene on NGS data and to explore the variability of alleles circulating in Italy in different honeybee subspecies. During summer 2017 and 2018, 125 colonies of seven different subspecies were sampled in Italy. Whole Genome sequences of the 125 worker bees were obtained through Illumina NextSeq with a 150 bp paired-end module. Sequences were mapped to the HAv3.1 *A. mellifera* genome and the hypervariable region of the csd gene was manually examined with the Integrative Genomics Viewer (IGV). Each strand was translated to obtain protein sequences for each allele of the heterozygous sequenced individuals. Nucleotide sequences have been constructed for 65 samples, belonging to 33 Ligustica, 20 Buckfast, 5 Carnica, 4 Sicula, 1 hybrid Carnica, 1 Cecropia and 1 Mellifera honeybees. In total 107 sequences were identified using identity as a criterion. For each subspecies, 58 sequences were identified in the Ligustica honeybees, 35 in Buckfast, 10 in Carnica, 8 in Sicula and 2 in hybrid Carnica, Cecropia and Mellifera. The most frequent sequence identified occurs in the 12% of the population and is present in four different subspecies (Ligustica, Buckfast, Carnica and hybrid carnica). Reconstructed sequences will be validated through Sanger sequencing in the near future. This study contributes to the characterization of the csd allelic variability in Italian honeybee colonies.

Session 03 Poster 14

Genomic assessment of the Romanov sheep using high density DNA chips

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The Romanov breed significantly differs by its phenotypic (thin tail, not fat) and proliferative traits (multiple fertility and off-season oestrus), as well as by its origin (European) from other Russian local coarse wool sheep breeds. The Romanov ewes are superior to other Russian and the most world breeds by the litter size per lambing in 2.5-3 times. Due to high prolificacy, the Romanov breed is widely known outside Russia. However, the Romanov breed is currently in a vulnerable state and is partially replaced by fat-tail breeds with a higher meat productivity (Karachaev, and Edilbaev). In this regard, it is relevant to analyse current genetic state of the Romanov breed using powerful DNA tools. A total of forty-eight individuals from the Romanov breed collected randomly in various farms were genotyped using Ovine Infinium® HD SNP BeadChip. The data processing was performed in PLINK 1.90 and in the R package 'diveRsity'. An analysis of genetic diversity showed that the observed heterozygosity was 0.368 in the Romanov sheep and exceeded the average value estimated for eleven coarse wool breeds (0.334). An insignificant heterozygote excess was recorded for all the studied sheep breeds, including the Romanov breed, which was 3% in the Romanov breed and averaged 1% for eleven breeds. Allelic richness was greater in the Romanov breed in comparison with other coarse wool fat-tailed sheep breeds: 1.918 vs 1.883, respectively. Thus, we found that the Romanov breed has a significant resource of genetic diversity and is not seemed to be endangered. Genomic assessment of the Romanov breed will be continued to search for genetic variants responsible for high proliferative traits which might be useful as targets for genome editing to improve the reproductive traits of low prolific sheep breeds. The SNP- profiles of fat-tailed sheep breeds were obtained with financial support of the RSF project No. 19-16-00070. The reported study was funded by RFBR and INSF, project number 20-516-56002.

Session 03 Poster 15

Genome scans for several morphological traits and defects in the autochthonous Reggiana cattle breed

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Autochthonous breeds are important reservoirs of genetic diversity. The production systems in which these populations have been developed resulted in the selection of specific exterior traits that are inherited and (almost) fixed in many breed populations. However, the existence of certain levels of phenotypic variability opens the possibility to understand the genetic basis for some of these traits. Reggiana is an Italian autochthonous cattle breed reared mainly in the Emilia Romagna region (North of Italy). The milk of this breed is almost exclusively utilized to produce a mono-breed Parmigiano-Reggiano cheese. We exploited phenotypic variability in Reggiana cattle to investigate at the whole genome level the genetic architecture of different exterior traits and defects, including grades of red coat colour, piedbaldism, muzzle colour (pink/gray/black), stature, presence/absence and number of supernumerary teats and teat length. DNA was extracted from blood and SNP genotyping was performed with the GeneSeek GGP Bovine 150k Array. A dataset of 1,776 animals, 124,482 SNPs and 404,061 haplotypes was used for genome-wide associations analyses (PLINK v1.9). Animals presented three main intensity of red of the coat colours (light-red 10.7%; normal-red, 83.7%; dark-red, 5.6%) that associated with DNA markers on the bovine chromosome (BTA) 5 and BTA13. Piedbaldism was observed in 4.3% cattle and associated with *KIT* gene markers (BTA6). Genome scans for muzzle colour pointed out the involvement of the *MC1R* gene markers (BTA18). Stature of the animals was associated with markers in two regions harbouring genes that have been already shown to affect this trait in several species: *NCAPG-LCORL* (BTA6) and PLAG1 (BTA14). Suggestive peaks of association for supernumerary teats and teat length were evidenced on BTA10 and BTA8, respectively. Overall, the identified genomic regions not only can be directly used in selection plans but also contributes to clarify the genetic mechanisms involved in determining exterior traits in cattle.

The inbreeding landscape of two Italian autochthonous cattle breeds from Parmigiano-Reggiano region

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Reggiana and Modenese are dual-purpose autochthonous cattle breeds reared in the North of Italy, mainly raised for the production of two mono-breed branded Protected Designation of Origin (PDO) Parmigiano-Reggiano cheese. The population size of these breeds experienced a drastic reduction in the 1980' and a subsequent slow recovery. Inbreeding is an important parameter to control in conservation programs, especially in small populations where few animals are unrelated. Inbreeding is traditionally estimated using pedigree data which are summarized in an inbreeding coefficient calculated at the individual level (F_{PED}). Commercial platforms for high-throughput genotyping allow more accurate estimates of inbreeding. Several approaches have been proposed to estimate the genomic inbreeding level and one of the most widely accepted is based on runs of homozygosity (ROH). In this study we compared pedigree and genomic inbreeding parameters in Reggiana and Modenese breeds over the last decades. A total of 1,726 Reggiana and 344 Modenese cattle (about two thirds of the whole living populations for the two breeds) were genotyped with the GGP HD 150k SNP chip. The increasing trend of genomic inbreeding rate estimated using ROH was significantly lower than that observed using pedigree-based inbreeding. A total of 15 and 14 ROH islands were detected in Reggiana and Modenese breeds, respectively. These chromosome regions include genes affecting performance and morphological traits that can explain differences and similarities between the two breeds. The obtained results are useful to define new conservation strategies for these two cattle genetic resources.

Faecal metabolites as biomarkers of diet and heat stress in dairy cows

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The intestinal microbiota contributes hundreds of metabolites which can influence host's metabolism and health. The analysis of faecal metabolite profile could provide novel insights into the effects of diet and heat stress on microbiota composition and gut health. Twelve multiparous Holstein cows (42.2±5.6 kg milk/d; 83.4±27.1 DIM) were used in a split-plot design testing the effects of mineral and vitamin supplementation on faecal metabolites during heat stress. The main plot was the level of dietary vitamin E and Se (Low; LESe; 20 IU/kg vitamin E, 0.3 ppm Se; vs high: 200 IU/kg vitamin E, 1.2 ppm Se; HESe). Within each plot, cows were randomly assigned to: (1) heat stress (HS; THI: 82); (2) pair-feeding (PF; THI=64); or (3) HS with vitamin D and Ca supplementation (HS+DCa; 1,820 IU/kg and 1.5% Ca; THI: 82) in a replicated Latin square design with 14-d periods. Faecal samples from d 14 were analysed for metabolites by LC-MS/MS. Data were log transformed and Pareto-scaled. Our targeted analysis detected 94 metabolites in faecal samples, including vitamins, amino acids, lipids, and biogenic amines. The One-way ANOVA identified 7 faecal metabolites that were influenced by treatment, including tridecylic and myristic acids, vitamin B7, histidine, p-hydroxyphenylacetic_acid, acetyl-ornithine and arginine (FDR<0.05). Relative to the LESe group, HESe increased a-tocopherol by 3-fold, whereas d-tocopherol was decreased by 78% (P<0.01). Correlation analysis between alpha tocopherol and all the others faecal metabolites did not show significant associations. Discriminant analysis revealed 15 biomarkers capable of predicting treatment groups (VIP score >1). We detected significant elevations in faecal homocysteine, hydroxypheny, tridecylic acid, caproic acid, myristic acid, methylhistidine, vitamin B7, 2-methylbutyril, retinol, and vitamin B9 in the HS+DCa group. The metabolome changes in HS+DCA were associated with lower rectal temperatures and respiration rates relative to HS. We conclude that vitamin D and Ca feeding can influence the faecal metabolome in cows experiencing heat stress.

Effects of heat stress on performance and gastrointestinal tract microbiome of weaned calves

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Our objectives were to evaluate the effects of heat stress (HS) on growth, health, and the gastrointestinal tract (GIT) microbiome in Holstein calves. In a completely randomized design, 35 bull and heifer calves were assigned to one of five groups (n=11-12/group): thermoneutral conditions (TN-Con), HS conditions (HS-Con), and TN conditions pair-fed to HS-Con (TN-PF). Weaned calves (62±2.5 d; 92±10.4 kg) were housed in TN conditions (temperature-humidity index [THI]: 60 to 69) for a 7-d covariate period. Subsequently, calves either remained in TN or were moved to HS conditions (THI: 75 to 83) for 19 d. Clinical assessments and BW were recorded. Blood, rumen fluid and faecal swabs were sampled. Organs from TN-Con and HS-Con calves were harvested on d 19. GIT bacteria were profiled using 16S rDNA sequencing. The statistical model included fixed effects of BW at birth, treatment, time, and their interaction. HS increased rectal and skin temperatures, and respiration rates ($P<0.01$). Dry matter intake (DMI) was higher in TN-Con, relative to HS-Con and TN-PF calves ($P<0.01$). Average daily gain was reduced in HS-Con calves relative to TN-Con 0.850 vs 1.32 kg/d, $P<0.01$); TN-PF was not different from TN- or HS-Con. Liver and small intestine were lighter in HS-Con relative to TN-Con ($P=0.03$ and 0.14, respectively). Although white blood cells and monocytes were reduced in HS-Con ($P=0.08$ and $P=0.03$, respectively), all animals were clinically healthy throughout the trial. Relative abundance of ruminal *Euryarchaeota* tended to increase over time in HS-Con ($P<0.10$). Jejunal *Euryaechaeota* and *Methanobacteriaceae* were greater in HS-Con calves ($P<0.05$). Ileal *Bacteroidetes* tended to be increased in HS-Con calves ($P\leq0.15$). Faecal *Proteobacteria* were increased in HS-Con compared to TN-Con and TN-PF calves ($P<0.01$). We conclude that DMI reductions account for growth deficit during HS. However, HS modulated the GIT microbiome independent of intake and increased the abundance of detrimental microbiota linked with gut dysbiosis, inflammation and methane production.

Milk fatty acids as markers of heat stress in cows fed diets with varying protein composition

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Milk fatty acid (FA) concentrations (conc.) could be a useful tool to identify risk of heat stress in cows. Twelve Holstein cows (42.2±10.6 kg milk/d; 83±28 days in milk) were used to evaluate the effect of balancing for Lys, Met, and His supply on milk fatty acid profile. Using two replicated 3×3 Latin squares (one primiparous and one multiparous), three treatments were tested: (1) heat stress (HS; 17% CP, THI: 82; Supply d7-14 (g/d): 107 Lys, 34 Met, 37 His, and 1,715 MP); (2) pair feeding in thermo-neutrality (PF; 17% CP; THI=64; AA and MP supply equal to HS group); or (3) HS with a diet balanced to supply 178, 64, and 43 g/d of Lys, Met, His, and 1,730 MP, respectively, based on DMI reductions (HS+AA; 17% CP; THI=82; observed DMI -32%) with 14-d periods. Milk FA acids were analysed by MIR from samples taken on d 0, 3, 7, 10, and 14. The statistical model included cow and period as random, and parity, treatment, day and their interactions as fixed effects. Dry matter intake decreased from 23.8 to 15.4 kg/d in all groups from d 0 to 7 (Time $P<0.001$). Milk <16C FA (de novo origin) concentrations progressively decreased over time in all treatments, reaching a nadir on d 7 ($P<0.05$). Milk 16C FA (mixed origin) conc. decreased over time (d 3 to 14) in PF cows relative to HS (-8%; $P<0.05$). On the other hand, HS+AA cows exhibited 4% lower ($P<0.05$) 16C FA relative to HS from d 7 to 14. Milk >16C-FA conc. (preformed) increased over time ($P<0.05$) reaching a plateau on d 7 in all treatments. However, PF cows showed greater (+8%; $P<0.05$) >16C FA conc. from d 7 to 14 relative to HS. No difference in >16C FA conc ($P>0.05$) was observed between HS and HS+AA groups. Changes in milk FA profile could be used to identify cows undergoing heat stress; however, protein composition of diets has an impact on milk FA concentrations under the conditions of the present study.

Session 04 Theatre 4

Early detection of heat stress in grazing dairy cows by use of behavioural and physiological traits

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The continuous direct exposure to the ambient climatic conditions coupled with their own metabolic heat production make grazing dairy cows particularly vulnerable to heat stress. Heat stress impairs performance as well as animal health and welfare. The objective of this study was to identify heat stress early in grazing dairy cows in a temperate climate using behavioural traits. The study was conducted with 24 primiparous and multiparous grazing Holstein dairy cows during summer 2018 and 2019 at Agroscope (Posieux, Switzerland). Climatic conditions were continuously recorded and were used to calculate the comprehensive climate index (CCI), which reflects experienced temperature in °C. The behaviour and respiration frequency were recorded over 12 periods of at least two days each with moderate but increasing CCI. The vaginal temperature (VT) which was continuously recorded with a temperature logger was used as a physiological indicator of heat stress. The data was analysed per day for morning (AM, 09:00-11:00) and afternoon (PM, 12:30-14:30). During AM, the VT changed little with increasing CCI and the cows showed small behavioural alterations. During PM, VT increased minimally up to CCI=25 °C and then rose steeply with increasing CCI. The VT correlated positively with the respiration frequency. The VT correlated negatively with the activity while standing and walking. With increasing VT, the animals were closer to the water trough and small inter-individual distances occurred more frequently. Alterations in VT in PM indicated that grazing dairy cows experienced heat stress under moderate climate conditions. Changes in behavioural parameters in relation to increasing VT seem to reflect behavioural adaptations to early heat stress.

Session 04 Theatre 5

Individual differences in the physiological response to heat stress of dairy goats

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Breeding strategies aiming to improve animal's resilience to global warming depend on the ability to identify robust indicators of resilience, and differences among individuals. This study aims to explore how indicators of heat stress (HS) evolved during a heat challenge, and to quantify individual responses in goats. A total of 20 dairy goats in mid-lactation were adapted to a climatic chamber and submitted to a temperature ramp consisting of thermal neutral (5 d; 15-20 °C; THI=63-65), transition (2 d; 25-30 °C) and HS (12 d; day, 37 °C; night, 30 °C; THI=79-88) conditions. THI was referred to NRC (1971). Breath rate (BR, breaths/min), rectal temperature (RT, °C) and milk yield (MY, kg) were recorded x2-daily, whereas water (W, kg) and feed (DMI, kg) intakes were recorded x1-daily. Reaction-norm models were used to study the evolution of indicators during the challenge. As a result of temperature ramp, d 3 and onwards showed a sharp increase in BR and RT, more evident in the afternoon, a drop in MY, an increase in W and a decrease in DMI. These negative effects were maintained until d 5 to 7 after the onset of HS, with BR, RT and W reaching a plateau, and DMI and MY returning to normal values. Clear individual differences were observed in the physiological response patterns, mainly in morning BR and RT, indicating that not all does recovered equally during the night. Thus, some were able to cope HS overnight, while others were not. Large W and DMI individual differences were observed. Some does barely modified their eating behaviour throughout the challenge, while others showed significant drops in DMI and marked increases in W. Finally, individual variability in MY was observed with smaller differences in the patterns of change along the HS challenge. These differences at the productive level could be associated with differences in the thermotolerance of individuals, as has been previously reported. Funding from project RTA2015-0035 is acknowledged.

Faecal microbiota and their association with heat stress in Chinese Holstein cows

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Heat stress negatively affects animal health, biological functions and reproduction. On the molecular level, heat stress changes the genes expression and therefore causes changes in proteome and metabolome. The importance of a microbiome in many studies showed that it is considered as individuals' 'second genome'. In the case of heat stress, the microbiome and its association with a host genome plays an important role in host cells' metabolism and thus changes on a physiological level. In this study, we identified and quantified bacteria associated with heat stress that was quantified by three metrics – rectal temperature, drooling, and respiratory scores. We amplified 136 faecal samples of 136 Chinese Holstein cows to isolate the V3-V4 regions of 16S rRNA gene. Lactating cows were examined in this study with an averaged 127.5 days in milk. Heat stress phenotype were expressed by Estimated Breeding Values for rectal temperature, drooling score, and respiratory score. Each EBV was corrected for effects of parity, lactation stage, and temperature humidity index. Sequence data were processed using a pipeline involving QIIME2 software and classified using SILVA 16S rRNA database. Statistical modelling was performed using a negative binomial regression implemented in the edgeR package. Significant results were filtered out using the False Discovery Rate (FDR)<0.05 criterion. The analysis revealed the total number of 24 genera associated with heat stress metrics. *Pseudobutyrivibrio* and *Succinivibrio* turned out to be the most significant. Other studies already identified that those genera had significantly increased abundance in mice exposed to stressor-induced changes. Moreover, identified microbiota significantly associated with heat stress measures were mostly mesophilic, so their association seems to be due to heat stress-induced secondary, metabolic changes, and not directly by temperature. Additionally, high fold changes of many genera suggest that they may be used as biomarkers for monitoring the level of heat stress in cattle.

Identification of heat stress responsive transcripts in Sprague-Dawley rats using mixed linear model

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In the study, the Norwegian rat was used as a model organism for a whole genome scan of transcript expression alterations due to heat stress. Rats from the heat-stressed group were exposed to 42 °C for 120 minutes, while rats from the control group were housed at 22±1 °C. Total RNA was isolated from blood (4 individuals), liver (5), and adrenal gland (5) for the control and heat-stressed groups of 8-week-old female rats. Illumina HiSeq 2000 was used to sequence reads in the paired-end mode. The bioinformatic pipeline consisted of: raw reads quality control, raw read editing based on reads' quality, transcript expression quantification and identification of differentially expressed transcripts. In the model, a dependent variable was log2(fold change)s in expression between control and heat-stressed animals. Transcript effects on heat stress were estimated accounting for the similarity between transcripts representing the same gene introduced by a nondiagonal transcript covariance matrix. The covariance was expressed by the Jaccard Index based on the similarity in exon composition of each transcript within the same gene, while independence between genes was assumed. The expectation-maximization algorithm was applied for the estimation of variance components of the model. To maximise the computational performance, a custom written Python program implementing Numba library was used. The effect of correlated transcripts explained 25.38% of the total variation of log2 in the adrenal gland tissue, 11.68% in blood, and 14.70% in liver. There was very little overlap among the top 10 most significant transcripts between tissue, indicating that the physiological response towards heat stress is tissue-dependent. Except for Gpx4, none of the identified genes has previously been reported for the association with heat stress response in rats in independent data sets, however some of the top 10 genes were reported in a previous analysis of this data.

Session 04 Theatre 8

Impact of heat stress on physiological characteristics and nitrogen utilisation in growing pigs

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Climate change may enhance extreme weather conditions with periods of high ambient temperature and humidity to which pigs have not been adapted. Heat stress (HS) may hamper animal well-being and performance as well as nutrient utilization and excretion into the environment. Studies have indicated that HS affects protein deposition and body composition of growing pigs. The reduction in feed intake and alterations in energy expenditure influence protein and lipid deposition in the body. In addition, HS can alter the intermediary metabolism, e.g. through changing insulin sensitivity of tissues, with direct impact on protein and lipid deposition. These processes may influence the response of the pig to dietary protein and amino acid supply under different climatic conditions. Better understanding of the impact of HS on protein retention is essential to optimise diet composition and nutrient utilisation. The present study was conducted to determine the influence of dietary protein and energy supply on nutrient digestibility, nitrogen retention and physiological characteristics in growing pigs in thermoneutral and hot conditions. The study was performed as a 2×2×2 factorial arrangement with two thermal conditions (24 and 32 °C), two dietary feeding levels (2.5 and 3.0 times energy requirements for maintenance) and two dietary protein levels (75 and 115% of requirements). The study was replicated over time in a cross-over design using 40 male pigs in two periods. After 9 d of adaptation, faeces and urine were collected throughout 5 d and subsamples subject to chemical analysis of proximate components allowing to calculate nutrient digestion and nitrogen retention in the body. Body temperature, heart rate, respiration rate, and blood characteristics were registered. The results, demonstrating the interaction between dietary protein and energy intake and HS on physiological characteristics, including nutrient utilization, and consequences for optimal nutrient supply, will be presented and discussed.

Session 04 Theatre 9

Betaine and zinc effect on growth and plasma variables of Iberian pigs under long term heat stress

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The objective of this study was to evaluate the effects of long term heat stress (HS) on growth and plasma metabolites of growing Iberian pigs as well as possible beneficial effects of dietary betaine or zinc (Zn) supplementation. Forty pure Iberian barrows (44±2kg BW) were randomly assigned to one of five treatments: (1) thermoneutral (TN, 20 °C) and *ad libitum* fed a control diet; (2) HS (30 °C) and *ad libitum* fed a control diet; (3) TN and pair-fed a control diet; (4) HS and *ad libitum* fed a betaine supplemented (5 g/kg) diet; and (5) HS and *ad libitum* fed a Zn-supplemented (0.120 g/kg) diet. The study consisted of a 28 d experimental period. Blood was collected at slaughter (61±1.3 kg BW) following an overnight fast. HS significantly ($P<0.01$) increased rectal temperatures compared with TN conditions and decreased (20%; $P<0.001$) feed intake. Weight gain of TN pigs fed *ad libitum* was greater ($P<0.001$) than HS pigs or pair fed TN pigs. HS decreased empty body weight (8%, $P<0.01$) and kidneys weight (17%, $P<0.05$) compared to TN counterparts fed *ad libitum*. Betaine and Zn had no effect on weight of viscera at 30 °C. Heat stress decreased albumin (13%, $P<0.05$) and HOMA-%B (-50%, $P<0.01$), increased glucose (41%, $P<0.05$) and tended to increase urea (22%, $0.05<P<0.10$) at similar feed intake. Betaine and Zn decreased plasma glucose under HS conditions (27 and 24%, respectively, $P<0.05$) and increased HOMA-%B (124 and 64%, respectively, $P<0.05$) indicating improved β-cell function. Insulin, QUICKI, HOMA-IR% and the rest of biochemical parameters were not affected ($P>0.10$) by treatments. In conclusion, chronic heat stress during the growing phase of Iberian pigs had consistent negative effects on performance which was explained by decreased feed intake. Long term HS induced a glucose sparing effect independently of feed intake and possibly increased protein catabolism. Furthermore, although no benefits for growth were observed after betaine or Zn supplementation, β-cell function was improved.

Relationships of stress during fall corral weaning with future productive performance in beef cattle

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Corral weaning is a popular and stressful husbandry practice in beef cattle operations, which is aggravated by the colder weather observed during late Fall in Canada. There is interest to learn about the individual variation of this response and the relationships with cattle performance over the production cycle. By using faecal cortisol metabolites (FCM, as an indicator of chronic stress during weaning), we monitored 146 crossbred calves (72 heifers, 30 bulls and 44 steers calves) with 4 to 6 months of age. At the weaning, calves were separated from the cowherd and trucked from the pastures to a handling facility. Calves were then weighed, and a rectal faecal grab was collected. Calves were commingled and housed in outdoor adjacent pens in groups of 10 to 12 calves. Three days after weaning calves were re-weighed and another faecal sample was collected. From this point, calves were submitted to their husbandry practice accordingly to each animal type category. Performance records were monitored in each category throughout their production cycle. Least square means comparisons were conducted to compare FCM and body weight at weaning and 72 h. Regression analysis, correlations and PLS will be applied to analyse the relationships with parameters measured around weaning with performance traits over the production cycle. Preliminary results, comparing FCM levels at weaning and 72 h later indicate an increase, from 38.7 to 67.8 ng/g across all the calves. Over this period, calves lost weight, shifting from 258 to 250 kg. Heifers had the highest (40.5 ng/g) increase in FCMs in response to weaning, followed by steers (26.1 ng/g) and bulls (14.9 ng/g). These results indicate that the FCM was sensitive to detect the stress due to weaning. Further analyses are under course to investigate the relationships of FCM with commercial traits in bulls, heifers and, in steers over the production cycle.

Energy balance and feed restriction effects on milk fatty acids and metabolic profile of beef cows

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Feeding winter diets at a flat rate is a common practice in beef cattle farms, all cows receiving the same diet irrespectively of individual requirements. This results in cows under a certain range of energy balance (EB), which can respond differently to a perturbation. This study aimed to evaluate the effect of a short feed restriction (4 d) in cows under different EB on milk fatty acid (FA) and the plasma profile. The study, funded by H2020 GenTORE, involved 31 lactating Parda de Montaña beef cows (626±48 kg body weight at calving). With d0 as the first day of restriction (58 days in milk), the cows received a diet that met 100% of the standard cow requirements (d-2 to d-1, Basal period), then 55% (d0 to d3, Restriction period) and then 100% again (d4 to d8, Refeeding period). Milk and blood samples were collected to determine the milk FA and plasma metabolites [glucose, non-esterified FA (NEFA), β-hydroxyburyrate (BHB), urea and malondialdehyde (MDA)]. The cows were clustered according to their previous performance and EB into 2 groups, Balanced (BAL) and Imbalanced (IMB) cows. Data were analysed with a mixed model considering the EB cluster, the period and their interaction. The milk FA were grouped by their origin (de novo, ≤C15; mixed, C16; and mobilization FA, C17). The de novo FA were affected by EB cluster, with greater values in BAL cows, whereas the mobilization FA were greater in IMB cows ($P<0.05$). During the Restriction, mobilization FA increased and de novo and mixed FA decreased ($P<0.001$), with opposite results in the Refeeding. Regarding the metabolic profile, only urea was affected by EB cluster, with greater content in IMB cows ($P=0.03$). NEFA increased in the Restriction; glucose increased and urea decreased in the Refeeding ($P<0.001$), with no clear differences in BHB and MDA ($P\geq0.10$). There was a moderate correlation between the individual EB and de novo, mixed and mobilization milk FA (r=0.68, 0.60 and -0.71, $P<0.001$) and between NEFA and de novo and mobilization milk FA (r=-0.60 and 0.53, $P<0.001$). Milk FA and NEFA were quick-responding indicators of energy status in beef cows under feed restriction and refeeding periods.

Effect of feeding and shelter management on age at first ejaculation in Murrah buffalo bulls

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Buffalo males generally considered late maturing and start semen donation at higher age, this investigation was aimed to study the effect of dietary energy and omega-3 fatty acid on growth, and age at first ejaculation (AFE) of Murrah males under different shelter management. A total of 30 Murrah buffalo male calves of 6-8 mo of age were selected and distributed randomly into five groups as T1, T2, T3, T4 and T5. Where, T1 (control) was provided with standard feeding (ICAR, 2013) under existing housing management. T2 with 10% higher energy (HE) (through molasses) diet till attainment of AFE under existing housing management. The males under T3 with 10% HE till 12 mo of age thereafter, supplemented with roasted linseed till attainment of AFE under existing housing management. Similarly, group T4 was provided with 10% HE and T5 with 10% HE and roasted linseed under modified housing management. The bull calves under existing housing only one time bathing during hotter part of the day and in modified housing they were provided with fans and high pressure foggers during summer at a regular interval and curtains during winter. The parameters viz. microclimatic condition of shed (THI), growth, digestibility, hormones and blood metabolites were recorded. The AFE was observed semen parameters were analysed. The results revealed that the average THI of modified house remained low ($P<0.01$) during summer and higher in winter compared to the existing housing management. The feeding and housing interventions influenced ($P<0.05$) the AFE; the bulls under group T5 ejaculated at an early age (15.58±0.41 m) followed by the T4 (16.98±0.50 m), T3 (17.08±0.26 m) and T2 (18.00±0.42 m) however, the bulls under T1 reached AFE late (18.67±0.64 m). The semen quality parameters (volume, concentration, motility, live %) were higher ($P<0.01$) in T5 compared to other groups, however, bulls under T1 had lower ($P<0.01$) quality compared to other groups. This study concluded that the Murrah buffalo males have the potential to grow faster, and can attain early puberty and AFE with better semen quality when provided with improved feeding and seasonal housing modifications.

Effect of feed restriction on lactating Charolais cows

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In a context of climate change where forage resources could be limited at certain times of the year, it becomes important to better understand how farm animals will adapt to this situation. In this study, 340 primiparous Charolais cows from two experimental farms were enrolled in a feed restriction challenge where half of the animals were fed 3 forage unit (UF) below their expected needs (around 30% below) from 10 days after their calving until being turn out on grass in April (average duration of 85 days). The animals were then conducted all together until mid-July (recovery period, average duration of 89 days). Cows and calves were weighted every two weeks and body condition score (BCS) of the dam was evaluated monthly. Milk production was estimated three times during the lactation (mid-restriction, end of restriction and mid-recovery) by weighting the calf before and after suckling for 24 hours. Finally, resumption of cyclicity was observed by progesterone sampling. In one of the farms, the animals performed their second and third lactations under the same conditions, for a total of 592 lactations. Generalized linear models were applied to correct farm and diet effects on the phenotyped traits. There was no difference between the diet groups before the restriction. At the end of the restriction period, restricted dams were on average 84 kg lighter than the unrestricted ones (which corresponds to 1.0 S.D.) and half a point of BCS bellow (0.8 S.D.). Calves from restricted dams were also lighter (20 kg difference corresponding to 0.68 S.D.) in association with a smaller milk production (-1.67 l at 90 DIM, corresponding to 0.77 S.D.). Finally, the resumption of cyclicity of restricted primiparous occurred on average a cycle later (difference of 18.4 days, which corresponds to 0.48 S.D.). The differences decreased during the recovery period but were still significant at its end. However, they disappeared on a longer period of time as no more difference was visible at the start of the next calving season.

Heat tolerance indices for local sheep breeds in the Mediterranean-Karagouniko & Barki case studies

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The study investigates the adaptability to heat stress conditions of two local sheep breeds (Karagouniko in Greece and Barki in Egypt), that are under selection programs. Karagouniko sheep trial was carried out on a dairy farm in Avra-Kalambaka, where animals grazed in natural and cultivated pastures. Environmental temperature and relative humidity during grazing were recorded at 5-minute intervals, skin and rectal temperature and respiratory rate were recorded after returning from the pasture on 12 ewes. Three visits were conducted on June-July-August. Thermal heat indices estimated include: environmental (ETHI), individual (ITHI), heat tolerance coefficient (HTC) by Iberia test and adaptability coefficient (AC) by Benezra test. ETHI was severe throughout the experiment (24.9), with extreme severe ITHI (28.3). Mean HTC was 108.1 in June, 108.3 in July and 103.1 in August, closer the values to 100 indicate adaptability. Mean AC was 2.17 in June, 2.14 in July and 2.25 in August, the closer the values to 2, the higher the adaptability. The study on Barki sheep was conducted on the Coastal Zone of Western Desert in Egypt on rams from 43 breeders. Rams were exposed to direct solar radiation at 12-2pm in August 2017. Estimated THI were 86.4 indicating that animals were under severe heat stress. HTI was estimated based on changes (±2SD) in physiological parameters; (respiratory rate, skin and rectal temperature). Animals scored 1 (no changes or change in 1 trait) were classified as high tolerant (HT,37.2%). Changes in the 3 traits classified animals as low tolerant (LT,20.9%). Changes in ST and RR with heat stress were significantly higher for LT and lower for HT animals. Changes in RT was highly significant for LT animals and insignificant for HT. Results indicated a highly desirable adaptation of local breeds of Karagouniko and Barki sheep in extreme severe heat stress conditions that could contribute in the discussion of adapting breeding programs under the climate change challenge. Research was carried out in the framework of 'PeRFORM' project (ARIMNet2).

Challenges of 16S rRNA analysis in Chinese Holstein cows under heat stress condition

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The development of modern molecular techniques delivered Big Data problems into biological sciences. Developments in bioinformatics allow one for insight into the more detailed molecular mechanisms by deploying sophisticated bioinformatics software. However, due to the numerous methods, in some cases, choosing a wrong method may result in erroneous results. Heat stress is a hot topic in animal science due to both, rising global temperatures and the optimisation of livestock physiology towards increase in the amount of product obtained from a single animal. Previous studies have confirmed that changes in the gut microbiome were associated with susceptibility to heat stress. The abundance of gut microorganisms can be quantified using 16S rRNA sequencing. In this study, we use 136 faecal samples of 136 lactating Chinese Holstein cows with an averaged 127.5 days in milk for whom heat stress was expressed by Estimated Breeding Values (EBV) for rectal temperature, drooling, and respiratory scores. EBVs were corrected for the effects of parity, lactation stage, and temperature humidity index. Since the reaction efficiency of the sequencing library is different for each sample, the normalisation step is required to compare all samples. After the normalisation step, the differences are comparable across samples and thus can be analysed e.g. using statistical models. In our study, we applied various normalisation techniques, including scaling, rarefying, and log-ratio normalisations to the 136 faecal microbiome samples. The results demonstrated a significant impact on normalisation on the results of statistical modelling of differential microbiota abundance. Since different statistical models were applied, including generalized linear models based on the negative binomial distribution as well as linear models including robust regression and mixed models, the final result defines a model, which is the most robust towards various normalisations in terms of the selection of taxa significantly associated with heat stress indicators.

Development of a gut barrier failure model in broiler using a rye-wheat based diet and heat stress

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An intact intestinal barrier in broiler is essential to avoid translocation of unwanted substances like toxins into the bloodstream, which can induce a systemic inflammatory response resulting in poor growth performance. The aim of the study was to evaluate the effect of a rye-wheat barley diet (RWB) in combination with heat stress (HS) on gut permeability and gut health. Twenty-four one day old Ross 308 broilers were randomly assigned to three groups. All animals were fed a corn-based starter diet until day 14. Thereafter, a rye-wheat-barley diet was fed to group two (RWB) and group three (RWB+HS), whereas group one was fed a corn-based diet (Control). On day 28, groups one and two were kept at thermo neutral conditions (23 °C), while group three was exposed to heat stress conditions (36 °C). After seven hours, all animals orally received fluorescein isothiocyanate (FITC)-dextran (2.2 mg/bird). Three hours after application animals were sacrificed to collect blood and intestinal tissue. The fluorescence signal of FITC-dextran was determined in the serum. The acute phase protein alpha-1-acid Glycoprotein (AGP) was measured with a commercial ELISA kit in the serum. Expression of selected genes in the duodenum, jejunum and ileum was measured with qPCR. The FITC-dextran concentration was increased in the RWB (1.25-fold), and RWB+HS treatment (1.99-fold) compared to the control. There was no effect of any treatment on AGP levels in the serum. In duodenum, RWB and RBW+HS treatment decreased the expression of HSP60 (-2.4-fold; -3.10-fold). In jejunum, RWB treatment decreased HSP60 (-3.0-fold), FABP2 (-2.8-fold), and increased HSP70 (2.2-fold), FABP6 (3.1-fold) and CLDN1 (2.8-fold). RWB+HS group decreased the expression of HSP60 (-3.7-fold), FABP2 (-2.3-fold), and increased FABP6 (3.2-fold) and CLDN1 (5.3-fold). In ileum, RBW+HS treatment reduced HSP60 (-2.20-fold), FABP6 (-7.5-fold) and CLDN3 (-2.1-fold). To conclude, the combination of rye-wheat based diet and heat stress showed the strongest effect on FITC-dextran translocation from the gut and expression of selected genes related to gut permeability and gut health. These findings indicate an impaired intestinal barrier in the model.

In silico prophylactic supplement for acute heat stress from expressed sequence tags of chicken

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The domesticated chicken breed is prone to acute heat stress, in particular, the fast-growth commercial chicken breed. Not only due to the environment but homeothermic nature and sensitivity for temperature ambient cause significant rearing cost and mortality rate. The *in silico* prophylactic supplement modelling was proposed to minimize the side-effect of acute heat stress. In the first stage, homology modelling, protein structures, and hypothetical proteins were constructed from expressed sequence tags (EST) of six genes associated with hypothalamic responses and thermoregulatory peptides. The available EST sequences were retrieved from the ftp database (dbEST) NCBI and EST DDBJ. The *in silico* EST analysis produced twenty-five consensus contigs and six hypothetical protein models as the following *AgRP*, *CART*, *CCK*, *HSP 4fl9*, *HSP 5uls*, and *NPY*. The first stage marks a footprint to identify the protein-ligand interaction, protein conformation, and molecular dynamics in future research.

The effects of HeatAntistress on dairy cattle performance during increased heat stress periods

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A commercial product (HeatAntistress; ASTR) containing potassium carbonate (50.5%), dry yeast (21%), vegetable fat (16.5%), calcium propionate (10.0%) and a mix of vitamins and minerals (2%) was tested during summer in a dairy farm. Dairy cows (n=96) were ranked based on their milk yield in two feeding groups: High (42±6.2 kg/day) and Low (32±4.7 kg/day). Then, cows within each group were randomly allocated to two treatments: control (no addition of ASTR) and ASTR (150 g/day of ASTR), forming four treatments: HCTR and HASTR, LCTR and LASTR (n=24/treatment). Cows within each group were fed the same diet with or without the addition of ASTR. The experiment period lasted for 9 weeks (from 6/7/20 to 28/8/20). Diets were fed *ad libitum* allowing 5-10% refusals and milk yield was recorded daily. Every three weeks samples were taken for 3 consecutive days to define dry matter intake, rumination time and milk yield. Statistical analysis was conducted with JMP using a mixed effects model for High and Low groups separately. Daily THI was on average 74 ranging from 64 to 86, and during the severe heat stress days was on average 81.5 ranging from 67 to 86 during the day. For both feeding groups milk yield was decreased during summer months but the decrease was more severe for CTR cows. Cows at the HASTR produced 2.1 kg more milk compared with HCTR cows (39.7 and 37.6 kg/d, respectively; $P<0.01$), while cows at the LASTR tended to produce 1.3 kg more milk compared with LCTR cows (31.8 and 30.5 kg/d, respectively; $P=0.08$). The rumination time (on average 571±12 min/d), body temperature (on average 38.3±0.1 min/d) and respiration rate (on average 55.8±1.7 breaths/min) were not different between treatments. However, during severe heat stress days (daily average THI>82) cows at the HASTR demonstrated lower respiration rate compared with HCTR cows at 8:00 and 11:00. In conclusion, the addition of HeatAntistress was beneficial for high producing dairy cows.

Prediction of the effects of heat stress on the farrowing rate in sows

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Seasonal variation of the climatic environment is known to adversely affect reproductive performance in sows. This study was designed to identify the period within the reproductive cycle when elevated ambient temperature has the greatest effect on the farrowing rate (FR) and to implement empirical relationships between temperature and FR according to the parity number of reproductive sows. The data consisted of 2,120,000 records on 497,541 sows from 780 commercial farms in mainland France and the island of Réunion collected from 2002 and 2012. Climatic parameters were provided by public meteorological stations located at proximity from pig farms (less than 10 km). Heat stress on a given day was measured in term of maximal temperature (Tmax). Pearson correlation coefficients were calculated between Tmax and FR for each day of the reproductive cycle and each sow. The pattern of the correlation coefficients during the reproductive cycle was fitted with a polynomial function. Consecutives days with greatest negative correlation between Tmax and FR were considered as the period for which Tmax has maximum effect on FR. Our results indicated that increasing Tmax between 26 and 5 days before the first insemination had largest impact on FR for primiparous sows. For multiparous sows, heat stress during 20 and 5 days before inseminations had largest effect on FR. The average of Tmax over these periods was then used to calculate polynomial relationships between heat load and average FR. In primiparous and multiparous sows, the relationship were as followed: $FR (\%) = 84.05 + 0.376\times Tmax - 0.015\times Tmax2$ (adjusted $R^2=0.85$) and $FR (\%) = 87.66 + 0.506\times Tmax - 0.020\times Tmax^2$ (adjusted $R^2=0.96$), respectively. In multiparous sows, the second parity sows seemed to be more susceptible to heat stress than sows in later parities. These empirical equations could represent an interesting tool for predicting the consequence of warming climate of reproductive performance of sows.

Session 05 Theatre 1

Insect-fed poultry value chains: trade-offs between opportunities and risks

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Insects such as fly larvae are valuable protein sources for feed that can replace soymeal or fishmeal in feed for livestock such as poultry. Because fly larvae can be produced on organic side streams their inclusion in feed contributes to circular agriculture. Commercialization of insect-fed poultry production requires the development of People-Planet-Profit (PPP) sustainable value chains. These range from side streams for substrate to final consumption of poultry products (meat and eggs), and have a high level of complexity, including various processes, actors and stakeholders. Careful design is therefore an important requirement for commercial success. Within the InsectFeed project (www.insectfeed.nl), all main aspects of these (future) value chains are included, and a basic qualitative conceptualization of the problem was carried out. First, a generic concept of an insect-fed poultry production value chain was designed, including major processes and transitions, main actors involved and stakeholders that might influence (beyond) legal decision making. Second, using the concept value chain, an inventory of opportunities and risks regarding the use of insects for poultry feed was conducted within the (appr. 35) consortium participants covering all relevant areas. Third, a classification of opportunities and risks was carried out, resulting in identifying trade-offs between opportunities and risks. The most important area was the use of (new) side streams for substrate to grow insects. Opportunities included side stream utilization and increasing the circularity (i.e. replacing soybean by insect protein in poultry feed), which coincide with risks on feed and food safety (side stream contamination). Moreover, this complex trade-off includes both technical, socio-economic and food-safety aspects, and is largely asymmetrically distributed along the value chain (e.g. between insect producers and poultry farmers). It was concluded that optimizing this trade-off in the context of the total value chain is pivotal for the commercial success of the value chain, and its implications for business model development are discussed.

Session 05 Theatre 2

Is the use of live insect larvae as environmental enrichments able to improve broiler welfare?

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The use of insect larvae as environmental enrichments has recently been tested in poultry, but implications for bird welfare and behaviour are poorly understood. This study investigated the effects of *Tenebrio molitor* (TM) and *Hermetia illucens* (HI) larvae on behaviour and faecal corticosterone of broiler chickens. A total of 180 4-day-old male broiler chicks were allotted to 3 treatments (6 pens/treatment, 10 birds/pen): control (C, commercial feed), HI (C + 5% of expected daily feed intake [DFI] HI live larvae) and TM (C + 5% of DFI TM live larvae). Three pens/treatment were filmed for 15 minutes every day (morning, larvae intake, and afternoon), and duration and frequency behaviours were analysed (BORIS). Faeces were also sampled and faecal corticosterone quantified by enzyme immunoassay (6 pools/treatment). Data were analysed by SPSS software ($P<0.05$). Insect-fed birds performed more stretching and wing flapping in the morning when compared to C, with TM group also showing higher frequency than HI ($P<0.001$). During larvae intake, scratching was greater in insect-fed birds when compared to C, and insect groups also displayed less grooming and leg stretching than C ($P<0.05$). In the morning, TM group spent more time ground pecking when compared to C, whereas more walking time was displayed by HI birds than C and TM ($P<0.001$). The C group also spent more time grooming when compared to insect-fed birds ($P<0.001$). During larvae intake, more ground pecking time was observed in TM group than HI, while TM birds spent less time grooming when compared to C ($P<0.001$). Less resting time was also identified in the insect-fed broilers than C ($P<0.001$). In the afternoon, TM birds spent less time ground pecking when compared to C and HI, with greater resting time being also observed in insect-fed groups than C ($P<0.001$). Faecal corticosterone was not affected by insect larvae administration ($P>0.05$). In conclusion, the use of live insect larvae stimulated the birds' activity, also increasing some positive behaviours.

Session 05 Theatre 3

Impact of dietary black soldier fly larvae on laying hen performance, egg quality and lipid profile

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Recently, the U.S. Food and Drug Administration and Association of American Feed Control Officials approved Black Soldier Fly larvae (BSFL) as a feed ingredient for poultry. The objectives of this work were: (1) to evaluate the nutritional profile of whole BSFL for laying hens; and (2) measure the impact of the dietary whole BSFL treatments on hen performance and egg quality. Replicate cages of commercial Single Comb White Leghorn hens were fed whole BSFL from 60-64 weeks of age. All diets were iso-caloric and iso-nitrogenous, with a corn-soy control (CON) compared to 3 diets containing whole larvae at 6, 12, and 18% of the diet. Data were analysed by one-factor ANOVA for the main effect of diet and Tukey's multiple comparison utilized for mean separation when the F test was significant. The results of the experiment indicated hen body weight averaged 1,737 g, egg production 85.02% and egg size 64.17 g and were not significantly impacted by the BSFL diets compared to the CON ($P>0.05$). However, feed intake and feed conversion (kg egg/kg feed) were significantly reduced by greater dietary levels of larvae ($P<0.05$). Measures of egg quality including blood spots, meat spots and yolk colour were not impacted by the treatment diets ($P>0.05$), although egg specific gravity was higher ($\bar{x}$=1.075 vs 1.073) and albumen height ($\bar{x}$=7.99 vs 8.55) and Haugh units ($\bar{x}$=87.54 vs 91.28) were lower among the BSFL treatment eggs compared to the CON ($P<0.05$). Egg yolk fatty acid profile was also impacted by the BSF larvae dietary treatments. Greater amounts of yolk fatty acids C12:0, C14:0, C14:1n5 and C21:0 were realized with increasing dietary levels of larvae ($P<0.05$), however, there was no impact of the treatments on C18:2, linoleic acid or C18:3n3, alpha-linolenic acid ($P>0.05$). These findings suggest that whole larvae of the BSF fed to laying hens can maintain body weight, egg production, egg weight, improve feed conversion and modify the fatty acid profile of the eggs.

Session 05 Theatre 4

Selenium biofortification of *Hermetia illucens* prepupae

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The aims of this study were: (1) the evaluation of selenium bioaccumulation capacity of black soldier fly (BSF) prepupae in order to create fortified insect meals for specific feed purposes; and (2) the investigation of selenium speciation applying an ICP-MS/HPLC-ICPMS method. The larvae were reared on three different substrates: (1) Gainesville diet, used as control substrate (CTR); () *Ascophyllum nodosum* diet (AN30%), with 30% substitution of the alfalfa meal with brown algae; and (3) selenium diet (Se), based on Gainesville diet fortified with 0.3 mg/kg of selenium in the form of sodium selenite. Eggs collected from a stable colony of BSF were placed for hatching on the three experimental substrates. All experiments were carried out under dark condition, at 25 °C with 70% relative humidity. Deriving prepupae were analysed for selenium concentrations and selenium species. Furtherly determination total Se in the soluble alkaline extract for inorganic selenium and soluble protease and soluble chitinase extracts for organic selenium were also carried out. The mean total selenium content in the prepupae reared on Se enriched substrate (Se diet) was more than 5 times higher compared to BSF larvae reared on CTR and seaweed enriched substrates (AN30% diet). Considering the selenium speciation analyses, in all experimental group the main part of selenium content (>50%) was in the form of organic selenium. Furthermore, the main part of inorganic selenium was in the form of selenite with an average percentage of 38.6%. Differences were also observed among groups in term of total Se in the soluble alkaline extract for inorganic selenium and soluble protease and soluble chitinase extracts for organic selenium. These results could indicate that BSF larvae reared on specific/selected rearing substrates are able to convert inorganic Se to organic Se. The overall results shows that both seaweed and sodium selenite inclusion affect the BSF prepupae total Se and selenium species.

Inclusion of *Tenebrio molitor* in broiler feeds as a partial substitution for hipro soybean meal

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Tenebrio molitor (yellow mealworms) have a similar protein content compared to traditional protein feed ingredients. There is therefore the potential to use them as a partial replacement, which could help alleviate some of the pressure to find alternative feed ingredients to improve sustainability in livestock production. The aim was to investigate growth, feed intake and feed conversion ratio of broiler chickens fed mealworms as a partial replacement of soya in the feed. Two isonitrogenous and isoenergetic crumb feeds were formulated and obtained from Target Feeds. One was the control (CO) diet based on a previous chicken trial and the other contained crushed dried mealworms (MW) to replace 10% of hipro soybean meal. 80 one-day old Ross 308 broiler chicks (PD Hook) were acclimatised for 6 days on the CO feed. On day 7 birds were randomly allocated to pens (n=4/pen) with 10 replicate pens per feed. Birds were housed according to ASPA and fed *ad libitum* until day 35/36 when birds were culled. Bird live weight and feed consumption were measured twice weekly. Data were analysed by repeated measures ANOVA, to determine the effects of feed, time, and their interaction, using Genstat. As expected, average daily gain (ADG) (g/bird/d) significantly increased over time ($P<0.001$), but there was no significant effect of feed. There was a significant interaction ($P=0.034$) between feed and time for average daily feed intake (ADFI) (g/bird/d), with a higher ADFI in the MW group up until the final 6 trial days, when there was higher intake of the CO group. Feed conversion ratio (FCR) significantly decreased with time ($P<0.001$) and the MW group was significantly higher compared to CO group ($P<0.001$). Overall, no significant differences in the ADG suggests that there is potential for mealworm to be used as a partial replacement for soybean meal in broiler feeds. However, the higher ADFI and subsequent increased FCR suggest that digestibility may be reduced. Further work is needed to investigate the effects of MW inclusion on feed digestibility.

Practical, semi-purified diets for protein requirement determination in *Hermetia illucens* larvae

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To effectively incorporate insect breeding into circular economy model, it is necessary to maximize production through waste. For this purpose, it is essential to breed insects that are able to efficiently convert waste into a resource, and the black soldier fly (BSF) appears to be one of the most promising species. This study aimed to determine BSF larvae protein requirement to optimize the waste-based diets formulation. Four practical semi-purified (PSP) and isoenergetic diets with increasing protein levels (10%, CP10; 14%, CP14; 16%, CP16; 19%, CP19) were tested (100 larvae/box; 4 box/replicate). The Gainesville diet (GA) was applied as environmental control and, subsequently, excluded from the statistical analysis. Larvae growth was determined at 10, 14 and 18 day-old, while survival rate was calculated at the end of the experiment. When a pupa was identified, it was placed in a numbered box to assess the adult emergence rate (ER) and the fly live weight. Fly life span, exuvia and dead fly weights (DFW) were evaluated at the fly death. Data were analysed by means of Kruskal-Wallis and One-way ANOVA tests and General Linear Mixed Model (IBM SPSS Statistics V20.0.0, $P<0.05$). At 10 d, CP10 larvae showed the worst weight ($P<0.001$). The CP16 and CP19 groups remained heavier than CP10 at 14 d ($P<0.001$). At 18 d, a difference between CP14 and CP19 larvae weight was observed (0.238 and 0.223 g; $P<0.001$). Considering the whole trial, CP16 treatment was found to be numerically the best, and statistically different from CP10 and CP14 larvae. The CP19 group showed an intermediate performance between CP14 and CP16. In the adult parameters, the only statistical difference was detected in the DFW, with CP10 flies weight being heavier than CP19, and CP14 and CP16 showing an intermediate weight ($P<0.01$). No difference was observed for ER. However, the GA showed a more than double ER when compared to the experimental diets. In conclusion, considering the larval stage, the optimal protein level appears to be 16%. Furthermore, given the effects of the PSP on adult emergence, additional researches should be carried out to evaluate the nutrient deficiency that led to this outcome.

Production and optimization of *Hermetia illucens* (L.) larvae reared on former foodstuffs

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New waste management practices able to upcycle food waste streams, such as former foodstuffs, are required. At the same time, the European feed sector requires new sustainable protein sources that have lower environmental and social impact than the conventional sources (soymeal and fishmeal). During WICE4Soil project, the production of *Hermetia illucens* larvae (L.) (BSFL) was conducted in 20 batches at pilot scale, and the quality and safety of BSFL and insect frass (residue from the production) were assessed. Based on the pilot production a Return-on-Investment business model was conducted. About 1,400 kg of former foodstuffs were bioconverted into 240 kg of BSFL suitable for pet food and 230 kg of insect frass intended for soil improvement. The bioconversion of former foodstuffs into BSFL was found to be highly efficient, with feed conversion rate (FCR) values ranging between 2.3 and 4.0 when assessed on dry-to-dry matter (DM) basis. The larval biomass was found to be rich in both crude protein (35% DM) and lipid (41% DM), and the NPK analysis revealed that insect frass is suitable for use as soil amendment. The separation of frass resulted in a high-quality insect frass fraction (80%) with very low impurities (packaging residues >2.mm) (<1%), and a dischargeable fraction. As expected, the heavy metals analysis revealed that the former foodstuffs, BSFL and insect frass have lower heavy metals concentrations then the maximum limits. Moreover, the business model shows that a Return on Investment of 4.7 years can be achieved in case of BSFL being reared on former foodstuffs. Thus, the results from WICE4Soil, indicate that former foodstuffs can be effectively and safely used as substrate in the production of BSFL and insect frass.

Effect of dietary crude protein on growth performance of mealworms (*Tenebrio molitor*)

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Intensive research about insects as food or feed has been conducted in recent years, but nutrient requirements of insects were merely investigated. Therefore, the present study aimed to determine the effects of varying dietary crude protein (CP) contents on the growth performance of mealworms. 50,000 growing mealworms (average bodyweight of 40 mg) were allocated randomly to 50 groups and were housed in plastic bowls in a climate cupboard. 9 isoenergetic diets with varying CP concentration (g per kg dry matter (DM): 90, 115, 139, 163, 186, 206, 230, 257, 280) were formulated on base of wheat flour, starch, gluten, and a vitamin-mineral mixture. A commercial layer feed (227 g CP per kg DM) served as control (standard). Each 5 mealworm groups were allocated to the 10 diets (9 experimental, 1 control). Biomass (BM) of mealworms and feed supply (FS), which was daily adjusted to feed consumption in order to estimate *ad libitum* feed intake, were recorded per bowl every 5 days starting with experimental day 10. On day 31, mealworms were euthanized by freezing. Final BM, weight gain (WG), and total FS were statistically analysed using ANOVA (all 10 variants) as well as regression analysis (9 experimental feed variants) using dietary CP concentration as regression determinant. BM, WG and FS responded to rising dietary CP according to a cubic function (R^2=0.99) with increasing values at very low dietary CP and maxima at around 120 g CP per kg DM, followed by a decrease at further rising CP concentrations ($P<0.01$). Interestingly, growth performance of the standard group (227 g CP per kg DM) was equal to animals fed the experimental diet containing 115 g CP per kg DM with a putatively low protein quality (predominantly gluten). This gives rise to the hypothesis that CP requirements of mealworms are significantly lower than currently assumed. The depressive effects of experimental diets with high CP contents might be caused by physiological reasons (e.g. amino acid imbalances) as well as physical problems (e.g. sticky consistency of feeds). In total, protein requirements of insects in terms of dietary concentrations as well as amino acid composition need to be investigated more in detail. Conclusions by analogy from common livestock species should be done cautiously.

Suitability of agro-by-products for the rearing of *Zophobas morio* and *Alphitobius diaperinus* larvae

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The aim of our study was to evaluate the suitability of by-products of various agro-industrial processes for the rearing of larvae of the superworm, *Zophobas morio*, and the lesser mealworm, *Alphitobius diaperinus*. The eleven by-products tested were derived from the cotton, sugar beet, sunflower production and processing, as well as from the seed cleaning process of major legumes and cereals widely cultivated in Greece. At first, we performed a screening of the by-products' potential to support complete larval development. Plastic cylindrical vials were filled with 4 g of each by-product for *Z. morio* and with 1 g for *A. diaperinus*. A mixture of wheat bran and dry yeast (9:1) served as control. Groups of 20 larvae were placed into the vials and allowed to feed for 4 weeks, after which larval survival and weight as a group were determined bi-weekly until pupation (*A. diaperinus*) or until a length >5 cm was reached (*Z. morio*). Development time, as well as feed utilisation parameters were calculated per by-product. Carrot slices were provided as a moisture source, whereas the substrate was refreshed when depleted. In a second series of bioassays, isonitrogenous diets (at two protein levels of 16.7 and 20%) based on the best-performing by-products were formulated and evaluated following the experimental design described above. Pure sunflower, barley and oat by-products efficiently supported larval growth of both species. Regarding the compound diets, survival rate was in most cases low and larval development was slowed for *Z. morio*. For *A. diaperinus*, larval performance in most of the diets was similar to control. Our results show promise for the efficient valorisation of some of the by-products and diets tested for the rearing of *Z. morio* and *A. diaperinus* larvae. Funding: Operational Program Competitiveness, Entrepreneurship and Innovation, Call: RESEARCH – CREATE – INNOVATE (T2EDK-01528).

Safety of black soldier fly (*Hermetia illucens*) larvae reared on substrates with veterinary drugs

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Manure could be used as substrate for black soldier fly larvae rearing (BSF, *Hermetia illucens*), to convert this organic waste stream into protein-rich ingredients for feed and food. However, manure often contains residues from veterinary drugs. For example, the antibiotics doxycycline, flumequine and sulfadiazine, and the antiparasitic drugs flubendazole and ivermectin are regularly found in pig or chicken manure. This study aimed to investigate the effects of veterinary drugs on black soldier fly larvae rearing, including growth and survival, as well as on the transfer of residues of these veterinary drugs from substrate into larvae. Five-days old larvae were exposed to either 0.05 and 0.5 mg/kg flubendazole, 0.05 and 0.5 mg/kg ivermectin, 0.5 and 5 mg/kg doxycycline, 0.5 and 5 mg/kg flumequine or 0.5 and 5 mg/kg sulfadiazine for one week. The growth and survival of the larvae was not affected in any of these treatments, except for the growth of larvae reared on substrate with the high ivermectin concentration, which was significantly lower than the control. In general, the concentrations of the veterinary drugs in the larvae were low after one week exposure. Only, the observed concentrations doxycycline in the larvae were high, compared to the European Commission maximum limit for doxycycline in meat products. Next to this, possible breakdown or conversion of the veterinary drugs by the larvae was observed, except for sulfadiazine. In conclusion, the investigated veterinary drugs as present in the substrate did not affect larval growth and survival of BSF larvae, except for the growth reducing effect of ivermectin. Veterinary drug concentrations in the larvae were generally low, except for doxycycline. Therefore, when rearing BSF larvae on substrates like manure, the possible presence of veterinary drugs should be determined to ensure optimal insect growth and safety of insect products.

Growth and nutrient conversion of BSF larvae fed substrates varying in chemical composition

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Growth performance and nutrient conversion in *Hermetia illucens* larvae (black soldier fly; BSF) were assessed in triplicate in seven substrates: chicken feed (CF; reference diet), liquid pig manure + silage grass (PMLSG), organic wet fraction of household waste (OWF), slaughter waste, sludge after separation of coarse fraction – water separated from fats and solid particles (SW), fast-food restaurant waste (FFW), mushroom feet (MF), and solid pig manure (PMS). On top of each substrate, 1,850 starter (8 days-old) BSF larvae per kilogram (wet) substrate were incubated in 21 plastic containers (75×47×15 cm) (each substrate in triplicate) in a climate chamber under dark conditions. Water or wood shavings were added to create a substrate with 35% dry matter. At the end of the experiment substrates were much drier and pH of the substrates had increased. Highest larval growth rate (17.2 mg/d) was observed in larvae fed FFW, which was even higher than in larvae fed the CF (8.1 mg/d). Growth rates of larvae fed other substrates were in descending order: OWF, PMS, MF, SW, PMLSG. Waste reduction index (WRI) was highest at FFW followed by CF and WRI and these values for other substrates were in descending order: SW, OWF, MF, PMS, PMLSG. The Efficiency of Conversion of Ingested substrate (ECI) was highest in FFW followed by PMS, PMLSG, OWF, CF, MF, SW. Crude protein conversion was calculated as crude protein in larvae mass divided by crude protein in substrate mass at the start (DM based) × 100. Crude protein conversion was highest in FFW (55%) followed by OWF (30%), MF (30%), CF (24%), PMS (13%), PMLSG (12%) and SW (7%). Crude fat conversion was highest in FFW (36%) followed by CF (26%), OWF (8%), SW (3%) and PMS (3%). PMLSG and MF substrates did not contain crude fat so its conversion could not be calculated for those substrates. These results implicate that crude protein and crude fat concentrations in substrates affect BSF larval growth performance and nutrient conversion. Further research on factors affecting nutrient conversion by BSF larvae is required.

Rearing density and dietary inclusion of cassava and taro leaves on growth performance in crickets

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In 2013 the Food and Agriculture Organization of the United Nations (FAO) said that insect production for food and feed could become an economic opportunity for rural women in developing countries. However, since then, just a few initiatives around the world had tried to implement this kind of projects in zones of tropical countries without previous experience in cricket production and insect human consumption such as Colombia. To achieve both roles it is necessary to standardize farming methodologies to be profitable to rural women of the municipality of La Mesa, Cundinamarca, Colombia. Therefore, we carried out two experiments: the first focusing on the effects of density on final weight and length, developing time and survival rate at different initial densities and the second focusing on the effects of replacement of 20% of cassava (*Manihot esculenta*) and taro (*Colocasia esculenta*) leaves powder on final weight and length, developing time, survival rate, fecundity, fertility, and proximate composition. The trials were carried out formulating four diets: (1) control receiving chicken feed diet (18% protein); (2) chicken feed with 20% replacement of taro leaves powder; (3) chicken feed with 20% replacement of cassava leaves powder; and (4) chicken feed with 20% replacement of taro and cassava (50:50) leaves powder. Results of first experiment showed that developing time, final weight and length are affected negatively with increased stocking density, however, a reduction of survival rate was not observed. In the second experiment, the results indicate potential of taro and cassava to alter the growth performance, reproductive parameters, and proximate composition of *Gryllodes sigillatus*. Additionally, the results of the elaboration of culinary recipes by rural women with cricket powder incorporating cultural aspects of the context of La Mesa, Cundinamarca, will be presented.

Replacement of conventional fat sources by black soldier fly larvae fat in weaned piglet nutrition

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Recently, insect derived feed ingredients have received increased attention and are considered sustainable raw materials for animal feed. Furthermore, among insect sources, black soldier fly larvae (BSFL) oil is regarded as a source of highly digestible fatty acids, with potential health benefits due to its high content in MCFA (40% lauric acid). The objective of this experiment was to evaluate the impact of conventional fat source replacement with BSFL oil in starter feed, on growth, faeces consistency and sanitary treatments in piglets. Thirty-two pens of 5 piglets (42-day-old, average body weight of 11.9±1.6 kg) were allocated to 4 different antibiotic-free diets comprising respectively 2% of insect fat, 2% of rapeseed oil, 2% of palm oil or 2% of soybean oil, for 28 days. Individual body weight (BW), average daily gain (ADG), pen-based daily feed intake (ADFI) and sanitary treatments were recorded. Faeces consistency per pen was scored daily (scale 0 to 3). Regarding sanitary status, few pigs were treated for diarrhoea (7%) during the trial, with no significant difference between groups. Faeces consistency was improved with insect fat compared to the other groups (score 2; $P<0.05$). No significant zootechnical difference was observed, although numerically higher ADG were obtained for pigs consuming diets with insect fat and soybean oil, reflecting higher ADFI in those 2 groups. Using BSFL oil as an alternative to plant-based fat sources allowed similar piglet growth performances with some potential benefits on sanitary status that need to be further investigated.

Effects of four mycotoxins on survival, growth and toxin accumulation in *Musca domestica* larvae

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Novel protein sources for animal feed are urgently needed, and the use of insects as feed ingredient is explored as a circular and sustainable approach to feed production. In order to make insect rearing sustainable and economically interesting insects could be reared on waste or side streams. Potential food or feed safety issues should be identified and mitigated in advance. Mycotoxins are natural contaminants which are typically and commonly found in agricultural crops worldwide. As part of the InsectFeed consortium, the effect of mycotoxin exposure on survival and growth of the housefly *Musca domestica* L. (Diptera: Muscidae) is examined, as well as possible accumulation of mycotoxins in the larvae. Larval feed is spiked with the mycotoxins aflatoxin B1, ochratoxin A, deoxynivalenol or zearalenone at concentrations of 1 and 10 times the maximum limits set for feed materials by the European Commission. Larval mortality and growth are recorded over five days of exposure. In order to determine whether the toxins accumulated in the larval body, a LC-MS/MS-based method is used to analyse the concentrations of the mycotoxins and several metabolites in the feed offered, the larvae and residual feed material. Mass balance calculations are performed to determine how much of the spiked mycotoxins was recovered in the larval body and the residual material.

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Effect of yellow mealworm meal on growth performance and some welfare traits of broilers

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Utilization of insects as animal feed is regarded as a promising developments, potentially serving as a viable alternative to increasingly fluctuating traditional commodities. This trial examined the dietary use of locally produced yellow mealworm (*Tenebrio molitor*) larvae meal on the growth performance and some welfare traits of broiler chickens. In a 35-day trial, 120 one-day-old broiler chicks (Ross-308) were randomly allocated to 3 treatments (10 chicks per floor pen). Commercial breeding, management and vaccination procedures were employed. Control treatment (TM00) was fed commercial maize and soybean meal diets in mash form. Diets of treatments TM05 and TM10 were supplemented with the desiccated and ground larvae meal at 5 and 10%, respectively. Performance parameters were evaluated for periods: 1-10, 11-24, 25-35 and 1-35 day. Litter, pododermatitis and feather scores, as well as dry matter of litter, were also assessed for the overall period. Results showed that final body weight was increased ($P\leq0.05$) in TM10 compared to TM05. Feed intake and feed conversion ratio did not differ significantly ($P>0.05$) between the treatments. Pododermatitis score was lower ($P\leq0.05$) in TM05 compared to TM10 group, but did not differ from the control treatment TM00. Litter score, dry matter in litter and feather score did not differ ($P>0.05$). This research has been co-financed by Greece and the European Union (European Regional Development Fund) in context 'Research-Create-Innovate' within the Operational Program (Competitiveness, Entrepreneurship and Innovation (EPAnEK) of the NSRF 2014-2020, Project Code: T2EΔK-02356. Acronym: InsectFeedAroma.

Session 05 Poster 16

Effects of dietary yellow mealworm on meat composition and liver genes' expression of broilers

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In this study locally produced yellow mealworm (*Tenebrio molitor*) larvae meal was examined on meat quality and liver genes expression of broilers. 120 one-day-old broiler chicks (Ross-308) were randomly allocated to 3 treatments (10 per floor pen). Control treatment (TM00) was fed commercial maize and soybean meal mash diets. The diets of treatments TM05 and TM10 were supplemented with 5 and 10% of the desiccated and ground larvae meal, respectively, resulting in higher protein, amino acid and fat content. At day 35, all birds were slaughtered. Breast and thigh meat chemical composition and oxidative stability were determined. Expression of target genes was evaluated in liver samples: methionine synthase (MTR), tyrosine aminotransferase (TAT), spermidine synthase (SMS), methionine sulfoxide reductase (MSRB1), betaine homocysteine S-methyltransferase (BHMT). Thigh meat of TM05 and TM10 had decreased ($P\leq0.05$) fat and increased ($P\leq0.05$) protein content compared to TM00. Breast meat malondialdehyde values (1 day of storage) were decreased ($P\leq0.05$) in TM05 and TM10, compared to TM00. After normalization to β-actin expression, quantitative real-time PCR analysis revealed an induction ($P\leq0.05$) in the expression of MTR, SMS and MSRB1 genes in the liver of TM05 and TM10. Greatest induction was observed for SMS (2.7-fold greater gene expression) in TM10. No differences ($P>0.05$) were observed for the TAT and BHMT genes. This research has been co-financed by Greece and the EU (European Regional Development Fund) in context 'Research-Create-Innovate', Operational Program Competitiveness, Entrepreneurship and Innovation (EPAnEK) of the NSRF 2014-2020. Project Code T2EΔK-02356. Acronym InsectFeedAroma.

Dietary yellow mealworm effects on intestine and liver architecture of broilers

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The future shortages of grains and proteinaceous feedstuffs and their continuously rising prices due to the increasing world demand and climate change influence the exploration of alternative protein sources. In a 35-day trial, 120 one-day-old broiler chicks (Ross-308) were randomly allocated to 3 treatments (10 chicks per floor pen). Commercial breeding, management and vaccination procedures were employed. Control treatment (TM00) was fed commercial maize and soybean meal diets in mash form. The diets of treatments TM05 and TM10 were further supplemented with 5 and 10% of the desiccated and ground larvae meal, respectively. At the end of the trial, samples of liver and intestine (8 chickens per treatment) were carefully acquired, fixed in a 10% buffered formalin solution, and subjected to histopathological evaluation. Histomorphometric analysis of duodenum, jejunum and ileum showed that TM05 treatment had higher($P \leq 0.05$) values of villus height compared to both TM00 and TM10. Crypt depth values did not differ ($P > 0.05$) between the experimental treatments. Liver histopathological findings were absent or only minimal lesions were observed. It is possible that the supplementation of broilers' diets with yellow mealworm may form a viable dietary strategy for broiler nutrition, however it needs further evaluation. This research has been co-financed by Greece and the European Union (European Regional Development Fund) in context 'Research-Create-Innovate' within the Operational Program (Competitiveness, Entrepreneurship and Innovation (EPAnEK) of the NSRF 2014-2020, Project Code: T2EΔK-02356. Acronym: InsectFeedAroma.

Effects of replacing soybean with black soldier fly (*Hermetia illucens*) in different swine tissues

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Pig farming has a considerable environmental impact and the need to find sustainable alternative feed ingredients is urgent. Recent studies indicate that some species of insects have great production potential and may be a source of both protein and lipids, which is the case of black soldier fly (BSF) (*Hermetia illucens*). However, it is necessary to evaluate if these kinds of dietary changes have a significant impact on animal health. The objective of the current study was to evaluate the effects of the replacement of soybean meal and oil with a BSF meal on different tissues. Following a randomized block experimental design twelve non castrated males (Landrace×Large White×Duroc crossbred pigs), with average live body weight of 62±3.6 kg, were individually allocated in metabolism cages and fed with experimental diets (BSF0 – Control, BSF50 – 50% replacement; BSF100 – 100% replacement), formulated to be isoenergetic and isoproteic (15% CP). The inclusion of larvae meal in diets was 87.8 and 175.6 kg/ton for respectively diets BSF50 and BSF100. To evaluate the impact of the different diets, after sacrifice, samples of the gastrointestinal tract (stomach, duodenum, jejunum, ileum, colon, cecum), liver, kidney, pancreas, spleen, mesenteric lymph node, lung, heart, testicle, and 3 different fat samples (retroperitoneal, mesenteric, coronary) were taken and fixed in 10% buffered formalin. After fixation, samples were processed for routine H&E staining. Histopathological assessment was performed, and inflammatory infiltrates were scored both in terms of severity and extension on the different tissues. Morphometrical studies (adipocyte cell average area) were also measured on the fat samples. No statistically significant differences were observed between the groups for any of the parameters studied. These results indicate that inclusion of larvae meal in in swine growing diet did not induce morphological lesions.

Consumption preference of feed including *Tenebrio molitor* flour in slow-growing chickens

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Insects are an attractive alternative for animal feeding. The aim of this study was to assess the consumption preference of 50-day-old slow-growing Coloriel broilers fed *Tenebrio molitor* larvae flour for 7 days as the main protein source. 256 chicks were divided into two homogeneous groups based on animal size: the heaviest group (G) had a liveweight of 1,825.41±106.39 g (n=128) and the lightest group (L) of 1,181.25±172.89 g (n=128). In turn, each one of these groups were subdivided into two groups: in one of them (n=8×8) a vanilla flavouring (F) was used as a method of increasing feed palatability, and in the other one (n=8×8) no flavouring (NF) was used. All groups were provided with two isoenergetic and isoproteic different rations, one including *T. molitor* flour (13%) and the other one without flour. Each chick group was daily weighed and the feed consumption was recorded. Flavouring use did not lead to significant differences in consumption, regardless of animal body condition or feed type. Considering feed preference, all chicks significantly chose the *T. molitor* diet (P=0.047) with a 53.71±6.49% compared to 46.28±6.49%. Group G significantly preferred insect flour diet (P=0.033), with 51.94±1.99% compared to 48.05±1.99%. Numerical differences in terms of palatability were observed, preferring the L group the insect flour diet with 55.48±9.28 vs 44.51±9.28%, but this result was statistically not significant (P>0.05). In conclusion, all chicks showed a predilection for *T. molitor* larvae flour diet, being more noticeable in heavier animals, regardless of the flavouring inclusion.

Partially defatted black soldier fly meal inclusion in juvenile Pacific white shrimp diets

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For several years, feed manufacturers are searching for new sources of proteins in order to respond to the growth of the sector and the challenges of sustainable development. At the same time, more than 30% of the world agricultural production is wasted. Recommended by the Food and Agriculture Organization of the United Nations, insects make it possible to valorise food waste. The black soldier fly (BSF) represents a particularly adapted resource to feed fish or shrimps because it is natural, safe, sustainably produced and has a good nutritional quality. The objective of the study carried out in the facilities of IMAQUA (Merelbeke, Belgium) between March 2020 and April 2020 is to evaluate the zootechnical performances of juvenile shrimps (*Penaeus vannamei*) when a part of fishmeal is replaced by a partially defatted BSF meal in comparison to a conventional feed (CTRL). A BSF meal, produced by MUTATEC, is incorporated in pelleted feeds at three inclusion rates (6.4; 12.7 and 19.1%), as replacement material for fishmeal (33; 66 and 100% of replacement). These feeds have been used to feed 600 Pacific white shrimps during 28 days of trial. Final weight (and related weight gain) was numerically higher in all dietary treatments including insect meal compared to CTRL group. Feed conversion ratios were also improved in shrimps fed with insect meal. The optimal inclusion level was 12.7%, the specific growth rate was significantly better than the CTRL for this group of shrimps. An increase in average final weight up till 16.8% for this group was observed after 28 days of feeding. Also, survival was slightly higher in all dietary treatments compared with the CTRL. These results suggest that the replacement of fishmeal with partially defatted BSF meal may positively affect growing performance in shrimp. It therefore seems possible to replace a significant portion (up to 100%) of fishmeal by BSF meal.

Effect of the protein level in diets for growth of *Tenebrio molitor* larvae

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Level of protein in diets for *Tenebrio molitor* larvae is not well established. In studies assessing low-quality feeds protein level may be at or below 12%, whereas in others it raises to 20%. Further, some works compare extreme levels that do not allow for making recommendations regarding larval growth. In this study, diets based on wheat grain and straw included increasing levels of soybean meal to reach protein levels of 99 (T10), 123 (T12), 145 (T14) and 166 (T16) mg/g dry matter (DM), maintaining the same level of starch (566 mg/g DM). Ingredients were ground to 2 mm. Four trays with 20 g feed and 40 larvae (2.80 g average weight) were prepared per treatment. Larvae were allowed to grow for 28 days in a dark room at 23 to 27 °C and 31 to 47% humidity, and fresh carrot was weekly provided as a source of water. Larval and residual weight were recorded weekly. At the end of the experiment, larvae were slaughtered by freezing and lyophilised. Initial weight was considered as covariable for statistical comparison of treatment results. Mortality was not affected by dietary treatments ($P>0.10$), ranging from 12 to 16%. Final larval mass produced did not differ among protein levels, expressed either in fresh or DM basis ($P>0.10$). However, once corrected by the number of surviving larvae, individual growth was higher in P10 and P14 than in P12 (1.37, 1.13, 1.30 and 1.125 mg DM/d for T10 to T16, $P=0.003$). Total DM intake of larvae given T10 was the highest ($P<0.001$), and consequently feed to gain ratio (F:G, on DM basis) was higher for T10 than for T14 and T16 (4.62, 4.18, 3.75 and 3.78 g/g for T10 to T16, $P=0.03$). The higher intake with T10 did not balance overall protein intake, which was lower than that with T16 and T14 ($P=0.002$); however, no differences in protein of fat retention in larvae were recorded ($P>0.10$). Protein efficiency (retained to ingested protein ratio) was higher with T10 than T12 and T14 ($P=0.042$). Within the range of this study, no major effect of the dietary protein level was detected in larval growth or protein retention. However, the higher F:G ratio with T10 and T12 suggests that a dietary level of 14% protein should be suitable for growing *T. molitor* larvae.

Can agro-based wastes be a sustainable approach to improve soil health and productivity?

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The FAO predicts that the global human population will reach 9.1 billion by 2050. To keep up, higher food production will be required to meet increasing food demand. Nowadays, inadequate anthropogenic practices related to agri-food production systems are preventing soil to function as expected by threatening ecosystems and imperilling delivery of ecosystem services. Developing sustainable methods to increase productivity without compromising public health and environmental integrity is vital. Recently, technological innovations have been developed to recover, recycle and value agro-industrial by-products. Special attention is being given to integrated strategies that promote nutrients' circularity (e.g. using insects to transform agricultural wastes into compost). These agro-waste based fertilizers (AWBF) offer the double environmental advantage of waste recycling and low expected environmental hazard, contributing to a circular and sustainable economy. Yet, little is known about AWBF agronomic efficiency and effect on soil biodiversity. This study aimed to evaluate the effectiveness and environmental impact of an AWBF derived from the digestion of vegetable by-products by *Hermetia illucens* larvae and provided by EntoGreen. The productivity of several plant species and impact in soil invertebrates fitness was assessed, as a proxy to soil functions Plants productivity was assessed through a modified version of the Phytotoxkit (MicroBioTestsInc., Belgium) and the fertilizer impact on soil invertebrates was evaluated at laboratory scale. So far, by amending Lufa 2.2 soil with AWBF, we observed no impact on both plants and soil invertebrates; in fact, by increasing AWBF in soil, both plants' aerial part and soil invertebrates' fitness (higher number of juveniles) was enhanced. This double approach highlighted its value for a more efficient application and by integrating agronomical studies with ecotoxicity assessments it may help refining waste-based fertilizers applications for better crop productivity and improved soil health.

Session 05 Poster 23

Isolation and identification of dominant bacteria from black soldier fly larvae for practical uses

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The black soldier fly (BSF; *Hermetia illucens*) belongs to the most promising farmed insect species currently being investigated. For the BSF larvae (BSFL), the gut microbiome is crucial for diverse aspects of insect physiology, affecting the health and survival. These properties can be exploited in industrial applications, but microbial strains are needed that are easy to cultivate and that can colonize the gut to exert their functions. Therefore, this study established a strain collection of 172 dominant aerobic bacteria from the interior of BSFL. The larvae were reared on either chicken feed or fibre-rich substrates to obtain a broad collection. To isolate abundantly present microorganisms, an approach only considering the highest serial dilutions of BSFL extract was considered. Our collection mainly includes members that have already been described in literature to be frequently present in the interior of BSFL, but their presence and abundance may vary depending on diet, biotic and abiotic factors. In total, twelve genera were collected with the main ones being *Enterococcus* (29.1%), *Escherichia* (22.1%), *Klebsiella* (19.8%), *Providencia* (11.6%), *Enterobacter* (7.6%), and *Morganella* (4.1%). For this representative collection, we consider three developmental prospects. First, *in vitro* digestion models used to predict larval growth and biowaste conversion can be improved by incorporating our isolates. Secondly, mock communities specifically designed for sequencing the bacterial microbiota of BSFL can be developed based on this collection. Lastly, the isolates can be added during rearing as probiotic symbionts to stimulate larval performance, for instance by enhancing the enzymatic potential for substrate digestion.

Session 05 Poster 24

Bioremediation of poultry and pig manure by black soldier fly (*Hermetia illucens*) larvae

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Manure valorisation by black soldier fly (BSF) larvae will be an opportunity to produce, in short periods, larvae for bioindustrial use and a high value organic fertilizer. The objectives of this study were to evaluate the potential of poultry (PT) and pig (PG) manure for BSF larvae growth and the larvae bioconversion capacity to produce a valuable fertilizer. For each manure five replicates of batches of 13.5 kg, were tested as follow: PTC (control, without poultry manure) and PTM (poultry manure); PGC (control, without pig manure) and PGM (pig manure). Each control was performed at the same time as experimental digestions. Whenever required in PTM and PGM, manure was mixed with a commercial larvae feed (EntoGreen®) and water, to obtain batches with mean values of 65% Dry Matter (DM) and adequate nutritional value. EntoGreen® was used as the only substrate in PTC and PGC. BSF eggs were inoculated in commercial larvae feed until 5 days old, being then inoculated in the different substrates. All juvenile larvae were hatchet in the same conditions. At days 4, 8, 11 and 14, larvae (n=60) were randomly sampled from 3 different places in each batch and weighted and batches temperatures recorded. Temperatures (°C) decreased in both manures from the 8th to the 11th days of incubation (PTC: 35.7 vs 23.5; PTM:29.8 vs 24.3; PGC: 45.1 vs 33.1; PGM: 48.7 vs 36.3). Larvae growth showed different tendencies. Mean Larvae weights varied between 2.85 and 1.91 g for PTC and PTM, and between 1.04 nd 1.41 g for PGC and PGM, respectively. To access the agronomic and environmental potential of the BSF Frass as an organic fertilizer, it was conducted a greenhouse experiment with ryegrass (*Lolium multiflorum*), using four treatments of BSFF. Under the experimental conditions, the results showed a significant effect of BSFF on the overall ryegrass production. This work was funded by PRD2020 through the FEADER, Project GOEFLUENTES (PDR2020-101-031831).

Session 05 Poster 25

Development of housefly larvae on manure from herbivores, omnivores and carnivores

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The demand for animal products is increasing due to increases in population and per capita income. This increases animal production and concomitant environmental problems. Better manure management can mitigate such problems for instance by biodegrading manure with housefly larvae (HFL). However, production of manure fed insects is currently prohibited in EU. If properly processed, HFL could be used as a protein rich feed ingredient. A test was conducted to gain insight in what determines manure suitability for HFL. Manure from several species varying in diet (herbivore, omnivore, carnivore) and digestive system (foregut and hindgut fermenters) was utilised. For each donor species 68 g of manure, with 34 g of water and seeded with 50 HF eggs was placed in a bug dorm container. A wheat bran mix was used as a control. HFL performance, in the form of survival, weight, and development time was determined after 5 days. Moreover, dry matter, carbon, and nitrogen content of both the larvae and the manures, and manure pH were determined. Overall, performance was best on manure from the hindgut fermenters orangutan, gorilla, tiger, lion and warthog, with values similar to the control. Poor performance was found for HFL on manure from brown bears and red pandas. Possibly, the latter manures were older than the others. Carnivore and herbivore manure yielded the same larval biomass as the control diets, whereas this was lower for omnivore diets. Manure from hindgut fermenters resulted in a higher survival and biomass than manure from foregut fermenters, with the values comparable to the controls. This study shows a high variability in manure suitability. Whereas larval parameters were not correlated to manure pH or C/N ratios, higher N content resulted in a higher larval biomass. The high suitability of manure from hindgut fermenters might indicate that increased microbial activity prior to defecation facilitates HFL development and manure utilisation. Further studies to optimise manure biodegradation using HFL should focus on identifying other relevant manure characteristics.

Session 05 Poster 26

Formulated fly perfume for oviposition control – developing FlyScent odour attractants using PTR-MS

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Industrial scale production of black soldier fly (BSF) eggs is a major bottleneck in the emerging field of insect farming. While the rearing and processing steps are available as complete turn-key solutions from several engineering companies, the breeding that supports such production is lagging and requires quantitative improvement and standardisation. The BSF oviposition today uses decaying organic materials as attractants and improvised egg collectors (dischargeable cardboard, or reused plastic and wood matrices), placed on the top of the decaying materials. Afterwards, the harvested eggs are either kept inside the cardboard until hatching or scrubbed with a spatula from the wood/plastic matrices before hatching. Such improvised solutions are often subject to high egg-loss rate, risk of mould and bacterial contamination and high egg infertility from scrubbing or pathogen contamination. The FlyScent project focuses on controlling and directing the egg laying behaviour (oviposition) and egg collection process of BSF, aiming to optimise egg production in breeding facilities. Different oviposition solutions, including odour attractant, egg collectors and dispersing system are being explored using proton transfer reaction – mass spectrometry (PTR-MS, Ionicon QMS300), which is an online instrument for measurement of volatile organic compounds (VOC). PTR-MS will be used to scan and obtain the chemical fingerprints of different potential materials known to lure fertilised BSF females and stimulate oviposition. Consequently, key marker compounds will be identified and used to develop synthetic odour attractants. It is planned that the developed attractants will be tested and optimised at a pilot scale, using a feedback loop approach before being demonstrated in a production setup and consequently reach the market.

Partially defatted black soldier fly meal inclusion in feed of different species

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For several years, feed manufacturers are searching for new sources of proteins in order to respond to the growth of the sector and the challenges of sustainable development. At the same time, more than 30% of the world agricultural production is wasted (unsold or expired products, by-products of agri-food industries). Recommended by the Food and Agriculture Organization of the United Nations (FAO), insects make it possible to valorise food waste (bioconversion). The black soldier fly (*Hermetia illucens*) represents a particularly adapted resource to feed fish, shrimps and even poultry because it is natural, safe, sustainably produced and has a good nutritional quality. Several trials have been conducted in order to evaluate the growth response of different species when a part of their feeds contained a partially defatted black soldier fly meal (BSF) as a source of proteins and at different inclusion levels. During the previous years, the BSF meal produced by MUTATEC have been incorporated in pelleted and extruded feed. These feeds have been used to feed rainbow trouts (*Oncorhynchus mykiss*) and pacific white shrimps (*Penaeus vannamei*) in lab trials and in commercial farms. Different stages of development were tested from juvenile to ready-to-eat size. All results were positive. Although not systematically significant, an increased final weight was often observed in treatments for which a conventional diet was replaced with a diet containing insect meal. The best observed inclusion levels were between 10 and 15%. All the farmers who had the opportunity to use a feed containing BSF meal gave a positive feedback. MUTATEC takes part to different research groups. From 2021, the NEXTGEN PROTEINS project is investigating the response of sea breams and salmons fed with insects. The trials will be conducted in labs and in commercial farms. The growth response will be investigated but also the effects on digestibility, health and final quality of the fish. Another project 'Poultry fed with Insects: Nutritional, Health & Sustainability issues' is ongoing with a different animal: the broiler. Although scarce, the literature suggests that the results of both projects should be positive as this was the case for trouts and shrimps.

Sensors for animals in future food systems – Farmworx approach

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Worldwide the demand for food continues to increase, while at the same time interest and concerns for welfare of our livestock animals is raising. These contrasting global trends result in challenges to prioritize animal welfare in parallel to optimizing production efficiency. Precise monitoring of health and physiological functioning of our animals is key in handling this challenge, as it will facilitate not only customized treatment of diseased animals, but also will provide early-warning signals to identify animals at risk for compromised health and welfare. In-depth longitudinal information concerning physiological capacity of animals can advance breeding strategies aiming at selection of the most resilient animals for the next generation. In this talk, I will introduce sensor platforms and approaches that were developed for measuring signals, behaviour and processes to understand the resilience and physiological functions and ways to measure emotions of farm animals. The faces of farm animals can be one of the richest channels for expressing emotions. We present WUR Wolf – a real-time facial expression recognition platform that can automatically code the emotions of farm animals. The Artificial Intelligence based facial coding platform developed by the Wageningen University has the ability to extract features such as eye white, ear postures and facial cues in determining the mental make-up of the farm animals such as cows and pigs. Resilient animals are able to adapt to stressors from their environment and rapidly return to their pre-stressor state. It is hard to find objective measurements for resilience traits due to the fact that resilience is a complex, multifactorial trait. We introduce SOLARIA – SensOr-driven resiLient and Adaptive monitoRIng of Farm Animals, and a 3-in-1 sensor patch which measures heart rate, respiration rate and the activity simultaneously in a non-invasive manner from the body of cows and pigs. Use of this multiplex sensing platform is being enabled by software which integrates physiological data from the sensor and attempts to quantify the resilience indicators and be displayed in the decision support farm management system.

Measuring sickness recovery with thermal imaging

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The burden of cumulative stress in current pig husbandry may reduce the future capacity of pigs to recover from challenges, i.e. their resilience. In the current study, the resilience of pigs was measured by following the recovery rate after a lipopolysaccharide (LPS) injection, which induces a sickness response. Sickness indicators are commonly obtained by highly invasive procedures. Acute inflammation is characterized by an instant vasoconstriction of blood vessels, which leads to a decrease in temperature of the extremities of the body, such as the tips of the ears. The objective was to use this metabolic process to measure the recovery rate of pigs with a non-invasive indicator, infrared imaging. At 15 weeks of age, 32 pigs were injected with 2 µg of LPS/kg of body weight while 16 other pigs received a saline solution. At 24 h before the LPS injection (baseline) and 1, 3, 5 and 24 h post injection blood samples were collected for cortisol concentrations. For each time point, rectal temperature was measured just before. Thermal images were taken with the FLIR T430sc camera at 24 h before (baseline) and at 1, 2, 3, 4, 5 and 6 h post injection. Ear temperatures (average temperature of the ¼ of the tip of the ear) were measured with the graphical software FLIR Tools. Pigs injected with LPS had lower ear temperatures at 1, 2, 3 and 4 h post injection compared to pigs injected with saline. At 6 h, the ear temperature was back to baseline. At climax of the sickness process, the temperature difference between the two groups reached 7.3 °C. Ear temperature at 1 h was highly correlated with the concentration of cortisol (r=-0.88) and with rectal temperature (r=-0.74). Thermal imaging appears to be a promising non invasive indicator of sickness recovery. The methodology is highly replicable and correlates with physiological indicators.

Use of thermal imaging in the detection of claw disorders in dairy cows

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Poor claw health is an economic and animal welfare issue in modern dairy farms. Tool for detecting cows in need of hoof trimming is sorely missed. As an inflammation commonly causes an increase in skin surface temperature, thermal imaging could provide an easy and fast tool to detect claw disorders in early stage. We followed in total of 60 dairy cows' claw health with routine and acute case claw check-ups over a period of 3-7 months depending on the cow. During the observation period, each cow was trimmed on average 2.4 times (range 1-4). Claw disorders were recorded according to ICAR Claw Health Atlas and classified (1-8) based on their assumed severity. Thermal images were captured every time just before the claw trimming with thermal camera (FLIR T540) having an infrared resolution of 464×348, thermal sensitivity of 0.03 °C and accuracy of ±2 °C. Air temperature and humidity were adjusted to the camera settings. Used emissivity was 0.95 and reflected temperature was a weighted average (3:1) of air temperature and estimated cow surface temperature (34 °C). All four legs were imaged from the distance of 0.5-1 m, inner and outer claw separately. From all the images the minimum, maximum and average temperature of the coronet band and claw horn were analysed (ThermaCAM Researcher Pro2) using a polygon tool. We used a linear mixed model to determine the effect of claw disorder and imaged leg (front or hind) (independent variables) on the claw surface temperature (dependent variable) with air temperature as a covariate and cow as a random effect. Only sole ulcer ($P=0.002$) and white line abscess ($P=0.015$) significantly raised the average surface temperature of the coronet band. This increase was on average 1.3 °C for sole ulcers and 2.3 °C for abscesses. Based on these results we assume that thermal imaging could provide a useful tool for detecting cows in the need of hoof trimming, at least in cases of severe claw disorders. However, also air temperature and the imaged leg (front or hind) affected the coronet band temperature (both $P<0.001$), and thus, these variables should be considered when interpreting thermal images of claws.

Using thermal imaging to detect tail tip alterations in dairy cows

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In fattening bulls tail tip alterations are described as health issues associated with (sub)acute rumen acidosis and lameness. Investigations of dairy cows' tail tips are scarce; however, there is evidence that tail tip alterations occur as a result of intensive feeding management and metabolic imbalance. In June 2020, we investigated 68 German Holstein cows for tail tip alterations using a handheld thermal camera (FLIR® T1030). Thermal images of the shaved tail tip were taken from two different perspectives: (p1) tail in front of the udder and (p2) held tail in front of the ground. Additionally, all cows were evaluated for any kind of tail tip alterations, body condition score (BCS), and locomotion score (LMS). Milk yield data, resulting from latest performance testing were collected, too. Images were analysed using FLIR Tool+ and a generalised linear model was used to correct measured temperatures for fixed effects. The prevalence of tail tip alterations, regardless the findings, was 94%. Raw temperature data showed high correlations of $r^2=0.79$ for maximum and average temperature and $r^2=0.70$ for minimum temperature between p1 and p2 ($P<0.001$). Tail tip necroses and BCS>3 decreased the tail's temperature, while swelling of the tail tip increased its temperature. Average, maximum and minimum temperatures for perspective 1 and 2 were 34.05 (±0.16) and 33.78 (±0.20) °C, 35.46 (±0.11) and 35.52 (±0.11) °C, and 30.30 (±0.30) and 29.22 (±0.38) °C, respectively. Temperatures of necrotic tissues (n=4) decreased on average by 8.38 °C compared to the average temperature and were close to the minimum. We concluded thermal imaging can be used for the detection of alterations regarding the tail's temperature, which could allow an early-warning system for swellings, probably caused by inflammation and possibly resulting in necroses. Further studies are needed to evaluate the prevalence of tail tip alterations in dairy cows on larger samples and to clarify their etiology. However, thermal imaging can be used as an early-warning system for tail alterations. Each early-warning system regarding health issues, improves animal welfare as it allows adapting management strategies early.

Interest of 3D imaging technology to study feed efficiency in dairy cows

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Three-dimensional (3D) imaging allows acquisition of morphological traits, surface and volume data. Focusing on a selected section of an animal, it is supposed that regular acquisitions of such images allowed to follow, for example, fat deposition or gastro-intestinal changes. This technology was applied to study abdominal volume (AV) changes of dairy cows, which was considered to be a good estimation of rumen contents (RC). It aims to study the links between these changes and feed efficiency (i.e. production per unit of dry matter ingested). To assess RC variations, the rumen was manually emptied (study 1) or artificially filled (study 2) on 17 and 18 Holstein cows, respectively. In study 3, 55 Holstein cows were fed individually successively 2 diets differing in energy and fibres contents. Feed efficiency of cows was determined on this latest diet. Retention of feed bolus was defined as the ratio between AV and feed intake. Including individual effect in the analysis indicated that the linear regression of the RC was accurate with errors of 1.6 to 2 (studies 1 & 2), whereas RC prediction using partial least squares regression was not so accurate (7.1% error). The 25% most efficient cows in study 3 had a 1.18 times retention higher than the 25% less efficient. These results suggested a longer retention of the food bolus and a higher digestibility. Preliminary results indicated that 3D-imaging opens opportunities to study rumen contents, which is of interest to study feed efficiency and select animals for a lower environment impact.

Session 06 Theatre 6

A preliminary study of changes in circadian rhythmicity of sheep around lambing

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Determine the actual day of lambing in extensive sheep systems, where ewes are in remote paddocks and cannot be individually monitored is an impossible task. With the help of Precision Livestock Farming, farmers can predict events such as parturition or illness, based on data collection and algorithms. Circadian rhythms show diagnostic value in physiological processes, with changes in the presence of stress. The aim of this work was to determine the utility of the study of changes in the circadian rhythms of temperature (T), heart rate (HR) and activity (ACT) of sheep, to predict lambing dates. Five ewes were subcutaneously implanted with a T, HR and ACT bio-logger (DST milli-HRT ACT, Star Oddi, Iceland), and programmed with data logging every 5 minutes for 13 days. Two of the ewes lambed 4 after the onset of the logging procedures. Cosinor rhythmometry (CR) was applied to the data recorded from the lambed ewes the day before, the day of lambing and the day after lambing. With the whole set of data of the rest of the animals, plus data of the lambed ewes from day after lambing onwards, the CR was applied to establish a gold standard to be compared with the cosinor curves around lambing. Mesor (mean level), Amplitude (half the range of oscillation) and the acrophase (time of peak) of the three parameters were estimated. Paired t-test for related samples were used to compare cosinor curves. The standard cosinor curve fitted a 24-h rhythm ($P<0.001$) for T, HR and ACT. Mesor values were 37.94 °C, 103.04 bpm, and 13.06 mg, resp. Amplitude 0.21 °C, 13.45 bpm, and 5.65 mg. Acrophase times 15:37 h for T, 13:27 h for HR, and 10:38 h for ACT. The CR of the lambed ewes revealed that the day before lambing for T, and the day after lambing for HR, did not fit the 24-h curve ($P>0.05$), revealing an alteration of the circadian rhythm of T and HR around lambing. The acrophase of T the day of lambing (18:20 h) was delayed ($P<0.05$) 3 hours compared with the acrophase of the standard curve. Considering this experiment as a preliminary study, it seems that the calculation of circadian rhythms of T and HR, and their changes, could be a useful tool to estimate lambing dates.

Session 06 Theatre 7

Inertial sensors to classify animal behaviour and quantify pasture intake in grazing ruminants

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Animal behaviour can be defined as the coordinated set of responses performed by an individual in response to stimuli such as infection, predation, hunger, social disruption, or the environment. This behaviour is often consistent, and predictable, and provides the basis for using animal behaviour as a guide for the optimal management of animal health, welfare, and productivity. However, measurement of animal behaviour at scale is not easily achieved because of the labour required to physically monitor large numbers of animals continuously or across extensive environments. Wearable sensor technologies offer a possible means of measuring the behaviour of large numbers of animals in remote environments, but this can only be successful if sensor outputs accurately reflect animal behaviour and deliver results in real time. For the last ten years our team has worked to develop machine learning behaviour classification models that accurately classify the behaviour of pasture-based cattle. The operation of these mathematical models has progressed from analysis of archival data in supercomputers to real time analysis on resource constrained devices. In addition, research is also underway to use data collected from inertial sensors to estimate individual pasture intake, animal health status and detection of a range of reproductive events. This presentation highlights the trials, tribulations, and successes of our research team.

Session 06 Theatre 8

Indirect approaches of digestive processes to determine feed efficiency in dairy cows

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In a dairy herd, all cows do not use a similar ration (amount, quality) in the same way. At the same level of performance, body reserves and live weight, they consume different amounts of the same ration, meaning that they do not have the same feed efficiency (FE). The differences in feed efficiency can be up to 10% of the feed intake difference between the most and least efficient cows. The main challenge is to be able to estimate FE without measuring individual feed intake, which is only available in a few research facilities. Another way consists in understanding the drivers of FE and directly use these drivers to estimate or improve it. This work focusses on digestion differences, which is known to be a driver of FE in dairy cattle. We hypothesised that the digestion differences can be approached indirectly thanks to gas analysis using Green Feed (GF) technology and the change in rumen volume, using 3D imaging technology. The 3D image data of the animals were used to calculate the volume of the abdomen (AV), considered as a proxy for rumen volume. For this purpose, data from 60 lactating Holstein cows was used, receiving successively two diets based on a low (L), or high (H) level of Neutral Detergent Fibres (35 vs 42%). L and H rations contained 14.5 and 16.7 crude protein, 0.83 and 0.84 UFL and 93.0 and 101.9 g/kg of PDIE, according to INRAE feeding system. The AV increases linearly with dry matter intake (DMI), which confirms the interest of AV. The comparison between the two periods indicated that the rumen volume increased with the diet H, which is consistent with the notion of rumen fill due to fibre content of diet. At the same level of DMI (L diet), the AV increases with FE of the cows, with a significant negative coefficient of Residual Feed Intake (RFI; -21.8 l/kg) to explain AV with DMI and RFI. If CH_4 yield (g/kg dry matter intake) was positively connected with feed efficiency, no significant relation was found between CH_4 yield and AV/DMI. These results suggest that FE seems driven rather by digestion and less by metabolic drivers. The better digestion for the most efficient cows could be due to a longer residence time of the ration in the rumen.

Session 06 Theatre 9

Evaluation of two wireless rumen pH sensors for dairy cows

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Wireless rumen pH sensors are a potentially useful tool in the detection of subacute rumen acidosis. The functionality and measurement accuracy of two models of such sensors were examined and compared over their stated lifetime of 150 days. For this purpose, 6 eCow (eCow Ltd., GB) and 6 Smaxtec boluses (Smaxtec GmbH, AT, SMA) were used 2016. Three rumen fistulated, lactating dairy cows each carried two boluses of each manufacturer in their rumen over 154 days. On day one, after activation and calibration, the boluses were placed in a buffer solution (BS) of pH 4 for two hours. This procedure was repeated with a BS pH 7 before placing the boluses in a cluster, via the fistula in the rumen. The checks of the boluses with the BS pH 4 and 7 were repeated every 14 days; pH of the BS were measured before and after the fortnightly control periods using a pH meter (HI98128, Hanna Instruments Inc., USA, resolution of 0.01). The average service life of the 6 eCow was 82 days and two of them worked only for 28 days. Moreover, read-out of the records of eCow was challenging. In total, 48% of the potential total of measurements were missing. In contrast, all SMA recorded the pH exactly during 150 days and the read-out was done automatically via a base station. The average pH differences, both BS, between the reference measures and eCow or SMA records amounted to 0.09 (standard deviation (SD) 0.48) or -0.29 (SD 0.22), respectively. For eCow, the 95% confidence interval (CI) of the mean pH difference indicated no systematic error. In contrast, CI of the mean pH difference (-0.33 to -0.25) suggested a systematic negative bias with SMA. The range between the limits of agreement (±1.96×SD) were enormous for eCow (-0.85 to 1.01) and was caused by obviously erroneous records. For SMA the range of agreement was between -0.73 and 0.15. Only for SMA a more in-depth evaluation using a linear mixed model was performed. The fixed factors, BS and date of measurement, had a significant effect (both $P<0.001$) on the pH differences between reference method and SMA. Even the random factor, individual bolus, showed a significant effect, which means, that the results may differ depending on the bolus used. To be of real use, eCow needs to function more reliably and SMA have to measure more precisely.

Associations between liver ultrasounds measurements and productivity indicators in Holstein cows

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Ultrasound (US) imaging is a non-invasive tool for monitoring liver dysfunction in dairy cows. This study aimed at investigating the associations between US predicted liver triacyglycerol content (pTAG, mg/g), liver depth (LD, mm), portal vein depth (PVD, mm) and area (PVA, mm^2), with body measurements and milk productivity indicators in 342 clinically healthy Holstein cows (days in milk [DIM]≤120 d). Data concerned US measures, milk traits, withers height, heart girth and body condition score (BCS). 73 cows were weighed to develop a regression equation to predict body weight (pBW), used to compute productivity indicators scaling milk production traits by pBW. Body and milk traits have been classified (low, medium and high) according to 0.75 standard deviation units of residuals generated from a linear model including the effects of parity (PAR), DIM and herd/date (H/D). pTAG, PVA, PVD and LD were analysed with a mixed model including the fixed effects of PAR, DIM, class of body and milk traits and productivity indicators and the random effect of H/D. The US traits resulted associated with body measurements and BCS. pTAG was inversely related to BCS ($P<0.01$), whereas PVD and LD increased at the increasing of body measures, BCS and pBW. Conversely, no relevant associations have been observed between US and milk traits, also when expressed in terms of productivity. In conclusion, in healthy cows dimensional US liver measurements seem related to the size of the cows, whereas pTAG concentration reflects body condition fluctuations, without particular implications in terms of milk production and productivity. The US imaging revealed to be a promising technique to assess differences in liver dimension and rate of body reserves mobilization, thus confirming its potential as non-invasive tool of monitoring liver conditions.

Use of thermal images to search for roe deer fawns before mowing

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Drones with infrared cameras can be used for fawn search before mowing. The ascertainment of thermal points (i.e. possible fawn positions) and their control are mostly performed directly during an overflight. The aim of the study was to test another method. Therefore, only thermal images are taken during the overflight. The search for fawns is performed according to GPS coordinates of thermal points obtained after evaluation of the images. The drone DJI Matrice 200 and thermal camera DJI Zenmuse XT2 were used to take thermal images. The software Agisoft was used to create orthomosaic maps of overflown areas. The software CoGIS was utilised to ascertain GPS-coordinates of thermal points visually detected on the orthomosaic maps. A smartphone connected with a real-time kinematic device was used for navigation to the ascertained GPS coordinates. The method was applied on 28 plots of 67 ha in total. Thereby 6 fawns were found on 5 plots, but only 3 of them on 3 plots on base of GPS coordinates. The other 3 fawns on 2 plots were found only by search performed directly during a new overflight. Unfortunately, several difficulties occurred applying this method. Not every time enough images were taken, and the overflight had to be repeated. Problems occurred also with creation of orthomosaic maps even if enough images were taken. Then single images had to be evaluated, and the GPS coordinates could be ascertained only for the midpoint of the image and not directly for the thermal point. Furthermore, difficulties occurred by the search of fawns, e.g. due to navigation inaccuracy of the used system and due to possible changes in position of animals in the field in the time between overflight and search. Furthermore, not all thermal points were shown on orthomosaic maps even they were seen on single images. Moreover, the evaluation process of thermal images is time consuming, and up to 30 min (or even more depending on number of images) were needed until search could start after the overflight. In conclusion, even if all technical problems could be solved, we still see the time lag between taking of images and the search and also the search according to the GPS coordinates of thermal points as problematic for effective search.

Vocal and behavioural differences of lambs between different sheep breeds

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In sheep individual recognition between parents and offspring has been clearly demonstrated and it is critical for the development and survival of the young since the lambs actively follow their mother from birth. Vocal responses have been used in smart farming technology as an indicator of stress, but in order for that to be accurate other factors affecting call characteristics, like breed, need to be clarified. The aim of the present study was to analyse the vocal response of lambs during a short separation from their mother close to artificial weaning (40±2 days post-partum). Lambs (n=59) of two different Greek dairy breeds were isolated from their mothers and vocalizations and behaviour were recorded. Statistical analysis showed that breed affected significantly ($P<0.05$) four acoustic parameters as well as two behaviours (trotting, leap) whose frequencies seemed to differ between the examined breeds. The study provides valuable novel information about lamb vocal and behavioural characteristics at a stressful situation and may contribute to future development of in-situ livestock applications that allow early identification of stressful situations under farming conditions.

Do the lambs selected for better feed efficiency have the same feeding behaviour?

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Feed efficiency is a trait of major interest for all livestock species because breeding efficient animals is not only cost-saving but also leads to a decrease of environmental impacts. So, residual feed intake (RFI), a selection criteria for improving farm animals efficiency, is increasingly used by breeding companies, but the correlated responses on feeding behaviour is rarely questioned. The aim of this study was to explore the feeding behaviour of the 2 last generations of Romane lambs divergently selected for RFI under a 100% concentrate diet, at INRAE Experimental Unit P3R. In the third generation, divergence on RFI reached nearly 2 genetic standard deviations. From 2017 to 2020, 329 lambs (half belonging to each of the two divergent lines) had their *ad libitum* intake controlled during 6 weeks with automatic concentrate feeders. Feeding behaviours were investigated with ANOVA through feed intake and feeding duration at 2 levels (per visit or per day), feeding rate, number of visits to the feeder, time between successive visits and evolution of the feed intake splitting. As expected, daily feed intake was significantly higher for the inefficient lambs compared to the efficient ones. Feed intake at the visit level was also smaller for the efficient animals, whereas a moderate negative genetic correlation (-0.33±0.14) was estimated between both traits in the past in the Romane base population. We confirmed with this dataset the independency between RFI and the feeding rate. Moreover, the number of visits per day did not differ between both lines but efficient animals increased their feed intake splitting during the six weeks of the test. This evolution of feed fractioning raises questions about the state of health of the animal's rumen. Finally, in a subset of the animals of the third generation of selection on RFI, feeding and drinking behaviours under a forage-based diet will be studied and compared to their feeding behaviour under a concentrate diet.

Session 06 Poster 14

Explaining cattle weights with environmental and animal traits

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Cattle farming contributes to climate change through greenhouse gas emitted from the animals. The emission per animal can be deduced from their live weight, but measuring live weight is cumbersome, which is why researchers sometimes predict it with other morphological measurements. If it is not important that at the time of prediction the animal is alive, a predictor that is more readily available is carcass weight. Here, we quantify how well carcass weight can predict live weight of adult female cattle. We further explore the influence of environmental and animal traits' influences on live weight, carcass weight and dressing percentage using linear models. Our Swiss Animal Movement Database provides us with data from more than 600,000 slaughtered adult females with breeds Holstein, Braunvieh and Limousin, from years 2010-2020. It contains information about the following environmental and animal traits: slaughter date, carcass weight, beefiness and fat scale categories, number of moves to different types of animal holdings and markets, the altitude zone of the different holdings, the animal's birth date and date of last calving. For a subset of the animals it also contains live weights measured at cattle markets. In order to quantify how well the carcass weight explains live weight, we trained the model on learning data and then applied it to test data. We found that carcass weight alone explains around 87% of the live weight variance. When more predictors are added, we reach >90% explained variance. We conclude that carcass weight is a good proxy for live weight. The prediction can be further improved by considering environmental and animal traits. We show that the most important factors for explaining live and slaughter weight are beefiness and fat scale. Further, the calving to culling interval, the zone before slaughter, the age at slaughter, the number of times an animal was brought to a market explain a statistically significant amount of the variance. Least-square means show that there has been a steady carcass weight increase for all breeds since 2014. They also reveal that the live weights generally decrease by at least 10 kg in the summer and only recover in the winter.

Session 06 Poster 15

Relation between milk urea nitrogen and urine nitrogen excretion in dairy cows as depending on breed

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Ammonia (NH_3) contributes approximately two thirds to the nitrogen load ending up in sensitive ecosystems in Switzerland at present where it induces eutrophication and acidification of the soil. The aim of this study was to model the major NH_3 substrate, urine nitrogen excretion (UN; g/day) based on an easy-to-determine proxy. One such proxy would be the milk urea nitrogen content (MUN; mg/dl), determined in tank milk samples from dairy farms. Therefore, we investigated urine nitrogen and milk urea values from 22 different feeding experiments performed with 175 Brown Swiss (BS), 368 Holstein (HO), 44 Jersey (JE) and 50 Swiss Fleckvieh (SF) cows. The relationship between the coefficients of UN and MUN was investigated by a linear mixed model. The model included the fixed effects MUN, breed and the interaction between them, as well as experiment as random effect. The coefficient of determination of the model was high (R^2=0.823). The regression coefficients ± standard errors of MUN were 7.28±0.74, 13.12±1.53, 11.72±0.87 and 10.75±1.49 g/day UN increase per mg/dl for BS, SF, HO and JE, respectively. The estimated fixed main breed effects ± standard errors were 41.4±18.0 (Intercept), -48.1±23.5, -18.7±14.0 and -39.0±22.0 g/day for BS, SF, HO and JE, respectively. The results are consistent with other studies and show MUN content of the milk is a useful tool to predict the UN in lactating dairy cows. This enables to improve the accuracy of mass flow models to estimate ammonia emissions which are based on the nitrogen excretion of livestock at the beginning of the manure management chain. The results also show that the prediction model has to consider breed where differences were particularly high between BS and HO.

Session 06 Poster 16

Determining colour in the CIELab space of bovine and porcine longissimus dorsi using computer vision

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Colour is the most crucial meat quality attribute perceived by consumers. The human judgment of the meat colour is subjective and inconsistent, and therefore, typically, a colorimeter is necessary. In recent years essential developments have been achieved in computer vision related to colour. Thus, this work aims to study the relationship between the colour values in the CIELab space of beef and pork meat obtained with a colorimeter and a flexible and straightforward computer vision system (CVS). For this study, 89 samples of bovine longissimus dorsi (LD) muscle and 110 swine LD samples were used. The L* for the lightness from black (0) to white (100), a* from green (-) to red (+), and b* from blue (-) to yellow (+) colour parameters. These colour parameters were measured through colorimetry with a Minolta CR-10 colorimeter and with a CVS. An Olympus EM-5 digital camera constitutes the CVS with a 16 Megapixel sensor equipped with lens EZ F3.5-6.3 M. Zuiko ED, 12-50 mm with 24 mm, aperture f8 and with a circular polarizing filter and A Macro Olympus OM T28 Double Flash mounted vertically on a support, at a distance of 30 cm from the samples. The L* parameter measured with the CVS in the swine LD muscle was more significant ($P<0.05$) than that obtained using the colorimeter. Parameters a* and b* also obtained high values ($P<0.05$) with CVS. The correlation coefficients for each colour parameter obtained by the two methods were higher than 0.85 for cattle (r=0.894; 0.878 and 0.955 for L*, a* and b* parameters, respectively). However, for the swine samples, the correlation coefficients ranging between 0.6 and 0.7. These results encourage the pursuit of the use of CVS to determine meat colour more quickly and efficiently.

Session 06 Poster 17

Biomarkers in milk as predictors for metabolic health status of dairy cows during early lactation

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The transition period of 60 to 90 days around calving is crucial for high yielding dairy cattle. Early identification of cows with an impaired metabolic status is important to limit negative effects on production, animal health and welfare. The gold standard to assess the metabolic status is blood sampling. However in commercial dairy farming this is not feasible, thus non-invasive biomarkers via milk sampling would facilitate a more rigorous follow-up of transition cows. The aim of this study is to evaluate the value of milk biomarkers for the prediction of the metabolic status. A monitoring program at the ILVO research institute (Flanders) has been set up from Nov 2018 until Oct 2020. Milk analytes (protein, lactose, urea, somatic cell count, fatty acids and ketones) were monitored daily (day 3 until 23) in freshly calved dairy cows (n=99 cows). Blood samples were taken on day 3, 6, 9 and 21 post partum (pp). Blood non-esterified fatty acids (NEFA), beta-hydroxybutyrate (BHB) and glucose were used in a k-means clustering to distinguish a metabolically balanced group vs animals with an impaired metabolic status (median values (mmol/l); BHB: 0.80 vs 1.39; NEFA: 0.49 vs 0.75 and glucose: 3.15 vs 2.82 respectively). Random forest models with a Boruta feature selection were constructed for day 3, 6, 9 and 21 pp and evaluated using leave one group out cross validation and area under precision recall curve (AUCpr). Prediction models using milk fatty acids (n max selected=25) resulted in the highest AUCpr on day 9 and 21 (AUCpr 0.81). Important predictors were: some short chain fatty acids, C15:0 and C18:1,c9. Lowest performance was noticed on day 3 (AUCpr 0.50). Predictions relying on fat, protein, urea content and fat protein ratio showed highest performance on day 21 (AUCpr 0.64). Adding additional parameters, which can be routinely determined (e.g. C18:1 based on MIR-spectra), improved the predictions to a maximum AUCpr of 0.70 on day 6. These results confirm the potential of milk biomarkers in early lactation to monitor metabolic health.

Multicolour flow cytometry for quality control of frozen bovine sperm and bull fertility prognostics

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Our study aimed: (1) to evaluate the use of multicolour flow cytometry for monitoring the quality of commercially produced frozen bovine sperm; and (2) to explore the value of flow cytometrically assessed sperm functional traits for the prediction of sire fertility after artificial insemination (AI). Thus, the annual fertility index (FI) of 151 sires with more than 300 first AI was recorded between November 2018 and November 2019. Sires lying at the extremes of the FI distribution (outside the range of mean FI±SD) were classified as low- (LF; n=16 bulls) and high-fertile (HF; n=17 bulls), showing a non-return rate of 61.7±6.8 and 73.1±1.2%, respectively. At least four cryopreserved semen batches per bull (31±13 batches per bull) were examined immediately after thawing. The percentage of sperm with high esterase activity (C_{pos}), intact plasma membrane (PI_{neg}), unstained acrosome (PNA_{neg}), low intracellular Ca^{2+} levels (F_{neg}) and high mitochondrial membrane potential (M_{pos}) was quantified using a five-color flow cytometric panel that included calcein violet, propidium iodide, peanut agglutinin, Fluo-4 AM and MitoProbe™ $DiIC1_{(5)}$, respectively. The Sperm Chromatin Structure Assay™ was performed to assess the percentage of sperm with high DNA fragmentation index (%DFI). Between-group differences of sperm functional traits were assessed through mixed-effects linear models, that included the random effect of batch (nested within bull) and the fixed effects of production month, sire fertility group and their interaction. HF and LF bulls showed similar $PI_{neg}PNA_{neg}$ (56.7±11.1 and 57.8±9.9%, respectively) and %DFI values (3.7±1.7 and 3.7±3.4%, respectively), but the fraction of $PI_{neg}F_{neg}$ sperm within the $C_{pos}M_{pos}$ (viable) subpopulation was significantly higher in HF than in LF bulls (74.2 vs 66.7%, respectively; b=7.45, P=0.008). Concluding, flow cytometric analysis revealed a heterogeneous ability of viable sperm to control their Ca^{2+} homeostasis; this ability appeared relevant to sire fertilizing potential.

How to re-implement synergies between crops and livestock farming?

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The Farm-to-Fork strategy opens the way towards a rejuvenated agriculture that stays within planetary boundaries. The 'Animal Task Force' and the 'Plants for the Future' European Technology Platform recognize the urgency of transition and published, in September 2019, R&I opportunities for the crop-livestock value chain. The proposals aim at developing a climate change-mitigating, circular, resource efficient agri-food system with closed nutrient cycles, healthy soils and ecosystems, restored biodiversity and attractive landscape. Local re-integration of livestock and cropping offers new opportunities to reduce the environmental footprint and restore ecosystems functions, soil quality and organic content. The ability of livestock to utilise a broad range of biomasses could help diversify crop rotations, close nutrient cycles, reduce pest pressure and chemical inputs, and enhance biodiversity. Three recommendations were developed. The first one is the upgrade of LCA to track progress towards more sustainable farming by assessing the interactions between crop and livestock sectors in a circular economy, and to proper evaluation of biodiversity, economic and social (employment, rural vitality) performances of value chains. The second one is the optimisation of synergies in circular livestock-cropping systems. It covers identification and development of innovative cropping systems allowing for permanent soil cover and livestock farming systems as well as genetic improvement of plants and animals to maximise resource use efficiency and development of biorefineries in support of circularity to maximise the utilization of products and by-products as well as N and protein from manure and green biomass. The third recommendation concerns the Governance, roles of stakeholders and public policies to promote changes over time. This concerns actors coordination to change the socio-technical system and design of public policies to guide and support transitions.

Development of plants with improved composition for animal nutrition and the environment

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Livestock breeding has significantly improved performance over the last 30 years with consequent increases in nutrient demands, which can outstrip those provided in typical feed crops. Equally, concern over environmental footprint and competition for land/sea resources has questioned the use of certain crops and food resources for animal feed. Here we investigate some successes and opportunities for ruminant and monogastric livestock systems for improving feed supply through plant genetic improvement. Breeding forages has provided a means of improving the efficiency of milk and meat production at the same time as reducing environmental pollution at a minimal cost to farmers. Genetic gains in early spring growth and annual dry matter yield have been a major focus. However, improving the nutritive value of forage (e.g. water-soluble carbohydrate) has also played a significant role through maximising microbial capture of nutrients in the rumen and improving efficiency and product quality. Novel plant traits such as polyphenol oxidase in red clover, which has been shown to improve nitrogen use- efficiency and protect polyunsaturated fatty acids (PUFA) through the rumen, such traits could be incorporated into future breeding programmes. For monogastric livestock (including farmed fish), supply of long chain omega-3 PUFA such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) currently relies on marine fish stocks, which has been questioned in light of sustainability concerns. Alternatively, oil seeds (*Camelina sativa*) PUFA profiles have been successfully modified through application of microsomal elongase activity technology. This technology offers great potential in providing a sustainable terrestrial source of fish oils, and represents the most sophisticated plant metabolic engineering to date to undergo environmental release. Such plant-based feed approaches to feed our livestock either to improve animal performance, reduce environmental pollution or address a sustainability feed supply issue, is critically needed to realise the true potential of livestock within future sustainable food systems.

Reducing the feed and food competition: perspectives and best practices

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What can plant breeding do to better feed animals? Innovative feed-livestock value chains with genetic improvement of plants for feed, aiming to the development of crops with improved nutritional and digestive properties. Sector findings on crops and animals to align productivity and resource use of crops & livestock, and achieve a better utilisation of more diversified crops and an increased metabolic conversion and uptake by farm animals.

Session 07 Theatre 4

Novel feed from crops and crop residues – vision from the feeding industry

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It is clear that the role of animal production is changing at this moment. Diets will change, with a reduction of the animal protein in the diets. Feed, food, biomass production are topics to face. Land utilization is going to be a key indicator concerning feeding the worlds ever growing population. Substantial challenges, connected to the animal production sector, have to be taken into account: Greenhouse gas emission reduction, a positive impact on biodiversity, animal welfare and health, the fight against zoonotic diseases and reduction of the risk of antibiotic resistance are important ones. At the same moment understanding is growing that animal production plays an important role in valorisation of non-edible products, and by that in optimal land use. And livestock farming is playing a very important role in generating income to low class population in many developing countries in Asia and Africa. So, what does this mean for feeding animals? At the end it is clear: We should not feed animals products which are in competition with food products. But what would is the practical consequence of this? Some first thoughts will be given. At the same moment part of the transition is clear: In Europe the Protein Plan is a very important topic, which will lead to different raw materials. But this transition is not an easy pathway. Because arable crop farmers are looking to optimal financial revenues in balance with the biodiversity and soil fertility. Some examples of the struggles and challenges in the development of European soy, faba beans and other crops will be given. It is important to realize that the amount of food waste in Europe is huge: 90 million tons. As a comparison: The entire compound feed market in Europe is 150 million tons. At present about 6 million tons of these former foodstuffs are being used as feed, so the potential is substantial. But what are the conditions under which this will be an affordable and most important safe feed stock for live animals. Many challenges, but at the same moment, also many opportunities are in front of the feed industry. Scientific development, together with developing new business models will play a key role in this development. This is an important path forward, in line with Agrifirm's vision: a responsible food chain for future generations.

Session 07 Theatre 5

Animal breeding towards the valuation of new feed

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Pig breeding has, so far, mainly focused on improvement of efficiency and robustness with high quality and digestible diets as a given. Since cost of feed is around 75% of cost of livestock production and pigs eat almost everything, recycling by-products of the human food industry can be a serious option. Feed efficiency is most often expressed as kg/kg or energy intake over weight gain. With restrictions on human edible resources and environmental influence of pork production, the definition of efficiency changes towards nutrient efficiency, i.e. nitrogen efficiency and fibre digestibility. After ingestion of the feed, most dietary nutrients are made available for absorption by the animal itself, whereas the fibrous part of the diet can be broken down by the intestinal microbiome. The digestion of dietary nutrients decreases at a higher feed intake, hence the *ad libitum* feeding as standard in worldwide pork production is suboptimal. Feed intake potential of animals should be high enough to allow for sufficient ingestion of less balanced feed in the future. But feed intake should also be resilient for changes in feed ingredient composition and quality. If management of farms will allow for restricted feeding, then genetic selection can focus on optimizing feed intake and increasing digestion of nutrients. Right now, as an immediate solution for resource efficiency, exploiting genetic variation in existing production populations could be considered for precision feeding. Sensory technologies can help to follow animals through their productive life, optimizing the combination between their genetic potential and the available feed resources.

Phenotypes for grass utilisation and persistence under grazing to improve grass breeding

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The proportion of grazed grass in dairy cow diets in Ireland is estimated to be 80%, with silage and concentrates accounting for a further 20%. Perennial ryegrass is the main forage species sown in Ireland, as under temperate conditions it produces large quantities of high quality feed, persisting for many years under correct management. Farmers conduct pasture reseeding to increase the proportion of sward perennial ryegrass. The pasture profit index (PPI) is used to select grass varieties for reseeding. The PPI is an economic variety ranking index which identifies the most profitable perennial ryegrass varieties for reseeding. Grassland farmers aim to optimise the proportion of grazed grass in animal diets as high pasture utilisation maximises farm profits. Perennial ryegrass varieties influence grazing management and utilisation but until recently no indication of a varieties ability to be grazed was available. As grazing is the predominant production system used in Ireland, farmers want to sow varieties suited to animal grazing, therefore a grazing sub-index was established and included in the PPI. Investigations into variety grazing efficiency were conducted in plot trials. Plots were grazed by dairy cows in rotational grazing systems. Within each variety plot, a number of pre-grazing measurements were made. Post-grazing height measured on each plot using a rising plate meter. The Residual Grazed Height concept was developed to accurately account for grazing efficiency differences between varieties. Increased grazing efficiency was correlated with higher levels of leaf, crude protein and organic matter digestibility. Tetraploid varieties tend to excel in these traits, therefore they were found to have better grazing efficiency than diploids. Selection based on the traits identified in this study will assist plant breeders to select for more grazing efficient varieties. Grazing data from this trial has been included in the PPI as the 'Grazing Utilisation' sub-index in 2021. This will help the grassland industry and more importantly farmers select better varieties for grazing systems, encouraging grass breeders to increase selections in their breeding programs for this trait.

Improving resilience of organic beef-sheep farming systems

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Mixed farming systems are gaining interest both as a risk management strategy and to apply agroecological principles. Diversity in organic farming systems is particularly important since those farms have limited access to external inputs and use more frequently direct marketing. The main objective of this study was to identify strategies that improve the resilience and the overall sustainability of organic beef-sheep farms according to farm characteristics and to their short-term flexibility. This study focuses on farms combing beef cattle, sheep for meat and some annual crops. Among the European farms surveyed for the MixEnable project, four French mixed beef-sheep farms were contacted. They were asked what were the main risks they were facing, which variations of yield and prices they consider as being very unfavourable, unfavourable, normal, good and very good and how frequent these variations occurred over the last 10 years. They were asked which levers they usually used to face hazards, namely weather hazards, and which long term adaptations could be considered on their farms. The states of nature of each hazard were crossed assuming independence between risks and considering each farmer declaration. The Orfee bioeconomic farm was used to simulate the impacts of those hazards on farm resilience. The short-term adaptations such as feed purchase, feed stock, modification of grassland use, animal produced and sold and intercropping were optimized by the model for each combination of risk within the range of possibilities specified by farmers. Different long-term adaptations such as the reduction of stocking rate, the modification of the mix of animal species or the increase of annual crops were tested. Farmers had different exposures to risks but all declared a much higher proportion of bad yields than good yields over the last 10 years. Flexibility regarding grassland management and intercropping varies greatly between farmers. Few of them made security stock and most of them prefer buying feed than reducing animal productions. The simulated results show that optimal strategies vary according to farms but reducing stocking rate and increasing annual and inter crops often appear promising.

Does integration promote sustainability in organic multi-species livestock farms

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Implementing agrobiodiversity is a core principle of agro-ecology and organic farming. It is often observed through the lens of crop-livestock integration. However, keeping two or more livestock species or breeds simultaneously on the same farm is a diversification option that has received little attention to date as an option for improving livestock farm sustainability. Here, we present a pan-European analysis of organic multi-species livestock farms to identify most sustainable management strategies. After characterising the sample of 95 farms combining two or more livestock species (cattle or small ruminants, for meat or milk, pig or poultry), we undertook a partial least square regression analysis to relate sustainability indicators (productivity, efficiency, human welfare) with indicators reflecting farm structures and management, including the type of marketing and the level of integration among farm enterprises. We show that unlike expectations, the most sustainable farms do not necessarily exhibit the highest levels of integration. We also elaborate on the methodological advances needed for an analysis covering various types of species and productions at the scale of farming system scale. This work is being finalized and has been carried out in the European project MIX-ENABLE provided by transnational funding bodies, being partners of the H2020 ERA net project, CORE Organic Cofund, and the cofund from the European Commission.

Pulses and oilseed rape for food and feed

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Plant breeding is always driven by breeding goals: as these two crop groups either offer oil and protein (oilseed rape) or starch and protein (pulses) a breeder has to make decisions about his priorities. In general the main components are negatively correlated. A too strong selection for one component will reduce the other one. Large seeded pulses like faba beans, peas and lupins for a long time were considered as pure feed components that provide protein and very often also starch thus substituting to a certain percentage soybean protein/cereals. As soy was cheap and plenty available on the world market the feed industry in Europe relied on soy imports. Thus these domestic crops were only marginally grown resulting in low breeding activities and also loss of knowledge about their suitability for the main groups of livestock. Due to growing concerns about GM soybean, carbon dioxide footprints and more awareness of locally produced fodder stuff, those pulses have undergone quite a renaissance in the last years. This is continuously supported by exploiting their potential as home grown food components which is more profitable than being used as feed component. This is enhancing breeding in general and will also potentially fuel research for the feed sector. In oilseed rape, there was a very successful story in breeding for food and feed: eliminating Erucic acid from the oil made the oil perfectly suitable for human consumption. After extracting the oil, the remaining extracted cake contains a high amount of protein with a very good amino acid composition. However, glucosinolates limited the use of the cake in animal fee. Breeding lowered the glucosinolate content significantly which makes the residues of oilseed rape extraction well suited as a feed stuff for various livestock species. Farmers in Germany until today are paid a premium for oil content which consequently led to the protein as a component in oilseed rape being of minor importance in Europe. Also, in oilseed rape we today see a shift to more attention to the protein content: this is driven by a sharp increase in demand for plant proteins, either through direct use for human consumption, or as feed for animal protein production.

Panel discussion

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Panel discussion, with speakers and the audience, moderated by Jean-Louis Peyraud, INRAE and Martin Scholten, WUR (ATF past-Presidents). What are the missing points in the discussion? What are gaps in R&I?

Does morphology make the athlete – genetic analysis of Argentine polo horses lines

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The Argentine Polo horse is a breed widely recognized for its sporting aptitude. Its main purpose is the practice of polo, but horses also participate in morphological competitions where there are evaluated for conformation and functional correctness. Since function and morphology are related, there may be only minor differences between animals that obtained prizes in one or another competition. The objective of this study was to evaluate the genetic variability of horses rewarded in morphologic (M, n=969) and sports competitions (S, n=1,570), according to the criteria of the breeders' association. Pedigree data (n=140,000 records) were used to calculate the inbreeding (F) and coancestry (f) coefficients, effective population size (Ne), the effective number of founders (Fe), and ancestors (Fa), and the proportion of effective founders (Fe/n). Both groups showed the same degree of average inbreeding (0.5% in M and S). However, f and Ne were higher in S than in M (0.89 vs 0.70%, $P<0.05$ in f and 472 vs 299,$P<0.01$ in Ne respectively). Although f reveals a closer relationship between individuals, Ne demonstrates a lower rate of inbreeding in S. This is consistent with the fact that removing S or M does not any produce any significant changes in the total genetic diversity. On the other hand, Fe and Fa in S were significantly lower than in M (Fe=319 vs 436, and Fa=78 vs 100), the proportion of effective founders was more than twice as high in S than in M, but Fe/Fa ratios were close to 4 in both groups indicating a strong genetic bottleneck in the Argentine Polo horse population. Although the most contributing ancestors were common in both groups, they showed different degrees of a marginal contribution. Our analysis showed that genetic variability in individuals rewarded for morphology or performance was similar, but sport horses showed a narrower genetic basis. The imbalance in the genetic contributions of ancestors may relate to selection with different objectives. However, the genetic flow between M and S groups is high, suggesting that morphologic selection is linked to sporting aptitude.

Estimation of genetic parameters depending on the distance in endurance competitions of horses

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Selection of horses for endurance competitions involves a challenge in breeding programs. A horse can perform well over shorter distances, but not maintain it over longer distances. Thus, assuming the same performance in short and long competitions could lead to an inadequate selection of horses. The main objective was to estimate the genetic parameters of the ranking, time of race and placing (1, unqualified horses; 2, horses ending the race) using records from races of different distances by applying a random regression model. The database contained 6,903 records from 1,672 horses. The pedigree contained 11,985 animals. A random regression model was applied for each trait using distance in kilometres as a random covariate. All models included the age, gender, race and a first order Legendre polynomial of the covariable distance in kilometres as systematic effects. The animal and the rider were fitted with Legendre polynomials of second order as a function of distance in kilometres with random coefficients in both. Software VCE6.0 was used to obtained solutions and estimations. Heritabilities from 0.06 to 0.21, 0.01 to 0.55, and 0.03 to 0.31 were obtained for the ranking, time and placing, respectively, varying across distances (60 to 192 km). Patterns of evolution of genetic value across distance differed among traits. Breeding value trends of animals was stable along different distances for ranking, with high correlations between all distances (0.83 to 0.99). Genetic values were stable for time in short distances, but animals highly differed in long races; thus, they were highly correlated in close distances (0.99 for 150 and 160 km) but null or low correlated in very different distances (-0.01 for 80 and 160 km). For placing trait it was possible to recommend specific animals for different distances (best in short races or best in long races); there was a null correlation between 80 and 130 km, but it was 0.98 between 99 km and 139 km. Moreover, there were some animals with constant performance in each trait, keeping its performance along different distances. Random regression models in endurance competitions allowed to distinguish the competitive ability of the horse in different distances of races.

Young horse competitions as predictors of adult horse performance in endurance races

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Genetic evaluation for endurance competitions of horses is routinely performed in Spain. Nowadays, the performance control is carried out in Young Horse competitions (YHC) designed ad hoc to promote an early race performance of horses and in the races organized by the Royal Equestrian Federation (REC). The objective of this study was to ascertain if the selection criteria in young horses are good predictors for performance in adult horses. Data sets included 2,054 records of 1,106 horses from YHC held between 2006 and 2019, and 6,903 records of 1,672 horses from REC held between 2000 and 2019. Pedigree contained 11,985 animals (54% Arab horses). The analysed traits in YHC were racing time, recovery time and placing whereas in REC the considered traits were ranking and placing. The YHC traits were solved using a multiple trait model including sex, age, and event as fixed effects and the rider as additional random effect. Regarding the REC traits, the model for placing was the same as for YHC traits and the model for ranking included also the rider-horse interaction and environmental permanent effect as additional random effects. TM and GIBBSTHUR software were used to predict the breeding values. Heritabilities for racing time, recovery time and placing in YHC were 0.18±0.04, 0.26±0.05 and 0.12±0.04, respectively. Regarding REC, heritabilities were for ranking 0.13±0.04 and for placing 0.12±0.03. Genetic index weights were: 50% racing time, 25% recovery time and 25% for placing for YHC, and 60% ranking and 40% placing for REC. Genetic correlations between genetic indexes for YHC and REC ranged between 0.38 and 0.40 depending on the animals included in the set (all animals included in the pedigree matrix, horses with YHC and REC records or a breeding value accuracy of at least 0.4). The more correlated breeding values were those between racing time and ranking (0.38-0.47) and both placing traits (0.31-0.41). These results suggest that the genetic index for early performance in horses is a good predictor of later performance in races.

Genetic relationship between free movements and under rider gaits in Pura Raza Española horse

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The Pura Raza Española (PRE) is among of the equine breeds with the largest census worldwide. One of the PRE breeding program's objectives is to obtain animals with favourable functional aptitudes that support outstanding performance in dressage competitions. The aim of this study was to determine genetic correlations between quality of movement (walk, trot and canter) across assessments of PRE horse to conclude on the possibility of early selection. Natural gaits when presented in hand (free movements, FM) during functionality tests and gaits under rider (UR) during dressage competitions were evaluated. Datasets for FM and UR were provided by the Asociación Nacional de Criadores de Caballos de Pura Raza Española (ANCCE). From a total of 13,743 horses, 13,091 were evaluated for FM, and 2,619 for UR. A sample of 886 horses had records for both FM and UR. Several factors potentially related to the animal's functionality were analysed using a general linear model (GLM). The final models included age as a covariate and sex, coat colour, geographic area of the breeder's stud farm, and year of evaluation as fixed factors. Coat colour was introduced due to its direct relationship with conformation and indirectly with functionality, studied in previous works of PRE horses. Rider, competition and competition level, were additionally analysed for UR. For the estimation of the genetic parameters a relationship matrix of 40,772 individuals was build (with a mean maximum generations of 15.10, a mean complete generations of 5.19 and a mean equivalent generations of 8.67). The results obtained for the heritability of the three natural gaits of the PRE horse, ranged from 0.11 for canter (UR) to 0.24 for trot (FM). Genetic correlations between analogous FM and UR traits were moderately positive; 0.61 for walk and trot and 0.55 for canter. The obtained genetic parameters highlight the possibility of preselecting horses at an early age for their aptitude to participate in dressage competitions.

Estimation of genetic parameters for 'early life' jumping traits in the Belgian Warmblood horse

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Belgian Warmblood horses are worldwide-known for their success in show jumping competitions. Their jumping capacity is assessed at young age either in freedom (free jumping, FJ) or under saddle (SJ) by using a linear scoring scheme. Data collection started in 2003 for free jumping and in 2014 for jumping under saddle. Eleven traits are scored by appraisers on a 9-point scale (scores ranging from -20 to 20): scope, take-off (power/quickness), technique of forelegs, technique of back, technique of haunches, attitude (willingness), care, stride length of canter, impulsion, elasticity of canter and balance. In this study, genetic parameters were estimated based on 2,164 free jumping (FJ) records, 1,745 jumping under saddle (JS) records and 12,730 horses in the pedigree. Two multivariate animal models were used (11 FJ traits, 11 JS traits) with fixed effects for age and sex of the horse and a random contemporary group (cg) effect (combination of the date, the location of the appraisal, and the appraiser). Genetic correlations of pairs of corresponding traits (e.g. scope assessed in free jumping and in jumping under saddle) were estimated with bivariate animal models. Estimated heritabilities of FJ traits varied from 0.04 (attitude (willingness)) to 0.30 (scope) and estimated genetic correlations were all positive, ranging from 0.29 to 0.96. Heritabilities of JS traits ranged from 0.08 (attitude (willingness)) to 0.29 (stride length). A negative correlation of -0.24 was found for scope and attitude (willingness). On average, estimated heritabilities of FJ and JS traits were comparable (0.17 and 0.16, respectively). However, the cg-effect was higher for jumping under saddle than for free jumping, which indicates that there is more variation between appraisers for JS. On top of that, genetic correlations of pairs of corresponding traits ranged from 0.54 to 0.87, implying that some of them are genetically different (rg<0.80). Further analyses will assess the correlations between these early performance measurements and rankings in competitions. This research builds on the development of a genetic evaluation based on these 'early life' jumping traits to predict later success in competitions.

Assessing the value of genetic linear profiles for selecting for sport performance of riding horses

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Routine linear profiling and estimation of breeding values (EBV) for linear traits allow refined characterization of the individual horse and its genetics with regard to conformation, gaits and jumping. When compared to genetic proofs for performance in sport competitions, genetic linear profiles become available earlier, but the value of individual linear traits as indicators for favourable performance in riding sport is unclear. The aim of this study was to determine association patterns between the set of 46 linear traits included in the routine genetic evaluation of the Oldenburg studbooks, OL and OS, and sport traits reflecting dressage (D) and show jumping (J) competition performance through rank-based (R) and summarizing (highest level achieved, L) trait definitions. Statistical analyses were performed with SAS software on the basis of EBV of the active mare population of OL and OS comprising together almost 7,200 mares. Correlation analyses and analyses of variance included between 604 and 629 mares which fulfilled the inclusion criteria of own phenotypic data and/or at least two progeny with phenotypic data for indicator and target traits (DR, DL, JR, JL) in the respective genetic evaluations. Associations patterns with the sport traits were derived considering the linear traits either directly (Pearson correlations, linear regression) or as class variables. Results indicated absent or low correlations of $|r^2|<0.2$ with EBV for sport performance for EBV for most of the 24 linear conformation traits (D n=16, J n=23). Conversely, significant correlations with $P<0.001$ were found for 11 of the 12 linear gait traits with regard to D and for 8 of the 10 linear jumping traits regarding to J. Analyses of variance revealed consistent results, indicating slightly different patterns for trait definitions L and R and high indicator value ($r^2>0.5$) of reach of hind limbs in walk, carrying power and reach of front limbs in trot and direction of movement in canter for D and jumping ability for J. Our results support the use of genetic linear profiles in breeding programs of riding horses.

Single-step genomic evaluation in German riding horses

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German horse breeding associations started in 2017 under the umbrella of the International Association of Future Horse Breeding (IAFH) a collaboration project with partners from science and practice to advance genomic applications in sport horse breeding. The joint genomic reference population of 5,000 horses, completed at the end of 2020, will now form the basis for implementing a system for estimating genomic breeding values (GEBVs) for a wide range of traits. Harmonization of the routinely used linear recording scheme among the participating associations has ensured that standardized phenotypes are available for all horses in the reference population, including conformation and performance (gaits, jumping) traits. In order to most comprehensively exploit all relevant information, a single step genomic BLUP model (ssGBLUP) was applied using all available phenotype, genotype and pedigree data. The same fixed effects were used as for conventional breeding value estimation for linear conformation and performance traits. First analyses referred to the horses with phenotypic data and the 1,034 stallions with progeny in the reference population. Comparison of phenotypic data between the top and bottom 10% of horses based on their GEBVs revealed promising results for both conformation (e.g. stance of forelimb pastern) and performance (e.g. mechanics of front limbs in trot). Since the reference population was built across breeding associations with varying numbers of pedigree links among each other, ongoing work on validation and optimization must particularly focus on reliability of GEBVs for all breeds.

Validating genomic selection for sport traits in Dutch warmblood horses

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In recent years, genomic selection has been successfully implemented in various animal species for a number of performance traits. Horse breeding has only marginally been part of this development. Meanwhile, a reasonable number of genotypes on horses using a high density SNP panel is available within the Royal Dutch Sport Horse-population. Horse breeders are still hesitant to use BLUP techniques over phenotypic selection which is still common practice. For decision making and to increase acceptance it is important to demonstrate and quantify the advantages of genomic selection. The aim of this study was to examine the added value of genomic breeding values compared to traditional breeding values for sport performance in warmblood sport horses by means of a validation. The accuracy of EBVs obtained with either BLUP or single-step genomic BLUP (ssGBLUP) were evaluated using a leave-one-out technique. For 52 breeding stallions born since 2003 in a specialized breeding programme with at least 30 offspring competing in sport, all information of all their offspring and potential grand offspring was iteratively removed from the dataset. This has been repeated but now also omitting stallion's own performance. The accuracy of the breeding values (r_{IH}) was determined by the weighted Pearson correlation between the (G)EBV of the stallion from the evaluation where its sources of information were removed and the average pre-corrected performance of his offspring. The full dataset used for this analysis comprised 109,089 records with performance data and known pedigree in five generations; 4,273 animals had SNP genotypes. A multivariate animal model was used to estimate the (G)EBVs for the sport trait and three other performance related traits using MiXBLUP software (version 2.2). The accuracies of (G)EBVs from ssGBLUP compared to BLUP improved by 7% when omitting both own performance and (grand-) offspring performance of the stallions (49% for BLUP, 56% for ssGBLUP), and by 5% when omitting the performance of offspring and potential grand offspring only (55% for BLUP, 59% for ssGBLUP). Despite the limited size of the training data, genomic selection showed an improved accuracy for a breeding goal trait in sport horse breeding.

Microsatellite alleles imputation from SNP genotypes for parentage verification in sport horses

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Nowadays in France, microsatellites markers (MS) are used for parentage verification among all horse breeds. Studies have shown that parentage verification based on Single Nucleotide Polymorphisms (SNPs) are less prone to error, cheaper and have shorter processing times. Moreover, ISAG is working on a SNP panel to allow the transition from MS to SNP for parentage verification. The principal constraint of this transition is the cost of re-genotyping all sires and mares that are already MS-genotyped. In this study, we wanted to test a method to impute MS alleles from SNP haplotypes. A total of 6,295 horses from 3 breeds (Arabs, Selle-Français and French Totters) were used. They were MS genotyped for parentage verification and have been SNP genotyped with 54K to 670K chips. Haplotypes for markers within 500 Kb on either side of each MS were obtained using FImpute 3 by considering MS alleles as pseudo-snp. To determine SNP haplotypes that best fit a MS allele, we started by selecting 10 SNP on each side of the MS genotype, with the possibility of extending it if haplotypes matched with several MS alleles. The objective was to obtain a haplotype that always fitted one MS allele (or as little MS as possible). The haplotypes were built within breeds and across breeds. First analysis of the 9 alleles of MS AHT4 and the 166 haplotypes with 20 flanking SNPs showed mixed results. Problematic results from phasing, i.e. missing MS allele or more than one MS allele on the same haplotype, represented less than 0.5% of phased samples. Rare association (1 case) between MS allele and SNP haplotypes represented 1% of the phased samples. After removing these cases, SNP haplotypes were able to attribute unambiguously MS allele in 84% of the cases considering all breeds together (from 50% for Selle Français alone to 100% for French Trotters alone). Longer haplotypes will be tested and influence on parentage verification will be checked.

Pseudoauthosomal región as a key factor for the molecular detection of Turner´s syndrome in horses

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The monosomy of the sex chromosome pair (known as Turner´s syndrome) is a frequent cause of infertility in the horse. This disease, characterized by individuals showing a mare phenotype with gonadal dysgenesis, was reported across breeds most commonly in two different forms: pure (63, X63,X) and mosaic (63,X/64,XX). Nowadays, its diagnosis is made by direct observation using classical or molecular (FISH) karyotyping. In this study, we analysed 6 horses previously diagnosed as carriers of Turner´s syndrome (3 pure and 3 mosaic) using a chromosomal number aberration approach based on medium density SNP genotyping. Individuals were genotyped using a 65K GGP array for horses. Copy number aberrations (CNA) were detected based on LRR intensity and heterozygosity (HET) values in two regions of the ECAX: 1:1,850,000 bp (Pseudoautosomal region PAR) and 1,850,001:128,200,000 bp (NON-PAR). Results were compared among groups using an ANOVA (LRR) and a Z-proportion test (HET). All the mares showed LRR and HET values close to 0 and 25%, respectively, in both regions (PAR and NON-PAR), indicating the presence of two ECAX. Stallions showed the same pattern in PAR, since this chromosome region is present in ECAY and ECAX. However, LRR and HET means decreased to -0.41 and 2% in NON-PAR, a pattern consistent with the presence of only one chromosome, since only one ECAX is present in stallions. Only 63,X individuals showed a single chromosome pattern (LRR=-0.42 and HET=1%) in PAR and NON-PAR, indicating absence of ECAY, and thus allowing its detection. In the case of 63,X/64,XX, PAR, and NON-PAR showed intermediate values of LRR (-0.23) and normal values of HET (21%), consistent with the coexistence of two cell lines with different chromosomal complements. In conclusion, the use of SNP genotyping seems to be a valid methodology for CAN screening in horses. In addition, the PAR region is the key to detect the ECAX monosomy. Further studies with larger datasets are necessary to validate this approach as a reliable methodology before including it in the genomic toolbox.

Screening for genomic association with fertility in PRE horses using high density genotyping data

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Reproductive traits are a key factor in the economic success of horse production systems. However, they are scarcely genetically analysed, probably due to the lack of reliable phenotypic data and the difficulty to model the environmental factors. Among them, the reproductive efficiency (RE) determined as the deviation between optimal and real parity number at age of each mare, was suggested as one of the most promising traits to evaluate individual fertility. In this study, we performed a genome-wide association study (GWAS) for RE in a population of 821 Pura Raza Español (PRE) horses. As targets, we used two RE pseudophenotypes, the first estimated using BLUP with a pedigree-based relationship matrix (A; DEBVRE) and the second using the relationship matrix H (ssDEBVRE) constructed blending the genomic-relationship matrix (G) with A matrix, using ssBLUP. The horses were genotyped using the Affymetrix Axiom Equine 670K, excluding the SNP markers with call-rate ≤0.95 and with a minor allele frequency <0.01. After filtering, SNP dataset used for the estimation of the G matrix included 449,967 markers. For GWAS, the genomic dataset included 148,073 SNPs from the autosomal chromosomes after linkage disequilibrium pruning. The GWAS analysis was performed using GEMMA software with a linear model association test. Our results showed that the model based on ssDEBVRE showed a better adjustment. Using ssDEBVRE, we detected 30 genomic regions with a significant association to the RE criteria. Among them, two regions, located in ECA5 and ECA6 included 3 candidate genes (*AKAP3, ATP2B4, ACRBP*) involved in different aspects related to fertility. To our knowledge, this is the first report of GWAS analysis for autosomal genomic regions on horse fertility using a large dataset of deregressed data. Further analysis are necessary to validate our findings.

Genomics to maintain diversity and robustness of Coldblood breeds

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German draught horse breeds were developed in the 19th century. Interbreeding of local working horses with other draught horses from neighbouring countries was practised to improve performance. The most important German draught horse population was the Rhenish German Coldblood. South German, Black Forest and Schleswig Coldblood horses were bred in Bavaria, Baden-Wurttemberg and Schleswig-Holstein, respectively. Even if these breeds were almost lost in the 1970s, governmental support, enthusiasm of breeders and the use of draught horses for work in forests, for milk and meat production as well as for leisure activities helped to maintain these breeds. We employed new genomic tools to test for genetic characteristics including disease conditions, estimate genomic relationships and inbreeding and measures of genetic diversity. Particularly, we performed whole genome sequencing of 50 draught horses from all German breeds. Our results showed that draught horses constituted a clearly distinct cluster compared to warmblood, primitive, Arabian and Thoroughbred. Whole genome sequencing data from modern horses and early domestic horses of bronze age from the Eurasian steppe and Europe were used for phylogenetic analyses, which revealed that draught horses may have their origin in early domesticated horses. Osteochondrosis, myopathies and chronic progressive lymphoedema are of major concern and impact breeding decisions. In order to maintain a broad range of breeding animals we are employing genotyping and whole genome sequencing data to support breeding decisions. Genomic tools proved powerful to estimate heritabilities and genotype by environment interactions, trends in effective population size and increase in inbreeding and genomic relationships among animals within and across breeds. We are offering draught horse breed organizations easy-to-use PC-software to reduce individual rate of inbreeding through optimizing mate allocation. In order to keep breeders enthusiastic, disease conditions severely impairing health and life expectancy will be given more emphasis based on genetic tests and genomic breeding values in breeding programs.

Genetic parameters for new phenotypes measured in Italian Heavy Draught Horse

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A joint breeding program named Equinbio has recently been launched by the Italian horse breeders' associations under the umbrella of the European Agricultural Fund for Rural Development. Within this program, new phenotypes have been defined and recorded aiming to evaluate their possible use for selection. Particularly, the Italian Heavy Draught Horse breeders' association has focussed on 5 new phenotypes to be recorded in young foals and 3 in adult horses. The new phenotypes measured on young foals (2-9 months of age) were neck length (NL), neck direction (ND), croup length (CL), croup direction (CD), and body condition score (BCS). The new phenotypes measured on adult horses (about 30 months of age) were shoulder slope (SS), shoulder length (SL), and body condition score (BCS). With the only exception of SLwhich was obtained by an objective measure, all new phenotypes were obtained by a linear score using a 9-point scale (1-5 scale with half points by skilled classifiers. Data were recoded from 2017 to 2019. The final dataset included for 1,330 records for young foals and 525 for adults. The number of animals in the pedigree files were 6,269 for foals and 4,165 for adults. The models adopted for the two datasets accounted for the effects of stud-year-classifier (single or groups in case of single observation assembled by geographical area, type of housing, production target, vaccination of foals and average body condition of the studfarm), sex, and age at evaluation. Age of the mare was additionally considered only for the foals' evaluation. Analyses were performed with AIREMLf90. New phenotypes obtained from foals showed heritabilities from low (0.08 for ND) to medium (0.16 for NL) or medium-high (0.32 for BCS and 0.36 for CL). In adults, heritability estimates were generally lower than in foals for body measures: 0.02 for SS and 0.07 for SL. BCS in adults showed a medium-high heritability of 0.50. Related to the limited amount of data especially for the adult horses, heritability estimates showed high standard errors, hence these results reported are suggestive.

GxE and selection response for fertility and linear type traits in Italian Heavy Draught Horse

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This study aimed to investigate genotype by environment interactions (GxE) and response to selection (R) for lifetime fertility and linear type traits under diverse environmental conditions (EC) in which the local Italian Heavy Draught Horse breed is reared. Target traits were lifetime foaling rate (LFR), that is the number of foals generated on the opportunities to do so after 6 breeding seasons, and 14 linear type traits (LTT) routinely collected on mares by trained classifiers. The LTT included morphological traits like fleshiness and hip-buttock distance, and aesthetic and handling-related traits like head expression and temperament. Data since 1992, including the whole pedigree of the breed were available. As EC, geographical area and rearing system were considered, since horses are typically reared in stables (mainly in the North of Italy) or in semiferal and feral conditions in low grade pasturelands (Centre and South of Italy, respectively). GxE were estimated in single-trait models using the reaction norm approach, i.e. a first step based on a single trait animal model (M1), and a subsequent step with a random regression sire model (M2) using the solutions of the stud-group classifier by year of birth (SYC) effect of M1. SYC was considered as environmental covariate to assess the intercept G and the slope of GxE across SYC levels. A multivariate response to selection (R) in different EC was estimated for the target traits using phenotypic genetic (co)variances. Solutions of M1 showed that EBVs of sires varied in different EC. The introduction of M2 showed that part of the phenotypic covariance was due to GxE, and heritability of traits changed consequently. Some re-ranking of sires according to their EBVs was observed when comparing between results from the classical model without GxE and the model including this term. R was also influenced by including GxE or not in the model, and by EC. This is likely due to the extremely different conditions under which horses of this breed are reared. Results suggest considering GxE in genetic evaluation of IHDH and other local horse breeds exposed to various rearing systems and management conditions.

Shape and gaits 2.0 – high-precision phenotyping for equine breeding

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Conformation and gait quality traits are important criteria for the selection of horses. Data of these traits mainly relies on subjective evaluation scores of judges of a specific breed. Therefore, these scores are often biased and can only with reservations be compared between breeds. Genetic analyses of these traits remain challenging due to the small breed-specific sample sizes and limited variation in scores. The standardized measurement of conformation and gait quality traits are more accurate and comparable between breeds, under the condition that the same methodology is used (e.g. selection of anatomical landmarks, kinematic measurement system). Such data collection simultaneously support the genetic dissection of complex traits. In the Shape and Gaits 2.0 project, we apply two novel phenotyping methods: the horse shape space model for conformation measurements and the inertial measurement unit system EquiMoves$^{(R)}$ for gait quality assessments, to better understand the associations between breed type, conformation and gait quality traits. The horse shape space model can quantify variation in shape using the principal component scores of 246 normalized landmark coordinates from a single photograph. To date, we have collected shape data of 541 Franches-Montagnes (FM), 224 Lipizzan, 32 Shagya Arabian, 21 Swiss Warmblood (WB) and 19 Pura Raza Espanol horses. For the gait quality analysis, 125 FM and 21 WB were additionally measured at walk and trot on a straight line using the EquiMoves$^{(R)}$ system. The majority of the horses (n=523) were also genotyped on the Affymetrix 670K genotyping array to identify genetic variants affecting conformation and gait quality traits. Based on the shape data of 300 FM and 224 Lipizzan horses we already identified a new QTL for the poll angle. In this study, we are currently sampling more horses to further elucidate the genetics of shape and gaits.

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Are conformation characteristics of Pura Raza Española adult horses predictable?

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Conformation is a predictor of performance and susceptibility to injuries and also important for reasons relating to aesthetics, health and welfare, durability and functionality. The Pura Raza Española (PRE) horse breeding program includes conformational and functional traits as selection criteria. Indirect selection by conformation traits is useful, since their heritabilities are often higher than those of performance traits. A total of 155,716 records from 142,244 PRE horses were available. Animals were grouped by sex (94,935 females and 60,781 males) and age (82,408 young horses, <4 years old, and 73,308 adults, ≥4 years old). Sixteen conformation traits (14 measurements and 2 linear traits related with limbs) were analysed. Differences between and within ages and sexes were evidenced in all traits. Coefficients of variation CV were medium-high in 94% of traits in both sexes and ages; only body length exhibited low CV (<5%). The effects of breeder, sex, coat, age and inbreeding were included in the estimation of genetic parameters using a univariate linear animal model with REML using VCE software, with at least 4 ancestral generations of the horses with record s in the pedigree. The heritabilities were of medium-high level for both age groups, with ranges of 0.18-0.80 in young horses and 0.27-0.89 in adult horses. The lowest estimates were obtained for chest width (0.18±0.006 in young horses and 0.27±0.004 in adult horses), and the highest for point of hip-stifle distance in young horses (0.80±0.010) and body length in adult horses (0.89±0.008). The genetic correlations between analogous traits in young and adult horses were estimated to evaluate the predictability of the traits in adult horses. Estimates were positive and >0.75 for all traits, except for chest width (0.52±0.024). The heritabilities imply high genetic progress through the breeding programs when information on young horse conformation is used for selection of breeding stock, with the genetic correlations ensuring efficiency of pre-selection of young horses because of the predictability of traits of adult horses.

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Phenotypic and genetic analysis of conformational defects of the knee in Pura Raza Española horse

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Diseases of the horse's limbs constitute an important group of defects, in particular including developmental and degenerative joint diseases. They are often underlaid by complex genetic effects, which may be breed-specific or common in certain types of horses. Disorders of the carpal joint or front-knee have a significant impact on these specific performance characteristics and thus severely affect the use of horses in equestrian sports. The aim of this study was to establish the within-breed prevalence, possible associated risk factors and heritability of four different carpal defects in PRE horses. Lateral view carpal defect was divided into 'over at the knee' and 'back at the knee' and frontal view carpal defect into 'bench-kneed' and 'knock-kneed'. Each defect has been classified in 5 classes: 1 having no defect and 2 to 5 having defects of increasing severity. A total of 42,158 Pura Raza Española horses were evaluated, and 55.7% were without any carpal defect. Prevalences of 6.4, 20.8, 3.7 and 26.2% were determined for over at the knee, back at the knee, bench-kneed and knock-kneed, respectively. Fixed effects were included in the model according to significant results in analyses of variance (GLM): gender, age, coat colour, geographical area and size of the breeder's stud farm for its relationship with the management system. Genetic parameters were estimated using a Bayesian procedure with the TM software. Heritability estimates ranged from 0.39 (knock-kneed) to 0.12 (bench-kneed). To study if there was a genetic relationship between the carpal defects, bivariate mixed models were applied: over at the knee vs bench-kneed, over at the knee vs knock kneed, back at the knee vs knock kneed, and back at the knee vs bench kneed. The closest correlation was found between 'back at the knee' and 'knock kneed' (0.67) and the weakest between 'over at the knee' and 'bench-kneed' (0.08). These results imply that selection against limb defects is possible and would allow reducing the genetic risk of the horses' offspring to suffer from them.

Exploratory whole genome association with fertility in PRE horse breed using chromosome X NGS data

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Although reproductive performance should be under selection pressure in most equine breeds, the genetic basis of reproductive traits in horses is still unknown. In this study, we analysed whole NGS data from 88 Pura Raza Español (PRE) horses, conducting an exploratory whole genome association study of reproductive efficiency (RE), defined as deviation between optimal and real parity number at age of each mare. The samples were sequenced using Igenomix Riptide™ high throughput library. Trimming and QC of raw reads was performed using Fastp and FastQC pipelines. Thereafter, remaining reads were aligned with EquCab3 reference using BWA MEM, pooled with Picard, and duplicates removal and VCF calling performed with GATK tools. Finally, VCF files were pruned based on two criteria (DP≥3 and GQ≥20), and missing markers imputed based on haplotype calls using BEAGLE.-v.5.1. From the final dataset including 11,317,780 SNPs, the360,869 SNPs located on ECAX were extracted for the GWAS analysis after LD pruning (r^2>0.5). Genome wide association study was performed with univariate linear mixed model in GEMMA software using deregressed breeding values for RE as pseudophenotypes. A total of 75 SNPs was found to be associated with the target trait. These were then analysed in terms of functionality using SnpEff software. One of them was located in the *FGF13* gene, involved in a variety of biological processes, including embryonic development in various species. To our knowledge, this is the first GWAS performed in horse fertility traits using a large SNP dataset obtained from whole NGS data. Further validation of our results is necessary.

Inbreeding coefficient estimation methodologies comparison in Pura Raza Española horse population

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The Pura Raza Española (PRE) is one of the oldest European horse breeds and the most well-known in the Iberian Peninsula with more than 250,000 active individuals. Although the large PRE horse population, genealogical analyses have demonstrated that inbreeding (F) levels are high. More than 45% of the total PRE population showed an F coefficient equal or higher than 6.25%. The objective of this study was to describe and compare inbreeding coefficients determined by different methodology in the PRE horse population and stablish behavioural differences between them in a long population breed when different levels of homozygosity are analysed. The analysis was based on the total pedigree (TP) information (328,706 horses) and the last generational interval (LGI) population (134,746 horses born in the last 10 years). The maximal number of generations in TP was 17.14 with an average equivalent of known generations of 9.44, whereas respective figures for LGI were 18.76 and 10.40, respectively. The numbers of founders and ancestors contributing to the current genetic pool were 1,056 and 1,026, respectively. Wright classical F (F_W), Ballou ancestral F (F_BAL), ancestral history coefficient (AHC), and Kalinowski ancestral F (F_KAL) were analysed. Average F values for TP were 7.5, 31.3, 44.4 and 4.9%, respectively. Results for LGI were similar with the largest difference observed for AHC (49.9%). Accordingly, correlations between F values were positive and significant ($P<0.05$) with similar results for TP and LGI. The closest correlations were found between F_BAL and AHC (0.98) and between F_KAL and F_W (0.90), the weakest between F_W and AHC (0.36) and between F_W and F_BAL (0.35). Nevertheless, when only animals with F_W>12.5% were considered, correlations with F_W decreased, while the further correlations increased or remained equal. Our results demonstrated different behaviour of most commonly estimated inbreeding coefficients when populations with different levels of homozygosity are evaluated.

Clustering of the faecal microbiome during stressful phases in carnitine supplemented dairy cows

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Transition from late gravidity to early lactation and multiple illnesses can pose outstanding challenges for dairy cows. L-carnitine (CAR) as a feed additive is supposed to support the cows' energy metabolism during these critical periods. The individual variability and long-term changes of the faecal microbiome and metabolome were studied here. Cows were fed with a CAR supplemented diet compared to control animals (CON) in a randomized experiment (n=55) around parturition and a lipopolysaccharide (LPS)-induced systemic inflammation. Trial started at day 42 antepartum and lasted until day 126 postpartum (pp). Animals were LPS-stimulated on day 111 pp, applying 0.5 µg LPS/kg body weight intravenously. Faecal samples were collected at 13 time points. Extracted DNA was used for 16S rRNA Illumina sequencing (V1-2 region) of bacteria. Volatile fatty acids (VFA) were measured in all samples, a subset from seven time points was selected for targeted metabolomics measurements using AbsoluteIDQ®p180Kit (Biocrates). Data were analysed by multivariate statistical analyses. Both challenges had clear impacts on total bacterial community and accompanied metabolite profiles, whereas there was no grouping effect for CAR. The individual animals were classified into three distinct microbiome clusters based on the relative abundances of bacterial members and the rate of dispersion. Three microbiome clusters (C1 to C3) were found according to the 'enterotype' algorithm known from human microbiome studies. C1 showed a dynamic pattern of the faecal bacterial community composition among all time points, whereas C3 showed a static pattern. Animals belonging to C1 showed lower bacterial diversity and highest numbers of residual energy intake as C2 and C3. Blood glucose, faecal VFA, milk quality as well as case and duration of illnesses seemed to be significantly influenced by or with the respective microbiome cluster of the cow. In summary, cow microbiome clusters vary in composition, responds differently to stress challenges, and seem to be associated with a 'health status' of the animals.

Microalgae *Schizochytrium* spp.: a game-changer in goats' rumen microbiome

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Fat rich microorganisms, such as microalgae *Schizochytrium* spp., are potential biotechnological tools in the modulation of rumen microbiome towards ecofriendly and nutritional value end-products. However, limited *in vivo* trials have been reported on the topic. The aim of this study was to contribute to the knowledge on the effect of fat rich microalgae: (1) on the methanogenic species adhering to the feed particle or floating in the rumen liquid by using a qPCR platform; and (2) on the rumen bacteriome in the liquid fraction using an NGS. For the trial, twenty-four goats were divided into four homogenous clusters (six goats/treatment) according to their fat corrected (4%) milk yield, body weight and age. The concentrate of the control group (CON) contained no microalgae, while those of the treated groups were supplemented daily with 20 (ALG20), 40 (ALG40), and 60 (ALG60) g of *Schizochytrium* spp./goat. The relative abundances of total Archaea, methanogens, Methanomassiliicoccales, *Methanobrevibacter* spp., *Methanosphaera stadmanae* and *Methanobacterium formicicum* were significantly ($P<0.05$) decreased in solid fraction of microalgae-fed goats compared to the CON group, while only *Methanobrevibacter spp.*, was significantly ($P<0.05$) decreased in the rumen liquid. bacteriome analysis unveiled imbalances between proteolytic, amylolytic and cellulolytic bacteria in goats' rumen liquid. Specifically, the abundance of the cellulolytic bacteria was decreased ($P<0.05$), while those of amylolytic and proteolytic species were increased ($P<0.05$) in the ALG40 group compared to the CON. In conclusion, goats' diets supplementation with *Schizochytrium* spp., could be considered a sustainable nutritional strategy for methanogens inhibition without further disturb the rumen fermentation balance. This research is co-financed by Greece and the European Union (European Social Fund- ESF) through the Operational Programme 'Human Resources Development, Education and Lifelong Learning' in the context of the project 'Reinforcement of Postdoctoral Researchers – 2nd Cycle' (MIS-5033021), implemented by the State Scholarships Foundation (IKY).

Session 09 Theatre 3

Unrevealing the effect of seaweeds on rumen bacterial and archaeal microbiota

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Feeding seaweed to ruminants might help methane emission mitigation by altering the microbial ecosystem of the rumen. Our goal was to study, in an *in vitro* system, the effect of supplementing five seaweeds (*Ascophyllum nodosum* and *Fucus vesiculosus* (both from two locations and *Asparagopsis taxiformis*) on the bacterial and archaeal rumen microbiota composition and methane production. A total mixed ration control and five seaweed treatments (supplemented at 2.5%) were analysed in the semi-continuous rumen simulation technique (Rusitec). The experimental run had a duration of 14 days for each seaweed, thereof 7 days of adaptation followed by 7 days of sampling. Samples of the fermenter liquid (FL) and fermenter effluent (E) (d 7 and d 14) and the feed residues (FR) (d 14) were characterized using target amplicon sequencing. Phylogenetic analysis was assessed using QIIME2, followed by multivariate statistical analysis. Statistically significant effects were detected in the bacterial community for sample, treatment, and day. Interactions between these factors were not significant. In archaea microbiota, significant effects were observed for the type of sample, treatment, day, and the interaction between treatment and day. The distribution of archaea showed a significant effect of treatment in FR and an interaction of day and treatment in E. The supplementation of *A. taxiformis* significantly reduced methane production and increased the abundance of *Methanomassilicoccus* in FR but reduced the abundance of *Methanomicrobium* compared to the other treatments. Effluent samples of the treatment supplemented with *A. taxiformis* showed no abundance of *Methanomicrobium* on d 7. On d 13, *Methanomicrobium* was reduced compared to d 7 in the treatments supplemented with *A. nodosum* and *F. vesiculosus*. *A. taxiformis* treatment showed a much higher abundance of *Methanomassilicoccus*. Seaweed supplementation has a more substantial effect on the archaeal community than on the bacteria.

Session 09 Theatre 4

Association between *in situ* rumen kinetics and *in vitro* gas production of corn silage nutrients

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This study evaluates the estimation of *in situ* rumen kinetics of corn silage from their chemical composition and the *in vitro* fermentation characteristics of fresh whole silage using an automated gas production (GP) system. A total of 42 samples were incubated, as is, in nylon bags (~6 g DM/bag) in the rumen of 3 cows for 3, 8, 16, 32, 56, 96 and 336 h in duplicate. The washable fraction (W) was determined using a washing machine rinse cycle for 35 minutes at 10 °C (0 h residue) and the degradable fraction (D) as the difference between the undegradable fraction (U; 336 h residue) and the 0 h residue (100-W). Degradation rate (Kd) of D was estimated using the model: $Y = U + D \times \exp(-Kd \times t)$ where Y is the fraction degraded over time. Effective degradability (ED) was calculated at a passage rate of 5%. GP with 600 ml of buffered rumen fluid from ~5 g of DM of sample was registered over 72 h. GP was fitted to a mono-phasic sigmoidal model: $Y = A/1 + (B/t)^C$ where A=asymptotic GP; B=time at half of the asymptotic GP; C=sharpness of the switching characteristics. Models with *in situ* values as the response variable, and proximate analysis, GP parameters and degradable OM (% DM) as predictors (x=20) were evaluated using partial least square regression with PROC PLS in SAS. A leave-one-out cross validation method was applied. For DM, the U, W and Kd was predicted with 9, 3 and 2 factors (k) which explained 86, 89 and 40% of the response variation (R^2) with a RMSEP of 4, 2 and 20% of the mean, respectively. For CP, only U (k=3, R^2=62%, RMSEP=12%) and W (k=3, R^2=57%, RMSEP=17%) yielded a prediction after cross-validation. U of starch was assumed to be 0, and only W (k=2, R^2=65%, RMSEP=34%) and Kd (k=10, R^2=75%, RMSEP=27%) were assessed. Despite the lower predictability of Kd, the ED *in situ* of DM (k=3, R^2=86%, RMSEP=2%), CP (k=4, R^2=64%, RMSEP=7%) and starch (k=4, R^2=73%, RMSEP=9%) can be predicted. *In vitro* GP together with chemical composition can be used to estimate *in situ* degradation parameters.

Determination of linoleic acid requirements for dairy cows using a meta-analysis approach

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Linoleic (LA) and α-linolenic (ALA) acids are essential fatty acids (EFA) involved in major body functions (e.g. reproduction, fluidity of cell membranes) and regulations (e.g. inflammatory response, platelet aggregation). As no EFA requirement values are available in dairy ruminants, we quantified LA requirements for productive (i.e. lactation) and non-productive (i.e. all other functions) expenditures in dairy cows by meta-analysis of a database including 73 *in vivo* experiments (273 treatments) providing LA intake and LA milk yield (MY) and body weight (BW). In accordance with the approach used in INRA (2018), between-experiment regressions of LA intake (g/d) in function of LA MY (g/d) and BW (kg) were studied. We quantified a non-productive requirement of 0.17 g/d of LA intake/kg BW and a requirement for milk LA secretion of 7.32 g/d of LA intake/g of LA MY (LA intake (g/d) = 0.17×BW (kg) + 7.32×LA MY (g/d); RMSE=111.2 g/d; R^2=88.3%). The residuals of that relation were not linked to the days in milk, the main diet characteristics, the milk yield and composition, but were positively correlated (r=0.7; $P<0.001$) with the diet total FA content (% DM) and negatively correlated (r=-0.5; $P<0.001$) with the milk fat content (MFC). Thus, we tested the effect of Milk Fat Depression (MFD) vs control (CTL) rations on LA requirements, with MFD rations characterized by a drop in MFC and milk fat yield greater than 15% compared to CTL and a pronounced increase in *t*10,*c*12 18:2 and/or *t*10 18:1 in milk fat. We observed a higher requirement for milk LA secretion with MFD (17 treatments) compared to CTL (256 treatments) rations, whereas the non-productive requirement was not affected (MFD: LA intake (g/d) = 0.13×BW (kg) + 13.80×LA MY (g/d); CTL: LA intake (g/d) = 0.13×BW (kg) + 7.42×LA MY (g/d); RMSE=95.8 g/d; R^2=91.3%). Our approach gives a first estimate of LA requirement for non-productive functions according to BW as well as for milk LA secretion. A similar approach is currently applied to ALA.

In vitro digestion and Ussing chamber to investigate nutrient effects on intestinal physiology

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The use of chestnut extracts (CHE) as feed ingredients affects gut ecosystem of livestock. Little is known about the effects of CHE on the digestibility of nutrients containing different levels of proteins and lipids and how CHE metabolites can affect the pig intestinal integrity. Thus, we investigated the effect of CHE on simulated monogastric *in vitro* digestibility (IVD) of soy protein isolate (SPI) and two different soybean meals extracts obtained by screw pressing (SM1) and solvent extraction (SM2). The CHE-derived metabolites were tested for their effects on jejunum integrity in pigs. Samples of SPI, SM1 and SM2 were digested *in vitro* with or without the addition of 3% CHE. The polyphenol content before and after IVD was quantified by HPLC. Trans-epithelial resistance (TER), was studied in porcine jejunum segments obtained from 100 kg Swiss Large White pigs and mounted in Ussing chambers in the presence of three different dilutions of CHE-derived metabolites (1:4, 1:8 and 1:16 v/v) or absence. The tissues were then lysed to determine claudin-1, occludin, zonula occludens-1 protein expression. The CHE (3% w/w) decreased ($P<0.05$) both the IVD and polyphenols content of both SPI (-3.2 and -60.3%), SM1 (-8.1 and -67.5%) and SM2 (-7.2 and -68.2%). No detrimental effects of CHE on TER were observed. The 1:8 dilution increased ($P<0.05$) both zonula occludens-1 and occludin protein expression (+60%) compared to the control, while the dilution 1:16 only increased zonula occludens-1 expression (+100%). In conclusion, CHE exert anti-nutritional effects on the IVD of soy-based products when included at the 3% w/w. The observed effects depend on the chemical composition of the soy meal. In addition, low concentrations of CHE derived metabolites exert protective effects on the intestinal epithelial cell integrity.

Ex vivo intestinal model for the evaluation of nutrient bioaccessibility

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Nowadays, the nutrition plays a pivotal role for both human and animal health, and increasingly requires science-based evidences to develop effective functional diets. In order to reduce the use of animals for experimental purposes innovative reliable and informative models, simulating the complex intestinal physiology, represent an expanding research field. In this scenario, the aim of this study was to develop and evaluate an intestinal swine *ex vivo* model for the evaluation of nutrient bioaccessibility and its functionality among time. A pig intestine was harvested, following Maastricht criteria, from a 100 kg pig at the slaughterhouse. The isolated duodenum tract was reperfused in normothermic conditions with heterologous blood after cold ischemic storage. *Ex vivo* duodenum perfusion model was maintained in controlled pressure conditions (flux pump 1.48 l/min, O_2 2 l/min, artery pressure 76/55 mmHg) through extracorporeal circulation for 4 hours. During this period, blood and luminal content samples were collected at regular intervals for the evaluation of the concentrations of glucose by glucometer, minerals (Na, Ca, Mg, K, P) by ICP-AES, lactate-dehydrogenase (LDH) and derivates of reactive oxygen metabolites (D-ROMs) by colorimetric methods. Histological examination was carried on three intestinal segments. The macroscopic observation showed peristaltic activity caused by intrinsic nerves. Measured glucose levels suggested its utilization at cellular level confirming the organ viability in line with histological examination. At the end of the experimental period, intestinal mineral concentrations resulted lower than their level in blood plasma highlighting their bioaccessibility ($P<0.001$). A progressive increase of D-ROMs and LDH concentration among time, in luminal content, was observed related to a slight oxidative stress ($P<0.05$). Isolated *ex vivo* duodenum perfusion developed model fulfils all the prerequisites to study bioaccessibility of nutrients, offering a variety of experimental possibilities in line with 3Rs principles.

Role of short chain fatty acids to counteract inflammatory stress in mucus secreting HT29-MTX cells

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The integrity of the gastrointestinal barrier represents the first step in maintaining the gut health, which affects production animals' performance and welfare. Although SCFAs, especially butyrate, are known to promote intestinal health, their role in the protection of the intestinal barrier integrity and function is poorly characterized. The main aim of the study was to set-up an *in vitro* model of colonic epithelium to study the role of SCFAs as protective nutrient metabolites for the intestinal epithelial cells exposed to a stress condition. Intestinal goblet HT29-MTX-E12 (E12) cells were differentiated and further stressed with Dextran Sodium Sulphate (DSS), to simulate intestinal inflammation. The effect of butyrate alone (BUT) and the SCFAs mixture (MIX) was tested on intestinal cell viability (LDH test), epithelial integrity (TEER) and permeability (FITC) of differentiated E12 cells exposed to inflammatory stress condition. MUC2 and MUC5AC gene expression modulation was also evaluated by RT-PCR. Results showed that the concentration of 10% DSS (24 hours) decreased the TEER about 50% compared to control (0% FCS). Treatment with a concentration of 10 mM of MIX for 1 and 24 hours significantly ($P<0.05$) counteracted the decrease of TEER. Treatment with a concentration of 10 mM of BUT for 24 hours significantly ($P<0.05$) counteracted the decrease of TEER induced with DSS. FITC data demonstrated that the treatment with concentrations of 0.1 mM and 10 mM of MIX for 1 hour significantly ($P<0.05$) reduced the epithelial permeability of HT29-MTX cells stressed with DSS. Finally, the concentration of 10% DSS for 24 hours significantly reduced MUC2 and MUC5AC gene expression, while treatment with 0.1 mM BUT and MIX for 24 hours significantly promoted MUC2 and MUC5AC gene expression ($P<0.05$). The present study demonstrates the suitability of E12 cells stressed with DSS as an inflammatory bowel diseases model to study the role of bioactive compounds in promoting intestinal health. SCFAs play an essential role in maintaining intestinal health by affecting epithelial integrity and mucus production.

Anti-protozoal efficacy of pine bark extracts against *Cryptosporidium parvum* in cell culture

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Cryptosporidium spp. is a common cause of severe diarrhoea in humans and animals worldwide. Treatment options are few and ineffective, and there is a need for alternatives. Previous research has shown anti-parasitic effects of condensed tannins (CT) against nematodes and protozoa. Objective: To assess the anti-cryptosporidial efficacy of CT-containing bark extract from Scots pine (*Pinus sylvestris*) against *Cryptosporidium parvum* by means of an *in vitro C. parvum* growth inhibition test. Methodology: Water, methanol, and acetone bark extracts were produced for the experiment. HCT-8 cells were seeded onto 96-well plates and incubated for 24 h, followed by inoculation with *C. parvum* oocysts. After 4 h, 100 µl of each bark extract was inoculated onto the cell monolayers (n=3) at concentrations of 50-300 µg extract/ml in 1% DMSO, and incubated for an additional 44 h. Each plate was set up in replicate, positive and negative controls included. DNA was extracted using QiaCube DNeasy® blood & tissue kit, and *C. parvum* DNA was quantified by qPCR-analysis. Results: The acetone and methanol extracts inhibited *C. parvum* growth in a dose-dependent manner. At the highest concentration, the effect was comparable to the anti-cryptosporidial effect of the positive control, paromomycin. The water extract had no significant effect on *C. parvum* growth. Conclusions: Acetone and methanol bark extracts of *P. sylvestris* showed anti-cryptosporidial properties in the highest concentrations tested. CT are less soluble in water than in the organic solvents, which may explain the lack of anti-cryptosporidial efficacy of the water extracts. Further studies are needed to establish if extracts of pine bark can be used *in vivo* to treat or prevent cryptosporidiosis.

Industrial by-products as alternative to cereals do not impair piglets gut microbiota

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An ongoing challenge is to find alternatives to conventional cereals to feed livestock animals. In this study, common cereal grains were partially replaced by bakery (FFP-B) and confectionary (FFP-C) by-products in post-weaning piglets' diets. The aim was to investigate the effects of these alternative ingredients on faecal microbial population in the post-weaning period. Thirty-six post-weaning piglets were housed for 21 days in individual pens and fed three different diets: a standard wheat-barley-corn meal diet, a diet containing 30% FFP-B and a diet containing 30% FFP-C, for a partial replacement of conventional cereals. The faecal microbiota was characterized by the next generation sequencing of the 16S rRNA gene. No significant differences in the abundance, biodiversity and phylogenetic diversity of microbial population have been observed between groups. Both the unweighted and weighted beta diversities were not affected by the experimental diets compared to the control. Different bacteria as potential biomarkers between the three groups have been identified. Compared to the control, the inclusion of FFP-B increased the amount of Proteobacteria phylum, while FFP-C increased the amount of Firmicutes phylum. Given the limited effects on the gut microbiota composition and diversity, FFP-B and FFP-C can be potentially used as alternatives to cereal grains in post-weaning piglets, but their effects on gut health need to be tested for a longer period and in different growth phases.

Silage type but not *Acacia mearnsii* supplementation influences rumen microbiota of dairy cows

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Microbiota is responsible for the degradation of crude protein (CP) in the rumen. Forage based diets often contain high CP levels, leading to high urinary N-losses and environmental problems. N-losses can be reduced through the reduction of protein degradation by supplementing tannins. However, the interaction between dietary CP, tannin supplementation and microbiota is not entirely understood. Therefore, this study investigated the effect of condensed tannins (CT) supplementation to different silage types on the rumen microbiota of dairy cows. Six lactating Holstein cows were blocked in a 6×6 Latin square according to milk yield (36.6±3.9 kg/d), lactation stage (70±13 d in milk), and body weight (649±47 kg) and assigned randomly to six diets. The diets were formulated (on dry matter basis) with 75% grass silage, 5% whole corn plants pellets, and 20% concentrate. The concentrate contained either a CT enriched extract from the bark of *Acacia mearnsii* or straw meal, counting for 2% of the total diet. The grass silages were either ryegrass-rich (R, low CP), clover-rich (C, high CP), or sainfoin (S, intermediate CP and rich in CT). Each of the six experimental periods lasted 21 d including a 14-d adaptation and a 7-d data collection period where feed intake was recorded daily and feed samples were pooled across each collection period. Ruminal fluid was collected twice per collection period using a stomach tube. Microbial DNA was extracted and amplicons of the 16S rRNA gene of bacteria and archaea were sequenced using Illumina. Multivariate statistics were done using PERMANOVA and ANOSIM. 3,172 OTUs of bacteria and 422 OTUs of archaea were obtained. Due to the different CP content, silage type affected CP intake considerably, which was greatest for C, intermediate for S and lowest for R ($P<0.001$). Silage type influenced bacteria and archaea community ($P<0.01$), while Acacia supplementation did not, suggesting that the strong effect of the basal diet may mask the influence of the tannin extract.

Modelling multiple scenarios of glyphosate speciation in rumen fluid of dairy cattle

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Glyphosate, the active ingredient in Roundup® agricultural herbicides, is used on crops that are commonly grown for animal feed. Even though there has not been empirical data to suggest that animal productivity and health are affected when glyphosate-based products are used as labelled, accusations based on hazard identification alone have circulated widely. One of these accusations originates by citing a patent claims chelation of minerals by related compounds, but does not include glyphosate. They hypothesized that glyphosate could chelate minerals in the gut and prevent absorption. Formation constants measure the strength of complexation between metal ions and ligands and can be used to model the speciation in mixtures of minerals, amino acids, volatile fatty acids (VFAs), and glyphosate as they exist in the rumen. The amine and carboxylic acid groups of all amino acids and functional groups such as the imidazole group of histidine are potential donor groups for binding minerals. Likewise, carboxylic acid groups from VFAs are prevalent sources for binding of minerals. Literature references of concentrations of minerals and amino acids were used along with a measurement of glyphosate, and speciation calculations were performed at three pH's commonly encountered in the rumen (5.8, 6.3 and 6.8). Different scenarios were used to predict realistic and worse-case scenarios for glyphosate residues on feed. Modelling for one case (pH=6.3) as an example, results in 55.1% of cobalt present as free Co2+, 28.6% bound to a VFA (acetate, propionate or butyrate), and 13.1% bound to histidine. The remaining 1.6% is distributed among Ala, Asp, Cys, Glu, Gly, Lys, Met, Ser, Thr, NH_3 and OH. Similar results will be presented for different minerals, and glyphosate concentrations. Data from these models demonstrate that glyphosate is a relatively weak chelator and they corroborate animal studies that indicate that label usage should not chelate minerals compared to other compounds in the rumen.

Session 09 Poster 13

In vitro study of the effect of riboflavin on hindgut microbial fermentation in pigs

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Dietary riboflavin (B2) is absorbed through the small intestine, but a beneficial outcome modulating gut microbiota has been recently demonstrated. The *in vitro* fermentation pattern and microbial community with 0, 25, 50, 75 and 100 mg B2/kg of a pepsin-pancreatin pre-incubated common substrate were studied. Five incubation runs with caecal inoculum from growing pigs were carried out, with 5 bottles/treatment. Gas production was measured every 2 h; volatile fatty acids (VFA), ammonia and B2 concentration were determined at 6 and 12 h, and microbial composition at 6 h. Gas production increased with T100 respect to T0 and T25, but no differences were recorded neither between T100 and T75 nor with T50 except at 4 h ($P<0.05$). The rate of gas production (ml/g OM per h) increased from 0 to 4 h for all treatments, and this and the linear increase in DM disappearance at 12 h ($P=0.006$) suggest an enhanced metabolic activity. No treatment differences were detected on ammonia and VFA, neither in absolute concentrations nor on molar proportions, but presence of B2 led to numerically higher proportions of propionate and butyrate compared with T0. Despite no differences were observed on final B2 concentration at 6 or 12 h, all treatments showed low values at 12 h, suggesting minimal bacterial B2 synthesis. Indexes of microbial diversity did not differ between treatments, although both linear ($P<0.001$) and quadratic ($P<0.001$) increasing trends were observed with B2 dose. A decrease ($P=0.043$) in the *Proteobacteria* to *Firmicutes* + *Bacteroidetes* ratio with B2 concentration was also observed, as well as a reduction of *Escherichia-Shigella* group at high B2 level (T50 and higher, $P=0.020$). *Faecalibacterium* spp. did not show differences between treatments, although relative abundance increased over time. In conclusion, results show that riboflavin addition of more than 75 mg/kg enhances hindgut microbial fermentation of pigs, probably through changes in the microbial structure and complexity.

Session 09 Poster 14

Low variability in gene expression of intestinal organoids and original tissue in pigs

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Organoids are an important *in vitro* model system that will help to understand underlying mechanisms of complex traits (like feed efficiency) and to be used as a phenotyping and screening tool in livestock research. The primary aim of this research was to investigate the within animal variability of produced intestinal organoids by gene expression. A secondary aim was to compare gene expression profiles of the produced organoids and the tissue of origin. The included intestinal segments were duodenum, ileum without Peyer's patches, ileum with Peyer's patches, and colon. One piglet was sacrificed, and three replicate tissue samples per intestinal segment were collected. One part of each tissue sample was put in RNA later for RNA analysis and an adjacent part of each tissue sample was used to produce organoids. RNA for RNAseq was isolated from the tissue and from derived organoids. RNAseq data was processed using a previous developed pipeline for our RNA-sequence work with organoids. Trimming of the RNA reads were performed in TrimGalore, alignment of the RNA reads were performed with STAR and quantification of the reads was performed with RSEM. Statistical analyses and visualization were performed within R. Distance plots with multidimensional scaling were generated to determine the sources of variation in the data. For this we have used the Transcripts per Million (TPM) values and the root mean square of the log2 Fold Change. Variation between the replicate organoids derived from samples within a specific intestinal segment was relatively low. Gene expression of the organoids was also compared with that of the tissue from the respective intestinal segment to identify cell-specific markers to characterize the organoids in comparison with the original tissue. The found low variability between biological replicates in this study shows that the organoids can be produced from intestinal segments in a reproducible fashion. A next step will be to investigate the variability within an intestinal segment, i.e. proximal vs distal tissue. This research will help us to apply the *in vitro* organoid model system in livestock research.

Session 10 Theatre 1

The level of hemicelluloses in lactating sow diets affects milk composition

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The inclusion of dietary fibre in sow diets, including the hemicelluloses (HC), has been shown to increase bacterial fermentation and ultimately enhances the production of volatile fatty acids (VFA) in the large intestine. Those VFA are absorbed and may be directly transferred to the milk or may serve as an energy source for milk synthesis in the udder. As VFA, especially butyrate, plays an important role for piglet intestinal health, increasing their level in sow milk could be beneficial for piglet development. This study aimed to evaluate the effect of different contents of HC during late gestation and lactation on milk quality and on offspring development. Four diets with decreasing levels of HC were formulated: T12% (HC: 120 g/kg), T11% (HC: 108 g/kg), T9% (HC: 86 g/kg) and T7% (HC: 72 g/kg). Sows ($n=40$) had access to one of the four diets from 110 days (d) of gestation to weaning (26±0.4 d post-farrowing). Milk was collected at d 3 and 17 of lactation. At birth, piglets were split into 2 groups according to their birthweight (BtW): Normal ($1.20<\text{BtW}\leq 2.47$ kg) or Low (0.80 $\text{kg}\leq\text{BtW}\leq 1.20$ kg). Piglet body weight was recorded at birth, 5 and 16 d after birth, at weaning and then 7 and 14 d post-weaning. Piglet performances, VFA profile and gross chemical composition of milk were analysed with an ANOVA followed by a Tukey test to evaluate mean differences between experimental groups. The T7% diet positively affected post-weaning growth of Low BtW piglets, resulting in 1.9 kg heavier body weights at d 14 post weaning compared to the three other diets. Furthermore, the T7% diet increased butyrate proportion ($P<0.05$) and total VFA concentration ($P<0.10$) in milk and decreased lactose content ($P<0.05$) compared to the T12% diet. The T11% diet increased the energy milk content ($P=0.05$) and tended to increase the fat content ($P<0.10$) compared to the T12% diet. In conclusion, the present findings highlighted the importance of dietary fibres in the maternal diet to modify milk quality and piglet development.

Session 10 Theatre 2

Sows diet enriched with BCAA and/or Arg lead to an improvement of sow' and piglet' performance

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This study aimed to evaluate if the dietary supply of branched-chain amino acids (BCAA; L-leucine, L-isoleucine and L-valine) and/or L-Arginine (Arg) to lactating sows enhanced litter performance pre- and post-weaning. 68 sows were divided into 4 groups according to parity and body weight (BW): (1) control (CO) (fed a corn lactating based diet); (2) CO + 22.5 g/d/sow of BCAA; (3) CO + 22.5 g/d/sow of Arg; (4) CO + 45.0 g/d/sow of BCAA and Arg. Amino acids (AA) were supplied on top. Diets were fed to the sows from 4 days before farrowing (d-4) until weaning (d27). Piglets were weighed at d0 (farrowing), d7, d14, d27, d34 and d41. Health status was followed during the study. Blood was sampled at d-4, d10 and d27 from sows (8 sows/group) and at d10 and d27 from their piglets (16 piglets/group) for haematological analyses, urea, glucose, insulin, prolactin and immunoglobulins (Igs) concentrations. Colostrum and milk were sampled at farrowing and at d10 and d20 and analysed for polyamines and Igs. A 2×2 model using GLM or GENMOD procedure including batch, parity, BCAA and Arg supply and their interaction as factors was applied. Both BCAA and Arg supply increased glucose and prolactin ($P<0.05$) in sows' serum at d27 and BCAA+Arg increased the spermine in milk at d27 ($P=0.048$). Arg increased IgM in sow's serum at d10 ($P=0.05$) and tended to increase it in colostrum ($P=0.08$). BCAA tended to increase IgA and IgM in colostrum ($P=0.06$), increased the IgA ($P=0.004$) in milk at d20 and tended to increase lymphocyte % in sows' blood at d27 ($P=0.07$). Arg supplementation increased the monocyte % in piglets' blood at d27 ($P=0.025$) and reduced piglets' mortality pre- (d7, d14) and post-weaning (d41) ($P<0.05$). At d41, piglets born from the 3 groups of sows supplemented with AA were heavier than piglets born from CO sows ($P=0.03$). The administration of BCAA affected the sows' blood and milk immunological profile, while Arg increased the sows' IgM in serum and colostrum, improving the piglets' survival rate and immune competence.

Differences in enzyme activity between piglets fed dry feed or liquid feed prior to weaning

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Increased creep feed intake before weaning is associated with promoting a more mature gastro-intestinal tract (GIT) and less post-weaning diarrhoea (PWD). However, up to 70% of piglets fail to consume sufficient amounts of creep feed prior to weaning. Interestingly, it has previously been suggested that liquid feed leads to increased dry matter (DM) intake prior to weaning. Therefore, the effect of two dietary treatments (liquid and dry) offered during the suckling period on feed disappearance and intestine enzymatic development at weaning, and PWD treatments after weaning was investigated. Three hundred forty-seven piglets distributed between 29 litters were allocated to one of two dietary treatments: (1) dry- (DCF, 14 litters); or (2) liquid (LCF, 15 litters) creep feed, offered from day 10-24 postpartum for 9 hours a day. Forty piglets (20 DCF and 20 LCF) were euthanized before weaning, and the small intestine was sampled to examine the effect on activity of selected disaccharidases (sucrase, maltase and lactase). The remaining pigs were on average weaned at 28 days of age and fed a standard dry starter diet. Feeding a liquid diet pre-weaning increased ($P<0.001$) the DM disappearance day 10-18 compared with dry diet (20.07 vs 5.43 g). Despite the lower feed disappearance, the DCF pigs displayed greater ($P=0.024$) average daily gain (ADG) (200 vs 176 g/day) pre-weaning, and a greater ($P<0.001$) BW post weaning at day 61 (21.6 vs 19.7 kg). The activity of lactase, maltase and sucrase in the proximal part of the small intestine were greatest ($P<0.001$) in the DCF pigs (~25, 16 and 7 vs ~14, 11 and 3.5 U/g). In conclusion, in this on-farm setup, liquid feeding increased the feed disappearance in suckling piglets from day 10-18. The DCF pigs displayed a greater enzyme activity in the proximal part of the small intestine and a higher weight gain, suggesting more mucosal maturation and adaptation to a vegetable-based diet. Further research is warranted to investigate effect of pre-weaning dietary strategies on degree of digestibility of nutrients and fate of indigested nutrients in the hindgut in relation to PWD.

Session 10 Theatre 4

Understanding piglets' intestinal microbiome changes caused by zinc oxide and apramycin

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In-feed antibiotics (ABs) and therapeutic zinc oxide (ZnO) have been widely used to prevent post-weaning diarrhoea (PWD) and control *Escherichia coli* infection. Previous studies have shown that ABs and ZnO treatments affect piglets gut microbiota composition. In this study, we used shotgun metagenomic sequencing to monitor the changes in faecal microbiota composition of weaned piglets linked to the use of these antimicrobials. Faecal samples were collected across three different trials. Animals used in each trial (268, 180 and 342 in trials 1, 2 and 3 respectively) were distributed to 3 treatments: Control diet (Ct); Ct + 3,000 ppm of ZnO (Zn), and Ct + Apramycin (Ab). Trial 2 and 3 also included the study of room cleaning procedures: power washing and disinfection (A), and pre-soaking, power washing, detergent application, disinfection and drying (B) for trial 2; and No washing (C), Pre-soaking and power washing (D), and D + disinfection (F) for trial 3. Sequenced microbiomes revealed differences in richness, diversity, and abundance patterns, mainly amongst dietary treatments but also between cleanings A and B, revealing the clustering of the samples in 4 groups defined by the dominance of families as *Enterobacteriaceae* (Ct); *Lactobacillaceae* and *Veilonellaceae* (mainly Ct); and *Bacteroidaceae*, *Ruminococcaceae*, *Acidaminococcaceae* and *Lactobacillaceae* (Ab and ZnO groups). Cleaning procedures showed no differences on microbiota composition besides of those found between A and B. The observed differences between treatment groups microbiomes address the effectivity of these two antimicrobials against *Escherichia coli*, as well as their impact in gut microbiota composition.

Blood serum metabolites and faecal VFA as tools to detect unbalanced diets in grower-finisher pigs

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The present study aimed to assess the performance, serum metabolites (SM) and faecal volatile fatty acids (VFA) on grower-finisher (GF) pigs fed unbalanced diets. Two batches (B) of pigs were used at the GF stage. B1 was weighed per pen (n=20; 11 pigs/pen; 50.1±3.44 kg) at 13 wks of age. B2 was weighed per pen (n=20; 11 pigs/pen; 87.0±4.10 kg) at 18 wks of age. Both B1 and B2, pens were assigned based on BW to 5 dietary treatments (T) and were followed for 10 d. Diets formulated were: control (C; 10.03 MJ/kg NE; 16.0% CP), low protein (LP; 10.03 MJ/kg NE; 13.2% CP), high protein (HP; 10.03 MJ/kg NE; 18.8% CP), low energy (LE; 9.61 MJ/kg NE; 16.0% CP) and high energy (HE; 10.45 MJ/kg NE; 16.0% CP). Both B1 and B2, BW and FI were recorded per pen at the end of the trial and ADG, ADFI and FCR were calculated. In B2, blood and faeces samples (n=2/pen) were collected at the end of the trial to obtain the SM and VFA profile. Data were analysed using GLM. In B1, HP pigs were 2.1 kg heavier and gained 209.9 g/d more than LP pigs ($P<0.05$). FCR was higher for LP pigs (2.31±0.05) versus the other T (1.86±0.05; $P<0.001$). In B2, there was no difference in BW, ADG, ADFI and FCR between T ($P>0.05$). In the SM profile, albumin and glucose did not differ between T ($P>0.05$). HE pigs had higher triglycerides (0.48±0.04 mmol/l) and creatinine (133.8±3.97 µmol/l) than LE pigs (0.28±0.04; 117.2±3.97; $P<0.05$); however, HE pigs had lower total protein (60.9±1.51 g/l) than C pigs (67.4±1.51; $P<0.05$). LP pigs had higher cholesterol (2.12±0.10 mmol/l) than C and LE pigs (2.07±0.10; $P<0.01$); while HP pigs had higher BUN (13.6±0.95 mg/dl) than the other T (7.5±0.95; $P<0.001$). VFA (mmol/kg) did not differ between T ($P>0.05$), but C pigs had higher branched chain fatty acids (11.9±0.93) than LP and LE pigs (7.7±0.93; $P<0.05$). Dietary T did not have an effect at late GF stage although T differed in the SM and VFA profile. SM and VFA analysis may be a fruitful area for further work to detect unbalance diets in GF pigs.

Can genotyping and breeding value estimation for hens increase efficiency of layer breeding?

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Optimization of layer breeding programs often focuses on breeding value estimation (BVE) and selection of the cocks. In this work, the effects of different selection techniques on the hen side on the response to selection were analysed with a simulation study conducted with the R-package MoBPS. We considered one line of a traditional layer breeding program accounting for nine traits that were only available for hens. Selecting males and females using pedigree based best linear unbiased prediction (BLUP) breeding values (BV) led to an increase of 21.6% in BV after 10 generations compared to phenotypic selection for hens. With only males being genotyped and male selection candidates being selected based on single-step genomic BLUP (GBLUP), while females were selected based on plain phenotypes, genomic selection has not shown a substantial benefit compared to pedigree BLUP. However, selecting the hens phenotypically, but genotyping also all female selection candidates led to an increase of 20.9% in total BV compared to only genotyping males. When also performing GBLUP for the selection of the hens the BVs increased further (+38.7%) while the ratio of increase in BV per increase in inbreeding level decreased. An additional opportunity when using BVs from GBLUP is the possibility of selecting individuals before phenotypes of the current generation are available and thus to reduce the generation interval. When performing early GBLUP for males and females, halving the generation interval and consequently just including the phenotypes of the previous generations, the total BV became even higher (+35.3%) despite 23.9% lower accuracies of BVE, but the ratio of increase in BV to increase in inbreeding became less favourable. When halving the generation interval and selecting the hens due to pedigree BLUP the lowest accuracy of BVE of all scenarios, as well as the worst ratio of BV to inbreeding was observed. The simulations demonstrated the benefit of genotyping hens and selecting them using GBLUP. Halving the generation interval further increases the genetic gain but has some price in an increased margin of inbreeding per unit of genetic gain.

Genetic study of social interaction models for the predictability of overall biting in gilts

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Pig biting is a trait with important economic and welfare implications. Overall biting can be defined as a combination of tail, ear, and vulva biting, which are known to be a result of animal social interaction. Although social interaction is usually taken as a fully environmental factor, it can be also associated to animal genetics. We aimed to evaluate genetic parameters and predictability of genetic models accounting or not for heritable social effects on the evaluation of overall biting in gilts. Information on the overall biting score (0 or 1) of 36,920 F1 growing gilts was evaluated with three models: (1) an animal model without any social effects; (2) an animal model extended with environmental social effect; and (3) an animal model extended with environmental and heritable social effects. Predictability was calculated as the correlation of EBV (models 1 and 2) or total EBV (TBV) (model 3) of young sires with the raw phenotypes of their progeny, and prediction accuracy was calculated using the Linear Regression (LR) method. Heritabilities for models 1 and 2 were low at 0.04, whereas the total heritable variance in model 3 was about 3-fold greater (0.14). This result indicates an important genetic influence on the social behaviour of gilts and underlines the possibility of exploiting an extra layer of genetic variance in the genetic selection against overall biting. The correlation of direct and social effects was close to zero (-0.07), suggesting no antagonist behaviour over the selection for direct or social genetic effects. Thus, the direct selection of animals that are genetically less likely to be bitten will not increase the number of genetically potential biters in the next generations. The results of predictability showed a significant advantage of model 3 (0.22) over models 1 (0.17) and 2 (0.10). Prediction accuracy was 0.25, 0.23, and 0.32 for models 1,2, and 3, respectively. Overall biting is significantly influenced by the heritable social effect, and its proper modelling might promote an increase in the genetic gain by selection.

Influence of sex on endogenous antioxidant defences in broiler chickens, a new perspective?

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Oxidative stress is sometimes considered to be the leading cause of losses in livestock farming. Mixed-sex or all-male are the main model used for the study of the antioxidant levels in broilers. Since glutathione peroxidase (GPx) is one of the most commonly endogenous antioxidant defences studied and plasma is the main biological fluid used to monitor the antioxidant defences in animals, the aim of the present study was to compare the GPx levels in all-male and all-female broiler chickens' cohorts raised contemporarily. Two trials were set up in an experimental farm in France, one in summer time and one in the autumn. In both trials, 2 groups of 600 broilers (day-old) were divided in 20 repeats each of 30 males or females in the same building. Birds were fed the same diet during both trials and blood samples were drawn on 80 males and 80 females (4 birds/pen) on the beginning of the finishing period (D21) in both groups in trial 1 and 40 individual per group in trial 2 (2 birds/pen). A further sampling occurred in trial 2 on 40 individually tagged females or males (2 birds/pen) on the day of slaughter (D35 and D43 respectively) to allow for the study of the individual evolution of GPx and glutathione (GSH) levels in time. Whole blood GPx content was analysed in both trials as well as GSH in the second trial. Statistical analysis was performed using ANOVA. Results showed a significant difference in the circulating GPx level between sexes on D21 in both trials, with females presenting significantly higher circulating levels of GPx than males ($P<0,01$ for trial 1 and $P<0.001$ for trial 2). GSH levels were however significantly higher in males on D21 ($P=0.01$). Trial 2 also showed a significant increase of the circulating GPx level in both sexes in time, with a stronger daily increase in females ($P<0.01$). The opposite was however observed for circulating GSH ($P<0.001$). Whilst the increase of the circulating levels of GPx in time has been shown in previous studies in broilers, the sexual difference of its level is original and illustrates the need to take this parameter into consideration in trial designs and broilers' nutritional strategy.

Dietary protein and ractopamine effects on boar taint and testes parameters of immunocastrated pigs

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The effects of immunocastration, dietary protein level (low, medium or high) and ractopamine hydrochloride supplementation (0 or 10 mg/kg) on the adipose concentrations of androstenone, skatole and indole, and testes functioning, were investigated in 120 male PIC© pigs. Animals were randomly allocated to the treatments at 16 weeks of age. Sixty pigs received 2 ml Improvac® at 16 and 20 weeks of age. Up until 20 weeks of age, all pigs were fed a commercial grower diet, after which they were fed one of the six experimental finisher diets. Live weight and backfat depth were measured at slaughter, as well as testes weight, size, and cut surface CIE colour. For a sub-sample of pigs, 100 seminiferous tubules were measured for their circumference and epithelium thickness. Subcutaneous backfat samples were also taken and analysed for androstenone, skatole and indole, using ultra-performance liquid chromatography with fluorescence. The correlations between these parameters were explored to ascertain whether they may be indicators of boar taint. Immunocastration decreased testes weight and length, seminiferous tubule circumference and epithelium thickness. Also, testes from immunocastrated pigs were paler, and less red in colour, indicating decreased activity. Immunocastration decreased androstenone and skatole concentrations but dietary factors had no influence on the parameters measured. The testes parameters were strong indicators of androstenone and skatole concentrations in the carcasses, while body weight and carcass fatness were weak indicators. Immunocastration together with the adjustment of dietary protein and ractopamine hydrochloride supplementation, successfully prevented boar taint while maintaining performance.

Effect of straw distribution method on pig behaviour

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Barren environments are known to reduce animal welfare through e.g. inducing abnormal behaviours such as tail biting and inactivity in pigs. Whereas tail biting has received a lot of attention, inactiveness has been assumed to be positive for production and, therefore, not been prioritized. Provision of straw reduces tail biting and possibly inactiveness. However, provision of straw solely on the floor may only supply inadequate rations for the pigs' explorative needs. The aim of this study was to investigate the effect of method of straw provision on pig behaviour and straw availability. The study was conducted within a Swedish commercial finishing pig unit holding 459 undocked pigs in 42 pens from 30~115 kg (rearing period of 14 weeks). Half of the pens were provided with long straw through racks (44l) (R) and the other half had only straw (25l) on the floor (F). Ten randomly assigned pens (5R, 5F) were scan sampled for activity (standing/sitting/lying) and exploring activities (fittings/straw floor/straw rack/other/nothing) during 24 h three periods during the study. The behaviour were analysed through SAS (proc mixed) model included effects of treatment, time of day, weekday, week, straw and light and the interactions treatment×period and treatment×straw. Pig (within pen) was considered a random effect, and consideration was taken of repeated observations within pig. Preliminary results showed that R pigs were more active than F pigs, in period 2 and 3. During period 1, R pigs did not interact with the straw racks which influenced the straw accessibility and hence also the behaviour. In general, R pigs were more active at the same amount of straw on the floor. Floor straw availability was higher in F during period 1 and 3. However, if including straw in the rack in the straw availability measure R had higher straw access throughout the study. Treatment did not have an effect on straw manipulation. Straw racks may be a way of increasing straw availability and activity levels in pigs, however more research is needed to understand how to design straw racks to promote use and hopefully also explorative behaviours.

Session 10 Theatre 11

Effect of lameness on feed intake and growth performance of grow-finisher pigs

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The health and welfare (HW) of pigs can affect feed intake behaviour and performance. The aim of this study was to determine associations between HW and performance of grow-finisher pigs. Sixty pigs born within one week were moved to the finisher accommodation at 11 weeks of age. Pigs were housed in 5 mixed sex pens (n=12 pigs/pen; 0.95 m^2/pig) with fully slatted plastic floor and individual feeding stations. Pigs were weighed at 11 (23.9±4.92 kg) and 22 (74.6±10.61 kg) weeks of age. Average daily gain, average daily feed intake and feed conversation ratio were calculated. HW status of each individual pig was assessed weekly from 14 to 22 weeks of age. Following the Welfare Quality® criteria, pigs were checked for tail, ear and body lesions, lameness, external abscesses, coughing, sneezing, hernias, and number of antibiotic treatments. Performance data were analysed using GLM. The prevalence of ear lesions decreased from 25.4% (14 weeks) to 0% (19 weeks). Prevalence of body lesions increased from 15.7% (14 weeks) to 42.4% (22 weeks). Prevalence of external abscesses increased from 3.4% (14 weeks) to 20.4% (19 weeks), while lameness increased from 0% (14 weeks) to 33.9% (22 weeks). Tail lesions, coughing, sneezing, hernias and injections were not prevalent (<15% of pigs affected). External abscesses and body lesions were not related to growth performance. However, lame pigs (1,492.8±247.54 g) consumed 223.7 g less feed per day than non-lame pigs (1,715.9±264.43 g; vs<0.01). Body weight and average daily gain were not significantly different between lame and non-lame pigs (vs>0.05) although lame pigs (72.5±9.71 kg) weighed 3.2 kg less and gained 40.3 g less per day than non-lame pigs (75.7±11.16 kg). Nevertheless, feed conversion ratio was 0.2 lower for lame pigs (2.35±0.24) versus non-lame pigs (2.57±0.39; vs<0.05) probably due to close-to-*ad libitum* conditions. Lameness affects feed intake and reduces growth in grow-finisher pigs. Thus, early treatment of lame pigs is key to reduce productive losses and improve animal welfare.

Session 10 Theatre 12

Applying metabolomics for revealing novel biomarkers and metabolic fingerprints of welfare in pigs

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Welfare of animals in the industry is a major increasing concern around the globe. In spite of the great need, there is a huge gap of knowledge in objective measures to assess welfare, stress and resilience. Metabolomics is the newest 'omic', following the eras of genomics and proteomics, a relatively new approach for the discovery of specific metabolic fingerprints of given conditions. It allows to analyse simultaneously a high number of metabolites, which their concentrations may change when the body is out of homeostasis. The objective of this research was to reveal novel biomarkers for welfare and stress and their altered metabolic pathways, using untargeted metabolomics in pig saliva. Saliva was collected from 200 pigs at a group level from each pen of 5-10 pigs, at two time points; first sampling one day before slaughter, at their regular pen (familiar environment), and second sampling after 24 hours later, under well-known stressful conditions for pigs (after transport to the slaughterhouse, regrouping, and overnight at the new pen with the unfamiliar new pen-mates). Metabolites were extracted and analysed using ultra high performance liquid chromatography coupled to a high resolution mass spectrometer. Overall, a total of 2,133 metabolites were observed, of which 846 were significantly different under the stressful conditions (P<0.05). 216 metabolites were upregulated and 630 metabolites were downregulated. Furthermore, several metabolic pathways found to be alternated after 24 hours in stressful conditions for the pigs. These results highlighted that performing metabolomics analyses is a promising direction in the future research of animal welfare in its broadest definition.

Session 10 Theatre 13

An economic and environmental optimization model for pig-fattening units using a carbon tax

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Economic and environmental sustainability is a major concern for pig production systems (PPS). Previous studies showed that formulating low-impact diets using a carbon tax could decrease the climate change (CC) impact of pig-fattening units. However, they did not consider the effect of interactions between feed formulas, feeding and shipping strategies (FFSS) on the environmental impacts of pig production. Consequently, the objective of this study was to investigate effects of a carbon tax on economically optimized FFSS and the resulting economic and environmental performances. We used a bi-level optimization model in which the upper level represents a bioeconomic model that simulates the growth of a batch of pigs and optimizes both the amino acid contents in growing and finishing feeds, the level of feed supply, and the shipping strategy. The lower level represents a linear least-cost feed formulation. The model's behaviour was investigated in four contexts of recent feed and pork prices (low price: L and high price: H; feed: F and pork: P) at different carbon tax level. Optimized FFSS were highly sensitive to both the economic context and the carbon tax level. Without carbon tax, CC impact was lower in LF-LP than in the other economic contexts. With HF, the optimal amino acid contents and feed supply decreased as tax level increased. With LF, the optimal amino acid contents in the finishing diet increased as tax level increased, to improve feed conversion ratio. With increasing tax level, peas and cereal by-products were replaced with cereals and oil meals in pig diets. The highest potential of CC mitigation was obtained with HF-LP context, whereas LF-LP context had the lowest potential of CC mitigation because it resulted in low CC impact, even without application of a carbon tax. Optimizing both levels (FFSS) while applying a carbon tax decreased CC by up to 43% and saved up to 26% of income compared to FFSS optimized without carbon tax. This model provides a valuable tool to investigate the adaptation potential of PPS to the application of a carbon tax.

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Attempts to assess the onset of sexual maturity in finishing boars

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Male piglets are castrated to ensure suitability for fattening and to avoid boar taint. Surgical castration without anaesthesia has been recently banned in Germany. Immunocastration and fattening of entire male pigs are alternatives to surgical castration, both involving drawbacks. Main disadvantages of finishing boars are the occurrence of unpleasant odour of the meat as well as behavioural problems affecting animal welfare. Boar taint and changes in the behaviour of entire male pigs are related to sexual maturity. Determining the onset of sexual maturation is difficult in live animals. It is not known, what proportion of entire male finishers actually reach sexual maturity until slaughter. The objective of this study was to evaluate characteristics related to sexual maturation to find parameters that correlate with the onset of sexual maturity in finishing boars. We analysed 2,961 entire male animals from different breeds on boar taint compounds, performance data and measures of testes and bulbourethral glands. Furthermore, we examined 47 entire males of the Piétrain breed × (Large White × German Landrace) during fattening and after slaughter on testosterone levels, preputial flushings, performance data, boar taint compounds, measures of testes and bulbourethral glands as well as histology of testes and epididymis. Testicular volume corrected for weight at slaughter was the most variable characteristic within both groups. Only two out of 47 boars didn't seem to have reached sexual maturity, showing predominantly spermatogonia and rarely spermatocytes and spermatids in the tubuli semeniferi and no spermatozoa in the epididymis.

A blend of active compounds (Fresh Up) to mitigate heat stress in lactating sows

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Heat stress, caused by high temperatures, can negatively influence the performance of lactating sows. The first consequence of heat stress is the reduction of feed intake in order to limit the production of metabolic heat due to digestion. As a consequence, milk production can be impaired and be detrimental to piglets' growth. For this purpose, a trial was designed to evaluate a blend of plant extracts, micro and macro minerals and flavouring agents (Fresh Up, Wisium) targeting inflammation, gut permeability, acid-base homeostasis and nutrient absorption in lactating sows in a commercial farm in Brazil during the hot season. In total, 80 sows (242.9 kg BW on average, mean parity: 1.8) housed in individual farrowing crate were assigned to the experiment. Sows were divided into two groups from parturition to weaning at 26 d: control group (CON) fed a conventional lactating diet with placebo at 5 kg/T (white phyllite) and experimental group (FRESH UP) fed the same lactating diet but supplemented with the blend of active compounds at 5 kg/T (Fresh Up, Wisium). Sows were housed in air-conditioned barns in order to maintain the inside temperature between 27 and 30 °C. Sows were fed *ad libitum* all over the trial. There was no significant difference in feed intake in lactation, but sows in the FRESH UP group had a significantly lower body weight loss compared to the CON sows (29.4 kg and 41.2 kg respectively, $P=0.01$), indicating a possible better metabolic response to heat stress. Litter size and litter weight at weaning did not differ significantly between the two groups, though piglet mortality in the FRESH UP group was significantly lower compared to the CON group (5.3 and 12.6% respectively, $P<0.0001$). This was due to different litter size at birth. Then, one might assume that litter size and litter weight at weaning would have been improved in the FRESH UP group if litter size at birth was comparable between the two groups. To conclude, a holistic approach dealing with the different damages induced by heat stress seems beneficial to reduce body weight loss in lactating sows and mortality in suckling piglets.

Genetic analysis of protein efficiency in Swiss Large White pigs

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Protein is an important nutrient in livestock feed needed primarily for growth, body repair and immune function. However, a non-negligible amount of the ingested protein in pigs is excreted, thereby contributing to environmental pollution. Thus, it is important to improve protein efficiency (PE) in pigs through selective breeding. The aim of this study was therefore to estimate the heritability of PE in Swiss Large White pigs and correlations with production traits. So far, 541 pigs from an ongoing experiment were used to estimate the heritability of PE and 276 pigs were used to estimate the genetic correlations. Pigs were slaughtered at 100 kg body weight and protein content of the carcass was calculated from lean mass measured with a Dual-Energy X-Ray Absorptiometry scanner. PE was calculated as the ratio of protein in the carcass to total dietary protein consumed. The production traits taken were average daily gain (ADG), feed conversion ratio (FCR), and average daily feed intake (ADFI). The genetic and environmental variance components of PE were estimated using a univariate animal model (corrected for the effects of sex, slaughter weight, slaughter age, and series) and genetic correlations were estimated using bivariate models with a Markov Chain Monte Carlo algorithm. The average PE ± SD was 0.38±0.02. The heritability (95% credible interval) and litter effect for PE was 0.40 (0.24, 0.59) and 0.21 (0.15, 0.34), respectively. PE had a significant genetic correlations (95% credible interval) with ADG (-0.58 (-0.674, -0.478)), FCR (-0.91 (-0.94, -0.86)) and ADFI (-0.93 (-0.95, -0.91)). The heritability of PE clearly indicates the potential for selective breeding for PE, and the litter effect shows that pigs in the same litter respond similarly compared to others in different litters. Furthermore, PE has a favourable relationship with ADFI and FCR. In future, we aim to identify SNPs associated with PE and investigate its genetic correlations with meat quality traits.

Enteric methane mitigation in dairy cattle via feeding strategies

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On a global level, cattle (beef and dairy) generate 65% of the total livestock greenhouse gas emissions, mainly due to the emission of enteric methane (CH_4). The enteric CH_4 production from ruminants is mainly driven by the feed composition and by the level of feed intake, and thus feeding strategies are considered most promising for CH_4 mitigation. Examples are the use of feed additives, dietary lipids, increasing starch content, reducing fibre content, etc. This talk will discuss recent successes and challenges of CH_4 mitigating feeding strategies for dairy cows. The following questions will be discussed: Is the reduction of enteric CH_4 through 3-NOP supplementation dependent on its dose, its mode of application and the dietary NDF content?; Is a maize silage-based diet always advantageous in terms of CH_4 emissions from dairy cattle as compared with a grass-silage based diet?; Does a compound feed with extruded linseed and linseed oil successfully reduce enteric CH_4 in a maize as well as in a grass silage-based diet?; Will replacement of soybean meal by rapeseed meal or by the combination of brewers' grains and rapeseed meal result in CH_4 reduction?; Can daily grazing decrease the enteric CH_4 emissions?; What is the effect of these feeding strategies on milk production?; How about the implementation in practice?; And how well do we know the working mechanism of each of the promising feeding strategies? Unravelling these working mechanisms can possibly offer perspectives to enlarge the set of CH_4 mitigating feeding strategies. Finally, the importance of the production of feeds for (dairy) cattle will be touched upon briefly, as it is the largest or second largest source of greenhouse gas emissions from the dairy sector.

Effect of feed strategies on the carbon footprint and economic variables of cow-calf systems

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A simulation model was developed to evaluate the impact of the incorporation of feed strategies on economic variables and the carbon footprint (CF) in a traditional cow-calf system. The use of creep feeding was one of the feeding strategies used. The supplement used corresponded to corn silage with quantities of 0.5 to 1 kg/animal/day. The incorporation into the corn silage of two feed additives: monensin with a dose of 30 mg/kg dry matter (DM) and canola oil with a treatment of 46 g/kg DM was also considered, using reductions in enteric methane emissions of 12 and 21% respectively. The simulation baseline scenario was the traditional cow-calf system in the Southern zone of Chile. The CF was defined as kg of CO_2-eq/kg of live weight (LW). As an economic variable, the average total cost, the average operational cost, and total and operational incomes were used. The model considers the possibility of incorporating randomness to the mortality and voluntary forage intake variables. All the experimental scenarios were analysed with and without consideration of carbon sequestration in rangelands. The results were evaluated using one-way and multifactorial ANOVA. The average CF was 22 kg of CO_2-eq/kg of LW, while the average unit total cost and average unit operational costs were US$ 1.2/kg of LW and US$ 1/kg of LW, respectively. The supplementation had an effect on the CF, average unit operational cost, and average unit total cost ($P<0.01$). The use of additive and the consideration of carbon sequestration affected the CF ($P<0.01$). CF decreased as the amount of supplement increased due to the significant increase in the calf's selling weight ($P<0.01$), but with an increase in the average unit costs. CF reduction resulting from the addition of corn silage additives was 3 and 1% for canola oil and monensin, respectively. The model revealed that the supplementation and use of additives would reduce the CF of the cow-calf system studied, with an increase in average unit costs. However, due to the higher production (kg LW sold), the effect of average costs on the total and operational incomes of the rise in average unit costs only becomes significant when the supplemented is above 0.5 kg/animal/day.

The effect of type of forage on the methane emission and performance of dairy cows: a meta-analysis

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Globally, enteric CH_4 from ruminants represents 6% of total GHG emissions of anthropic origin. Dietary manipulation is considered the most direct and effective strategy to mitigate CH_4 emissions. Forages are important feedstuffs for ruminants, so the present study aims to evaluate the effects of the different types of forage included in TMR on enteric CH_4 emission and milk production of lactating cows by meta-analysis. The forage types included in this analysis were corn silage (CS), alfalfa silage (AS), grass silage (GS), and green forage (GF). A total of 64 *in vivo* studies without the use of feed additives or lipid supplementation in lactating dairy cows were included in the final database for statistical analysis. Mixed model was applied with SAS, weighting the observation by the inverse of pooled SEM squared, and the 'study' was considered a random effect. Methane production was higher in the cows with the silage-based diets (CS, AS, and GS) (averaging 437 g/d) than that fed GF diet (334 g/d, $P<0.01$). However, GF resulted in lower milk production than the silage-based diets (21.2 kg/d vs 31.0 of CS, 30.8 of AS, and 26.7 of GS; $P<0.05$), but higher CH_4 per kg of milk (18.0 g/kg, $P<0.01$), except for the GS diet (15.9 g/kg milk). Among silage-based diets, the GS diet did not differ from AS (14.9 g/kg milk). The CS diet resulted in the lowest CH_4 emission (14.1 g/kg milk) but not different than AS (14.9 g/kg milk). Dry matter intake was higher for CS and AS (22.4 kg/d, on average) than for GS and GF (18.1 kg/d on average, $P<0.05$). Except for GS diet, dairy efficiency (kg of milk/DMI) was negatively correlated with CH_4/kg of milk for all the forage types ($P<0.01$). Dietary starch was negatively correlated with CH_4/DMI for CS (slope=-0.19; P=0.03) and GF (slope=- 0.22; P=0.02). The present study underlined the potential of the forage basis, dairy efficiency and dietary chemical composition in affecting CH_4 emission and lactation performance. However, more data are needed for better prediction models, especially for forages like alfalfa hay or winter cereals widely used in the European region.

Tannins and essential oils as dietary ingredients to mitigate rumen methane and ammonia production

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In the last years, many studies have focused on feeding strategies targeting the reduction of the environmental impact of livestock. The mitigating effects on rumen methane and ammonia formation of individual tannins and essential oil compounds (EOC) were previously described. However, the presence or absence of a synergic effect when simultaneously using tannins and EOC was rarely tested. This study aimed to select the best mixtures of hydrolysable or condensed tannins with different EOC blends. In six runs 50 treatments were tested with the Hohenheim gas test apparatus (n=6 per treatment). In every run, control diet, pure tannins, pure EOC, and mixtures were included as well. The mixtures or the pure compounds were added, together with 4% of extruded linseed, to a control diet with a forage: concentrate ratio of 70:30. The mixtures were prepared by combining 2% of tannins (quebracho or chestnut extract or a mixture of them) with 1.5% of an EOC blend. A total of twelve EOC blends were formulated by combining 0.5% of carvacrol/oregano oil, 0.5% thymol/thyme oil, and 0.5% of eugenol, clove oil, limonene, α-pinene or bornyl acetate. Each treatment was evaluated for methane and ammonia formation, *in vitro* organic matter digestibility and volatile fatty acids (VFA) production after 24 h of *in vitro* fermentation. All mixtures reduced ammonia production by up to 31%. The depressive effect on ammonia was sometimes related to a reduction of total VFA (from 2 to 7%). Some mixtures showed a significant reduction of methane yield up to 15%, while the additives alone did not influence either methane or ammonia production. In conclusion, three out of thirty-six mixtures are promising for concomitant mitigation of both methane and ammonia, mainly without interfering with feeding values. *In vivo* trials are needed to confirm the reduction potentials of these mixtures.

In vitro rumen total gas and methane formation from Northern hemisphere macroalgae

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The effects of 18 Northern hemisphere macroalgae on *in vitro* feed degradability and associated methane formation were determined using the Ankom^{RF} system. Furthermore, effects of ensiling method of *Saccharina latissima* on anti-methanogenic activity were also investigated. After harvest, *S. latissima* was either frozen directly (unensiled, U) or vacuum packed for ensiling for 28 days using four different additives (A-D). Dried samples of the 18 macroalgae or *S. latissima* silages (0.1 g) were incubated with maize silage (0.5 g) as a basal feed in buffered rumen fluid for 48 h. Total gas production (TGP) was measured and emitted gas was collected for end-point determination of contents of CH_4. Pure maize silage was incubated as a control. The content of halomethanes in the 18 macroalgae was measured using methanol extraction and GC-MS with a detection level of 1 µg/g dry weight. *Asparagopsis taxiformis* was used as a positive control. Degradable (d) DM, dOM, TGP and methane formation data were analysed by using a mixed procedure of R. The screening study of the 18 macroalgae showed the largest reduction in methane formation when *Fucus spiralis*, *Dictyota dichotoma*, *Ulva* sp., *Sargassum muticum* and *Fucus serratus* were added to maize silage. Furthermore, none of the 18 macroalgae contained detectable levels of halomethanes. For the *S. latissima* silages, TGP after 48 h was significantly lower for silage A and B and higher for U. There were no differences in dDM and dOM among U, the silage types or maize silage. Methane formation was significantly lower for silage A and B, 3.38±1.11 and 3.98±1.10 ml/g DM, respectively, while the formation was greater from pure maize silage (22.2±1.36 ml/g DM). Thus, it can be concluded that ensiled *S. latissima* significantly decreased TGP and the methane formation *in vitro* without affecting dDM or dOM of the basal feed. In addition, several of the Northern hemisphere macroalgae had potential to reduce methane formation without containing halomethanes or affecting the degradability of the basal feed negatively.

Could aquatic resources help to mitigate enteric methane from ruminants?

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Aquatic resources like seaweeds, microalgae and Lemna are perceived as novel feed materials. Although their energy and protein value can offer potential for use in livestock diets, there are still several limitations for large scale application. For aquatic resources harvested from nature, limiting factors include processing needs, unstable quality and feed safety. Moreover, the costs per ton dry matter are still high compared to terrestrial alternatives. Therefore, aquatic resources cannot yet be seen as commodities for large scale inclusion in livestock diets. If aquatic resources have specific bio-functionalities, their potential as livestock diet ingredient can be much higher. The use of seaweeds at low inclusion levels in ruminant diets to mitigate enteric methane emission is an example of such bio-functionality. This application will be presented in more detail. An overview will be given of methane emission reduction potential as well as food safety and health risks for a selection of European seaweeds, and for the (sub)tropical red seaweed *Asparagopsis* spp. Data on methane emission include results from *in vitro* gas production tests with simultaneous methane production determination, as well as data from a feeding trial with lactating dairy cows with individual methane emissions measurements using a GreenFeed automated emission monitoring system. Data on food safety and health risks include results of an *in vivo* study focussing on the potential transfer of bromoform ($CHBr_3$) present in *Asparagopsis taxiformis* to milk and urine of lactating dairy cows.

Feeding mixtures of seaweeds has no effect on methane emission of beef cattle

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Feeding seaweed to ruminants has been shown to decrease methane emission, but the effect is dependent on the seaweed fed. Based on *in vitro* evaluation of methane inhibition by a variety of seaweeds in the SeaCH4nge project (www.eitfood.eu), our objective was to measure effects of feeding mixtures of *Ascophyllum nodosum* (AN) and *Fucus vesiculosus* (FV) without or with *Asparagopsis taxiformis* (AT) on methane emission of growing beef cattle. In a first study 8 Holstein steers (498 kg body weight [BW]) were fed *ad libitum* a total mixed ration (TMR; 75:25 grass:maize silage, dry matter [DM] basis) containing one of 4 levels (0, 15, 30 or 45 g/kg diet DM) of seaweed (70:30 AN:FV, DM basis) with seaweed replacing grass silage DM. Steers received treatments according to a replicated 4×4 Latin Square design with 2 week periods. Feed DM intake (DMI) and methane emission were measured for the last 4 days of each period using respiration chambers. Feed DMI (8.2±0.4 kg/d), methane emission (242±10 g/d) and methane yield (29.9±0.9 g/kg DMI) were not affected by treatment (P>0.49). In a second study, 36 Holstein steers (460 kg initial BW) were individually fed *ad libitum* one of 3 TMR diets (45:15:40 maize silage:grass silage:concentrates, DM basis) containing 0, 13 (1.3 AN, 11.7 FV), or 1.3 (0.065 AN, 0.585 FV, 0.65 AT) g seaweed/kg DM (seaweed replacing grass silage DM) for 12 weeks, with BW measured weekly to determine average daily gain (ADG, kg/d) and feed conversion rate (FCR; ADG/DMI). During weeks 6 and 12 methane emission from 4 steers on each treatment was measured using respiration chambers. There was no effect of treatments (P>0.45) on DMI (11.8±0.3 kg/d), ADG (1.25±0.05 kg/d), or FCR (9.63±0.45 kg/kg). During methane measurements there was no effect of treatments (P>0.32) on DMI (9.0±0.6 kg/d), DM digestion (6.61±0.43 kg/d), methane emission (238±10 g/d), or methane yield (26.7±2.1 g/kg DMI). At the levels fed these seaweed mixtures had no effect on methane emission of growing cattle.

Mitigation extent of GHG emissions in Sardinia dairy sheep farms by forage quality improvement

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In Sardinia (Italy), standard haymaking often results in low quality forages, compelling farmers to feed high levels of concentrates. This study aimed to quantify the effect of improving forage quality on predicted GHG emissions at farm level. For this purpose, a dairy sheep farm was considered as case study and surveyed for a complete annual life cycle inventory which included information on feed production, feed purchased, animal diets, flock composition, etc., in a cradle-to-gate perspective. Total emissions were expressed per kg of fat and protein corrected milk (FPCM) and estimated by the Environmental Footprint Method 2.0 (2018). The case study farm was 73 ha in size, with flock of 500 heads, stocking rate of 5.5 head/ha and with an average flock milk production of 156 kg FPCM/head/year. The effect of three different haymaking techniques tested in the same farm was considered: (1) production of standard (low quality) hay (DM: 83%, as fed; CP: 14%, NDF: 67%, on DM); (2) production of early cut hay (ECH; DM: 87%, as fed; CP: 13%, NDF: 47%, on DM); (3) production of baled and wrapped haylage (WBHL; DM: 39% as fed; CP: 11%, NDF: 56%, on DM). In the first scenario emission intensity was equal to 5.04 kg CO_2eq/kg FPCM, mostly dependent on enteric CH_4 and N_2O emissions (57%), on-farm feeds (23%) and purchased feeds (13%). Improved forage quality in the scenarios 2 and 3 decreased GHG emissions to 4.70 and 4.58 kg CO_2eq/kg FPCM in the scenarios 2 and 3, respectively. Furthermore, the production of ECH and WBHL lowered on average by 7 and 9% the total GHG emissions and by 52 and 42% the GHG emissions from purchased feeds, respectively. In conclusion, the improvement of forage quality proves to be a good practice to reduce purchased feed and total GHG emissions in Sardinian sheep farms. The EU is gratefully acknowledged for its financial support (Project SheeptoShip LIFE 15 CCM/IT/000123).

Life cycle assessment (LCA) of Simmenthal cattle organic farms in Italy

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The European Parliament has recently issued the 848/2018 regulation (starting since 1st January 2021) which defines in detail the rules of the organic farming. Italy is one of the most important nations in Europe for the number of organic farms (more than 69,000, about 25% of all European producers) and 20,000 organic transformation companies. In Italy, in the last decade, the land used for the organic supply chain has increased and the number of organic farms has reduced but with an increase in the number of animals. According to Federbio (Organic Association), 34% of consumers are driven to purchase organic products in relation to their low environmental impact (reduced use of pesticides and synthetic fertilizers) and perceived better quality. In theoretical terms, organic methods make it possible to reduce GHG emissions by 20% for each product, in the case of agricultural crops, and by 4 percent, in the case of livestock. In this study, a comparative analysis was carried out at 5 organic Simmethal dairy farms in different Italian regions. The location, size and farming method were chosen to be representative of the national average. A conventional Simmenthal cattle farm of similar size and characteristics and simulated data were used for the comparison of the organic farms. Environmental performance was assessed with the LCA approach taking into account global warming (GW), acidification of air and water (AC) and eutrophication of water (EU). The impacts were related to 1 kg of FPCM and to 1 ha of land used. No differences were observed regarding the environmental impact when referring to the production unit, while the biological system showed lower values of GW, AC and EU when referring to the surface. Organic milk showed, on average, a unitary CO_2 emission of 10% lower compared to the conventional milk thanks to the lower use of feeds, absence of synthetic products in the fodder production and a reduction in the nitrogen distribution for surface unit. The study highlighted the differences between Italy and Central and Northern Europe farming systems, in terms of lower share of animal grazing and greater contribution of the maize feed to nutrition.

Methane emission by alpacas and sheep grazing Andean natural grasslands during dry season

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Alpacas and sheep in South America live mainly in the Andes at altitudes above 3,800 m.a.s.l in a semi-arid climate with a short wet season and a long dry season characterized by poor biomass production and nutritional quality. Feed selectivity and cell-wall plant digestibility are greater in alpacas than sheep, mainly attributed to a longer rumen retention time. It was hypothesized that under harsh conditions, the diet selected by alpacas has a lower concentration of neutral detergent fibre (NDF) and greater crude protein (CP), organic matter digestibility (OMD), and methane (CH_4) emissions compared to that of sheep. Nine male Huacaya alpacas (body weight (BW) 35.5±standard deviation 4.7 kg) and nine male Criollo sheep (BW 30.1±2.4 kg) were used. Dry matter intake (DMI) was estimated from faecal CP. Diet selectivity was assessed using the manual simulation technique. The CH_4 emission was measured using the sulphur hexafluoride technique. Data were analysed by ANOVA and Tukey means comparison test (MINITAB 19). The DMI was the same between species, but DMI expressed in function of BW was greater in sheep than alpacas (86.4 vs 57.2 g/kg $BW^{0.75}$; 3.7 vs 2.3 DMI%BW). Diet selected by alpacas showed a lower concentration of NDF (543.8 vs 705 g/kg dry matter (DM)) and greater OMD (0.52 vs 0.62) compared with that of sheep. The concentration of CP (76.2 vs 73.1 g/kg DM) was the same between diets. The CH_4 production was the same between alpacas and sheep (21.8 vs 22.7 g CH_4/day; 1.4 vs 1.8 g CH_4/day/kg $BW^{0.75}$; 25.0 vs 22.0 g CH_4/kg DMI; 7.2 kg CH_4/kg fibre vs 5.5 kg CH_4/kg of wool). Under harsh conditions, alpacas show greater selectivity but the same CH_4 emissions as sheep.

The effect of natural essential oils and synthetic essential oils on ruminal fermentation

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Secondary compounds of plants which are present in essential oils have shown a marked antimicrobial effect, so they are of great interest in the field of animal nutrition (e.g. ruminal microbiota modification, decrease of methane production). This experiment describes the effects of two different natural essential oils of oregano (NEO1 and NEO2) and their corresponding synthetic formula (SEO1 and SEO2), manufactured using the pure compounds, and considering the composition obtained by chromatographic analysis of NEO1 (carvacrol 70.62%; p-cymene 7.06%; γ-terpinene 7.58%) and NEO2 (carvacrol 57.57%; p-cymene 12.26%, γ-terpinene 10.43%). The same inoculum (bovine ruminal liquid) was used in all the 100 ml vials, which contained 0.5 g of dry matter (complete pelleted diet formulated). Five doses (0, 25, 50, 75 and 150 mg/l) were tested for each of the four oils, and three replicates were made. After 24 hours of incubation, liquid samples were taken to measure volatile fatty acids (VFA) and gas samples were collected for methane analysis. The four oils decreased the total VFA at 150 mg/l, thus indicating inhibition of ruminal fermentation at high doses. Moreover, NEO1 did not affect the acetate:propionate ratio nor methane production for the lowest doses (25, 50 and 75 mg/l), whereas SEO1 caused an increased acetate:propionate ratio even at 50 mg/l. Both parameters were increased ($P<0.05$) for NEO2 at 75 mg/l, with comparable results for SEO2 at 50 mg/l. In conclusion, synthetic EOs have similar effects to natural EOs, although all of them can inhibit ruminal fermentation above 75 mg/l. Furthermore, they may increase methane production, so it will be necessary to characterize the effects on the ruminal microbiota, even for each particular compound.

Tradilin™ in dairy cow diet also leads to a decrease in enteric methane emission

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Adding lipids in dairy cow diets is a known solution to reduce methane emission. Tradilin™ is a range of products composed of selected linseed with high content of fat and linolenic acid and processed under specific conditions of temperature and pressure (process patented with the number EP1155626). A kilogram of Tradilin with 100% linseed contains 39% of ether extract and 220 g of omega-3 fatty acids (on a raw matter basis). Tradilin is already known to increase milk production and fertility. To study the effect of Tradilin on methane emission in dairy cows, a linear regression between the amount of Tradilin in diet and measured methane emissions was established thanks to the data of twelve treatments from five publications using Tradilin as a methane mitigation strategy in dairy cow diets. For that, a standardized Tradilin product with 100% linseed was considered. Variation of methane emissions have been calculated compared to control diets (without Tradilin) to establish relationship between amount of Tradilin and decrease of methane emission. Amount of Tradilin in these experiments were between 0.6 and 3 kg/d/cow, which do not represent what is usually done in farms: amount of Tradilin conventionally distributed in farms up to a dose of 1,500 g/d/cow, with an average of 297 g/d/cow (Meignan, 2018). Thus, to extrapolate experimental results to what is actually done in farms, the regression between amount of Tradilin and decrease of methane emission was forced to go through the intercept to integrate the amount less than 1 kg/d/cow of Tradilin. This regression shows that when 1 kg of Tradilin is added in dairy cow diets (on a DM basis), the expected reduction of methane emission is 12.0% for methane expressed in g/kg of milk. This regression is highly significant, with a P-value under the threshold of 0.001.

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Condensed tannins from Sainfoin (*Onobrychis viciifolia*): effects on *in vitro* ruminal parameters

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There is an increasing interest in using local legumes in ruminant feeding. Sainfoin (SF) is a perennial legume extensively used in the Mediterranean area, but it presents condensed tannins (CT). The aim of this study was evaluate the effect of CT from fresh SF using polyethylene glycol (PEG), as blocking agent of CT. For that, in spring 2019, SF was cut three times a week during 4 weeks (mid to late vegetative stage), and then a composite sample per week was obtained. An *in vitro* assay to study the fermentation was carried out with an Ankom system during 72 h. Samples were incubated with buffered solution:rumen fluid (2:1 v/v). To make the SF+PEG samples, PEG-4000 was added to the buffered rumen fluid at a concentration of 2.3 g/l. Samples were incubated in triplicate and three runs were conducted. The interaction of the presence of CT and the week was not significant in any parameter studied, therefore, results are presented separately by each factor. The presence of CT decreased the potential gas production (87 vs 92 ml), the rate of gas production (0.14 vs 0.16 h^{-1}) and the methane production (62.7 vs 65.1 ml/g dry matter (DM)) ($P<0.05$) but did not affect *in vitro* dry matter digestibility (IVDMD). The total volatile fatty acid (VFA) production was unaffected ($P>0.05$) but the individual VFA proportions varied with the presence of CT ($P<0.05$), which increased the proportion of acetic (64.4 vs 63.1%) and decreased the proportions of propionic (16.3 vs 16.6%), butyric (11.6 vs 12.2%) and valeric acid (1.9 vs 2.1%). In addition, the presence of CT decreased the content of ammonia (393 vs 450 mg/l; $P<0.01$), confirmed the inhibition elicited by CT from SF in the ruminal degradation of dietary proteins. Regarding the effect of the week, methane production varied among weeks without a clear pattern, with values between 61.6 vs 66.1 ml/g DM, in week 2 and 4 ($P<0.05$) while the IVDMD decreased linearly (87, 84, 80 and 77% for week 1, 2, 3 and 4, respectively; $P<0.001$), as the maturity of SF advanced. The use of sainfoin in ruminant diets is recommend as their CT can reduce the potential gas production, methane emissions and ammonia content with similar IVDMD.

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Rumen ciliates and methane production of beef bulls fed a biodiverse haylage, low-starch Mixed Diets

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This study evaluated the rumen ciliate protozoa (PTZ) and methane (CH_4) production of 20 crossbred young bulls (crossbred Charolais × crossbred Alentejana breeds) fed for 126 days with 4 total mixed diets including a legume-grass haylage as forage source and differing in forage:concentrate ratio (F:C 60:40 vs 75:25 (DM)), in the level of starch by replacing 50% of cereals in concentrate by low-starch agro-industrial by-products (LSBP) and in the addition of 6% (DM) of sunflower seeds (SS) as lipid supplement. Diets were: (1) 60C: 60:40 for F:C, concentrate with cereals; (2) 60S: 60:40 for F:C, concentrate with LSBP; (3) 60SG: 60:40 to F:C, LSBP and SS; (3) 75SG: 75:25 to F:C, LSBP and SS. Samples of rumen fluid were collected at slaughter and were incubated *in vitro* for 48 h to estimate CH_4 production with the Ankom System. PTZ were evaluated by microscopic counting. ANOVA was used to evaluate the diet effects on total PTZ counts and individual genus proportions (% in total PTZ), total gas (Totgas) and CH_4 production ($TotCH_4$) and CH_4% (% of total gas). Relationships between PTZ, gas and CH_4 productions were evaluated by the Spearman´s correlation coefficient. Diets did not affect total PTZ, but *Entodinium* was higher in bulls fed with 60C diet than with 60SG and 75SG ($P=0.011$) and *Epidinium* was lower with 60C than with 60SG and 75SG ($P=0.008$). Totgas and $TotCH_4$ productions were higher with 60C and 60S and lower with 75SG ($P=0.029$ and $P=0.048$, respectively). Total PTZ was not correlated with Totgas, $TotCH_4$ or CH_4%, but *Isotricha* ($P=0.047$) and *Eudiplodinium* ($P=0.030$) were correlate negatively with Totgas and *Epidinium* correlate negatively with Totgas ($P=0.010$), $TotCH_4$ ($P=0.002$) and CH_4% ($P=0.025$). *Entodinium* correlate positively with Totgas ($P=0.030$), $TotCH_4$ ($P=0.007$) and CH_4% ($P=0.004$). Conjugating LSBP with SS in the diets changed PTZ community composition and increasing haylage to 75% and supplementing with 6% SS reduced *in vitro* Gas and CH_4 productions. Rumen PTZ community was apparently related with gas and CH_4 determinations. Work funded by PDR2020 program through the FEADER under the project LegForBov (PDR2020-101-031179).

How much the introduction of legume-based forage can mitigate climate change in a sheep farm?

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This study aimed to provide insight on the impact of improving forage quality, through the introduction of legume species (*Hedysarum coronarium*), on predicted GHG emissions at farm level. For this purpose, a dairy sheep farm located in Sardinia (Italy) was considered as case study and surveyed for a complete annual life cycle inventory in a cradle to gate perspective. Total emissions were expressed per kg of fat and protein corrected milk (FPCM) and estimated by the Environmental Footprint Method 2.0 (2018). The case study farm was 85 ha in size, with 98% of feed self-sufficiency, 950 heads and an average flock milk production of 237 kg FPCM/head/year. Two scenarios were considered: (1) survey data (maize-based silage); (2) after the production of baled and wrapped haylage of *H. coronarium* (DM: 35.3%, as fed; CP: 16.6%, NDF: 42.9% on DM). In survey scenario, emission intensity was equal to 5.08 kg CO_2eq/kg FPCM and depended mostly on enteric and barn emissions (43%) and emissions from crops (41%). Introducing legume-based forage decreased GHG emissions to 4.12 kg CO_2eq/kg FPCM. Furthermore, the production of baled and wrapped haylage lowered on average the total GHG emissions by 19%, the GHG emissions from crops by 45% (mainly due to lower diesel use and fertilizers) and the GHG emissions from off-farm feeds by 24% (since it replaced off-farm rich-protein feeds and by-products). In conclusion, the predicted mitigation extents (-19%) of total GHG emissions observed in this study suggested that the introduction of legume-based forages could be considered a good farming practice to mitigate climate change in the Sardinian sheep farming sector. The EU is gratefully acknowledged for its financial support (Project Forage4Climate LIFE 15 CCM/IT/000039).

Response in blood methemoglobin level in dairy cows supplemented with dietary nitrate

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Dietary nitrate supplementation is an efficient methane mitigation strategy for dairy cows. However, nitrate supplementation may lead to methemoglobinemia. As part of a larger experiment, it was investigated if dietary nitrate supplementation and other methane mitigation additives affected blood total haemoglobin (tHb), methemoglobin (MetHb, % of tHb) and haematocrit (Hct) levels. Forty-eight lactating Danish Holstein cows were included in an incomplete 8×8 Latin square design with 6 periods of 21 days each. Cows were assigned to 6 blocks of 8 cows each, according to parity (24 1^{st} parity and 24 $>1^{st}$ parity) and days in milk (DIM). These cows were randomly assigned to 6 of 8 diets. Diets were arranged in a 2×2×2 factorial design; 2 levels of fat (low fat; 30 g CF/kg DM, or high fat; 63 g CF/kg DM), 2 levels of nitrate (0 g/kg DM, UREA, or 10 g/kg DM, NIT; diets were isonitrogenous) and 2 levels of 3-NOP (0 mg/kg DM, BLANK, or 80 mg/kg DM, 3-NOP). A blood sample was taken from the tail vein once on day 21 in the four last periods (3-6) and was immediately analysed for tHb, MetHb and Hct by oximetry by using an acid base laboratory analyser (Radiometer ABL90 Flex). A total of 191 samples were included in the statistical analysis using a mixed procedure of R. The model included fixed effects and interaction effects of dietary treatments, fixed effects of parity, DIM, and period, and a random effect of cow. Blood tHb was unaffected by dietary supplementation and averaged 6.95±0.07 mmol/l (LSM±SEM). A significant interaction effect between fat, nitrate and 3-NOP ($P<0.01$) on MetHb was found. In addition, only NIT fed cows had significant higher MetHb proportion in blood compared to cows receiving UREA (2.41±0.06 vs 2.26±0.06% of tHb; $P=0.03$). The highest proportion of MetHb found in NIT fed cows reached 3.7% of tHb compared to 3.1% of tHb in UREA fed cows. Hct values were unaffected by NIT supplementation, while addition of 3-NOP increased Hct value ($P=0.05$), although the numerical increase was small and within normal ranges. These results demonstrate a significant response in MetHb by nitrate supplementation, although the numerical increase was minor and thus did not pose a risk of methemoglobinemia.

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Effect of tannins in *Onobrychis* and *Quercus robur* on *in vitro* ruminal gas and ammonia formation

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Mitigation of methane and ammonia contribute to greenhouse gas abatement objectives and can have a positive effect on animal production efficiency. Reducing the production of methane and ammonia can take place by influencing ruminal methanogenesis through feedstuff. In this regard, the secondary plant metabolites tannins, which are polyphenolic compounds, have shown promising results. Numerous studies demonstrated mitigating effects of tannins on *in vitro* and *in vivo* methane and ammonia formation. In the present study the potential of the Swiss indigenous tannin-containing plants sainfoin (*Onobrychis*) and oak bark (*Quercus robur*) was explored. Four different cuts of sainfoin from three different agricultural sites in Switzerland and one production unit of oak bark powder were tested *in vitro* using the Hohenheim Gas test (HGT) method. Ruminal fluid from four Original Brown Swiss cows was taken and tested separately in four consecutive runs. For every run the same mixed ration, containing maize silage, grass silage, hay, alfalfa, concentrate mixture and UFA 249 (32 : 60 : 2 : 2 : 2.6 : 1.5) was incubated and served as control. Every feedstuff was tested in different dosages (sainfoin: 20, 30, 40, 50 and 60 mg DM; oak bark: 1, 2, 3 and 4 mg DM) which replaced the respective amount of the mixed ration. The dosages were tested twice per run to evaluate the consistency of their effect resulting in a total of 8 replicates per treatment. In a further step, statistical analysis will take place to investigate if tannins in indigenous sainfoin and oak bark have the ability to affect *in vitro* methane and ammonia formation as well as digestibility. Sainfoin and oak bark can be produced on Swiss soil. This presents the possibility for farmers to orientate their cultivation in this direction and helps to diminish emission due to transportation to a minimum. Hence, the relevance of our findings lies in the potential to propose a practical and cost-efficient solution for the reduction of greenhouse gas emissions in the Swiss dairy production sector.

Session 12 Theatre 1

Detecting effective starting point of genomic selection by divergent trends from BLUP and ssGBLUP

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Genomic selection has been adopted nationally and internationally in different livestock and plant species. However, understanding whether genomic selection has been effective or not is an essential question for both industry and academia. Once genomic evaluation started being used, estimation of breeding values with pedigree BLUP became biased because this method does not consider selection using genomic information. Hence, the point of divergence of genetic trends obtained with BLUP and Single-step genomic BLUP (ssGBLUP) may indicate the start date of genomic selection. This study aimed to find the start date of genomic selection for a set of economically important traits in broiler chickens, pigs, and Angus cattle by comparing genetic and Realized Mendelian sampling (RMS) trends obtained using BLUP and ssGBLUP. In broiler chickens, the genetic trends from ssGBLUP and BLUP diverged for two traits and did not diverge for one trait, which was selected for before genotyping. The genetic trends for pigs diverged for al traits although with different intensity. In beef cattle, the trends started diverging in 2009 for weaning weight and in 2016 for postweaning gain, with little divergence for birth weight. In broiler chickens, the RMS trends from ssGBLUP and BLUP diverged strongly for the trait selected without the genomic information, weakly for the second trait, with no divergence for the third trait. The RMS trends for pigs diverged strongly for one trait and weakly for another trait. In beef cattle, the RMS trends started diverging in 2009 for weaning weight and in 2016 for postweaning gain, with little trend for birth weight. Divergence of the genetic trends from ssGBLUP and BLUP indicates onset of the genomic selection. Presence of trends for RMS indicates selective genotyping, with or without the genomic selection. The onset of genomic selection and genotyping strategies agree with industry practices across the 3 species. In summary, the effective start of genomic selection can be detected by the divergence between genetic and RMS trends from BLUP and ssGBLUP.

Session 12 Theatre 2

Impact of preselection on subsequent ssGBLUP evaluations of pigs

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Empirically assessing the impact of preselection on subsequent evaluation of preselected animals requires comparison of scenarios with and without preselection. However, preselection almost always takes place in animal breeding programs, and so it is difficult, if not impossible, to have a scenario without preselection. Hence most studies on preselection used simulated datasets, concluding that subsequent genomic estimated breeding values (GEBV) from single-step genomic best linear unbiased prediction (ssGBLUP) are unbiased. In real breeding programs, additional sources of bias to GEBV from ssGBLUP, such as inaccurate pedigree and inaccurately estimated additive genetic (co)variances, may be present and their impact on bias may be confounded with the impact of preselection. This may explain the observation that in practice GEBV obtained from ssGBLUP are sometimes biased. The aim of this study was to investigate the impact of preselection, using real data, on accuracy and bias of GEBV from subsequent ssGBLUP. We obtained data from a pig sire line on average daily gain during performance testing, average daily gain throughout life, backfat thickness, and loin depth. The data were already preselected, with similar intensities of preselection among males and females. We ran ssGBLUP with the entire data until validation generation and considered this the scenario without (additional) preselection. We then implemented scenarios with additional layers of preselection by removing all animals without offspring either; (1) only in the validation generation; or (2) in all generations. The last scenario is the most severe preselection that can be retrospectively imposed. In estimating accuracy and bias, we compared GEBV against mean offspring performance of animals in the validation generation. Results show only a limited loss in accuracy due to the additional preselection. The observed bias was also limited, and generally very similar between scenarios with or without the additional preselection. More results will be presented and discussed at the conference.

Session 12 Theatre 3

Modelling genetic differences of combined broiler chicken populations in single-step GBLUP

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The objectives of this study were to model the inclusion of a group of external birds into a local broiler chicken population for the purpose of genomic evaluations and to evaluate the behaviour of two accuracy estimators under different model specifications. The pedigree was composed by 242,413 birds and genotypes were available for 107,216 birds. A five-trait model that included one growth, two yield, and two efficiency traits was used for the analyses. The strategies to model the introduction of external birds were to include a fixed effect representing the origin of parents and to use UPG or metafounders. Genomic estimated breeding values (GEBV) were obtained with single-step GBLUP (ssGBLUP) using the Algorithm for Proven and Young (APY). Bias, dispersion, and accuracy of GEBV for the validation birds, i.e. from the most recent generation, were computed. The bias and dispersion were estimated with the linear regression (LR) method, whereas accuracy was estimated by the LR method and predictive ability. Models with fixed UPG with estimated inbreeding for UPG or random UPG resulted in similar GEBV. The inclusion of an extra fixed effect in the model resulted in unbiased GEBV and reduced inflation, whereas models without such an effect were significantly biased. Genomic predictions with metafounders were slightly biased and inflated due to the unbalanced number of observations assigned to each metafounder. When combining local and external populations, the greatest accuracy and smallest bias can be obtained by adding an extra fixed effect to account for the origin of parents plus fixed UPG with estimated inbreeding or random UPG. When changing model specification, the largest variation for the LR accuracy was 20%, whereas for predictive ability it was 110%. To estimate the accuracy, the LR method is more consistent among models, whereas accuracy by predictive ability can be inflated when phenotypes are not adjusted for origin of founders.

Session 12 Theatre 4

Unknown-parent group and metafounder models for missing pedigree in single-step genomic BLUP

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Pedigree information is often missing for some animals in dairy cattle. Missing pedigree may cause some biases and inflation in genomic EBV predicted with single-step genomic BLUP (ssGBLUP), which combines matrices of pedigree (A) and genomic (G) relationships into a single unified relationship matrix. Unknown-parent groups (UPG) are assigned to the missing parents to avoid biases in EBV. Although the UPG model is well-accepted for pedigree-based BLUP, it is uncertain how UPGs are integrated into the inverse of the unified matrix (H-inverse). The metafounder (MF) model is a generalization of the UPG model, but similarities and differences between UPG and MF models have not been discussed well. This study aimed to derive several H-inverses, compare genetic trends among UPG and MF models in simulated and actual purebred populations, and discuss the MF model from a UPG's point of view. All H-inverses were derived with the joint-density function of the random genetic groups and breeding values. The Quaas-Pollak-transformed (QP) H-inverse had a good convergence behaviour, but it produced biased genetic trends and underestimated UPG solutions due to confounding among GEBV and UPG effects. The Altered-QP model was available by removing the genomic relationships among GEBV and UPG effects. Although the model resulted in biased genetic trends when the pedigree has many missing parents, the model showed less bias in genetic trends and less inflation in predictions, compared with the QP model in real data. The UPG-encapsulated (EUPG) model with the inverse of the additive relationship matrix for genotypes (A22-inverse), which absorbed the UPG contributions. This new model was found to be the same structure as the MF H-inverse. The A-inverse with MF was shown to be a special case of the A-inverse with UPG. The models with EUPG and MF showed no bias in genetic trends in a simulated population. Although the EUPG and MF models are preferred because of the possibility of reducing biases and theoretical justification, more research should be needed to confirm their ability to remove the bias in practice. The QP and Altered-QP models are expected to give reasonable genetic trends in real populations with enough data.

Session 12 Theatre 5

GEBV and h2 of milk traits in a nucleus of Sarda ewes depend on a matrix weight in ssGBLUP

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Efficiency of selective breeding in most dairy sheep has been limited by scarce diffusion of AI and the low accuracy of the phenotype recording. The difficulty to measure on large scale innovative traits has made crucial to assess the feasibility of a genomic selection approach based on a female informative population (FIP). The aim of this work was to assess the effect of different weights of A matrix into H matrix on h^2, predictive ability (PA) and GEBV accuracy for milk traits estimated with ssGBLUP using FIP as reference population and rams of the herd book (HB) as selection candidates. On 2000, around 900 Sarda × Lacaune backcross ewes were generated in a nucleus. Successively, 25% replacement ewes were yearly generated with HB rams. Ewes born from 2000 to 2020 (4,489), 272 sires and male ancestors and 734 HB rams (SHB) were genotyped with the OvineSNP50 Beadchip. Data were 15,008 lactation records of milk yield (MY), protein (PC) and fat contents (FC). Estimates of h^2 were obtained with ssGPLUP using different weights of A matrix into H matrix (from 0 to 1 by 0.2). Cross-validation was used to assess PA either on 300 ewes out of those with 4 records or 275 ewes of the 2 last generations. GEBV and their theoretical accuracies (Rg) were calculated for SHB. Rg values were correlated with the amount of information on relatives in the nucleus. The highest h^2 were reached at 0.80 A weight for MY (0.44), PC (0.72) and FC (0.58). The lowest values were reached at 0.05 A weight for MY (0.32), PC (0.61) and FC (0,51). PA patterns were less definite depending on the validation sample. The mean Rg of SHB were 0.32 (0.20 to 0.66) for MY, 0.37 (0.24 to 0.71) for PC and 0.35 (0.24 to 0.70) for FC. The overall results show that blending A matrix into H matrix is important in this population to adjust for genomic relationships in the G matrix which are partly due to IBS rather than IBD genome sharing and improve h^2 and PA. Rg of SHB show that FIP approach is feasible as long as the size of the nucleus and the flow of sires from and toward the nucleus are managed to achieve sufficient amounts of information on relatives for most selection candidates.

Integration of MACE information into single-step evaluations for conformation in Brown Swiss

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The German-Austrian official genomic evaluation for conformation traits in Brown Swiss has used information from foreign bulls since its introduction in 2011. Access to genotypes of foreign bulls has been possible through the participation in the InterGenomics consortium. Initially, genotypes and de-regressed MACE breeding values of foreign bulls were integrated in a calibration set of a two-step genomic evaluation system. In April 2021, the first official genomic breeding values for conformation traits from a single-step system including foreign information were published. The objective of this work is to present an overview of the effects of the inclusion of foreign information in the new single-step system. Data from the December 2020 run of the German-Austrian official genomic evaluation for 29 conformation traits in Brown Swiss were used. Benefits from the inclusion of external MACE information were assessed in a minus 4 years validation framework and quantified in terms of validation reliability. For that, data was truncated to the status of December 2016 and evaluations were conducted with and without external MACE breeding values. The truncated dataset included 23,992 genotyped animals, from which 5,757 originated from the exchange with InterGenomics partners. External MACE breeding values were corrected to remove information on domestic daughters and then de-regressed. Final observations used in the analyses comprised yield deviations of domestic cows and de-regressed corrected MACE breeding values as pseudo-phenotype of foreign bulls. The number of foreign bulls contributing a phenotype to the evaluations ranged from 2,968 to 6,607, with an average of 5,270. Validation reliabilities with the inclusion of external MACE breeding values increased by on average 6 reliability percentage points. The gains ranged from 2 to 13 depending on the trait. In udder score, for example, the reliability increased from 0.40 to 0.45. In conclusion, integration of foreign information in the new single-step system resulted in notable increase in validation reliabilities.

Interim genomic prediction for young genotyped animals for different single-step evaluations

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Single-step genomic evaluations simultaneously combine all available phenotypic, genotypic, and pedigree information. However, it often occurs that genotypes of young animals without phenotype become available after the routine evaluation. Therefore, the aim of this study was to develop and test a method to compute interim genomic enhanced breeding values (GEBV) for genotyped but non-phenotyped young animals, without solving a single-step genomic evaluation with the additional genotypes. Two different versions of single-step genomic evaluations were tested: a single-step system that relies on the Woodbury matrix identity applied to the inverse of the genomic relationship matrix (so-called ssGTBLUP), and a single-step system that estimates directly the single nucleotide polymorphism (SNP) effects (so-called ssSNPBLUP). The developed method relies on the decomposition of GEBV of all animals included in the single-step evaluation into its different components, such as the residual polygenic effects and the direct genomic values. The method was tested on real data from a single-step evaluation for reproduction of Topigs Norsvin. An initial single-step evaluation for one trait with maternal effects included about 18 million animals, 9.6 million records, and 343 thousand genotypes. Interim GEBV for 20,000 young genotyped animals were computed from the solutions obtained for the two different versions of single-step evaluations. For both single-step approaches, Pearson correlations between interim GEBV and GEBV obtained from a single-step evaluation that included young genotyped animals, were higher than 0.999 for young animals with both parents genotyped, 0.996 for animals with only one parent genotyped, and 0.986 for animals with no genotyped parents for the direct effect. All coefficients of the regression of GEBV obtained from a single-step evaluation on interim GEBV were equal to virtually 1. Similar correlations and regression coefficients were obtained with GEBV for the maternal effect.

Session 12 Theatre 8

Single step evaluation for resistance to ketosis in a Brown Swiss cattle population

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In early lactation, the feed intake capacity of dairy cows is often limited compared to their milk production needs. This limitation leads to an increased frequency of metabolic diseases such as ketosis. Genetic selection of animals less prone to ketosis is possible. In Switzerland, routine traditional genetic evaluation for resistance to ketosis was recently implemented with a multi-traits animal model comprising acetone in milk (mAC_AA), blood non esterified fatty acid (bNEFA_MIR), and fat/protein ratio in milk (mFPR). Although traditional genetic evaluation results provided first improvements towards better resistance to ketosis, genomic selection for these traits proved to be a challenging task. This was mainly due to difficulties in defining a reference group of genotyped animals with high reliabilities of traditionally estimated breeding values. Multi-step genomic evaluation reliability estimates were computed predicting the 10% youngest proven bulls and reached 0.66, 0.70 and 0.70 for mAC_AA, bNEFA_MIR, and mFPR, respectively. In this study, the implementation of a single-step method for resistance to ketosis is investigated. Using the same phenotypic inputs and models as for the animal model, all genotypic information of the Swiss Brown Swiss population was added to set up the relationship matrix. Single-step was implemented with full inversion of the genomic relationship matrix and not optimised for computational efficiency so far. Reliability estimates of the single-step were estimated for the same young bulls and reached 0.70 for mAC_AA, 0.75 for bNEFA_MIR and 0.73 for mFPR. Following these results, BV estimated with the single-step approach will be further investigated in parallel with methods increasing computational efficiency prior to a possible routine implementation of genomic selection for resistance to ketosis for the Brown Swiss cattle population in Switzerland.

Session 12 Theatre 9

Implementation of single-step evaluations for fitness traits in Fleckvieh and Brown Swiss

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The official genomic evaluations for the German-Austrian-Czech Fleckvieh and German-Austrian Brown Swiss populations were implemented in 2011 and since then only bull genotypes were used in the calibration set of the two-step system. In April 2021, the first genomic breeding values from a single-step system for almost all traits in the breeding program were published. The single-step system includes roughly 300,000 Fleckvieh and 70,000 Brown Swiss genotypes, of which up to 250,000 animals in Fleckvieh and 50,000 in Brown Swiss had direct phenotypes for some traits. The objective of this work is to give an overview of the most important issues that came up in the process of implementation of the single-step system for fitness traits. Additionally, there is a more detailed focus on the effect of different genomic relationship matrices (G-matrices) used in the single-step runs. Concerning the general implementation process an important point is to have a suitable validation strategy. Here the LR-method, which Legarra and Reverter published in 2018, is used. Single-step showed clear advantages in reliability and bias reduction. Still, a key aspect is the detection and subsequent dealing with bias in specific groups of animals. In this regard, the effects of different genomic relationship matrices (G-matrix) are investigated. For the construction of the G-matrices, either estimated base allele frequencies or allele frequencies equal to 0.5 were used. In addition, methods to prevent singularity of the G-matrix and scaling of the G-matrix have an influence on the systematic bias in specific families.

Multi-breed genomic evaluation for dairy cattle in the US using single-step GBLUP

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Official multibreed genomic evaluations for dairy cattle in the US are based on multibreed BLUP evaluation followed by single-breed estimation of SNP effects. Single-step GBLUP (ssGBLUP) allows the straight computation of genomic (G)EBV in a multibreed context. The objective of this study was to develop ssGBLUP multibreed genomic predictions for US dairy cattle. This involved the use of unknown parent groups (UPG) to model the difference in genetic base caused by breed, year of birth, and sex. We used only purebred Ayrshire (AY), Brown Swiss (BS), Guernsey (GU), Holstein (HO), and Jersey (JE). A total of 45M phenotypes for milk (MY), fat (FY), and protein (PY) yields recorded from January 2000 to June 2020 were available for 19.4M cows. Pedigree information was recorded on 29.5M animals, of which 3.4M were genotyped. A 3-trait repeatability model was applied to a complete (reduced) dataset with phenotypes of cows born from 1992 to 2018 (2014). All the effects in the model were breed-specific. Validation for cows was based on correlations between (G)EBV and adjusted phenotypes, whereas for bulls, the latter was replaced by daughter yield deviation. Evaluations were done for each breed separately, AY-BS-GU, and all five breeds together. Reliabilities for bulls and predictability for cows were similar between single-breed and five-breed BLUP. Under ssGBLUP, predictability (reliability) for AY, BS, and GU was on average 21% (9%) lower in the five-breed compared to single-breed model. No changes were observed for HO in the five-breed model. Combining AY-BS-GU into one evaluation resulted in predictions similar to the ones from single-breed. Single-step large-scale multibreed evaluations are computationally feasible but fine-tuning is needed to avoid a reduction in reliability when numerically dominant breeds are combined.

Breeding value reliabilities for multiple-trait single-step genomic BLUP

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An approximate method with three steps was developed to calculate reliabilities for estimated breeding values (EBV) in a multiple-trait single-step genomic BLUP (ssGBLUP) model. First, a traditional animal model was used to estimate the amount of non-genomic information for the genotyped animals. Second, this information was used with genomic data in SNP-BLUP to approximate the total amount of information and ssGBLUP EBV reliabilities for the genotyped animals. Finally, reliabilities for non-genotyped animals were calculated using a traditional animal model where the increased information due to genomic data is accounted by pseudo-observations for the genotyped animals. The approach was tested using a three-trait ssGBLUP model. Data had 46,535 first, 35,290 second, and 23,780 third lactation 305-d milk yield records from 47,124 Finnish Red dairy cows. The pedigree comprised 136,593 animals, of which 19,757 were genotyped. Correlations between the exact and the approximated ssGBLUP reliabilities of EBV for the genotyped (non-genotyped) animals were 0.989 (0.992), 0.978 (0.990), and 0.945 (0.989) for first, second, and third lactation, respectively. These correlations were higher than those between the exact ssGBLUP model and the traditional animal model within both the groups and traits. The computing time for the approximation method was approximately 78% less than with a direct exact approach. In conclusion, the developed approximate approach allows calculating EBV reliabilities in the ssGBLUP model even for large data sets.

Evaluation of accuracy and bias of beef cattle international single-step genomic evaluations

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In beef cattle, international evaluations led by Interbeef involve 13 countries and 5 breeds. Beef cattle national genomic evaluations have been implemented in some countries, while others will follow soon. Therefore, the Interbeef pedigree-based evaluations need to be adapted to include genomic information. A single-step model where pedigree, phenotypes and genomic data from participating countries are used simultaneously at an international level is considered to be the optimal approach. However, the impact of including genotypes in beef cattle international evaluations on estimated breeding value accuracy and bias is currently unknown. Therefore, the objective of this study was to investigate the impact on accuracy and level and dispersion bias due to including genomic information at the international level. Accuracy and bias were assessed using the Linear Regression method. Weaning weight phenotypes for more than 330,000 Limousin male and females were available from 7 countries. Genotypes imputed to a panel of 57,899 SNP were available for 17,607 animals from 5 countries. We implemented two international scenarios where countries were modelled as different correlated traits: $PBLUP_{int}$ being the current international pedigree BLUP evaluation, and $ssSNPBLUP_{int}$ being the international genomic single-step SNPBLUP evaluation. Moreover, we compared the results of international scenarios with two national scenarios where only nationally submitted phenotypes and genotypes were used: $PBLUP_{nat}$ and $ssSNPBLUP_{nat}$ for pedigree and genomic evaluations, respectively. In general, less level and dispersion bias was detected in $ssSNPBLUP_{int}$ than $PBLUP_{int}$. Overall, the observed level and dispersion bias was similar between national and international scenarios. On average across countries, accuracies for direct and maternal effects of genotyped animals with phenotypes increased by 26 and 5%, respectively, when using $ssSNPBLUP_{int}$ instead of $PBLUP_{int}$.

A fast method for approximating reliabilities in genomic BLUP

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The purpose of this study was to implement an algorithm to approximate reliabilities in large single-step genomic evaluations. A critical part of such an algorithm is obtaining the diagonal elements of the inverse of the coefficient matrix of GBLUP for possibly millions of animals. The current approach calculates reliabilities of markers in a SNP-BLUP model and transform them into reliabilities for genotyped animals. The computational complexity of such a method increases cubically with the number of markers and quadratically with the number of genotyped animals. Here, we propose to obtain the diagonal of the inverse of the GBLUP coefficient matrix, in which the inverse of the genomic relationship matrix was constructed based on the algorithm for proven and young, using a combination of an iterative method and stochastic estimation. The method works as follows: (1) compute reliabilities for a small subset of reference individuals using an iterative method, (2) compute all the reliabilities with a stochastic estimator, (3) compare results from (1) and (2) for the small subset of animals; if the stochastic estimates reach a certain degree of accuracy, rescale reliabilities for all animals; otherwise, increase the number of samples. The iterative method in (1) is Gaussian Quadrature Lanczos, which allows the estimation of reliabilities for a subset of animals. Computing cost is less than quadratic with the number of noncore animals. The stochastic estimator in (2) uses conjugate gradient with multiple right-hand sides to obtain a Monte Carlo estimate of the diagonal of the inverse of the GBLUP coefficient matrix. Overall, computing cost is linear with the number of noncore animals and quadratic with the number of core animals. For genetic evaluations, the latter remains constant. This approach also allows for parallelization and acceleration through the use of single-precision arithmetic. For a set of 4k core animals (typical for pigs and chicken) and 46k noncore animals, the algorithm required 4 GB of memory and 15 minutes (wall clock time). Extrapolating to 1 million animals with the same core size would require 5-6 hours. An accurate and feasible approximation of reliabilities in single-step GBLUP is possible for very large datasets.

An approach to reduce computing time in multi-trait single-step evaluations

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Single-Step (SS) evaluations are planned to replace current genetic and genomic evaluations in France. SS evaluations consider all phenotypic and genotypic information available as well as their pedigree. However, multi-trait (MT) SS evaluations can be very time-consuming, in particular due to complex underlying models leading to slow convergence. An alternative solving strategy consists in running univariate SS evaluations first, in order to provide better starting values for GEBV or SNP effects as well as corrected phenotypes for fixed effects and other complicating features. Then a much simpler multi-trait SS evaluation can be envisioned as a second step. Such a strategy was successfully implemented on a Montbéliarde dataset with about 1.6 million animals with performance on a group of 8 correlated type traits. On the one hand, a multi-trait SS was launched to obtain GEBV of animals for each trait with a stringent convergence criterion. On the other hand, a univariate SS evaluation was first run for each trait and after correction for fixed effects, followed by a simplified multi-trait SS evaluation. GEBV obtained were similar to the ones of a conventional run multi-trait SS but with a strong reduction in overall computing time (by a factor of about 4). The strategy was extended to other situations – with missing performances on some traits or accounting for heterogeneous residual variances.

Fitting the mean of genotyped individuals per breed had limited benefit for single-step evaluations

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Fitting the mean of genotyped individuals as a fixed regression has been shown to increase accuracy and reduce bias in single-step evaluations. The value of the associated covariate (J-factor) for genotyped animals are equal to -1, and ranges between -2 and zero for ungenotyped relatives. To date the mean of genotyped animals has been considered only in purebred populations, but in crossbred evaluations different breeds have different base populations. Our aim was to determine if there is a benefit to fitting a separate J-factor for each breed. To test this, we used data from a three-way crossbred experiment with 150 sires in the purebred sire line and 5,000 crossbred progeny. For two traits (body weight at 7 or 35 days), the data was filtered for different genotyping rates (100, 75, 50 and 25%). The filtering was either random or selective of the top performing phenotypes. The animal models fitted either one J-factor (across all breeds), three J-factors based on the expected breed proportion (50% for the sire line and 25% for each of the dam lines), or three J-factors calculated with the actual breed proportions derived from genotypes. In total 100 validation replicates with five cross validation folds were performed for each combination of J-factors, genotyping rate, and genotyping selection method. Results indicate there is no difference for sire EBVs, accuracy or bias of prediction when using one or three expected breed J-factors. The correlation of EBVs using actual breed J-factors and one J-factor is between 0.87 and 0.91. The accuracy is marginally higher with actual breed contributions (±1%) and there is no consistent pattern for bias. If there is no selective genotyping we recommend one J-factor be fitted. With selective genotyping, particularly at lower genotyping rates, it may be considered to fit multiple J-factors.

The European genetic resources strategy – joining forces in conservation and sustainable use

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European agro-food systems and value chains are dependent on the continued availability of diverse genetic resources. Negative trends in the conservation and use of genetic diversity in agriculture may reduce options for the future. The aim of the EU Horizon 2020 GenRes Bridge Coordination and Support Action is to develop an integrated European Genetic Resources Strategy (EGRS), and to accelerate collaborative efforts and widen capacities in plant, forest and animal genetic resources conservation and sustainable use. The three genetic resources networks (ECPGR, ERFP and EUFORGEN for plant, animal and forest genetic resources, respectively) are central to region-wide efforts. The implementation of the EGRS will minimize the loss of genetic resources, support diversification and innovation, and build resilience in agriculture and forestry. It will enable adaptation to changes in climate, production systems and consumer preferences, and ensure that these vital resources are available for future generations. The EGRS is pan-European in scope and complementary to policies of the EU Green Deal. This challenge session includes presentations and reflections of representatives of major stakeholder groups, from the GenResBridge consortium and from different genetic resources domains. Presentations will be followed by a round table discussion about collaboration opportunities between actors and domains implementing the European Genetic Resources Strategy.

Conservation of genetic resources at landscape level through demonstration cases

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This study was realised in the context of the GenRes Bridge project (EU Horizon 2020 R&I programme, grant agreement No 817580). The general objective of the GenRes Bridge project is to strengthen and improve integration of the conservation and sustainable use of genetic resources in the plant, animal and forest genetic resources and three government-backed regional programmes (ECPGR, ERFP, and EUFORGEN) in Europe and neighbouring countries. One of the key actions of the project is the development of an over-arching European genetic resources strategy, that will form the backbone of the future approach to genetic resources conservation and management in the region. One element of which is to identify landscapes with high levels of genetic resources of livestock breeds, agricultural crops and forest trees that coexist and are managed jointly. This approach is in line with the Strategic Priority 5 of the Global Plan of Action for Animal Genetic Resources, which aims to promote agroecosystems approaches to the management of AnGR. The landscapes selected for the demonstration cases cover three broad areas: Aurland, in Norway; Ehden Natural Reserve in Lebanon; three sites on Alps. Using the selected demo-cases we investigated domestication, management, inter-dependencies between plant, animal and forest genetic resources and the interaction both within the three genetic resources communities and with wider biodiversity. The analysis results from the selected landscapes are used to demonstrate the value of integrated conservation action and help raise awareness of the value of genetic resources. Different target messages were formulated from each demo-case. However, as a general conclusion, the results provide an evidence-base that establishes the advantage of taking an integrated approach to genetic resources conservation, which improves landscape resilience and the production systems sustainability collectively and within each domain. Conservation within Genetically Diverse Landscapes place the focus on the importance of the GenRes, which in turn underpin our global food security and ecosystem services.

Transboundary breeds in Europe: promote concerted action between countries

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This study is part of the European Regional Focal Point (ERFP) for Animal Genetic Resources (AnGR) and its Ad Hoc Action on Transboundary breeds in Europe. The history of the European region, its countries and farmers, national and international regulations played and still play an important role in the management of Animal Genetic Resources (AnGR). Conservation of AnGR in each country is mostly organized around its native and locally adapted breeds. Efficient breeding (and conservation) programmes, especially for small populations, could present difficulties due to several reasons, both technical and economical. The presence of breeds in two or more countries (transboundary breeds) could be an opportunity to improve breed management when all populations are considered together. The general objective of this Ad Hoc Action is to strengthen coordination between countries (not only EU Member States) and Breeders Associations for the conservation and sustainable management of endangered transboundary breeds. This is also fully integrated with the development of EUGENA and it is based on the issues raised during the Dialogue Forum of the IMAGE project. The overall objective is to propose ways to develop recommendations for the efficient management of transboundary breeds (especially endangered) through the development of appropriate ERFP guidelines. A variety of cases is presented, based on specific classification criteria, representing a diversity of species, geographical areas and management practices are discussed. This work improves the exchange of knowledge between countries and contributes to the definition of common actions that could be undertaken.

Update on TechCare: innovative technologies to improve small ruminant welfare management

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This paper presents the over-arching latest advances of the multi-actor approach project, TechCare, which aims at developing appropriate early warning systems and business models using innovative technologies to improve welfare management in EU small ruminant production systems. Small ruminants play a key socio-economic role in Europe, especially in harsh environments where innovative technology is not yet commonly adopted. Small ruminants are often managed as a flock/herd, allowing only average welfare states to be considered. Innovative technologies are a unique opportunity to improve small ruminant welfare management at the individual or flock/herd level, along the value chain. To this aim, in each participant country, stakeholders will be involved to prioritize the most relevant welfare issues and corresponding indicators. In a second step, they will contribute to assess an array of country and production system-tailored innovative technologies and early warning systems with potential for managing the prioritized welfare issues. These will then be tested on pilot farms in different conditions and validated at large-scale. The best performing technologies and systems which successfully pass all these steps will be evaluated for their viability in terms of business models. Whilst other consortium authors will present details of specific project activities (namely – stakeholders' engagement, welfare challenges identification and innovative technologies inventory), this paper focus on presenting preliminary activities and goals of the project to ensure awareness to a wide range of small ruminant stakeholders.

Developing a welfare prioritization for small ruminants – the TechCare project

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The welfare of small ruminants (SR) is often perceived to be good, particularly in comparison to more intensively farmed species. However, the extensive environment can reduce opportunities to observe and detect welfare issues, and SR are often not managed as individuals. The TechCare project aims to provide solutions to these issues by developing methods to manage welfare through the use of sensors and PLF approaches. SR management is very diverse in the EU: with production systems, ranging from very intensive dairy systems to very extensive meat systems. Both production purpose and system have significant impacts on potential welfare issues for SR. As a first step for TechCare, a welfare prioritization exercise was conducted. Building on previous EU funded work in the AWIN project and a literature review, a preliminary list of 80 welfare issues per species was developed, considering all the issues along the production chain. Within the TechCare experts, the lists were then prioritized to develop a shorter list of approximately 30 issues per species and management systems. The most important issues that span systems include: disease, lack of sufficient colostrum in lambs/kids, and heat stress, with issues of gastrointestinal and ectoparasites and undernutrition being important in grazing systems, and environment and competition between animals more relevant in housed systems. The final prioritization will be achieved through structured discussion with stakeholders and will guide the future development of the TechCare project.

A stakeholder co-design approach for improving small ruminant welfare: the TechCare Project

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The small ruminant (SR) value chain in Europe is a complex matrix of stakeholders with very different interests and profiles where it is not possible to establish one-size-fits-all solutions. The aim of this paper is to present the TechCare strategies to manage animal welfare using innovative technologies, that are investigated through a multi-actor approach which takes into account the local SR production systems and involves all relevant stakeholders, from farmers to transporters, slaughterhouse, consumers and regulators. This co-design approach aims to: (1) Identify all relevant actors in SR value chain and train facilitators to engage stakeholders in continuous interaction and feedback to the rest of the project activities; (2) explore attitudes, behavioural dynamics and perceptions of stakeholders and their implications for prioritizing welfare issues and using innovative technologies to manage animal welfare at different production stages, and analyse the sustainability of the identified technologies by exploring their environmental, social, economic and cultural impacts. A series of engagements (i.e. one-on-one interviews, focus groups, questionnaires, nominal group techniques, concept mapping, etc.) will be set at regular stages in the project, for consultation and exchange of ideas and thus allowing co-design and co-innovation in a multi-actor approach. Among those, two national workshops per year are planned in each of the nine partner countries to allow feedback and to effectively design business models and develop training and dissemination activities.

State-of-the-art in precision livestock farming technologies for monitoring small ruminant welfare

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Knowledge on the use of PLF technologies (Tech) at different levels of the value chain for small ruminants (SR) was updated. Their uses for addressing animal welfare (Care) were analysed with a scientific literature review (SciRev). Keywords (n=67) were identified and SciRev was launched by TechCare partners, using several engines. A total of 991 abstracts were identified. Records entailing both Tech and Care (TC papers) were retained and classified according to the Tech and targeted Care indicators, animal species, production type and farming system. The outcome was a shared database compiling details of 368 TC for which a corresponding Mendeley PDF database was built and made available online. Preliminary analyses shows that most frequent Care issues are related to malnutrition (monitored by behavioural sensors), general or chronic fear, transport and heat stress. Image analysis (video camera) is the most quoted Tech, followed by GPS (mainly for animal geo-referencing), accelerometry, heart rate measures and ultrasonography. Many technologies are used to monitor the same Care issue. The range of Tech is narrower for adequate housing, where environmental sensors are often coupled with body thermal condition/emission. There are few Tech for detecting mastitis, lameness and endoparasites. Preliminary results suggest that early warning systems based on different interoperating sensors are needed to effectively monitor welfare issues such as malnutrition. Further research is required to match effectively Tech and prioritised Care issues in the context of TechCare project.

Validation of a 3D imaging device to measure new morphological phenotype on ewes

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Monitoring of body condition and/or morphological changes is essential for optimal management of ewe health and welfare, but also production and reproduction performance. However, due to implementation difficulties (handling, workload, skills and training), body condition scoring is rarely implemented on commercial farms. Modern technologies based on three-dimensional (3D) shape analysis combined with electronic identification could address this issue. The purpose of the present study was to develop, test and validate a device that can record and analyse 3D body shapes of shorn ewes. Manual measurements on 12 Vendéen breed ewes (gold standard) were compared to measurements from the 3D images. Height at withers (HW), chest circumference (CC), chest depth (CD) and chest width (CW) were registered. Correlations between 3D device and manual measurements were 0.37 for HW, 0.80 for CC, 0.80 for CD and 0.82 for CW. For the 3D system, the repeatability standard deviation ranged from $1.53E^{-03}$ to 1.65 (coefficient of variation (CV) from 1.54 to 3.77%) and the reproducibility standard deviation ranged from $2.36E^{-04}$ to 0.77 (CV from 0.3 to 1.17%). Repeatability values are very close between the two methods, and 3D device measurements are more reproducible than manual measurements. In the future, automatic determination of ewes body condition score thanks to this technology will be tested, as well as the possibility of measuring new phenotypes such as the volume or the surface, which are of many interest in ewe selection and production.

Animal-based indicators to improve small ruminant welfare using precision-livestock farming

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Extensive livestock management systems are perceived by consumers as providing high animal welfare, as animals' behavioural needs are more easily met. In reality, a changing environment and rare opportunities for monitoring, diagnosis and treatment lead to unique welfare risks. Sheep producers farming extensively face many challenges to the regular assessment of flock welfare, including a dwindling labour supply, thin profit margins and animals spread across vast areas. Despite the availability of technology to monitor the behaviour of other species, such as cattle, the development of technology for use on sheep farms is at an earlier stage. Welfare can be assessed by animal-based measures, such as behaviour and physiological traits, which are useful in providing insight into animals' experiences. This PhD project focuses on identifying valid, reliable and feasible sheep welfare indicators that can be measured by PLF technology in extensive systems. This will be achieved by examining three examples of sheep welfare concerns: lameness, gastrointestinal parasitism and mastitis. The behaviour of sheep affected by these conditions will be assessed using quantitative and qualitative methods; behaviour sampling and scan sampling will be carried out in person, as well as qualitative behaviour assessments. Video recordings will be analysed using tracking software to quantify behaviour. Additionally, sensors such as accelerometers and proximity loggers will collect behavioural data. This project will partly consist in continuing the ongoing validation work for these types of wearable technology. However, it also aims to gain understanding on the application of more passive sensor systems, which are less costly and thought to be more acceptable to sheep farmers. Bringing these types of tools to market for sheep producers could contribute to improving the lives of millions of sheep farmed across Europe and answer consumers' calls for high animal welfare production systems.

Monitoring post-weaning behaviour in Merinos d'Arles ewelambs at grazing with a walk-over-weighing

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One-hundred weaned Merinos d'Arles female lambs (24.8±3.44 kg of liveweight, LW), were allocated in a grazing experiment under Mediterranean conditions. The objective was to study: (1) their adaptive behaviour during the transition period (i.e. after being separated from their mothers) and; (2) the adaptation of such naïve ewelambs to voluntarily cross through a walk-over-weighing (WoW), strategically placed in the paddock for the automatic monitoring of the individual LW. The experiment lasted five weeks. Animals grazed a mixed sward (ryegrass and native herbaceous legumes), and were submitted to a rotational system in paddocks (0.33 ha) grazed during 5 days each. The progressive voluntary daily number of passages through the WoW (N°Pass), looking to visit the water trough, molasse and the mineral salt blocs, was assessed, as it was the daily kinetic of visits (CrossK) and the number of biologically plausible LW records (PlausLW) registered by the WoW. With valid LW records the average daily gain (ADG, g/d) was calculated. The N°Pass increased with time, attaining the last three weeks around 340 passages/d. CrossK shows a normal distribution from 8 am to 6 pm, with a peak of passages between 7 and 8 am. During the first week of the experiment, PlausLW (records/d) was low (28), but improved with time (130, 174, 170 records/d last 3 weeks, respectively). Variability in the intra-group ADGs increased between the first and the last week (i.e. sd=0.06 kg/d, sd=0.13 kg/d). According to the overall behaviour and growth performance, it was concluded that these ewelambs were considered adapted to the grazing system as well as to the use of the WoW after 21 d. Using the WoW, under the conditions of this study miming real-life production conditions, allowed us to perform a close monitoring of the grazing behaviour and performance of these growing animals.

Sm@RT: small ruminant technology – PLF and digital technology for small ruminants

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Rural economies across Europe, and associated countries, heavily rely on small ruminant (SR) systems, particularly in areas unsuitable for other agriculture livestock production systems. Despite recent advances in precision livestock farming (PLF) and digital technologies (DT) to facilitate farm management, there is a slow rate of uptake by SR producers. This paper presents the project Sm@RT, which aims to improve the understanding, awareness and uptake of different technologies currently available to the SR sector, and further to facilitate 'solutions to needs' and identification of technology gaps. Sm@RT focus is on the use of PLF and DT across all types of sheep and goat management systems and environments, to develop a long-term, self-sustainable European/International network to encourage the use of PLF and DT across the sectors. Eight countries (UK, Ireland, Norway, Estonia, France, Italy, Hungary, Israel) are involved in Sm@rt. The paper presents the original project's approach: an interactive transdisciplinary and multi-actor approach relying on well-equipped demonstration farms ('digifarms') and innovative commercial farms to foster exchanges within the SR industry. It is an iterative step-by-step approach to engage relevant actors from all stakeholder groups at the appropriate level in the knowledge exchange process, crucial to boost dissemination in a trusting environment. Sm@RT will create opportunities to collect and exchange technical and practical knowledge, with information and guidelines on how to use PLF and DT to gain the best benefits for SR systems of production. This will increase efficiency and sustainability of SR farming systems in Europe and beyond.

SmartPigHealth, flagship innovation experiment in the SmartAgriHubs project: outline and insights

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The SmartAgriHubs project aims to accelerate the digital transformation of the European agrifood sector. A broad network of Digital Innovation Hubs and competence centres will be built throughout Europe. This multi-layer approach is supported by 28 Flagship Innovation Experiments (FIEs), in which innovative ideas, concepts and prototypes are further developed and introduced into the market. SmartPigHealth (SPH), a FIE, intends to lower the abundant use of antimicrobials in pigs. This is to be achieved by improving animal health and welfare. SPH aims both for an investigation of the interactions between animal health and climate criteria as well as the interactions between climate criteria. Predictive analytic methods will be developed to give a prognosis of upcoming diseases and mal-conditions. To examine animal health, veterinarians assessed health of the pigs every other day during a period of 6 waves. To gather information about the situation 24/7 in the barn, digital technology was implemented to measure the temperature (18 sensors), luminosity (4 sensors), humidity (4 sensors), NH_3, CO_2, and the water consumption (9 sensors). The production data of 1,900 piglets was recorded. The barn was divided into 8 pens with a central aisle. The results reveal large differences in the relationship between sensor data with respect to type of sensor and location of the sensor. The relationship between the data of the 16 temperature sensors placed in the pens is very strong ($r>0.94$). The correlation between sensor data of NH_3 and CO_2 is 0.53. The interactions between NH_3 and the remaining sensor data are depending on the position of the temperature and humidity sensor. A decreasing aisle temperature is indicating an increasing NH_3 value. The proportion of water intake of fluid feed and free water is changing during the wave. This is impacting on water supply management.

Session 15 Theatre 1

Iceberg indicators for sow and piglet welfare

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Animal welfare is a multifactorial concept. In order to find general acceptance, an animal welfare assessment scheme should account for all the different components of animal welfare. However, existing welfare assessment schemes such as the Welfare Quality® protocols have been criticised for being too time-consuming. Variable reduction is one possibility to enhance feasibility in this sense, however, it must be assured that still all different components of welfare are assessed. One possibility to achieve this is the identification of indicators with special significance, hence, indicators that display multiple components of welfare, so-called iceberg indicators. Hence, focussing on welfare assessment in sows and piglets, this study identifies iceberg indicators. Data collection took place between September 2016 and April 2018. In 65 farm visits, the Welfare Quality® protocol and a national protocol were carried out. Data were analysed using partial least square structural equation modelling (PLS-SEM). A hierarchical component model was built with animal welfare representing the higher-order and the principles of the Welfare Quality® protocol the lower-order components. In sows, welfare was revealed to be most influenced by the principles good housing, good health and appropriate behaviour (path coefficients=0.77, 0.86, 0.91). A large amount of variance was explained as indicated by high coefficients of determination R2 (good housing R2=0.59, good health R2=0.75, appropriate behaviour R2=0.83). Stereotypies was the indicator most valuable to assess sow welfare. Additionally, the final model included the indicators panting, shoulder sores, metritis, mortality and an indicator assessing stereotypies in resting animals (indicator reliabilities 0.54-0.88). However, the model did not include the indicators lameness and body condition, which may be due to the farm sample. Welfare of piglets was most explained by the indicators carpal joint lesions, mortality, sneezing and undersized animals (indicator reliabilities 0.48-0.86).

Session 15 Theatre 2

Tail posture as an early indicator of tail biting – a comparison of animal and pen level

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One of the major welfare issues in rearing and fattening pigs is the occurrence of tail biting. The tail posture is potentially an early indicator of such an issue. The aim of the study was to investigate, whether there are differences between monitoring of tail posture at animal or at pen level. This study used the data of two trials, in which 480 (trial one) and 368 (trial two) piglets were observed for their tail biting behaviour for forty days during rearing period. In this period the pigs' tails were scored twice a week for tail posture (lifted or lowered) and tail lesions, which resulted in twelve scoring days. With a random regression model (Legendre polynomials), the random animal and pen effects were calculated at every scoring day for tail posture and tail lesions. Using the Pearson Correlation Coefficient between the random effects, the correlation between tail posture and tail lesions for every scoring day was calculated. All correlations were significant ($P<0.05$). The correlations between tail posture on scoring day x and tail lesions on the following scoring day x+1 increased from scoring day two to scoring day ten on trial one at animal level (0.26 to 0.52) and at pen level (0.65 to 0.70). In trial two, the correlations ranged from 0.32 to 0.44 at animal level and from 0.61 to 0.82 at pen level. The highest correlations (>0.9) were determined in trial two at pen level on scoring days five to nine. The correlations between tail posture on scoring day x and tail lesions on scoring day x+2 were nearly at the same level as mentioned before. Therefore, there is a relationship between a lowered tail posture and consequent tail lesions. Thus, the tail posture serves as an early indicator of tail biting up to a week before the first tail lesion occurs. Furthermore, the correlations in both trials are higher at pen level than at animal level. Therefore, the farmer should observe the tail posture at pen level to be able to intervene before or in the early phases of tail biting occurrences.

Iceberg indicators for finishing pigs' welfare

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The evaluation of finishing pigs' welfare by the Welfare Quality® protocol provides an acceptable opportunity of assessing their welfare status. Nevertheless, the implementation of the protocol is complex, so that it is necessary to identify possibilities, to improve feasibility. Therefore, this study examined collected data (n=197) of the Welfare Quality® protocol in finishing pigs by using multidimensional decision trees (J48) in MEKA 1.9.1 or the procedure PROC PLS in SAS® 9.4 to identify Iceberg indicators which can predict the welfare principles good feeding, housing and health and appropriate behaviour. The processed data set contained the criterions recorded on farms and slaughterhouses that were observed with a prevalence ≥0.4%. Thus, the implemented decision trees revealed the indicators pericarditis, mortality, white spots on liver, bursitis (score 0), absence of manure on the body (score 0), tail docking, pneumonia, qualitative behaviour assessment (QBA), exploratory behaviour and fear of humans as most suitable to predict the four welfare principles (accuracies: 0.64-0.83; hamming loss: 0.28). These results largely agree with those of the PLS procedure as the variable importance plot (VIP) (threshold for the VIP was set at ≥0.8) showed the criterions bursitis (score 0), absence of manure on the body (score 0), wounds on the body (score 1), tail docking, negative social behaviour, exploratory behaviour and QBA as the most suitable indicators to estimate the welfare principles good feeding, housing and health and appropriate behaviour. In consideration of reliability studies, these identified Iceberg indicators could improve the feasibility of the objective animal welfare assessment in finishing pigs on farms.

Animal welfare indicators for pigs – guideline and tools for on-farm self-assessment by farmers

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Since 2014, German pig farmers have to assess and evaluate their own animals' welfare with animal welfare indicators in accordance with the German Welfare Act. However, the legislation does not provide hints on how to implement this legal requirement. In 2016, the Association for Technology and Structures in Agriculture (KTBL) published the practical guideline 'Animal welfare indicators – pigs'. It presents several indicators for on-farm self-assessment targeting relevant animal welfare problems in practice. Furthermore, it describes how farmers can implement self-assessment on-farm with a random sample of animals. In a collaborative project, further application tools were developed and the indicators of the guideline were tested regarding their feasibility with 34 pig farmers. All pig farmers were trained (online or live) and afterwards they assessed the indicators on their own animals in parallel with a scientist. After on-farm assessment, farmers provided feedback that most of the indicators were easy to assess. However, they express their concerns on the high effort for the assessment. Thus, the Excel® application 'Animal Welfare Indicators – Assessment' was developed as a first offer for digital data collection and calculation aiming to reduce the effort for the assessment. Finally, based on the on-farm tests of indicators and the feedback from farmers and stakeholders, the guideline was revised and the second edition was published in the end of 2020. In addition, an open access online training was developed allowing all interested parties to obtain information on animal welfare indicators and to practise their assessment. Furthermore, reference values were published in 2020, which enable farmers to evaluate their results of on-farm self-assessment in terms of good, early warning and alert animal welfare situation. These reference values are based on a Delphi-survey and a literature review and were agreed in a multi-stage process including numerous stakeholders. All application tools are available on: https://www.ktbl.de/themen/tierwohlbewertung (in German).

Smart animal health – data-driven planning of animal welfare inspections

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The goals of the Smart Animal Health project are to develop data-driven methods to assess and foster the ever-more important health and welfare of livestock. This work focusses on leveraging the existing Swiss governmental databases and registries to build a metric that represents the health and welfare state of a farm. We present proof-of-concept methodologies aimed at optimising the on-farm controls by proposing a list of priority holdings that are more at risk of animal welfare violations. Since the data collection and coverage are best for cattle and pigs, we focused on these livestock species. After data pseudonymisation, the different databases were linked to extract as much information as possible on the holdings. Data for individual animals were aggregated at the herd level. The results of previous on-farm inspections were used to derive a simple welfare index, which is the prediction goal. Several types of supervised machine-learning techniques were used to estimate the risk of violations. This approach is based (mostly) on proxy information such as the participation in health and welfare promoting programs, herd type and size, notifications of major events in the herd and farm structural data. Most of the techniques reach similarly good classification performances. The explainability of the model is important both for public acceptance of such a data-driven index and the planification of on-farm inspections. The performance of random forests, for which the decision process can be understood, were investigated in depth. This study highlights that data-driven monitoring of the livestock population is already possible with the existing datasets in Switzerland. We recommend to systematically register or make available several additional proxy parameters. The goal is to increase the performance of our models and to allow further development of data-driven methods, including but not limited to the monitoring of animal health and welfare.

Smart animal health – health indicators for livestock

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Animal health and welfare are important topics in modern livestock and food systems. The aim of this project was to develop a method to assess animal health and welfare for different livestock species in Switzerland, focusing on animal-based indicators as well as data-driven metrics that can serve as proxies for the health and welfare status of a given farm. The indicators shall allow to assess the animal health and welfare status at the level of individual farms, groups of farms and the Swiss livestock population as a whole. In a first step, the scientific literature was systematically reviewed to identify potential indicators of animal health for cattle, sheep, goats, pigs and poultry. The identified indicators were then categorized and integrated into four categories, based on feedback from relevant stakeholders from the field: Husbandry and feeding; Absence of clinical signs; Appropriate behaviour and Freedom from pain, suffering, harm and anxiety. The method was applied in the field for different farm types and compared with outcomes of the Welfare Quality scores from the corresponding farms. To investigate data-driven metrics, different databases and national registries that provide information on farm demographics and on animal identification, traceability, diagnostic results and animal welfare inspections were pseudonymised and interconnected. Supervised machine learning techniques were applied to model the status of the farms and to identify and suggest farms for risk-based animal welfare inspections. The developed method will allow to: observe changes in the health status of the livestock population over an extended period, assess the effectiveness of measures to improve animal health and welfare, recognize and promote especially good livestock farms with financial incentives and carry out more targeted animal welfare inspections. The research project will be concluded by the time of the conference and results will be available for presentation.

Session 15 Theatre 7

Moving hens from cages to floor: is this an economically sustainable option?

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A considerable number of retailers and food companies have committed to supply only cage-free eggs by 2025 or even earlier. In addition, EU legislation increasingly supports laying hen welfare improvement and several Member States have already banned all types of cages for laying hens. The aim of this study was to estimate a decision-making model for the economic sustainability of moving laying hens from cages to a floor system. More specifically, the model simulates the transition of a 12,000-laying-hens farm from enriched colony to deep litter – barn production system. Input data were based on available literature whereas egg and infrastructure prices and costs were estimated based on survey in the Greek retailers' and suppliers' market. The year of transition was considered to be the 5th year of operation and the analysis was examined over a 10-year period. The differences in revenues and expenses between the two production systems were analysed using the Partial Budget method and the decision criteria of net present value (NPV), the internal rate of return (IRR) and payback period (PBP) were also estimated for assessing financial viability of the transition under investigation. Financial analysis and partial budgeting showed positive economic results. Partial budgeting showed that transition from cages to floor system led to a positive change in net income of € 32,985 in the first year of operation. Income was improved in all periods throughout the 10-year analysis. Both NPV (€ 12,972.67) and IRR (9.27%) criteria showed that the investment can be considered acceptable. In conclusion, transition from a caged to a floor production system in laying hen farms is financially viable. However, further market analysis is required in order to establish egg producing farmers transitioning to improved welfare production systems.

Session 15 Theatre 8

Providing environmental enrichments affects activity and performance but not leg health in broilers

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Leg problems are one of the most important factors affecting health and welfare of broilers. Previous studies showed that providing enrichments can reduce the risk of leg problems, but it remains unknown whether or not these effects are similar for fast and slower-growing broilers. Therefore, the aim of this study was to identify effects of environmental enrichment on behaviour, walking ability, contact dermatitis and performance in fast and slower-growing broilers. Day-old male broilers, 420 fast-growing (Ross 308) and 420 slower-growing broilers (Hubbard JA757), were housed in a 2×2 factorial arrangement, using a complete randomized design. Broilers of both strains were randomly allocated to enriched (EE) or non-enriched (NE) pens (total of 28 pens, 3 m^2). EE pens included barrier perches, ramps, platforms and a dustbathing area. In addition, black soldier fly larvae were provided daily in the dustbathing area and broilers in NE pens received an additional protein-fat mix, to achieve similar energy and nutrient intake compared to the EE treatment. Behaviour was observed by scan sampling and focal sampling in weeks 2, 4, 5 and 7 of age. Walking ability and contact dermatitis were measured in 3 broilers per pen at a body weight of 1.7 and 2.6 kg. Performance was determined weekly. Broilers of both strains in EE pens showed longer duration of activity compared to broilers in NE pens. Behaviour analyses based on comparable body weight of both strains are in progress. No effects of enrichment were observed for walking ability and contact dermatitis. Broilers in NE pens had a higher average body weight, a higher average daily gain and a higher feed intake than broilers in EE pens. In conclusion, providing enrichments increased activity to a similar extent in both strains, but did not improve walking ability and had an adverse effect on performance in both strains.

Session 15 Theatre 9

Welfare assessment in veal calves fattened in 'outdoor veal calf' versus conventional operations

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The 'outdoor veal calf' system was developed to encounter the demand for a veal fattening system that allows for reducing antimicrobial use without impairing animal welfare. Management improvements including direct purchase, short transportation, vaccination, quarantine in individual hutches during three weeks, and open-air housing in small groups in a roofed, straw-bedded paddock with a group hutch were implemented in a prospective intervention study (1,905 calves, 19 intervention and 19 control farms, one year): antimicrobial use was five times lower in 'outdoor veal' farms compared to control farms ($P<0.001$), but it was crucial to ensure that antimicrobial treatment reduction was not associated with decreased animal welfare, i.e. that sick animals were not left untreated. Welfare was assessed monthly on the farms and organs of 339 calves were examined after slaughter. Cough and nasal discharge were observed significantly ($P\leq0.05$) less often in 'outdoor veal calf' than in control farms, mortality (3.1 vs 6.3%, $P=0.020$) and lung lesion prevalence (26 vs 46%, $P<0.001$) were lower; no group difference was seen in abomasal lesion prevalence (65 vs 72%). Thus, beside reduced antimicrobial use, calf health and welfare were improved in 'outdoor veal calf' farms in comparison to traditional operations.

Session 15 Theatre 10

A model for economic impact of animal welfare improvements at slaughter of cattle and pig

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Stress in animals prior to slaughter affects animal welfare as well as meat and carcass qualities, and may generate direct costs for the slaughterhouse business and the meat process industry due to reduced meat quality and condemnation of meat. Suboptimal design of slaughterhouse interior layouts and fittings acts to reduce animal welfare, but also leads to suboptimal workflow, impaired flow of animals through the slaughter process and thus reduced production efficiency for the slaughterhouse operator. In order to stay competitive, food business operators such as slaughterhouses need to optimise their economic returns. A literature review on the economic effects of animal welfare aspects at slaughter was performed, however only few studies previously published in this area were found. The overall aim of this study was to map the possible economic impact of improved animal welfare at slaughter at slaughterhouse business level. The specific aims were first, to develop an economic model, second, to map the impact of animal welfare improving practices based on scientific literature and third, to verify and adjust the economic model based on focus groups interviews. The results of our study can be used to support and improve the understanding of the economic aspects of animal welfare at slaughter and furthermore, enable informed decisions by policy makers.

Effects of slaughtering methods on stress and *post mortem* changes in rainbow trout

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The study evaluated the effects on stress and freshness evolution in rainbow trout (*Oncorhynchus mykiss*) slaughtered by percussion or immersion in cold saline water (-4/-8 °C; 5‰ NaCl) during 5 minutes. A total of 100 rainbow trout were sampled for measuring stress indicators (cortisol, malondialdheyde-MDA) in plasma and tissues (30 fish; 15 per treatment) and for evaluating freshness (pH, rigor mortis index, fillet and skin colour and muscular texture) at 3, 48, 168 and 264 h of storage (70 fish; 35 per treatment). When cold immersion was used compared to percussion, trout showed lower cortisol in skin mucus (4.33 vs10.4 ng/ml; $P<0.001$), muscle (14.8 vs 31.4 ng/g; $P<0.01$) and fin (34.9 vs 70.6 ng/g; $P<0.01$), and higher muscle MDA (20.0 vs 10.5 nmol; $P<0.001$). Plasma cortisol (184 ng/ml, on average) and MDA (13.7 nmol) were not affected. As for freshness evolution, the cold immersion sped up the onset of rigor mortis compared with percussion, but no differences were found between slaughtering methods in fillet pH (6.46), colour (L* 47.3; a* −0.89; b* 10.1), TVB-N (17.6 mg/100 g of fillet), fatty acids composition (% total FA) (EPA: 0.73%; DHA: 2.66%; PUFA n-3: 7.67%) or texture profile analysis. Some minor changes were observed according to storage time, that is TVB-N showed the highest value at 264 h of storage (15.8, 13.8, 14.2 vs 26.5 mg/100 g of fillet); pH changed from 6.57 at 3 h of storage to 6.39 and 6.37 at 48 and 168 h to raise at 6.50 at 264 h ($P<0.001$). In conclusion, based on cortisol changes, cold immersion was likely to be a less stressful slaughtering method compared to percussion. As for freshness, some differences were found on rigor mortis onset between the two methods, which however did not produce relevant effects on fish quality traits.

Citizen's opinions regarding pig welfare conditions detected at ante-mortem inspections

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The aim of this study was to assess the opinion of Chilean citizens with different level of involvement in animal production and different animal product consumption regarding welfare conditions detected in pigs at ante-mortem (AM) inspections. All participants filled the same survey. Citizens involved (I; n=157) and not involved (NI; n=551) in animal production were recruited in public places. Participants were asked about their level of agreement using Likert scale (1 to 4): (Q1) 'To what extent the presence of tail lesion, ear lesion, lameness, bruising, wound, and hernia produce suffering/pain in the pigs?' (Q2) 'To what extent each outcome can affect the quality of the meat?'. (Q3) Participants were also asked what they considered to be the most important investment to improve on farm animal welfare (among 4 options). Among participants, 103 were vegan, vegetarian or pescatarian (V). Mean value (±SD) for Q1 and Q2 were calculated and descriptive data are presented. The responses of citizens involved and not involved in animal production were similar in Q1 (I: 3.0±0.35; NI: 3.1±0.34) and Q2 (I: 2.3±0.87; NI: 2.4±0.66). Similarly, the responses of meat consumers (MC) and V were similar in Q1 (MC: 3.1±0.34; V: 3.1±0.36) and Q2 (MC: 2.5±0.72; V: 2.1±0.66). The most important investment chosen by participants were 'to improve the ability to detect and treat diseases on farm (33%)', followed by 'the usage of anaesthesia for male pig castration (27%)', 'implementation of training courses in animal management for farm workers (20%)', and 'provision of straw as environmental enrichment (20%)'. These results suggest that citizens have similar opinions regarding welfare conditions detected in pigs at AM inspections regardless of their involvement with animal production or animal products.

Location of burrows in housing: effects on their occupation rate and health of growing rabbits

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Providing growing rabbits with a 'burrow like' area may enhance their wellbeing, by allowing animals the possibility to search for a refuge and hide. Here, we studied the effect of burrow location inside a pen on its occupancy rate, health status and activity of 180 growing rabbits from 28 to 77 days of age. Animals were housed by 30 in large pens (152×90×80 cm, W×L×H) equipped with two burrows (58×37×30 cm with a circular opening; Ø 15 cm). Experimental groups differed in the location of burrows: two ground burrows (2GB, n=60 rabbits), two raised burrows (2RB, n=60 rabbits) accessible by a platform and one ground and one raised burrow (GRB, n=60 rabbits). Spatial distribution of rabbits was recorded by direct observation twice daily (morning and afternoon), 3 days a week for 7 weeks. The rate of active rabbits and the dynamic use of burrows were measured through video recordings (40 minutes, both in the morning and in the afternoon) at 69 days of age. The percentage of rabbits inside the burrows was higher in the 2GB (23.7%) than 2RB group (5.2%), the GRB group being intermediate (13.7%; $P<0.01$). In GRB group, rabbits clearly prefer the ground burrow to the raised one (78.1 vs 21.9%; $P<0.001$). The percentage of rabbits inside the burrows significantly ($P<0.001$) increased from week 1 (9.1%) to week 7 (18.5%). The rate of active rabbit was higher in 2GB group vs 2RB and GRB (52.6 vs 35.9%; $P<0.05$). The mean interval between two exits was 4.3 min for ground burrows and 14.7 min for raised ones. Average live weight at 77 days was 2,235 g and mortality rate was 12.2%, being similar among groups. Morbidity rate, however, tended to be higher in the 2GB group vs 2RB and GRB (15.0 vs 5.0%). This study demonstrated a significant use of burrow and a clear preference for its location on the ground. However, the high occupation of ground burrows combined with a flat solid floor (faeces accumulation) seems to degrade the hygiene condition, affecting the morbidity rate.

Management practices associated with footpad dermatitis in turkey flocks

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Footpad dermatitis (FPD), an ulcerative lesion of the footpad, is one of the most frequent diseases in turkey production. In addition to welfare concerns, birds severely affected by FPD demonstrate fewer behaviours, decrease feed intake and this reduces growth. FPD is a multifactorial problem and influenced by many aspects of production. The aim of this study was identifying factors associated with FPD prevalence in turkey flocks. A questionnaire and flock health-scoring guide was circulated to 500 turkey farmers across Canada. Farmers scored FPD on 30 birds in their flock using a 0-2 scoring scale based on severity. Prevalence of FPD in the flock was estimated as the percentage of affected birds (score 1 or 2). Univariable linear regression modelling was used to identify variables to be retained for multivariable analysis ($P<0.25$ or biologically relevant). Forward-stepping multivariable linear regression modelling then identified factors associated with the prevalence of FPD. Four variables were included in the final model and accounted for 26.7% of the variation in FPD prevalence: bird weight, litter type, picking up birds and using feed/water additives. As average bird weight (kg) increased, the prevalence of FPD was higher (3.6±1.13). FPD was also higher in flocks bedded with straw (12.1±7.9) and higher in flocks where the birds were examined individually less frequently during daily inspections (11.6±8.10). Lastly, FPD was higher in flocks that used feed/water additives to reduce litter moisture (20.5±10.59). These findings are an exploratory assessment of risk factors related to FPD prevalence on Canadian turkey farms and emphasizes the importance of litter management and the stockperson in reducing FPD. However, the estimates from this study need to be interpreted with caution and further longitudinal studies needed to assess the identified variables influence on FPD more accurately.

Using an omics-based strategy to unravel seasonal weight loss tolerance in small ruminants

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The use of Omics such as Proteomics, Metabolomics or Transcriptomics is gaining international recognition in the framework of animal sciences as a broad and high throughput research tool. Omics have multiple applications and have been used in very diverse topics, from establishing metabolic disease biomarkers to productive characterization or breed differentiation. Seasonal weight loss (SWL) is a pressing issue in animal production. Animals selected in SWL prone areas are well adapted to SWL. Understanding the molecular mechanisms of SWL adaptation is of high importance in animal selection. Over the last 20 years we have been studying the way different breeds of sheep and goat have adapted to SWL by using an Omics based approach combining proteomics, metabolomics and transcriptomics with production traits. Our research was mostly focused on different tissues: muscle and liver in meat producing sheep and the mammary gland secretory tissue in dairy goats. Herein, a retrospective of the work conducted over the last 20 years is presented, highlighting major findings within this research line and possible avenues for the future. Furthermore, we provide an emphasis on how Omics-based research can be of interest in small ruminant research activities, as well as the major advantages and drawbacks of this exciting novel field.

Liver transcriptomics of sensitive and heat-tolerant dairy goat phenotypes

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The objective was to evaluate liver transcriptomics response to heat stress (HS) in dairy goats differing in their phenotypical heat tolerance. Murciano-Granadina dairy goats classified as HS resistant (R; n=8) or HS sensitive (S; n=8) were used. The design was a crossover of 2 periods (2 weeks each) and 2 climatic conditions: (1) thermo-neutral (TN; 15 to 20 °C day-night); and (2) HS (day, 37 °C; night, 30 °C). Humidity (50%) and dark-light (12-12 h) were constant. Liver biopsies were obtained at the last day of each period using Tru-Cut 14G × 15 cm needles under sedation. Liver tissues were snap frozen in liquid N and stored at -80 °C until RNA extraction. RNA sequencing (2×101 bp) was performed with a HiSeq 4000 instrument. Sequencing reads were trimmed using TrimGalore, aligned to the goat reference genome with HISAT2, and quantified by StringTie. Differential gene expression (DGE) analyses were subsequently carried out by using DESeq2 (R software), in which the effects of condition (TN-R, TN-S, HS-R and HS-S), period (1 and 2) and their interaction were included. DGE was considered at $\log_2$ fold change >1.5 and adjusted P<0.05. Additionally, DAVID Bioinformatics Resources database was used for the functional enrichment of DEG. No differences were detected in the transcriptomic profile under TN conditions. However, in HS we detected 487 DGE (315 down- and 172 up-regulated genes) between HS-S and HS-R. These DEG were related to RNA transport, proteasome, metabolism of alanine, aspartate, glutamate and sphingolipid, non-alcoholic fatty liver disease, hepatitis C, and hepatitis B pathways. In conclusion, heat tolerance phenotype conditioned liver transcriptomic response to heat stress as evidenced by the significant changes in the expression of genes participating in several biological processes. These processes included amino acid and sphingolipid metabolism as well as pathways related to fatty liver and hepatitis diseases.

Single-step genetic evaluation of resistance to parasites in the Swiss Alpine goat population

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Increased resistance of gastrointestinal nematodes (GIN) against anthelmintics across the world raises the need for alternative approaches to control GIN in small ruminants. Next to flock or pasture management strategies, breeding for more resistant and resilient animals must be investigated. The heritability of three traits linked to goat resistance against GIN and their genetic correlations were estimated in the Swiss Alpine goats in a previous experimental study. Three traits were recorded as repeated measurements: faecal egg counts (FEC), FAMACHA© eye score (FAA), and packed cell volume (PCV). Due to being labour and cost intensive, the recording of these traits cannot be implemented in population-wide routine data collection. Consequently, the phenotypic datasets for these traits will remain small and breeding value (BV) estimation models must be developed that tap into the full potential of the available information. The current dataset comprised 948 animals genotyped and phenotyped for FEC, FAA and PCV. An additional 329 animals were genotyped in the context of routine SNP-based parentage verification. Due to the small data size, a genetic evaluation of each trait using a BLUP animal model limited the BV estimates to reliabilities between 0.10 and 0.20 for selection candidates. Any selection on these BV remains uncertain. Multi-step genomic selection was impossible to set up as no reference population of sufficient size could be defined. Single-step GBLUP models were evaluated for each trait to overcome the BLUP selection accuracy limitations. Estimated BV of young animals without phenotype nor progeny had reliability estimates above 0.30 already. Following those promising first results and in the context of the H2020 project SMARTER (EU grant no. 772787), further development at several steps, including model definition and ongoing phenotyping / genotyping, are required before implementation of a genetic evaluation for resistance against GIN in the Swiss Alpine goat national breeding program.

Using proteomics to differentiate wool fibres in Portuguese sheep: two case studies

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Wool properties and value vary considerably between ovine breeds. In Portugal, three major ovine groups exist: Churros, Bordaleiros and Merinos. Regarding the latter, there are two major breeds, the white and the black merinos. Such fibres have different uses and physical traits. This work studies the effect of the ovine genotype on the wool proteome of such groups. Wool was collected from 15 ewes/breed and genetic groups: Churra da Terra Quente (CTQ) or Churro, Serra da Estrela (SE) or Bordaleiro, Merino Branco (MB) and Merino Preto (MP). Proteins were extracted and subjected to two different experiments: (1) label-free proteomics analysis (CTQ vs SE vs MB); and (2) iTRAQ (MB vs MP). Over 50 keratinous protein groups were identified in all the samples, divided into type I and II keratins and the keratin associated proteins: high-glycine-tyrosine proteins, ultra-high sulphur proteins and high-sulphur proteins. Regarding experiment (1) major differences were found between MB and CTQ with respect to K75 and K38, both medullar proteins and to a lesser extent between SE and CTQ. Regarding experiment (2) the expression of HSPs, KAP2-3 and KAP2-4, decreased in the pigmented animals, whereas KAP13-1 increased in white Merinos. These differences indicate that such proteins may be suggested as putative wool quality markers, with relevance for the textile industry.

Genome-wide characterization of performance resilience to climate volatility in dairy sheep

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The Mediterranean basin is considered particularly vulnerable to climate change implying adverse impact on livestock sustainability. Milk production of semi-intensively raised small ruminants is of major economic importance and selection for resilient animals to climate change may constitute a plausible mitigation option. We studied the genomic architecture of performance resilience to temperature fluctuations under hot weather conditions (temperatures above 25 °C) in Chios dairy sheep. Reaction norm functions were fitted to random regression models to derive resilience phenotypes representing milk yield change in response to temperature volatility, utilizing daily milk records obtained from the Chios Sheep Breeders' Cooperative Macedonia. A total of 538 milking ewes raised in 3 flocks were genotyped with the Ovine SNP50K Bead Chip. Genetic parameter estimation, genome-wide association (GWAS) and regional heritability (RHM) analyses were conducted with mixed models. Genomic heritability of animal resilience was moderate ($h2=0.20+0.09$). Phenotypic and genetic correlations with milk yield were strongly negative (-0.79, -0.94). One genome-wide and five suggestive significant SNP markers were detected with GWAS on chromosomes 5 and 19. Furthermore, RHM detected two genome-wide regions and one suggestive significant genomic region harbouring these SNP markers. Subsequent gene annotation and functional enrichment analyses identified 94 candidate genes and biological processes relevant to adaptation to ambient temperature fluctuations and animal olfactory activity. Results support the notion to selectively breed for enhanced sheep resilience to climate change after the correlation of the latter with other breeding goal traits has been clarified.

Genomic inbreeding trends and the impact of ongoing selection in the Slovak Original Valachian sheep

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This is the first study regarding the genomic analysis, based on SNP data, done on the Slovak Original Valachian population. This study focused on analysing the inbreeding levels using runs of homozygosity (ROH) and identifying highly conserved regions based on ROH islands. A total of 94 animals and 42,686 SNPs remaining after quality control were analysed. As the main goal was to analyse ROH segments, the minor allele frequency was not filtered. The recent and historic genomic inbreeding coefficient was computed, accounting for the common ancestors from three to 50 generations in the past. These thresholds correspond to a minimum length of ROH set to 16 and 1 Mb, respectively. The average historic inbreeding coefficient considering 50 generations was 8%, but with high variable individual inbreeding levels (standard deviation 6.7%). The recent inbreeding considering ancestors up to three generations was 2% (standard deviation 2.7%). Such inbreeding levels were similar to other European populations, but such relatively high inbreeding levels could justify interventions and breeding managements to prevent biodiversity loss. The top 1% overlapping ROH regions, so-called ROH islands, were further analysed. Quantitative trait loci (QTLs) found in the 21 ROH islands across 12 chromosomes confirmed the multi-purpose character of the breed. Several QTLs for parasite resistance, somatic cell count and reproductive traits were also found, highlighting the fitness and adaptability traits of the breed, utilised in marginal, high altitude pasture areas.

Implementation of genomic selection in Norwegian Dairy goats increases breeding value accuracies

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Genomic selection (GS) is currently under implementation in Norwegian Dairy goats (NDG). All traits included in the breeding goal are measured on daughters with production records and the sire of a doe is 2.5 years of age when his estimated breeding value is based on progeny test. Only progeny tested bucks are currently accepted for artificial insemination (AI), but many bucks are culled prior to progeny test results. The main aim of the implementation of GS is to be able to select breeding animals at a higher accuracy at an earlier age. The current reference population counts 3,777 lactating does born from 2012 to 2019, and 771 bucks with daughters in production. Herds for genotyping of does were selected based on their phenotypic recording and use of AI. Genotyping was done using Genotyping by Sequencing (GBS). Single Nucleotide Polymorphism (SNP)s were kept if they were autosomal (when mapped to the ARS1 goat assembly) and if they passed appropriate Hardy-Weinberg criteria. A breed-based genomic relationship matrix was calculated using the KGD method, based on the VanRaden method I but accounting for read depth, and individual allele frequencies based on breed proportions of NDG and French Alpine. Fifteen years of phenotypic information of test day records of kg milk, gram fat, gram protein, gram lactose, fat pct, protein pct, lactose pct, milk somatic cell counts, milk free fatty acids, udder conformation and milking speed were extracted from the dairy database along with pedigree information counting 1.5×10^6 test day records and 0.5×10^6 animals in the pedigree. Genomic enhanced breeding values were computed jointly for all 11 traits using single-step genomic best linear unbiased prediction (ssGBLUP) in the DMU package. A weight of 10% on pedigree relationships were applied to all traits. Model accuracies were computed for one trait at a time and showed 3-4% increase in accuracy for proven bucks, while the increase in accuracy was 20-25% for the youngest year class. This indicates that it is possible to select bucks for AI at a higher accuracy at a younger age when introducing GS.

Variation in the CCR5 gene in Swedish goats

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Swedish landrace goat is the most common goat dairy breed in Sweden. There are also three more rare local breeds (Göinge goat, Jämt goat and Lapp goat) originating from different parts of Sweden. The disease caused by the Caprine Arthritis Encephalitis Virus (CAEV) is a problem within the Swedish goat production. There is a control program but only a small part of the Swedish goat herds are enrolled in it. The obstacles for the control program have been administrative problems with enrolling small herds and also that many owners of the rare breeds do not want to joint the control program since they do not want to slaughter the whole herd if the disease is detected. An alternative way to tackle the problem with the disease would be to breed for goats that are resistant to the disease. A recent study in Chamois coloured goats found a mutation (g.1059 T) in the promoter region of the CCR5 gene that is associated with the proviral load. We wanted to investigate the genotype at this site in the Swedish goat breeds and sequenced a 329 base pairs of the promoter of CCR5 in samples from the four Swedish breeds. Our results showed the unfavourable T allele at position g.1059 at high frequencies in Swedish goats. Although the mutation g.1059 T was present in all four breeds, the frequency differed a lot between the studied breeds. In the Swedish landrace goat from which we had most samples we could also see that the allele frequency differed between herds. The fact that there is variation in this site in Swedish goats suggests that it would be possible to breed for resistance to CAEV. We also found a mutation that occurred in quite high frequency in the Swedish goats. The relationship between this mutation and CAE remains to be studied.

Comparative rumen microbiomics of livestock breeds from Portugal and Africa

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It is widely accepted that the microbiome composition of livestock animals is key in affecting a number of physiological processes underlying animal health and production performances. While this awareness sparked the interest mostly towards commercial breeds of utmost socioeconomic relevance, local breeds from marginal areas remain largely neglected in this respect. However, the latter are agrobiodiversity resources of primary importance at regional level and are part of the cultural and historic heritage of the rural communities who selected them over millennia. Moreover, these breeds often developed unique adaptations to extreme environments, which makes them commercially interesting in an epoch of unprecedented climate change and could contribute to the attainment of UN 2030 Agenda goals. In this study we described and compared the rumen microbiome composition of local livestock breeds from Portugal (which despite its limited extension hosts a wide variety of habitats and a sharp latitudinal gradient from the cool and rainy Atlantic regions in the north to the warm and dry Mediterranean regions in the south), Tunisia (encompassing milder region along the coast and hot-dry regions in the south), and Angola (hosting extremely arid internal region). The study focused on the bacterial components characterized by metabarcoding and shotgun sequencing. The genomic data produced will pave the way for future transcriptomic analyses and cell culture processes to disentangle which genes are actively expressed and by which microbial taxa.

Genomics to breed sheep resistant to footrot

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Ovine footrot is a complex disease mainly caused by *Dichelobacter nodosus*, which is clinically characterized by interdigital dermatitis and under-running footrot. The objectives of our research project are to elucidate genomics of resistance to footrot within and across sheep breeds. In a large number of flocks in Germany, we recorded the prevalence of footrot using clinical data and the load of benign and virulent strains of *D. nodosus*. On farm data recording in 207 flocks comprising 11,500 sheep was done using a mobile electronic hand-held system for individual animal ear tags and data input. We employed qRT-PCR to differentiate benign and virulent *D. nodosus* strains for classification of the footrot status of flocks. Based on flock prevalences for *D. nodosus*, we were able to distinguish resistant, tolerant and susceptible animals. Genotyping was done for approximately 4,500 sheep on ovine SNP50, ovine GGP50 and ovine infinium HD SNP beadchips in Merino, Leine, Suffolk and East Friesian for 250-650 animals and across several breeds for at least 50-100 animals including Bentheimer, Dorper, Grey Heath, Forest sheep, Romney Marsh, Texel, White Polled Heath, White Hornless Heath and Pomeranian coarsewool. We found heritabilities for resistance to footrot using mixed models with genomic relationship matrices at h^2=0.6-0.8 for the different breeds. Heritabilities for footrot scores were at 0.20-0.40. Genome wide association studies (GWAS) with mixed models showed significantly associated regions within breeds. Breeds with highest similarities in genomic relationship matrices were simultaneously analysed using across-breed GWAS. Whole genome sequencing data from 385 individuals were employed to filter for highly associated variants. On whole genome sequences imputed genotyping data allowed us to validate highly associated regions and variants within and across breeds.

Sequence-based runs of homozygosity in goats reveal genetic changes associated with domestication

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The domestication of goat (*Capra hircus*) started 11,000 years ago in the fertile crescent. Breed formation in the 19th century and recent developments resulted in 10 modern goat breeds in Switzerland. We analysed whole genome sequences (WGS) from 217 domesticated goats and 9 wild bezoar goats (BEZ). After quality control 27,728,288 single nucleotide variants were used for the elaboration of runs of homozygosity (ROH) and the detection of ROH-islands. Average genomic inbreeding differed between breeds and ranged from 13.74 to 26.39%. Among the 217 individuals from modern breeds we found 15 ROH-islands on 12 chromosomes. ROH-islands of the modern breeds were contrasted with ROH-islands in BEZ, which revealed ROH-islands specific to domesticated goats. We identified a missense variant in the stanniocalcin 1 (*STC1*) gene, that is nearly fixated in the local, modern goat breeds, while it is totally absent in the BEZ-sample. We reported a second protein coding variant of moderate effect with divergent allelic frequencies between BEZ and the modern goat breeds in the thyroid stimulating hormone receptor (*TSHR*) gene on chromosome 10. These missense variants might represent the causative functional variants for two domestication signatures. The information from ROH-islands using WGS-data is suitable for the analysis of signatures of selection and allowed the detection of protein coding variants that may have conferred beneficial phenotypes during goat domestication.

Partitioning of genetic trends by flock in Istrian sheep breed

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Genetic trends play an important role in monitoring success of selection and partition of breeding values is an effective tool for detecting main providers of selection gain. Istrian sheep have been traditionally selected for milk yield, while little or no attention, has been paid to fat (FC) and protein (PC) content until recently. The objectives of this study were to: (1) estimate genetic trends for FC and PC from 2000 to 2019; and (2) partition genetic trends (BV's) by flock. A single-trait repeatability fixed regression test-day model was used to estimate BV's. Parity, litter size, season of lambing, flock, stage of lactation, and age at lambing were fitted as fixed effects, while additive genetic, permanent environment, and flock-test-day as random effects. Decomposition of the overall genetic trend was conducted by quantifying the sources of genetic gain related to a particular flock. This task was conducted using R package 'AlphaPart' especially designed for this purpose. There was no consistent genetic trend for PC and FC neither for the population as a whole nor for any of the flocks under study. The obtained results implicate absence of systematic selection for PC and FC, regardless of regular provision of breeders with BV's for these traits. We hope that upgrading the existing pedigree based BLUP to a single step genomic BLUP in the near future should be sufficiently simulative for breeders to pay more attention in making selection decisions.

Genetic diversity study in the Merino da Beira Baixa Portuguese sheep using SNP data

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Merino da Beira Baixa (MBB) is a local endangered breed from the centre of Portugal perfectly adapted to its environmental conditions being an important genetics resources and farming sustainability. Genetic characterization is a very important step to have a picture of population's dynamics, relationship and structure, status of genetic diversity, and inbreeding. High density SNPs arrays brought new opportunities to these studies bringing also the possibility to investigate polymorphisms associate with unique population characteristics as rusticity or productive ones, a robust tool for conservation programs aiming the protection and improvement of these genetic resources. This work developed in collaboration with the breed Herd Book, aimed to assess genomic diversity of 48 MBB individuals using high density 50K SNPs array (Axiom technology, Inatega, Spain). In order to give the relationships with breeds of different countries, data from HAPMAP consortium were used. After quality control excluding SNPs with call rates<90%, sex chromosomes, unmapped SNPs, MAF<0.05 and missingness>10% and after merging with public dataset, of starting 49,702 SNPs, 42,132 *loci* remained for further analysis. Results showed high level of informative loci with a MAF medium value of 0.41, with 77% of marker beyond the value of 0.20. Population values for observed heterozygosity of 0.38 (0.39 per SNP), were similar or larger than most population compared, as well as a medium coefficient of inbreeding (F) of 0.06. MBB revealed genetic proximity with three Spanish populations, Castellana, Aragonesa, and Ojalada and also shared the same root with Merino Landchaf and Lacune, being at higher distances with ovine populations geographically separated. This preliminary study is the first having this approach and offers novel insight into the genetic diversity of MBB breed and its relationship with other sheep breed over the world.

Genetic differentiation of mainland-island Greek sheep: implications on detection of adaptive loci

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In Greece, a variety of local sheep breeds are raised in a highly diverse geographic and climatic landscape across the country. These breeds can be used as a model to identify genetic loci contributing to environmental adaptation. To this end, 50k SNP genotypes of 90 local sheep originating from mainland (Epirus (n=35) and Peloponnesus (n=55)) along with 147 genotypes of sheep originating from island Greece (Skyros (n=21), Lemnos (n=36) and Lesvos (n=90)) were used. Principal components analysis, admixture analysis and spatial point patterns analysis suggested genetic differentiation of 'mainland-island' populations. Genome scans for signatures of selection along with genome-wide association analysis (GWAS) pinpointed one marker (*s25289.1)* on OAR4 (F_{ST}=0.39>0.355 (99.999th percentile), FLK=21.93, FDR P=0.10) that also displayed genome wide significance (FDR P=0.002) during GWAS. A total number of 6 positional candidate genes (*LOC106990429*, *ZNF804B*, *TEX47*, *STEAP4*, *SRI* and *ADAM22*) were identified within 500 kb flanking regions of the significant marker while two QTLs related to fat tail deposition are reported in genomic regions 800 kb downstream the marker. Of the positional candidate genes, *STEAP4* was considered as the most plausible functional candidate gene for long-term local adaptation, due to its documented involvement in human and sheep adiposity. Adipose tissue has a major role in the regulation of systemic metabolic homeostasis as a response to nutrient excess or fasting, cold exposure or physiological status.

Scan for signatures of selection in Russian fat-tailed sheep breeds via detection of ROH islands

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Local fat-tailed sheep breeds originated in the mountain terrain of Southern Russia are multipurpose breeds which produce milk, meat, as well as coarse wool and sheepskins. An understanding of how natural and artificial selection shaped their genomes and identification of relevant candidate genes will provide significant data to their maintenance and future prosperity. In this regard, we performed a search for genes involved in formation of adaption and productive traits in local fat-tailed breeds based on detection of signatures of selection via ROH islands. Ninety-seven samples from local fat-tailed sheep breeds including Karachaev, Andean, Tushin, Osetin, and Lezgin breeds were genotyped using Ovine Infinium® HD SNP BeadChip (Illumina, CA, USA). For ROH estimation, a window-free method for consecutive SNP-based detection implemented in the R package 'detectRUNS' was used. ROH islands were defined as the runs of homozygosity shared in more than 50% of animals. The genes found in the ROH islands were annotated with using Ensembl. ROH islands were found at OAR1 (three islands) and at OAR10 (one island). The identified genes have been reported to be potentiality involved in lipid metabolism including transportation of mevalonate and ketone bodies (MCT1) and regulation of fat deposition in the rump (VEPH1). In addition, we found genes associated with immune functions (TAFA3), involved in nervous system development (PCDH9), play a role in the oocyte formation and in endocrine processes (PTX3). The study was financially supported by RSF No. 19-16-00070.

Nutritional valorisation from plants to humans: towards One Nutrition?

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Improving nutrient efficiency is a key objective of the Farm to Fork strategy and is emblematic of the key political aspiration boosting the circular economy. The FEFAC Feed Sustainability Charter 2030 contains five key ambitions, providing the common perspective of the EU feed industry on how to meet the key Green deal policy objectives for the livestock and aquaculture through the targeted feed sustainability initiatives. The Charter contains a series of concrete feed sector actions that can help increase the sustainability of aquaculture and livestock operations. The FEFAC Sustainability Charter 2030 addresses as well EU but also global (SDGs) objectives linked to aquaculture & livestock farming. Ambition 2: Foster Sustainable Food Systems Through Increased Resource & Nutrient Efficiency aims to Assess the nutrient availability in the wide range of potential ingredient resources & look for new dynamics to improve the system. The „One nutrition approach' seeks to connect the scientific progress of plant, animal and human nutrition in order to determine cross-sectoral feed and food chain options to optimised nutrient efficiency along the whole food chain. The integration of resource-efficient nutrient management concept provides the basis foundation for delivering impactful solutions to enhance sustainable food system in the context of the future Farm to Fork policy. The „One nutrition approach' takes its inspiration of the successful implementation of 'One Health' strategy building on cross sectorial scientific excellence of both human and veterinary medicine as well as environmental risk assessment.

Session 17 Theatre 2

Adjusting feed efficiency methodologies to valorise inedible feed and biomass from marginal land

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Novel concepts & methodologies to assess the role of livestock farming in sustainable food systems. Adjusting feed efficiency methodologies considering the added value of animals to valorise inedible feed and biomass from marginal land. Novel feed efficiency methodologies considering inedible feed, quantifying flaws of by-products, potential of biomass growing on marginal agricultural lands that are not suitable for food crop production (such a grasslands, potential of grassland management to increase productivity and the nutritional value of products.

Session 17 Theatre 3

Evaluate the land-use efficiency of regional livestock systems from a food systems perspective

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The aim of this study is to evaluate the land-use efficiency of four different livestock systems from a food systems perspective. To this end, we quantified the land-use ratio (LUR) of livestock systems in seven regions of Ireland across different soil types and climatic conditions. The LUR is a metric that quantifies the level of feed-food competition occurring in livestock systems. It compares the quantity of human digestible protein (HDP) produced by the livestock system against the HDP of the potential alternative crop from the land used to grow the system's feed and fodder. The source of this feed may be in-situ pasture or feed imported from crop systems at a national and international level. A LUR of <1 demonstrates that the livestock system is an efficient user of its land; producing more HDP than could be grown in the alternative crop. In contrast, a LUR of >1 in a livestock system demonstrates that the system is land-use inefficient. The four livestock systems studied include three pasture-based ruminant systems: the dairy and its surplus beef calf system; a suckler beef system, a sheep-meat system, and a monogastric pig system. The different biophysical features in the regions as studied include; different pasture yields, different proportion of pasture suitable for crop systems, and different crop yields. In this study, only the dairy beef system had a LUR value <1 in every region; LUR values ranged from 0.21 to 0.71. The sheep-meat system is land-efficient in only three out of seven regions, LUR ranged from 0.52 to 2.16. The suckler beef system was land-efficient in only two out of seven regions, LUR values ranged from 0.77 to 2.78. Lastly, the pig system was not land-use efficient in any region, LUR values ranged from 1.89 to 2.00. Hence, our results demonstrate that certain regions are more suitable for ruminant systems based on their suitability for cropping and pig systems that were based human edible feed were consistently land-use inefficient.

A partial life cycle assessment of smallholder livestock systems in Western Kenya

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Ruminants are central to the economic and nutritional life of much of sub-Saharan Africa, but cattle are now blamed for having disproportionately large negative environmental impact through emissions of greenhouse gases. However, the exact mechanism behind these emissions is not well-understood and indeed accurate estimates are lacking due to a paucity of reliable data. Smallholder mixed cropping/livestock are the dominant livestock keeping systems in the highlands of East Africa. Employing individual animal records obtained at regular farm visits, this study quantified farm-level emissions intensities (EIs) of greenhouse gases for smallholder farms in three counties of Western Kenya. Crude protein (CP) was chosen as the functional unit to capture outputs of both milk and meat. The results showed farm EI ranged widely from 20->1000 kg CO_2-eq/kg CP and median EIs were 60, 71 and 90 kg CO_2-eq/kg CP for Nandi, Bomet and Nyando respectively. Enteric CH_4 contributed >95% of emissions and manure ~4%, with negligible emissions attributed to input to the production system. Collecting data from individual animals on smallholder farms enabled the demonstration of an extremely heterogenous EI status amongst ostensibly similar smallholder farms and provides clear indicators on how low EIs may be achieved in these environments. Contrary to current belief, our data show that industrial- style intensification isn't required to achieve low EI, but that those are associated with more output-oriented smallholder systems.

Restoring or reinventing the virtuous cycle of crop-livestock integration?

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Crop-livestock systems are ubiquitous worldwide, and far more common across the developing world where they provide at over half the cereals, meat and milk as well as supporting livelihoods of about half a billion people. In Europe some 30% of farms are less than 20 ha, and often integrated crop-livestock units. As future food systems are contemplated with the multiple challenges of planetary health, human health – or better still, One Health, mitigating waste, addressing environmental sustainability and so on, the place of these small, integrated farms must be carefully evaluated. In much of the developing world, the multiple, essential roles of small crop-livestock farms for food and nutrition security, livelihoods, income and much more will need to respond in the context of rapidly rising demand for milk, meat and eggs in particular. There needs to be transformation without losing the benefits of integration. In Europe, and across much of the developed world, a production system transformation has taken place, with small, mixed farms being replaced by larger but less integrated units – enterprises producing feed, animals, and food are often very separate spatially. And while production may have greater efficiency the downsides of environmental pollution, waste products, animal health and welfare among other challenges have raised concerns leading to a new focus on returning to the smaller integrated crop-livestock farming units. This presentation will review ongoing work undertaken in the context of the Action Networks of the Global Agenda for Sustainable Livestock along with integrated research solutions from CGIAR and partners targeting transformation of the crop-livestock systems across the developing world. It will consider the diverse pathways towards integrated production for future food systems that fully benefit from a circular bioeconomy.

Session 17 Theatre 6

Panel discussion: how to better re-implement synergies between crops and livestock farming?

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Going beyond the Feed vs Food competition: crops and animals together to address food and nutrition security. One of the controversial aspects of livestock production is the resource competition for feed and food production that requires a disruptive shift in resourcing animal feeding and in European agriculture. Only 20-25% of annually produced terrestrial agricultural biomass (crops and grasslands) is edible as human food. Thus, animals are very useful to convert the remaining 75% into edible food and manure that is a source of nutrients and carbon for plants and soils. This is basically a virtuous circle. However, in response to increased market demand and economic pressure, some agricultural systems and territories have become increasingly specialized. At the same time, productivity in the agricultural sector has largely increased and mixed farming systems integrating crop and livestock production have strongly declined in many countries or regions. Modern agriculture has developed with livestock and crop production becoming more intensive, and at some places more specialised and spatially separated which has resulted in an imbalance in nutrient flows with negative impact on the environment. To achieve the conditions required to deliver sustainable farming systems, it is essential to develop science-based management strategies that reduce the current reliance on non-renewable resources and securing the production in an increasingly unpredictable climate. Such solutions focus on sustainable land use and the interconnection of arable and livestock systems as part of a circular and sustainable bio-economy at different scales. Panel discussion, with speakers and the audience. Moderated by Jean-Louis Peyraud, INRAE and Martin Scholten, WUR (ATF past-Presidents). With also Tommy Bolland, University College Dublin, co-chair of the LFS Study commission of the EAAP: What are the missing points in the discussion? What are gaps in R&I?

Session 18 Theatre 1

Ideas & solutions for mountain livestock farming – the EAAP Working Group

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The EAAP across commission on Mountain Livestock Farming (MLF) was established in 2018, aiming at creating an international team of experts in different fields of livestock farming in mountain areas. The targets of the working group (WG) are the fostering of transdisciplinary collaborations, the development of new ideas and solutions to the emerging challenges and the dissemination of relevant projects and results through dedicated events. As a starting point, the WG published a study that mapped the world research on MLF over the last 40 years using text-mining and topic modelling analysis. The main topics identified represent a useful source of information for further and more detailed analysis which are carried out in sub-groups within the WG. Recently, an opinion paper on new research approaches for sustainable mountain agroecosystems has been drafted and it has been submitted to peer-reviewing. The WG also promotes dissemination initiatives focused on the mountain agriculture such as the 1st Joint Meeting with FAO-CIHEAM Mountain Pastures Sub-Network 'Mountains are agroecosystems for people' which took place in Bled (Slovenia) in June 2021. This event had the goal of sharing the common interest for a sustainable use of vegetation, animal and human resources, in mountain areas. The WG philosophy is that a truly holistic and interdisciplinary research approach is needed to identify drivers of change and to understand current and future challenges faced by MLF. In mountain area, livestock research must be specific, multidisciplinary and practical, in order to give the answers in demand and support proper political actions needed to sustainably preserve mountain livestock (eco-)systems.

The relationship of local Swiss cattle populations with other local breeds from the Alps

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In a previous study, using 50k SNP data from nine Swiss dairy cattle populations, we showed that the local cattle populations Original Brown (OB), Simmental (SI), Eringer (ER) and Evolène (EV) are genetically clearly distinct from the major dairy cattle populations. Individuals from local breeds are traditionally shared within geographical regions across borders. To disclose the genomic relationship of local Swiss cattle populations with alpine cattle breeds from other countries, we used SNP genotyping data of 14 local autochthonous breeds from the alpine area. In total we analysed 4,920 individuals of 18 local breeds with genotypes from medium to high density SNP chips. The results based on ~32,000 common SNP showed a clear separation of SI and OB from all other populations. For the ER and EV breeds the known genetic link with the neighbouring breeds Valdostana Black Pied and Valdostana Castana could be confirmed at genomic level. Average genomic inbreeding (FROH) and breed specific Runs of Homozygosity (ROH) islands were derived. This study confirms the previously described ROH island on chromosome 11 (~67.6 Mb) for OB. This analysis will be further developed by balancing sample sizes from different breeds and by describing ROH-islands using information from high-density SNP data.

Grazing with robust cattle breeds: an opportunity to sustainably use mountain grassland

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Livestock breeding strongly enhanced the productivity of cattle. If these modifications unintentionally altered the foraging and movement behaviour, too, there would be far-reaching consequences for vegetation, especially on biodiverse mountain pastures. To analyse the impact of productivity on grazing, we compared suckler cows of low-productive Highland cattle (HC) with medium-productive Original Braunvieh and high-productive Angus×Holstein in a controlled experiment in the Eastern Swiss Alps. We weighed the cows, measured their claws, tracked their movement by GPS and pedometers and recorded the botanical composition of the diet while grazing species-rich alpine pastures. A second study compared the vegetation of 25 pastures grazed by HC for at least 5 years with 25 similar, neighbouring pastures of high-productive cattle. HC differed from more productive breeds in almost all parameters: (1) They were significantly lighter, but had relatively large claws. Thus, the pressure is spread over a large area, which protects the sward. (2) HC covered less distance, which further reduces trampling impact. Consequently, trampling-adapted plant species were far more common on the pastures of productive breeds. These plants outcompete more-susceptible species, thereby decreasing biodiversity. (3) The more productive a breed is, the more selectively it grazed. High-productive cattle preferred nutrient-rich, easily digestible forage plants, whilst low-productive cattle more often fed on unattractive plants. Thereby, they reduce the dominance of problem plants, which in turn promotes pasture biodiversity and forage quality. Even woody plant species were consumed frequently by HC, which makes them an attractive option for shrub control in mountain areas. (4) HC used the pasture more evenly by visiting steep slopes and areas of low forage quality. Consequently, there were fewer over- and underused areas. (5) The productive breeds lost weight on the marginal, alpine pastures whereas HC gained weight, indicating a more efficient roughage conversion. Low-productive cattle can make efficient use of mountain grassland, thereby sustaining these pastures and promoting biodiversity. On many mountain farms, the existing livestock could be supplemented by an extensively reared 'service herd' with minimal effort and expense.

Dairy calves suckled by their dams: which compromise between animal performances and welfare?

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In dairy farms, early separation of cows and calves is increasingly questioned by consumers. Two trials were set up to test dairy farming practices that allow cow-calf contact, while limiting milk losses, and improving calf growth and animal welfare. In trial 1, a group of 14 male and female calves were maintained with their dam (n=14; D1) between morning and evening milkings until weaning (±11 weeks). In trial 2, a group of 14 calves were maintained with their dam (n=14; D2), until weaning for 9 female calves and sale at 3 weeks for 5 males. In another group (M), 9 female calves and 5 male were kept with their dam (n=14) for 3 weeks, when males were sold and females were fed with an automatic milk feeder. These groups were compared to a control group (C) of 14 cows and 14 calves, separated at birth and fed with an automatic milk feeder. Until weaning, D1 and D2 cows produced less milk than C cows (-40% and -35%), with higher protein content (+1.3 and +1.0 g/kg) and lower fat content (-5.8 and -4.0 g/kg). Cows in group M had intermediate results between those in groups D and C (-32%, +1.6 g/kg and -0.9 g/kg, respectively), but on a shorter period. No difference was observed between groups on milk SCC. All groups returned to the same milk production and milk composition as group C, from 2 weeks after weaning (D2) to 5 weeks after separation (M). Calves D1 and D2 had faster growth (+9.4 kg at weaning, on average), while calves M had similar growth to group C. Stress at separation/weaning was assessed by counting vocalizations during daytime. All calves vocalized at weaning, up to 7 days, but D1 and D2 calves vocalized longer than C calves. M calves vocalized at dam separation and 7 weeks later at weaning. We also showed that D calves were able to express a more natural repertoire of behaviour and had more social relationships than C calves, due to the presence of other calves and adult cows in the barn. For farmers, these practices should be compensated by a higher milk price, based on a possible 'ethical' label. Further research is needed on the possibility of making separation/weaning more gradual and reducing the level of stress for animals in these critical phases.

Economic weights for beef production traits in three different fattening systems in Switzerland

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The goal of a breeding program is to improve the biological potential of a population in order to increase efficiency of production and optimise economic returns. This is achieved by selecting for multiple traits that contribute economic merit, simultaneously. Selection for an index which gives proper weight to several traits is more efficient than selecting for individual traits. One method for this involves defining a breeding objective that comprises the values for the respective traits into one single selection criterion by weighting the genetic value of each trait with its economic value. The economic value of a trait depends on the amount of profit it is expected to generate for each unit of improvement of that trait. Selection indexes that weight the genetic merit of traits with their economic values are valuable in livestock breeding. However, due to the large range of factors that influence the economy of beef cattle production, a major challenge that remains in determining an appropriate selection index is the weighted assessment of the economic values of the different traits. The aim of this study is to calculate the economic values for growth, carcass and functional traits in three beef production systems in Switzerland. A bio-economic model is used to estimate economic values for Limousin cattle traits, with an emphasis on carcass conformation, carcass fat covering and carcass weight. Calculations are evaluated separately for veal, Natura-Beef and classical beef fattening systems. The structure of the cow herd is calculated using Markov chains. The marginal economic value of a trait is defined as the partial derivative of the profit function with respect to the trait mean in the population. All parameter values pertaining to income and expense for determination of the profit function were collected according to Swiss fattening systems and input into the software ECOWEIGHT for calculation of economic values.

Feed efficiency and digestibility values of three crossbreeds in fattening bulls

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Feed efficiency and digestibility values as one of its putative determinants were investigated in beef meat production systems, representative of hill regions of Switzerland. The three most widespread crossbreeds used in Switzerland were included, in order to develop recommendations for practice regarding the choice of the crossbreed and its adaptation to the system under study. Fattening bulls (n=68; 154±13 kg BW) born from dairy Brown Swiss mothers and sires of the meat breeds Angus (AN), Limousin (LI) or Simmental (SI) received until slaughter at 520 kg BW, one of two *ad libitum* iso-energetic and iso-proteinous TMR, made of corn and grass silages, differing in botanical composition and concentrate. Two successive residual feed intake (RFI) and feed conversion ratio (FCR) measurement periods (P1, P2) computed over 70 days each, initiating at 197±17 and 342±20 kg BW, as well as a one-week individual digestibility measurement using an internal marker method (chloridric-acid insoluble ashes) at 330±20 kg BW were carried out. The relationships between RFI or FCR and digestibility were explored by linear regressions. Data were analysed by ANOVA with diet, breed type and their interaction as fixed effects. SI achieved the highest average daily gain (ADG), with 1.80 vs 1.78 (AN, ns) and 1.69 (LI, $P<0.001$) kg/d in P1 and 1.65 vs 1.39 (AN) and 1.29 (LI), (both $P<0.001$) kg/d in P2 and a better feed efficiency according to their FCR with 3.47 vs 3.82 (AN) and 3.75 (LI) kg DM/kg ADG ($P<0.01$) in P1 and 5.01 vs 5.98 (AN) and 6.00 (LI) kg DM/kg ADG ($P<0.001$) in P2. AN had a higher dry matter (DM) intake with 6.79 vs 6.22 kg/d in P1 ($P<0.001$) and a higher fibre digestibility than SI, with CF 68.2 vs 64.7% ($P<0.05$), ADF 64.2 vs 60.4% ($P<0.05$) and NDF 60.1 vs 56.6% ($P=0.06$), LI having lower DM intake than AN in P1and than AN and SI in P2 and being intermediate for digestibility between both other breeds. Dry matter digestibility did not appear to be a determinant of feed efficiency, even though by an intra-breed analysis, the most efficient AN animals (according to their RFI) also had the highest DM digestibility ($P<0.10$).

Opinion: a new research approach is needed to secure viable mountain livestock (eco-)systems

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Mountain areas are diverse and multifunctional landscapes largely influenced by mountain livestock (ML). ML (eco-) systems provide numerous services, e.g. sustainably produced food, most biodiverse habitats, an attractive landscape for tourism, cultural heritage, soil stabilisation and avalanche protection. Today, ML (eco-)systems are threatened by abandonment of unfavourable locations leading to losses of biodiversity, cultural heritage and production potential as well as by intensification in favourable areas (e.g. high stocking density, concentrate feeds, fertilisation) leading to long-term degradation, eutrophication, soil instability and biodiversity loss. To answer the relevant questions and to secure ML (eco-)systems, a new research approach is urgently needed: (1) ML research must not longer mistakenly transfer solutions from the lowlands to mountains. Complex mountain (eco-)systems require mountain-specific solutions. (2) ML research must be site-specific, since mountains are extremely heterogeneous. (3) ML research must measure the most relevant parameters instead of the parameters most easy to quantify. Specific sustainability indicators must be developed. (4) ML research must overcome fragmentation administratively (among regions and countries), spatially (to avoid problems of upscaling and generalisation) and among disciplines (to establish a holistic and multidisciplinary approach). (5) ML research must be put into practice. Researchers first have to listen to practitioners, second communicate understandably and finally clearly advice policy makers. In this paper, we will provide justification for these claims by reviewing good-practices examples in the ML literature. We will demonstrate that if ML research becomes specific, precise, multidisciplinary and practical, it will give the answers in demand and initiate the political action needed to sustainably preserve ML (eco-)systems.

Reducing concentrate feeding in organic dairy cows with the help of body condition-monitoring

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Starting in 2022 it will be mandatory for Swiss organic farms to feed no more than 5% of concentrates in yearly rations to ruminants; so to put the 'feed no food'-strategy into effect. It will be a challenge to feed high yielding dairy cows adequately. In the frame of two term papers we tested on two commercial farms whether it would be a suitable method to reduce or to cease concentrate feeding as soon as cows stop mobilising body fat. In 2020 we observed milk production- and body condition (BCS) development after 100%-concentrates reduction (12 Swiss Fleckvieh (SF) and 2 Red Holstein (RH) cows, first lactation) or 50%-reduction (6 multiparous RH cows). Amounts of concentrates were compared to the (fictive) amounts of concentrates that would have been fed in 2020 if the former feeding regime (concentrate amounts according to daily milk yield) would have been used. And we compared milk yields of each cow in 2019 and 2020. In average 35% less concentrates were fed per animal than would have been fed with the former feeding regimes. Average yearly concentrate amounts per cow were reduced from 123 kg to 64 kg and from 348 to 260 kg in SF and RH cows, respectively. 76% of the study animals showed a higher milk production in 2020 than in 2019, in 52% also milk fat content was higher and in 52% milk protein content was higher. 75% of the study animals did not reduce milk production after concentrates had been reduced in their ration. 25% did reduce milk production and 25% started to mobilize body fat again. 40% of the cows did not reduce milk production nor body fat. No animal reduced both. Lactation number was positively and number of concentrate feeding days was negatively correlated with body fat mobilization after concentrates reduction. The method of reducing concentrates after body condition score has become stable seems to be well practicable, but animals mobilising body fat after the reduction might not cope well with that system; they have to be well observed. Further studies with more high yielding animals are needed to prove these results.

Replacing maize silage and concentrate by grass silage and corn-cob mix in beef cattle diets

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The use of high amounts of grass silage is limited in intensive beef production as an impaired performance is expected from the lower energy supply when replacing starch-rich maize silage by grass silage. However, feeds from grassland are promoted in various countries including Switzerland. Besides, meat lipids from animals fed grass silage have been reported to be favourably rich in n-3 fatty acids (FA) and to have a low n-6/n-3 FA ratio. We studied the effects when replacing maize silage and part of the concentrate by grass silage and corn-cob mix (CCM), on performance and the FA profile of meat lipids. Three different diets containing grass silage, maize silage and concentrate in ratios of 0.1:0.6:0.3 (G10; control), 0.3:0.5:0.2 (G30) and 0.5:0.3:0.2 (G50), or grass silage, CCM and concentrate at 0.5:0.3:0.2 ($G50_{CCM}$) and 0.75:0.15:0.1 ($G75_{CCM}$), respectively, were tested in 30 Limousin-sired bulls initially weighing 164±18 kg. A protein-based concentrate fed to G10 was replaced by a wheat-based concentrate (14 instead of 27% crude protein) in the remaining diets. Bulls were randomly assigned to the five diets. At 521±11 kg body weight, bulls were slaughtered, and carcasses were graded for conformation and fat cover according to CH-TAX (equivalent to EUROP). Animals fed G10 and $G50_{CCM}$ had higher average daily gains (1.43 and 1.34 kg, respectively) and shorter fattening periods (247 and 270 days, respectively) than those of the remaining groups. Total dry matter intake was not affected, while that from silage and concentrate varied according to the experimental design. Dressing percentage and carcass quality (weight, conformation, and fat cover score) were similar between groups. The proportion of n-3 FA in the meat lipids increased with increasing proportions of dietary grass silage. Thus, the ratio of n-6/n-3 FA declined from 6.57 in G10, to 3.80 and 2.96 in G50 and $G50_{CCM}$, respectively to 2.14 in $G75_{CCM}$. The results show that complementing grass silage with energy-rich forages can help to maintain the performance ($G50_{CCM}$) while improving the nutritional value of the beef.

The of use haylage-based diets for fattening of young bulls and heifers

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High-quality forage-based diets reduce the competition between humans and cattle for the use of cereals and are expected to reduce the carbon footprint of the meat produced. Thirty two veal's, 16 females and 16 males, weaned with 7 months, were submitted to a trial until an average live weight of 400 and 500 kg, respectively. The animals were blocked by sex and weight and distributed in pairs by 16 pens. The Control diet (C) was based in concentrate, and 3 Total Mixed Diets, with haylage : concentrate ratio of 1:1 (dry matter), were formulated to a target protein level of 16% in DM. The haylages used were two biodiverse mixtures harvested in January (MW) or April (MS) and *Triffolium suaveolens* (TS) harvested in April. All diets were offered *ad libitum*. Methane production was evaluated *in vitro* using the Ankom System. Meat quality was evaluated in *Longissimus lumborum* at days 3, 7, 14 and 28 *post mortem*. Dry matter intake was not affected by diet and was 16.2% higher in males. The intake of starch was higher and of NDF and total phenols were lower in C than in MW, MA and TS diets. Daily weight gain was 43.9% higher in males and was higher in C and MW diets than in MS and TS. Dry matter conversion ratio was higher for TS and similar for the other diets. Feeding costs were not affected by diet and were higher for females. Diets did not influence the methane production which presented an average value of 19.5 ml/g DM. Carcass weight was higher for C and MW than for MS and SP, and dressing percentage was unaffected by the diet. Meat and subcutaneous fat colour were unaffected by diet. Meat redness decreased and yellowness and chroma increased linearly with ageing. Lightness changed quadratically with ageing with a maximum value at 14 days and hue was not affected. Shear force decreased with ageing and was higher for TS. High-quality forage based diets may be a sustainable alternative to concentrates for the fattening phase of young cattle, considering production costs, productivity and meat quality. Work funded by PDR2020 program through the FEADER under the project LegForBov (PDR2020-101-031179).

Estimation of crossbreed beef carcass chemical composition by DXA scan of half-carcass or 11th rib

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The aim was to estimate beef half carcass (HC) composition by using dual X-ray absorptiometry (DXA), from either HC or 11th rib analysis. 42 cold left HC (94±49 kg, 17 to 157 kg) and their 11th rib were obtained from bulls of the three most Swiss-widespread crossbreeds in dairy herds (♀ Brown Swiss × ♂ Angus, Limousin or Simmental). Lean, fat, bone mineral content (BMC) and total masses of cold HC and 11th rib were determined with a DXA scanner (iLunar, GE, 'Right Arm' mode). HC was then grinded, before chemical analyses (lipid: Soxhlet, protein: Dumas, ash: 550 °C). Regressions (R software, v3.6.3) were tested between the HC and 11th rib DXA values and HC chemical composition. HC contained 11±8.3 (0.8 to 29.1) kg lipid, 18±9.2 (3.1 to 30.6) kg protein and 4±1.8 (0.8 to 6.5) kg ash. The cold HC weight explained a large part of chemical component masses (lipid: R^2=0.884, residual coefficient of variation (rCV)=25.3%; protein R^2=0.996, rCV=3.4%; ash R^2=0.971, rCV=7.8%). It was also well estimated from HC DXA total mass with a R^2 of 0.999 (rCV=1.3%). Estimations of lipid, protein and ash masses with a single HC DXA variable were precise with fat (R^2=0.983, rCV=9.7%), lean (R^2=0.996, rCV=3.4%) and BMC (R^2=0.975, rCV=7.8%), respectively. Multiple models were even more accurate with HC DXA fat, lean and BMC masses for lipid mass (R^2=0.990, rCV=7.7%), cold HC weight and DXA fat mass for protein mass (R^2=0.997 and rCV=2.9%), and cold HC weight, DXA fat and BMC masses for ash mass (R^2=0.979 and rCV=7.2%). Multiple regressions of HC lipid and protein masses with 11th rib DXA variables provided R^2 very close to the previous ones established from HC DXA scan. But the rCV was twice higher for lipid mass (rCV=14.8%) and remained equal for protein mass (rCV=2.9%). Crossbreed effect was rarely significant in models and only improved slightly their accuracy (maximum of +1% for the R^2 and -1.5% for rCV for HC lipid mass from 11th rib DXA variables). A single rib DXA scan appears to be a promising method to estimate carcass composition in a simple, quick, reproducible, accurate and non-destructive way.

Session 18 Theatre 12

Body composition estimation in cattle: comparison of imaging and adipose cell size methods

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The aim of present study was to compare three methods for the estimation of empty body (EB) or carcass composition for lactating and growing cattle. 12 Simmental cows (589±45.6 kg of body weight (BW), 290±4.7 DIM) and 10 of their pre-weaning offsprings (10±0.2 mo, 354±32.7 kg of BW), were scanned with ultrasound (US; 9L-RS Linear-Sonde (4.0 MHz); GE) on ischium-sacrum location (adipose tissue thickness). At slaughter, tail-head subcutaneous and perirenal adipose tissues were sampled for cell size measurements (ACS). Cold (4 °C, 24 h) left half-carcasses were scanned by dual energy X-ray absorptiometry (DXA; iLunar, GE, 'Right Arm' mode) to record fat, lean, bone mineral content (BMC) and total masses. Half carcass, blood and the rest of EB (5th quarter fully collected after exsanguination and digestive content removal) was grinded before chemical analyses (lipid, Soxhlet; protein, Dumas). Regressions (R software, v3.6.3) were tested between the variables and lipid and protein masses in EB or carcass. EB contained 73±23.0 (24.2 to 122.2) kg lipid and 72±17.4 (44.3 to 96.1) kg protein. Carcass contained 41±14.2 (13.5 to 69.9) kg lipid and 43±11.2 (25.9 to 58.2) kg protein. EB lipid mass was estimated from BW including the fixed effect of animal type combined with the US adipose tissue thickness (R^2=0.88 and residual coefficient of variation (rCV)=12.0%) or the subcutaneous ACS (R^2=0.82 and rCV=14.3%). Carcass lipid mass was estimated *in vivo* from BW including the fixed effect of animal type combined with the US adipose tissue thickness (R^2=0.91 and rCV=11.7%) or *post mortem* from carcass weight combined with DXA BMC and fat masses (R^2=0.99 and 4.4%) or the perirenal ACS (R^2=0.83 and rCV=15.0%). EB protein mass was estimated *in vivo* only with the BW (R^2=0.98 and rCV=3.7%) and no variables derived of the *in vivo* or *post mortem* methods improved the model. Carcass protein mass was estimated *post mortem* from the cold half carcass weight combined with DXA BMC mass (R^2=0.99 and rCV=3.1%).

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Resumption of postpartal ovarian activity in herbage feeding systems of dairy cows

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Milk production in Switzerland is mainly based on herbage feeding with little input of concentrates. The present study investigated the effects of a solely herbage-based diet with (C) and without concentrate (nC) supplementation on luteal activity, milk production and metabolic status in 23 multiparous Holstein dairy cows with early or delayed resumption of ovarian cyclicity post partum (pp). Cows were retrospectively assigned either to a group with early (until d 25 pp, EOV) or delayed resumption of ovarian activity (>d 30 pp, DOV), resulting in four subgroups depending on concentrate feeding: DOV-C, DOV-nC, EOV-C, EOV-nC. Milk progesterone (P4) concentration was measured every 3 d, and different metabolites were analysed in weekly blood samples. Resumption of ovarian activity was detected between d 19 and 25 pp in EOV, and between d 30 and 60 pp in DOV. In DOV-C, resumption of cyclicity tended to start earlier (38.3±1.7 d pp) compared to DOV-nC (45.2±6.3 d pp; P=0.10). Independent of grouping, the ovarian activity occurred later in cows with greater plasma -hydroxybutyrate (BHB) concentrations (P<0.05). Milk P4 concentration at the first rise >1 ng/ml did not differ between groups (P>0.05), but milk P4 peaks during the experiment were higher in EOV compared to DOV (P<0.05). Cows of DOV-nC experienced the most negative energy balance during the first 3 weeks pp with concomitantly lowest plasma glucose and highest concentrations of non-esterified fatty acids and BHB. Insulin-like growth factor-1 concentrations were lower in cows without concentrate supplementation, but not related to EOV or DOV (P=0.61). Overall, concentrate supplementation caused an earlier onset of luteal activity in cows in herbage-based feeding systems. Resumption of ovarian activity in cows with DOV was further delayed if energy and nutrient supply were limited.

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Key figures of energy and protein metabolism in blood serum of suckler cows compared to winter feedi

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The objective of the studies was to examine the concentration of blood serum metabolites in contrast of the grazing season and during winter-feeding period. The aim of the study reported here was to establish reference values for parameters in blood serum of suckler cow under different feeding systems. The data from the study were used from investigations in suckler cow herd in the last 13 years. Results from 3 farms could be used to compare the grazing period and winter-feeding time. For statistical analysis within each farm were estimated the average concentration of blood serum concentrations per month to minimize the effect of different herd sizes. Statistical analysis took place with ANOVA with fixed effects of farms (1-3) and period (grazing season vs winter-feeding period) using SPSS. An alpha of 0.05 was used for all statistical tests. The serum NEFA concentration was significant higher during the winter-feeding period (878 µmol/l) than in the grazing season (600 µmol/l). Increased NEFA can indicate a ketosis and negative energy balance or a problem of the cows with stress and adrenaline during the samples were taken (FÜRLL, 2004; STAUFENBIEL, 2020). The concentration of BHB was similar between the grazing season (0.42 mmol/l) and the winter-feeding period (0.38 mmol/l). UREA was average 5.0 mmol/l and show a significant difference between the winter-feeding period (3.5 mmol/l) and grazing season (7.1 mmol/l). In contrast was a higher concentration of TP during winter (76 g/l) than the grazing season (69 g/l), however, the values were within the reference values according to Fürll (2004) and Staufenbiel (2008). Activity of ASAT and GLDH show no differences between the two periods. Cholesterol was significant higher during the grazing season (3.4 mmol/l) than winter-feeding period (3.0 mmol/l). The results indicated that different periods of feeding effects the metabolism of energy and protein in suckler cows. Reference values can within certain limits adapted from dairy cows and should be adapted to the conditions of extensive animal husbandry.

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Genetic background and rearing conditions: finding the best compromise to optimize meat quality

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The aim of this study was to assess sensorial and nutritional qualities of meat from 71 crossbred fattening bulls of Brown Swiss dairy mothers and sires of Angus (AN), Limousin (LI) and Simmental (SI) breeds. Bulls received one of two corn silage-based diets (A with alfalfa and B with beet pulp and grass silage) from 170 kg until 480 kg ('Swiss Quality Beef', SQB only for A) or 530 kg body weight (Swiss standard, STD A and B). 48 h post mortem, Longissimus thoracis slices were excised from the 10th to 12th ribs and analysed for intramuscular fat (IMF, Soxlhet without acid hydrolysis) and fatty acids (FA) composition (GC-FID), or directly stored under vacuum at 2 °C for 14 d before freezing. After thawing, slices were cooked and measurement of water holding capacity, shear force (Warner-Bratzler), and oxidation susceptibility (TBARS) as well as sensory analysis was (trained panel (n=8) evaluating the intensity of overall flavour, tenderness and juiciness on a 10 cm unstructured line scale) performed. Data were analysed by MANOVA with a linear model. Shear force from AN meat tended to be lower as compared to SI (AN 26.4, LI 27.7 and SI 29.3 N, $P=0.08$), which was confirmed by sensory analysis (tenderness: 5.2, 5.3 and 4.8, $P<0.03$; juiciness: 5.8, 5.6, 5.0, $P=0.06$, for AN, LI and SI, respectively). Angus meat IMF and mono-unsaturated FA contents were higher, and poly-unsaturated FA was lower than in LI and SI meat (IMF: 1.6, 1.1 and 1.0%; mono-unsaturated: 44, 41 and 40%; poly-unsaturated FA: 11, 16 and 17% total FA, for AN, LI and SI, respectively; $P<0.05$). Treatment SQB-A decreased water holding capacity during cooking, IMF and flavour compared to STD-A and STD-B (water loss: 20, 18.5 and 17.8%; IMF: 1.0, 1.3 and 1.4%; flavour: 5.4, 5.8 and 5.7, for SQB-A, STD-A and STD-B, respectively; $P<0.05$). Increased IMF rendered the meat of STD-B more susceptible to oxidation during cooking than SQB-A (SQB-A 0.21, STD-A 0.27 and STD-B 0.34 mg equiv. MDA/kg, $P<0.05$). In conclusion, for intensive feeding and low to mid slaughter body weight, LI crossbreed bulls may represent a good compromise between AN and SI, combining both, desired meat sensorial quality and nutritional value.

Gestation length in Swiss Fleckvieh cows

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This study examines the impact of the sire breed on the gestation length in Swiss Fleckvieh (SF) cows. Data from a total of 345,779 SF cows from the years 2000-2020 with a total of 18 different sire breeds were provided by the breeding association Swissherdbook. A random sample of 25,467 observations was taken from this data set, with the various sire breeds represented as follows: Purebred Simmental Cattle descending from swiss genetics (SI60) 2,419, Purebred Simmental Cattle descending from international genetics (SI70) 352, Angus (AN) 377, Belgian Blue (BB) 363, Blonde d'Aquitaine (BD) 110, Swiss Brown (BV) 35, Charolais (CH) 81, Holstein (HO) 270, Eringer (HR) 52, Jersey (JE) 23, Limousin (LM) 4,929, Montbéliard (MO) 629, Normande (NO) 55, Original Swiss Brown (OB) 30, Pinzgauer (PI) 149, Red factor carrier (RF) 356, Red Holstein (RH) 5,266 and Swiss Fleckvieh (SF) 9,971. The relation between the gestation length and sire breed has been investigated by a linear mixed model. The model included the fixed effects month of calving, milk linear and squared, calf age linear and squared, number of calves born by gender (litter size), sire breed, as well as sire as random effects and residual effect. The longest gestation length occurred with the sire breed Blonde d'Aquitaine of 291.5 days, followed by the sire breeds PI, SI60, OB and LM at 289.7, 289.6, 289.6 and 289.4 days, respectively. The shortest gestation length resulted from the sire breed Jersey at 281.5 days, followed by the breeds HO, RF, RH, AN and BB, at 282.2, 283.5, 283.7, 283.7 and 283.7 days, respectively. The remaining breeds had gestation lengths of between 284 and 288 days. Both the gender and the number of calves born have an impact on the gestation length. The gestation length of male calves is approximately a day longer than of female calves (f=285.5 days; m=286.8 days). In the case of twins, the gestation length of female twins and male and female twins was 281.4 and 281.3 days, respectively. The gestation length of male twins was 282.9 days. The results show that breeders can influence the gestation length by their selection of sire breed.

Prediction of cow diet composition using mid-infrared spectroscopy on milk

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The ability of mid-infrared spectroscopy (MIR) to predict indicators of diet composition of dairy herds was tested on 7,607 bulk milk spectra from 1,355 farms located in the Massif Central area (France). MIR spectra (FT-Plus equipment, Foss, Hillerød, Denmark) of bulk milk used for routine milk payment analysis were collected from April 2018 to March 2019. At each milk sample was associated the corresponding cow diet composition data, obtained through on-farm surveys routinely and monthly performed by farmers' advisors. Cow diet composition varied largely (i.e. from full grazing for extensive farming system to corn silage based diets, typical of more intensive farming systems). The dataset was divided into a calibration (n=6,107) and a validation sets (n=1,500). Partial least square regression were performed using the raw spectra, on the segments between 2,989 and 2,561 cm^{-1}, between 1,809 and 1,712 cm^{-1}, and between 1,600 and 926 cm^{-1}. The pasture proportion in cow diet was predicted by MIR with a model characterized by an R2 in external validation (R2V)=0.81 and standard error of prediction (SEP) of 11.7% dry matter diet. Pasture + hay, corn silage, conserved herbage, fermented forages and total herbage proportion in cow diet were predicted with R2V>0.61 and SEP<14.8. Prediction models with R2V<0.5 were found for hay, grass silage, concentrates, fermented herbage, hay + haylage and total forages proportions in cow diet. The proportion of several of the main feedstuff in cow diet were predicted with residual error derived by the supposed high estimation error of the reference data issued through on-farm surveys. Prediction performances of our models MIR predictions can be considered suitable for an application on bulk milk for authentication purposes and can provide to producers and consumers (farmers, dairy plants) useful indicators on cow diet composition.

Let there be light – blue, red or white light for dairy cows

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Artificial light can be used as a management tool to increase milk yield in dairy production and improve the working conditions for barn staff. The aim of this study was to investigate effects of light wavelength on milk production, endocrinology, lying behaviour and feed intake in dairy cows. A tie-stall barn with controlled light environment, equipped with light-emitting diode light (LED) fixtures with variable spectral composition, was used to create the experimental light environments. Four iso-photonic light treatments with 16 h daylight dominated by Blue, Red, White or White-Blue light in combination with dim night light was tested on pregnant lactating cows for five weeks. Particular focus was given to possible carry-over effects of blue light during the day on activity at night since this has been demonstrated in humans. Milk yield was maintained during the study period, during a phase of the lactation when milk yield is expected to decline. Plasma levels of melatonin was higher in dim night-light than during daylight for all treatments ($P<0.001$), and there was no difference between treatments during daylight. There was no effect of treatment on the daily duration or distribution of lying behaviour. In conclusion, the diurnal pattern of melatonin in dairy cows was not significantly different when blue, red or white daylight comprising the same number of photons was used. Blue light was not superior to red or white light in terms of improving milk yield and did not affect cow activity or feed intake in the tie-stall barn used in this study.

Effects of the novel concept 'outdoor veal calf' on antimicrobial use, mortality and weight gain

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The aim of the intervention study 'outdoor veal calf' was to evaluate a novel concept for calf fattening which aimed at reducing antimicrobial use without compromising animal health. Management practices such as commingling of calves from multiple birth farms, crowding, and suboptimal barn climate are responsible for high antimicrobial use and mortality. The risk of selecting bacteria resistant to antimicrobials and of economic losses is accordingly elevated. The concept, implemented in nineteen intervention farms (IF), is based on three main measures: (1) purchased calves are transported directly from neighbouring birth farms to the fattening facility; (2) each calf is vaccinated against pneumonia and completes a three-week quarantine; and (3) the calves spend the rest of the fattening period in outdoor hutches in groups not exceeding 10 calves. The covered and bedded paddock and the group hutches provide shelter; constant access to fresh air is warranted. Nineteen conventional calf fattening operations of similar size served as controls (CF). Every farm was visited once a month for a one-year period, and data regarding animal health, treatments, and production parameters were collected. Treatment intensity was assessed by use of the defined daily dose method (TIDDD in days per animal year). Mean TIDDD was 5.3-fold lower in IF compared to CF (5.9 ± 6.5 vs 31.5 ± 27.4 days per animal year; $P<0.001$). Mortality was 2.1-fold lower in IF than in CF ($3.1\%\pm2.3$ vs $6.3\%\pm4.9$; $P=0.020$). Average daily gain did not differ between groups (1.29 ± 0.17 kg/day in IF vs 1.35 ± 0.16 kg/day in CF; $P=0.244$). A drastic reduction in antimicrobial use and mortality was achieved in the novel system without compromising animal health. The principles of risk reduction used in designing the system can be used to improve management and animal health, decrease the need for antimicrobial treatments and thus selection pressure on bacteria in veal operations.

Feed characteristics and potential effects on rumen microbiome of ensiled seaweeds for dairy cows

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Seaweed silage has potential to be an alternative feed ingredient for dairy cows. This study aims to evaluate the feed characteristics of ensiled seaweed, by performing chemical analysis and digestion examinations on *Saccharina latissima* and *Alaria esculenta*, preserved frozen or using various ensiling treatments (16 °C, 3 months) including: (1) NoA: no additives; (2) FA: 0.4% formic acids; (3) BI: lactic acid bacteria (LAB) inoculant; (4) BI30: LAB inoculant, and the biomass was prewilted to 30% dry matter (DM). Further, the microbiome of rumen fluid after 48 hours *in vitro* incubation with seaweed silages was analysed using16S rRNA sequencing. The seaweed was seeded at northern Norway (N68, E15) in autumn 2018, harvested in June 2019, and processed within 24 hours. Average DM content was low (9.4% without prewilting). The overall ash and crude protein contents were 243 and 111 g/kg DM, respectively. Without additives (NoA), silage pH failed to reach below 4.5. Acetate was the major volatile fatty acids found in all silages, while lactate was only detected in silages with LAB inoculant. The organic matter *in vitro* digestibility using rumen fluid (OMD) and indigestible NDF (iNDF) varied between species ($P<0.001$) and treatments ($P<0.05$). In FA treatments, the highest OMD (Alaria: 319 g/kg DM, Saccharina: 546 g/kg DM) and the lowest iNDF were observed (Alaria: 165 g/kg DM, Saccharina: 76.4 g/kg DM). The effective protein degradation at 5% passage rate (EPD5) and total protein digestibility using mobile bag were both low and not improved by silage treatments. The highest EPD5 was found in BI30 Saccharina (460 g/kg CP), while the highest digestible rumen bypass protein was found in frozen Saccharina (180 g/kg CP). Overall, *S. latissima* demonstrated higher digestibility and thus nutrients value comparing to *A. esculenta.* The microbiome analysis is still ongoing and expected to be available before the conference.

Milk yield and fertility in a randomized controlled trial of extended voluntary waiting period

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By extending the voluntary waiting period (VWP) for the first insemination, the transition period during which the cows are considered to be at higher disease risk, becomes a smaller proportion of the dairy cow's calving interval. Due to a longer VWP the energy balance at insemination and thus its outcome is expected to be improved. Previous studies have shown that extended VWP might be beneficial for several fertility measures. Primiparous cows often show a flatter lactation curve, which may make them suitable for longer calving intervals. As part of an ongoing study, we aimed at determining effects on milk production and fertility from prolonging the VWP in primiparous cows. Data was collected from the Swedish milk recording system regarding calvings, test milkings, inseminations, and parentage, from 16 commercial farms in southern Sweden. All primiparous cows, having their first calf within the first six months of the study period ($n=641$), were included and randomly allocated to either a traditional VWP of <96 days or extended VWP of >144 days, resulting in approximately 12 and 15 months calving intervals. All full lactations i.e. from cows that had both their first and second calf during the study period (August 2018 to July 2020), and where the assigned VWP was complied with, were included in the final dataset ($n=386$). The preliminary results show that first service conception rate, was significantly lower ($P<0.001$) for cows with a traditional VWP (51.8%, $n=218$) than for cows with extended VWP (72.0%, $n=168$). Milk yield per day in the calving interval did not differ significantly between the groups (25.4 vs 25.2 kg/day, $P=0.72$). In conclusion, these preliminary results suggest that milk yield per day in the calving interval is sustained and first service conception rate is increased for primiparous cows randomized to receive an extended VWP compared to a control group with traditional VWP.

Effect of milk β-casein variants on cheese making traits

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Caseins are a heterogeneous class of milk proteins and, on average, they represent 82% of total protein content of bovine milk. They are the main protein component of cheese and are responsible of milk coagulation process, forming a reticulum in response to acidification and specific enzymatic proteolysis. Caseins family mainly includes four different fractions: α_{s1}, α_{s2}, β and κ. In particular, β-casein represents approximately 45% of total casein content. Specific β-casein variants, presenting a histidine at the amino acid position 67, have been largely studied for the supposed adverse role on the digestive process of milk. Moreover, β-casein variants are known to affect milk technological traits. Specifically, milk of cows with A1 and B alleles has better coagulation properties than milk of cows with the A2 allele. This study aimed to quantitatively investigate the effect of β-casein variants A1, A2 and B on quality and coagulation traits of bulk milk. A total of 132 commercial herds located in northern and central Italy were visited and bulk milk was collected after morning milking. Traditional milk quality traits (fat, protein, casein, lactose, somatic cell count and urea nitrogen), major minerals content and coagulation properties were assessed. Major minerals were determined by inductively coupled plasma optical emission spectrometry, and milk protein composition and the genetic variants of β-casein were determined using high pressure liquid chromatography. Effects of β-casein variants on milk quality and coagulation traits were estimated fitting a mixed model, with the concentrations of β-casein variants as covariates, and the day of analysis and herd as random effects. Results indicated that β-casein variants differently affected milk quality, major minerals content and milk coagulation properties. In particular, greater amounts of variants A1 and B had positive effects on rennet coagulation time and curd firmness, and were linked to greater Ca and P content compared with A2 variant. This project was funded by Sabelli SPA under grant agreement No [C38I19000000001 – LaTraZione 2021 Sabelli].

CNGB3 missense variant causes recessive day-blindness (achromatopsia) in Original Braunvieh cattle

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In this study we characterize the phenotype and the genetic aetiology of congenital day-blindness observed in several cases of purebred Original Braunvieh cattle. Electroretinography in an affected calf revealed absent cone-mediated function, whereas the rods continue to function normally. Brain areas involved in vision were morphologically normal. When targeting cones by immunofluorescence a decrease in cone number and an accumulation of beta subunits of cone cyclic-nucleotide gated channel (CNGB3) in the outer plexiform layer was obvious. After SNP genotyping and subsequent homozygosity mapping, we performed whole-genome sequencing and variant calling. Thereby, we identified a single missense variant in the bovine *CNGB3* gene within a case-related run of homozygosity on chromosome 14. All cases are homozygous carriers of the p.Asp251Asn mutation that was predicted deleterious affecting an evolutionary conserved residue. In conclusion, we have evidence for the occurrence of a breed-specific novel *CNGB3*-related form of recessively inherited achromatopsia in Original Braunvieh cattle segregating at an allele frequency of 7%.

Effects of a low protein diet with rumen protected Met, Lys and His on the performance of dairy cows

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Lowering the dietary protein content can reduce nitrogen (N) excretions and emissions and increases milk N efficiency (MNE). However, milk yield (MY) and composition can be compromised due to a deficiency in amino acids (AA). Methionine (Met) and lysine (Lys) are known as first limiting AA for dairy cows, but recently also histidine (His) was mentioned as limiting, especially in grass-based or low protein diets. To examine this hypothesis an experiment was started with a 3-week adaptation period (diet 16.5% CP), followed by a depletion period of 4 weeks in which all cows (n=39; average ± SD: 116±29 DIM, 1.8±1.2 lactations, 638±73 kg BW, 32.7±5.8 kg milk/d) received a low protein diet (14.5% CP). Then, cows were blocked by lactation number, His blood concentration and MY, and randomly assigned to one of 3 treatments during the experimental period of 7 weeks; (1) Low protein diet (CTRL); (2) CTRL + RP-Lys (AjiPro®-L, Ajinomoto H&N, USA) + RP-Met (Excential Rumenpass MET, Orffa Additives) (MetLys); (3) CTRL + RP-Lys + RP-Met + RP-His (exp. prod., Ajinomoto Co., Japan) (MetLysHis). Products were dosed assuming requirements for digestible Lys, Met and His being respectively 7.2, 2.4 and 2.4% of metabolizable protein. In the cross-back period of 5 weeks, all cows received the CTRL diet. During the last week of each period a N balance was conducted. Decreasing CP intake by 2%-points, increased MNE from 30.4 to 34.5% and lowered blood and milk urea by 29 and 20%, respectively. Compared to the CTRL group (21 µM Met; 71 µM Lys), Met and Lys blood levels were significantly ($P<0.05$) or tended ($P<0.10$) to be higher for the MetLys (26 µM Met; 88 µM Lys) and MetLysHis (26 µM Met; 87 µM Lys) group. The His blood level of the MetLysHis group (60 µM) was higher ($P<0.05$) in comparison to the CTRL (36 µM) and MetLys (43 µM) group. Although the blood results prove good absorption of the RP-AA, no effect on animal performance nor N balance was observed. Total blood AA profile indicated that other essential AA may have been limiting.

Do dairy cows feeding on organic wastes burp less methane?

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To tackle climate change, a major challenge in dairy production is reducing enteric methane emissions. Because of the relationship between productivity and emission intensity, low-yielding dairy cows in the Global South contribute disproportionally to greenhouse gas emissions worldwide. In several countries of the Global South, demand for animal products is rapidly increasing, which in turn leads to a rapid intensification of dairy production. In India, rapid urbanization also drives a rise in (peri-)urban dairy production and fosters a surprising opportunity to improve the sustainability of its dairy sector: an extensive feeding strategy relying on the upcycling by dairy cows of organic (peri-)urban wastes into high quality animal products. Organic wastes are easily available in (peri-)urban areas and furthermore, cheap. This study was thus conducted in Bengaluru, India, with the aim to compare the feeding strategy and productivity of nine (peri-)urban dairy producers, who included or omitted organic wastes in the diet of their dairy cows; Thereby we focused on the feeding strategy's impact on enteric methane emissions per cow. Results show that, despite a high average organic matter digestibility of the diet (71%, $P<0.05$), cows eating organic wastes (n=41) had a higher carbon footprint (2.9 kg CO_2-e per kg of milk, $P<0.05$) attributable to their low productivity (7.6 kg of milk per day, $P<0.05$). In contrast, despite a low average diet digestibility (64%), cows eating a more conventional diet (n=160) had a lower carbon footprint (1.9 kg CO_2-e per kg of milk) attributable to their higher productivity (10.0 kg of milk per day). Due to their high nutritional quality, organic wastes thus have the potential to reduce enteric methane emissions of (peri-)urban dairy cows. However, their use must go hand in hand with a further increase in productivity and better dairy farming practices in the rapidly urbanizing Global South to reduce the environmental impact of dairy production worldwide.

Exploring the bovine colostrum microbiome in Holstein Friesian and double muscled Belgian Blue

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The first milk a cow produces after calving, better known as colostrum, is of vital importance for the neonate. Besides supplying IgGs, referred to as 'passive transfer of immunity', colostrum contains beneficial bacteria as well. In humans, these beneficial bacteria are thought to express immune modulatory function after colonizing the infant's gut. The present study defines the colostral microbial composition from Holstein Friesian (HF) and Double Muscled Belgian Blue (DMBB) cows and investigates its link with colostrum quality (i.e. IgG level) and the transfer of passive immunity in the calf. Colostrum samples were taken from 64 HF and 46 DMBB cows at the ILVO research farm within 1 hr postpartum. Calves received 3 times 2 L of colostrum within 2, 6 and 24 hrs after birth, respectively. At 3 days of age, blood samples were taken from calves to measure serum IgG levels. Microbial DNA was extracted from colostrum with the Powerfood microbial kit (Qiagen, Germany). Library preparation and amplicon sequencing of the bacterial V3-V4 region of the 16S rRNA gene was performed using the Illumina MiSeq V3-technology (Macrogen, South-Korea). Taxonomy was assigned using the SILVA database with aligned rRNA gene sequences. Both colostrum as well as serum IgG concentration was higher in DMBB vs HF ($P<0.001$). Colostrum IgG level was on average 70.9±13.9 g/l for DMBB and 60.6±9.8 g/l for HF. Serum IgG was on average 24.5±10.1 g/l for DMBB and 17.8±7.5 for HF. Colostral microbial composition differed significantly between DMBB and HF. Moreover, within each breed, there was a significant difference in microbial composition between colostrum from good vs bad quality. Only in HF cows, microbial composition differed in colostrum administered to calves that obtained low vs high serum IgG. In addition, we observed a seasonal effect on the colostral microbial composition. These results suggest that microbiota of colostrum affects the transfer of passive immunity. Acknowledgement: I. Van Hese is an SB PhD fellow at FWO, Research Foundation – Flanders, project number 1S20220N.

Associations between udder health traits and milk protein fractions in Holstein cows

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Together with somatic cell count (SCC), the differential somatic cell count (DSCC) has recently been proposed as a novel indicator to deeply investigate udder health dynamics. While the association between these two parameters and milk quality has already been evaluated, less information is available for the fine milk composition (i.e. protein fractions), that could also be useful for understanding the relationships between udder health and milk technological characteristics. Therefore, this study aimed at gaining more insights on the associations between SCC and DSCC and the detailed milk protein profile in 1,102 Holstein cattle reared in 5 herds. Four caseins (CN) namely αs1-, αs2-, κ- and β-CN and 3 whey proteins, namely β-LG, α-LA and LF were quantified using a validated RP-HPLC method. All traits were analysed with a linear mixed model including as fixed effects cows' days in milk, parity, SCC and DSCC, expressed both as a proportion (%) and as a count of polymorphonuclear neutrophils plus lymphocytes (PMN-LYM) and macrophages (MAC), and the herd/date as a random effect. SCC was negatively associated with almost all the CNs ($P<0.001$), with the only exception of κ-CN, and positively associated with α-LA ($P<0.01$) and β-LG ($P<0.001$). On the other hand, DSCC (%) showed an opposite trend towards the protein fractions respect to the one observed for SCC. In particular, DSCC was positively associated with β-CN proportion and negatively with β-LG content ($P<0.001$). For DSCC expressed as count, the results showed that MAC was negatively associated with β-CN ($P<0.001$) since it decreased its content by ~13%, while the PMN-LYM was positively associated with β-CN ($P<0.05$). In conclusion, the study added new insights on the associations between DSCC and milk protein fractions, which could be useful to better assess the impact of mammary gland inflammation on the milk technological characteristics. Acknowledgements. The study was part of the BENELAT project funded by Regione Lombardia (bando d.d.s. n. 4403 del 28/03/2018) and the LATSAN project funded by MIPAAF.

Session 19 Theatre 11

Milk microbiota investigation during and after summer Alpine transhumance and relation with cheese

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Temporary summer alpine farms, called 'Malga' in Italy, contribute to the maintenance of the environment, landscape, local traditions and typical dairy production. Malga cheese is made with raw cow's milk and is characterized by a peculiar flavour. Our aim was to investigate milk microbiota in summer pasture and in autumn, after the transhumance, in lowland during indoor farming and to characterize milk composition, cheese-making properties and quality. The project involved 4 permanent farms (PF) moving the cows on 4 summer farms located at Passo Vezzena (Trentino). Milk samples were collected in mid-July in Malga, from 26 Italian Simmental cows (5-7 per farm), and in mid-October, during lowland indoor feeding based on hay and compound feed. Each milk sample was investigated for the total aerobic and anaerobic bacteria, for the technological potential (lactic acid bacteria, LAB), presence of putative healthy bacteria (bifidobacteria, propionibacteria) and pathogenic bacteria (coliforms, haemolytic streptococci, prototheca). Over 200 bacterial isolates from MRS, MRS-cys, YELA, and WC plates were considered for the identification by 16S rDNA gene sequencing. Individual milk samples were also analysed for microbiota composition by Miseq Illumina, milk composition, milk coagulation properties, nutrient recovery in curd, cheese yield and composition. The data were analysed with a mixed model including the fixed effects of farm, period and their interaction, and the random effects of individual cows and residual. The results revealed high variability among milk plate counts. Coliform, LAB, total bacterial counts, propionibacteria, bifidobacteria, β-haemolytic streptococci were significantly lower in Malga compared to PF, by contrast α-haemolytic streptococci counts were higher. The metagenomics analysis confirmed the high variability of the microbiota in milk produced during and after summer highland pasture. Moreover, we confirmed that the milk microbiota has implications for the change in the cheese-making attitude of milk and the quality of cheese obtained.

Session 19 Theatre 12

Modelling the impact of feeding strategies on greenhouse gas emissions using process-based models

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Feed management decisions are an important element of managing greenhouse gas (GHG) and nitrogen (N) emissions in livestock farming systems. Statistical and empirical models are well-suited for practical applications when evaluating mitigation strategies, such as GHG calculator tools for farmers and for inventory purposes, whilst process-based simulation models (which according to the Intergovernmental Panel on Climate Change (IPCC) are to be recommended when developing more refined methods) are more likely to provide insights into the impact of biotic and abiotic drivers on GHG and N emissions. These models allow to delineate underlying processes and their drivers within the system and to evaluate the integral effect of feeding management on GHG emissions and simulate the variation in GHG and N emissions and the associated whole farm budget. However, combining a whole set of process-based models has not been used yet. This study aims to describe how to capture the impact of diet on on-farm GHG and N emissions using process-oriented modelling approaches, and the steps of the modelling process. Two well-monitored case study farms, a confinement system in Germany and a grazing system in New Zealand will be assessed using this methodology and will be compared to evaluate the variation encountered in the Carbon (C) footprint of the two systems. The research is still ongoing, but the preliminary results from animal simulations show substantial differences between the outputs from process-based models between the farm cases and the current default emission factors (EF's) applied in national inventories.

AEA enhanced bovine vascular endothelial cell barrier integrity during LPS challenge mediated by CB1

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Dysfunctional inflammation associated with coliform mastitis is a major contributor to the severity and potential lethality of systemic infections. Targeted breakdown of the vascular endothelium is part of the pathogenesis of coliform mastitis. The ability of the endocannabinoid (EC) system to modulate inflammation was shown in several non-bovine species. The EC system is comprised of the cannabinoid receptor 1 and 2 (CB1/CB2, respectively) and their ligands, fatty acid ethanol amides and glycerols. The EC arachidonoylethanolamide (AEA) improves network formation and proliferation in human and rodent endothelial cell models via activation of several receptors, including CB1. Increased plasma AEA during systemic coliform mastitis *in vivo* and elevated CB1 and 2 expression in cultured bovine aortic endothelial cells (BAEC) challenged with endotoxin (LPS) support the bovine EC system involvement in inflammation. Prior experiments using the electric cell-substrate impedance sensing (ECIS) technology showed a dose dependent increase in barrier resistance of BAEC challenged with LPS. Rimonabant, a CB1 inverse agonist, was used to elucidate the involvement of CB1 in the increased barrier resistance observed in BAEC treated with AEA during LPS challenge. Primary BAEC cell lines were grown to confluence in ECIS 96-well arrays and treated with 25 ng/ml of LPS for 6- 8 hours before addition of AEA/rimonabant. Rimonabant doses of 0.1, 0.5, and 1 µM decreased barrier resistance within the first 2 hours of treatment, with a loss of effect at 4 hours. Timing and effect of rimonabant treatment indicate that AEA related increase in barrier resistance is CB1 mediated. However, AEA and rimonabant were shown to activate non-CB receptors involved in network formation and proliferation. Therefore, further *in vitro* modelling is necessary to fully elucidate the mechanism of increased barrier resistance of BAEC in response to AEA. Identification of regulatory points of AEA enhanced endothelial cell barrier resistance, may lead to new therapeutic targets to optimize inflammatory responses during acute disease events and reduce incidence and severity of systemic infections, thus reducing dairy cow mortality.

β-κ-casein haplotypes and its association with milk production traits in Spanish Holstein cows

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The A2 milk, that contains only the A2 type of β-casein, is gaining popularity in some markets. This work aimed to analyse the β-κ-casein (*CSN2* and *CSN3*) haplotypes and to study their associations with milk yield and composition traits as well as to evaluate the implications of selecting cows exclusively by *CSN2* genotype instead of *CSN2*-*CSN3* haplotypes. A total of 1,747 Spanish Holstein cows, genotyped with a chip developed by EuroGenomics and with predicted genomic breeding values (GEBVs), were considered. Haplotype analyses were done using *SimWalk2* considering all the genealogical information available (3,413 animals, of which 254 were bulls). The results indicated that the most frequent haplotype for β-κ-casein was A2-A (33%) in the analysed population. The association analysis showed that haplotype A1-A was negatively associated with GEBVs for milk and protein yield, with 100 kg ($P<0.001$) and 2.9 kg ($P<0.001$) less milk and protein yield, respectively, than other haplotypes. Likewise, haplotype A1-B was negatively associated with milk yield ($P<0.001$) but positively associated with fat ($P=0.004$) and protein percentage ($P<0.001$). Also, haplotype A2-B was positively associated with protein percentage ($P=0.002$). In the analysed population, the preferential selection for β-casein A2 aimed at a conversion of dairy farms towards A2 milk production does not seem it could negatively affect neither the protein content nor the milk properties for cheese-making. Though no evidences for genetic linkage were obtained, due to linkage disequilibrium between β-κ-casein variants observed in the population the impact of selection for β-casein A2 on κ-casein was considered. In this sense, the frequency of B allele for κ-CN -overexpressed compared with A allele- was similar and the frequency of E allele -known for having unfavourable milk clotting properties- was clearly lower (30%) for the animals carrying the A2 allele than A1 allele carriers for β-CN. However, analysis considering αs1- and αs2-caseins (*CSN1S1*-*CSN2*-*CSN1S2* haplotypes) should be done before recommending selection exclusively for *CSN2*. Also, results may not be generalizable to other populations, considering the differences in the linkage disequilibrium between *CSN2* and *CSN3*.

Application of a bio-economic model for economic estimation of Lithuanian dairy breeds

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Assessing the economic importance of traits is crucial for defining appropriate breeding goals in dairy cattle breeding. The objective of the present study was to calculate economic values (EV) for the two dairy cattle breeds Lithuanian Black and White (LBW) and Lithuanian Red (LR). For this purpose, the stochastic bio-economic model SimHerd was used, which simulates the expected monetary gain in dairy herds. The simulation model was calibrated for the two breeds, taking into account breed-specific phenotypic and economic data from productivity reports of Lithuanian breeds. For each trait, two scenarios were simulated with the respective trait at different phenotypic levels. To obtain the EV, the scenarios were compared with each other in terms of their economic outcomes. In order to avoid double counting of effects, the economic outcome was corrected using a multiple regression analysis. The EV were derived for traits related to production, fertility, direct health and calving traits. The EV for energy-corrected milk (ECM) was 0.16€/kg ECM for both breeds. The results showed high economic importance of functional traits in the cattle breeds. The largest EV (in € per change in trait unit and cow-year) were found for conception rate (EV_{LBW}=3.82€, EV_{LR}=1.95€) and heat observation rate (EV_{LBW}=2.74€, EV_{LR}=1.63€). The highest economic loss was found for cow mortality (-11.44€ and -10.77€ per change in trait unit and cow-year for LR and LBW, respectively). The highest EV for direct health traits were found for mastitis (173€ to 182€ per case) and lameness (122€ to 127€ per case). Overall, the results of this study allow to set up new breeding goals for the regarded breeds.

Nutrition of hyperprolific sows to achieve survival and growth of their offspring

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Nutrition of sows during the transition period, i.e. around parturition, and throughout lactation has a major impact on productivity of sows and piglets. Feeding strategy and feed composition in late gestation is important for colostrum production and the farrowing process and for the milk production of the sows during lactation. Especially the colostrum production and the farrowing process is of great importance, because breeding for hyper-prolific genotypes has accelerated the need for greater colostrum yield and reduced farrowing duration. With respect to colostrum production, piglet removal of colostrum is the limiting factor in litters with less than 15 piglets, but due to the continuous breeding for higher prolificacy, the sow is now the limiting factor for colostrum yield and piglet intake of colostrum. The nutritional needs of sows differ around parturition and at peak lactation, which challenges formulation of an optimal diet for sows in the farrowing unit. Dietary fibres are in general beneficial for sow productivity, health and welfare around parturition, and has been shown to improve traits like colostrum production, reduce physical activity, increase satiety and improve gut health, and decrease stillbirth rate. However, a high content of dietary fibre becomes an obstacle at peak lactation, where it may reduce the sow feed intake and/or reduce the nutrient digestibility. With respect to dietary protein, the requirement is greater around parturition as compared with the gestation period but lower than that required during lactation. As lactation progress, more and more protein (and lysine) is needed in the diet until peak lactation is reached to match the milk production, but dynamic changes in nutrient requirements are not possible to match using a single lactation diet. This talk will present updated knowledge on nutrition of hyper-prolific sows and special attention is given to traits like colostrum production, farrowing process and milk production, because they are of major importance for the piglet survival and growth.

Short-term fixation of sows in farrowing pens among different opening procedures

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Permanent fixation of pregnant sows in farrowing crates has long been under discussion for animal welfare aspects. Alternative farrowing systems have been researched but are often related to high piglet losses due to crushing. This study investigated a short-term fixation of sows in farrowing pens five days around farrowing compared to conventional farrowing crates with permanent fixation. A main aim was to examine whether the time of opening the crate has an influence on piglet losses, especially on crushed piglets after crate opening in the farrowing pens. Therefore, crates of the farrowing pens were either opened three days after farrowing at 6 am (AM: n=26) or at 6 pm (PM: n=25) or were closed full time for conventional crated sows in the control group (CL: n=23). All litters were standardized to 14 piglets within the first two days' postpartum. First results showed no significant differences of total piglet losses as well as piglet losses due to crushing in comparison of the AM- and PM-group and control group (CL) in crates (AM: 16.9%; PM: 15.3%; CL: 12.2% respectively AM: 8.21%; PM: 7.42%; CL: 5.58%). In pens with morning opening time, 25.7% of all piglet losses were caused within the first two days after crate opening and were significant higher compared to the other groups in this time period (AM: 25.7% vs PM: 12.5% vs CL: 6.38%; $P<0.05$). Furthermore, piglet losses due to crushing within the first two days after crate opening were significantly higher in the PM-group then in the CL-group (AM: 36.4% vs PM: 15.38% vs CL: 9.52%; $P<0.05$). The results showed that sows might adapt better to non-fixation when crates are opened in the evening and there is no disturbance due to stockpersons. In the ongoing study, sows are videorecorded to examine laying down and rolling over behaviour as well as the influence of sows' pre-experience in farrowing pens after crate opening among different times. In addition, moving sensors are installed to investigate the nest-building behaviour and to predict the farrowing date.

Modelling piglet pre-weaning mortality using litter records in technical management databases

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Piglet survival is a key issue in pig production. The variability observed among farms allows us to envision room for improvement for many of them. The objective of this study was to assess the effects of the main factors associated with maternity losses (stillborn and liveborn mortality rates). The analyses comprised nearly one million litters extracted from the French Technical Management of Sow Herds database (GTTT) managed by IFIP, which corresponded to the careers of sows that had weaned at least one litter in 2019. In a first step, analysis of average performances of farms made it possible to identify characteristics that influenced losses in the maternity unit, such as the size of the farm, its geographical location and the age at weaning of piglets, and to specify their relations. The proportions of stillborn piglets and piglets dead during suckling calculated per litter were then modelled with linear models by integrating the effects identified for the litter itself (litter size and sow parity, gestation and lactation durations, year and season of farrowing), the sow (commercial line, age at first farrowing) and the farm (size, region). The results obtained confirmed the major factors that influence piglet mortality, in particular prolificacy and sow parity. Indeed, liveborn mortality rate was increased by one percentage point for each extra piglet at birth. Furthermore liveborn mortality rate was significantly lower for multiparous sows than primiparous sows (contrast of -1.5 point of %). The results highlighted too the importance of characteristics of the sow and the farm. This study also demonstrated the value of analysing data recorded in the GTTT database to perform in-depth investigations related to sow reproduction issues.

Effects of the farrowing and rearing system on skin lesions, tail lesions and losses of piglets

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This study investigated the effects of farrowing systems followed by rearing in the same pen with a reduced regrouping at weaning on skin lesions, tail lesions and tail losses of growing and finishing pigs. In 8 batches, 1,106 piglets were weaned on day 26 from three different farrowing systems: (1) conventional farrowing crates (FC), (2) single-housing in free-farrowing pens (FF), and (3) group housing of lactating sows (GH). At weaning, they were divided into two rearing systems: (1) piglets were relocated and regrouped into conventional rearing pens (Conv) or (2) piglets stayed in their farrowing pens for the rearing period (FP). After 37 days of rearing, all piglets were relocated and regrouped for fattening period. Batchwise, tails were docked or left intact. Starting after weaning, pigs were scored for skin lesions biweekly during rearing and 4-weekly during the fattening period. Tail lesions and losses were scored weekly. After weaning, Conv-GH pigs had significantly less skin lesions than conv-FC and conv-FF pigs. After regrouping for fattening period, Conv pigs did not differ significantly anymore while FP-GH pigs had significantly less skin lesions than FP-FF and FP-FC pigs. Soon after weaning, the frequency of tail lesions of undocked Conv pigs increased (20.5%), while undocked FP pigs remained at the preceding level (10.4%). Tail lesions of undocked Conv pigs peaked in week 4 after weaning (66.8%) while FP undocked pigs reached their maximum (36.2%) two weeks later. At the end of the rearing period, already two thirds of Conv animals (65.3%) had lost parts of their tails, while most of FP pigs (96.3%) still had intact tails. At the end of fattening, only few Conv pigs had an intact tail while 56.9% of FP pigs had intact tails. In conclusion, group housing during lactation and litterwise rearing has immediate advantages on the incidence of skin lesions after regrouping events. With regard to the rearing of undocked pigs, rearing in the farrowing pen can be recommended.

Water consumption of lactating sows

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Water is essential for all life, but still this is often the forgotten ‘feed ingredient’ when we discuss optimum feeding strategies in our production animals. For lactating sows, the rule of thumb is that you need four litres of water to produce one litre of milk. But still there are more assumption than facts presented when we talk about water needs for our modern highly prolific sows’. As part of a project where the overall aim was to use current and new technology to collect large-scale feed and performance data from litter producing sows, we installed a water meter system, at a commercial pig farm, that enabled us to register the water consumption of lactating sows’. Water consumption was registered as l/hour, and one pen would thus give us 24 records per day. The animals included in the trial were Topigs Norsvin TN70 sows. Preliminary analysis shows that sows consume around 40 l/d±6.1 during lactation regardless of parity and production performance in terms of litter size and litter weaning weights, and there seems to be a moderate positive correlation between total water consumption and total feed consumed during lactation (0.40 P=0.0519. Average length of lactation in Norway is around 33 days. The data also suggest that sows consume less water around parturition and the first 24 h after farrowing before stabilizing on a daily intake ranging between 40-46 l/d throughout lactation regardless of their feed intake. Further analysis will aim at a more in-depth study of water consumption patterns in late gestation and during lactation with correlation to feed and performance data in a larger data set.

Session 20 Theatre 6

The effect of cross-fostering on pre- and post-weaning piglet performance

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Due to increasing litter sizes, piglet uniformity at birth has been decreasing which is accompanied by higher pre-weaning mortality that mostly affects low birth weight piglets. Piglet survival in large litters can be increased if surplus piglets are cross-fostered to smaller litters, however, little is known about the performance of cross-fostered (CF) piglets. A study performed at De Heus' Swine Nutrition Center aimed to establish: (1) what are the effects of cross-fostering on growth performance of CF piglets and of receiving litters; and (2) which piglets (light, regular or heavy at birth) need to be CF into what type of receiving litter (low or high uniformity) to achieve higher growth performance CF piglets. Piglets were CF when the number of liveborn piglets exceeded the number of functional teats, within 24-48 hours after birth. Cross-fostering was based on individual birth weight (light (≤1.2 kg), regular (1.2-1.6 kg) or heavy (≥1.6 kg)) and mean weight of receiving litter to create either low or high uniform litters. All piglets (n=483) were again individually weighted at day 7, 14 and at weaning. In addition, CF piglets (n=38) and their paired control (piglet from the same birth sow with similar birth weight) were individually weighed at day 7 and 14 post-weaning. Data was analysed using a Mixed Linear Model to evaluate the litter effect on piglet performance (control vs receiving litters) and analyse individual piglet performance (CF piglets vs paired control). Piglets from receiving litters had a lower body weight (BW) at day 7, 14 and at weaning and a lower average daily gain (ADG) in every period compared to piglets from control litters. Compared to paired control, CF piglets had a lower BW at day 7, 14, weaning and day 7 and 14 post-weaning and a lower ADG in every period except for the period from weaning until day 7 post-weaning. There was no effect of low or high uniform litters and birth weight category on the performance of CF piglets. In conclusion, this study showed that: (1) cross-fostering piglets causes not only a lower BW and ADG for the CF piglets, but also for the receiving litters; and (2) there is no effect of low or high uniform litters and birth weight category on the performance of cross-fostered piglets.

Session 20 Theatre 7

The course of parturition and periparturient behaviour of sows to predict the expulsion of a piglet

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The farrowing process is a crucial aspect in piglet production and forms the basis for an optimized start of the suckling period for both the sow and her piglets. Due to the extraordinary importance of farrowing, the aim of the present study was the analysis of sow body movements with the objective to record behavioural pattern that could indicate the imminent birth of a piglet and thus support the early detection of a piglet's birth. Therefore, the pattern of farrowing processes and the behaviour traits of 41 sows with the birth of in total 659 piglets were observed by use of a continuous video monitoring during farrowing. The recorded sow behaviours included: (1) posture changes; (2) striking with the hind legs; (3) pulling the back leg forward; and (4) tail flapping. The piglet births occurred almost solely in a lateral lying posture of the sow (92.6%), which was occasionally changed by taking up another posture. Changes in posture were recorded more frequently in the first quarter of farrowing. Neither the striking with the back leg nor pulling the back leg forward could be used in this study as characteristics of an imminent birth of a piglet. However, it could be determined, that the sow's tail flapping could serve as a potential behavioural trait indicating an imminent birth event and thus might support early birth detection. In conclusion, monitoring the sow's tail flapping can be recommended to include in automatic monitoring systems for the farrowing process.

PertMat: a new tool for automatic determination of risk factors and improvement in breeding

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The IFIP – Institut du Porc developed a new expert tool (PertMat) designed to perform automatic mortality profiling and identification of specific areas for improvement. This tool uses individual farm data stored in the French National Pig Management database (GTTT) and is supported by IFIP GT-DIRECT Web platform. The mortality profiles is established for each farm, considering mummies, stillborn and liveborn mortality rates. Based on contemporary or historical litters in the national database (more than 600,000 litters each year), PertMat identifies the mortality's explanatory factors and suggests areas for improvement. More than 12 risk factors are evaluated, in relation with the characteristics of the sows (litter sizes, parity, gestation length, genetics, age at first farrowing, etc.), and management (season, month and day of farrowing, day of birth, etc.). Simple within-farm statistical tests (Kruskal-Wallis) performed by PertMat identify significant factors associated with different mortality criteria. PertMat allows an exhaustive analysis of all the factors likely to affect the rate of losses among those available in GTTT databases. Without collecting additional data, it provides descriptive results and automatically identifies risk factors specific to each farm. This first step allows to enrich exchanges between technical and veterinary breeders and to suggest areas for progress. A study on 250 farms with a very high level of prolificacy conducted in 2019 by Boulot *et al*. confirmed the relevance of the factors tested by Pertmat. The situation of each farm is unique. Certain factors almost always contribute to the explanation of pre-weaning mortality, such as the size of the litter. Others act only in certain farms given a particular context, and sometimes occasionally over time such as genetics, season, month and day of farrowing. To support changes in prolificacy, PertMat will add new analyses on management practices for supernumerary piglets and their efficiency and crossed factors.

Evaluation of point-of-care tests to characterize the vaginal discharge of sows after parturition

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The lochia is the physiological uterine discharge postpartum. Pathological changes indicate puerperal disorders in sows, which might negatively influence the further reproductive performance. Therefore, the aim of this study was to characterize the lochia in sows using feasible and reliable point-of-care tests, and to correlate their results with the subsequent reproductive performance of the sows. The birth process of 48 clinically healthy sows accommodated in free farrowing pens was monitored, and several parameters characterizing the vaginal discharge such as total amount, colour, amount of cells (somatic cell count) and cell characteristics (cytology) were collected daily from first to fifth day after parturition. Finally, the reproductive performance of the subsequent gestation was evaluated and corelated with characteristics of the lochia. The amount of vaginal discharge was significantly increased on the second ($P<0.01$), third ($P=0.019$) and fourth ($P=0.011$) day post-partum compared to day one. Furthermore, a decrease in the percentage of neutrophilic granulocytes from day one to three ($P=0.038$), four ($P=0.038$) and five ($P=0.048$) post-partum was observed. The percentage of neutrophilic granulocytes in yellowish vaginal discharge was increased compared to whitish ($P=0.02$) or clear ($P=0.027$) vaginal discharge. In addition, the amount of vaginal discharge was significantly increased, when obstetrics ($P=0.003$) or an extended farrowing duration ($P=0.017$) was noted. Sows with a high amount of vaginal discharge had a significant higher body temperature than sows with no ($P=0.014$) or low amount ($P<0.01$) of vaginal discharge. No correlation was detected between the evaluated parameters of the lochia and the subsequent reproductive performance. It is hypothesised that the amount of vaginal discharge alone is not predictive for the performance of sows during their next gestation. However, it might serve as indicator for acute endometritis. In summary, the different parameters of the vaginal discharge determined by means of point-of-care tests might be useful to strengthen a presumptive diagnosis of endometritis in sows during the first five days after parturition.

Implementation of a pig toilet in a nursery pen with straw-bedded lying area

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The emission of ammonia is one of the largest problems of intensive pig husbandry. Currently, installing air filter systems is a suitable measure to keep ammonia pollution levels as low as possible, thus protecting the environment. However, in order to reduce the formation of ammonia itself and to improve animal welfare, it is necessary to reconsider and optimise pig housing conditions. In this study, a pig toilet was installed on an organic pig farm in the Netherlands, which enabled the pigs to use a lying area littered with straw and keep it clean. For this purpose, the natural excretory behaviour of the animals, which leave the nest when they are only a few days old to defecate in a suitable place far away from the nest was used. Sixteen animals (nine female and seven male pigs) of the Berkshire breed were weaned at six to seven weeks of age and housed in the experimental system for four weeks. Four trials, each with four pigs per group, were performed, and videos were recorded on two days per week during the entire four-week housing period. A total of 1,500 elimination processes were recorded and analysed during that time. The analysis showed that 96.4% of all urinations and 97.6% of all defecations took place in the installed toilet. Video analysis also revealed that the animals immediately divided their new environment into functional areas and strictly adhered to them after the first day. We conclude that installing a pig toilet offers the possibility to create littered lying areas in pig farming, possibly reducing ammonia emissions and increasing animal welfare. Further studies are needed to find out whether, and to what extent the system can be transferred to commercial pig farming.

Survey on farrowing management and reproductive performance in sows in a free farrowing system

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Over the last three decades, the litter size of sows has considerably increased. Therefore, an adequate farrowing management is necessary to increase the sows` health and the vitality of the piglets. The aim of this study was to investigate different management factors, which are routinely implemented on farms with free farrowing systems during the peripartal period in Switzerland using an online survey. In addition, an association between the measurements and the performance characteristics were analysed to identify factors, which positively influence the reproductive performance. The questionnaire comprised of a total of 40 questions covering general information about the herd, reproductive performance and farrowing management and was distributed to 140 farms across Switzerland. The statistical processing of all data was performed in R, considering the level of statistical significance being $P<0.05$. In total, 43 farms (response rate 31%) answered the survey. Overall, a mean of 86 sows (SD±50.5) were housed per farm. On average, the sows were moved on day 5 (SD±1.5) before farrowing into the free farrowing pen, which had a mean size of 7.1 m^2 (SD±1.1 m^2). A nestbuilding material was available on all farms. A minimum of 1 kg nestbuilding material per day significantly increased the liveborn piglets ($P<0.01$), decreased the stillborn piglets ($P=0.03$) and reduced the farrowing ($P=0.01$) and placenta delivery ($P=0.01$) duration. In 89% of the farms management procedures of new born piglets are implemented. Estimated duration of birth from the first to the last piglet was 260 minutes (SD±78 min). In 5% of the farms painkiller were administered routinely. The use of painkillers negatively correlated with the number of stillborn piglets ($P=0.004$). Taken together, to our best knowledge this is the first survey evaluating the farrowing management on farms with free farrowing systems in Switzerland. Several management procedures should be implemented on farm to improve animal welfare and therefore increases productivity.

Maternal effects of the leptin receptor gene in pigs

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Maternal effects can impact genetic change in natural and selected populations. There are many examples of how maternal environment may affect offspring development and growth. One of the best-known in pigs is the favourable effect of the sow feeding status on piglet growth. Lactation increases energy demands for sows since they must simultaneously cope with self-maintenance and milk production. However, although genetic correlation estimates often support a negative relationship between maternal and direct effects, there is still a lack of evidence for causal genetic mechanisms that may help to decipher such antagonism. We hypothesized that variants in genes that impact feeding status and body composition may be a source of maternal influences for piglet growth. A recessive missense mutation (C>T) in the porcine leptin receptor (*LEPR*) gene (rs709596309) has been identified as a possible causal polymorphism for increased feed intake and fatness. Using data from a Duroc line, we show that the TT sows exerted a negative impact on the body weight of their offspring at the end of the growing period of similar extent to the positive direct effect of the TT genotype over each individual. Thus, TT pigs from TT dams were about as heavy as CC and CT (C–) pigs from C– dams, but TT pigs from C– dams were around 5% heavier than C– pigs from TT dams. In contrast, body composition was only influenced by *LEPR* direct effects. Results also indicate that TT pigs consumed more feed, favoured fatty acid uptake over release, and produced lighter piglets at weaning than their C– counterparts. We conclude that the antagonistic maternal and direct effects of the leptin receptor gene on body weight are due to a higher propensity of TT pigs for self-maintenance rather than for offspring investment. The *LEPR* gene provides evidence for a negative correlation between direct and maternal effects in body weight.

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Urinary creatinine concentration in lactating sows in relation to body weight and amount of daily urine

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Creatinine has commonly been used as an index compound to express the creatinine rate of urinary metabolites in different species. Feeds for sows is composed mainly of cereals and by-products of cereals. They are frequently contaminated with zearalenone (ZEA) or deoxynivalenol (DON) and so affect for example feed intake or fertility. The diagnosis of ZEA and DON often based on an analysis of feed. But a major part of absorbed ZEA and DON is excreted via the kidney. The combination of creatinine level in urine and the concentration of mycotoxins in urine maybe can be used as an indicator of exposure in sow´s. The objective of the present study was to validate urinary creatinine concentration to predict the amount of daily urine in lactating sow´s. The urinary creatinine excretion was obtained by an experiment with cross breed sows (F1) during lactation (first and third week of lactation). Daily creatinine excretion (g/d) was calculated with concentration in urine with amount of urine per day. For this purpose, the amount (litres) per urine output was recorded and samples of the urine were taken. The sows were weighed and the fat thickness was recorded. The urine samples were analysed for the content of creatinine, allantonin and hippuric acid as well as mycotoxins. The data was evaluated using a one-way analysis of variance. Statistical significance was accepted at $P \leq 0.05$. The total amount of urine excreted per day was 21 litres. In the investigations, a average excretion of creatinine in the urine of the sows of 0.68 g/l could be determined, the coefficient of variation was 78%. The average daily amount of creatinine was 12.9 g/day. If the creatinine excretions are related to the live weight of the sows, it can be observed that a strong variation from 30 to 74 mg/kg could be observed. A creatinine excretion of 48 mg/kg live weight was documented on average. In conclusion, the results of the present study show that the urinary creatinine concentration can´t use for controlling of the feeding systems and metabolism of lactating sows.

New functional traits in selection of the Czech dam pig breeds

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Breeding for animal functional traits can improve acceptability and long-term sustainability of the pig sector. Therefore, farrowing interval (FI) and number of sow teats (NST) were added to the current breeding objectives and selection criteria of the local dam pig breeds as the first step of the present index enrichment. In total, 5 traits as breeding objective and 7 traits as selection criteria were evaluated. Regarding the litter size, the number of piglets born alive (defined as breeding objective) was also considered in the index along with the total number of piglets born and weaned. Genetic and economic parameters of the traits of interest and general principles for selection index construction and for selection response calculation were applied. Under the current production and economic conditions, economic values of the new goal traits expressed per sow and year were -2.11 € per day of FI and 32.52 € per one functional teat. The trait breeding values were estimated with reliability of 22 and 60%, respectively. The currently constructed index indirectly resulted in unfavourable genetic changes in both traits (FI increased by 0.07 days and NST slightly reduced by -0.01 teats, both expressed per sow and generation interval). When the enhanced selection index was settled on actual traits ratio with making a space for the new traits (5, 5, 8 and 10% for piglets born total, piglets weaned, FI and NST, respectively), selection responses for the new goal traits were more favourable (+0.01 day and +0.05 functional teats) preserving the index reliability on 43%. The same was true when the index coefficients were optimised to reach the maximal selection response in breeding goal traits. In this case, responses of +0.01 day and +0.22 functional teats and a higher index reliability of 57% was reached. Proportion of growth was there reduced (up to 8%) in favour of the functional teats ratio (increased on 30%). The results showed that enhanced selection index results in a more favourable selection response in the new functional traits while keeping genetic gains in the current objective traits on acceptable levels. The study was supported by project MZE-RO0718-V003 and QK1910217 of the Czech Republic.

Survey on mycotoxin content in feed and colostrum from sows

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Animal species, age and type of production can variously modify the risk that mycotoxins may have on animal production, depending on the type of mould. During lactation, mycotoxins can be transferred from the feed consumed to colostrum and milk. Research is mainly focused on mycotoxins and especially aflatoxin transfer from dairy cows, for the risk of dairy products. Conversely, studies on the transfer of mycotoxins from sow feed to colostrum are scarce and not extended to several mycotoxins. A sample of complete feed for gestating-lactating sows and one of colostrum were collected from 2 or 3 sows (total 49) from each of the 19 included Italian farms. The farms were selected to: (1) have an in-house feed mill; (2) administer the same batch of feed at least from 5 days before the farrowing until the early lactation); (3) do not use chelating additives. Samples of colostrum were obtained at farrowing, without use of oxytocin. The feed contents of aflatoxins B1/B2 (AFs), fumonisins FB1/FB2 (FUs), deoxynivalenol (DON) and zearalenone (ZEA) were assessed using ELISA and confirmed by liquid chromatography-mass spectrometry. Levels of mycotoxins present in the feeds were in general low (10; 12; 17; 2 positive samples for AF, FU, DON and ZEA, rsp.), always within the risk limits of the EU law, except for 2 samples (1 for AF, 1 for DON). Based on feed values, colostrum samples from 13 farms were tested for at least one mycotoxin (total 35). M1/M2 and AFs were not detected in any sample. A signal for FUs was observed in 5 of 11 colostra, despite the low feed values; DON was frequently present in the colostra (10/14). A value of colostrum content of 10.9 µg/kg DON was seen in the farm where the feed exceeded the European suggested limit, but this is approximately 1/69 of the value showing toxicity in young pigs. The lack of reference values for neonate pigs, their reduced immunocompetence, the risk of higher and longer ingestion of DON by sows, suggest that increasing routinely controls of sow feeds are needed. More research on DON transfer and toxicity in piglet is advisable. Attention should be paid to the control of feeds for sows also for fumonisins content.

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Genetic evaluation for gestation length and stillborn piglets in Swiss Large White sows

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The aim of this study was to estimate genetic parameters and genetic trends of reproductive traits, especially gestation length and stillborn piglets in Swiss Large White sows. The 36,181 litter records of 9,553 sows over a 10-year period 2010-2019 from 6 biggest herdbook farms were used for parameter estimation by REML. Gestation length (GL) and percentage of stillborn piglets (PSB) were analysed together with number born alive (NBA), percentage of piglets below 1 kg (PB1), percentage of piglet survival during suckling (PS) and days weaning to oestrus (WO). The animal model included permanent environmental sow and service sire effects as additional random effects. Heritabilities (h^2) for NBA, PB1, PS and WO were 0.12, 0.09, 0.06 and 0.10, respectively and for PSB and GL were 0.10 and 0.40, respectively. The h^2 for PSB is in similar order of magnitude to NBA. PSB is genetically unrelated to NBA (0.07), however it has favourable negative genetic correlation with PS (-0.21). Stillborn piglets cause wasting of valuable resources and should be included in the breeding goal. Inducing births is rarely (approx. 10%) practiced in Switzerland. Therefore, variability and h^2 of GL is of interest. GL is more heritable than the other reproductive traits with the strongest genetic correlation to PS (+0.30) and small negative genetic correlations to NBA (-0.18) and PSB (-0.09). Phenotypic and genetic trends for reproductive traits were estimated using a 253,035 litter records from 62,491 Large White sows with litter years 2010-2020 from the herdbook. Trends were estimated as regression coefficients of year of the litter on phenotypes or EBVs of the sow. The yearly genetic trend estimates for NBA, PSB, PB1, PS, WO and GL were 0.15, -0.19, 0.01, 0.34, -0.08 and 0.03, respectively, the phenotypic trends for these traits were 0.07, 0.03, -0.14, 0.25, -0.08 and 0.08, respectively. In our balanced breeding goal, the highest selection pressure is on PS, resulting in corresponding selection response. As a consequence of high selection pressure on PS, GL of sows has increased yearly by 0.08 day since 2010. The genetic trend of GL was positive but lower than its phenotypic trend. Genetic parameters suggest that it might be possible to keep GL genetically stable with limited negative impact on selection response for PS.

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Effect of an arginine supplementation in gestation and lactation on sow performances over two cycles

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L-arginine is a functional amino acid involved in numerous biological processes such as embryonic and foetal development during gestation and is also essential for the development of suckling piglets. The present study aimed to determine the effect of a L-arginine supplementation in late gestation and lactation on sow and litter performances over two reproductive cycles (RC). At insemination, 36 sows (12 primiparous and 24 multiparous) with an average body weight (BW) of 204±24 4 kg were allocated in one of the two treatment group and followed over two RC. From day 70 of gestation onwards, half of them were offered L-Arginine supplemented gestation and lactation diets (ARG+; dArg/dLys=1.8) while the other half received a standard gestation and lactation diets (ARG-; dArg/dLys=1). Their BW and back-fat thickness were recorded at insemination, on days 84 and 110 of gestation, at farrowing, on days 7, 14 and 21 of lactation and at weaning (d 28±3) over the two RC. On day 20 of lactation, milk samples were collected. Data were analysed with the MIXED procedure of SAS (9.4). The REPEATED statement was used to account for the multiple measurements on sows over the two RC. There were no interactions ($P>0.10$) between the L-arginine supplementation and the time. The L-arginine supplementation did not affect ($P>0.10$) back-fat thickness or BW over the two RC. However, ARG+ sows had a lower ($P=0.01$) back-fat gain between insemination and day 110 of gestation, lost less ($P=0.007$) back-fat between day 110 of gestation and the day of farrowing but had similar back-fat loss in lactation compared to ARG- sows. The L-arginine supplementation markedly reduced ($P<0.001$) BW loss in lactation. Over the two RC, the L-arginine supplementation had no impact ($P>0.10$) on the number of total born, stillborn, born alive and weaned piglets. Moreover, nor the litter weight at birth, or the litter weight gain until weaning were affected ($P>0.10$) by the L-arginine supplementation. Finally, the arginine content in the milk expressed in a dry matter basis tended to be greater ($P=0.08$) in ARG+ than ARG- sows. The present results suggest that L-arginine supplementation is effective in limiting sow weight loss in lactation without affecting piglet performances.

A 39, XYY-trisomy case found in a Minipig

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For several decades now, mainly since the 1980s, many studies have been carried out to identify chromosomal rearrangements in pigs and their impact on reproductive indexes. The presence of structural chromosomal alterations is common in pigs, mainly the reciprocal translocations, whose incidence has been estimated to be around 0.47% or even higher when systematic detection studies for chromosomal aberrations are not carried out on the population. By contrast, anomalies in the number of chromosomes in the pig rarely have been found, being especially uncommon on the sex chromosomes, in which only a few cases have been reported: X-monosomy (37, XO), Klinefelter syndrome (39, XXY trisomy; 40, XXXY aneuploidy) and mosaic karyotypes (39,XXY/40,XXXY; 37,X/38,XY/39, XYY). This study describes a 39 XYY trisomy case found in a Minipig whose karyotype was analysed both with classical cytogenetics and by FISH. This aneuploidy in the sex chromosomes had already been described in a pig mosaicism case (37, X / 38, XY / 39, XYY) but not as trisomy in an individual. Reproductive consequences of such trisomies in carriers may be testicular hypoplasia and dysfunctions of the secretory role of sexual glands, thus, usually these animals present abnormalities in the gonads or the external genitalia that could affect their fertility. The individual studied 39, XYY, presented phenotypic characteristics of a male, and although its seminal quality or hormonal profiles could not be studied, a testicular histological study was performed to evaluate the status of the seminiferous tubules. It was observed that the diameter of the seminiferous tubules of the male with 39, XYY trisomy, was significantly smaller than in its contemporary ones that did not present altered karyotype. The presence of chromosomal aberrations in pigs (both numerical and structural) can have a negative effect on their fertility and development. For this reason, it is crucial to establish an early detection cytogenetic screening, mainly in animals that are going to be used as breeders.

The impact of dietary potassium diformate on the milk yield in sows

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Optimising the sow condition and growth rates of suckling piglets are key requirements for success in today's pig production systems. Organic acids, particularly salts of organic acids, have been reported by many experts to enhance growth performance in swine production sustainably. Potassium diformate (traded as FORMI, ADDCON, hereafter referred to as KDF), a double-salt of formic acid, has been shown in numerous trials to improve health and performance in piglets, growing-finishing pigs and sows. The impact of KDF on sows and their suckling piglets is noteworthy and has been studied recently in more detail worldwide. However, to gain more insights into the impact of the additive onto the performance of sows during late gestation and lactation, more data are needed, especially regarding estimation of milk yield during lactation. It is therefore essential to understand the magnitude of the impact of feed intake on sow milk production, as well as the milk energy output, since insufficient sow feed intake will result in a reduction in milk produced – leading to smaller litters of lighter pigs at weaning. To estimate the impact of feeding KDF in the lactation diet of sows on their milk production, a meta-analysis was performed. The final dataset contained the results of 5 documented studies with KDF-inclusion in the lactation diet, ranging from 0.8 to 1.2%. Including KDF in the sow diet lasted from one week before farrowing until piglets were weaned (28 days). The average dietary KDF level included across the dataset was 0.96%. Daily milk production and daily milk energy output were calculated considering litter weight gain, litter size and milk dry matter content (19%) applied to the equations of Noblet and Etienne (1989). Data were analysed and a confidence level of 95% defined. Over the lactation period, milk production increased significantly ($P<0.01$) by more than 5.3% from 9.1 kg/d to 9.6 kg/d, while milk energy output rose ($P<0.01$) from 47.1 to 49.7 MJ GE/d in sows fed with KDF. This finding agrees with studies in sows, which found an increased feed intake of the lactation diet by the sow. It is therefore concluded, that FORMI in sows can improve conditions during the suckling period, when it comes to the supply of their piglets with milk.

Additive and dominant genomic analysis for litter size in pure and crossbreed Iberian pigs

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White pig production relies on crossbreeding of purebred populations to increase the phenotypic yield thanks to heterosis and complementarity. In contrast, Iberian pig production has been developed using purebred lines. In the last decade, INGA FOOD S. A. has produced a hybrid sow by crossing the Retinto and Entrepelado strains. Until now, selection of parental populations has been developed using purebred information under the hypothesis of a high genetic correlation with crossbred performance. This study aims to estimate the additive and dominant variances of the pure and crossbred populations for litter size (total number born). We used 2,394 litter size data from the Entrepelado population, 2,350 from the Retinto population, and 2,220 from the crossbred population, corresponding to 373, 362 and 582 sows, respectively. All individuals were genotyped with the GeneSeek® GGP Porcine70K HD chip. The model of analysis was a 'biological' multivariate mixed model with additive and dominant SNP effects. The variance of the additive genotypic effect ranged between 0.136 (Retinto) to 0.544 (Crossbred population). In contrast, the variance of the dominance genotypic effect varied between 0.266 (Retinto) to 0.332 (Crossbred population). The additive and dominance genotypic correlations were all positive and over 0.5. After backsolving to calculate the variance associated with SNP effects, we computed the additive and dominance variances in pure and crossbred populations. The additive variance was higher in the crossbred population (0.411) compared to the purebred (0.153 and 0.106) and the dominance variance ranged between 0.073 (Retinto) to 0.111 (Entrepelado).

Reproductive effects in a boar with various C-Band polymorphisms

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Constitutive heterochromatin is highly polymorphic, both in size and in the location of heterochromatin, but apparently it has no phenotypic effect and appears intensely stained using the C-band technique. Due to the role that centromeres play in spindle attachment, chromosome pairing, and cell division, any perturbation in these heterochromatic regions could have consequences on gene expression affecting gamete formation, fertilization and embryogenesis. The karyotype of a Pietrain boar was analysed from a peripheral blood lymphocyte culture using the GTL-Banding technique. We found that three of its acrocentric chromosomes (chromosomes 14, 16 and 18) presented heteromorphism with its homolog. We carried out a C-band staining to stain constitutive heterochromatin of centromeres and we observed the difference in the size of the centromeric heterochromatin between homologous chromosomes. Polymorphisms in the amount of centromeric heterochromatin were found in all acrocentric chromosomes of the pig, but in our laboratory, we had previously identified the polymorphism in chromosome 14 only in one animal. Therefore, we ruled out that it could be a small translocation by performing Fluorescent *in situ* hybridization (FISH), marking chromosome 14 with a whole chromosome painting probe and observing that no area with fluorescence appeared on any other chromosome. This boar has made a total of 161 mattings with a fertility rate of 78.8% and an average of total piglets born alive per litter of 14.6. Analysing the reproductive data of this boar and statistically comparing them with those of the rest of the males of the insemination centre during the same period of time, we verified that there were no significant differences in these reproductive indexes. Therefore, we can affirm that these C-band polymorphisms of the centromere constitutive heterochromatin had no impact on the reproductive capacity of this boar.

Reproductive qualities of pigs of Irish selection in the conditions of industrial production in Ukraine

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In recent years, a large number of breeding pigs of foreign origin are imported to Ukraine without taking into account of their adaptability to new climatic conditions. Different studies were conducted to determine the reproductive qualities of purebred and hybreed sows of Irish origin in the Ukrainian industrial farms. For this study, 4 groups of sows 60 heads each were formed: group I 'control' (♀Yorkshire × ♂Yorkshire), group II (♀Landrace × ♂Landrace), group III (♀Yorkshire × ♂Landrace), group IV (♀Landrace × ♂ Yorkshire). The maintenance and feeding of sows during the experiment were identical. The experiment took into account the total number of piglets born, the number of piglets born alive, the weight of the litter at birth, the weight of the litter and one piglet at weaning at 27 days. Sows from group III had a higher total number of piglets born (17.2±0.35 heads) and the number of piglets born alive (15.8±0.34 heads) ($P \leq 0.01$) comparing to control group. The part of stillborn piglets was higher in sows of group I – 12.4±0.93% ($P \leq 0.01$-0.001). The best level of survival of piglets was indicated in sows of group IV – 12.8±0.12 ($P \leq 0.001$). Purebred Landrace sows covered with Landrace boars had a higher weight of weaned piglets than their Yorkshire-crossed analogues. It was found that the litter weight of piglets at weaning of sows III (82.7±1.9) and IV groups (85.6±1.71) were higher compared with the control group ($P \leq 0.05$).

Perfect condition at farrowing is necessary to maximize the performance of first parity sows

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Lactation imposes a heavy burden for first parity sows which are still growing, have a lower feed intake capacity and have fewer body reserves compared to older sows. Meanwhile, modern sows, including first parity, are leaner, produce larger litters and wean heavier piglets. Extensive body weight (BW) losses during the first lactation reduce reproductive performance in the next cycle and reduce sow longevity. This study at De Heus' Swine Nutrition Center aimed to gain insight into factors influencing lactation performance of first parity hyper-prolific sows. Records of 226 TN70 sows, housed in farrowing crates from day 108 of gestation until weaning (25 days) were used. All sows were provided with the same diet and feed strategy during their gestation and lactation. A correlation-based principal component analysis (PCA) was conducted to explore the variation in the dataset, which includes 22 variables. The first two components were responsible for 63.5% of the total variation, PC1 accounted for 33.9% and PC2 accounted for 29.6. The PCA correlation matrix indicated that average daily feed intake (ADFI) during lactation was negatively correlated with backfat thickness (BFt) at farrowing. BW and BFt at farrowing were positively correlated with BW loss during lactation. Litter growth (LG) was positively correlated with ADFI and with BW loss during lactation. The effect of BW, BFt at farrowing and lactation ADFI on sow performance was also analysed using the GLM procedure of ANOVA. Sows were divided into three categories for each parameter. BW at farrowing: light (<223 kg), average (224-240 kg) and heavy (>240 kg), BFt at farrowing: lean (<15 mm), average (15-18 mm) and fat (>18 mm) and lactation ADFI: low (<5.5 kg), average (5.5-6.1 kg) and high (>6.1 kg). Heavy sows had similar ADFI as average and light sows but lost more weight during lactation ($P<0.05$). Lean sows had the highest ADFI ($P<0.05$) and tended to lose less weight and BFt during lactation ($P<0.10$). LG was not affected by BW and BFt at farrowing. Sows with a low ADFI had lower LG ($P<0.05$) and higher BW losses ($P<0.05$) during lactation. This study indicates that first parity sows have a high feed intake potential. Optimal body condition at farrowing is necessary to maximize ADFI and to limit BW losses during lactation.

Session 21 Theatre 1

The role of livestock production in global food security

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Livestock farming is found in a variety of ecosystems around the world. Permanent pastures and grasslands cover about 25% of the world's land. Production systems and products are also very diverse. Meat, milk and eggs account for 34 and 17% of the global protein and calorie consumption respectively. They are also dense in variety of essential micronutrients. Livestock also make a significant contribution to food security through manure production, animal traction and transport and income. They have a key role to play in women's empowerment and gender equality, both in rural and urban areas. Demand for meat, milk and eggs is increasing due to population growth, rising incomes and urbanization. This trend is expected to continue, particularly in low and middle income countries. The sustainable development of the sector must consider a range of current issues, starting with animal feed and the ability of livestock to convert grass and swill into protein. Contrary to often cited figures, only about 3 kg of cereals are needed to produce 1 kg of meat on average globally. The efficiency of monogastrics, often highlighted in terms of GHGs emitted per kg of meat, is questionable if the consumption of forage and non-edible food by humans is included in the balance. The entire livestock sector uses about 2.5 billion hectares, 77% of which are grasslands, with a large part of it non-cultivable and therefore only for grazing animals. Livestock contribute to greenhouse gas emissions, particularly through enteric fermentation, but the sector has a high mitigation potential, notably through carbon sequestration. The role of livestock in the circular bioeconomy needs to be strengthened by promoting the use of by-products and residues as feed for livestock and recycling manure for energy and nutrients. The sustainable development of the sector requires adequate policies, and there are already a variety of mechanisms available, including regulations, conditionality systems, payments for environmental services and research and development. Priority areas for policy makers should be aligned with the Sustainable Development Goals and include: (1) food security and nutrition; (2) economic development and livelihoods; (3) animal and human health; and finally, (4) the environment, climate and natural resources.

Session 21 Theatre 2

Animal products in healthy diets

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The place of animal source foods in healthy diets has become the topic of heated arguments, also within the scientific community. Whereas some authors recommend to drastically reduce (or in some more extreme cases, to eliminate) their intake for health reasons, others argue that this may come with its own problems as these foods are important sources of quality protein and 'difficult' micronutrients, besides a long list of other bioactive compounds that may be overlooked in conventional assessments. There are reasons to assume that animal source foods are not simply interchangeable with so-called 'plant-based alternatives', whereby promotion of the latter often represents a reductionist approach to nutrition and food. Low intake (or avoidance) of animal source foods may make diets less robust, which is particularly worrisome for the more vulnerable populations, including the young, elderly, and diseased. From an evolutionary perspective, various anatomical and metabolic adaptations suggest that such foods have played a key role in ancestral health and nutrient security for most of the existence of the human genus, making it very unlikely that they would *cause* diseases of modernity. The latter are more likely to be ascribed to the impact of the 'Western diet', typified by a disproportionate intake of highly refined and ultra-processed foods within an overall context of contemporary lifestyles. Evaluation of the data from nutritional epidemiology of chronic disease should be done with care, especially with respect to the use of causal language, as confounding and bias are intrinsic problems within the discipline. The latter is especially the case for red meat and saturated fat, which both have been excessively accused of being unhealthy food choices during the last decades. Even if their intake may serve as a *marker* for problematic diets in the Western(ized) areas, being associated with more unhealthy lifestyles in general, their specific effects need to be disentangled and properly contextualized. Finally, the nutritional angle also has very important repercussions for the sustainability debate, as any environmental comparison of foods or diets should also take into account nutritional value.

Session 21 Theatre 3

Sustainability in high producing animals

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High producing food animals produce more products per annum. Productivity of food animals can be compared among countries by dividing each country's percentage of global output of a product (milk, pork meat, eggs, wool, etc.) by that country's percentage of the global animal species (dairy cows, pigs, egg-laying chickens, sheep, etc.) This provides a productivity index where the world average is 1.0. Countries with indexes above 3.0 are primarily in East and West Europe and North America, with a few in East Asia and Oceania. Among food animals, greatest annual yield by single animal may be 10-12 standard deviations above a country's mean. Improvements in productivity over the last 5 decades have been driven by genetic selection and improved management. There is now more genetic emphasis on health and welfare in high-producing animals and genetic longevity has increased. Yet, genomic selection for yield traits has reduced generation interval, leading to a herd life that is lower than its true genetic value. There is a positive relationship between herd size and productivity and larger herds provide better healthcare and biosecurity and are more likely to utilize professional nutritionists and veterinarians. High producing animals must be fed to meet their nutritional needs and are more likely to require higher levels of certain nutrients such as rate-limiting amino acids. High producing animals generate less greenhouse gases per unit of food product, and therefore lower environmental impact of food animal production. Lifetime productivity of high producing animals can be affected by their development *in utero* and their care and management during the first several months of life – these impacts are likely mediated by epigenetic effects on stem cells or developing germ cells, and some of these effects are passed to future generations. Significant issues with high productivity include greater risk of disease in the peripartum period, increases in death rates during certain production stages, lameness in animals housed on concrete or abrasive surfaces, overstocking in some facilities and limited access to pastures. Increasingly, food animal products are produced on larger farms, and this makes it challenging for smaller farms to be profitable and sustainable, especially in today's global marketplace.

Session 21 Theatre 4

Environmental responsibility of animal production: some answers to questions from society

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Society is asking many questions about the responsibility of the animal production chain for its negative impacts on the environment, and the mass media and politicians often respond in an emotional vein rather than with rational analysis. We should distinguish: renewable vs non-renewable resources (NRR), short-term reversible vs long-term irreversible damages, and local vs global impacts. Fossil fuels are NRR and CO_2 emitted has a global and irreversible impact on climate change, so the only solution would be to reduce their use to zero. Biogenic CO_2 (from the respiration of living beings, slurry fermentation, etc.) is renewable, and its impact is zero. Biogenic CH_4 (enteric and slurry fermentation) has a warming potential 28 times greater than CO_2, but its average life is short (12 years), and becomes biogenic CO_2: its impact is global but it is temporary and reversible. Biogenic N_2O has a warming potential 265 times greater than CO_2 and a very long life (120 years), so its impact is global, long-term, and almost irreversible. Water is a renewable resource and shortage of it is a local, temporary and reversible problem, although it is aggravated by increases in the human population and the demands of industry. Land is a NRR that could be almost irreversibly lost with urbanization, infrastructure construction, and erosion, or used for different purposes. The greatest competition for land use in the coming decades will be food vs renewable energy production (biomasses for heating and electricity). Forests are important carbon sinks, but their O_2:CO_2 balance is substantially zero. We – humans and farm animals – are part of the anthrome ecosystem and the O_2 we consume is produced by cultivated fields and grazed areas, which also capture the biogenic CO_2 we emit and produce the feed and food we need. Concluding, animal scientists should prioritize reducing the use of NNR (fossil fuels, land, P, etc.). Reducing N_2O seems more important than mitigating CH_4. Reducing the use of N is also essential for mitigating water eutrophication, acidification, water drinkability, etc. Increasing the efficiency of feed and food production is essential, but only if based on reducing the use of NRR and developing a circular food system.

Session 21 Theatre 5

Linearity, circularity, control, and robustness in biology and livestock production systems

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The production of animal-derived products can be seen as a linear process in which inputs such as feed are converted to outputs such as meat, milk, or eggs. Feed efficiency is then just the ratio of outputs over inputs. However, there are many more inputs and outputs in the production process, and not all are desirable. The idea behind a circular (bio)economy is to consider undesirable outputs from one subsystem as useful inputs for another subsystem, thereby eliminating waste and closing the loops. The circular flow of material and energy has been described by concepts such as 'urban metabolism' or 'agricultural metabolism'. The metaphor with metabolism is interesting, because circularity exists widely in biology. The Krebs cycle and the urea cycle are examples of this, but the storage and mobilization of nutrients can also be seen as circular processes. However, these circular processes are linked to rather 'linear' objectives such as the production of ATP from acetyl CoA, the elimination of ammonia as urea, or to account for time differences between nutrient supply and demand. Circularity in metabolism is thus not necessarily a goal by itself, but a means to achieve a specific linear goal. Metabolism is also very well controlled, involving circular feedback loops, which occur at different levels of organization. For example, the functioning of an adipocyte is controlled both locally and centrally. Adipocytes can have a life of their own (e.g. they can be cultured *in vitro*), but they also respond to the energy supply and demand of the organism. The combination of linearity and circularity allows us to control our metabolism within very narrow limits, making the system remarkably robust. However, a 0.5% difference between energy intake and expenditure (i.e. the equivalent of one peanut per day), results in gaining roughly 20 kg of lipid between 20 and 60 years of age. Our metabolic system is thus very robust, but not fail-proof. Agricultural metabolism and livestock production systems are probably also very robust, but nether fail-proof nor fool-proof. Because we have some control over it, let us try not to be the fools that caused the disruption.

Session 22 Theatre 1

Robustness scores in pigs estimated from routine phenotypes: definition and genetic parameters

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The objective of this study was to define robustness scores based on data collected routinely on farm to allow phenotyping of this trait in fattening pigs. A total of 7,542 pigs, from three male lines (Piétrain, Piétrain NN and Duroc), were controlled at the Axiom boar testing station (Azay-sur-Indre, France), in 2019-2020. During the fattening period (from 75 to 150 days of age), individual performance indicators were recorded (growth, backfat, loin depth, feed conversion ratio) together with indicators such as mortality, clinical signs and antibiotic injections. These indicators were combined to estimate a set of categorical robustness traits. Genetic parameters were estimated using an animal linear model. The robustness score (R3) that combines information from antibiotic injection and mortality presented the most interesting heritability, from 0.03 (±0.03) to 0.10 (±0.04) depending of the breed. The genetic correlations between R3 and average daily gain, feed conversion ratio and loin depth were null or slightly favourable (ranging from 0±0.14 to 0.22±0.20). Estimates of genetic correlations of R3 with backfat depth were unfavourable (0.33±0.16). The results show the interest of using routine phenotypes collected on farm to build simple robustness indicators that can be applied in breeding. These first results, that are of low precision due to the number of data available, have to be confirmed on large datasets.

How to breed successfully against tail biting – a simulation study in MoBPS

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Breeding for behaviour traits is important both in terms of animal welfare and animal health. Breeding for behaviour traits such as tail biting is a challenge since they often occur at low frequency, typically have low heritability, and are complex and/or costly to phenotype directly. Assessing the success of breeding strategies from real-world breeding stocks is further complicated by the effects of management, housing conditions, and for traits with low incidence also the variance of the outcome. In this work, we suggest tackling such design questions by the use of stochastic simulations with the R-package MoBPS. For this real data of 25.295 young boars and breeding animals was used to replicate a large-scale pig breeding scheme including complex trait architecture for a variety of performance traits, tail biting, and other behaviour traits that are easier or cheaper to phenotype. An example of this is would be a grade for the reaction when lifted via a hydraulic beam ranging from 1 (extremely calm) to 6 (extremely restless). We assumed this trait to have $h^2=0.29$ and a correlation of $r=0.5$ to the target trait tail biting ($h^2=0.3$). By including such a correlated behaviour trait in the selection index, the genetic gain for the performance trait was reduced by 5.4% while reducing share of culprit animals from 1.04 to 0.24% after 6 breeding cycles. Note that assessing this via a simulation study not only provides information on the expected gains but also on the distribution of the outcome. As such, the share of culprit animals in the reference ranges from 0.4 to 2.2% in 10 independent runs compared to at most 0.4% when including the correlated behaviour trait in the selection index. In comparison, when only having access to a mildly correlated behaviour trait ($r=0.1$), the tail biting frequency reduced to 0.88%, with a similar impact on the performance trait. Note that a direct measurement via the use of video recordings could also become more realistic in the future and is another scenario to consider and weigh if the added detection precision outweighs the additional costs of setting up such an advanced phenotyping platform.

Genetic parameters of piglet mortality in Danish organic pigs

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Piglet mortality from pre-farrowing to weaning is a major concern, especially in outdoor organic production systems. This may result in impaired animal welfare and economic losses for the farmer. Management tools that are commonly used in indoor pig production systems are difficult to apply in the organic or outdoor production systems. Using genetics and breeding to improve piglet survival might be especially favourable in this situation. However, little is known about the genetic background underlying survival traits in organic pigs. Therefore, this study aimed at estimating genetic parameters for piglet mortality in a Danish organic pig population. The analysed dataset included mortality records of piglets from pre-farrowing to weaning (around 70 days of age). Pedigree-based threshold models were used to analyse the mortality traits of piglets at 0 to 3 days of age, 4 to 11, and 12 to weaning. Stillborn piglets were included in the group of piglets dying at 0 to 3 days of age. Heritability estimates on the liability scale were calculated. We found that the mortality rate of piglets was highest at 0 to 3 days of age, accounting for 68% of all piglet deaths from pre-farrowing to weaning. Mortality from days 0 to 3 had a direct heritability close to zero, whereas the maternal heritability was 0.023. For piglet mortality from day 4 to 11 and from day 12 to weaning, the direct heritability was 0.002 and 0.017, respectively; and the maternal heritability was 0.016 and 0.004, respectively. These results suggest that when the piglets are young, piglet mortality is predominantly due to the sows' genotype, but at a later age, it is due to the piglets' own genotype. In conclusion, selection must focus more on the genotypes of the sows rather than on the piglets when breeding for reduced mortality of piglets in organic pig production.

Functional longevity in Latxa dairy sheep breed

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Genetic selection of functional and long-lived animals on farm is relevant, as well as the quantification of the culling risk caused by non-genetic. The Latxa breeding program, implemented in the 80s, was initially focused on milk yield, but over the years included milk composition and udder morphology. However, longevity-related traits have not yet been explored. A Weibull proportional hazard model was used to investigate the effect of genetic and non-genetic factors on functional longevity. Records of 32.809 Latxa Cara Rubia (LCR), 29.240 Latxa Cara Negra from Euskadi (LCN_EAE) and 12.196 from Navarre (LCN_NAV) ewes, collected between 2000 and 2018, were used. From these data, about 30% records were censored, i.e. animals were still alive at the end of data collection. All ewes were required to have a known sire, and the age at first lambing registered. The length of productive life was defined as the number of days from the first lambing until culling or censoring date. The culling risk linked to the age at first lambing, number of lambs born alive per birth and total milk yield produced per lactation were estimated using likelihood ratio test. The genetic component was estimated using a sire-maternal grandsire model. Results show that the culling risk increased about 30% in ewes with their age at first lambing being higher than 1 year old, and 21 and 35% in those who gave birth to 2/3 alive lambs, regardless of the Latxa ecotype. On the other hand, a culling risk reduction of about 50% was found when the milk yield increased in all the Latxa ecotypes. Regarding genetic parameters, the highest sire genetic variance and heritability was observed for LCR (0.065 for σ^2s and 0.123 for h^2), followed by LCN_NAV (0.040 for σ^2s and 0.085 for h^2) and LCN_EAE (0.036 for σ^2s and 0.075 for h^2). The results of this first longevity study approach in Latxa allow inferring that both a reproductive delay and a high prolificacy increase the culling likelihood, whereas the higher the milk production, the lower this risk. Furthermore, the genetic findings indicate that it is possible to select by longevity, although its additive component is quite low. Further studies are required to establish the genetic correlation between longevity and other production traits.

Genetic parameters for liveability traits in turkeys

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Mortality in poults and male turkeys close to slaughter is a challenge for the industry, with prevalence in some cases reaching upwards of 20%. The aim of this study was to define and estimate genetic parameters for traits that describe liveability of turkeys up to 20 weeks of age. Two binary traits were defined: juvenile survival between 2-6 weeks of age (JSURV) and late survival in males between 7-20 weeks of age (LMSURV). Data from a male line hatched between 2010-2018 was used containing 232,547 (JSURV) and 107,738 (LMSURV) records. Prevalence of mortality was 4.6% for JSURV and 14.5% for LMSURV. The pedigree file contained 714,031 animals. Genotypes on 49,780 SNPs were available for 20,445 birds with records. Genetic parameters were estimated by linear and threshold models using single-step genomic BLUP. Records were adjusted for fixed effects of hatch week (JSURV and LMSURV) and sex (JSURV). Bivariate models were used to calculate genetic correlations between survival and body weight at 20 weeks (BW), feed conversion ratio (FCR), breast meat yield (BMY) and walking ability (WS). Heritability estimates for linear (threshold) models were 0.06±0.01 (0.12±0.02) for JSURV and 0.04±0.01(0.07±0.01) for LMSURV. Genetic correlations and were 0.08±0.02 and -0.30±0.07 (BW); -0.03±0.01 and -0.05±0.02 (FCR); 0.19±0.05 and 0.22±0.07 (BMY); and -0.04±0.01 and 0.20±0.05 (WS) with JSURV and LMSURV, respectively. Genetic correlations between JSURV and LMSURV was 0.36±0.08. Utilizing a threshold model is advantageous as it can better capture additive genetic variance. The low heritability of JSURV and LMSURV suggests selection for improved survival in turkeys is possible but expected progress will be slow. LMSURV was unfavourably correlated with BW, but favourably correlated with FCR, BMY, and WS which emphasizes the importance of balanced breeding. The implementation of direct selection for liveability will help the breeding program to achieve product excellence through raising more healthy animals and maximizing the number of birds that can go to market.

Session 22 Theatre 6

Genetic and non-genetic effects on tail injuries in pigs

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Tail biting as well as tail docking impair the welfare of pigs. Improved management, e.g. fresh water, sufficient space and straw, can avoid the necessity of tail docking. Breeding might contribute to reduce tail biting, too. The objective of the study was to quantify the prevalence of tail lesions, assess and quantify influencing factors and estimate genetic parameters. Data on tail injuries were available from ~18,600 purebred and crossbred pigs conventionally kept at the quarantine and test stations of the Bavarian State Research Center for Agriculture between 2017 and 2019. The pigs' tails with different docking status were assessed 5 times for the traits lesion, bleeding, necrosis, partial loss of the tail and swelling: twice at the quarantine station (Q1, Q2) and 3 times at the test station (T1 to T3). Generalized linear models were used to estimate least squares means for each of the five appraisals including the effects of docking status, sex, breed of dam / sire, year-month of arrival at quarantine station (only Q1 and Q2), station /station-compartment, year-month of test begin (only T1 to T3). A similar model was applied for the estimation of genetic parameters, including the same fixed effects and the random effects of animal, litter (Q1 and Q2 only) and pen (T1 to T3). Highest prevalence was observed at T1, i.e. shortly after the arrival from the quarantine station, with 14% lesions and 1.6% bleeding; prevalence of necrosis, partial loss of the tail and swelling were all below 1%. Docking status was one of the most important factors affecting the prevalence of lesions with prevalence decreasing with increasing docking status. Prevalence in 1/3 docked pigs was more than 30% higher than in 2/3 docked pigs. Heritability estimates for tail lesions ranged from 0 to 0.11, depending on the breed of the sire and the development stage of the pigs, indicating that breeding has the potential to reduce tail biting in the long term. Tail biting is difficult to record, and the value of selection against lesions remains unclear. For defusing the problem in the short term, management measures are particularly suitable.

Session 22 Theatre 7

Genetic parameters for birth weight and litter size in Danish organic pigs

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Denmark is the leading European country in organic pig production. Currently, 3.7% of all living pigs in Denmark is kept under organic conditions, whereas the European average is 1%. Compared to conventional pigs, organic pigs have more allocated space, they have access to different feed types, and they have a higher infection pressure due to outdoor conditions. Hence, organic pig farmers prefer smaller litters with stronger piglets to large litters with smaller piglets. The current organic pig breeding program is highly dependent on germ plasm from conventional pig breeding programs. Conventional breeding programs aim at increasing litter size. Consequently, the organic pig population follows this genetic trend. The strong differences between the conventional and organic production system demand for a reevaluation of the breeding strategy. Yet, little is known about the genetic parameters for traits under outdoor or organic conditions, such as litter size or birth weight. This study aimed at estimating genetic parameters for litter size and birth weight in organic pigs. Data were collected from March 2018 through October 2020 from a Danish organic multiplier pig farm. For birth weight, 18,971 records were available. For litter size, 2,985 litter records from 963 sows were available, with information on total litter size, number of live-born, and number of stillborn. Statistical analyses were performed with DMU software, using a maternal effects model for birth weight (piglet records), and a repeatability model for litter size (sow records). For birth weight, the heritability estimate of the direct effects of the piglet estimate was low (0.02), whereas maternal heritability was higher (0.19). Heritability for total litter size was 0.13, for live born 0.10, and for stillborn 0.06. In conclusion, heritability for litter size traits under outdoor conditions varied from low to moderate. Direct heritability for birth weight was negligible compared to the high maternal heritability. Moreover, these results show that birth weight in organic pigs is a maternal trait, rather than a piglet trait. This is the first time that genetic parameters were estimated for litter size and birth weight in pigs under organic or outdoor conditions.

Genetic analysis of novel behaviour traits in pigs derived from social network analysis

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Selective breeding may provide long-term solutions for reducing harmful social behaviour in pigs. Social network analysis (SNA) has shown potential in providing novel behavioural traits that describe the direct and indirect role of each animal in social interactions. However, it is not known to what extent these traits are genetically controlled. Using aggression as a focus, the objectives were to (1) estimate the genetic parameters for SNA traits previously identified to play a role in pen level aggression, (2) quantify the genetic association between SNA and skin lesion traits as a measure of the outcome of aggression, and (3) determine the magnitude of the reduction in skin lesions using SNA traits as selection criteria and vice versa. Animals were video recorded for 24 h post-mixing. The observed fight and bullying behaviour of each animal was used as input for the SNA. Skin lesions were counted on the anterior, central, and posterior area of the body at 24 h (SL24h) and 3wk (SL3w) post-mixing. A Bayesian approach estimated the genetic parameters of SNA traits and their association with skin lesions. The expected response to selection in SNA traits on skin lesions and vice versa was estimated based on standardized estimated breeding values (EBVs) of the relevant trait for selecting 10% of the population. All SNA traits were heritable, ranging between 0.09 and 0.26 and strongly genetically correlated, exceeding 0.88. Moderate to high positive genetic correlations were observed between SNA centrality traits and anterior SL24h (rg>0.64). Our results suggest that selection against eigenvector centrality could reduce anterior SL24h, although it would increase SL3wk. Selection against anterior SL24h is expected to be the most efficient in reducing SNA centrality traits. Incorporation of the SNA traits along with other behaviour and performance traits could help in developing a multi-trait index to improve performance and welfare of pigs.

Development of a new selection index for assessing the rearing performance of lactating sows

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In recent years, a tremendous increase in fertility performance has been achieved in sow herds. The further increase in the number of piglets born and the resulting lower birth weights require a targeted selection of efficient and long-lived sows to remain in the herd. The aim of the present study was to investigate the suitability and reliability of performance parameters of lactating sows that could in long-term be used to select proper sows for new rearing periods. Therefore, in this study 2400 litters from different parities sows (parity 1 to 14) housed in three conventional pig farms were analysed. Sows were divided into the parity classes primiparous sows (1st litter (P1)), gilts (2nd litter (P2)), high performers (3rd-6th litter (P3)) and old sows (7th-14th litter (P4)). Milk yield was estimated by the daily weight gain of the piglets. Each piglet was weighed within the first 24 hours after birth and at the peak of lactation (Day 15 till 18). The evaluation has shown that multiparous sows from parity class P4 had on average the lowest number of piglets born alive (mean=14.66, SD=3.10) compared to P3 sows (mean=16.72, SD=3.32). This is one crucial parameter which influence the culling risk for a sow. However, P4 sows showed on average higher birth weights of the piglets (1.42 kg, SD=0.48) and higher daily weight gains (mean=275 g, SD=6 2 g) related to all parity classes. Sows of P3 achieved an average daily weight gain of 244 g per piglet (SD=53 g). Piglets of P1 sows (mean=196 g, SD=45 g) and P2 sows (mean=233 g, SD=47 g) had the lowest daily weight gain. The new selection index will serve as a management tool by supporting the farmer to optimize his culling decisions towards an improved longevity.

Novel traits on the horizon: conceptual and theoretical aspects of Turkey breeding

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Remarkable advances in poultry production have been achieved through breeding for faster growth rate, improvement of production and reproductive traits, improved biosecurity and improvement of nutritional management. The identification and selection of animals with higher genetic potential for observable phenotypes has been crucial for this improvement, but this hinges on recording high quality phenotypes and developing statistical methodology and indexes for balanced genetic gain. For example, the implementation of single-step genomic BLUP (ssGBLUP) selection programs can increase the accuracy of breeding values, therefore improving the ability to estimate the genetic merit of birds. In this presentation, we describe statistical methodology, including ssGBLUP, Structural Causation Models, and TRD for application to different conventional and novel traits in commercial turkey lines (15,000 turkeys genotyped at 65K). This reference population was used to assess the increase in accuracy of selection using genomic information, which ranged from 0 to +0.3, depending on the trait. A further goal of the study was to monitor inbreeding within the different lines. A large number of high-quality phenotypes related to fertility, growth, production, carcass composition, meat quality, health, behaviour and stress were also developed and collected. With improved methodology, more detailed phenotypic information, and comprehensive data collection and integration, we present more accurate selection of parent stock for application in applied poultry breeding programs.

Genome-wide association study for resilience in growing pigs

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There is a growing concern about the genetic determinism of resilience and its possible implementation in breeding programs. However, resilience has numerous definitions and cannot be directly measured. The first key step is to consistently define this concept and establish a straightforward quantifying method. In this work, resilience was defined as the ability of pigs to withstand stressors and maintain normal growth. Commercial Duroc pigs were challenged with an attenuated viral vaccine at 12 weeks of age. Deviation from the expected body weight given the growth curve of non-vaccinated pigs (ΔBW) and the increment of the acute phase protein haptoglobin (ΔH) 4 days after the challenge were both used as resilience phenotypes. Pigs that were minimally affected by disturbance, showing high and positive values of ΔBW and the lowest ΔH, were deemed resilient. The study aimed to identify genomic regions and candidate genes associated with these resilient phenotypes. Association analyses were carried out on 264 pigs and 41,868 SNPs using two approaches: a single-marker and Bayesian multiple-marker regression. Genomic regions at SCC1 (6.6-8.6 Mb), SSC2 (10.6-12.6 Mb), SSC9 (9.5-11.6 Mb) and SSC13 (132.8-136.7 Mb) were found to be associated with ΔBW in both analyses. The genomic regions at SSC8 (7.7-9.7 and 17-19 Mb), SSC10 (24.7-26.7 and 42.9-44.9 Mb), SSC11 (8-10 Mb) and SSC13 (5.7-7.7 Mb) were related to ΔH. These regions harbour several genes (*PTGDR2*, *MPEG1*, *CD5*, *CD6*, *ADIPOR1*, *TFRC*, *LRRC32*, etc.) involved in the immune system, stress response and cellular signalling. Our study identified important candidate genes for resilience in growing pigs, some of which are currently being studied in more detail in our group.

Preventing pathogen co-evolution when breeding animals for lower infectious disease prevalence

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The possibility to breed animals for improved resistance to infectious diseases has been recognized by animal breeders for a long time. Recent theoretical results show that the potential of such genetic selection for reducing the prevalence of infectious diseases is much greater than predicted by conventional quantitative genetic models. Although these results are very promising, there is a risk that pathogens co-evolve to become more virulent again for resistant animals, diminishing the benefit of breeding against infectious diseases. Pathogens are known to be able to adapt to virtually any applied intervention, with the widespread antibiotic resistance as the most prominent example. It is therefore essential to take co-evolution of pathogens into account in the development of strategies to breed for lower prevalence. A key condition to stay ahead of pathogen co-evolution is that eradication of a pathogen from the animal (sub)population should be achieved before resistant variants evolve and spread through the population. Here we investigate how breeding strategies should be designed to avoid or limit the risk of co-evolution of pathogens. Preliminary results show that greater selection intensities reduce the risk of co-evolution, because it reduces the time-window for the pathogen to co-evolve. Furthermore, the risk of pathogen co-evolution is reduced when multiple mechanisms underly disease resistance (polygenic resistance). Because herd-immunity is an essential element in the control of infectious diseases, selection is most effective when an entire herd is replaced all at once by a new generation of animals with greater disease resistance. This prevents the pathogen from maintaining itself in the older animals with lower resistance. Although not every option is feasible in each animal population, these strategies are of great relevance to sustainably breed animals for improved disease resistance.

Relationship between somatic cell score and female fertility in Florida goat breed

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Selection for high milk production, ignoring its relationship with other traits in selection programs of Spanish dairy goats could lead to undesirable outcomes in the correlated response of other traits (e.g.: fertility and health). Therefore, this study aimed to estimate genetic parameters of somatic cell score (SCS) and female fertility and to determine their genetic relationship. Pedigree and production records were provided by the National Association of Florida Goat Breeders. Test-day somatic cell count was transformed to SCS by a classical logarithmic transformation to achieve normality distribution, and a mean lactation somatic cell score (LSCS) was computed as the geometric mean of SCS. Female fertility was defined as the reproductive efficiency (RE) calculated as the deviation between optimal and real parity number of age of each goat. In total, 87,298 lactation records from 35,678 Florida females were analysed and the number of animals in the pedigree was 109,583. Genetic parameters were estimated by fitting a bivariate repeatability animal model using the restricted maximum likelihood (REML) approach with AIREMLF90 software. Non-genetic effects for LSCS and RE were: herd-year-season of parity, age at parity, milk yield, litter size, and as random effects the additive genetic and permanent environmental effects. Heritability estimates were 0.24±0.009 and 0.38±0.011 for LSCS and RE, respectively. This result shows that SCS and RE are heritable traits in the Florida breed and can be used as selection criteria for the genetic improvement of resistance to mastitis and fertility in Spanish goat breeds, respectively. The phenotypic correlation between LSCS and RE was -0.013 whereas the genetic correlation was -0.083±0.03 indicating that assuming a linear relationship, both traits are not correlated. More studies considering a nonlinear association are required.

Genetic trends in the behaviour of sows and piglets in early lactation

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Changes in animal behaviour in relation to 21 years of genetic improvement were studied. Performances between two groups (G77 and G98) of Large White sows obtained by insemination with semen from Large White boars born in 1977 or 1998 were compared. The objective was to analyse the evolution of the mother-young relationship and to understand it with regard to the evolution of mortality and growth of piglets all left with their biological mother. The two lines did not differ in litter size (11.5 vs 11.3 piglets born alive, P=0.69) or overall mortality during the first week (12.4% vs 13.6%, P=0.63). Video recordings were analysed to study (1) the postural activity of sows from D1 to D4, and (2) the reaction of the sow and piglets to a 20 min separation 24 hours after farrowing (D1). Competition of piglets for udder access was assessed by characterizing facial injuries at D1. From D1 to D4, sows progressively resumed activity in both lines. At D1 and D2, G98 sows spent twice as much time lying on the belly as G77 sows, which decreased access to the udder. At D4, however, they were less likely to shorten their lactation than G77 sows. At D2, G77 piglets slept more in contact with the udder than G98 piglets (P=0.10). At D1 and D2, in comparison to G77 piglets, G98 piglets fought more with each other during lactation, resulting in more facial injuries (P<0.10) and were more likely to suckle upon their return after separation (P=0.013). As G98 piglets have higher nutritional requirements, they are more demanding of their mother for suckling and are more combative at the udder than G77 piglets. This may explain the evolution of the behaviour of sows, which hide their udder more frequently, and of piglets, which sleep less frequently in contact with their mother.

Total number born at 1st and 2nd parities are predictors of sow lifetime productivity development

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The study was on Large White (n=570) and Landrace (L, n=4,688) from Farm of Russia. The traits included the Total number born (TNB), Total number born alive (NBA), Litter weight, piglets born alive (BALWT); totals for lifetime of sows: (TNB_All), (NBA_All), (BALWT_All) and (P_All). The calculations were performed using the method of mixed linear models, the fixed factors (birth year of sow, number of parity) and random factor (individual sow genotype, father genotype, etc.). For the analysis, all sows were divided into groups depending on their productivity – the number of piglets at the first parity (P1) and at the second parity (P2) considering quartiles (25 and 75%) and median. As a result, the sows were divided into 4 groups according to the first parity: O1L – low productivity, O1M – medium, O1H – high, O1VH – very high. In a similar vein, 4 groups were formed according to the results of the second parity: O2L, O2M, O2H and O2VH; and 16 groups for the first and second parities. The data obtained have demonstrated the significant effect of piglets' number at the first and second parities on the subsequent reproductive ability in Landrace and Large white sows. The high litter size at the first and second parities is maintained at the next parities. The differences in reproductive abilities between groups O1L and O1VH in Large white are 31.7 for TNB_All; 29,3 for NBA_All and 26.9 for BALWT_All (P≤0.001) and in Landrace 13.0 for TNB_All; 12,5 for NBA_All and 12.0 for BALWT_All (P≤0.001) throughout the period of economic use. Based on the results of the second parity low productive sows retained significant differences comparing to the high productive sows. The advantages of the O2VH comparing to O2L are 28.6 for TNB_All; 26.4 for NBA_All and 25.8 for BALWT_All (P≤0.001) in Large white and 17.6 for TNB_All; 17.5 for NBA_All and 18.4 for BALWT_All (P≤0.001).Therefore the data on the number of newborn piglets at the first two parities could be considered as reliable predictors of the subsequent reproductive ability in Landrace and Large white sows. Funding: This research was supported by the Russian Scientific Foundation (RSF) within Project No. 19-76-10012.

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Embryo and foetal mortality in divergent selected lines for homogeneity in mice

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Selecting for homogeneity is becoming one of the selection objectives in genetic breeding programs because produces more robust animals. A divergent selection experiment for birth weight variability in mice during 25 generations has shown that weight of homogeneous animals is lower, but having higher litter size, survival and feed efficiency. The higher litter size of low variability line (L) could be attributed to morphological differences in the female reproductive tract, the ovulation rate and/or embryo/foetal survival originated by the divergent selection process. The objective of this work was to compare the foetal survival between 14 days and birth and the embryo survival from fecundation to 14 days assessing also the ovulation rate in both lines. A total of 138 H and 240 L ultrasound scans were performed at day 14 of the first gestation in order to address foetal mortality as the difference between number of foetuses in the scan and litter size at birth. Also, in order to determinate ovulation rate and embryo survival, a total of 50 L and 48 H females were sacrificed in their third gestation at day 14. The genital tract was measured and the number of foetuses were counted. Ovaries were examined in a stereo microscope and the corpora lutea were counted (ovulation rate). The ratio between number of foetuses and number of corpora lutea were computed (embryo survival). There were no significant differences in the number of foetuses observed at 14 days between lines, but litter size at birth was significant higher in L (9.82 vs 8.36 pups). Regarding the *post mortem* analysis, there were not differences in number of foetuses at 14 days. However, significant differences were found (H vs L) in: number of morula of the uterus (2.69), corpora lutea (2.34), embryo survival (-0.25) and ovules lost from corpora lutea to foetuses at 14 days (3.79). Finally, the horn lengths were significantly shorter in H, -7.76 mm (sum of the horn lengths). According to these results, the higher litter size in L is due to important advantages in terms of higher embryo and foetal survival.

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Comparison of the productivity and welfare of sows in individual stalls and group housing

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This study was conducted to provide commercial pig farms with information about group housing systems for sows in accordance with the amendment of the prohibition law for individual stalls for sows in South Korea. Therefore, this experiment was performed to compare the effects of individual stalls (IS) and group housing systems (GS) on the productivity and welfare of sows with the overall aim to investigate the feasibility of replacing individual stalls with group housing systems in commercial sow units. Forty primiparous sows (Landrace × Yorkshire; 210.67±2.22 kg average initial body weight) were randomly assigned to four treatments with restricted feeding after 8 weeks from artificial insemination. The four treatments were: (1) individual stalls (IS; housed in pen stalls while pregnant); (2) short stalls (SS; sows housed in pens with non-gated feeding stalls); (3) free access stalls (FAS; a non-competitive housing system); and (4) electronic sow feeders (ESF; used with radio frequency identification technology to allow individual sow management without individual confinement). All sows were transferred to farrowing crates at 110 days of gestation. There were no differences on sow productive performance, reproductive performance, colostrum composition and lameness between IS and GS and among GS. However, the skin injuries of sows in GS treatment were higher than that of sows in IS. The GS treatment did not negatively affect productivity parameters of primiparous sows compared with IS, but studies to reduce sow competition are considered necessary.

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Genetic parameters of variability of piglet birth weight in Swiss Large White pigs

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In piglet production, the homogenization of litter size and especially of piglet birth weight (BtW) is desired because the production of a large proportion of light-weighted piglets is disadvantageous from an ethical and economical viewpoint. Selection for hyperprolificacy in sows has led to an increase in the heterogeneity of BtW and to a greater number of low BtW piglets within litters. The survival of those piglets is compromised, their meat quality is impaired and they incur higher management costs. Estimating heritability of BtW variation will allow assessing the potential to improve sow prolificacy through selective breeding in a more sustainable way. We estimated genetic parameters of BtW and its heterogeneity, conditional on litter size, parity, year of birth and proportion of stillborn piglets, in the litter of 2,777 litters born between December 2004 to January 2021 from 863 sows. The average litter size (± SD) was 13.7±3.8) and the average BtW was 1,420.5±380.6 g. Within-litter standard deviations and CV of BtW ranged from 35.4 to 640.2 g and 1.7 to 46.5%, respectively. On the phenotypical level, BtW heterogeneity was positively correlated with the proportion of stillborn piglets (rho=0.27, $P<0.001$) and negatively with the proportion of weaned piglets (rho=-0.22, $P<0.001$). Thus, BtW heterogeneity was indeed unfavourably related to survival in this population. The heritability (and 95% credible interval) of BtW and the mean within-litter BtW was 0.14 (0.1, 0.19) and 0.39 (0.26, 0.49), respectively. We failed to detect a non-zero heritability of within-litter standard deviation of BtW, a commonly used measure of heterogeneity. However, the heritability of within-litter CV of BtW was low, but clearly different from zero (0.14 (0.08, 0.21)), which is line with heritability estimates of BtW SD reported in the literature. Repeatability, i.e. the amount of variance explained by the sum of the sow ID effect and the additive genetic effect, was 0.18 (0.13, 0.22). To conclude, BtW heterogeneity in terms of CV is a heritable trait that can potentially be exploited in genetic selection and offers the possibility to reduce piglet mortality.

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Repeatability of udder health and welfare traits of semi-extensively reared dairy goats in Greece

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The objective was to estimate the repeatability of udder health and welfare traits in semi-extensively reared dairy goats in Greece. A total of 1,210 dairy goats from seven farms were used. They belonged to two native (Eghoria; n=418 and Skopelos; n=429) and one foreign (Damascus; n=363) breed. Udder health and welfare traits were recorded monthly on individual animals for two consecutive milking periods. Udder health records included milk somatic cell count (SCC) and total viable count (TVC). Thresholds were set for SCC and TVC at $>10^6$ cells/ml and $>20\times10^3$ cfu/ml, respectively. The latter were used to define three udder health phenotypes: (1) UHP1, scored as 1 or 2 if at least one of the traits was below or both were above thresholds, respectively; (2) UHP2, scored as 1-3 if both traits were below thresholds, one of the two was above, or both exceeded thresholds (3) UHP3 scored as 1-4 if both traits were below, only TVC was above, only SCC was above, or both were above thresholds. Welfare records included presence of ear and horn injuries, head skin lesions, udder asymmetry and abscess, arthritis, overgrown claws and body abscess. Trait repeatability was estimated with mixed linear and non-linear models. Significant ($P<0.05$) estimates were derived for all traits except for arthritis and head skin lesions in Skopelos, and UHP2 in Damascus. Repeatability estimates for udder health traits were 0.22-0.53, 0.27-0.52 and 0.12-0.22 in Eghoria, Skopelos and Damascus, respectively. Repeatability estimates for welfare traits were 0.05-0.78, 0.12-0.79 and 0.15-0.72 for Eghoria, Skopelos and Damascus, respectively. Across-breed, the most repeatable traits were horn injuries, arthritis, ear injuries and UHP3 (0.69, 0.60, 0.57 and 0.41, respectively) and the less repeatable was head skin lesions (0.08). Significant variation between individuals indicates potential for selection to improve udder health and welfare in dairy goats. The work was funded by H2020-SMARTER (772787) and FP7-SOLID (266367) projects.

Estimating the heritability of broiler activity throughout life as recorded by using an RFID system

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Health, welfare and performance data at the individual level can be difficult to collect for broilers, as they are commonly housed in large groups and have a uniform appearance. Individual activity levels of broilers have been suggested to be related to different health, welfare and performance traits, and may have potential to serve as a proxy for these traits. In order to implement activity as a proxy in breeding programs, it has to be assessed whether activity of broilers is heritable. In the current study, we implemented a passive radio frequency identification (RFID) tracking system to record broiler locomotor activity throughout life. Over 300 purebred broilers were fitted with an RFID leg tag at one-day-old and their activity in the home pen was recorded until slaughter age. Activity in the home pen was recorded continuously. Broilers originated from 31 sires and 96 dams and were kept with approximately 80 birds in the home pen (4.7 m^2). The average distance moved per hour was used as the measure of locomotor activity for each animal. A linear animal model was used to estimate genetic parameters. The average distance moved was 9.5±1.7 m/h over the whole testing period. Preliminary results indicate that activity in broilers is moderately heritable. The heritability of average distance moved was 0.38±0.12. So far, average activity from day-old until slaughter has been used, whereas results show that activity decreased over time. Therefore, future research will focus on the heritability of activity at different ages. This study indicates that there is potential to select for increased activity in broilers. However, potential adverse effects of selecting for increased activity remain to be investigated.

Resilience and efficiency in monogastrics: results from Feed-a-Gene

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The objective of the Feed-a-Gene project (2015-2020; H2020 grant agreement 633531) was to adapt the feed, the animal, and the feeding techniques to improve the efficiency and sustainability of monogastric livestock production systems. For ‘feed’, we concluded that non-GMO European soybeans can be processed in small-scale processing plants to obtain soybean meals with similar or even better nutritional quality as imported soybean meals. Also, there is a potential for the use of green biomass to extract protein for monogastric animals to replace imported soybean meal in organic farming. Concerning the ‘animal’, monogastric animals have to rely more on alternative feed resources that are less or not in competition with other uses. These resources are typically more variable and of poorer nutritional quality. With new molecular and automated records, animals can be selected that do best with feed that is worse and transform these alternative feeds into meat, respecting their welfare and robustness and limiting environmental impacts. Precision livestock feeding is a novel technology that can be using to provide tailored diets to animals according to their (individual) requirements, thereby reducing nutrient excretion and the environmental impact of livestock production. The potential of precision livestock farming is much larger than what we explored in Feed-a-Gene (e.g. precision medication, design of housing systems). It is not a question of ‘if’ but of ‘when’ these systems will be implemented, and ‘how’ society will perceive trade-offs of improved resource use, reduced environmental impact, improved welfare versus a perceived further industrialization of livestock production.

Impact of feed efficiency in young beef animals on adult resilience, from field challenge experiment

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Feed efficiency and resilience are often studied separately but rarely together and the link between them remains largely unknown. In the GenTORE H2020 project, following work on FE from one side and resilience on the other side, we try now to address the interplay between those two by different approaches. First, experimental data of FE of 569 heifers of 22 months of age was estimated on a 70 d period. Then a dietary challenge was applied to 340 of these animals after their first calving, half of them being fed 30% below requirements from 10 d after calving up to the start of the grazing period. When possible, animals were kept until 3rd lactation for a total of 592 lactations. Dam weight, BCS, and calf weight were measured regularly, dam milk production was estimated 3 times during the lactation, resumption of cyclicity was phenotyped as well. Using linear models, the effect of heifer efficiency was tested, heifer efficiency being divided into two extreme classes (RFI<-0.4 = efficient; RFI>0.4 = inefficient). Preliminary results suggest that the efficient animals are slightly heavier when fed *ad libitum* but no weight difference was observed during the restriction. No difference was observed for BCS regardless of the diet. Within both diets, efficient animals produced less milk than inefficient dams, with a subsequent effect on the calf weight. Finally, the resumption of cyclicity was 1 week shorter for the efficient animals when fed *ad libitum* but the opposite was observed within the restricted group. Second, modelling approaches were tested using a beef cow model that incorporates the dynamic interplay between life functions. We have used the experimental data to calibrate the model parameters that define acquisition and allocation of resources. Finally, we have tested the effect of different genetic correlations between three parameters of the model on the efficiency-resilience interplay to explore the basis of the experimental results.

Session 23 Theatre 3

Modelling GxE to find efficient, robust and resilient cattle

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Selecting robust and resilient animals is important when genotype by environment interaction (GxE) exists. Developing methodology to improve efficiency and resilience in dairy and beef cattle is one of the major goals of the Horizon2020 project GenTORE (grant agreement no 727213). GxE is usually estimated with: (1) multi-trait models, where the same trait measured in different environments is considered being a genetically different, but correlated trait; or (2) reaction norm models, where the breeding values are modelled as a function of the environment defined on a continuous scale. We will present results of pedigree-based GxE analyses for two populations. Firstly for somatic cell score (SCS) and clinical mastitis in the French Normande dairy breed estimated with multi-trait and reaction norm models where herd levels of production or SCS were used as environmental descriptors. Secondly for production traits in German dual purpose Fleckvieh cattle where e.g. height above sea level of farm location, temperature humidity index or fat-to-protein-ratio have been used as environmental descriptors. Genomic prediction equivalents of both GxE models can be implemented by using a genomic rather than a pedigree based relationship matrix. Within environments, the genomic prediction equivalents of both models assume homogeneous SNP variances across the genome, which in reality may not be the case. We have extended both models, to allow SNP variances within environments and SNP co-variances between environments to be different across the genome. These models have been tested on simulated data showing an increase in accuracy of genomic breeding values compared to models assuming homogeneous SNP variances. The approach of modelling heterogeneous SNP variances will be applied in genomic multi-trait and reaction norm models in German Fleckvieh cattle (production traits) and Irish crossbred beef cattle (age at slaughter).

Genotype by temperature-humidity index interactions on production in the Montbeliarde cattle breed

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Global warming has already caused an increase in average temperature and heat peaks and this phenomenon is expected to continue in the coming decades. This trend will affect European cattle with an expected decrease in welfare and overall performances. The objective of the study was to estimate the magnitude of genotype-by-environment interactions on milk production in Montbéliarde dairy cattle breed for a range of temperature-humidity index (THI). The French genetic database was combined with the Safran database from Meteo-France. This database provides daily estimated meteorological measurements since 1958 on a grid of 9,892 8×8 km squares. Both databases were merged according to the commune code of each farm. First lactation test-day records from 2015-2016 were associated with the THI of the day and with the average THI of the 3, 4, and 7 days before the test day. These years were selected according to their contrasted climatic conditions. In total, 266,094 test-day records from 33,296 cows in 1,292 herds were analysed. The 3-day mean THI presented the strongest effect on milk yield and was considered subsequently. Milk yield was analysed with a mixed model including fixed (herd-year, days in milk, age at calving, length of gestation, and THI) and random effects (additive genetic and permanent environmental (PE) effect of the cows). Milk yield was highest around 50 (i.e. ~7-13 °C), with a slope of +0.02 kg and -0.10 kg/THI unit below and above 50. Individual response to THI was estimated by a reaction norm model. This random regression model included THI Legendre polynomials for both genetic and PE random effects. This model estimates a set of regression coefficients for each animal and thus provides genetic variances all along the THI trajectory as well as genetic correlations between THI conditions. Meteo-France is acknowledged for the Safran database, and Apis-Gene for funding the CAICalor project.

Long-term impacts of selecting for resilience: combined genetic and systemic modelling

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Breeding for economically efficient farm animals has been the cornerstone of livestock sector strategies. This objective is challenged by the need for more resilient animals, able to cope with changing and more extreme environments. Breeding for both resilience and economic efficiency implies dealing with complex biological traits that interact dynamically and depend on diverse environmental conditions. The challenge is to predict long-term effects of selection without unwanted side effects and to ensure that breeding goals are successful in various environments. To tackle this issue, we need to make the best use of knowledge from different disciplines. The objective of this talk is to present the benefits from combining a systemic model (AQAL), which incorporates the biological relationships between efficiency and resilience at animal level, with a stochastic model (ADAM), which incorporates the evolution of traits depending on selection strategy at population level. AQAL integrates parameters of feed resource acquisition and allocation to biological functions as base traits and simulates phenotypic trajectories, depending on potential trade-offs and environmental constraints. Simulations generate different levels of functional traits and resilience of cows, depending on acquisition and allocation. Combined with a simple genetic module, it showed that both changing the genetic (co) variance structure of the base traits and the environmental constraints changed the genetic parameters for the traditional traits. For simulations of breeding schemes including resilience traits the stochastics program ADAM was used. These simulations showed that resilience can be included in the breeding scheme via indicator traits such as SCS or milk yield variation, without harming the overall economic gain too much. Next step is to combine AQAL and ADAM to test different breeding strategies where impacts of selection on allocation and acquisition is modelled for both traditional and resilience traits given different environmental constraints.

Session 23 Theatre 6

Farm system characterization across Europe, and climate change impacts on farm system resilience

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European dairy farm systems usually rely on their own forage production as the primary feed source, therefore changes in forage yields due to climate change could severely impact their production and economic performance. Using the FADN based database, dairy enterprise output was assessed within a range of defined climatic regions, and within multiple system types to account for variation in forages and reliance on external feeds. Incorporation of FAO GAEZ potential yield data for medium- and long-term time periods allowed an estimation of future forage yields, obtained using a regression equation developed from baseline data. The results show that whilst forage yields and therefore milk production would likely decline in southern European regions, more northern regions such as Boreal and North Atlantic and upland areas such as the Alps could see up to 12.5% yield increases per hectare. However, this raises questions as to the suitability of these landscapes to support higher stocking rates, so alternatively, less reliance on concentrates maybe a better option. In central and west Atlantic regions changes are expected to be less stark, but changing the current forage crops could result in greater productivity, due to better drought tolerance. Changing forage crop could be a successful mitigation decision in areas with a yield reduction, especially as relying on an increase in external feedstuffs to replace lower yielding forages may not be possible due to increasing competition for commodities to feed a growing human population.

Session 23 Theatre 7

Breeding for resilient dairy cattle – opportunities and challenges

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Global demand for dairy products is expanding, however, the dairy industry is facing several issues related to human and animal health, sustainability, and social acceptability. The Resilient Dairy Genome Project (RDGP) is a large-scale collaboration between various international research and industry partners. This project aims to integrate genomic approaches to enhance dairy cattle resilience, defined as the capacity of an animal to adapt rapidly to changing environmental conditions, without compromising its productivity, health or fertility, while becoming more resource-efficient and reducing its environmental burden. Collection and analysis of phenotypes in the key areas of 'closer-to-biology' fertility (e.g. oestrous expression and embryo survival), enhanced disease resistance (e.g. fertility disorders, Johne's disease, leukosis, and calf health), and environmental efficiency (e.g. feed efficiency and methane emissions) are explained in this contribution. Genomic and environmental relationships between both novel and existing traits using multigenerational analyses and epigenetic information are examined. We present a path for integration of these traits into a new resilience index, and investigate producers, market, and public perspectives to optimize traits for maximizing resilience and societal acceptance. We would like to acknowledge the support and funding from all the partners involved in the RDGP project through the Large Scale Applied Research Project program from Genome Canada and Genome Alberta.

Session 23 Theatre 8

Validation of resilience indicators based on daily milk yield in dairy cows

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Resilience is the ability to be minimally affected by disturbances or to quickly recover. Resilience indicators have been developed for genetic selection in cows. These are variance and autocorrelation of daily deviations from a predicted milk yield curve. Low variance and autocorrelation indicate stable milk yield with little dependency between subsequent days and are genetically correlated with good health and longevity. However, it is unknown if they reflect minimal response to and quick recovery from actual disturbances. The aim was to estimate genetic correlations of variance and autocorrelation with traits describing production response to an actual disturbance at herd level. A dataset with daily milk yield of primiparous cows was used to identify disturbances affecting the entire herd, by selecting dates where the mean yield of all cows in the herd dropped. Production response of individual cows was then quantified by the depth of the production drop during the disturbance in kg, the length of the drop in days and the total yield loss during the drop in kg. These 'response traits' were available on ~71,000 cows. Resilience indicators were available on ~79,000 cows. Genetic correlations were estimated using a bivariate sire-maternal grandsire model in ASReml. Partial genetic correlations between variance and the response traits, adjusted for production level, were calculated because of strong genetic correlations with yield level. Variance had strong partial genetic correlations with drop depth (0.82) and total yield loss (0.74), but not with length of the drop (-0.18). These results suggest that variance contains information about strength of response to disturbances that cannot be explained by milk yield level. Autocorrelation had weak genetic correlations with drop depth (r_g -0.13) and total yield loss (-0.01), but a strong genetic correlation with length of the drop (r_g 0.97). These results show that variance and autocorrelation contain information about resilience, but both cover different aspects. Therefore, they should be combined to breed for improved overall resilience.

Session 23 Theatre 9

Analysis of milk solid production and mid-lactation bodyweight to evaluate cow lactation efficiency

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The urge of genetically elite animals to efficiently covert grazed pasture to milk fat and protein (milk solids; MS) has been widely substantiated. Access to routine cost-effective measures are required for further development of robust cow efficiency measures both in terms breeding programs and on-farm selection intensity. Mid-lactation bodyweight (BW) and milk recording data were available from high genetic merit Holstein-Friesian (HF) and Jersey × Holstein-Friesian crossbred (JFX) cows across 80 commercial spring-calving herds. Milk solid efficiency (MSeff) was calculated by dividing the 305-day MS yield by the BW and energy efficiency (ENeff) was calculated by adding up the energy requirements for maintenance, milk and growth and dividing these by the 305-day MS produced. The analyses were carried out using PROC MIXED in SAS. Parity and breed had a significant effect on MS production, BW and lactation efficiency parameters. Overall, JFX had a greater ($P<0.001$) MS yield (+5 kg), lower BW (-42 kg), and as a result a greater MSeff (+0.09 kg MS/kg BW) and ENeff (-0.06 UFL/kg MS) compared to HF (503 kg, 544 kg, 0.92 kg MS/kg BW, and 5.96 UFL/kg MS, respectively). A significant breed by parity interaction indicates the greater MS increase for JFX between parity 1 and 5 (+153 kg) in comparison to HF (+145 kg). Although largely variable, efficient dairy cattle were characterized by a combination of higher genetic merit, greater MS production and lower mid-lactation BW. This study highlights the suitability of genetically elite JFX cattle due to the ability to maintain superior lactation efficiency throughout parities. Further research is required to evaluate specific characteristics of high and low MSeff/ENeff animals within controlled experiments. Routinely weighing and milk recording the herd can maximize selection intensity, identify the most efficient dairy cows and drive animal performance and farm profitability.

Session 23 Poster 10

Milk natural isotopic composition, feed efficiency, and body mobilization in early lactation

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Early lactation of dairy cows is characterized by body reserve mobilization and increased risk of metabolic disorders. An efficient and robust dairy cow has the ability to produce milk, to reproduce and to maintain good health by consuming less feed resources than other cows. Compared to the ingested diet, body fat reserves are depleted in ^{13}C while body protein reserves are enriched in ^{15}N, thus the milk isotopic signatures may capture the extent of body mobilization in early lactation. Here, we propose a non-invasive proxy based on milk natural abundance of stable isotopes to assess body mobilization in dairy cow, and test whether this proxy can reflect between-animal variation in feed efficiency. The natural abundance of ^{13}C (δ^{13}C) and ^{15}N (δ^{15}N) in milk was then analysed in 27 dairy cows (14 Holstein, 13 Montbéliarde) all fed the same diet. Milk isotopic analyses were performed on weeks 1, 2, 3, 4, 6, 8 and 12 and plasma samples analysed for non-esterified fatty acids (NEFA) at the same times. The results showed a strong link between the changes across time of δ^{13}C, δ^{15}N and NEFA. As expected, a decrease in milk δ^{13}C was observed during the first weeks of lactation, and was closely associated to an increase of δ^{15}N in milk and plasma NEFA concentrations. The areas under the curve (AUC) of δ^{13}C and δ^{15}N were strongly correlated ($r=0.76$; $P<0.001$). A correlation between the AUC of δ^{13}C and plasma NEFA ($r=0.55$; $P=0.03$) was also observed. These results show that time changes in milk δ^{13}C and δ^{15}N may reflect body mobilization in early lactation dairy cows. At this stage, no clear link was found between δ^{13}C or δ^{15}N and feed efficiency parameters (i.e. milk yield/dry matter intake; residual feed intake; milk energy/energy intake). Further research is underway to analyse these data and model trajectories.

Session 23 Poster 11

Heart rate of grazing Hereford heifers classified by paternal RFI

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This work aimed to evaluate the effect of sires' efficiency on HR variation along the day and its repeatability in different seasons (fall and spring) in Hereford heifers grazing rangelands (Campos biome). Heifers (n=74; 190.8 and 250.8 months of age and 26,926 and 27,926 kg BW for fall and spring, respectively) classified into three groups according to their paternal EBV for RFI (percentiles$\leq$20% for high, 30-50% for medium, $\geq$80% for low) were used in this experiment. Heifers grazed native pastures with an average herbage allowance of 35,081,154 kg DM/ha for fall and 20,271,285 kg DM/ha for spring. Individual HR was measured every 5s intervals for at least two periods of 4 consecutive days during each season. Data quality was assessed using sliding window functions and later analysed using repeated measures with a mixed model with the paternal RFI group, the moment of the day (MOD; morning, afternoon, night and the hours corresponding to each time adjusted according to the sunset and sunrise of each season) and their interaction as fixed effects and paternal EBV as a random effect. On both seasons, HR was the lowest at night and the greatest in the afternoon (64, 69 and 74 5 beats/min and 71, 76 and 81 6 beats/min, for night, morning and afternoon of fall and spring, respectively; $P<0.001$). Neither in fall nor spring HR was affected by paternal RFI groups ($P>0.30$) or the interaction between the paternal RFI group and MOD ($P>0.17$). However, if only the progeny of the two most (percentile$\leq$10) and two least (percentile$\geq$80) efficient sires were considered, in fall, HR tended ($P=0.075$) to be greater for the daughters of low-efficiency sires. The Spearman correlation between individual fall and spring HR was 0.379 ($P=0.0019$), suggesting there wasn´t a significant re ranking of the animals: individual HR relative to the group tended to be stable between seasons. These results indicated that HR increased in spring as animal BW and intake increased, that independently of the season varied along the day, probably reflecting grazing activity, and that although a greater number of animals with more divergent paternal EBVs might be needed, HR may be related to feeding efficiency.

Climate effects on birth weight environmental variability in a divergently selected mice population

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There is an increasing interest on homogeneity since the reduction of variability of certain animal traits benefits the welfare and reduces mortality as well as decreases the cost of handling and production, resulting in higher profits for the farm. Data of 25 generations from a selection experiment in mice that included two divergent lines for high (H-line) and low (L-line) variability for birth weight (BW) showed higher survival and litter size which suggests higher animal welfare and robustness in L-line than H-line. Also it has been shown that L-line is less sensitive to changes in the food intake amount than the H-line. In order to continue tracing the differences between divergent lines, the aim of this study was to analyse their response to changes against climate conditions such as temperature (T), humidity (H) and TH index. Maximum, minimum and mean of T (°C), H (%) and TH index at three moments (the fecundation, a week before the parturition and the parturition) were linked to 22,614 BW data from 30,628 pedigree records, totalling 27 climatic variables. An analysis using a heterogeneous model that included the generation, sex, parity number and litter size as systematic effects, the litter and the additive genetic effect as random effects, and each of the climatic variables, showed that the maximum T a week before birth (TSMX) had an important effect on BW. A second analysis with a heterogeneous model with random regression and the same effects as previously with a Legendre polynomial of order 2 on TSMX applied to the trait and to the genetic effect was performed. In the last generation, the predicted breeding values for H-line had a mean of 0.30 and a range of 0.26 between maximum and minimum across TSMX, and for L-line the mean was -0.14 within a range of 0.12. Comparing the ranges between the two lines, a higher sensitivity to changes in TSMX was shown in H-line for BW suggesting that L-line presents more stability to environmental changes and showing global robustness.

Beef cattle with superior feed conversion efficiency present higher whole-body protein turnover

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Protein turnover (PT), the continual synthesis and degradation of body proteins not leading to protein deposition, is a high energy-demanding process used for maintenance. It can be expected that lower PT may spare dietary energy for growth, contributing to explain the individual variations of feed efficiency in livestock. We tested whether protein degradation (PD) rates decreases as feed conversion efficiency (FCE = ADG / DMI) improves in 32 young Charolais bulls (≈543±56 kg BW and 377±13 days of age) fed two contrasting diets (n=16 per diet) based on corn- and grass-silage, respectively. The PD was assessed with two methods: (1) the urinary 3-methyl-hystidine to creatinine ratio (3MHC), as a biomarker of the skeletal-muscle PD; and (2) the isotopic N turnover rate measured in urine, as an alternative new proxy of the whole-body PD rate. Both 3-methyl-hystidine and creatinine excretion were determined from a 10 d total urine collection at the beginning of the efficiency test. In contrast, the isotopic N turnover rate in urine was evaluated across the whole efficiency test (n=14 sampling days)by modelling the urinary 15N depletion rate following an isotopic N dietary change. With both methods the PD was higher with corn vs grass silage diets (16 to 21%; $P \leq 0.02$), as expected based on their higher protein and energy intakes (+10%, $P=0.001$). Within diet, individual variation of FCE was positively related to whole body PD ($r=0.49$; $P<0.01$) but not to skeletal muscle PD ($r=0.03$; $P=0.98$) in both diets. Differences between methods could be due to differences in time scales (10 vs 112 d) and the fact that FCE is not a repeatable trait across time. In addition, 3MHC mostly reflect the PD of the skeletal-muscle pool while the isotopic N turnover rate measured in urine is proposed as a proxy of the whole-body protein PD, including also the PD of some visceral organs. Our results showed a positive rather than a negative relationship at the individual level between FCE and the whole-body PD evaluated in the long term (112 days).

CowBhave open-source acceleration tag hardware and firmware for cow behaviour monitoring

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Cow behaviour monitoring systems based on tags measuring acceleration are widespread in research and commercial farm. They dramatically increase the efficiency of management of the barns by early recognition of diseases, calving, oestrus, etc. Nevertheless, the disadvantages of the existing commercial tag system are in inability to access raw acceleration data required for development of new behavioural models in research, and relatively high cost of equipment and maintenance for commercial barns. We developed a low-cost tag system consisting of freely available parts with detailed instruction for installing and using and open software. The system consists of acceleration sensors transferring data by the BLE protocol, microcontrollers as the data receiving stations, router providing a local network and a computer processing and storing the data. The tag acceleration can be measured with a frequency up to 25 Hz and resolution of 0.001 g. A system with about 100 tags was tested in experiments in research dairy barn. The data produced by the tags is processed by a machine learning algorithm classifying cow behaviour. An average cost of the system is about 50 euros for a tag with minimal maintenance. Its installation is easy and doesn't require changes in the barn infrastructure. We'll present the firmware of the tag, software for receiving data from multiple base stations and results from validation experiment. The system represents a platform for the further development of equipment and behavioural models for different types of animals. A dataset library with labelled data is in open access.

Using deep neural networks to detect cow behaviour from CowBhave accelerometer collar

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Open source wireless accelerometer system capable of continuously recording raw acceleration data was developed in the CowBhave project. Accelerometer based systems are used widely in monitoring the behaviour of dairy cows, however most of the existing systems have been trained using limited reference data and with traditional machine learning methods. The aim of this paper was to investigate whether the accuracy of behavioural classification can be improved using deep neural networks. We used the developed measurement system to record acceleration at 25 Hz from tags attached to leg and neck collar in total of 80 cows in 2 different research barns. We used automatically recorded reference data for training convolutional neural network model (CNN) to predict feeding time and lying time. Feeding time of the cows was obtained from Insentec RIC feeding system and lying behaviour was calculated based on the leg tag. More than 100 total measurement days were collected with the system. The performance of different CNN architectures and the effect of the amount of used reference data on model learning will be reported. The uncertainty on model performance between different animals and barns will be reported. Developed model will be openly published and can used on new datasets or as pre-trained acceleration model for transfer learning.

Session 24 Theatre 3

Effect of sward height on the behaviour of grazing dairy cows

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Pasture management affects quantity and quality of the feed provided to grazed animals. Therefore, it has an impact on animal welfare, but is often perceived by the farmer to be labour-intensive and might be challenging due to its complexity and interdisciplinarity. One challenge is estimating the amount of feed available and when to move to the next paddock without causing too many losses or impair animals' welfare. Tools based on behavioural measurements might assist the farmer in this decision process. To investigate this approach, we aimed to determine if the behaviour of dairy cows changes with decreasing sward height during grazing. Forty-four cows (German Holstein) were allocated two areas of different pre-grazing sward height (12.0 and 8.0 cm, respectively). The cows grazed until a sward height of 4.0 cm, which took two to six days. Sward height was measured four times a day using a rising plate meter (Grasshopper®, True North Technologies). Feeding and rumination behaviour was measured by RumiWatch® (ITIN + HOCH GmbH) and lying behaviour by IceTag3D™ (IceRobotics Ltd.). Weather data were obtained from a weather station of the research farm. Based on a principal component analysis, we identified three components that could be associated with different behavioural patterns and explained 70.4% of the variance in the dataset. For each pattern, representative variables were selected. Eating and lying time were chosen for the 'general behaviour' at pasture, number of activity changes and number of lying bouts were representative of the pattern 'activity', and 'ruminating' was represented by rumination bout duration. We found significant effects of compressed sward height on eating time, lying time, and number of activity changes, which, however, only occurred when further effects like the time of the day and the weather were taken into account. The factors that we investigated in this study described only a part of the data variation. Therefore, further aspects need to be considered to develop a reliable decision support tool for practical application on farms.

Session 24 Theatre 4

Detecting feed scarcity on pasture by a drop in milk yield and behavioural changes of grazing cows

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Most of the time, dropping milk yields are the initial alarming sign to recognize a potential problem with feed intake of dairy cows. The present study aimed at determining the changes in milk yield and behavioural patterns of grazing dairy cows that occur as soon as the allocated feed in a plot becomes scarce. A part-time rotational grazing system was set up in 2019 where twenty lactating cows grazed in two plots on permanent grassland for 9-11 h/d over six consecutive days during six experimental periods. The available feed within a plot was 80% of all cow's dry matter feed demand for each 6 d. The same restriction was applied to the feed supply in the barn. To verify changes in daily feed intake on pasture, rumen fill scores were evaluated visually in each cow following the daily time on pasture. Milk yields were recorded by the milking system and chaw movement sensors measured grazing and rumination behaviour of the cows. Both was summarized by day. The next-day's milk yield was considered to correspond to the cow's feed supply on the present day. The first three days of each experimental period were used to create a cow-individual milk yield baseline. As a first result, the mean relative decrease in daily milk yields of cows during days 4 to 7 was -1.6, -7.3, -10.8 and -9.7% from the baseline, respectively. The mean relative decrease in rumen fill scores was more pronounced and gradually increased towards the end of the 6-d-periods, when it was -19.7% lower than the mean rumen fill score on days 1 and 2. The mean standard deviation of the milk yield baselines across all cows and periods was 6.3%. In a next step, we will consider the feed allowance of individual cows as scarce, if their milk yield drops below the 99% confidence interval, and analyse the behavioural patterns that occur in such cases. We hypothesize that behavioural patterns can be used to inform farmers about scarce feed availability on pastures without a one-day delay such as in the response of milk yield, which would support decision-making related to the allocation of new grazing plots to cows in commercial dairy farming.

Session 24 Theatre 5

Digital monitoring technologies – from supply to implementation on Swiss farms

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Digital technologies provide an opportunity for modern farm management. To be able to profit from digitalization, knowledge about supply and demand for new technologies is needed. Therefore, we set out to collate information and provide an overview of current available Smart Livestock monitoring technologies. In addition, we investigated the uptake of smart technologies on Swiss farms. To identify commercially available systems grey literature and websites as well as relevant databases such as PubMed and Scopus were scanned. Our search considered systems that are at least partially automated and that capture animal-based information such as physiological or behavioural parameters at individual or group level. To assess the state of mechanisation and digitalisation in Swiss agriculture, Agroscope conducted a large-scale survey in 2018. *Inter alia*, farmers in the dairy cattle, dairy goat, suckler cow, beef cattle and meat sheep sectors were surveyed regarding their use of sensors and measurement devices, electronic controls and data processing options on their farms. For the livestock part of the survey, 1,497 returned questionnaires were analysed. Additional farm and farmers' characteristics were used, to identify which characteristics are associated with the uptake in ruminant farming. A binary logistic regression was chosen for analysis. The literature analyses showed that the greatest range of smart technologies was available for dairy cows. Fattening pigs and broilers fallowed in second place, whereas only a few technologies were found for other enterprises. The survey revealed that easy-to-use sensors and measuring devices built into the milking parlour are more commonly used than other technologies. Moreover, the use of digital technologies varies between different enterprises of ruminant husbandry. The binary logistic regression identified that farms with higher number of livestock units are more likely to use digital technologies. It was also demonstrated that farms in mountainous regions are less likely to use these technologies. To conclude, the technology range and implementation rate of smart technologies is most advanced in the dairy sector. Moreover, technology adoption occurs in the upper part of the farm size distribution, which varies country-specific.

Session 24 Theatre 6

How are animal health monitoring tools used – the point of view of French livestock farmers

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Agroecological transition requires numerous changes in animal health management. Monitoring health disorders to prevent diseases, is a way to improve health management in this context. Precision livestock farming being a tremendous field of innovations for livestock farms, it is interesting to study how much it contributes to health monitoring. In this study, our objective is to improve advisory tools toward more preventive and participative approaches. Therefore, we led a comprehensive study to understand how do farmers use these health monitoring tools? what do they suggest to improve them? Three types of surveys have been realized: (1) an online survey with 178 answers from French specialists of Monogastric and Ruminant sectors to identify the main tools in dairy and meat, cattle and smallstock production; (2) 3 focus group with 21 farmers; (3) on-farm surveys with 24 other cattle meat and dairy farmers. Results of these 3 types of data show that despite existence of numerous monitoring tools only a few are used by livestock farmers for health management. First, farmers use mainly sensitive indicators (visual, olfactive, etc.), or tools that help daily observation of their herds, for health monitoring. 4 main types of sensitive indicators are cited as used for animal health monitoring. On the contrary, monitoring tools requiring data input, and based on periodic analysis, are less used. For example, half of meat farmers consider that monitoring tools are not useful for health management; 2/3 dairy farmers use numeric monitoring tools but most of them present them as only in addition of 'farmer's eye', that remains the most important way to detect health disorders. These results question the necessity for numerous innovators to consider interactions between digital monitoring tools and more sensitive, informal and subjective indicators that remain important for farmers.

Value creation for animal health and welfare by real time surveillance (FAS) in broiler production

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In recent years, digitalization and precision livestock farming (PLF) technologies have changed workload and management in some sectors of livestock production. In broiler farming however, automatic data collection is mostly limited to some stationary climate control devices. Management, care, welfare and early disease detection fully depend on the knowledge, skills and time of the animal caretaker and his observations, and issues are recognised rather late because of the low surveillance frequency and lack of permanent monitoring. This paper reports about a novel mobile robot, called farmer assistant system (FAS), which runs permanently on ceiling-based rails and monitors continuously and in real time bird distribution, quality of excrements and identifies dead birds by visual and infrared cameras. Sensors measure permanently temperature, relative humidity, wind velocity, carbon dioxide, ammonia, light and noise close above the heads of the animals. All data are stored in a protected, cloud-based system, mapped across the barn floor and presented to the farmer via PC or smart phone at any time. This enables him to survey his animals continuously and take early action in case of animal disorders or indisposition. Data mapping leads him directly to the points of an animal health or welfare issue in the barn. Experiences in broiler farms in Germany and Spain show the great potential of the FAS to improve the quality of life of the broilers. The value of this new intelligent PLF technology is that it enhances the confidence of the farmer in his production because of the green – yellow – red warning system. It increases understanding of animals, improves close surveillance of health and welfare of the animals, stabilizes the productivity and may create more confidence among consumers by the transparency. Estimates show that the system can result in savings from lower labour, potentially less health costs, lower mortality and improved productivity.

Development and use of insemination values to aid decision-making in precision dairy breeding

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Dairy farmers are faced with many operational decisions regarding inseminations of dairy cattle that affect profitability. Important are the timing of insemination and which sire to use. Economically optimal decisions depend on semen type and cost, sire conception rate, genetic merit of the semen, inbreeding, etc. The probability of conception can be more accurately predicted using algorithms that include data from sensors, milk production, disease status, etc. These male and female aspects of the insemination opportunity interact and the best decision is not always clear. Our objective was to develop a decision aid that quantifies the economic values of all insemination alternatives given an insemination opportunity for individual cows. We calculated future cash flows of insemination alternatives by building and using a stochastic discrete dynamic programming model. The model included weekly steps. Cow states vary by lactation number, week of lactation, week of gestation or oestrus cycle, dry period length, levels of milk production, feed efficiency, body weight, disease status, female genetic merit, fertility, risk of culling, and season. Default inputs such as lactation curves, feed intake functions, and prices complete the female aspects in the model. For this study, we offered 5 insemination alternatives when a cow had an insemination opportunity: delay (no insemination), sexed semen, conventional semen, beef semen, and embryo transfer. Insemination values were calculated as the cash flows of the 4 insemination types minus the cash flow of the decision to delay. Insemination values ranged from negative to several hundreds of dollars. The probability of conception affected the insemination values and could change the preferred semen type. The model can assist dairy farmers with economic decision making regarding insemination alternatives. The model is also useful to quantify the economic value of better predictions of the probability of conception such as through precision dairy farming methods.

Session 24 Theatre 9

GPS tracking indicates high variability in grazing patterns of lactating cows in Alpine summer farms

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Understanding grazing patterns is necessary to manage semi-natural grasslands for both productivity and conservation of biodiversity with the associated Ecosystem Services. This study used GPS tracking to characterize the daily movement patterns and use of Alpine pastures by lactating Brown Swiss cows in three summer farms located at an average elevation of 1,900 m a.s.l. (SD: 90) in the eastern Italian Alps. Herd management was similar for all summer farms. Each day, after the morning milking in the barn, cows were driven to graze in different areas of the pastures, where they were then left free until the return to the barn for the evening milking, after which they were again released free to spend the night outdoor. From July 5th to September 5th in 2019 and 2020, 12 and 10 lactating cows per each farm, respectively, were equipped with GPS 'collars' scheduled to collect a position every 2 minutes. After editing data to exclude individual milking periods in the barns and outlier positions, the total daily distances walked by cows were on average close to 7 km but ranged from less than 2 km to around 15 km, varying greatly between dates, but also farms and years, mostly in relation the farmers daily decisions for grazing areas. Slopes steeper than 30° were very seldom used, most likely reflecting the cows choices of single movement steps. The total surface used by the cows of each summer farm (average: 211 ha; SD: 29 ha) varied by 4 to 30% between years, because of pasture patches being used only in one year. Internal use of such surface was highly heterogeneous: the quartile of surface with the lowest locations density contained <1% of total locations, while that with the highest density contained around 80% of the locations and was concentrated in the proximity of the barns. Use of high-frequency GPS tracking outlined the great variability of movement patterns and pasture use of the cows in the extensive conditions of Alpine summer farms, and helped to outline the role of farmers and animals choices. This knowledge is necessary to assess movement costs for the animals, and the fine scale spatial distribution of animals load and potential impacts on the grazed area.

Session 24 Theatre 10

Lean start-up principles in the development of walk over weight system for outdoor reared pigs

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The development of PLF technologies is generally considered a long and complex process requiring multidisciplinary collaborations which increase the price of final products. To deal with this, lean start-up principles evolved in favour of fast testing and development of a MVP (minimal valuable product) with minimal costs in terms of time and money thanks to rapid prototyping and manufacturing techniques. The MVP is then exposed to the 'early adopters' customer segment in order to obtain direct market feedback. In the current work, lean start-up principles were applied in developing a PLF solution for extensively reared pigs with the aim of reducing antibiotics use. The main PLF component was a walk over weight (WOW) system, integrated with temperature and humidity sensors. Single animal monitoring was performed by RFID ear tags and identification antenna (LF). Structural frame and casing were predominantly made up of 3D printed components (both in polymer and metal) while GSM communication modules were all 'on shelf' open source systems. The data collected were managed by a dedicated algorithm for the following parameters: group average weight (daily), average single animal weight (daily), n. of measurements per day, and microclimatic conditions. The trend over time was tracked for individual animals, as well as its deviation from the group average. User interface (UI) was based on available open-source form and was developed in collaboration with farmers and veterinarians. Production took 40 days with key thresholds included 3D design submission (day 7), frame assembly (day 21), limited trials (day 25), and MVP deployment (day 30). Overall costs included: materials= 500€, software& communication= 200€, labour= 1,200€ with ROI= 2.62 production cycles. ROI estimation was based on current meat prices (1.5€/kg or 225€/150 kg animal), reduction in mortality (from 7.5 to 2%), and subtraction of one antibiotics treatment (123.6€/50 pigs). In conclusion, the application of lean start-up principles to the development of PLF products can reduce development time and overall costs while allowing faster customer feedback and increasing the involvement of farmers in the development process.

Session 24 Theatre 11

Machine learning algorithms can predict tail biting outbreaks in pigs using feeding behaviour records

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Tail biting is a detrimental behaviour that affects the welfare and health of pigs. Early detection of tail biting precursor signs allow preventive measures, thus potentially avoiding the tail biting event. This study aimed to build a machine-learning algorithm for real time detection of upcoming tail biting outbreaks, using feeding behaviour recorded by electronic feeders. The data set consists of daily feeding data from 65 pens originating from two herds of grower-finisher pigs (25-100 kg), in which 27 tail-biting events occurred. Seven machine-learning algorithms were evaluated for their capacity to correctly predict upcoming tail biting events. For this, data were divided in training and testing data, either by randomly splitting data to 75% (training set) and 25% (testing set), or by randomly selecting pens to constitute the testing set. Algorithms were first built on the training set and their prediction performances were evaluated on the testing set. In the first random data set, the model made predictions for the testing set based on previous records of the pen. In the second data set, the algorithm made predictions for a hitherto unknown pen. Of the algorithms evaluated, the random forest algorithm was able to predict 70% of the upcoming event with an accuracy of 94%, when predicting events in pens for which it had previous data. The detection of events for unknown pens was less sensitive, and the neural network model was only able to detect 14% of the upcoming events with an accuracy of 63%. Our results indicate that machine learning algorithms, especially if based on continuous implementation of the model, could be considered for implementation into automatic feeder systems on farms for real time prediction of tail-biting events.

Session 24 Poster 12

Education to enhance knowledge on precision livestock farming to improve livestock sector competence

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Precision livestock farming (PLF) has, as a scientific subject has been around for more than 20 years. PLF has a major role in the digital transformation of agriculture. PLF is complex, needs an understanding of several perspectives and collaboration between different competencies. To improve the livestock production sector's knowledge, there have been several initiatives to improve and encourage the adoption of new technologies, like 4D4F.eu. Initiatives in academic education have been scarce, with few actors giving courses on a basic and advanced level with a PLF profile. A gap of comprehensive knowledge of PLF within the livestock sector from technology providers, extension services, authorities, and end-users, has been identified. This has urged the need for life long learning initiatives. In the Erasmus+ consortium USAGE (BOKU, UniBZ, TUM, and SLU), they have addressed the lack of competence in smart or digital farming technologies among companies and organisations by offering courses in digital farming. At SLU, activities have included pedagogic training for teachers on online teaching with students with different backgrounds and the development of a course 'Precision livestock farming for sustainable production' on an advanced level for regular students. The course is also offered to students from companies, who are not meeting the entry requirements, in a commissioned training. The course's learning outcomes were matched with modules including an introduction to PLF, from sensors to information (including advanced mathematics), the role of SOP, examples for companies, end-user perspectives, and a final project where students describe the R&D cycle of a PLF technology. For a distance course to be suitable for both professional participants and regular students, most of the teaching was given in asynchronous learning activities with recorded lectures, student feedback to lectures, hand-ins, and assignments. Synchronous activities took only place when students presented and discussed their assignments. Web-meetings with social purpose were arranged a couple of times.

Body condition monitoring in dairy farms: survey among farmers and advisors of West France

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In dairy farm, the preservation of reproductive performances is a pillar of the sustainability of the system. These performances are strongly impacted by the level and dynamics of the evolution of body reserves, reflected by the evolution of the body condition score (BCS). For this, the body condition score could be an indicator for an individual reproductive management. Unfortunately, there is little knowledge on how farmers use body condition score to manage the herd. For that purpose, 29 interviews have been conducted with 14 advisors and 15 farmers located in west part of France. We aimed at understand by whom, how and for what purpose body condition is monitored in dairy farms. The results highlight that few advisors (3) and no farmers score the body condition. However, 11 advisors and all the farmers observe body condition through precise anatomical points (9 advisors, 8 farmers) or anatomical points of a scoring grid (5 advisors). Six farmers say they observe the whole body of the cow. The body condition is seen as an indicator for overall herd monitoring to prevent a problem: it is used to evaluate rations and about 2/3 of the farmers use it as a criterion for reproductive management. Individual monitoring is not systematic but is carried out punctually on the animals that come out of the batch. To correct body condition of cow, feeding management is the first lever (10 farmers at individual level, 5 farmers and 11 advisors at herd scale), then comes the change of the drying time of the cows (8 farmers). For 9 advisors and 10 farmers, body condition is a criterion to inseminate if the cow has recovered body condition. This study has shown that the body condition is not really monitored and scored in farm while it appears as an indicator to manage animal feeding, drying period and insemination.

A data infrastructure that automatically collects animal identification real time

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With the developments in precision dairy farming, streams of collected data are increasing rapidly. Data are collected in many places, both inside the barn or out in pasture to capture behaviour, health, welfare, and performance information. To fully utilize these data, different data streams needs to be combined using animal ID as key identifier. However, not every data stream records animal ID and if they do, it is not straightforward to retrieve animal ID automatically and real-time. To monitor or manage a dairy farm with precision, a system is needed that continuously retrieves animal ID and makes this available in real-time for transmission, publication or storage along with other data being collected at the same time. We developed a system that makes the ear tag identification of the cow continuously available through the use of a camera, so that this essential information can be added to other data streams in real-time. The system contains two main parts: ear tag detection and number recognition, and an infrastructure on farm. The steps involved in the first part are: (1) detection of the ear tag area using openCV in python; (2) digit segmentation using an image processing technique; and (3) number recognition by transfer learning from the pre-trained house number model, a convolutional neural network classifier. This model is retrained with ear tag numbers, which increased the accuracy of a test set from 80 to 87%. The second part involves an infrastructure composing of a video station involving a Raspberry Pi, fitted with a Raspberry Pi High Quality Camera. The video station will be installed in the milking robot as a place where all milking cows are known to pass several times per day. The Raspberry Pi is programmed to collect, and process the live-stream of the camera, to push just the ear tag number to an Arduino. The Arduino is subsequently programmed to push the ear tag number onto the cloud services Microsoft Azure. This infrastructure is currently rolled out to be validated at DairyCampus. This data infrastructure will enable researchers to link different data resources in real-time.

Session 24 Poster 15

CowBhave project: open-source accelerometer-based systems for dairy behaviour monitoring

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CowBhave is a project financed within the ICT-AGRI Call 2017 'Farm Management Systems for Precision Farming'. The overall objectives of the CowBhave project derive from the need of making available to the European farmers a low cost open-source cow behaviour monitoring system that would facilitate herd management by discriminating dairy cow behavioural activities and deviating behaviour. To this aim, the system allows discriminating lying from standing and walking and deviating behaviour by using leg tags and collar tags. To explore different use cases, two firmware versions of a MEMS device and behavioural classification algorithms were developed to build: (1) a real-time system based on an accelerometer fixed at the cows' leg and a threshold-based algorithm; (2) a real-time system based on a firmware for raw acceleration data acquisition, which transmits data to a base station at 25 Hz frequency, and on an artificial neural network to classify cows' behaviours. Furthermore, an algorithm for positioning of the cow based on Bluetooth signal strength was developed. A graphical user interface completes the system and open API for the system, using widely accepted internet technologies, have been implemented to export the data. Validation of the system has been performed by using ultra-wide-band systems and video-recordings. Deliverables of the project include: guidelines of the low cost open-source cow behaviour monitoring system; algorithms, validation data and API software released in open repositories; API specification whitepaper; and scientific publications.

Session 24 Poster 16

CowBhave system components for automated cow behaviour discrimination from accelerometers at the leg

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Automated monitoring of dairy cow behaviour based on non-invasive sensors offers a great potential to improve the monitoring processes of its welfare and health in the context of precision livestock farming (PLF). Utilisation of micro-electro mechanical systems (MEMS) in in-barn PLF activities requires a certain degree of adaptation of the device and its firmware to fulfil the requirements connected to the actual conditions of application. In this study, the development of a new firmware was conducted for a general-purpose MEMS device equipped with an accelerometer sensor (Ruuvitag by Ruuvi Innovations LTD, Finland) used during the experiments. The firmware acquired acceleration at 10 Hz, computed a variable suitable for the discrimination of cows' behavioural activities and sent it every 5 sec to a single board computer (Raspberry Pi). Data were stored in files handled by a PostgreSQL relational database that was designed to perform scheduled data processing. The Graphical User Interface and the API functions were also defined to allow user-friendly use of the system and data export from CowBhave system to other sharing systems. By the use of this system, the outcomes of some in-barn experiments carried out within the CowBhave project are summarised in this paper. The results showed the suitability of the system to perform discrimination of the behaviours with minimum data loss. Moreover, many advantages derive from the adoption of a client-server architecture (the server side could be moved on a different device when the Raspberry's workload increases with the number of connected Ruuvitags, as well as the client side, when required), and from the cross-platform feature connected to the use of Python scripts and PostgreSQL.

Sustainable productivity of livestock in European and Asian alpine grassland: a case study roadmap

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Global grassland areas are often referred to as important resources for agricultural production of animal sourced food avoiding land-use pressure on arable soils. However, real utilisation of grassland is often not at optimum, for varying reasons of economic, social and political implications. This concerns also mountain grassland areas, which are in many cases poorly utilised. In the European mountains, too high labour costs at too small revenues are a frequent reason for slackening of mountain pasture utilisation and ultimately abandonment of the land. In Central Asian regions the opposite is frequently the case: due to poverty and social structures, stocking density on mountainous pastures is too high, however at insufficient herd management. Both tendencies lead to grassland degradation. Consequences are ecological damage as well as low productivity on the scales of animals or land. We hypothesise, that in the European as well as in the Central Asian mountainous pasturelands increase of productivity and improvement of ecological sustainability are possible as synergistic effects of improved herd, pasture and fodder management. To challenge the hypothesis and in in order to identify measures of action and the potential margins of productivity raise, case-studies are needed. Two proposed case studies in the European Alps and the Kyrgyz Tian Shan are aiming at: (1) developing applicable strategies of action based on the pilot character of research and development work; and (2) providing sound data to calculate the potential contribution of grassland-based food production to future global nutrition demands under improved management conditions. The role of climate change is a further crucial issue requiring assessment in this context. The overall target is to value the political, economic and ecological importance of mountain grasslands in a demanding future. Based on the exemplary in-depth description of the status quo in the two regions mentioned, this paper will deduce the general necessity and delineate a roadmap for community-based long-term regional research and development projects regarding herd and forage management in global grasslands.

Two-decade evolution of livestock farming systems in Mediterranean arid rangelands

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In a context of limited resources and opportunities in arid rangelands, livestock systems are a key source of income for local communities. These systems have experienced relevant changes in the last decades, which although might affect farm's vulnerability, have not been given much attention in the scientific literature. Understanding farming systems trajectories of evolution at farm level will help informing the development of policies and programmes to face future challenges. This is particularly crucial in arid rangelands that will be severely impacted by climate and global change. We aimed to analyse the main changes occurred since 2004 in livestock farming systems in El Ouara rangelands, a paradigmatic example of arid rangelands in South-East Tunisia. A face-to-face survey was implemented to 73 farmers in 2004 and 2019 to gather information about farm structure, production orientation, feed management, and economic performance. We identified the trajectories of evolution of farming systems using multivariate statistics. The number of livestock units of sheep has doubled and agriculture (i.e. cereal) areas increased by 66% ($P<0.001$), while there was a one-month and two-month average reduction of the period herds spend in rangelands and transhumance, respectively ($P<0.05$ and $P<0.001$). We identified three farming systems types, which were similar across years: (1) small sheep-specialized farms; (2i) large sheep-focused farms; and (3) camel-focused farms. Farms shifted among farm typologies from 2004 to 2019, with small sheep-focused farms being more common in 2004 and large sheep-focused farms more common in 2019. Our results showed a clear relationship between the geographical location and the evolution of farming systems. Camel-focused farms were found in the most marginal areas, while farm in areas close to main cities evolved from pure sheep farms to mixed cereal-sheep farms. Our work showed a slow but steady substitution of feed resources from arid rangelands with by-products from agriculture, whose long-term sustainability cannot be assured.

Robust breeds grazing green alder: integrating meat production and biodiversity conservation

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In the past decades, the decline of traditional agriculture has caused an abandonment of marginal pastures in many mountain areas of Europe. In the Swiss Alps, green alder is the most abundant successional shrub. A survey of 24 pasture-shrub gradients across the Swiss Alps showed that the encroachment by green alder, in contrast to other shrubs, is associated with a substantial decline in plant biodiversity. The understorey of alder is primarily populated by very few, broad-leaved herbs, which benefit from the atmospheric nitrogen fixed symbiotically by green alder. A two-year grazing trial with cattle (Dexter), sheep (Engadine sheep) and mixed-breed goats in the Eastern Swiss Alps was carried out to investigate how grazing in alder-encroached pastures affects animal performance and vegetation composition. The trial demonstrated that green alder and its understory vegetation provided an underestimated forage source, rich in protein and comparable in digestibility to vegetation on nearby open pastures. Sensor data and field observations indicated that the animals readily penetrated the thickets and spent on average 32% of their foraging time in stands of green alder. The Engadine sheep and the goats consumed green alder bark, damaging the shrub and thus actively counteracted the encroachment. Dexter cattle did not forage on alder bark but on leaves and opened the thickets by their movement through them. Since goats preferred other woody species to green alder and depleted them before the alder, they may impair the regeneration of late-successional forest. Surprisingly, grazing pastures with high shrub cover and on open pastures did not result in differences in average daily weight gain, carcass and meat quality in Dexter heifers and Engadine lambs. Lambs grazing green-alder encroached pasture even had higher carcass proportion of body weight than those on open pastures (45 vs 40%, on average). In this way, low-input grazing systems utilizing adapted breeds, especially Engadine sheep, can add to conservation goals and sustain a viable meat production in marginal areas.

Milk yield and quality of Original Brown cattle reared in Italian alpine region

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The present study aimed to investigate non-genetic sources of variation of milk production and quality traits of dual-purpose Original Brown (OB) cows farmed in Italian alpine region by using milk test-day records (n=4034). Except for milk yield and somatic cell count, all phenotypes were predicted from milk mid-infrared spectra and included traditional composition traits, coagulation properties, casein fractions, minerals and fatty acids. Data were analysed using a mixed linear model which included calving season, parity, stage of lactation and first-order interactions as fixed effects, and cow and herd-test-date as random effects. Milk yield averaged 18.07 kg/d and means of fat, protein and casein content (%) were 4.03, 3.40 and 2.68, respectively. Primiparous OB cows (32.93 g/110 g FA) produced milk with a greater content of unsaturated fatty acids compared with multiparous (31.79 g/110 g FA). Except for Na, minerals content was generally greater in primiparous than multiparous cows. The comparison with the literature demonstrated that OB cows produce milk with fatty acid profile similar to that of Alpine Grey and with more favourable coagulation properties and mineral composition than Pinzgauer cows. Considering that OB milk exhibited good coagulation properties, a prerequisite for cheesemaking, strategies to valorise this endangered breed should mainly focus on the manufacturing of OB mountain cheeses. In general, results for several milk-related performance coupled with the well-known strong adaptability of the breed to extensive mountain farming systems are the starting point towards the valorisation and potential expansion of this local dual-purpose genetic resource, with the ultimate goal of contributing to its conservation.

How do suckling dairy calves impact their dams' milk production – insights from the Ladybug project

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Separating dairy calves from their dam at birth is increasingly criticized in terms of animal behaviour and welfare. As part of the 'Ladybug' project, which aims to design 'eco-citizen' dairy systems (promoting product quality, environmental preservation, farmer and animal welfare), a low-input farmlet was set up from spring 2020 in a mountain grassland area (1,100 masl). It included 28 Holstein and Montbéliarde cows which reared their calves until weaning (15 female) or slaughter (12 male). Impacts on welfare starts to be well documented, but there is little knowledge regarding herd milk performance. During the first 15 weeks of lactation, milk yield and composition of Ladybug (L) cows were compared to those of Conventional (C) cows, whose calves were separated at birth. Calvings occurred between March 1 and May 15 for both groups. Prior to grazing, L cows were fed hay and 2 kg/d of concentrate, whereas C cows received hay, beetpulp and 5 kg/d of concentrate. At pasture L cows were fed only grazed grass, whereas C cows received an additional 1.5 kg/d of wrapped silage and 3 kg/d of concentrate. L calves were kept with their dam to suckle during the day, separated for the night, and L cows were milked only once a day at 7 AM. Milk collected at parlour was lower for L cows than for C cows (11.0 vs 25.4 kg/d). Nevertheless, the total production appears to be only slightly reduced in L system because the difference in milk collected is mainly due to the amount drunk by the calves (about 11% of their BW i.e. -10 kg/d on average) and the cows' diet (about -3.2 kg/d). Regarding milk composition, the impacts for L cows are a lower fat content (28.7 vs 38.7 g/kg), a higher protein content (31.9 vs 29.9 g/kg), and no difference on SCC. The lower fat content in collected milk is mainly due to the suckling of calves, which allows a better extraction of the alveolar milk, richer in fat. The higher protein content reflects a lower mobilisation of body reserves after calving, confirmed by a higher weight recovery of the L cows 15 weeks after calving (+20 kg). To promote cow-calf contact systems, further investigation is needed in order to understand the underlying mechanisms and the social and economic impact of such practices.

How the presence of pig enterprise improves the sustainability of French mountain cattle farms?

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Despite the low density of pigs in the French Massif Central, they play an essential role in maintaining slaughter and processing tools and cultural identity. Most of these pigs are produced in cattle farms. Consequently, the future of pig production in this area is intertwined with the future of mixed pig-cattle farms. The objectives of this study were to: (1) assess whether the presence of a pig enterprise improves the sustainability of beef and dairy cattle farms in the Massif Central; (2) propose an aggregation method to assess the overall sustainability; (3) identify in which types of farms the pig enterprise improve the most cattle farm sustainability. The Orfee bioeconomic farm was modified to simulate both mixed pig-cattle farms and specialized cattle farms. The production and economic characteristics of 17 mixed farms surveyed in 2017 were used to parameterize simulations. We tested for each farm two settings: the current setting with both pigs and cattle (beef or dairy) and an alternative setting with cattle only. The grasslands and crop areas were kept unchanged between the two settings but the grassland management, fertilization and number of workers associated were adjusted by the model. The sustainability indicators calculated in Orfee were aggregated in three scores (economic, environmental and social) by an econometric method based on the data envelopment analysis principles. The determinants of the score difference between the two settings were analysed by a Bayesian regression. We found that the average income (+4 k€/WU) and the 20% of the lowest income (+2 k€/WU) per Worker Unit were higher in mixed farms especially for the beef cattle farms (+7 k€/WU), for pig enterprise fattening their pigs and for large farms that had higher least favoured area subsidies due the additional WU related to the pig enterprise. However, income was not more stable in mixed farm when considering only price variability over the period 2010-2018. Mixed farm enables to pay more WU per ha and to produce more food but feed-food competition was not reduced. Global warming potential per protein produced, fertilization and biodiversity scores were improved. Globally, the presence of a pig enterprise improved the three scores of sustainability.

Indicators to assess the sustainability of sheep and goat farming systems on Epirus mountains

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Although, transhumance farming is steadily reducing in the last decades, it continues to play a vital (economic, environmental and social) role in mountain areas. In the frame of the DoMEsTIc project (ARIMNET ERANET) a set of indicators to assess the sustainability of sheep and goat farming was developed based on the information collected through the specific designed questionnaires addressed to farmers. We applied this list of criteria and indicators on the data collected from the 32 farms in the Ioannina district (Epirus region) to assess the sustainability of the system. We worked on a part of the extended list of criteria, classified into three pillars: economic, social and environmental. Specifically, we assess farming systems sustainability based on the following criteria: market channels and price satisfaction, price differentiation on quality (economical level), farm continuity, farmer commitment and farm ability to cope with changes (social level) and breed diversity / *in situ* conservation and breed adaptation (environmental level). The results are contrasting and underline the vulnerability of the systems studied. Thus, although sufficient level of economic sustainability is observed based on the indicator link with the price satisfaction, at the same time indicators on price differentiation imply low level of sustainability. Similarly, the environmental criteria reveal the gap between the recognition of breeds characteristics and the actions taken to support their diversity and sustainability. Furthermore, the scores concerning social sustainability are moderate to low, underlying the urgent need to re-design the structures and enhance capacities supporting the mountain farming systems implementing innovative participative approaches. The cooperation that is started within Highlands3 HORIZON 2020 project provides an excellent opportunity to exchange experience and to co-design methodological approaches aiming to the rural development actions, in the mountain communities that will ensure their viable future.

Sheep/cattle mixed grazing: parasitism dilution improves sheep growth more than grazing facilitation

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Sheep liveweight gain (LWG) can be higher in sheep/cattle mixed-grazing than in monospecific sheep grazing. Sheep LWG can benefit from mechanisms related with parasitism dilution and forage facilitation. For a given stocking rate, parasitism dilution can operate through a reduction of sheep heads per ha under mixed grazing conditions, compared with monospecific ones. Regarding forage facilitation, the quality of forage ingested by sheep can be improved by the fact that this species, which is more selective than cattle, can focus on the most nutritive plants. Cattle can also maintain patches of short grass in early growth stage, which is more nutritive that old senescent vegetation. These patches can be grazed subsequently by sheep. In a two-year experiment (2019-2020), we compared the performance of young ewes (between 6-12 months) in mixed vs monospecific grazing conditions. The experiment was carried out in central uplands of France (Massif Central), in a pasture grazed continuously from mid-May to late October. The pasture was a mesic grassland with very few legumes and forbs. Monospecific and mixed configurations had the same stocking rate (0.8 LU/ha) and we monitored LWG on a monthly basis. We also followed parasitism through the number of eggs of parasitic worms per gram of faeces (EPG), and faecal nitrogen content (N), as a proxy of ingested vegetation quality. LWG was higher in mixed grazing than in monospecific grazing (~40 g higher per day, $P<0.001$), EPG was half lower (641 vs 1,284, $P<0.001$), whereas N was similar, around 2% of faecal mass. Under our experimental conditions (continuous grazing of a grass-dominated pasture), parasitism dilution therefore explains LWG improvement. Our results can help provide solutions to reduce chemical deworming, thereby improving the sustainability of sheep farming. They can also help design farming systems adapted to vast pastures of mountainous areas.

Session 25 Theatre 9

Sustainable control of gastrointestinal parasites in goats of hilly areas of Jammu (India)

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Parasitic nematodes of the digestive tract remain one of the main constraints to goat production both in temperate and tropical countries. The climatic factor may favour the development of helminth parasites during nutritional stress and wet season in tropical and semitropical areas. Gastrointestinal parasitism especially *Haemonchus contortus* is a major problem in goat production worldwide, these parasites cause diarrhoea, anaemia, reduced weight gain and increased production costs. A total of 60 goats 1-3 years of age naturally suffering from gastrointestinal infection were selected and divided into 3 groups Group A containing 25 goats and group B containing 25 goats and Group C goats contain control group of 10 goats. Goats in group A were given 3% Morantel citrate oral solution (Banminth) and goats in group B were given Closantel bolus (Zyclose) and Group C goats were not given any treatment. The efficacy of the drugs was evaluated on the basis of reduction and absence of eggs as well as clinical improvement. The EPG count and clinical examination was made on 0th, 7th, 14th and 21st day of post treatment. General condition like anaemia, diarrhoea, loss of weight, alertness, appetite, feed consumption physical appearance, etc. was also observed. The observation revealed that EPG of group A and B goats was 380±30 before treatment which came down to almost 0±2 on day 21st post treatment and goats in group C still had high EPG till 14th and 21st day. It was also seen that some goats in group B showing symptoms of Liver fluke infection also responded to the treatment of Closantel. This indicated that there was mixed parasitic infection in these goats. The goats in hilly area mainly suffer from mixed parasitic infection which cannot be controlled by a single drug so there is a need of holistic approach for sustainable control of these parasites which can be done by regular deworming, improved pasture management and regular and timely check up.

Session 25 Theatre 10

Genetic improvement of alpacas and llamas in Peru

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Alpacas and llamas play a vital role in many rural families' livelihood in the High Andes of Peru. The last census of 2012 indicates a population of 3 million alpacas and 746,269 llamas. Both species are kept in extensive, low-input, pasture-based systems in altitudes between 3,800 m and up to 5,000 meters above sea level. Alpacas are mainly kept for their highly-valued fibre, whereas llamas provide meat, to less extent fibre, and are still used as pack animals. There is no national breeding program for alpacas in place, but there are many individual initiatives from private companies, NGOs, and farmers' cooperatives aiming to improve fibre quality due to the textile industry's demand. The common breeding objective is to reduce the fibre diameter, but more recently, the medullation of fibre has been discussed as a possible new objective. As farmers' income still depends more on the quantity of fibre sold than on quality, fleece weight also plays a role in breeding decisions. Recently, an alpaca SNP-Chip has been developed, and the possible implementation of genomic selection is currently evaluated. A major challenge in the coming years will be to bring the various initiatives to the negotiating table and to achieve harmonisation and consolidation of the individual breeding programmes. There is no national breeding program for llamas, and llama farmers have received little attention and support from the national government. There are some isolated initiatives to improve meat production, but they are confronted with an unsecure funding situation. Llamas are a neglected species also by research, despite their great potential for sustainable meat production and their high adaptation potential to the predicted climate changes.

Session 25 Poster 11

Attitudes of Peruvian llama farmers towards genetic improvement

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Farmers' active participation is mentioned as an essential factor for the sustainable implementation of breeding programs. Technical expertise, know-how, access to information, but also personal attitudes towards breeding issues influence farmers' decisions. Therefore, this study aims to better understand llama farmers' attitudes towards genetic improvement. An online survey was carried from November to December 2020. A snowball sampling technique was applied as the invitation was sent by e-mail, or WhatsApp and participants were asked to forward to further interested persons. A total of 80 respondents completed the survey. The questionnaire consisted of 11 questions related to attitudes. Data on-farm management practices and farm characteristics were collected. Attitudes were measured using a Likert-Scale. Participants were on average 38 years old, and 55% of them had completed higher education. The majority of the farmers kept two different types of llamas, namely K'ara (meat-type) and Chaqu (fibre type). 57% of the respondents reported collecting performance data, and 77% also performed controlled matings in their herds. Pedigree information was collected by only 18% of the farmers, and artificial insemination and embryo transfer are currently not used. A common practice to replace breeding males is purchasing males from another farmer (48%), followed by replacement within own herd (29%). Current purchasing decisions for breeding animals are based on performance data, phenotypic appearance, but also the prestige of the owner, and advice from colleagues are taken into account. Two-third of the respondents stated that crossbreeding of the two types should be avoided, and it is relevant to maintain the two types separately. Although technologies (artificial insemination, genotyping of animals) are not yet readily available, farmers considered them as possible useful strategies. Exchange of breeding males, comparison of animals of different herds, participation in a breeding organization were ranked as important elements of a breeding program by more than 60% of the respondents. In general, a very positive attitude towards breeding and different elements of a breeding program was documented.

Session 25 Poster 12

The future of llama keeping in Peru: a sustainability assessment

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Llamas play a vital role for about 95,000 rural families in the Peruvian Andes. They provide meat, fibre, manure for agricultural production, are used as pack animals, and form part of the cultural heritage. Meat is mostly used for home consumption as access to formal markets is limited. The production system can be described as a low-input, pasture-based system at an altitude of 4,000 m and above. High poverty rates and a low human development index are characteristics of llama rearing regions. These figures show that these farmers are marginalized not only geographically but also socially. Although llama husbandry's importance for rural areas is repeatedly emphasized, there is no functioning agricultural extension service, hardly any investment, and no longer-term development plans. Nowadays, farmers see their livelihoods more and more challenged by environmental and economic changes. Many, especially young people, see migration to urban regions as the only alternative for their future. There has been no comprehensive assessment of llama husbandry's sustainability to develop possible strategies for a successful future. Therefore, this study takes a case study approach and aims to evaluate the sustainability of the llama keeping activities of a cooperative in the Pasco region in the central Peruvian Andes. The cooperative owners are 200 smallholder farmers who jointly manage their llamas on communal grazing land. The sustainability assessment of food and agriculture (SAFA) guidelines of FAO were used to assess the four dimensions: good governance, social well-being, environmental integrity, and economic resilience. Out of the 116 proposed indicators, 67 were considered relevant. For evaluating each indicator, information was taken from management documents of the cooperative and reports. Interviews with the management board and staff members were carried out. The first results indicate shortcomings in all four themes, but especially in the economic one. Although according to the FAO, this method is also suitable for smallholders, the evaluation of some indicators was challenging due to missing data. The study shows that farmers' efforts alone are not enough, but that public long-term support such as creating sales markets and the provision of technical know-how is also needed.

Session 26 Theatre 1

Opportunities for the insect sector under the European Union (EU) regulatory framework

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Since the establishment of the European insect sector, its first regulatory priorities were built around steps that would aim creating a legislative level-playing field in the European Union (EU). Notably, this was considered a prerequisite in upscaling the capacity of the sector, while maximising the impact of European insect farming to key agri-food challenges. In the recent years, certain legislative developments paved the way for the creation of a regulatory level-playing field in the European Union – in both food (e.g. 'new' novel food Regulation) and feed (e.g. authorisation of insect processed animal proteins; PAPs). In the context of the developments from 2021 (e.g. the first novel food opinion/authorisation of an insect species and the approval of insect PAPs in poultry and pig feed), this presentation will update the audience on the new opportunities under the EU regulatory framework, possible implications, as well as upcoming targets of the sector (e.g. organic production). More specifically, the speaker will focus on the IPIFF and its members' priorities, as presented in the IPIFF Regulatory Brochure.

Session 26 Theatre 2

A roadmap for black soldier fly breeding and genetics

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Black soldier fly (BSF) has been only recently domesticated for farming but is already proving to be a very valuable protein producer. Looking at the history of improvement in other agricultural species, with gains of 10-100× compared to wild ancestors, we envision extraordinary future for BSF. The BSF sector is developing with a lightning speed and differentiating into small- and large-scale producers, technology providers and genetics providers. BSF has similar properties as species in aquaculture and plant breeding. In these settings breeding programmes are based on population improvement and product development. Population improvement is driven by defining breeding targets (larval and fly traits), data recording, intense and accurate selection, management of genetic diversity and fast breeding. BSF has immense potential for population improvement due to its large prolificacy, high genetic diversity and short generation interval. Practical challenges lie in managing numerous, fast and short-living individuals as well as recording and leveraging data to drive accurate breeding decisions. These challenges will be addressed with specialised breeding technology and know-how, high-throughput phenotyping at scale using automation, vision, possibly genome-wide genotyping, and data science. Product development is driven by defining production targets and assembling genotypes that deliver these targets. In BSF we envision crossbreeding that will leverage the wealth of BSF strains to target a range of global production settings. Population improvement of strains into high-performance breeds will drive continuous product development and protection of intellectual property. Breeders will have to manage three major risks. First, intense selection in prolific species can deplete genetic diversity and lead to inbreeding depression. Second, diseases of BSF are largely unknown. Third, microbiome seems to play key role in some phenotypes and breeders will have to account for these interactions. Finally, there is also an immense potential in developing specialised reproductive technology and leveraging the power of genome editing.

Deciphering the evolutionary history of the black soldier fly, *Hermetia illucens*, on a global scale

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Insects are considered promising candidates for improving agronomic sustainability. Contrary to conventional livestock, however, genetic resources of farmed insects remain poorly characterized. We genotyped almost 3,000 black soldier fly (BSF) specimens from 150 wild and captive populations collected in 57 countries worldwide with newly developed microsatellite markers, providing individual genetic fingerprints throughout. Substantial global population genetic structure was detected by various complementary approaches such as analyses of molecular variance, pairwise population differentiation, isolation by distance, cluster analyses, and coalescence-based modelling, which further revealed complex sub-structuring shaped by naturally derived geographic distribution and human-mediated breed formation. In-depth reconstruction of the evolutionary history of the BSF using our novel highly resolving molecular tool kit infers ancient indigenous range expansions emanating from genetic hotspots within the Americas, and further dynamic demographic trajectories to all other non-native continents in recent history, resulting in pronounced diversification of genetically unique wild lineages via colonization from single-sources or intra-specific admixture events. Conversely, globally predominant captive populations show reduced genetic diversity and, despite featuring distinct breeding-mediated footprints, trace back to a single origin that exhibits marked signatures of domestication. Fuelled by common mass-rearing operations, presently ongoing introgression between commercialized domesticated strains and wild populations was found to threaten the genetic integrity of local populations in some regions. Our comprehensive worldwide population genetic inventory of the BSF provides a benchmark for necessary investigation of this fly's evolutionary ecology, and particularly allows fast and reliable genetic characterization in applied BSF research, with implications on harnessing the potential of this insect for solving urgent socio-economic challenges.

Comparison of pedigree genetic evaluation methods for black soldier fly breeding

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This study describes comparison of different methods for pedigree genetic evaluation in a black soldier fly (BSF) breeding programme. Tracking individual insects and their parentage is resource intensive due to their small size and large number. However, some traits can be phenotyped on a per individual basis and pedigree between expected full-sib groups can be established, albeit with uncertain parentage. Here we present three alternative methods of pedigree genetic evaluation in such a setting. In the first method we estimate simplified additive relationship between groups of individuals – ignoring the within group variation. In the second method we estimate relationship between individuals and allow for uncertain parentage. In the third method we assume that the pedigree is known. To compare these methods, we have conducted a life-cycle analysis of the BSF and simulated a breeding programme with the R package AlphaSimR. The simulation generated individual genomes, breeding values and phenotypes influenced by breeding value, management, year-season and other environmental effects. Using these data, we compared the different methods on how accurately they estimate variance components with airemlf90 and breeding values with blupf90. We further used the estimated breeding values to drive selection decisions and compared in silico achieved genetic gain and depleted genetic variance over generations. This work will guide the development of state-of-the-art breeding programmes for BSF.

Session 26 Theatre 5

Revivable eggs by cryopreservation for insect production enhancement

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The current production of *Hermetia illucens* (BSF) is centralized. Each factory is producing the entire fly lifecycle, maintaining on-site colonies to supply their production. However, reproduction of BSF in captivity requires high investments and profound biological knowledge. The number of eggs produced from a BSF colony also varies from week to week, and the larvae are known to be very fragile and prone to high mortality during the first five days of life (5 DOL). Therefore, current BSF factories must produce surplus eggs and neonates to maintain their planned production capacity. Some large-scale BSF producers are developing new genetic strains of BSF with enhanced traits. Without a reliable preservation system, specialized genetic strains may loose some of their traits due to colony collapses, diseases or naturally occurring genetic drifts. The RECIPE project aims to develop innovative technologies for cryopreservation of eggs and suspension of neonates that can enhance and consolidate breeding sites and allow for the implementation of smarter and smaller rearing sites in the proximity of waste streams. The preservation of eggs and neonates will ensure a constant supply and thus help to develop a stable and efficient insect industry, while long-term cryopreservation of BSF eggs will secure the specifically bred BSF strains. RECIPE will develop, optimize and validate cryopreservation of BSF eggs and suspended neonates at pilot scale, allowing for easy supply with eggs/neonates directly at the rearing site or at an intermediate location (nursing hub). Furthermore, a business evaluation of different production value chains: (1) breeding site (production of cryopreserved eggs) -> rearing site (nursing of neonates to 5DOL and production of BSFL); (2) breeding site (production of suspended neonates) -> rearing site (nursing of suspended neonates to 5DOL and production of BSFL); (3) breeding site (production of cryopreserved eggs) -> nursing hub (nursing of neonates to 5DOL) -> rearing site (production of BSFL) will be made and compared to the current centralized production scenario.

Session 26 Theatre 6

Female-biased sex ratio in production colonies of the common housefly, *Musca domestica*

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The aim of insect mass rearing is to maximise quality and yield. As only females produce eggs, it is often beneficial to rear more females than males. However, altering the proportion of females and males is not easy as most insect populations are selected for an equal sex ratio, dictated by the sex determination mechanism and environmental factors. Insect industry thus requires knowledge for manipulation of population sex ratio and optimal male/female ratio in production colonies. The housefly polymorphic sex determination system may allow for population sex ratio manipulation to increase yield in mass rearing facilities. We established experimental populations with XY males and Md-traD (dominant female-determiner) females and recorded the percentage of females and their egg production over multiple generations. Initial increase in egg quantities as function of deviated sex ratio was registered but was not stable as sex ratio converged towards 50% females over five successive generations. We performed fitness assays under female-biased conditions to determine the optimal proportion of males in housefly production colonies. A correlation between male body size and mating rate was found, and males at 25 °C tended to mate more often than males at 32 °C. Lifetime fecundity was inversely related to higher mating frequencies of males, and the observed paternal effect on egg production therefore plays an important role in unbalanced sex ratio housefly populations. We artificially selected for increased protandry to separate the housefly sexes. The experimental design included crosses between first emerging males and last emerging females over six successive generations. We registered insignificant improvement in the time interval between male and female emergence by comparing selected lines with unselected control strains, and noticeable body size differences between the first and last emerging flies. We conclude that the diversity of sex determination systems and knowledge on the mechanisms controlling reproductive biology may provide valuable information for housefly industrialization.

Molecular and phenotypic polymorphism in *Tenebrio molitor*: how to characterize strains?

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In recent years, the production of insects for feed and food has experienced a genuine worldwide expansion. Nowadays, the activity is developing at industrial scale projecting to produce several dozens kilotons of insects per year. With the multiplication and transfers of insect populations, the question arises about the genetic diversity and the performance of the strains currently farmed. Very little is known on this topic. However, the success of the insect industry implies a good mastery of the genetic dimension which is currently in its infancy. The yellow mealworm (*Tenebrio molitor*) is an insect model species traditionally reared in laboratories or by hobbyists for exotic pet, and now massively farmed worldwide as source of high-quality proteins for both feed and food. There must be many strains created voluntarily or not through foundation effects, breeding practices or deliberate artificial selection, but there is no method to differentiate them yet. This lecture proposes to address this issue by presenting recent advances in polymorphism study of several mealworm populations from different origins. Ÿnsect's R&D team has indeed developed standard methods to assess phenotypic variances for several agronomical traits at individual and populational level. Phenotypes variability and estimated genetic parameters of these traits were compared to analyse the possible statistical distinction between populations. In parallel, the molecular polymorphism of theses populations has also been investigated using a genomic approach. This study is based on the recent high-quality assembly of the *T. molitor*'s genome performed by CEA-Genoscope in the European BBI-JU Project FarmŸng. Molecular characterization of yellow mealworm strains is a major step forward towards a better understanding of the global cartography of the current farmed populations, as well as for the preservation and the exploitation of their genetic diversity in the growing insect industry.

Utility of pooled whole-genome sequences to determine genomic inbreeding in honey bees

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The Western honey bee, *Apis mellifera*, is an important pollinator for agriculture. In Switzerland, hybridisation with imported honey bees, particularly *A. m. carnica* (CAR) and Buckfast threatens the native *A. m. mellifera* (MEL, dark honey bee), as honey bees are polyandrous and often open-mated. Therefore, several conservation areas have been established in remote alpine valleys to maintain the genetic diversity of MEL. To ensure the effectiveness of the conservatories, hybridized colonies are identified and replaced based on genetic marker tests using a panel of ancestry informative markers, while the extent of inbreeding is currently not assessed. In this study, we used pooled whole-genome sequences of ~500 workers to determine genomic inbreeding (FROH) in honey bees. In total, we sampled 265 honey bee colonies consisting of 145 selected MEL from Switzerland (SL_CH) including 4 different patrilines (P1-P4), 66 conserved MEL from Switzerland (CS_CH, 45 colonies) and France (CS_FR, 21 colonies) and 54 CAR colonies sampled in Europe and North America. The population structure was ascertained with network-based clustering (NetView) and the programme Admixture 1.23, while FROH was calculated in Plink using the function --homozyg. Based on pooled whole-genome sequences, it was possible to clearly distinguish MEL from CAR and to ascertain fine-scale population structures within MEL according to conservation origin and patriline. Within MEL, the mean FROH was lowest in CS_FR (FROH=0.023), but still comparable to SL_CH and CS_CH (FROH=0.025). Surprisingly, the mean FROH of CAR was twice as high (FROH=0.050). The pedigree-based inbreeding of 74 SL_CH colonies was not correlated with FROH. Therefore, pooled genome sequences provide a valuable, complementary information to determine inbreeding and genetic diversity of honey bees.

Why and how to estimate genetic parameters for black soldier flies?

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Selection in black soldier flies is currently done based on phenotypic variation, such as selecting the fast developing larvae or the biggest from a population to use as parents of the next generation. If the traits under selection are highly heritable this is a very practical strategy that will lead to genetic response. However, if the traits have a low heritability, the animals exposed to the best environmental factors are selected and limited to no genetic response is achieved. In addition, the impact on genetically correlated traits, favourable or unfavourable, are unknown and hence other traits may be impaired along the phenotypic selection process. It is hence very important to know the heritability of traits and the genetic correlation of such traits with other relevant traits. In livestock it is easy to identify individuals and measure all sorts of phenotypes over the course of their life, and recording their pedigree. In BSF this is complicated as individual identification over time is basically impossible, and measurements on individuals are also complicated due to their limited size. To estimate heritabilities and genetic correlations data has to be collected only once on a subset of the population. We investigated what is needed to get good genetic parameter estimations for BSF, considering practical limitations of the species. Calculations based on selection theory show that with 5-10 individuals from 30-50 families good estimates of heritability can be achieved. Simulation based results show that genetic response can be achieved with phenotypic selection when heritabilities are higher. However, genetic response will be lower when selecting on traits with a high unfavourable genetic correlation. Such dataset and the resulting genetic parameters are basic values needed to discuss genetic selection strategies and allow for further development of improved selection strategies. Measuring phenotypes on BSF is labour intensive and hence costly, with accurate genetic parameters we can estimate if the gains from a selection program outweigh the costs.

Chinese consumers' perspective and potential acceptance toward artificial meat

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This study aimed to investigate Chinese consumers' potential acceptance of artificial meat, known as cultured meat, which can be grown from live stem cells rather than from farm animals, due to its growing interest in many countries including China. A survey was thus carried out in China and inputs from 4,666 people concluded that 20% of the respondents were definitely willing to try artificial meat, whereas 10% were definitely unwilling to try, 47.2% were not willing to eat it regularly and 87% were willing to pay less for artificial meat compared to conventional meat. Despite that around half of the respondents believe that conventional meat production causes environmental and ethical problems and that artificial meat appears more eco-friendly and ethical than conventionally produced meat, environment and ethics are not the first drivers for most of the current respondents to accept artificial meat unlike in Western countries, such as in France. In fact, for Chinese consumers, food safety is the most important issue. However, perceived unnaturalness of artificial meat can induce a feeling of low safety and of emotional resistance with subsequently, a feeling of 'absurdity and/or disgusting' (for 15% of the respondents). This might further lead to the rejection of this product with no willingness to eat regularly. In addition, 36% of the respondents consider that artificial meat is 'promising and/or acceptable' whereas 49% consider it is 'fun and/or intriguing'. Apart from safety, taste and nutritional value are the main expectations for Chinese consumers towards artificial meat. In light of the Chinese catering culture, 34% of the current respondents would still prefer to consume plant-based meat substitutes rather than accepting artificial meat to be a viable meat alternative. On the contrary, 17% would prefer artificial meat than plant-based meat substitutes. Finally, 53% of the respondents may accept artificial meat as an alternative to the conventional meat in the future.

Feasibility of circular food systems leveraging beef and dairy cattle

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Circular food systems (CFS) are increasingly investigated as a strategy to balance the nutritional needs of the growing population with the essentiality of reducing anthropogenic environmental impacts. Circular food systems aim to generate no waste. Ruminant animals, such as beef and dairy cattle, are particularly well suited to CFS because they have the physiological capacity to convert human-inedible waste materials to fertilizer and human-edible products high in essential amino acids, vitamins, minerals and fatty acids. As such, these animals fit well within the concept of a 0-waste food system. Several factors must be considered when assessing the benefits of CFS in terms of human food supplies and environmental impacts. Extant assessments of different food systems rarely evaluate the capacity of agriculture- driven shifts in food production. This work evaluates the capacity of unconsumed food resources in Europe and Northern America to sustain highly productive beef and dairy cattle populations in these regions. Based on FAOStat data obtained from 2010 to 2019 in Europe and Northern America, and food composition and edible portion data obtained from the USDA Foods List, the ruminant-edible metabolizable energy supplies of inedible food portions were calculated for each region in each year. The whole-system energetic costs associated with modern beef and dairy production systems were estimated based on a cradle-to-farm gate model of these systems leveraging contemporary production parameters. Based on these data, a CFS in Northern America could sustain a population of 84 to 99 million cows. Similarly, a CFS in Europe could sustain 213 to 259 million cows. In both calculations, population sizes are defined in numbers of mature breeding females. Assuming current production efficiencies, these populations could generate similar meat and milk quantities as realized today, given sufficient advancements in ration formulation with novel feed sources, genetic improvement, and optimized reproductive efficiencies. Additional challenges include sustainable sourcing, collection, transportation, distribution, and processing of unconsumed food resources for repurposing as animal feed. Irrespective of these challenges, the data demonstrate tremendous opportunity to incorporate work toward CFS utilizing ruminants.

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Effect of quarantine on *post mortem* performances of Charolaise young bulls

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Biosecurity measures are important to reduce the spread of diseases. This in turn results in a reduction of antimicrobial consumption and limits the phenomenon of antimicrobial resistance. Little is known about the role of the quarantine on *post mortem* performances and antimicrobial use in beef cattle. Therefore, the aim of this study was to investigate the effect of quarantine on *post mortem* traits of beef bulls. Data on 575 Charolaise young bulls were collected from July 2018 to March 2020 in 2 specialised Italian fattening farms. Within each farm, animals were split into two groups: one followed the standard period of the fattening farm (NO-QUA group) and the other followed a 30-day period of quarantine before moving to the standard fattening pens (QUA group). Live weight at slaughter and *post mortem* performances, namely carcass weight, SEUROP category, colour indexes (L*, a*, b*) and pH of *M. gracilis* were collected. Also, information on the number of treatments per animal and reasons for treating were recorded. A general linear model was used to analyse the aforementioned traits. The model included the fixed effects of farm, group (QUA and NO-QUA), season, body weight at the arrival to the fattening farm and the interaction between group and season. Results showed that live weight at slaughter was greater for QUA than NO-QUA animals (719.8 vs 710.8 kg; $P<0.05$), whereas carcass weight (441.2 vs 438.8 kg) and colour were not significantly affected by quarantine. NO-QUA animals had higher pH ($P<0.01$) and reached inferior SEUROP category than QUA animals. Also, NO-QUA animals were much more treated than QUA animals (139 vs 56) for respiratory diseases. Overall, the strategy of quarantine improved both *post mortem* performances and meat quality traits of Charolaise beef bulls. [This study is part of the 'AntibioticFreeBeef' project funded by PSR (Programma di Sviluppo Rurale) of Veneto region (Italy), grant number 3556074.]

Multivariate analysis of nutritional profile of human milk compared with milk from 6 farm species

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Milk is the first food for humans (and mammals). More than 80% of the global milk production derives from cattle, the remaining is provided by buffaloes, goats, sheep, camels, equids, and few other species. The present study aimed to explore and compare the nutritional profile of human milk in comparison with milk from 6 farm species (cows, buffaloes, sheep, goats, dromedary camels, donkeys). Samples of bulk milk from different farms (≥10 samples/species) were collected from different herds located in Italy, and from two different Algerian areas for individual camel milk samples. Ten individual human milk samples were donated by the human milk bank (BLUD of Vicenza, Italy). A complete macronutrient profile of these samples was obtained through gold standard ISO-IDF procedures and a detailed mineral profile was obtained using ICP-OES instrumentation. The data were analysed using multivariate analysis: principal component (PCA), factor (FA), and cluster analysis. About the PCA, the first PC explained more than 50% of the total variance and the second PC only 11%. Both showed that lactose had an opposite direction compared to all the other macronutrients and minerals (in particular K and Fe). The FA showed that 3 latent explanatory variables controlled 70% of the total variance. In particular, the first factor 'concentration of milk' discriminated milk with high or low content of the major macronutrients, except lactose, and some minerals linked to them. The second 'lactose vs K, Na, ash' was related to the osmotic pressure of milk. The third factor 'micro-minerals' was mainly related to milk Fe, Al, and Si. Finally, the dendrogram obtained from the cluster analysis showed that human milk was very close to donkey milk. Goat milk clustered together with cow milk and they were close to the camel milk. Sheep and buffalo milk were close to each other and most distant from human and donkey milk. Further analyses are scheduled to explore possible effects on human nutrition.

Effects of multiple grass-fed and grain-fed beef systems on fatty acid composition

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Retail sales of labelled fresh grass-fed beef in the U.S. have grown from $17 million in 2012 to $272 million in 2016. The objective of present study was to investigate the effect of four typical grass-fed and conventional beef systems raised in the western United States on beef fatty-acid composition. Treatments included: (1) steers stocked on pasture then finished in a feedyard (CON); (2) steers grass-fed for 20 months (GF20); (3) steers grass-fed for 20 months with a 45-day grain finish (GR45); and (4) steers grass-fed for 25 months (GF25). Fatty acids were analysed with GC using a 100 m CPSil 88 capillary column. All statistical analyses were performed using a GLM procedure in R. Final body weight varied significantly between treatments ($P<0.05$) with CON finishing at 632 kg, followed by GF25 at 570 kg, GR45 at 551 kg, and GF20 478 kg. Saturated fatty acid (SFA) concentrations were significantly different across treatments ($P<0.05$) with GR45 having the lowest SFA of 43% and GF25 having the highest SFA at 46%. Cis-monounsaturated fatty acid (c-MUFA) particularly oleic acid, which is known as a heart healthy fatty acids, were highest for CON at 44% and lowest for GF20 at 37% ($P<0.05$). Conjugated linoleic acids (CLA) were significantly lower for the grain-fed treatments (0.54 CON and 0.63 GR45; ($P<0.05$) compared to grass-fed treatments (0.93 GF20 and 0.86 GF25; $P<0.05$). For t11,c15-18:2, the precursor to conjugated linolenic acid (ClnA), was twice as high in the grass-fed treatments (0.39 GF20 and 0.40 GF25) than the grain-fed treatments (0.12 CON and 0.20 GR45; $P<0.05$). The grass-fed treatments were higher in n-3 PUFA (3.67 GF20 and 2.29 GF25) than CON (0.79; $P<0.05$), however feeding grain for a short period of time did not decrease n-3 PUFA concentrations to the CON level (GF45 2.51; $P<0.05$). In conclusion, our findings show that beef from grain-fed beef management systems is higher in c-MUFA, while grass-fed is significantly higher in bioactive fatty acids including CLA and branched chain fatty acids. Additional studies are required to find out if the differences in the fatty acid profiles between grass-fed and grain-fed beef would result in different health outcomes for the consumers.

Prediction of cheese-making properties of bulk milk according to farming practices

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Final cheese product results from the combination of ripening phase, transformation process and cheese-making properties of initial milk. To better manage these latter properties, it is necessary to understand what farming practices influence them. The aim of this study was thus to predict rennet coagulation time, gel firmness and caseins content from farming practices. Bulk milk samplings and surveys were collected in summer/autumn 2020 in 83 French dairy cow farms. Milk samples were analysed for rennet coagulation time, gel firmness, caseins, urea concentration and somatic cells. Surveys focused on feed management, herd characteristics and conditions of milking and milk storage on day of milk collection. Data used in the models were either quantitative [milk production, % of primiparous, lactation stage, somatic cells (indicator of udder health), urea concentration (indicator of proper ruminal function) and % of concentrates in diet] or qualitative [milking machine type, storage temperature in tank, and a 6-modalities 'Race-Diet' variable combining dominant breed (Prim'holstein PH/Montbéliarde MB) and dominant forage distributed in diet: PH/maize silage, PH/grass silage, PH/pasture, MB/grass silage, MB/pasture and MB/hay]. Linear models were developed for each cheese-making properties by testing all farming practices with a step-by-step approach and a cross-validation was applied (R^2 reported). Only the best models are reported here. Model for gel firmness included milk production and Race-Diet with significant difference between PH- and MB-groups (n=83, R^2=0.58). Model for caseins contained % of primiparous and Race-Diet (n=81, R^2=0.32). Model for rennet coagulation time included lactation stage and Race-Diet (n=83, R^2=0.25).This work led to original models for predicting cheese-making properties of bulk milk from survey data easily accessible in farms. However, the accuracy of prediction is not entirely satisfactory and results deserve to be further developed with the aggregation of these data with others.

The future of the meat industry and of meat substitutes in China

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China has a thousand-year history of producing and eating meat substitutes made with plant materials such as soybean proteins. Plant-based meat substitutes have a large market in China with a high acceptance by Chinese consumers. Nowadays, the population of vegetarian or vegan in China is more than 50 million, equivalent to 4-5% of the total population. Furthermore, China was projected to be the fastest-growing market for vegan products with a growth rate of 18% from 2015 to 2020. In addition, Chinese people are increasingly aware of the negative consequences of consuming extensive animal products. Hence, they are more open to eat plant-based substitutes and probably cell-based meat, which can be grown from live animal stem cells rather than from farm animals. With the increasing worldwide interest about cell-based meat, China is regarded as a potential market by Western private companies to develop cell-based meat. However, the significant role that plant-based substitutes played in the diverse Chinese catering culture might be a potential barrier to the development of cell-based meat. Therefore, this review aims first to introduce the plant-based substitutes in the Chinese context, which will help to better understand the traditional meat substitutes culture and market in China. Second, this review will provide barriers and motives for the future development of any type of meat substitutes including cell-based meat in China.

Insight into the genetic background of sialic acid metabolism in bovine milk

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Milk is an important food for human, however, for many reasons, not everyone considers it a healthy food. To contrast this opinion, many studies have been carried out to show the presence of bioactive compounds, with beneficial effects on human health. The improvement of milk quality can be achieved by the use of association studies, to identify genes correlated with the functional compounds, and precision breeding, to accelerate the genetic progress. Sialic acids (N-acetylneuraminic acid (Neu5Ac) and N-glycolylneuraminic acid (Neu5Gc)), play a fundamental role in the protection of proteins from protease activity, cell-cell interactions, in the effector functions of IgG, and also have receptor functions. Humans are not able to synthetize Neu5GC, and its ingestion with food (milk or meat) cause health problems. Neu5Ac and Neu5Gc, were analysed in milk samples collected around 60 and 120 days after calving in 113 cows from four breeds (Holstein; HO, Simmenthal; SM, Simmenthal × Holstein crossbred; SM×HO, and Podolica; POD). POD animals showed a lower level compared to HO, SM×HO and SM. Specific genes of sialic acid metabolism pathways were investigated, through SNP- and gene- based association study. A SNP nearby ST6GALNAC6 and ST6GALNAC4 genes was significant associated with Neu5Gc in SM, and a SNP nearby ST8SIA1 gene and two genes (ST6GALNAC5 and SLC17A5) were suggestively associated with Neu5Gc in POD. A signal on ST3GAL6 gene was significant associated with Neu5Ac content in SM. All the identified signals were part of the conjugation pathway in which sialic acids are transferred to glicoconjugates. These preliminary results can be helpful to understand the sialic acid metabolism in cow, and represent a base for a breeding program focused on milk healthiness. Acknowledgement: this research was funded by MIPAAF in the national research project MIQUALAT (D.M. 16844/7100/2019), and partly supported by CEF Highlander project (INEA/CEF/ICT/A2018/1815462).

Effect of supplementation of dairy cow with fat encapsulated linseed oil on milk fatty acids

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Encapsulating vegetable oils with hard saturated fats can be a strategy to protect polyunsaturated fatty acids (PUFA) from rumen biohydrogenation, increasing its absorption and transfer to milk. This study evaluates dairy cows' supplementation with an encapsulated linseed oil on milk fatty acid (FA) composition. Sixty Holstein cows (30 primiparous and 30 multiparous), with 11±0.3 DIM and fed corn silage-based total mixed ration were allocated to one of the two treatments. The treatments differed in the type of lipid supplement used, palm oil calcium soap as Control (CON) and fat-encapsulated linseed oil treatment NatOmega-3 (NO-3) offered (1.2 kg/d) to the cows through an automatic feeding system (DairyFeed C-8000, GEA Farm Technologies, Bönen, Germany) that registered individual supplement intake. The NO-3 supplement was designed to provide 64 g/d of 18:3n-3. Milk was collected weekly for nine weeks, and its FA composition was determined by gas chromatography. The milk FA data were analysed with a repeated-measures model that included the DIM and supplement (ING) intake as continuous independent variables and parity and treatment as fixed effects and their interactions. The intake of both supplemented differs widely between cows, ranging from zero to the maximum offered. Thus, the treatment effect could only be evaluated by the interaction between the type of supplement and its intake. The treatments did not affect the milk yield (48.6 l/d), but NO-3 increased (6.7%) the milk fat content. Increased intake of NO-3 supplement supplementation of NO-3 increased the proportions of 18:1t9, 18:2c9t15, 18:2t11c15, and 18:3n-3 in milk. For each g of NO-3 intake, the 18:3n-3 increased 0.00055 percent points ($P<0.01$). The increase of milk 18:3n-3 achieved with NO-3 supplementation had no practical relevance to increase the omega-3 content in milk. Work funded by FCT – Fundação para a Ciência e a Tecnologia through UID/CVT/00276/2021 (CIISA), and Project NATOMEGA3 (PDR2020-101-031461).

Specific heat treatment reduced ruminal biohydrogenation of oilseeds and improved milk fat profile

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ADM developed some years ago a specific technological process, using heat + sugar addition (3P process), able to reduce ruminal protein fermentescibility of meals. For oilseeds, as fat is often bound to protein, it was hypothesized that this process could also be efficient to protect the unsaturated fatty acids (FA) from ruminal biohydrogenation. A first trial aimed at assessing the effect of this 3P-process applied to linseed, rapeseed, camelina seed on the biohydrogenation rate (BHR) of C18:3 and C18:2 in an *in vitro* fermentation model. 1.2 g of a balanced diet + 1.2 g of the tested seed were incubated with 24 ml of ruminal juice and 216 ml of buffer during 24 h. BHR was calculated for the untreated vs 3P-treated (60 min heating, with or without 4% sugar) seeds by measuring C18:3 and C18:2 contents before fermentation and after 24 h of fermentation. The 3P treatment reduced the BHR of C18:3 of all tested seeds: raw vs 3P BHR was resp. for linseed 70 vs 35% ($P<0.01$), for rapeseed: 69 vs 21% ($P<0.01$), for camelina seed: 60 vs 48% (NS). Similar trends were observed for C18:2. A second trial aimed at measuring the effects of supplying raw crushed, 3P heat-treated or extruded linseed on milk FA profile and C18:2 and C18:3 transfer rate from feed to milk. 2×10 cows were supplemented resp. with 0.67 kg of crushed or 3P-treated linseed for 3 weeks. After a wash-out period, 17 of these cows received the same quantity of extruded linseed for 3 further weeks. Milk FA profile was measured individually before and after the 3 weeks of supplementation. Linseed supplementation, whatever its form, improved significantly milk FA profile. 3P-treated linseed, compared to crushed linseed, improved total omega 3 (0.98 vs 0.95%, $P<0.10$) and CLA contents (0.72 vs 0.63%, $P<0.05$), increased numerically total polyunsaturated FA (PUFA) content (4.55 vs 4.37%), and reduced n-6/n-3 ratio (1.51 vs 1.57, $P<0.10$). For C18:3, the transfer rate appeared significantly higher with the 3P treated seed supplementation (3.8%), compared to the raw crushed (3.2%) or extruded (3.2%) seed supplementation. Similar trends were observed for C18:2. The results of this study confirm the positive effect of the 3P process applied to linseed to reduce ruminal bio hydrogenation, therefore enabling to enrich milk PUFA content.

The concentration of major minerals in bovine bulk milk can be predicted using infrared spectroscopy

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Bovine milk is a dietary source of important minerals. Moreover, major minerals such as Ca, Mg and P are known to be associated with milk technological properties related to cheese-making. On the other hand, Na and K levels in milk have been associated to problems at udder level and unbalanced diets. For such reasons, monitoring mineral composition of bulk milk during periodical milk quality controls is of great relevance for the livestock and cheese industry. Within the project 'Innovamilk' ('Innovations in Italian dairy industry for the enhancement of farm sustainability, milk technological traits and cheese quality') funded by AGER (Grant N° 20171153), the present study evaluated the possibility to use mid-infrared spectroscopy for the determination of milk Ca, Mg, P, K and Na content in bulk milk. For this purpose, bulk milk samples from 179 Holstein farms located in northern Italy were collected; the herd size ranged from 60 to 200. Herds were representative of different management systems, feeding rations, and milking equipment. Farms were sampled 1 to 5 times, during different seasons. For each bulk milk sample, infrared spectrum was collected using a mid-infrared spectroscopy device (FT7) and the concentration of each mineral was determined through the gold standard ICP-OES. Prediction models were developed applying different spectral pre-treatments and final equations were selected using cross-validation. Obtained results suggested that minerals of interest for the Italian dairy industry can be accurately predicted from bulk milk spectral data, with a determination coefficient in cross validation higher than 0.80.

Session 27 Poster 12

Prediction of milk quality lipid indices in sheep using partial least squares regression

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The desired fatty acid (FA) profile of animal derived products represents an actual objective of the producers and researchers, since the interest of consumers in buying high-quality and healthy foods has increased. The aim of this study was to evaluate the possibility to predict quality lipid indices, estimated from the fatty acids (FA) composition, in sheep milk by applying partial least squares regression (PLSR) regression on mid-infrared (MIR) spectra. Two indexes, atherogenic (AI) and thrombogenic (TI) indexes, and the ratio hypo/hypercholesterolemic FA (hH) were calculated. Detailed FA profile of 968 individual sheep milk samples collected from 45 farms were obtained by gas chromatographic determination. The quality lipid indices were calculated as follow: AI = [C12:0 + (4 × C14:0) + C16:0]/[(sum of polyunsaturated fatty acids, PUFA) + (sum of monounsaturated fatty acids, MUFA)]; TI = (C14:0 + C16:0)/[(0.5 × MUFA) + (0.5 × PUFA omega-6) + (3 × PUFA omega-3) + (PUFA n-3/PUFA n-6)]; h/H = [(sum of C18:1cis-9, C18:1 cis-11, C18:2n-6, C18:3n-6, C18:3n-3, C20:3n-6, C20:4n-6, C20:5n-3, C22:4n-6, C22:5n-3, and C22:6n-3)/(C14:0 + C16:0)]. A Furier-transform Infrared (FTIR) milk-analyser was used for the recording of MIR spectrum. A cross-validation approach was used to validate the predictions. The dataset was divided into two sub-datasets: (1) training, with 90% of the observations; (2) validation, with the 10% of the observations. The training dataset was used to estimate prediction model parameters, whereas the validation dataset was used as external validation. This routine was performed 100 times by randomly allocating the animals in the two datasets. Good PLS prediction statistics were obtained for the three variables investigated, with the average coefficient of determination (R^2) being 0.78±0.04, 0.81±0.04 and 0.82±0.04, for TI, hH and AI, respectively, and the mean of RMSE, 0.02±0.01 for all the three considered traits. In conclusion, PLS achieves good accuracy of prediction for the health-related fatty acid indexes and ratio, allowing to provide useful milk quality information without additional time consuming and costly analytical procedures to these routinely carried out during the functional controls of the farms.

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Feasibility of pocket-sized near-infrared spectrometer for the prediction of cheese quality traits

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Laboratory near-infrared (NIR) spectrometers require an important initial investment and cost of maintenance that is not always affordable by cheese manufacturers. Thus, this study aimed to evaluate the feasibility of a low-cost and pocket-sized NIR spectrometers to predict protein (%), total protein, soluble protein, ripening index, total fatty acids (TFA; g/100 g of cheese), main FA groups (g/100 g of cheese) and minerals (%) – Ca, K, Mg, Na, P – content in soft and semi-hard cheese. The NIR device SCiO™ is a reduced handheld device (67.7×40.2×18.8 mm; 35 g) with a small sensor module (27.5×9.5×3.15 mm) linked to a mobile App to get scan data, sent information to the cloud and display the results. The cloud stores the reference database and chemometric models allowing to apply pre-established models or to develop new ones. A total of 103 ground cheeses samples from 8 cheese varieties were scanned with the pocket-sized NIR every 1 nm from 740 to 1,070 nm at room temperature. The shade accessory helped to avoid the influence of external light and to keep the same distance for all the measurements. Each sample was scanned 5 times and abnormal scans excluded to improve signal-to-noise ratio. Each spectrum was matched with the reference data to develop the prediction models. Modified partial least squares regressions were validated through a leave-one-out cross-validation. Several scatter corrections and mathematical pre-treatments combinations were tested and the best prediction was selected for each trait. Latent factors ranged from 1 to 13. The best models were obtained for protein (coefficient of determination of cross-validation, R_2cv, 0.97; residual prediction deviation, RDP, 6.21) and TFA (R2cv 0.98; RPD, 6.67). Saturated FA content revealed a R_2cv of 0.88 (RPD, 2.89). Among all minerals content, Ca (R_2cv, 0.88; RPD, 2.87), Na (R_2cv, 0.90; RPD, 3.14), and P (R_2cv, 0.94 RPD, 4.18) showed the best prediction performance. The results demonstrated that miniaturization of the NIR instruments represents an opportunity in dairy industry. Funded by AGER GA No. 2017-1153-Innovamilk.

Meat quality of beef obtained from Wagyu bulls fed a diet supplemented with Se and vitamin E

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This study investigated meat quality, antioxidant effect, microbiological quality and consumer preference after ageing in m. longissimus lumborum et thoracis (MLLT) (Ribeye) in a vacuum ageing bags for 23 days. We compared two groups of Wagyu-sired bulls and crossbreds with Charolais, Simmental, Limousine reared on farm with extensive fattening system. Control group diet was corn silage (9.1%), grass silage (59.1%), hay (27.3%) and concentrated feed (4.5%). Meat quality was performed by pH, Warner-Bratzler shear force (WBSF), drip loss of water. The total number of animals (n=22) was divided into two groups: control and experimental. The experimental group (n=12) had fortified diet with Se supplement (Solplex 2000) and vitamin E, control group (n=10) had a standard diet. In experimental and control group was balanced number of Wagyu-sired bulls and crossbreds. No significant differences were found in pH, smell, microbiological quality, WBFS and colour between experimental and control group. Wagyu-sired bulls showed higher content of intramuscular fat and lower WBFS.

Can dietary linolenic acid increase t11-18:1 and c9t11-18:2 in meat of lambs fed cereal based diets?

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The major ruminal biohydrogenation pathway of linolenic acid (18:3n-3, LNA) is known to produce vaccenic acid (t11-18:1) as the main biohydrogenation intermediate. The t11-18:1 can be further absorbed and used as the substrate for the synthesis of c9,t11-18:2 (CLA) in tissues, a health promoting fatty acid to humans. However, attempts to enrich meat from intensively fed ruminants with t11-18:1 and c9,t11-CLA using dietary lipid supplementation fails consistently due to the extensive shift of rumen biohydrogenation pathways (i.e. t10-shift), resulting in a strong accumulation of the deleterious t10-18:1 isomer instead of the desirable t11-18:1 isomer. In this experiment, we aim to evaluate if supplementary 18:3n-3 contributes to the accumulation of t10-18:1 or t11-18:1 in rumen. For that, we designed 3 diets supplemented with oil blends intended to isolate the effects of 18:3n-3 and 18:2n-6 on the formation of t10-18:1 or t11-18:1: Control, Basal diet with low 18:3n-3 content and average 18:2n-6 content; +LA, diet with high 18:2n-6 (LA) content; +LNA, diet rich 18:3n-3 and average 18:2n-6 content. Lambs were fed for 6 weeks and slaughtered. Muscle longissimus lumborum was collected for fatty acid analysis. Our results showed no effect of diet in the saturated and polyunsaturated fatty acids, as well as in both t11-18:1 and t10-18:1 proportions in muscle. However, t10-18:1 was highest in all groups. The most relevant differences among groups were the highest accumulation of 18:3n-3 in meat of animals fed +LNA diet, but no differences were observed for 20:5n-3 or 22:6n-3. In summary, our results confirmed that dietary 18:3n-3 did not contribute to the accumulation of t11-18:1 or CLA in meat of animals fed concentrate based diets. This work was supported by FCT – Fundação para a Ciência e Tecnologia IP, grant UIDB/00276/2020.

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Early metabolic imprinting of Hanwoo with rumen protected fat increased beef quality at slaughter

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The objective of this study was to demonstrate that early metabolic imprinting of growing cattle (4 to 7 month of age) via supplementation of rumen-protected fat (RPF) will affect the adipogenesis in early stage and improve meat quality grade of cattle slaughtered at 30 months of age. Sixteen male Hanwoo calf (Korean beef cattle) aging 4 months with an average initial BW of 91±9.0 kg were allocated to 4 pens, balanced for BW, and assigned to one of two treatment groups; a control (only basal diet), and a test diet (basal diet with 200 g of RPF a day) for 120 days (4 to 7 months). After finishing RPF supplementation, both groups were fed same basal diet without RPF until slaughter (average shrunk BW of 796 kg). Supplementation of RPF during early growing stage did not affect final BW and back fat thickness. However, it improved the area of marbling, marbling fineness and intramuscular fat content at slaughter stage ($P<0.1$). The gene expression study revealed that early metabolic imprinting using RPF up-regulated the expression of Seipin lipid droplet biogenesis associated (*BSCL*2) gene at slaughter stage. While other genes involved in adipogenesis and lipid metabolism were not altered by RPF supplementation, expression of *BSCL2* suggested an increase in adipocytes maturation, which thereby improves adipogenesis. This study revealed that early metabolic imprinting of Hanwoo by feeding RPF before fattening period, could lead to higher intramuscular fat content with fine marbling when compared to the traditional feeding strategy in Korea.

Session 28 Theatre 1

Equine registration system and (non)use of horsemeat for human consumption: a survey in EU countries

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The EAAP (European Federation of Animal Science) Horse Commission and the European Horse Network (EHN) have taken the initiative to explore the current equine registration system. The main problem is described by EU Regulation 2015/262 on the second page, point 5, of Introduction: ‘Investigations carried out by Member States have revealed that the identification document is the target of significant fraud. The main risk represents the illegal reintroduction into the food chain of equidae previously excluded from slaughter for human consumption and treated with medicinal products not authorized for food producing animals. Following the adoption of Commission Decision 2000/68/EC (1), thousands of equidae have been irreversibly excluded from slaughter for human consumption. The lack of alternatives to the life-long keeping of redundant and unwanted equidae, which have been excluded from slaughter for human consumption, has led to neglect and abandonment with serious animal health and welfare consequences. This situation has been exacerbated by the current economic situation, which, in many cases, makes the keeping of equidae at the end of their productive life unaffordable.‘ In order to understand the situation, a survey is being conducted in European countries among national or breeding organizations. It aims to determine the real situation in various national contexts and to collect ideas. In particular, it focuses on the influences of the equine registration system on the (non) use of horsemeat for human consumption. Among treated themes, we can quote slaughtering contexts, requirements for human consumption, other solutions for end of life, illegal practices in the country, etc. The results will be used as arguments to help facilitate dialogue with the European parliament for improvement of EU regulation.

Equine end of life in Europe

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In 2019, the FVE/FEEVA position statement on 'End of Life for Horses' was unanimously adopted by the FVE General Assembly. This statement was prepared to address the welfare issues that arise when horses are no longer 'fit for purpose' for medical or other reasons, forcing retirement from sport/work. Horses may have many years in this 'retirement' stage and are at risk of becoming unwanted and neglected, but end of life options are limited. The statement included a set of recommendations for policy makers, owners, breeders, and veterinarians. These included promoting responsible breeding and ownership, the discussion of the irreversible exclusion of horses from the food chain, appropriate and humane use of euthanasia, the need for appropriate rendering/disposal services, and the need to educate owners and veterinarians on the available resources. The presentation will discuss the development of the position statement and any progress on the topic that has been made since the time it was adopted.

Self-organized equine users: key factors and value of professional services

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French equestrian is mainly organized around professional structures such as riding schools or livery stables. Faced with a decrease in the number of federal memberships and with sanitary issues, the French equine industry is interested in people who organize themselves to take care of their equine outside professional structures. Like in other sport sectors, practices in autonomy and outdoors are developing especially during the current pandemic. The services offered by professionals seem to have difficulties to adapt to a changing demand. In this context, this research focuses on equine users' characterization and behaviour concerning management and practices and on the value creation between individuals or with professionals. The analysis relies on semi-structured interviews conducted among 30 equine users between 17 to 68 years of age, in various regions. The results highlight the key factors to leave professionals' structures and self-organize, such as buying a horse or having resources. The main reasons for self-organization are the gain of autonomy, the desire to deepen the relationship with the equine and a dissatisfaction towards the management or well-being of equine in professional stables. Moreover, professional centres are seen as a place to learn to ride and not to learn equine management. This study also shows the lack of services concerning trusty equine sitters, infrastructure's access and a need for knowledge about pastures' agricultural management. These first research elements lead towards the possibility of value creation in partnership with local farmers' networks to help the development of local territories and promote equine green asset values. However, groups of individuals could also help each other to mutualize infrastructures or transportation. From a managerial perspective, the results invite to adapt the service offer to the self-organized equine users. Through co-creation, consumers and professionals would value services that are difficult to access depending on the living areas. This diversification of the professional services market could allow institutions such as the equestrian sports federation to bring back consumers in order to increase technical and managerial skills.

Hay preferences in horses versus selection by their owners

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The horse is physiologically adapted to high-fibre and low-protein feedstuffs. Hence, domestic horses should be fed roughages such as grass, haylage or hay, rather than concentrate feeds. The objective of the present study was to explore the preferences of three different types of hay by 36 horses of three different breeds (12 French mountain, FM horses, 12 Icelandic, IS horses, 12 warmblood, WB horses) to in a free choice experiment over a period of six consecutive days (baseline choice on day 1, adaption period from day 2-5 and a final preference test on day 6). Additionally, the owners of the horses selected among the three hay types the hay which they assumed would best meet their horses nutritional requirements. Consequently, influencing factors that had an impact on the preferred hay by the horse as well as by the owners were determined. Hay types (A, B and C) showed distinct characteristics based on colour, texture morphology (stems, leaves and flowers) and odour and nutrient contents were analysed with near-infrared spectroscopy. Most horses (69%) preferred hay type B and 31% preferred hay type C while horses avoided hay type A. Hay preferences by horses were not manifested in the same way in the three stables and tests. No significant association was found between horse and owner's preferences (P>0.05). While a large proportion of the horses in this study chose hay type B, the hay richest in energy and protein, 50% of the owners of the FM horses and 42% of the owners of the WB horses preferred hay type A, which, in contrast, contained the least amount of energy and protein among the three hay types. The contents of protein seemed to be one of the factors influencing the preference by the horses (r=0.680) and was also positively correlated with the portion of leaves in the hay (r=0.810). The stem portion was negatively correlated with the choices of the horse (r=-0.481) and with protein contents (r=-0.810). The B and C hays preferred by the horses in our study are the two hays with the highest protein and energy content which seems to confirm that horses choose feeds according to specific nutrients.

Influence of two feeding managements on behaviour and welfare in horses reared for meat production

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Horses reared for meat production can be kept in intensive breeding farms where they are fed with high amounts of cereals. The present study aimed to evaluate the effects of two feeding managements on horses' behavioural activities and subsequently on their welfare. Eighteen Bardigiano horses (11 females and 7 stallions), 14.3±0.7 (mean ± standard deviation) months of age were randomly divided into two group pens – one fed with high amounts of cereals (HCG; n=9) vs one fed with high amounts of forage (HFG; n=9). One 2D camera was installed on each pen. An ethogram of 13 mutually exclusive behavioural activities was used. Behavioural observations were carried out over a 96 h period by using scan sampling (n=144 scans/horse/day for a total of 10,368 scans sampled). The mean frequency (%) for each behavioural activity was calculated and behavioural data were checked for normality, employing the Shapiro-Wilk test. One-way ANOVA or Wilcoxon test were used to analysed data according to their distribution. The significance level was set at P>0.05. The results showed that the behavioural changes generated by feeding horses with a forage-based diet indicated an increased welfare, according to the increased expression of the feeding behaviour and the reduced frequencies of standing and locomotion in HFG compared to HCG. Moreover, feeding horses with a forage-based diet resulted in a lower expression of stereotypic behaviour compared with horses fed with a cereal-based diet. Further scientific studies are encouraged to improve the welfare of horses reared for meat production focusing attention on the feeding management adopted in order to reach the adequate compromise between horse welfare and meat production performances.

An international comparison of prevalence of headshaking syndrome in horses

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Equine headshaking syndrome (EHS) is defined as spontaneous shaking of the horse's head and neck without external stimulus. Although EHS has been described in literature since 100 years, aetiology and pathogenesis of the syndrome is not fully explained yet. The aim of this research was to generate an overview of causes for EHS in order to identify possible treatments, reduce distress of horses and potentially enhance therapeutic approaches for horse owners and veterinarians. For this purpose, an overview of affected horses in Europe was created, as comparative study on prevalence of EHS determined in Anglo-American countries. Based on a German study, an online survey was translated and distributed via newsletters in European countries. The questionnaire contains 27 questions and answers were quantitatively evaluated. Reliability was tested using split half consistency. The statistical evaluation was carried out descriptively. Moreover, to examine categorical relationships chi-square test was performed and to check expected frequencies fisher exact test was used. The objective of this study is to compare results of questionnaires carried out in Switzerland, France and Norway with results of the previous study from Germany. Contrary to the assumption that mainly thoroughbreds are affected by EHS, it was determinable that in Europe warmbloods are mainly affected (Germany (n_G=163) 55.4% warmbloods; Norway (n_N=333) 46.5% warmbloods, 2.4% thoroughbreds; France (n_F=804): 32% warmbloods, 26.4% thoroughbreds; Switzerland (n_{CH}=129): 55.8% warmbloods, 7.7% thoroughbreds). The gender distribution showed that geldings were affected more often in Germany (64.4%), France (58.5%) and Switzerland (57.4%), while in Norway geldings and mares were equally affected. Vertical movements of the head were the most frequently described symptoms in 75.5% of all participating countries. Mostly, the nose cover was used as therapeutic measurement to reduce symptoms but without treating the trigger of EHS. This study contributes to providing an overview of the current situations of EHS in Europe and allows drawing conclusions about the role of national factors such as breed distribution, common husbandry systems and therapeutic measures.

Character testing in Haflinger horses

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The aim of this study was, first to assess temperament and behavioural traits in Haflinger horses by implementing a character test into breeding performance testing in South Tyrol and, second, to check the practical suitability and reproducibility of the test procedure used. The temperament and behaviour of the horses are regularly requested by buyers and mentioned as an important purchase criterion. The results indicate a high variability in the character traits of Haflinger mares, which can be considered in the short to medium term for describing the personality and determining the intended use (e.g. pleasure riding, equestrian sports) of a horse. Furthermore, in the long term, the collected information on the character traits of the Haflinger could be used for breeding purposes, provided that sufficient data on the maternal and paternal side are available. Moreover, the results from the first character test indicate that the Haflinger can be described as a calm, interested and work-willing horse breed. The test design used proved to be practical, as on the one hand the data can be collected in an objective and repeatable way and on the other hand the time effort of the test procedure and material costs for the installation of the applied temperament test are moderate to low. Accordingly, the results reveal that an implementation of a character test in the form carried out is reasonable for Haflinger breeding in South Tyrol.

Session 28 Theatre 8

Determining objective kinematic parameters to assess ground coverage in Franches-Montagnes horses

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Ground coverage is a routinely assessed gait quality trait in equine breeding, which is particularly associated with stride length (SL). To better understand the criteria used by breeding experts, the association between kinematic parameters and SL were investigated at walk and trot. Limb and body kinematics of 24 Franches-Montagnes stallions were measured with 3D optical motion capture on a treadmill during an incremental speed test at walk (1.4-2.0 m/s) and trot (3.3-6.5 m/s), with four increments per horse and gait. SL was measured with the force plate integrated in the treadmill. In this study, the kinematic parameters were limited to those measurable with wireless sensors: maximal metacarpal (MC) and metatarsal (MT) pro-retraction angles, and the pelvis movements (range of motion and maxima for pelvic pitch, roll, yaw) for walk and trot, including stance duration, suspension duration, and the difference between diagonal MC and MT protraction angles for trot. Six breeding experts scored video recordings of the trials. The scores were compared with kinematic measurements using Pearson correlations (r). Significance and effect size (η) of the kinematic parameters on SL at walk and trot were estimated using linear mixed effect models, with speed and horse as random effects. At walk, the significant parameters with medium to large effect sizes (η>0.05) on SL were the maximal MC protraction angle (η=0.17) and the range of pelvic pitch (η=0.12). At trot, forelimb stance duration (η=0.25), suspension duration (η=0.18), maximal pelvic pitch (η=0.09) and the maximal MC protraction angles (η=0.07) showed the largest effect size on SL. At walk, expert scores were significantly (r>0.41) correlated with the aforementioned kinematic parameters, while at trot only suspension duration was significantly (r>0.41) correlated. The results demonstrate that objective kinematic measurements can complement expert scoring in the field.

Session 28 Theatre 9

Traits of interest in performance recording harmonisation of Equus genus – survey results

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Performance recording in horses and donkeys have not yet been standardised worldwide. In order to establish a list of breeding associations (BA) interested in harmonisation of Equid recording and genetic evaluation, the Survey of interests has been proposed. The Survey consists of basic information about BA and questions regarding breed and breed type, number of breeding animals (mare, stallion, and foals), parentage verification, main and optional use of the breed, traits important for selection, and potential traits of interest. The Survey is available at the following link: https://rodica.bf.uni-lj.si/EquidAC. So far, 33 BA fulfil the Survey. Around 150K animals were included in the breeding work of these organisations, containing around 59K mares and 5.5K stallions. Main and optional use of breed, according to economic use and breeding objectives, was sport riding school followed by leisure and leisure riding. Current method of parentage verification was mostly based on ISAG standard microsatellite (MS) test followed by own MS test conducted for mostly all registered animals. From the selection point of view, which is based on phenotype, the most important traits were temperament/character, health, and longevity traits. Traits of interest to be included in the breeding program in the future are body conformation and temperament/character. Selection should be based on breeding values. Future parentage verification should be conducted using MS or SNP based test. Most of the breeding organizations showed interest to harmonize all recording systems, parentage verification and breeding value evaluation methods.

Recent demographic history and genetic diversity in Martina Franca donkey population

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The Martina Franca (MF) donkey breed ranks fourth among the eight recognized Italian donkey breeds. It originated in Apulia, well-known for its use in the production of mules that, until a few decades ago, were used, by the Italian army. The advent of mechanization has led to a significant demographic contraction, associated with population fragmentation, and increase in consanguinity. In recent years, however, the breed has experienced a revival of interest thanks to the introduction of alternative uses such as in onotherapy and use of milk in cosmetics and infant feeding. The most recent demo-genetic survey on the MF genealogical records dates to a decades ago, when the registered population counted less than 500 animals. Sponsored by the local government of the Apulia region, we performed a follow-up study to monitor the status of the breed. Our dataset included 2,211 animals (790 males and 1,421 female), including 1,413 live animals (LA). The average inbreeding was 7.67% (3.95% in 2011) and the average relatedness was 14.37% (7.35% in 2011) while, in the current population, these were 8.25% (6.87% in 2011) and 14.89% (9.80% in 2011), respectively. The maximum number of traced generations in the LA was 8.53±3.09 (7.81±3.39 in the whole population) while the maximum number of complete generations was 2.65±1.53 (2.52±1.52 in the whole population). The MF, while showing a population expansion in the last decade, still needs to benefit from an accurate genetic management plan to limit the increase of inbreeding. The option for a molecular characterization using genome-wide SNPs, planned as a next step in this study, will allow: (1) to compare the average genealogical-estimated inbreeding coefficient with the one estimated from genomic data; (2) to assess the incidence of mis-recording of true genealogical relationships in the overall pedigree; (3) to provide an overview of the genomic structure and diversity of the breed; and, possibly, (4) to design a soft selection program in order to systematically take into account the traits of current interest.

Preventive compression measures on horses' front legs during resting affected surface temperature

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Swollen legs and particularly lymph oedema represent an increasingly serious impairment of animal welfare in horses kept for sport and leisure activities. Bandages are often applied during the resting phase for prevention of these issues. However, previous studies question the positive effect of bandages. Thermoregulation in the equine leg predominantly works through circulation by peripheral vasoconstriction, as well as through the release of heat via the skin surface. The aim of this study was to investigate the effects of two selected preventive compression measures applied to the horse's front legs during resting on the surface temperature and circumference of the treated legs. For this, the front legs of 11 healthy dressage horses were either wrapped with polo fleece bandages surrounding a spongy inner layer or with NoLeaf.comPress®Equi for 4 hours when kept in boxes for resting. The circumference of the metacarpus was determined immediately before, after and 1 hour after applying the compression measures. Skin temperatures were assessed every 3 minutes for the first 30 min after application between the leg and the compression device. Data were analysed with a mixed model in SAS 9.4 with the compression measure (fleece bandages versus NoLeaf.comPress®Equi) and time as fixed factors and individual horse as random factor. Both compression measures applied to front legs during resting in healthy horses failed to affect the circumference of the metacarpus. While the surface temperature increased after application of both compression measures ($P<0.001$), fleece bandages lead to higher temperatures than NoLeaf.comPress®Equi ($P<0.001$). Circumference measurements at different locations might be more useful to observe the expected effect of compression. Body surface temperatures will be measured with thermographic imaging in subsequent studies to further investigate the role of temperature with regard to efficiency and suitability of preventive compression applications for horses.

The chicory: a potential crop against summer drought for horses

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Current context of climate change tends to make summer drought more intense and longer. In these conditions, forage crops production is often challenged and animals grazing is confronted to difficult periods. In this context, chicory could be a solution since the resistance of this plant to drought is better than current grasses crops, notably because of its roots growing deeper. To test the resistance of this crop to equine grazing, five growing horses were kept during two months in pastures where only chicory have been seeded. Five other horses of the same age were kept in ordinary natural pastures as control. Both herds were managed with rotational grazing. The evolution of the health status was monitored regularly by observations and body condition scoring was performed using a scale from 1 to 5 (INRAE). Moreover, height of the different type of plants and their abundance were recorded before and after each passage of horses. No differences of health or body condition were observed between the two herds, expect that horses in chicory had more slack faeces than control horses. We observed that percentage of chicory varied during the grazing season between 35 and 60% during the complete grazing season. It was under 45% for only one passage and then increased again. It tends to decrease after more than a month of drought and then increase after rainfall. At the same time, percentage of grasses tend to decrease all along the season. In addition, whereas grasses were, on average, higher than chicory at the beginning of the season, chicory became higher for the last rotations. The higher height for chicory compared to grasses is even more interesting knowing that chicory is shorter than grasses after grazing. In conclusion, chicory manages to maintain itself on the pasture in drought period and with a quite intensive grazing. It also allowed horses to grow normally.

Effect of grazing on subcutaneous fat distribution in foals by ultrasound assessment

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The aim of this work was to study the effect of grazing on subcutaneous fat distribution (SFT) in foal by ultrasound assessment. For this study, 46 foals (19 Burguete (BU) and 27 Jaca Navarra (JN) breeds) were obtained from local farms after weaning and reared in natural grasslands until 17 months of age. One group of 23 foals were raised in high-mountain grasslands (HMG) (10 and 13 from BU and JN breeds, respectively) and 23 foals in lowland grasslands (LLG) (9 and 14 from BU and JN breeds, respectively). The weight (WGHT) and the depth of seven local fat deposits: three of them along the neck length (SFT-N25%; SFT-N50%; SFT-N75%), and four over the trunk (area just caudal to the shoulder (SFT-S), the area between 12th to 13th ribs (SFT-R12-13), the rump (SFT-R), and the tailhead (SFT-TH)) were measured by a transcutaneous ultrasound equipment. These measurements were taken in the last two months, while the animals were grazing. There were significant differences due to the effect of the grazing on the foal WGHT and on the SFT parameters. In particular, the foals raised in LLG showed significantly higher values than foals raised in HMG on WGHT ($P<0.01$) (346.2±45.3 vs 328.8±60.4 kg), on SFT-N25% ($P<0.05$) (2.8±0.7 vs 3.0±0.6 mm), SFT-N50% ($P<0.01$) (2.4±0.8 vs 2.7±0.7 mm), on SFT-N75% ($P<0.1$) (2.9±0.6 vs 3.3±0.9 mm), on SFT-R12-13 ($P<0.05$) (2.8±1.0 vs 3.3±0.8 mm) and on SFT-TH ($P<0.1$) (2.0±0.6 vs 2.3±0.7 mm). Regarding breed effect, only WGHT presented significant differences between foals ($P<0.01$). In this sense, BU foals displayed higher values than JN foals (384.7±29.2 vs 304.2±40.5 kg). These results were expected due to BU breed has a greater intrinsic size compared to JN breed. It could be concluded that although the foals raised in LLG were heavier and fatter than the foals raised in HMG, it is necessary to study if there is a relationship between these results and the organoleptic quality of the product, therefore this is a further study to be explored.

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Impact of essential oils on insulin sensitivity and the metabolome of insulin dysregulated horses

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Plant derived essential oils (EO) contain biologically active compounds that exhibit both antioxidant and anti-inflammatory properties and have shown to improve insulin sensitivity in humans and rodents. To test the hypothesis that EO supplementation improves glucose tolerance and insulin sensitivity (IS) in insulin dysregulated (ID) horses, 20 ID mares were blocked by degree of IS and assigned to treatment: oral daily bolus (50 ml) of either a plant derived EO (Cavalor LaminAid®) or carrier (CON). Mares were housed in dry lots with *ad libitum* access to grass hay and supplemented individually twice daily with a concentrate to meet nutrient requirements for mature, sedentary horses. Before and after 6 wks of treatment, mares underwent a combined glucose-insulin tolerance test (CGIT; i.v. glucose plus insulin,150 mg and 0.1 U/kg BW, respectively) and an oral sugar test (OST; 150 mg sugar/kg BW) on separate days. Data were analysed with treatment as a fixed effect and respective pre-treatment values used as a covariate. Global metabolome analysis was conducted on baseline plasma samples using chemical isotope labelling LCMS, which allowed the relative quantification of over 4,000 metabolites. Although treatment did not affect ($P>0.4$) AUC or glucose clearance during CGIT, there was a treatment × covariate interaction ($P\leq0.08$) for insulin concentrations at 75 min (INS75) and positive phase time (PT) with EO decreasing both INS75 ($P\leq0.002$) and PT ($P=0.05$) in horses with pre-treatment values at or above the observed mean. Similarly, EO treatment reduced ($P\leq0.006$) insulinemic response to the OST in horses exhibiting higher pre-treatment responses (treatment × covariate, $P=0.004$). A timewise (pre vs post treatment) effect was observed on the metabolome, however, 251 metabolites, including branched-chain amino acid- and glutamine-related metabolites, had fold changes of >1.2 with q-value of <0.1, that were unique to EO treated horses. These data show that EO altered the metabolome and improved hyperinsulinemia and certain measures of insulin sensitivity in horses with more severe initial ID.

Session 28 Poster 15

Mare's udder secretory capacity from 3 days to 6 months of lactation

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From ten years, consumers' interest toward mare's milk is growing, particularly due to its health-related properties. Production, however, remains limited in eastern countries. To develop this production, more information about milk yield is necessary. In this study, we focused on the udder secretory capacity. The experiment was conducted from 2017 to 2019 and included 72 mares (22 primiparous, 50 multiparous) of 8.3±2.6 years of age. They were kept in pasture in group with water available *ad libitum*. They were milked 5 times during the lactation at 3 days (D3), 1 month (M1), 2 months (M2), 3 months (3M) and 6 months (6M). At each time, foals were muzzled and, thus, were not able to suckle during the procedure. After three hours without suckling, a first milking (T1) was performed and a second milking (T2) occurred 28.3±2.7 minutes after. Milkings were performed manually with the mare able to touch her foal, starting by the right side of the udder, and without oxytocin injection. Thus, we were able to estimate the quantity of milk secreted by the mare between the two milkings for the left and right side of the udder (respectively, LS and RS). To normalize the data, quantities of milk obtained from LS and RS were divided by time between milkings to obtain a quantity of milk produced per minute. Mean quantities of LS and RS over lactation were 4.90±2.34 g/min and 5.32±2.54 g/min. Then, for each studied period, they were added to obtain the total quantity of milk (D3: 9.84±4.85 g/min; M1: 11.26±4.81 g/min; M2: 10.89±4.07 g/min; M3: 10.10±3.38 g/min; M6: 9.05±3.58 g/min). Using Spearman comparison, we were able to determine a positive correlation between LS and RS (rho=0.65, $P<0.05$). To study udder secretory capacity during lactation, a Kruskal-Wallis test was performed on T2 data. It indicated significant difference regarding lactation period ($P=0.02$). To go further, Dunn test was applied and using a Bonferroni-Holm correction on P-values, and no differences between period had been identified. In conclusion, despite important inter-individual variation, udder secretory capacity in the mare seems constant throughout the lactation period.

Times between foaling and the first standing and suckling of the new-born foal

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In mammals, maternal care is essential for survival and normal growth of the young. Mares establish the bond with their foal during the first hours after birth. Thus, this moment is primordial and will be important for the social and physical development of the young. Physical heath of the foal depends on its capacity to suckle enough, and early enough, colostrum to ensure himself a good immunity. To establish which factors may influence times of the first standing (St) and suckling (Su), we studied 76 foals from 2018 to 2020 (44 females and 32 males; average weight at birth: 51.55±6.20 kg). For the mares, 19 were primiparous and 57 were multiparous, their average weight 24 hours after foaling was 556.70±40.60 kg. We considered 2 type of housing of equal size, 'boxes' with wooden walls (51 foalings) and 'tubular' with tubular structure walls and an outdoor access (25 foalings). A ratio of the foal's birth weight regarding mare weight after foaling (BW/LWmare) was calculated (9.27±0.96%). Hours of St and Su were recorded either by the person in charge of the foalings or by video analysis. St and Su were both studied using linear mixed models with mare's parity, foal sex, type of housing and the ratio BW/LWmare as fixed effects and individuals as random variable. Then, Pearson correlation was performed to study the relationship between both criterions. Time of the first standing is significantly influenced ($P<0.05$) by the ratio BW/LWmare, foals with the lowest ratio being the longest to stand, and by the sex of the foal ($P=0.05$), male taking in average 19.60±8.32 minutes more to stand. Time of the first suckling was not significantly influenced by the studied variables. The two considered times were significantly correlated ($P<0.05$; correlation coefficient: 0.51). In conclusion, smaller foals seem to stand latter than bigger ones and females to stand quicker than males. Thus, extra-attention should be paid to small foals and males to make sure they get colostrum at the appropriate time.

Whether the mares milking process stresses the mares ?

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The population of cold-blooded horses in Poland was dramatically reduced during last decades. To avoid decline in numbers of a local population, a there are projects to promote their alternative use, e.g. dairy use. The mares of the local population of Sokolski cold-blooded horses were used in the experiment. This breed is characterized by a very calm temper and good character. The aim of the project was to evaluate their milk useful and factors influencing the amount of milk obtained. 9 mares were monitored under aspect of environmental factors on factors on quantity of milk yield, average daily length of milking. It was observed behaviour during milking and behaviour of foals during milking. A significant drop in performance in stressful situations was found. Results shows that the performance was significantly influenced by environmental conditions as well as factors such as the presence of strangers in the stable and unexpected situations.

Distribution of minor trace elements in different fractions of donkey milk

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The use of donkey milk for human consumption has been recently revalued as an alternative dietary ingredient for sensitive consumers, for example infants with cow milk protein allergy, adults with inflammatory and allergic ailments, or aged healthy persons. In depth information on nitrogen and fat components and on major minerals are now available in literature but less attention has been devoted to minor trace elements in donkey milk. The aim of this trial was to study the concentration of Li, B, Ti, Cr, Rb, Sr and Cs in donkey milk and their distribution among fat, casein, whey proteins and aqueous phase. Mechanical milking was used to provide individual milk samples from 16 lactating donkeys (6 Amiata and 10 Ragusana breed). Subsequent centrifugation, ultracentrifugation, and ultrafiltration (cut-off 3kDa) were carried out to remove fat, casein and whey proteins, to obtain skimmed milk, a supernatant whey fraction and the aqueous phase of milk, respectively. An inductively coupled plasma-mass spectrometer was used to measure the concentration of the aforementioned elements in whole milk and fractions, and the concentration of elements associated with fat, casein and whey proteins was then calculated. The effect of removal of fat, casein, and whey proteins was determined by analysis of variance for repeated measures. The milk concentration of Li, B, Ti, Cr, Rb, Sr and Cs was 5.88 l, 209.3, 79.7, 2.17, 1,434, 370.4 and 2.50 µg/l, respectively. Li and B were entirely present in the aqueous phase of donkey milk, together with the large majority of Cs (96.3%) and Rb (87.2%). The effect of fat removal was significant for Ti, Cr, and Sr, of which the fat fraction carried 7.3, 10.4 and 4.5% of total milk concentration. Half of milk Ti (50.0%) and the majority of Sr (69.8%) were associated with the casein fraction, as well as 20.2% of Cr and 7.9% of Rb. A significant but low amount of milk Rb (4.0%), Sr (3.04%) and Cs (3.9%) was associated with the whey proteins fraction.

Oxidative stability and volatolomic profile of dry vs vacuum aged horse meat

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Aging leads to the conversion of muscle to meat. It depends on several factors and it improves eating quality affecting tenderness, juiciness, taste, odour and flavour. During aging, there is a release of peptides, free amino acids, and free fatty acids due to proteolytic and lipolytic phenomena, leading to an increase of concentration and number of flavour compounds. The aim of the present work is to study the effect of dry and vacuum aging both lasting 14 days. 10 IHDH horses were included in the trial. Longissimus lumborum muscle of both half carcasses (from the 15th to 18th dorsal vertebra) was sampled by 10 male 18-months IHDH horses. The two samples by each head have been randomly assigned to vacuum or dry aging treatment. Vacuum aging was done storing samples after vacuum packaging at 4 °C; dry aging was done in an aging cabinet set at 2 °C, 82% of humidity and 0.2 m/s of ventilation. TBARS, hydroperoxides (HY) and protein carbonyls (PC) concentration, superoxide dismutase, catalase and glutathione peroxidase activity were measured. Finally, grilled samples were used for volatolomic profile, performed by solid-phase microextraction (SPME) and gas chromatography-mass spectrometry (GC-MS). Data were analysed using a one-way ANOVA, where ageing method was set as independent variable and single horse as random effect. Enzymes activity resulted higher in vacuum aged meat ($P<0.01$), associated to lower TBARS, HY and PC production ($P<0.01$). Moreover, dry aged meat showed higher aldehydes production (4,066 vs 2,198 ng/g of meat; $P<0.01$). Hexanal was the most concentrated VOC (2,083 vs 3,762 ng/g for vacuum and dry aging respectively). Hexanal, commonly considered a meat oxidation marker of lipids, is characterized by high aromatic potential, providing freshly cut-grass and green aroma notes. Vacuum aging, associated to low film permeability, resulted in lower oxidation processes, reducing VOCs amount, but preserving a higher antioxidant enzymatic activity.

Session 29 Theatre 1

Role of the researcher in facilitating innovation

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Drawing from real-life project experience, this presentation will describe the implementation and relative effectiveness of a network approach to working with farmers, involving the researcher as a free actor to facilitate the innovation process.

Session 29 Theatre 2

Fostering impacts through interdisciplinary science approaches with multi-stakeholder involvement

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Much research over time has focused on technical improvements without considering possible barriers, lock-ins and motivations to implement the results, apparently ignoring the important influences of structural incentives, social and societal factors to this. Participatory research emerged in many forms in the 1960s, and partly offered ways of contextually relevant research and implementing. This presentation will focus on how interdisciplinary science approaches (focusing on veterinary, agricultural and social sciences) can foster impacts through innovations in and development of farming and food systems. After having introduced key concepts ('participation' a.o.), different levels of impacts on farm, sector, community (including communities of practice (CoPs) and beyond will be explored and discussed, based on experienced examples. Two decades of mainly international research projects will be used as examples, where whole systems are the arenas of change and transition, including Stable Schools, Farmer Family Learning groups, organic farming research, animal health planning research and development, Living Lab involvement, cow-calf contact systems, and research in the meetings between governmental inspectors and farmers. Critical views on the use of the term 'participation' will be analysed together with aspects of social learning, social capital building, and Community of Practice theories. Furthermore, potentials to identify context relevant places and levers to intervene in a (farming or food) system, provides an interesting framework which can be unfolded when the body of common experience and learning builds up within and between the involved actors with different backgrounds, interests, and influences. The shared interest and understanding between research partners and other stakeholders to meet current societal challenges such as antimicrobial resistance, animal or human-animal ethical issues, climate change and increasingly broken food systems needs to be present in enabling environments. Interdisciplinary participatory science approaches can facilitate commonality of interests.

Session 29 Theatre 3

Reaping the rewards of livestock science at grassroots level

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Livestock farming has benefited hugely from the application of science in the fields of nutrition, reproduction, genetics, product quality, health, environmental impact, etc. But could previous and current investment in research and knowledge transfer yield an even greater return? This question is particularly relevant given increasing demands on the sector for sustainable economic development as well as delivery on societal needs and other public goods. Simple solutions taken in isolation are not appropriate to complex challenges, which in many cases do not begin and end at the farm gate. The person ultimately responsible for practical implementation is the livestock farmer or manager. He/she must work out which are the most appropriate technical developments, how best to customise these to the particular circumstances, monitor impacts and deal effectively with any trade-offs which might occur. The ability of the traditional top-down linear approach to deliver change is limited. The main constraint is frequently not the availability of technical information, but its customisation and translation into practice. Therefore, innovation is required both on the part of the deliverer of science and technology, as well as recipient. This was the area of focus for the EuroDairy Thematic Network, which followed the interactive model of the European Innovation Partnership (EIP). The EIP puts farmers at the centre of practice-based innovation, to adapt and develop new and existing scientific knowledge, and produce implementable solutions. Key to success is a multi-actor approach, notably between progressive farmers, advisors, scientists, customers, supply chain, and similarly minded peers. Involving end-users and other actors along the innovation chain, can be shown to be a more effective in developing implementable local or system-adapted solutions. Human factors such as attitude, motivation and business acumen, also have a major influence on positive or negative outcomes. This presentation will draw lessons from the results of the EuroDairy project, illustrated with real-life examples taken from 120 participating dairy farmers, where the sustained deployment and intelligent adaptation of applied science have led to remarkable results.

Session 29 Theatre 4

BovINE: beef innovation network Europe

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BovINE is an EC funded thematic network focussing on knowledge exchange to tackle sustainability challenges in the beef cattle sector. The project connects with farmers across Europe by providing an open platform – https://hub.bovine-eu.net – where all relevant stakeholders can exchange knowledge and share experiences to drive awareness and adoption of innovative and proven practices. BovINE focuses on 4 key themes: socio-economic resilience (SER), animal health & welfare, production efficiency & meat quality (PE-MQ), and environmental sustainability (ES). Based on these themes, BovINE identifies farmers' grassroots needs, providing the basis for priority topics tackled each year. For these topics, research findings are identified and their feasibility in practice is examined, including demonstrations on beef farms across Europe. In the coming months, several on-farm demonstrations will be organised to show the feasibility of the innovations and to undertake a cost-benefit analysis. On SER, the Teagasc eProfit monitor, a tool to benchmark and monitor farm performance based on previous years' activity or relative to other farm will be implemented in different EU regions. To improve health and welfare of dummy calves, a squeeze technique that induces a slow wave sleep and hormone change will be demonstrated during open days and tested on several farms. Automated weighing systems in fattening units will be demonstrated on commercial farms to illustrate its benefits in terms of PE-MQ. For ES, the use of nitrate as a feed additive to lower enteric methane emissions will be tested on a research farm and results will be presented during discussion groups with farmers. Each year, all knowledge gathered is shared in national stakeholder meetings to provide space for discussion between beef farmers and other stakeholders across member states and to inspire them to improve on-farm practices. During these meetings, needs are also collected to identify topics on which farmers would like to have more information. The authors thank the EC for project funding H2020 Project No: 862590 and all project partners for their contributions.

PROTECOW: French-Belgian exchange on cost efficient replacement of soybean meal in dairy diets

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Use of soybean meal in dairy cattle is under pressure. Because of high and volatile prices, farmers are eager to find European and/or on farm alternatives that are cost-efficient. Over 4 years researchers, advisers and a core cross-border group of 18 dairy farmers have worked together to explore new solutions using different research, communication and interaction approaches. Five alternatives for imported soybean were identified from research and implemented on farm. A farm trial investigated the entire replacement of soybean meal (8.1% of DMI) in a control diet (SBM) by rapeseed meal (10.9% of DMI) supplemented with rumen protected lysine (RM+L) to correct for lower lysine in the soybean free diet. RM+L resulted in increased milk production (+0.8 g, $P<0.001$) and protein content (+25 g/d, $P<0.01$). Milk urea decreased from 21.3 mg/100 g in SBM to 17.7 mg/100 g in RM+L ($P<0.001$). Impacts on ammonia emission were simulated using the KringLoopWijzer (WUR). The RM+L diet showed a small reduction of ammonia emission on farm (-1%) and per kg milk (-4%). Greater impact was seen on carbon footprint per kg milk, mainly caused by land use change effects of concentrate (SBM:1.28 kg CO_2eq/kg milk; RM+L:1.07 kg CO_2eq/kg milk). The project focused on improving (protein) autonomy of dairy farmers, which was summarized in 9 factsheets. For example: French farmers implemented earlier harvesting of grass silages. Belgian farmers learnt that more considered use of concentrates is ecologically and economically beneficial. Comparison and exchange of economic results within the cross-border group allowed them to learn from each other in a very interactive way. Researchers and advisers summarized important project findings in factsheets, videos, presentations and testimonials, which are available at the project website in French and Dutch (https://www.interreg-protecow.eu/).

Creation of a semantic resource responding to the principles of open science for the meat sector

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The OECD describes open science as a new approach based on cooperative work and new ways of diffusing knowledge by using digital technologies and new collaborative tools. In this context of transfer and innovation, it is more than ever necessary to have terminological repositories to facilitate the collaboration between disciplinary and linguistic communities, the discovery of new information and data from various sources and the development of tools based on knowledge of the field. This implies that repositories are available in formats that can be used by software. The Meat Dictionary from the French Meat Academy served as a repository of terms for the construction of a thesaurus on meat. A thesaurus is a controlled and structured vocabulary in which concepts are represented by terms, relationships between concepts are made explicit, and preferred terms are accompanied by lead-in entries for synonyms or quasi-synonyms. To achieve this, we had to transform the original dictionary in Word format (.docx) into a thesaurus in SKOS (Simple Knowledge Organization System) standard format. The definitions were then separated individually as different concepts, and a new file was loaded in another software to transform, clean and correct the data. Finally, the data were then extracted to build a correspondence scheme between a tabulated format and a representation in SKOS to identify concepts using URIs and to link them into informal hierarchies and association networks. After the format transformation, we imported the data into a semantic resource editor. Using this editor, the dictionary data was structured by four experts in meat science who organised the hierarchy, checked all concepts and added new ones when necessary. Not all thesauri present definitions for their different entries. The result is the meat thesaurus which contains 1,552 concepts organised in 12 main categories and 145 sub-categories. This thesaurus will soon be made available on a public repository in French, so to serve as an essential tool to share information among researchers, industries, journalists or consumers.

Prediction of marbling score in slaughterhouse using a pocket-sized near-infrared spectrophotometer

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The evaluation of meat eating quality is a crucial challenge for the beef industry. This is the reason why the MSA (Meat Standards Australia) system has been developed from 1996 onwards to predict beef eating quality in Australia. It is based on the combination of key criteria to predict palatability for individual cuts. Marbling is one of these key criteria which is routinely assessed in different countries and now recognised as important by the French beef industry. Despite this, it is still a challenge to evaluate marbling score in an easy, fast, cheap and non-destructive way. In the recent years, near-infrared (NIR) approaches have widely been used in food analyses because it is non-invasive, non-destructive, quick and cheap. Several attempts have been done in order to miniaturize the NIR technology. This has led to the development of low cost and pocket-sized tools for rapid evaluation in a slaughter house as they are easy to handle and do not require any knowledge in physics or chemistry. Nevertheless, the performance of these devices still needs to be evaluated. This study aimed to evaluate the potential of pocket-sized spectrometer NIR for the prediction of MSA marbling scores. In this work, 113 carcasses were used and spectra NIR information were collected in the wavelength ranges from 740 nm to 1,070 nm. Five spectra were collected for each carcass and the average spectra was used for each sample. The average MSA score was 346 with Standard Deviation of 103,05 on a 1-1,100 scale. The prediction models were developed using OPLS methods (R package, caret). The data was first pre-treated with a 2nd derivative. The validation method used the 'Leave-One-Out' on the 113 samples. Overall, an R2 value and RMSE were 0.65 and of 60.60, respectively. In conclusion, the NIR pocked device has the potential to be used in slaughterhouse to routinely assess marbling although with moderate performances so far.

New grading systems of beef to ensure consumer satisfaction and farm gate returns to producers

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First carcass classification standards or grading systems were designed to trade carcasses. Therefore, initially, almost all countries designed classification systems to describe the carcass to the purchaser with the definition of quality mainly based on carcass characteristics and varying for each destination market. In reality, as in Europe, conformation and fat scores of bovine carcasses are poorly related to marbling or to eating quality. However, some grading schemes (such as in the USDA) were based on both a yield grade and a quality grade for each carcass. Other quality labels are based on strict compliance with certain specifications throughout the chain, and/or on consumer taste panels to control eating quality of the labelled products. In addition, a unique grading system called Meat Standards Australia (MSA) was developed in Australia. Its principle is to grade individual muscles or cuts in interaction with the cooking method instead of the whole carcass. All these grading schemes should guarantee both enjoyable consumer experience and added-value for the producers. At the moment, these objectives are not achieved in the current market except in Australia where the MSA grading scheme successfully delivered farm gate returns to beef producers. To better spread international knowledge on beef eating quality and success stories for both producers and consumers, the International Meat Research 3G Foundation has been set up as a collaborative, independent, not-for-profit international eating quality research platform linked to the United Nations Economic Commission for Europe Specialized Section on Meat.

Session 29 Theatre 9

Assessment of needs for data integration and digitalisation in Austrian dairy cattle farms

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To investigate the needs and expectations of smart dairy farming, a series of personal and online surveys and workshops in Austrian dairy farms was analysed. Online surveys reached between approx. 2,900 and 3,700 farmers, representing about 19-24% of farms under performance recording in Austria. The responses gave insights into the current degree of adoption of automatization and digitalisation technologies on dairy farms and reflected the expectation of farmers depending on the farm characteristics. The results showed that the relevance of automatization systems increases with herd size and in farms that explicitly follow an optimisation or growth strategy. For the latter, animal sensors are of great relevance, with a majority of these farms already use or consider investing in sensor systems. The primary goal of farmers investing in automatization and digital solutions is the reduction of labour. More than 80% of the participating farmers considered this as important or very important. Another motivation is the improvement of precision and the support for decisions based on own knowledge. To reach these goals, more than half of the surveyed farmers see a need for extended data integration between the systems on their farms. Key areas are integration of data for herd management systems, animal sensors and equipment for feeding. There is awareness of critical aspects of digitalisation. About one third of the farmers in the survey feel overwhelmed by digital technologies, and data protection is a key issue for trust in digital systems. The survey showed that farmer-owned organisations (e.g. performance recording organisations) are highly trusted as keeper of farm data. Concluding, farmers are increasingly using equipment and services relying on data integration. There is also a need for solutions and advice from trusted, non-commercial organisations to speed up digitalisation in dairy farming.

Session 31 Theatre 1

Animal nutrition strategies to cope with EU Green Deal: GMO, additives and novel feeds

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Different strategies can be adopted to reduce the environmental impact of animal husbandry, so to cope with the objectives of EU Green Deal. Animal nutrition plays a crucial role in this context. Improving feed digestibility and feed conversion can reduce emission of pollutants in the environment; genetically modified crops resistant to insects and tolerant to herbicides allows farmers to spray a lower amount of pesticides to protect plants, and to better preserve health soil and to reduce fuel consumption and labour. Beyond classic approach to create GM crops, synthetic biology (SynBio) will open new insights in the construction of plants used for feed purposes, that could help to improve EU environmental sustainability. Feed additives have been proven to be efficient in improving feed utilization, and they can offer also some solutions to maintain good health conditions of animals considering the reduced use of antibiotics. An emerging approach in the feed sector is the use of feed sources that allow reducing the competition with conventional raw materials used for human animal nutrition, e.g. insects and algae, and feeds that derive from food no more usable for human nutrition and food waste, the so called former food. These last two categories of feeds are usually named novel feeds. The European Food Safety Authority (EFSA) assesses the safety of GMO crops and additives used in feed prior to its authorization. Currently different EU vertical legislations determine how risk assessment is performed for different hazards in in feed commodities Novel feed may drive the emergence of new hazards or increased exposure to known hazards, for both animal and human health, posing new challenges to assessors and regulators.

Duckweed as fishmeal-protein replacement for omnivorous and carnivorous fish fry

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Duckweed are fast growing floating aquatic plants with the potential of higher protein productions per unit time and area than most terrestrial plants including soy beans. Liquid animal manures and slurries can efficiently be recycled with high N and P uptake efficiencies of duckweed. As protein feedstuff, duckweed has successfully been used for a variety of animals, including pigs, poultry and fish. Here, we present the results of three feeding studies utilizing graded duckweed (*Spirodela polyrhiza*) concentrations as fishmeal-protein replacement for fry of common carp (*Cyprinus carpio*), Eurasian perch (*Perca fluviatilis*) and rainbow trout (*Oncorhynchus mykiss*), all of them important aquaculture species. Two different duckweed types, dried and fermented, were utilized to replace up to 45% of fishmeal-protein in carp and up to 35% in trout and perch. Fish were kept in a recirculating system in four replicates per treatment and hand fed for four weeks. Once a week the fish were weighed to adapt the feed allowance. Evaluated effects included changes of whole body chemical composition, growth performance, feed, protein and lipid utilization. Results were analysed by one-way ANOVA and subsequent Tukey HSD post-hoc test. Duckweed has been well utilized by carps and surprisingly also by rainbow trout with replacement levels up to 45% in dried and 30% in fermented duckweed for carp fry and 24% of dried and fermented duckweed for trout fry without significant reductions in performance. Perch fry, however, showed even at lowest duckweed concentrations of either dried or fermented duckweed significant reductions compared to control fish with stronger effects for fermented duckweed. Our results show, that for the omnivorous carp and for the carnivorous rainbow trout a certain replacement of fishmeal-protein is possible even for fry, the most sensitive life-stage. For Eurasian perch fry, however, duckweed is not a suitable source of protein.

Inclusion of silage in pig diets – effect on production and health

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Grass silage has potential as a locally produced protein and energy source for fattening pigs and could have positive effects on their gastric health. Feeding technique and pre-treatment of the silage will affect the consumption level and the pigs' potential to utilise the nutrients in the silage. The aim of this study was to study the effect of grass/clover silage, with different pre-treatments, included in total mixed ration (TMR) diets, on pig growth performance, carcass traits and occurrence of stomach ulcers. In total 128 fattening pigs were randomly allocated to one of four diets. The pigs were fed either commercial feed + intensively processed fresh silage, mixed and fed together (SE); commercial feed + chopped fresh silage, mixed and fed together (SC); commercial feed + ground silage, mixed and pelleted (SP); or commercial pelleted feed alone (C). In the SE diet, the silage was processed (1-3 mm) in a bio-extruder whereas in the SC diet, the silage was kept intact (chopped at harvest, 4-15 mm). The silage in SE, SC and SP diets were included to replace 20% of the dietary crude protein (g/kg DM). Diet had a significant effect on growth performance of the pigs (P=0.001) and the occurrence of stomach ulcers (P=0.001). The average daily weight gain (kg) was higher for pigs in SP, C and SE compared with SC (1.084, 1.023, 0.996 vs 0.951 (P=0.001). Pigs in SC had the lowest carcass weight followed by pigs in SE, C and SP (81, 82, 84 and 85 kg, respectively). Lean meat content (%) did not differ among diets, however pigs in C had higher dressing percentage (74%) compared with the SE and SC (73%) (P=0.016). Feed conversation ratio (FCR) differed (P=0.001) with highest FCR in pigs in SP and lowest in SC, but with no difference between pigs in SE and C (P=0.153). Pigs in SC and SE were fed more kg feed (P=0.001), but there was no difference in energy consumption between the diets (P=0.981). Thus, the results show that silage can replace parts of the diet, but that the pre-treatment of the silage might affect the nutrient utilization. Pigs in SE and SC had lower occurrence of stomach ulcers than SP and C (P=0.001), indicating that inclusion of fresh silage has a positive effect on pig gastric health.

Winery by-products as a functional feed to modulate ruminal fermentation *in vitro*

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Turning food wastes and by-products into feed for livestock is a promising approach to reduce the competition for human food sources and simultaneously reduce wastes generated from food production. Winery by-products not only have nutritive values for cattle but also contain functional compounds like polyphenols. We tested grape pomace (GP) and grapeseed meal (GS) used in combination with high-quality (HQ) hay and expected the positive effects on reducing excessive protein degradation and methane formation. Six dietary treatments with similar contents of organic matter (OM, 93%) and crude protein (CP, 18.3-19.1%) on a dry matter (DM) basis were tested including Control (70% HQ hay + 30% concentrate), Ext (Control diet + grapeseed extract at 3.7% of basal diet DM), GS-low (70% HQ hay + 25% concentrate + 5% GS), GS-high (65% HQ hay + 25% concentrate + 10% GS), GP-low (65% HQ hay + 25% concentrate + 10% GP) and GP-high (56% HQ hay + 24% concentrate + 20% GP). All treatments were performed in duplicate in each of the 4 runs (n=8 per treatment) using the rumen simulation technique. Each run consisted of 10 days and the last 5 days served as the measurement period. All by-products and Ext treatments decreased ammonia concentrations compared to Control ($P<0.05$), whereas they did not decrease methane formation. Both GP treatments and GS-high decreased short-chain fatty acid concentrations (6-8%) compared to Control ($P<0.05$). Ext and GP-high resulted in lower butyrate at the expense of acetate and propionate percentages compared to Control ($P<0.05$). Ext and by-product treatments except GS-low decreased the degradation of DM and OM (4-6%) compared to Control ($P<0.05$). Ext and by-product treatments decreased ammonia concentration per gram degraded CP by 14-18% ($P<0.05$). Degradation of acid and neutral detergent fibre was unaffected. The present *in vitro* study suggests the potential of using winery by-products as a replacement for high-quality feedstuffs for cattle with an added value related to their functional effect on ruminal protein metabolism.

Effect of forage source and processing conditions of fava bean on amino acid digestion in dairy cows

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Due to the relative low P content and the non-GM origin, fava beans is a suitable alternative protein source for dairy cows to substitute rapeseed and soybean when P load or origin has to be taken into account. In order to maximize the utilization of protein from fava beans, feasible and efficient approaches to process the fava beans are required. The aim of this study was to investigate the effect of forage source and fava beans processing conditions on supply of amino acids (AA) digested in the small intestine. Six multi-fistulated Holstein dairy cows were used in a 6×4 incomplete Latin square design. Diets were either high in grass-clover silage (45% grass-clover, 15% maize) or high in maize silage (45% maize, 15% grass-clover) of feed DM combined with 30% of either ground, toasted and ground, or rolled fava beans. Duodenal and ileal flow of AA were measured using Cr_2O_3 and TiO_2 as markers. Treatment effects were estimated by use of orthogonal contrast in R Studio (version 3.6.3) using the linear mixed-effects models procedure. Rolling of fava beans tended to lower small intestinal digestibility of total AA with 712 and 708 g/kg for diets high in grass silage and diets high in maize silage, respectively, compared to ground fava beans with 728 and 748 g/kg ($P=0.05$). Likewise, rolling of fava beans resulted in lower digestibility of Arg, Asp, Glu, Gly, Lys in the small intestine compared to ground fava beans ($P\leq0.05$). Forage source had no effect on AA digestion but resulted in a higher Gly small intestinal digestibility for diets high in maize silage compared diets high in grass silage ($P=0.02$). Toasting had no effect ($P>0.05$) on the digestion and digestibility of amino acids in the small intestine. There was no effect of treatments on total AA digested in the small intestine per kg of DM intake ($P>0.05$) with the numerically lowest (102 g/kg DMI) for diets high in maize silage with toasted fava beans and highest (112 g/kg DMI) for diet high in grass silage with toasted fava beans. In conclusion, treatments did not significantly improve AA digestion in the small intestine, but grinding resulted in the highest AA digestibility in the small intestine.

Session 31 Theatre 6

Novel concepts and methodologies in assessing sustainability in livestock farming: One nutrition

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The linear approach of nutrient efficiency is no longer sufficient on its own to evaluate the sustainability performance of a food production system in a circular way and new dynamics can be created as many crops and livestock production parameters are interdependent. Therefore 'One nutrition' concept is bringing together sciences of plant, animal and human nutrition so that the transfer of nutrients is optimised. Microbes, whether microbiota in soil and animals gut or as pathways to recover nutrients and generate biomass are critical elements of the equation to optimise nutrients use. The concept of 'One Nutrition' is similar to the concept of 'One Health' strategy of the EU aiming at reducing the use of antibiotics in livestock and companion animals along the principle 'as much as needed, as little as possible'.

Session 31 Theatre 7

Impact of dietary protein content and methionine supplementation in dairy ewes in early lactation

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Multiparous Lacaune dairy ewes (n=47; 77.2±1.2 kg BW) in early lactation (37±2 DIM), were used to assess the effects of the level of CP (14.8 vs 16.6%; DM basis) and supplementation with rumen protected Met (0 vs 5 g/d; Smartamine, Adisseo, FR) in the diet to cover 100% of metabolizable Met based on cow requirements (INRA 2007). Ewes were in balanced groups of 6 per pen (8 groups), milked 2×-daily and fed a TMR *ad libitum* (44:56% forage:concentrate; DM basis). Met was supplemented mixed with 40 g of cracked corn grain fed individually at the a.m. milking. The trial was a cross-over split-plot design with two periods of 3 wk with a 2 wk washout period. Intake (group) and milk yield were measured daily, and milk sampled for composition on d 16 and 17 of each period. Individual intake was assessed by using 30 g/d of PEG6000 as external marker. Blood samples were taken on d 18 of each period for metabolites analyses. BW, BCS and wool growth (shearing 200 cm^2/ewe) were recorded at the start and the end of each period. Data were analysed by using the GLIMMIX of SAS (v.9.4). No differences in DM intake (3.50±0.12 kg DM/d), milk yield (2.52±0.17 kg/d), milk protein (5.42±0.07%) and casein (4.02±0.06%) were detected either by CP or Met treatments. Milk urea content increased ($P<0.01$) with CP (47.0 vs 54.9±1.2 g/l), milk fat (6.54 vs 6.31±0. 13%) and solids (17.7 vs 17.4±0.2%) decreased ($P<0.01$) with Met. Blood urea increased (33 vs 48±2 mg/dl; $P<0.01$) and glycemia decreased (66 vs 62±2 mg/dl; $P<0.05$) with CP, whereas BOHB tended to increase (0.585 vs 0.640±0.042 mmol/l; $P=0.06$) with Met. Blood NEFA, cholesterol, insulin or greasy wool growth were not affected by treatment, but BW gain tended to increase with CP ($P=0.06$). Plasma Met concentration did not change with CP but increased with Met supplementation. CP or Met had no effect on N efficiency (24.8±2.4%). Increasing CP level did not improve lactating dairy ewe performance. An excess of metabolizable protein supply could explain the poor milk protein yield responses in Met balanced diets.

Replacement of soybean meal with poultry-based processed animal proteins in weaned pigs

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Reintroduction of Processed Animal Proteins (PAPs) as ingredient in animal feed fits in the EU policy to reduce dependency on imported protein and would contribute to sustainability of animal sourced food. In this study, we determined the effect of replacing soybean meal (SBM) with poultry PAPs on growth performance and intestinal characteristics related to protein fermentation and gut integrity of weaned pigs. The study was conducted during a 5-week period with 360 weaned pigs (age 26 d, BW 7.69 kg) in 5 dietary treatment groups, each comprising 12 pens with 6 pigs. Control pigs received a pre-starter (0-14 d) and starter (14-35 d) diet with 10 and 14% SBM, respectively. SBM (7.5%) was replaced by 5% high- or low-ash poultry meal (HPM and LPM), or 4% feather meal (FM) or blood meal (BM). Treatments did not alter ADG and ADFI ($P>0.05$) during the pre-starter phase, but FCR ($P=0.005$) was lower for Control and LPM compared to FM and BM (1.29 vs 1.39 g/g) and intermediate for HPM (1.34 g/g). Treatments affected ADG and ADFI ($P<0.02$), but not FCR ($P=0.47$) during the starter phase, with a lower ADG for HPM and FM than Control, LPM and BM. In the whole period (0-35 d), the ADG of LPM, BM and Control (419, 411 and 424 g/d) was higher than HPM and FM (387 and 389 g/d). The FCR of HPM, FM and BM (1.44 g/g) was higher than that of Control (1.40 g/g). Tail-biting scores, registered on d 7, 14, and 35 as indicator of adverse behaviour were not consistently affected by dietary treatment. The pH and concentration of ammonia and short chain fatty acids of chyme in ileum, caecum and colon harvested in one pig/pen on d 14 were affected by diet, with the main differences between FM and other PAPs. Expression of claudin 12 mRNA, but not of other claudins in ileal mucosa, was enhanced with LPM. Expression data of other genes in ileal mucosa, related to intestinal integrity, immunity, and inflammation will be available at the meeting. In conclusion, LPM and BM can replace 7.5% of SBM in weaned pig diets without loss in performance but FM and HPM may reduce growth performance, whereas FM may enhance ileal and colon (protein) fermentation. Consequences for intestinal integrity will be discussed.

Giving a second life to food waste: what to expect from the use of former foodstuff in animal feed?

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According to the Commission Regulation (EU) No 2017/1017, former foodstuffs are foodstuffs, other than catering reflux, which were manufactured for human consumption but which are no longer intended for human consumption. Their exclusion from human consumption market can be for practical or logistical reasons or due to problems of manufacturing, packaging defects or other defects. These products are typically produced based on biscuits, bread, breakfast cereals, pasta, snacks or sweets. Former foodstuffs retain a significant nutritional value and thus constitute a very promising alternative feed, completely in line with the current trend of circular economy. However, its use in animal feed remains currently limited in the European Union. One of the reasons for this limited use is probably that, in a safety point of view, former foodstuffs containing animal products follow the same rule that other products of animal origin for their use in animal feed and shall fulfil the requirements of the Commission Regulation (EC) No 1069/2009. Only former foodstuffs containing or deriving from milk products, egg products and non-ruminant gelatine are allowed to be used in farmed ruminant and non-ruminant feed in accordance to the restrictions described by Commission Regulation (EC) No 999/2001. Ingredients containing ruminant gelatine cannot currently be used in feed for farmed animals. The objectives of this study was to evaluate 12 samples regarding: (1) their nutritional value; and (2) their potential impact on official analyses for the detection of animal proteins. For this purpose, samples were considered using four different analytical techniques: Infrared spectroscopy was used to characterize the nutritional value of the samples. In order to determine the presence of constituents of animal origin, samples were analysed by light microscopy and PCR as described by Commission Regulation (EC) No 152/2009. Moreover, mass spectrometry-based proteomics was performed for the detection of ruminant proteins. Results and the strategy of combination of these different approaches will be presented and discussed during the meeting.

Session 31 Poster 10

Effects of maize kernel particle size on the gas production kinetics *in vitro* of maize silage

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The objective of this experiment was to assess the relationship between maize kernel particle size in maize silage and rumen fermentability using *in vitro* gas production (GP). Treatments consisted in 3 different processing roll clearances (between 1 to 14 mm) during harvesting of 15 fresh maize crops. Crops varied on country of origin (Spain, Canada, The Netherlands), field, harvesting date and hybrid. After ensiling, maize kernel particle size was evaluated by dry (maize silage processing score; CSPS) and wet sieving (WCSPS), and by the mean particle size (MPS) from the bottom pan of the Penn State Particle Separator. GP during 72 h of fermentation was measured using 1 l fermentation bottles with 600 ml of buffered rumen fluid and ~5 g of DM of sample (42 samples total). Rumen fluid was collected in a pre-warmed thermos flask flushed with CO_2 from 4 fistulated Holstein cows 2 hours before feeding. GP was fitted to a mono-phasic Sigmoidal model: $Y = A/1 + (B/t)^C$ where A = asymptotic GP; B = time of incubation at which half of the asymptotic has been formed; C = sharpness of the switching characteristic for the profile. The maximum rate of GP (R_{max}) was calculated by $R_{max} = A*B^C*C*t_{Rmax}{}^{(-C-1)} / (1+B^C*t_{Rmax}{}^{-C})^2$ where t_{Rmax} is the time when R_{max} is reached. All GP characteristics were compared using PROC MIXED with either CSPS, WCSPS or MPS, plus NDF, ADF, CP, DM, ash and starch of each sample as covariates, with the original sample, hybrid and harvesting date nested within field and location as random factors. As a percentage of total GP, GP from 3 to 20 h increased with CSPS (P=0.02) and WCSPS (P<0.01) while GP from 20 to 72 h decreased (P<0.01). The C decreased (P=0.01) with CSPS and WCSPS. Higher CSPS (P<0.02) and WCSPS (P<0.01) increased maximal fractional degradation rate (%/h) and R_{max} (ml/h). Degradable organic matter increased with CSPS (+ 1.3%; P=0.02) and WCSPS (+ 1.2%; P=0.01). Reducing maize kernel particle size, which can be estimated by CSPS and WCSPS, accelerated GP and degradability *in vitro* of maize silage.

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Replacement of soybean meal with poultry based processed animal proteins in growing pigs

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The EU Parliament aims to reduce the dependency on protein crops imported from outside the EU. Reintroduction of Processed Animal Proteins (PAPs) as ingredient in animal feed fits in this policy and would substantially contribute to circularity and sustainability of animal sourced food. Processing of PAPs has been improved in the last decades to assure optimal biosecurity and nutritional value. The latter was already studied and presented at the 70th Annual EAAP meeting. The removal of PAPs from pig diets led the perception of an increased incidence of adverse behaviour, like tail biting. The present study was conducted to determine the effect of replacing SBM with poultry PAPs on the growth performance and on tail biting of weaned pigs. A total of 240 male and female growing pigs with an average BW of 22.5 kg and undocked tails were randomly allocated (10 piglets/pen) to 4 dietary treatments and 6 pens per treatment. Pigs in the Control treatment were fed a diet containing 9.4% SBM. A total of 7.5% SBM was replaced by 5% high- or low-ash poultry meal (PM-H and PM-L), or by 4% feather meal (FM) in the other three treatments. Experimental diets were fed for 35 days. Pigs and residual feed were weighed, and tail-biting damage scored on 0-, 14- and 35-days of experiment. Faecal scores were determined weekly on a pen basis. Data were analysed with a one-way ANOVA and significant differences were estimated with a Fisher's test. Significance was declared at P<0.05. All treatments had a similar ADG, ADFI and FCR in the intermediate (0-14, 14-35 d) and overall (0-35 d) periods. The average ADG, ADFI and FCR of the overall period were 804, 1,490 and 1.86 g/g, respectively). The faecal consistency and tail biting scores were not significantly different between treatments. Nonetheless, numerically higher scores (i.e. more damage) were observed in the Control treatment on day 14. In conclusion, the three tested PAPs can replace 7.5% SBM when included at 4-5% in growing pig diets without negative effects on growth performance and tail biting. The results did not substantiate the perception that aggressive behaviour (i.e. tail biting) is affected by the inclusion of PAPs in pig diets.

Growth performance of Large White × Landrace crossbred pigs fed diets containing Marula nut meal

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In sub-Saharan Africa, soybean meal (SBM) is a major dietary ingredient in pig feeds. However, soybean production fails to meet soybean meal (SBM) requirements for the pig feed industry. Availability of agro-industrial by-products have compelled pig farmers to use alternative protein sources to replace cereals in pig diets. By-products of industrial Marula processing such as marula (*Sclerocarya birrea caffra*) nut meal (MNM), are a potential protein resource, which could be included in traditional pig diets. An estimated 100 tons of MNM is produced per day. This study evaluated the growth performance of pigs fed diets containing degraded levels of Marula (*S. birrea* subsp. *caffra*) nut meal (MNM). Dietary treatments were control (commercial diet with no MNM, 50 (M5), 100 (M10) and 150 (M15) g/kg inclusion of MNM. These diets were formulated to provide 14 MJ/kg digestible energy (DE), 180 g crude protein (CP)/kg and 11.6 g lysine/kg. The diets were fed *ad libitum* to 32 Large White × Landrace crossbred grower pigs (25±2.3 kg body mass) that were individually housed in 1.54×0.8 m pens. Pigs were allocated to diets in complete randomized design. The experiment lasted 50 days during which average daily feed intake (ADFI) and average daily gains (ADG) were recorded. Pigs fed the control diet had higher ($P<0.001$) final weight, ADG and lower ($P<0.001$) ADFI and feed conversion ratio (FCR) compared to pigs fed diets containing MNM. It was concluded that dietary inclusion of MNM at >100 g/kg reduced the growth performance of pigs. Further work that determines the digestibility of these diets in pigs is warranted.

Effects of dietary inclusion of *Spirulina platensis* on the reproductive performance of female minks

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The objective of the study was to assess the impact of administering Spirulina (*Spirulina platensis*), a nutrient rich feed supplement, on female minks during periods of high metabolic demand (mating, gestation, lactation). The study was carried out in a commercial mink farm in Greece. A total of 100 adult brown female minks (Mustela vison), individually housed in standard cages, were randomly and equally allocated to a Control group (C), where minks were fed the basic diet and a Spirulina group (Sp), where the basic diet was supplemented with 100 mg of Spirulina/kg of body weight. The experimental period lasted 5 months, from 1 month prior to mating to kit weaning. Dams were weighed monthly and their reproductive performance was recorded. Data was analysed using SPSS v25. Weight gain during the first month was higher in Sp compared to C ($P<0.001$). Females in the Sp group remained heavier ($P<0.001$) until the onset of lactation (month 4 of the experiment). Subsequently, they lost more weight than those in group C ($P<0.001$) but without an adverse effect on kit survival. A tendency for a higher whelping rate was detected in the Sp group (93.61%) compared to C (81.25%) ($P=0.07$). Litter size did not differ ($P>0.10$) between groups which at birth, day 10 post-partum and weaning was 7.41, 5.48 and 5.10 and 6.95, 5.36 and 4.88 for groups C and Sp, respectively. Results obtained here showed a beneficial anabolic effect of the dietary inclusion of spirulina on female minks as evidenced by the increase in their whelping rate. The consequent increased total number of weaned animals in the Sp group is also promising and warrants further research on the level of spirulina dietary inclusion and other nutrient co-supplementation.

Proposition for a concept to exploit alfalfa as protein source for monogastric animals

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Alfalfa is a local and, when grown in the European Union, a GMO-free forage plant, which provides various ecosystem services. In temperate climates, alfalfa has the potential to produce high yields of crude protein (CP) and dry matter per hectare, while hot air drying is an established procedure to preserve nutrients and ensure storage stability. During the 2019 harvest season a wide range of samples (n=236) from different locations were collected at a commercial drying plant in Northern Bavaria, Germany. Samples were taken from freshly harvested alfalfa and after processing (hot air drying, fractionation and pelleting), including three different cuts and different vegetation stages. Feed value including *in vitro* analysis of total tract digestibility (ttd) was analysed. Samples revealed a large variation in the feed value depending on cut, vegetation stage and type of processing. The content of CP, lysine, methionine, and *in vitro* ttd of CP and organic matter (OM) were highest in the samples of the third cut and in the fine fraction after sieving. CP ranged from 108 to 343 g/kg DM, lysine from 3.7 to 10.7 g/kg DM, methionine from 1.1 to 3.6 g/kg DM, *in vitro* ttd of CP from 58.0 to 89.9% and OM from 34.0 to 67.8%. So far, alfalfa-based materials are not sold together with an indication of nutrient values. In contrast, it is common practice to specify minimum levels and mix qualities, and thus ‘diluting’ batches with higher nutrient values which would fit the requirements of younger monogastric animals. Therefore, alfalfa remains under-utilised because different farm animals cannot be fed effectively with material that does not meet specifications. In order to rectify this situation, we propose a system of nutritional categories to differentiate the wide range of alfalfa qualities and relate these categories to the nutritional requirements of different species and development stages. By such an approach, the different quality grades of alfalfa could be made available for different species and utilisations. Long-term effects could be the promotion of alfalfa cultivation in Europe and an increasing focus on protein content and quality rather than dry matter yield.

A review on how local amino acid solutions can support the achievement of the EU Green Deal targets

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Many of the EU Green Deal targets are relevant for the EU livestock industry and linked to the sustainability of livestock protein nutrition in its broad sense. Reducing dietary crude protein (CP) level is recognized to improve sustainability of poultry and swine production. Reduction of dietary CP typically and gradually replaces protein-rich ingredients, such as soybean meal, by cereals and feed -grade amino acids. Appropriate amino acid supplementation allows to maintain optimal growth, feed intake, feed efficiency, as well as meat quality & yields. The dietary changes impact the animal metabolism, lowering excretion of nitrogen, especially excess nitrogen in urea or uric acid form, improving nitrogen efficiency, reducing water intake and excretion. Nitrogen emissions from manure, such as ammonia, nitrates and nitrous oxide, are responsible for a wide range of environmental impacts: global warming, acidification, eutrophication, air and water pollution, all of them part of the EU Green Deal objectives. By reducing nitrogen excretion, low CP diets reduce those emissions at the source. Reduction of dietary CP can reduce the carbon footprint of feed diets. It is mostly due to reduction of the Land-Use Change associated with soybean meal. This strategy also increases use of local feedstuffs, improving protein autonomy. Furthermore, reducing dietary CP lowers environmental impact of manure management, storage and spreading. Low CP diets and amino acids are an easy to implement, efficient solution to reduce environmental impacts of pig and poultry, acting at the source of emissions, both on feed and manure production. This review summarizes the current knowledge on mitigation of environmental impacts with low crude protein diets supplemented with amino acids in poultry and swine, its quantification and the biological mechanisms involved. It provides science-based, quantifiable and readily implementable solutions to contribute to the EU Green Deal objectives, from the perspective of the EU livestock industry as well as policy makers.

Ileal amino acids digestibility in organic protein feedstuffs for pigs

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The objective of this study was to determine the standardized ileal digestibility (SID) of crude protein (CP) and amino acids (AA) in a whole seed soya bean, a soybean meal (SBM), two forage peas grain (FP), two rapeseed meals (RM) and two sunflower meals (SM). A total of 8 male growing pigs (35 kg BW) individually fitted with ileorectal anastomosis were used into two parallel 5×5 Latin square designs with 5 diets and 5 periods each. A total of nine casein-corn starch based diets were prepared with 25% of the each protein sources (diets 2 to 9) or with casein as sole source of CP and AA (diet 1). On a 6th period, a N-free diet was used to measure the non-specific basal CP and AA endogenous losses. Within each collection period, ileal digesta were collected during three consecutive days after a 4-d adaptation period to the experimental diets. When compared to the whole seed soya bean, the SID of CP and AA was significantly higher in SBM (49.1 vs 82.8% for lysine). Within the FP sources, the SID of lysine varied from 71.5 to 84.4%. This variation was related to a difference in their fibre contents (15.2 vs 13.7% NDF) and in their antitrypsin factors activity (2,908 vs 1,895 TUI/g). In RM and SM sources, the SID of lysine were rather similar despite significant changes in NDF contents suggesting that others factors would affect the bioavailability of lysine for pigs. In SM, lysine has the lower SID compared to the others AA resulting in a very low amount of SID lysine (7.5 g/kg). This low availability of lysine could be related to high temperature during the oil extraction. The comparison of the AA SID values in organic rich protein sources to those available in the INRAE tables for non-organic feedstuffs showed some nutritional specificities especially for organic sources of SBM and FP that require to be addressed in furthers studies.

Metabolizable methionine balanced diets improved Lacaune dairy ewe performance

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The objective of this study was to determine the effect of supplementing 3 levels of metabolizable Met (MetDi as %PDI) on milk yield and composition in early lactation dairy ewes. Fifty-four multiparous Lacaune ewes (81.4±2.37 kg BW; 3.1±0.09 body condition) were used from the beginning of lactation. Ewes were fed *ad libitum* a 42:58 forage:concentrate total mixed ration formulated to meet nutrient requirements. Treatments were: control, 1.8 (CTR), 2.5 (Met25) and 2.7% (Met27) of MetDig/PDI. The rumen-protected Met (RPM; isopropyl ester of 2-hydroxy-4-methylthio butanoic acid; HMBi, Metasmart, Adisseo SAS, FR) was administered in the milking parlour mixed with 100 g/ewe/d concentrate offered in 2 equal portions during the a.m. and p.m. milkings. Ewes were divided into 9 balanced groups of 6 animals, according to BW and previous lactation milk yield, and milked twice daily. Treatments were randomly applied to 3 groups per treatment from lambing to d 70 in lactation. Intake was measured during wk 3, 6, and 10 using an indigestible marker (50 g/d PEG6000). Milk yield and composition was measured in 2 consecutive days within sampling weeks. Data were analysed using the PROC MIXED procedure of SAS. No differences ($P<0.10$) were observed in intake (2.89±0.19 kg DM/d), milk production (2.53±0.31 kg/d) and fat content (5.33±0.23%), although there were quantitatively relevant differences in milk yield: (2.32, 2.60 and 2.68 kg/d for CTR, Met25 and Met27, respectively). Milk protein content was 4.94, 5.18 and 5.06% ($P<0.07$) and milk urea was 464, 426, and 476 mg/l ($P<0.06$) for CTR, Met25 and Met27, respectively. The significant treatment × week interactions in fat content reflected a reduction in fat content as Met intake increased only in week 10 ($P<0.05$); and that in protein content reflected a higher level in Met25 in wk 6 and 10 ($P<0.05$). The increased supply of Met improved milk protein content. Therefore, dietary MetDi recommendation in dairy cows (2.5% metabolizable protein) may be extended to dairy ewes.

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Nutritive value and palatability of microalgae species in dog feeding

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Pets, especially cats and dogs, are kept as companion animals in most of households in developed countries, and, as a result of the continuous growth of the global population, their number is increasing worldwide. This increase has been accompanied by a growing concern about the quality and sustainability of pet food. Microalgae are highly efficient in converting inorganic or organic carbon sources into biomass rich in protein, lipids, and bioactive compounds. Studies that evaluated the use of microalgae in pet food are almost non-existent. Therefore, the present study evaluated the nutritive value and palatability of three microalgae species (*Nannochloropsis oceanica*, Chlorella vulgaris, and *Tetradesmus obliquus*) offered to adult Beagle dogs at different levels (0.5, 1.0 and 1.5% in substitution of the basal diet). Proximate composition, amino acids, fatty acids and minerals profile, and antioxidant activity confirmed microalgae as valuable sources of macro and micronutrients with functional properties. The inclusion of *N. oceanica* and *T. obliquus* up to 1.5% did not affect diet digestibility and metabolizable energy (ME) content whereas inclusion of *Chlorella vulgaris* linearly increased diet organic matter and energy digestibility and ME content. Inclusion of 1.5% microalgae did not affect first approach and choice, but decreased intake ratio of diets with *N. oceanica* and *C. vulgaris*, no differences being observed for *T. obliquus*. Overall, results suggest that microalgae have high nutritive value for dogs, but strategies to increase palatability should be envisaged. Financial support of Fundação para a Ciência e a Tecnologia (FCT) and Soja de Portugal to JGF (PD/BDE/150527/2019), of FCT to SCL (CEECIND/01620/2017) and MRGM (DL 57/2016 – Norma transitória) and LAQV (UIDB/50006/2020) are acknowledge.

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Quantification of unvalorised protein based on two methodologies evaluating protein self-sufficiency

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Today's high levels of milk productivity are based on energy and protein rich and stable diets. However, protein concentrates are scarcely produced on farm, and even at the regional level in the case of Europe, inducing a dependency of livestock systems towards imported protein rich feed. As a result of this, enhancing its protein autonomy, the ratio between the on-farm produced feed protein and the protein feed needs, is often put forward as a strategy for farmers to conciliate economic and environmental performances. A double methodology, based on available accountability data, was elaborated in the frame of the Interreg project AUTOPROT, to evaluate crude protein (CP) autonomy in dairy farming. It was tested on 205 conventional (CONV) and 12 organic (ORG) farms of the Great Region (France, Belgium, Luxembourg, Germany). The first methodology is based on the intake capacity, on a dry matter basis. The ingested proteins are then calculated by multiplying each feed quantity by its theoretical protein content. The second methodology is based on the proteins needs of the herd for maintenance, growth, gestation and milk production. The difference between the two protein quantities obtained through these two approaches; based on intake capacity or metabolic needs; allow us to estimate a quantity of protein that is ingested and not fully valorised, reflecting a lost, an inefficiency. For our sample, 'ingested' protein autonomy was of 67±14% (CONV) and 89±7% (ORG), while the results for the 'valorised' protein autonomy were lower; 54±25% (CONV) and 83±13% (ORG). Anyhow, the adjusted R^2 between the two methods is of 0.91. Protein losses resulted in 30±6% (CONV) and 39±5% (BIO) of the total ingested proteins and 444±152 kg CP/ha (CONV) and 354±131 kg CP/ha (BIO) at the farm surface level.

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Using cauliflower in dairy sheep feeding: effect on blood biochemical profile

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Due to the demand cauliflower market has suffered in recent years, European yield has increased significantly with subsequent oversupply. Therefore, it is necessary to seek strategies to reduce these leftovers, with their use in livestock feeding being an environmentally appropriate strategy. The aim of this work was to study the possible changes that the inclusion of cauliflower florets could cause on blood biochemical profile. Twenty dairy sheep were divided in 2 groups and one of them (CON group) was fed *ad libitum* with total mixed ration (50% forage – 50% concentrate), while the other received the same ration plus a daily supplement of 1.5 kg of fresh cauliflower florets (CAU group). The experiment lasted 42 days and blood samples were collected on days 1, 21 and 42 to analyse the biochemical profile in plasma and the acid-base status. Data were subjected to a repeated measures ANOVA using the lme4 R package. The animals of CAU group ate all the cauliflower offered and there was not difference ($P>0.05$) in total dry matter intake between groups (3.07 and 3.16 kg DM/sheep/day for CON and CAU groups, respectively). No differences were observed between the groups in the biochemical profile of the plasma or in the acid-base status, with the exception of the concentration of non-esterified fatty acids, which was higher in the CON group than in the CAU group (0.158 vs 0.105 mmol/l, respectively, $P=0.045$). Despite this, the values obtained were within the normal range for these parameters reported in the literature for sheep under similar physiological conditions. This study shows that cauliflower flowers can be used in dairy sheep feed without detrimental effects on animals' health.

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Cauliflower as a by-product in dairy sheep feeding: effect on milk production and composition

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Fruit and vegetable sector in Spain produces a massive amount of agro-industrial wastes with a high contamination potential. The use of such by-products in animal feeding would contribute to minimize their environmental impact, as well as to reduce the livestock production costs. The aim of the present study was to evaluate the effect of the inclusion of cauliflower in the diets of lactating ewes on milk production and composition. Twenty Assaf sheep in the second third of lactation were used (two groups of 10 animals each), which received *ad libitum* a standard total mixed diet (CON group) or supplemented with 1.5 kg of cauliflower (CAU group) for six weeks (three weeks of adaptation and three of sampling). During the sampling period, daily food consumption and milk production were recorded, and samples of milk were taken for analysis of their composition. For the statistical analysis, an ANOVA of repeated measures was performed using the lme4 R package. The animals of CAU group ate all the by-product offered and there was not difference ($P>0.05$) in total dry matter intake between groups (3.07±0.83 and 3.16±0.61 kg DM/ewe/day for CON and CAU groups, respectively). No differences were observed ($P>0.05$) between groups in milk yield (1.84±0.55 and 1.75±0.66 kg/ewe/day for CON and CAU group, respectively), milk composition and somatic cells counts. The results suggest that cauliflower by-products could be used in the feeding of dairy sheep without negative effects on animal performance and milk composition.

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Nitrogen discharge reduction in fattening cattle by a dietary blend of essential oils and spices

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In 2020 European governments committed to produce more sustainable agricultural goods and animal products. Dairy and beef productions are particularly concerned as sources of nitrogenous waste and greenhouse gases. Improving feed nutrients utilization by cattle is a way to meet animal production and consumers' expectations being reflected by Green Deal. The objective of the present study was to evaluate the efficacy of N utilization in fattening beef cattle fed a blend of essential oils and spices compared to a control fattening diet. One thousand one hundred and eighty-five Angus steers were enrolled in this study and housed in a commercial feedlot where they were paired by age, conformation and live weights on their arrival. Each group of the 4 pairs were assigned to 1 of 2 treatments diets: (1) control TMR (control=569 steers); or (2) control TMR with sensory feed ingredients (Phodé, Terssac, France) (SFI=616 steers). Animals were fed their experimental diets for 189 days up to culling target weights. Individual live weights were recorded at the beginning and the end of the period. Feed intake was measured daily. N efficiency was evaluated by N balance between N feed intake and N retention estimated from live weight gain. The 4 pairs thus formed were compared by a paired test of Student. The average live weight at the beginning of the study period was not different between SFI and control group: 410±35 vs 388±20 kg ($P>0.180$). During the study period, FI was significantly lower in the SFI group than in the control one: 3.71 vs 3.84% LW respectively ($P<0.002$). However DWG was not modified: 1.393 vs 1.395 kg/d ($P>0.427$). As a consequence, FCR was significantly improved by SFI: 7.80 vs 8.10 ($P<0.05$). The N balance analysis exhibited a significantly better N retention by SFI animals: 21.31 vs 20.41% ($P<0.05$) and lower N losses: 6.02 vs 6.34 kg of N for 100 kg live weight gain ($P<0.05$). These results strongly suggest that the SFI blend contributed to a better environment preservation by improving Nutrient efficiency and by the way reducing N waste. Further investigations have to be conducted to analyse the effect of this SFI blend on phosphorus efficiency and enteric CH_4 production.

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Rumen protected lysine improved serum lysine status in Fleckvieh bulls fed a nitrogen deficient diet

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We conducted a feeding trial to test the effects of rumen protected (RP) lysine (Lys) and methionine (Met) under the condition of dietary nitrogen deficiency. The feeding trial comprised 67 German Fleckvieh bulls with an average age of 156 days and 223 kg weight on d0 of the experiment. The bulls were randomly allocated to 3 different diets: CON was sufficient in crude protein (CP) (14% CP of DM), MET was reduced in CP (11% CP of DM) and supplemented with RP Met (0.11% of DM) and MET+LYS was also reduced in CP (11% CP of DM) and supplemented with 0.11% RP Met + 0.42% of RP Lys. All diets were isoenergetic (11.64 MJ ME/kg DM). Feed consumption was measured daily for the individual animal. From d63 onwards, a balanced number of bulls were slaughtered from each group at weekly intervals until d119. Blood samples were collected from the jugular vein at each slaughtering date. AA in blood serum were analysed by LC-ESI-MS/MS(MRM). Data analysis comprised 3-way ANOVA (treatment group (GRP), days in experiment (DAYS), LW at experimental start, GRP×DAYS). Warm carcass weight was higher ($P<0.01$) in CON compared to MET and MET+LYS and higher ($P=0.01$) in MET+LYS compared to MET. Serum Lys concentrations were comparable between CON and MET+LYS (CON: 259 µmol/l; MET+LYS: 249 µmol/l) but significantly reduced in MET (196 µmol/l; $P=0.01$). Met concentration in serum of MET+LYS bulls was significantly higher compared to MET bulls (62.3 vs 50.0 µmol/l; $P<0.01$). In conclusion, the feeding of RP Lys improved the serum Lys status of MET+LYS bulls compared to MET bulls, presumably due to an increased Lys availability at the duodenum. This was associated with a mitigation of nitrogen deficiency-associated performance losses.

A novel ammonia treatment of barley to optimize rumen function in sheep

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Meeting the energy and nitrogen requirements of high-performing ruminants while avoiding digestive disturbances (i.e. rumen acidosis) is a key priority in ruminant nutrition. This study evaluated the effect of a cereal ammoniation treatment, in which barley grains are combined with urea and enzymes that catalyse the conversion of urea to ammonia to optimize rumen function. Twelve rumen cannulated sheep were randomly divided in two groups and fed a diet containing 60% of ammoniated barley (AMM), or conventional barley supplemented with urea (CTL) to study the impact on rumen fermentation and feed utilization. Ammoniated barley had higher total N content and effective rumen degradable N fraction than conventional barely. AMM sheep had a consistently higher rumen pH throughout the day (6.31 vs 6.03) and tended to have a lower post-prandial ammonia peak and higher acetate molar proportion (+5.1%) than CTL sheep. The rumen environment in AMM sheep favoured the colonization and utilization of agro-industrial by-products (i.e. orange pulp) by the rumen microbes leading to a higher feed degradability. AMM sheep also had higher total tract apparent N digestibility (+21.7%) and urinary excretion of purine derivatives (+34%) suggesting higher N uptake and microbial protein synthesis than CTL sheep. The inclusion of ammoniated barley in ruminants' diet represents a valid strategy to maintain rumen pH within a physiological range and to improve N utilization by the rumen microbes which could have positive effects on animal´s health and productivity in intensive production systems. These findings warrant further studies under conventional farm conditions.

Inclusion of olive cake in the diet of Rusitec fermenters: effect on archaeal community

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By-products are an important waste in agro-food industry and they could represent an environmental problem in many countries, being difficult to eliminate, in most of the cases. The inclusion of these by-products in the diets for ruminants in substitution of commonly offered feedstuff could have both environmental and economic benefits, but the possible effects on ruminal microbes must be evaluated. Olive cake results from the extraction of oil and has bioactive compounds that might influence microbial communities present in the rumen. The aim of this study was to assess the changes in the archaeal community of the rumen promoted by the inclusion olive cake in a diet incubated in Rusitec fermenters. Two different diets were formulated, a control diet (CON) representative of those offered to dairy sheep (50% forage), and a diet where corn silage and barley straw were partially replaced by olive cake (16.7% of olive cake; OC). Four Rusitec fermenters were used in a cross-over design with two 14-days incubation periods and solid and liquid digesta were sampled at the end of each experimental period. DNA was extracted and archaeal community was assessed by high throughput sequencing. The number of archaeal OTU observed was lower for OC than for CON diet (17.4 vs 10.8, $P<0.05$) and the Shannon index tended to be lower for the OC diet (1.91 vs 1.65; $P=0.08$). When analysing beta diversity using Bray-Curtis distance, OC samples appear to be represented more disperse than CON samples that were closer to each other. There were detected 20 different archaeal OTU across the full set of samples. Three OTU (2 *Methanobrevibacte*r and 1 *Methanosphaera*) were more abundant in CON diet and 2 OTU (1 *Methanobrevibacter* and 1 *Methanomethylophilaceae*) were more abundant in OC diet. Under the conditions of this study, the inclusion of olive cake in the diet replacing part of the forage affected the structure of the rumen archaeal community of Rusitec fermenters.

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Effects of replacement of soybean meal with peas on fatty acid composition in growing turkeys

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The aim of this study was to investigate the effects of replacement of soybean meal (SBM) with an increasing amount of peas (from 10 to 40%) in turkey diets on muscle fatty acid s composition. Ninety-six turkeys were allotted into Control and 3 Experimental groups of twenty-four birds each. The Control diet contained 26.5-43.6% SBM, whereas in the experimental diets SBM was replaced by peas, the amount of which increased depending on the age of the birds: Experimental group 1 (EP1) – 10% at the age of 0 to 4 weeks, 15% at the age 5-8 weeks and 20% at the age 9-12 weeks; Experimental group 2 (EP2) – 15, 20, and 25% respectively; Experimental group 3 (EP3) – 20, 25, and 30% respectively. From the age of 13 weeks SBM was completely replaced by 40% of peas in the diets of all experimental groups (EG). The results of the study did not show any significant differences in the fatty acid composition between the EG with slightly different amounts of peas in their diets, but some differences were found in comparison with the control SBM diet. Thigh muscles of EG, contained lower amounts of harmful fatty acids Myristic, Palmitic and Margarine respectively 1.28-1.52 ($P<0.05$), 1.05-1.22 ($P>0.05$-$P<0.05$) and 1.36-2.05 ($P>0.05$-$P<0.05$) compared to Control group. The content of Oleic fatty acid was found to be 1.03-1.18 times ($P>0.05$-$P<0.05$) higher than in Control group. The study also indicated that the diets with peas had different effects depending on the sex of the birds. In the EG, 1.21 to 1.24 times ($P<0.05$) lower content of Oleic fatty acid was detected in the breast muscles of male turkeys, and in contrast, female breast muscles, contained 1.16 to 1.19 ($P<0.05$) times more Oleic acid. Similarly, the content of Eicatosriene fatty acid was 1.45 to 1.7 times ($P>0.05$-$P<0.05$) higher in male and 1.39 to 1.68 times ($P>0.05$- $P<0.05$) lower in female turkeys. Also, a decrease in the content of Docosahexaenoic fatty acid ($P<0.05$) was found compared to Control. In summary, it can be concluded that replacement of SBM with peas in turkey diets had no negative influence on the muscle fatty acids composition and can reduce the content of some fatty acids harmful to human health.

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Interaction between season and feeding level or amino acid supplies on pigs' growth performance

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Growth performance of pigs obtained with three feeding strategies were compared. Strategies AL_A, AL_B and RA_A differed by the feeding level (AL: *ad libitum*, RA: restricted) and the 3-phase feeding sequence that met amino acid requirements or not (subscripts A and B, respectively). Two batches (b1 and b2), each with 4 pens of 6 gilts or barrows per treatment, were used. Batch 1 was studied in winter and b2 in summer with the outdoor temperature being on average 10 °C higher so that it was not possible to keep the indoor condition at the thermoneutral level and the daily feed intake (DFI) of AL_A decreased by 20% (AL_A/b1: 2.12 vs AL_A/b2: 2.49 kg/d, $P<0.05$) over the 26-105 kg body weight (BW) range. With reference to $AL_{A/}$b1, the similar decrease in DFI due to feed restriction (RA_A/b1: 2.13 kg/d) or to season (AL_A/b2: 2.12 kg/d) was associated to a similar decrease in average daily gain (ADG, 1,034^a, 866^b and 888^b d in AL_A/b1, RA_A/b1 and AL_A/b2, respectively). The additional decrease in DFI due to cumulated effect of feed restriction and ambient condition (RA_A/b2: 1.79 kg/d) induced a marked drop in ADG (698 g/d). Subsequently, feed restriction did not affect the feed conversion ratio (FCR) in b1 (2.46 vs 2.40), but increased it in b2 (2.56 vs 2.39, $P<0.05$). Sequence B did not affect DFI ($P>0.05$, AL_B/b1: 2.42 vs AL_A/b1: 2.38 and AL_B/b2: 2.13 vs AL_A/b2: 2.00 kg/d) but decreased ADG, resulting in a higher FCR ($P<0.05$, 2.55 vs 2.40 in b1, and 2.54 vs 2.39 in b2) especially in gilts (sex interaction: $P<0.01$). Carcass leanness assessed from backfat and muscle thicknesses was 1.5 percentage point higher in RA_A than in AL_A (62.4 vs 60.9, $P>0.01$) on average in both batches, with a larger difference in gilts, especially under warm conditions (b2). This study demonstrated that both FCR and carcass leanness were improved by a more adequate AA nutrition but that only carcass leanness was improved by feed restriction. It may be due to a different partition of energy intake between different functions (growth vs activity) and could motivate a more precise grading of the entire carcass to assess more accurately consequences of feeding strategy and ambient conditions on the composition of BW gain and final carcass value.

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In vitro cell oxidative protection, improved fermentation and *Salmonella* mitigation via a postbiotic

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Intestinal modes of action of a soluble postbiotic (SP) for pre-weaned calves, manufactured from *Saccharomyces cerevisiae* fermentation (Dia-V™ SC), were assessed by laboratory models. An epithelial cell-culture model compared the addition of SP (500 mg/l in media) to a Control (media only). Trans-epithelial electrical resistance (TEER, 12 replicates) and localization of a tight junction protein, zonula occludens (ZO-1, 2 replicates), were determined in a monolayer of intestinal porcine enterocytes (IPEC-J2), before and after an induced oxidative stress by H_2O_2 (1 mM). Control cells exposed to peroxide for 1 h decreased TEER by 56% ($P<0.05$) and confirmed the stress effect. Cells pre-treated with SP had 50% higher resistance ($P<0.05$) and retained more ZO-1 membrane localization than Control cells after oxidative stress. Separately, an anaerobic intestinal model (IAMM, Diamond V SOP RIC-Lab-0008) was performed using a buffered faecal inoculum from 1-d old calves. Calf milk replacer substrate (150 mg) was incubated for 24 h alone (Control) or with 5, 12.5, 25 or 50 mg of SP in 5 replicates, all challenged with *Salmonella* Typhimurium (target 3.5 log cfu/ml). Diluted aliquots were analysed for volatile fatty acids (VFA), pH and *Salmonella* cfu. Regression analysis of each parameter to level of SP (0, 5, 12.5, 25 and 50 mg) fit for linear response ($P\leq0.001$) and generated a dose response correlation for VFA in mM (Acetate: 22.53, 22.37, 22.86, 23.75, 25.02; Propionate: 8.63, 8.80, 9.10, 9.64, 10.3; Butyrate: 4.21, 4.24, 4.28, 4.42, 4.64; Total: 36.51, 36.64, 37.30, 38.85, 41.19), pH (7.13, 7.11, 7.09, 7.07, 7.02) and *Salmonella* log cfu/ml (3.68, 3.71, 3.28, 3.17, 2.94). Results indicate that SP protected the epithelial cell layer after an oxidative stress challenge, measured by increased resistance and membrane localization of ZO-1 protein. Inclusion of SP linearly increased production of individual and total VFA, reduced pH and *Salmonella* Typhimurium numbers. SP addition to liquid feeding of pre-weaned calves may be enhancing the barrier function of the intestinal epithelial lining and protecting it from oxidative damage, as well as improving VFA production and limiting pathogenic *Salmonella* growth.

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Calculation of digestibility parameters in laying hens using NIR on faecal samples

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NIR analysis is a non-destructive method that allows simultaneous measurement of several components (e.g. dry matter (dm) and nitrogen). Results of NIR analysis can be used to calculate digestibility of different components and to understand differences in feed efficiency between animals and populations. In poultry, no studies are available using NIR on faecal samples for laying hens. The aim of this study is to use NIR results to calculate digestibility parameters in laying hens by using NIR on faecal samples and to investigate differences in digestibility between two lines that differ genetically and phenotypically. Faecal samples from 100 laying hens from two purebred white Leghorn layer lines (50 line A and 50 line B) were collected for four consecutive days and two times per day (morning and afternoon, last day only morning), resulting in 7 faecal samples per hen. From each sample 20-25% was combined in a pooled faecal sample per laying hen. In total, there were 100 pooled faecal samples which were used in the NIR analysis. In addition, performance data of the laying hens during the whole sampling period were collected and new traits were defined. The statistical analyses were carried out in the R environment. For the performance traits, hens in line A had a significant ($P<0.05$) higher daily feed consumed, a higher residual feed consumption, lower number of eggs laid, lower daily manure and lower feed efficiency compared to hens in line B. For the NIR traits, hens in line B had a significantly ($P<0.05$) higher dm in fresh manure and a higher dm in airdried manure compared to hens in line A. First results show that hens in line B are more efficient in producing eggs from 1 kg of feed compared to laying hens in line A. Further research will focus on: (1) digestibility for each NIR component; (2) chemical analyses to validate NIR results and to investigate the use of NIR results in the calculation of digestibility parameters in laying hens; and (3) the use of digestibility components as a prediction for feed efficiency. This study will help to understand digestibility in laying hens and will help to improve feed efficiency.

Faecal near-infrared spectral data as a tool to predict digestibility in pigs

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Increasing production efficiency in livestock is important due to population growth, competition with land use to produce food and biofuel, and to decrease the environmental impact of livestock production. Breeding more feed-efficient animals using feed efficiency (FE) related traits would be beneficial to increase production efficiency and decrease nitrogen (N) excretion to the environment. In this study, our hypothesis was that near-infrared spectroscopy (NIRS) of faeces can be used to predict apparent total-tract digestibility (ATTD) of N, dry matter (DM), organic matter (OM), and crude fat (CF) in Duroc pigs. The data materials were collected at Topigs Norsvin's boar testing station in Norway between the years 2019 and 2020. Faecal samples were sampled from each animal one time during the test, in pens where the animals were kept individually for 24 h. Total nitrogen content, dry matter, organic matter, crude fat, and acid insoluble ash (AIA) were measured on both feed and faeces for NIRS calibration. The number of records with both a phenotypic value and NIR was 1000 on 298 to 878 boars depending on the trait. Partial Least Squares (PLS) regression analysis was used to relate faeces NIR to ATTD of N, DM, OM, and CF using one-at-a-time cross-validation and 5-fold external validation. The number of fitted factors in the PLS varied between 2 and 18. The proportion of the variability explained by faeces NIR for ATTD of nitrogen, dry matter, organic matter, and crude fat was 0.84, 0.87, 0.87, and 0.44, respectively. Faeces chemical analysis of crude fat was done without acid-hydrolysis which explains the poor prediction accuracy of crude fat. Our results show that NIRS of faeces is an effective tool to predict ATTD of nitrogen, dry matter, and organic matter in Duroc pigs.

Digestibility and palatability of algal blend in dogs

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Increasing concern of the pet food sustainability and tutors desire to improve their companion animals' health has leverage the quest for functional foods for pets. In this sense, macroalgae and microalgae emerged as alternative ingredients in pet food. Alongside with the low carbon and water footprint, algae are rich in nutrient and bioactive compounds reported to have health-promoting effects, as antioxidant, immunomodulatory, prebiotic and antidiabetic properties. Brown macroalgae, particularly *Ascophyllum*, and microalgae, mainly *Chlorella* and *Arthrospira*, species are currently used in commercial complete diets to improve technological properties, palatability, oral health and functionality of dog foods. However, few studies have evaluated the effects of algae in dog foods and none has yet evaluated the combination of macro and microalgae species as alternative food ingredient. Therefore, this study aimed to evaluate the nutritive value of a commercial algae blend composed of two macroalgae (*Fucus* sp. and *Ulva* sp.) and one microalgae (*Chlorella* sp.) species (Algaessence feed™; ALGAplus/Allmicroalgae, Portugal) offered at 0.5, 1.0 and 1.5% dry matter basis in replacement of the complete diet to adult Beagle dogs. Palatability was assessed using a two-plate test, comparing 0 and 1.5% algae inclusion. Algae blend improved the crude protein digestibility of the diet and tended to increase dry matter and organic matter digestibility. First choice and approach were not affected by 1.5% algae blend supplementation, but consumption ratio was lower for the diet with algae blend. Proximate composition, amino acids, fatty acids and minerals profile, and nutrient digestibility suggest the high nutritive value and bioactive composition of algae blend for dog nutrition, however low palatability must be addressed. Financial support of Fundação para a Ciência e a Tecnologia (FCT), ALGAplus and Allmicroalgae to CSCM (PD/BDE/150585/2020), of FCT to MRGM (DL 57/2016 – Norma transitória) and LAQV (UIDB/50006/2020) are acknowledge.

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Digestibility of grass fibre increased by screw pressing in a bio-refinery

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In this study, the effects of screw-pressing prior to ensiling and days of regrowth of perennial ryegrass on fibre digestibility were investigated. Six rumen and duodenal fistulated, multiparous Holstein cows (176±93 days in milk; mean ± SD) were used in an incomplete 6×4 Latin square design with four periods and a 2×3 factorial arrangement of treatments. The factors were developmental stage of grass at harvest [early (ERL; 35 days of regrowth) and late (LAT; 44 days of regrowth)] and processing prior to ensiling [traditional grass silage (GS; mowed, wilted, and chopped), pulp pressed once (1×P; fresh grass processed using twin-screw press producing the two fractions pulp and green juice), and pulp pressed twice (2×P; half of 1×P was soaked in water and processed a second time)]. Grass of all six treatments ensiled for four months before the digestibility experiment started. A total mixed ration with experimental silages, soybean meal, rolled wheat, and minerals (65.0, 6.60, 26.6, and 1.80% of DM, respectively) was fed in periods of 21 days duration. Two markers (titanium dioxide and chromic oxide) were dosed in the rumen twice a day to determine flow of digesta in duodenum and output in faeces. A model including developmental stage, processing, developmental stage × processing, and period as fixed effects, and cow as random effect was analysed in R. Across developmental stage, DM intake was lower (14.9 vs 17.5 kg/d; $P<0.01$) and NDF intake was higher (7.07 vs 5.92 kg/d; $P<0.01$) for 2×P compared to GS. For rumen and total tract NDF digestibility, an interaction was found between developmental stage and processing ($P<0.05$). No effect of processing was observed for ERL (average rumen and total tract NDF digestibility were 73.3 and 76.5%), but an increase was observed with increased processing intensity for LAT (66.2, 71.5, and 76.6% for rumen, and 71.6, 73.8, and 78.3% for total tract NDF digestibility, for GS, 1×P, and 2×P, respectively). Across developmental stage, the amount of NDF digested in the total tract was higher for 2×P compared to GS (5.47 vs 4.36 kg/d; $P<0.01$). The study showed that processing can increase intake of fibre and that processing has the greatest effect on fibre digestibility in more developed grass.

Session 32 Theatre 1

Improving pork quality by means of breeding – a Swiss perspective

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It started with a disaster. The successful breeding for lean, heavy muscled carcasses led to high frequencies of a mutation in a major gene with pleiotropic effects: beside increased muscle growth and improved feed conversion it caused the porcine stress syndrome along with enormous economic losses and severely impaired meat quality. The action taken to fight the defect of this pale, soft, and exudative (PSE) meat may be seen as the origin of the strong focus on meat quality in the Swiss pig breeding program. The unfavourable mutation was consequently eliminated in Swiss pig breeds, but several traits, originally used as indicators of PSE, like early post mortem pH, meat colour, driploss, and intramuscular fat content (IMF), are still recorded in the central performance test and in part considered in the estimation of breeding values. This led to a decrease in driploss and increased IMF while at the same time carcass fat was reduced. To date, mean values close to optimum have been reached for these traits. This opened the opportunity to further improve pork quality by introducing new meat quality traits. Shear force as an indicator of tenderness and cook loss as a relevant additional marker of water holding capacity showed promising heritabilities and were recently included in the Swiss breeding scheme. Further attention, however, should be turned to ultimate pH, which is expected to correlate with meat quality characteristics and shows an undesirably decreasing trend. Quality problems also were a driver in another sector of pork quality. Soft pig fat was a huge problem for the meat processing industry in Switzerland, until the fat score was introduced as part of the payment system for pig carcasses in the late 80ies. As the body fat composition largely depends on the fat composition of the feed, it was the feed industry which had to cope with this problem in the first instance. However, it was shown that the fat composition also has a strong genetic compound, with medium to high heritabilities for the proportion of mono- and polyunsaturated fatty acids in the backfat. These fat quality traits are currently measured in the central performance test and breeding values can be estimated. This can be particularly useful for the selection of sires for organic pig production were the use of various feedstuffs is restricted.

Combining genetic and nutritional strategies improve the intrinsic and extrinsic qualities of pork

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To develop pig production practices that aim to improve the intrinsic (sensory, nutritional) and extrinsic qualities of pork (relocation of feed resources), we evaluated the effects of genetic and nutritional factors on growth performance and on carcass and pork quality. A total of 60 female pigs from two genotypes [LW × LD] × Piétrain NN (P, non-carrier of the n allele of the RYR1 (halothane) gene) or [LW × LD] × Duroc (D) were submitted to two different feeding regimen. Pigs were divided into 4 experimental groups (n=15/group) at 30 kg body weight (BW). The PC and DC groups received a control diet (C) including oilseed meal, while the PR and DR groups received a 'Roc+' (R) diet including extruded faba bean as major protein source and extruded linseed as source of n-3 fatty acids (FA). The animals were raised in individual pens and fed *ad libitum* from 30-115 kg BW, with nutritional intake (digestible lysine : net energy ratio) being adjusted weekly to the average requirements of pigs within each group. Growth rate, feed efficiency and slaughter BW were higher for D vs P pigs ($P<0.001$) and, to a lesser extent, for R vs C ($P<0.05$) pigs. D pigs exhibited lower carcass lean meat content, lower proportions of ham and loin and higher proportions of belly and backfat than P pigs ($P<0.01$), whereas carcass traits were not influenced by the diet. Loin meat (Longissimus muscle) from D pigs had lower drip loss, higher ultimate pH and higher intramuscular fat content ($P<0.05$), and was judged to be more marbled, tender and juicer by trained panellists ($P<0.01$) than that from P pigs. The diet did not influence the sensory traits but improved the nutritional value of pork by markedly reducing the n-6:n-3 and saturated:n-3 FA ratios ($P<0.001$) in both loin and backfat tissue. The combination of D genotype and R diet improves the sensory and nutritional properties of meat while contributing to the relocation of the production of feed resources, thus improving the intrinsic and extrinsic qualities of pork in a farm to fork strategy.

Multicriteria performance of fattening pigs assessed with the bioclimatic model ThermiPig

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The dynamic model ThermiPig was developed to simulate thermal balance at the fattening room scale, as the result of the heat produced by the group of pigs or provided by incoming air and equipment and that lost due to air renewal or thermal conductivity of the walls and ceiling. A growth model (assuming thermoneutral conditions, InraPorc) and a bioclimatic model (ThermiSim) were combined to create ThermiPig. It considers a common time step (1 hour), definition of the pig thermoneutral zone (based on body weight and type of floor), evaluation of impacts of cold and hot exposure on the amount of available energy for growth, and the circadian distribution of heat produced daily by each pig (based on meal partition over the day and post-prandial thermic effect of feed). Data collected *in vivo* from a group of 96 pigs of the IFIP experimental station were used to evaluate the accuracy of the prediction of ambient temperature (T). Characteristics of the fattening room, regulation rules of the climate box control of the ventilation system, nutritional characteristics of the 2-phase feeding sequence, and outdoor conditions (hourly T and relative hygrometry) observed during the *in vivo* trial were used as inputs of the model, and simulation were performed on 30 virtual groups of pigs from the same genders and crossbreed. With average *in silico* growth performance of the 30 groups similar to observed ones, the error of prediction (RMSEP) on ambient T averaged 0.6 °C. Thereafter, ThermiPig was used to simulate the impacts of alternative regulations for ventilation or the use of new equipment (heater, pad cooling) on growth performance of pigs, N excretion, and indirect energy (from feed intake) and direct energy (from electricity consumed by equipment) consumption. Comparison of multi-criteria performance *in silico* helps identify the most interesting option. Economic performance depends on carcass value minus costs of feed intake and electricity (depending on the energy source). Therefore, more accurate prediction of carcass value is expected in the next version of the model by considering effects of ambient temperature on the distribution of body fat and protein metabolism.

Effect of high ambient temperature on carcass composition of pigs slaughtered at the same age

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The study objective was to evaluate the effects of elevated temperature on carcass composition and the relative importance of each primal cut of pigs slaughtered at the same age (140 d). A sample of 48 females was reared for 60 days in two groups: one at thermoneutrality (TN, 18-24 °C) and the other in hot conditions (HS, 28-34 °C). *Ad libitum* access to a standard growing-finishing feed was provided. One day after the slaughter, half-carcasses were scanned by X-ray tomography to determine the tissue's contents, and then cut according to a standard commercial procedure. Rind content was assumed to be 4% whatever the treatment. Head and feet were considered as bone. HS decreased feed intake ($P<0.01$), resulting in lower growth ($P<0.01$), and final body weight (96.9 vs 104.8 kg; $P<0.001$). The decrease of 5.4 kg in carcass weight (73.8 vs 79.2 kg; $P<0.001$) affected the three main tissues. It was divided between tissues as follows: -2,3 kg of muscle (46.1 vs 48.4 kg; $P<0.01$), -2.1 kg of fat (11.9 vs 14.1 kg; $P<0.01$), and -0.7 kg of bone (12.7 vs 13.4 kg; $P<0.001$). This change in distribution resulted in a decreased fat content (-1.5 percent point; $P<0.05$), and an increased muscle (+1.2 percent point; $P=0.05$) and bone (+0.3 percent point; $P=0.09$) contents. Carcass fat content, backfat weight, and rump fat thickness decreased ($P<0.05$) by about 10, 12 and 18%, respectively. Ham percentage increased by +0.9% ($P<0.001$), but the loin percentage decreased by -0.7% ($P<0.05$); this divergent trend between two lean cuts might be associated with a change in conformation as seen in shorter HS carcasses (-2.4 cm; $P<0.01$). In summary, heat stress decreased live, carcass and cuts weights at the same age. The lower fatness, counterbalanced by a higher muscularity and bone content, could be explained by feed restriction.

Effect of restricted feeding or amino acid deficiency on the muscle content of pig carcases and cuts

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A sample of 288 pigs was used in a 3×2 factorial design based on 2 sexes (gilts, castrates) and 3 feeding strategies to quantify accurately the increase in muscle content due to restricted feeding (RF, 85% *ad libitum*) or decrease due to amino acid deficiency in a 3-phase sequence used *ad libitum* (AD: 0.74/0.66/0.56 vs Control and RF: 0.94/0.81/0.73 g of digestible lysine/MJ net energy). Two batches, one in winter and the other in summer, were used with four pens of six gilts or castrates per treatment each. The pigs were crossbred between Piétrain boars and Large White × Landrace sows and were heterozygous for the halothane gene. Half-carcases were scanned by computed tomography. The four primal joints, ham, shoulder, loin and belly, were also scanned for a subsample of 140 carcases. Least squares means of muscle contents were assessed by a general linear model; differences between treatment and control were tested by an unilateral Dunnett test. Treatment and gender had a significant effect on all variables. RF increased the muscle content of carcase by 3.5 percent points (68.5 vs 65.0; $P<0.001$). The increase ($P<0.001$) was higher in loin (+3.5) and belly (+3.7) than in ham (+2.2) and shoulder (+2.7). AD decreased the muscle content in all the cuts by 1.9 percent point ($P<0.001$) in ham, 2.0 ($P<0.001$) in shoulder, 2.3 ($P<0.01$) in belly, and 2.7 ($P<0.001$) in loin. In the carcase there was an interaction between treatment and batch. The decrease was significant ($P<0.01$) only in the summer batch (-2.6 percent points, 63.0 vs 65.6). RF and AD seem to cause more marked effects in the middle cuts than in ham and shoulder. The actual impact of restricted feeding or amino acid deficiency on the muscle content of carcases was about twice that is estimated by the current grading method. In order to get accurate estimates of effects on body composition the use of computed tomography should be promoted in zootechnical trials.

Muscle and backfat thickness in Bísaro pigs with different immunocastration protocols

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Real-time ultrasound (RTU) subcutaneous fat (SF) and *Longissimus thoracis et lumborum* muscle (LM) depths measurements were performed in 43 Bísaro male pigs (29 weeks old, LW 83.5±10.2 kg). Animals have been submitted to two different immunocastration protocols (Improvac®, Zoetis): IC1 (n=6) – two shots (at 9 and 13 weeks of age); and IC2 (n=11) – three shots (at 13, 17 and 19 weeks of age). The study also included a group of surgically castrated males (SC; n=12) and a control, age-matched boars (B; n=14). An Aloka 500 V scanner and a 5 MHz probe were used to capture RTU images. For image capture, animals were individually restrained in a crate. The probe was placed perpendicular to the last thoracic vertebra and a gel was used as a coupling medium. The RTU images were analysed to determine SF and LM depths using the Fiji software. To overcome variations in the SF thickness over the LM muscle, an average of three SF measurements were considered. No differences were found in LM depth among the four groups (3.26±0.36 cm; P>0.05). Regarding SF depths, no differences were found between the IC1 (1.44±0.11 cm), IC2 (1.72±0.08 cm) and SC (1.68±0.08 cm) groups whereas boars had a thinner layer of SF (1.04±0.07 cm) compared to all the other groups (P<0.001). These results support that compared to surgical castration immunocastration has no effect on the LM and SF depths. Thus, immunocastration provides a reliable substitute for surgical castration, safeguarding a significant income source for Bísaro pig farmers. This work was supported by the project Icas- Bísaro (reference n°. PDR 2020-101-031029) and the project UIDB/CVT/00772/2020 funded by the Fundação para a Ciência e Tecnologia.

Last supper – the effect of feed intake before slaughter on pork quality traits

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As part of the central performance test in the Swiss pig breeding program various meat quality traits are measured, including early *post mortem* and ultimate pH, drip and cook loss. These traits can be affected by the course of the post-mortal muscle metabolism, which in turn might be affected by various environmental factors. It was speculated that energy supply and thus feed intake during the period before slaughter might be such a factor, which could possibly be controlled by applying a standardized fasting period. In the Swiss performance testing station, pigs are kept in groups up to 12 animals and feed is provided *ad libitum*. The individual feed intake is recorded by two types of transponder-controlled feeders (FIRE pig feeder, Osborne Industries; Compident MLP II Station, Schauer Agrotonic AG). Feed intake during the last 6, 12, 18 or 24 hours before stunning (at 6:00 am) and meat quality data of 11,157 pigs slaughtered between Jan. 2017 and Jan. 2021 was used. Early *post mortem* and ultimate pH were measured 90 min and 24 h after stunning in *m. long. dorsi* (LD) and *m. semimembranosus* (SM) of all pigs, including Swiss Landrace (SL) and Large White (LW). Drip and cook loss were only measured in LD samples of sire lines (LW sire line, Duroc, Piétrain) and their 3-way-crosses with F1 sows (SL×LW). The generalized linear model included sex, breed, and type of feeder as fixed effects, slaughter date as random factor, and the feed intake during the specified periods as covariate. The analysis of variance indicated significant effects (P<0.05) of the feed intake on ultimate pH in the SM and on cook loss. However, the proportion of variance explained by feed intake never reached 0.5% while the coefficient of determination of the models ranged between 22-42% with slaughter date and breed as strongest factors. It is concluded that the feed intake pattern hardly affected the examined meat quality traits, indicating no need to consider feed intake shortly before slaughter in the breeding value estimations or to introduce a defined fasting period preceding slaughter. A short feed deprivation could nevertheless be recommended to reduce feed waste and enhance the valorisation of organs.

Effect of malted wheat and varying dietary phosphorus supply on bone mineral status of broilers

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We conducted a feeding trial to investigate the effect of malted and non-malted wheat in combination with varying dietary phosphorus (P) levels on bone mineral status of slowly growing broiler chickens. Four different diets were formulated for this trial containing either wheat or malted wheat in combination with and without P supplementation from 1.5% Monocalcium-phosphate (0.3 vs 0.4% P in diet), respectively. 200 1-day-old broiler chickens (Ranger Classic) were used for this experiment. From d1 to d16 the birds were fed with an organic starter diet. On d17 the equal number of birds from both sexes were randomly allocated to the four experimental diets (5 pens per diet, 10 birds each) comprising a completely randomized design. On d57 birds were slaughtered and both tarsometatarsus bones of each bird were taken and pooled within each pen. P in bones was determined photometrical and calcium (Ca), magnesium (Mg) manganese (Mn), iron (Fe) copper (Cu) and zinc (Zn) using Inductively Coupled Plasma Mass Spectrometry (ICP-MS). Statistical analyses comprised two- way ANOVA (wheat variant, P-level and interaction). P supply had no significant influence on P and Mg status of bones. However, birds fed with wheat had significantly more P and Mg in bones ash ($P=0.003$; $P=0.012$), than birds fed with malted wheat. Furthermore, P supplementation reduced concentrations of Zn, Fe and Mn in bone ash ($P=0.044$; $P=0.004$; $P<0.001$). Since the P supply did not affect the mineral composition of bone nor the zootechnical response (data not shown), we propose the applied broiler genotype may not need mineral P addition under the present dietary conditions.

The quality and health-promoting value of meat from pigs of the Złotnicka Spotted breed

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The popularity of meat from animals of native breeds is growing all over the world due to the belief of consumers about the higher quality of such meat over meat from industrial farm pigs. In addition, the living conditions (welfare) are also of great importance for consumers. The aim of the study was to compare the meat quality from pigs of the native Złotnicka Spotted breed, fattened intensively or extensively (with conventional farm-produced compound feed and acorns). The study involved 13 fattening gilts of the Złotnicka Spotted breed, which were reared on two farms in different fattening systems. Upon reaching their designated age or body weight (depending on the type of fattening), all animals were slaughtered. After slaughter and 24-h chilling at +4 °C, a 100 g slice of meat was cut from the middle part of the m. longissimus dorsi muscle of each carcass, labelled and frozen at -18 °C until analyses. The fatty acid profile was determined after 2 weeks of freezing, immediately after thawing. We stated, that the meat of extensively fed free-range pigs fed on silage and small amounts of acorns, compared to the meat of pigs of the same breed from intensive fattening, is characterized by a higher content of fat, which acts as a carrier for flavour and, indirectly juiciness. The meat from the extensively fed pigs can be described as healthy, due to the significantly higher MUFA content ($P \leq 0.05$) and the low atherogenic and thrombogenic indices ($P \leq 0.05$), which show the potential for reducing the risk of atherosclerosis and cardiovascular diseases. Moreover, the significantly ($P \leq 0.05$) lower peroxidisability index (PI) shows the lower susceptibility of this meat to rancidity and thus to deterioration of organoleptic traits during storage, which is particularly valuable for cured meats and traditional long matured or dried products, for which the meat of the native breeds of pigs is recommended. The work financed from the National Research Institute of Animal Production (Task no. 01-11-04-11) and the Ministry of Science and Higher Education of the Republic of Poland (DS-3705/1/KPPZ/2020).

Health and performance data in professional Swiss meat rabbit production

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For animal welfare reasons, Swiss legislation prohibits housing on wire mesh floor and group housing in fattening units is mandatory. In addition, all farms of the two major producers in Switzerland provide ground covering litter. Despite these animal-friendly husbandry standards, animal losses appear to be a significant problem. Reliable data on professional Swiss meat rabbit production has been lacking until now. The aim of this study was to generate reliable health and performance data, as a first step towards improving health of Swiss meat rabbits. 52 farms of the two major producers with a total of 4,440 does and 49,160 fatting spaces participated in the study. For one year, each litter and each fattening group was documented by the farmers using a standardised form, with special interest in animal losses and medication. In case of increased mortality, deceased rabbits underwent necropsies and laboratory testing. The average pre-weaning mortality was 14.9% (range: 1.0-30.0%), post-weaning mortality reached 17.3% (range: 4.3-31.8%). Intestinal disorders represented the most common disease complex with 68.2%, these were mainly dysenteries, followed by coccidiosis and mucoid enteropathies. Manly due to *Eimeria* spp., *Escherichia coli* and *Clostridium perfringens*. Respiratory diseases were found in 18.7% of the rabbits examined, often in combination with the detection of *Pasteurella multocida*. 60.0% of the farms used antibiotics during the study period, 34.7% of all examined fattening groups were medicated. The results confirm that diseases and animal losses represent a considerable problem in the Swiss rabbit production. To improve rabbit health and thus reduce the mortality rates and the use of antibiotics, a further step should be to identify risk factors for increased morbidity and mortality to develop specific prevention strategies.

Genomic regions affecting body size related traits in two pig populations

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20 years ago, the Swiss Large White pig population was split into the Large White maternal line (LWM) and the Large White paternal line (LWP). Breeding objectives differ between the two lines, while selection decisions in both rely on genomic breeding values estimated with a single-step GBLUP-model. With the introduction of genomic selection, the number of genotyped individuals has markedly increased. These data build a valuable pool for the identification of genomic regions affecting relevant traits in modern pig populations. Therefore, we analysed 60k-genotypes of 6,273 individuals from LWM and 8,722 individuals from LWP and their de-regressed BLUP breeding values ignoring genomic information for totally 33 traits (6 reproduction-, 15 production- and 12 exterior-traits) in genome-wide association studies. For the three traits carcass length, number of teats and number of intermediate teats we observed a region on SSC7 highly significant in both breeds. The top SNP (SSC7, 97,652,632 bp) is in proximity of the *VRTN* gene which is known to be associated with vertebrae development. For carcass length we found a further significant region on SSC17 (top SNP at 15,659,761 bp, near bone morphogenetic protein *BMP2*) and for number of teats on SSC10 (top SNP at 47,805,800 bp, within *FRMD4A*) in LWM. This region on SSC10 was also significant for all three traits in LWP. After the completion of the study, we will likely be able to identify QTL that underpin the distinct breeding objectives of the two populations or could be exploited for the acceleration of selection response.

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Effect of swine immunocastration on salts and volatile compounds of Teruel dry-cured hams

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Two experiments were carried out to evaluate the effect of immunocastration (immunization against GnRH) on the quality of Teruel dry-cured hams, proceeding from (Landrace × Large White) × Duroc pigs slaughtered around 135 kg. In the first trial, 20 hams from entire and immunocastrated gilts (EG; IG) were compared (n=10) and, in the second one, 14 hams from surgically castrated and immunocastrated males (SCM; ICM) were tested (n=7). All pigs, carcasses and hams had received the same management at farm, slaughterhouse and cellar. Once hams were cured (19 months), concentrations of sodium chloride, potassium nitrate, sodium nitrite, α-tocopherol, γ-tocopherol, δ-tocopherol, retinol, cholesterol and volatile compounds were measured in the *Biceps femoris* muscle. Data were analysed using the GLM procedure of SAS. In the first trial, IG presented greater ($P<0.05$) sodium chloride and sodium nitrite concentrations than EG, being in all cases normal values for this kind of product. About volatile compounds, IG presented lower ($P<0.05$) proportion of alcohols and furans, having both groups little influence on ham flavour. However, the 1-octen-3-ol alcohol and the 2-pentylfuran furan were also lower ($P<0.05$) in IG, which could imply lower mushroom and rancid notes and lower pleasant fruit and flower scents. In the second trial, ICM had lower ($P<0.05$) potassium nitrate and retinol concentrations than SCM. Also, ICM showed lower ($P<0.05$) percentage of alcohols (including 1-octen-3-ol) and sulphur compounds than SCM. This last group plays an important role in meat flavour and causes an unpleasant strong odour. Besides, ICM showed higher ($P=0.012$) proportion of acids, which are associated with fatty and cheesy notes. It can be concluded that immunocastration produced hams with more salt and nitrites in gilts and less nitrates and retinol levels in males. Besides, immunocastration affected some volatile compounds, which could have some influence on ham flavour. Project funded by MINECO (AGL2016-78532-R) and by Gobierno de Aragón (FITE and FEDER).

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Effects of immunocastration protocols in the meat quality of female Bísaro pigs

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Different immunocastration protocols were tested to determine whether the moment of the first inoculation would impact meat quality. This study used twenty multiparous female Portuguese *Bísaro* pigs (carcass weight, CW, 161.8±29.8 kg), reared in similar conditions, which were selected for culling after weaning. As these females present a marked body condition loss, immunocastration was decided to avoid reproductive function during the post-weaning recovery of body condition. Three protocols were applied, each consisting of two inoculations (Improvac®, Zoetis) four weeks apart. Three protocols were tested that differed in the moment of the Improvac® first inoculation: at the onset of oestrus (BE; n=5; CW 158.5±25.8 kg); one week after the beginning of oestrus (OE; n=5; CW; 158.6±30.3 kg); and in the middle of the suckling period (SP; n=5; CW 167±38,2 kg). A control non-treated group was also included (C; n=5; CW 163.4±33.2 kg). Sows were slaughtered four weeks after the treatment, at a municipal abattoir. After slaughter, various meat quality traits were assessed in the *Longissimus thoracis et lumborum* muscle: pH, measured at 45 min and 24 h *post mortem*; drip loss; L*a*b* meat colour (measured in the fresh-cut and after 60 min of blooming); cooking loss; and shear force. There were no differences between treatments in any of the traits assessed ($P>0.05$), suggesting that neither protocol tested affected the sow meat quality. This work was supported by the project Icas- Bísaro (reference n°. PDR 2020-101-031029) and the project UIDB/CVT/00772/2020 funded by the Fundação para a Ciência e Tecnologia.

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Carcass traits of two commercial broiler strains fed diet supplemented with essential oil

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Essential oils are one of the phytogenic feed additives (PFA) that has been widely used in broiler diet as an alternative to the antibiotics. The aim of this study was to evaluate the effect of using essential oils supplementation on carcass traits of two commercial chicken strains fed reduced protein and metabolizable energy ration. A total of 384 one-day-old chicks were obtained from a local hatchery (192 Indian River and 192 Hubbard). Birds were allocated randomly according to their genotype into three groups: control, Eos (received essential oil from day one up to day 14), and Eos grower (received essential oil from day 14 up to day 35) with inclusion rate 125 g/ton for both treatments. Body weight was recorded at the beginning of the study and then on weekly intervals. Feed intake was calculated at the end of each week. At the end of the trial, 16 birds from each strain-essential oils combination were chosen randomly to evaluate carcass characteristics. Data were analysed using the generalized procedure of SAS, strain, essential oil, and their interactions were treated the main effects. Carcass cuts percentages were only affected by strain ($P<0.05$). Shear force was lower for the Hubbard strain ($P<0.01$). Essential oils showed a significant effect on cooking loss. A significant strain by essential oil interactions were obtained for cooking loss, shear force, and colour coordinate a. In conclusion, the inclusion of essential oils in broiler's diet did not compensate for the protein and energy reduction for carcass traits. A future study with greater inclusion rate may merit different results.

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Association (ACLY) gene polymorphism and fattening, slaughter and pork quality traits in Polish pigs

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The aim of this study was to estimate associations between ACLY polymorphism (c.*523 T>C) and fattening, slaughter and meat quality traits in three polish pig breeds. c.*523 T>C polymorphism is located in the last exon of ACLY gene (Chromosome 12: 20,840,865, Sscrofa11.1:CM000823.5) in region which does not undergo translation (3'-UTR). UTRs jointly with other factors interacting with RNA may be responsible for stability, transport, and sub-cellular localization of mRNA as well as efficiency of translation, which affects total amount of synthesized protein. The ACLY gene is localized on the porcine chromosome (SSC) 12 (NCBI, Gene ID: 100125957). The chromosome (SSC12) is relevant to the genetics of fatty acids (FA) composition because of genes involved in biosynthesis of FA. Some functional candidate genes (ACLY, ACOX1, SREBF1, ACACA) have been analysed for explaining the QTL effects. Investigations were carried out on 526 pigs represented by three breeds: Polish Landrace (n=269), Polish Large White (n=189) and Puławska (n=68). ACLY genotypes were determined by PCR-RFLP method. Digestion of PCR amplicons (319 bp) with BsrDI enzyme allowed determination of three genotypes, based on the following restriction fragments lengths: TT – 319 bp, CC – 168 and 151 bp and TC – 319, 168 and 151 bp. The present study shows similar frequency of ACLY (c.*523 T>C) genotypes and alleles between the breeds investigated. In all groups, the heterozygous genotype was the most frequent, while the homozygous CC genotype occurred with lowest frequency. In Puławska breed the CC genotype was not observed, probably due to the small number of animals in the study. It was demonstrated that the analysed polymorphism had significant influence ($P\leq0.05$ and $P\leq0.01$) on several economically important traits in pigs e.g. average daily gains, average backfat thickness, lean meat percentage. The results obtained allow for application of c.*523 T>C polymorphism in breeding programs to improve the pig population in terms of fattening and slaughter traits. However, this breeding program have a slight negative effect on meat texture parameters.

Thermography to assess testicular temperature in immunocastrated bísaro pigs

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Immunocastration may provide a reliable substitute for surgical castration, and it is essential to provide information about its impact on pig's physiology. This work aims to do a preliminary validation of thermography as a non-invasive method of testicular temperature determination in pigs subjected to different immunocastration protocols – a total of 33 Bísaro pigs (29 weeks old, LW 83.5±10.2 kg). The main objective is to understand if thermography captures differences that otherwise would have to be measured through more invasive methods, such as measure testicular length and width. These animals were distributed by the following treatments: immunocastration at 9 and 13 weeks old (IC1; n=9), immunocastration at 13, 17 and 19 weeks old (IC2; n=10) and boars (n=14). Thermal images of both testicles were obtained using a thermography camera. Each animal was photographed twice, and the mean temperature was measured in two different spots of each testicle. The data were pooled and analysed using one-way ANOVA. There were significant differences between treatments ($P<0.01$). Boars presented the lowest testicular temperature (23.3±1.5 °C) compared to IC1 and IC2 ($P<0.01$), which also differenced from each other (26.3±3 vs 24.7±3.5 °C; $P<0.01$). Although significant differences were found between treatments, the standard deviations on treatments IC1 and IC2 are very high. These preliminary results show the potential of thermography to evaluate differences in testicular temperature, although a more thorough analysis needs to be done using more replicates. In the future, this data will be confronted with objective measurements of testicular size. This work was supported by the project Icas – Bísaro (reference n°. PDR 2020-101-031029) and the project UIDB/CVT/00772/2020 funded by the Fundação para a Ciência e Tecnologia (FCT).

Preparation for identification of ATAC-seq signals associated with high fat level in Złotnicka White

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ATAC-seq is a molecular technique, fast and easy to prepare method in comparison to FAIR-seq, CHIP-seq or DNAse-seq, which sequenced reads can be used to infer regions of increased accessibility and to map regions of transcription factor binding sites and nucleosome positions. The present study aimed to identify ATAC-seq signals in liver tissue associated with an increased fat level in the pigs. In the investigation, Złotnicka White pig breed was used that is native Polish breed. Among the group of 50 pigs chose 16 (8 per group) that presented high and low-fat level. The subcutaneous fat was measured in the ten points 20 minutes after slaughter cut liver tissue was collected into tubes with William's E medium with 1x hepatocyte plating/thawing medium A (Thermofisher), were transported to laboratory at 4 °C, centrifuged, undergone 10-15 minutes digestion in 37 °C using 1xAcutase, filtered by using 70um cell strainer (Sigma), washed with 10% FCS DMEM/F12 and then slowly frozen in cryotubes with cryoprotectant Cryostor 10 (Stemcell) and Mr Frosty™ freezing container to the temperature -80 °C. The vitality evaluation was performed by using 4% Trypan blue and samples exhibited the vitality ≥80% were subsequently qualified for further analysis. The cell preparation before ATAC-seq libraries was done according to Buenstro *et al*. protocol and TruPrepTM DNA Library Preparation Kit V2 (Vazyme) workflow. This kit contains new-type transposase, which converts the complex step of DNA fragmentation. The quality and quantity control of ATAC-seq libraries were estimated by using D1000 TapeStation2200 and Qubit® (Invitrogen). ATAC-seq libraries were ATAC-seq fractionalized for A (nucleosome-free fragments, NFR, 175-275 bp) and B (mono-, di- and trinucleosome fragments 275-675 bp) fractions. The ATAC-seq libraries were sequenced on Hiseq3000 in pair-end 150-cycle reads. During bioinformatics analysis, to boost genome accessible chromatin signals, the mtDNA background was removed. Obtained data might provide information about chromatin-accessible regions for transcription factors (TF) relevant to the up-regulation of genes involved in fatty acid metabolism and liver-specific TF motifs.

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Salmonella and parasites in French alternative pig farms: first results in 35 herds

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A study was carried out in 35 alternative farms to assess *Salmonella* shedding by growing and finishing pigs and their level of parasitism. An alternative farm was defined as a farm with outdoor access or deep litter housing. In each herd, faeces were collected from 10 growers (10-12 weeks of age) and 10 finishers (≥22 weeks of age). *Salmonella* was detected with a four-step protocol adapted from the NF-U47-102 standard. Isolates were serotyped by agglutination following the ISO/TR 6579-3. Worm eggs and coccidia oocysts were detected and quantified in faeces by the McMaster technique using saturated NaCl as a flotation fluid. Among the 35 farms, 71.4% of the herds were organic and 22.9% were involved in the French quality programme 'Label Rouge'. Growers and finishers had outdoor access in 40.0 and 57.1% of the herds respectively. Pigs received a deworming treatment in 85.7% of the herds. *Salmonella* was detected in 42.9% of the herds; 34.3% of the herds were positive at the growing step, 34.3% at the finishing step and 25.7% at the two steps. On the 700 samples of faeces, 17.9% were *Salmonella* positive, 18.3% at the growing step and 17.4% at the finishing step. Eight serotypes were found in positive samples. *Salmonella* Derby (45.0% of the isolates) was the most frequent serotype. Parasites were detected in 42.9% of the herds; 45.7, 65.7 and 40.0% of the herds were positive at the growing, finishing and at the two steps respectively. Parasites were detected in 22.3 and 41.4% of the faeces at the growing and finishing step respectively. Oocysts of coccidia were found in 25.1% of faeces (mean=14,176.1 eggs/g, σ=64,338.1). *Oesophagostomum dentatum*/*Hyostrongylus rubidus*, *Trichuris suis* and *Ascaris suum* eggs were quantified in 7.1, 5.1 and 1.3% of faeces respectively (mean=1,536.0 eggs/g σ=2,695.6; mean=252.7 eggs/g σ=301.9 and mean=433.3 eggs/g σ=545.4 respectively). In 17.1% of the herds neither *Salmonella* nor parasites were detected. These findings show that parasitism and *Salmonella* shedding are encountered in alternative pig farms. Identifying herd-factors associated with these two infections may help optimise herd health management programs and food safety.

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Xanthohumol reduces incidence of white striations and affects tissue oxidative status in broilers

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Emerging concerns have come related to the presence of breast muscle myopathies such as white striping (WS) and wooden breast (WB) in broilers. While the incidence is erratic, it has become an important issue for the poultry industry. Oxidative stress and metabolic perturbations have been reported as underlying causes. Xanthohumol is a plant metabolite found in the female inflorescence of *Humulus lupulus*, known as hop and has shown to possess strong antioxidant potential. Therefore, the aim was to evaluate the supplementation of xanthohumol, formulated to increase its solubility and hence bioavailability, to the diet on the incidence of breast meat myopathies and oxidative status. A total of 120 male broiler chickens were fed either a pelleted control wheat-soybean meal diet or diets supplemented with increasing levels of xanthohumol, i.e. 50 and 100 mg/kg (2 pens per treatment with 20 chickens each). At the end of the 39 day rearing period, all birds were euthanised and the severity of WS (0, normal; 1, mild; 2, severe; 3, extreme) and WB (0, normal; 1, mild; 2, severe) in the left breast muscle was assessed (n=40), next to malondialdehyde and glutathione peroxidase in plasma, liver and jejunal mucosa (n=16). The chi-square test revealed a significant effect for WS (P=0.014), but not for WB (P=0.662). Dose-dependently, the percentage of birds with absence of WS (score 0) increased from 74.4 to 97.4%, while the percentage with mild WS dropped from 20.5 to 2.6%. Malondialdehyde in plasma, a marker for lipid peroxidation, was linearly reduced with increasing supplementation with xanthohumol (18.1 in control vs 16.7 nmol/ml with xanthohumol at 100 mg/kg) (P=0.028). Likewise, malondialdehyde in liver was linearly decreased by adding xanthohumol to the diet (P=0.001). Glutathione peroxidase was not affected in plasma and liver, but showed a linear decrease in jejunal mucosa (P=0.028). Altogether, it illustrates that the addition of xanthohumol, in particular at 100 mg/kg, was able to reduce incidence and severity of white striations in breast muscle, associated with reduced lipid peroxidation in various tissues.

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Impact of swine immunocastration on fat quality of Teruel dry-cured hams

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Two experiments were carried out to evaluate the effect of immunocastration on the quality of Teruel dry-cured hams. It is a Spanish Protected Designation of Origin of hams from (Landrace × Large White) × Duroc pigs slaughtered around 135 kg. In the first trial, 20 hams from entire and immunocastrated gilts (EG; IG) were compared (n=10) and, in the second one, 14 hams from surgically castrated and immunocastrated males (SCM; ICM) were tested (n=7). All pigs, carcasses and hams had received the same management at farm, slaughterhouse and cellar. Once hams were cured (19 months), colour and thickness of the subcutaneous fat and marbling, lipid oxidation and fatty acid profile of the *Biceps femoris* muscle (intramuscular fat) were analysed. Besides, in the hams from males, boar taint compounds (androstenone, skatole and indole) concentration was also determined. Data were analysed using the GLM procedure of SAS. In the first trial, no effect ($P>0.10$) of immunocastration was observed on fat colour traits, marbling, lipid oxidation or fatty acid profile, but subcutaneous fat thickness was thicker ($P<0.05$) in IG than in EG. In the second trial, immunocastration did not influence ($P>0.10$) colour traits and thickness of the subcutaneous fat or marbling, lipid oxidation and intramuscular fat composition; however, ICM showed higher ($P<0.05$) skatole and indole concentrations than SCM, although these levels were under the thresholds above which consumers would negatively react to hams. Besides, androstenone concentration in both treatments was under the quantification limit of the equipment used. Therefore, it can be concluded that immunocastration, both in female and male pigs, had limited effect on fat quality of Teruel dry-cured hams. However, it is worth noting that in females it increased subcutaneous fat thickness, which is a positive aspect for the curing process, and in the case of males it failed to reduce the levels of skatole and indole as much as with surgical castration. Project funded by MINECO (AGL2016-78532-R) and by Gobierno de Aragón (FITE and FEDER).

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Assessment of an *in vivo* method for measuring *de novo* lipogenesis using stable isotopes in pigs

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The use of radioactive isotopes to measure de novo lipogenesis in pigs has been well established. In contrast to radioactive isotopes, stable isotopes present little or no risk to human and animal subjects. Therefore, the objective of this study was to adapt the bolus injection of glucose labelled with radioactive 14C method to use glucose labelled with stable 13C to estimate de novo lipogenesis in finishing pigs. Five gilts were fed a commercial diet (3.0 kg/day, as is) for 2 weeks; the last 4 d, they received their daily allowance in 6 equal portions. On the 4th day, gilts received an intra-jugular bolus injection of [U-13C] glucose (12 mg/kg BW). Then, blood was sampled at 2, 4, 6, 9, 12, 15, 20, 30, 40, 60, 80, 100, 120, 150, 180, 210, 240 min after the injection to determine plasma glucose isotopic enrichment (IE). The IE of lipids was determined from adipose tissue biopsies collected at 1, 2, and 3 h after the bolus injection, and from adipose tissue collected after pig's euthanasia 4 h after the bolus. Lipogenesis was calculated from the incorporation of 13C into adipose tissue lipids. The rate of disappearance (Rd) of [U-13C] glucose estimated using a double exponential function of glucose IE vs time averaged 5.4±1.4 mmol/min. Results showed that the IE of lipids linearly increased during the 4 hours following the bolus injection ($P<0.05$). The rate of lipogenesis estimated at 4 hours after the bolus injection averaged 9.0±3.4 µg glucose/(min×g of lipids), which is within the range of values reported by previous studies using radioactive isotopes. In conclusion, the *in vivo* method of a bolus injection of [U-13C] glucose allows a successful estimation of de novo lipogenesis in finishing pigs.

Intra- and inter-observer reliability when assessing white striping in turkey breast muscle

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Meat quality and myopathies are increasingly being considered in breeding programs to reduce product downgrading and increase consumer acceptance. As part of a larger project to incorporate meat quality traits in turkey breeding, 10,521 male turkey Pectoralis major muscles were photographed at commercial poultry abattoir. The aim of this study was to examine the intra- and inter-observer reliability of 6 scorers for white striping in turkeys. Pictures were assessed and scored using a 0-3 scale adapted from broilers chickens. Following initial discussion of the scoring system, the same set of 50 pictures were scored in two sessions with two scoring rounds per session. This was followed by a final session, also including two scoring rounds, with a new set of 50 pictures. Quadratic weighted kappa values were calculated for each scorer within one session (comparison of two scoring rounds) as a measure of intra-observer reliability. Inter-observer reliability was measured by calculating the overall Gwets agreement coefficient for ordinal data between all 6 scorers within each scoring round. The quadratic weighted kappa for intra-observer reliability ranged from 0.67 to 0.88, showing substantial to good agreement in the final session. The Gwets agreement coefficient ranged from 0.74-0.91 as a measure of inter-observer reliability. The main source of disagreement between scorers occurred when assigning scores 1 and 2, while the extreme scores 0 and 3 were more easily identified. This preliminary analysis provides first insights into intra- and inter-observer reliability of visual white striping assessment by independent scorers in turkeys. Further work will assess the potential of automated methods to assess white striping and the incorporation of white striping phenotypes into turkey breeding programs.

A major QTL at LHCGR for multiple birth in Holstein cattle

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Twin and multiple births are rare in cattle and have a negative impact on the performance and health of cows and calves. Therefore, selection against multiple birth might be desirable in dairy cattle breeds such as Holstein. We applied different methods to decipher the genetic architecture of this trait using de-regressed breeding values of around 3,000 Holstein animals for genome-wide association analyses of approximately 600K imputed SNP markers. Estimated breeding values do not show a significant genetic trend over time for the studied population which indicates no selection for this trait in the past. Besides suggestive non-significant QTL on different chromosomes, we identified a major QTL on chromosome 11 for maternal multiple birth, explaining a proportion of ~6% of the total genetic variance. Using a haplotype-based approach, this QTL was fine-mapped to a 20 kb window on chromosome 11 from 31.07 to 31.09 Mb harbouring two functional candidate genes (*LHCGR* and *FSHR*). Exploring whole-genome sequence data by linkage-disequilibrium estimation revealed several regulatory variants in the 5'-region of *LHCGR* as candidate causal variants for the identified major QTL. Furthermore, the identified haplotype showed significant effects on stillbirth, calving ease, and non-return rate in cows. QTL detection and subsequent identification of causal variants in livestock species remain challenging despite the availability of large-scale genotype and phenotype data. Here we report for the first time a major QTL for the trait maternal multiple birth on chromosome 11 in Holstein cattle and provide evidence for linked variants in the non-coding region of a functional candidate gene. This discovery, which begins to decipher the genetic architecture of this polygenic trait, opens the way for future selection against this undesirable trait and thus contributes to increased animal health and welfare.

Selection against infectious diseases: genetic variation and response to selection

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Pathogens have profound effects on livestock. Despite the availability of well-established epidemiological theory, however, quantitative genetic theory for infectious diseases is almost entirely lacking. Here we present a quantitative genetic theory for endemic infectious diseases, focussing on the genetic factors that determine the endemic prevalence (P; the mean fraction of the population that is infected). We define an additive model for the logarithm of individual susceptibility, infectivity and rate of recovery, resulting in a breeding value for the logarithm of the basic reproduction number (R0). Building on this breeding value, we present simple expressions for breeding values and genetic parameters for the endemic prevalence. Without genetic variation in infectivity, breeding values for prevalence are a factor 1/P greater than the ordinary breeding values for binary disease status (0/1). Hence, even though prevalence is the simple average of individual binary disease status, breeding values for prevalence show much greater variation than our ordinary breeding values. This implies that the genetic variance that determines the potential response of prevalence to selection is largely due to indirect genetic effects (IGE), and thus hidden to ordinary genetic analysis and selection. For disease susceptibility and recovery, a fraction 1-P of the genetic effect is due to IGE; For infectivity the entire effect is due to IGE. Hence, the genetic variance that determines the potential of livestock populations to respond to selection must be much greater than currently believed, particularly at low prevalence. We evaluated this implication using simulation of endemics following standard methods in epidemiology. Results show that response of prevalence to selection increases very strongly when prevalence decreases, and is much greater than predicted by our ordinary breeding values. These results supports our theoretical findings, and show that selection against infectious diseases is much more promising than currently believed.

Genetic relationships between multiple ketosis related traits in Swiss Brown Swiss dairy cattle

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Ketosis is a major health issue in dairy cows. Genetic selection to increase resistance to ketosis could increase profitability for dairy farmers and improve animal welfare. The aim of this study was to assess genetic relationships between ketosis related traits in order to gain insight into their applicability in a genetic evaluation for resistance to ketosis. In the Swiss milk recording scheme seven indicators linked to ketosis are recorded: milk acetone (mAC_FOSS) and milk β-hydroxybutyrate (mBHB_FOSS) concentrations as well as milk fat/protein ratio (mFPR) estimated with MilkoScan FT 6000 or MilkoScan 7 RM, milk acetone (mAC_MIR), blood non-esterified fatty acids (bNEFA_MIR) and blood BHB (bBHB_MIR) concentrations based on collaboratively developed MIR spectral models and milk acetone concentration measured on a AutoAnalyzer 3 (mAC_AA). The mAC_MIR, bNEFA_MIR and bNEFA_MIR equations were developed using standardized MIR spectra. For each ketosis indicator an associated ketosis trait has been defined as the highest available ln-transformed (except mFPR) record between 5 to 60 DIM. The amount of available data varied, depending on the trait combination, from 232,344-234,518 lactations of 122,760-123,820 Brown Swiss cows from 1,960-1,982 herds. Genetic variance and covariance components were estimated by REML with bivariate linear animal repeatability models. Heritabilities were 0.077-0.083 (mAC_AA), 0.100-0.104 (mFPR), 0.106-0.112 (mAC_FOSS), 0.111-0.124 (bNEFA_MIR), 0.146-0.151 (mAC_MIR), 0.147-0.168 (mBHB_FOSS) and 0.157-0.169 (bBHB_MIR). Genetic correlations between the traits ranged from 0.09-0.93 and were lowest between mFPR and all other traits (0.09-0.33) and highest between mAC_FOSS and mBHB_FOSS (0.93). Genetic correlations between traits based on ketone bodies (acetone and β-hydroxybutyrate) were higher compared to genetic correlations between those traits and mFPR and bNEFA_MIR. mAC_AA is an exception to that pattern. These results indicate that there is genetic variability in all the traits and that there are considerable genetic differences between the traits. Analyses are ongoing to identify the traits which best reflect resistance to ketosis.

Prevalence of known recessive disease alleles in Italian beef cattle

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In the last decade, five different genetic recessive disorders with known molecular causes were reported in Chianina, Marchigiana and Romagnola cattle. Targeted genotyping of the following pathogenic variants affecting five different genes became possible: the two forms of ATP2A1-related pseudomyotonia (PMT) in Chianina and Romagnola, KDM2B-related paunch calf syndrome (PCS) in Marchigiana and Romagnola, NID1-related congenital bilateral cataract (CC) in Romagnola, ABCA12-related ichthyosis fetalis (IF) and FA2H-related ichthyosis congenita (IC) in Chianina. This study aimed to characterize the frequency of the recessive alleles associated with PMT, PCS, CC, IF and IC and to investigate whether the causal variants are breed specific. Top sires used for artificial insemination and young bulls short-listed for the performance test, for a total of 7,283 Chianina, 2,696 Marchigiana and 1,574 Romagnola animals were considered. Carriers rates (CR) considered on the base of the year of birth of the animals ranged as following: CR of PMT-ATP2A1 exon 6 from 8.1 to 22.3% in Chianina, 7.4 to 14.3% in Marchigiana and 0 to 8.1% in Romagnola; CR of PMT-ATP2A1 exon 8 in Romagnola from 3.9 to 13%; CR of CC-NID1 in Romagnola from 3.5 to 8.1%;CR of PCS-KDM2B from 0 to 14.3% in Marchigiana and from 9 to 55.6% in Romagnola; CR of IF-ABCA12 in Chianina from 1.8 to 6.3%; CR of IC-FA2H in Chianina from 3.8 to 31.8%. Considering only the top sires, the CR resulted as following: CR of PMT-ATP2A1 exon 6 was 3.5% in Chianina, and 9.7% in Marchigiana; CR of PMT-ATP2A1 exon 8 in Romagnola was 4%; CR of CC-NID1 in Romagnola was 6%; CR of PCS-KDM2B was 2.2% in Marchigiana and 27.8% in Romagnola; CR of IF-ABCA12 in Chianina was 1.7%; CR of IC-FA2H in Chianina was 15.2%. This study validates the concern regarding the impact of recessively inherited diseases on breeding programmes. It also reports the first evidence of the segregation of PMT-ATP2A1 variant initially found in Chianina also in Marchigiana. Systematic genotyping of sires for these alleles is recommended to prevent risky mating.

Resilience indicators calculated from daily activity in dairy cows

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Resilience is the ability to cope with disturbances and can potentially be improved by genetic selection. Many diseases in cows are associated with reductions in activity. Therefore, the aim was to develop resilience indicators from activity data. Daily step count measured by Nedap Smarttags were obtained from 86 Dutch farms between 2016 and 2019. Data from parity 1-3 were selected, which were 4,854,856 daily records on 19,751 lactations. Daily step count was first corrected for seasonal and pregnancy effects by fitting an ANOVA (oestrus days excluded), and taking the residuals. Potential resilience indicators were then calculated, which were the mean of corrected daily step count in the following periods of the lactation: prepartum (2 weeks before calving), early lactation (month after calving), later lactation (75-450 days after calving), and complete lactation. A genetic analysis was performed in ASReml using a repeatability model. In addition, the phenotypic effect of four diseases on the potential resilience indicators were estimated. Diseases were mastitis, ketosis, claw disorders and uterus disorders registered by farmers or indicated by milk production recording. Heritability and repeatability ranged from 0.21 and 0.38, respectively, for mean steps prepartum, to 0.41 and 0.73, respectively, for mean of the complete lactation. All diseases were significantly ($P<0.05$) or close to significantly ($P<0.10$) associated with reduced daily activity in at least part of the lactation, up to a reduction of 143 steps per day in early lactation for ketosis ($P<0.001$). These results suggest that diseases generally coincide with reduction in activity, in part of the lactation or the complete lactation. Therefore, it may be possible to breed for improved (disease) resilience by selecting for higher activity level. However, genetic correlations with disease traits and other functional traits should be estimated to investigate if the association between activity level and disease is also genetic, and if unfavourable genetic correlations with other traits exist. Nevertheless, this study is an important step towards resilience indicators based on activity level.

Do we really breed for healthier cows, and can we do better?

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Dairy cattle breeding programmes aim at simultaneously improving productivity and functional traits, such as fertility, ease of calving and longevity. Based on the given weights, genetic parameters and accuracies of genomic breeding values, we used an advanced selection index framework to derive the expected genetic gains in German Holsteins for the last decade and find that the observed genetic gains for productivity and longevity were lower than expected, while the expectations for fertility, calving ease and type traits were exceeded. Considering longevity, somatic cell score and calving ease as welfare-related traits, their joint weight in the overall selection index was 30% and they were expected to contribute 26.9% to the overall genetic progress, but actually only made up for 24.4% of the observed total genetic gain in the last decade. The observed differences can be explained by individual selection decisions that give more weight to certain traits and also may be due to the application of some forms of trait-specific threshold selection. With the assumed correlation structure, the system appears to be rather 'buffered' so that changes in the a priori trait weights have little effect on the expected composition of the genetic gain. Applying retrospectively an inverse desired gains index, we find that the observed trends over the past 10 years are consistent with an index that basically assigns no direct economic value to longevity. In fact, breeders assigned the highest 'effective' weight (42%) to production traits, medium weights were assigned to fertility (26%) and type (20%), while somatic cell score, ease of calving and longevity were all below 5%, giving the welfare-related trait complex a combined effective weight of 10.7% only. From April 2021, a new index will be introduced that includes more direct health traits and gives the welfare-related traits a higher total weight (42%). If selection will be consistently based on the new index, we expect 45.8% of total genetic progress to be made in welfare-related traits and 38.4, 3.4 and 12.4% in milk, fertility and type, respectively.

Exploiting massive genotyping and sequencing data to improve fertility in Swiss dairy cattle

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Breeding programs including genomic selection (GS) offer a unique opportunity, as the single nucleotide variants (SNV) data generated represents the current and recent living breeding population. While GS decreased the generation interval and increased the genetic gain, an increasing negative trend in fertility in today's dairy populations can be observed. The negative correlation between the intensive selection for production traits, and reproduction and survival are known, however, GS showed that health and production traits can be improved simultaneously. Nevertheless, through the so-called hitchhiking effect, recessive lethal alleles affecting fertility might have increased in frequency even though selection pressure was put to improve fertility traits. Therefore, we currently explore the available genotyping data for candidate haplotypes that are associated with impaired fertility and rearing success. Thus, we search for haplotypes showing a significant depletion from the Hardy-Weinberg equilibrium by applying a trio-based approach in the Swiss dairy cattle breeds Brown Swiss, Original Braunvieh, Original Simmental and Holstein. For these haplotypes, association studies including various production and reproduction traits were carried out. In addition, genome-wide association studies were used to evaluate if overlapping phenotypically interesting genomic regions exist. Furthermore, over 400 animals carrying the identified haplotypes had been whole-genome sequenced, in order to mine the data for putative candidate variants potentially responsible for fertility traits such as embryonic lethality, abortions, stillbirths and other undesired phenotypes such as illthrift. In conclusion, the screening of the local Brown Swiss population with 48,807 genotyped animals on the SNV array including 114,890 SNV led to the detection of 36 different genome regions harbouring such haplotypes. For example, we observe six haplotypes with a significant negative association to non-return rate, one to live birth and four to reduced rearing success. Therefore, the current analyses aim to identify potential causal variants in these regions.

Impact of Holstein haplotypes

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Since genomic selection became the new standard in dairy breeding, Holstein haplotypes have been discovered by various researchers. Such haplotypes result from a mutation in an ancestor which has spread in the population. Discovery of carrier animals is now part of weekly routine genomic evaluation. Seventeen haplotypes are analysed on over 450.000 animals. These haplotypes are 13 lethal disorders, 3 hair colours and polled. Animals are assigned as homozygous affected, heterozygous carrier or homozygous non-carrier. Many of the lethal haplotypes result in abortion during the gestation or in stillbirth. While functional mutations have been discovered for all of these haplotypes, these are not always present on DNA chips, so verification of haplotype segments remains needed. Trends are computed for each semester of birth for % of carriers, and for lethal haplotypes the expected number of lethal cases and expected percentage of lethal cases. From the Italian animals 72% was non-carrier for all of the 13 lethal haplotypes, whereas 24% carried one, and 3% more than one. HH3 and HCD have been greatly reduced, however HH5 has become quite frequent with around 7% carriers leading to nearly 3,000 abortions/year. Furthermore, recessive red has a carrier frequency of 7%. Cumulative frequencies of lethal haplotypes have reduced by 44%, and lethal cases by 50% since 2012. This corresponds with a reduction from around 8,000 to around 4,000 lethal cases per year in a population of a million females.

New insights into resistance to paratuberculosis from sequence-based GWAS in Holstein cattle

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Bovine paratuberculosis is a contagious and incurable disease, caused by *Mycobacterium avium* subsp. *paratuberculosis* (MAP), with adverse effects on animal welfare and serious economic consequences. In a previous GWAS conducted on 1,644 Holstein cows with an accurately defined status for MAP (controls, non-clinical shedders and clinical cases) and whole genome sequences (WGS) imputed using the 6th Run of the 1000 bull genomes (1kBG) project (multibreed population, UMD3.1 reference genome), we identified three genomic regions with effects on resistance to MAP infection on chromosomes 12, 13, and 23. The objective of the present study was to conduct further investigations using the 7th Run of the 1kBG, which includes a larger Holstein population with WGS based on the recent ARS-UCD1.2 reference genome. Thus, 50K genotypes of the 1,644 Holstein cows were imputed to the high-density (HD) level with FImpute and then up to WGS with Minimac using reference sets of 776 and 700 Holstein bulls, respectively. GWAS was carried out with GCTA, testing the individual effect of ~25M variant and accounting for the population structure through a HD-based genomic relationship matrix. After filtering variants for low imputation accuracy ($R^2<0.3$) or frequency (MAF<0.001), 4,815 variants had significant effects ($6\leq-\log(P)\leq34.5$) on resistance to MAP infection. They were located in eight different genomic regions on chromosomes 6, 9, 12, 13 (2 regions), 14, 18, and 23. Two-thirds of these variants (3,194) were located in genes, mainly in introns (2,170), upstream or downstream regions (907), and exons (101 including 65 non-synonymous). In each region, the variants with the most significant effects were located in the *TMPRSS11F*, *HS6T3*, *ABCC4*, *SNTA1*, *SNTB1*, *NOTCH4*, *NFKBTL1*, *ATP6V1G2*, *DDX39B*, and *ELOVL5* genes. This study confirms the three genomic regions initially detected and reveals five novel regions for resistance to MAP infection.

Is longevity still needed in dairy cattle breeding goals?

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Longevity is an important trait in dairy cattle breeding that is included in many national breeding indices with proper emphasis. The primary aim when selecting for longevity is to reduce involuntary culling and thus to increase the probability of a cow staying longer in the productive herd. It is non-controversial that longevity is positively associated with profitability of a dairy herd. Additionally, improvements in productive lifetime of cows reduce the environmental impact of dairy farming and are crucial with respect to animal welfare. However, longevity is only a proxy for healthy and fertile cows. Nowadays, breeding goals increasingly consist of functional traits such as fertility and direct health traits, which are primary risk factors for involuntary culling. Furthermore, different studies have been reported that fertility and health traits are genetically favourable correlated with functional longevity. Therefore, the genetic improvement of direct traits is possible and the inclusion of longevity in selection indices must be rethought. The aim of this study was to answer the question whether longevity is still needed in breeding goals of dairy cattle. A suitable approach is presented that allocates the economic consequences of reduced involuntary culling in a dairy herd to direct traits rather than including longevity itself in the breeding goal. This procedure offers mainly two advantages. First, the economic weight of longevity is being attached to traits where it actually belongs. Consequently, more emphasis is given to direct traits in the breeding goal and the importance of longevity is reduced. Second, double counting of economic effects in relation to longevity is avoided. Stochastic simulation is used to demonstrate the economic and genetic consequences of omitting longevity from breeding goals in dairy cattle.

Across countries genomic prediction using national breeding values versus MACE breeding values

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In across countries genomic prediction for dairy cattle, two kinds of bull information can be used as dependent variables. The first is estimated breeding values from multi-trait across-countries evaluation (MACE EBVs), assuming genetic correlations between countries equalling 1. The second is estimated breeding values (EBVs) from the national genetic evaluations, assuming genetic correlations between countries less than 1. In the present study, the level of bias and reliability of across-countries genomic predictions using national EBVs or MACE EBVs as dependent variable were investigated. Data from Brown Swiss organizations joining the InterGenomics service of Interbull Centre were used. National and MACE EBVs for 3 traits (protein yield, fertility rate and fertility interval) from 7, 5 and 4 countries, respectively, were used, resulting into 16 trait-country combinations. Genotypes for 45,473 SNP markers and de-regressed EBVs (national EBVs or MACE EBVs) of 7,490, 5,833 and 5,177 bulls were used in analysis of protein yield, fertility rate and fertility interval, respectively. For most of the traits, the use of MACE EBVs via single trait approach resulted in less biased and more reliable across-countries genomic predictions. In case some of the MACE EBVs are inflated, the resulting single-trait genomic predictions are inflated as well. For these specific cases, the use of national EBVs via multi-trait approach provided less biased and more reliable across-countries genomic predictions.

Mapping of multiple loci affecting rearing success in four Swiss dairy cattle populations

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The aim of this study was the investigation of the genomic background of rearing success in four Swiss dairy cattle populations including Holstein, Simmental, Brown Swiss and Original Braunvieh. Rearing success is defined as the proportion of animals that successfully survive from birth till slaughter or production period. For genetic evaluation of this trait complex, three sub-traits are defined according to age and sex: period 1 (p1; survival from day 3 up to 30th day of life), heifer period 2 (hp2; survival of heifers from day 31 up to 458 days), and bull period 2 (bp2; survival of young bulls from 31 days up to 183 days). All three traits are evaluated together using a multitrait animal model. For each of the traits, breeding values were estimated, which were subsequently de-regressed for downstream analyses if BLUP reliability exceeded 0.2%. Animals were genotyped or imputed to approximately 310K markers based on a custom SNP array. Window-based association analyses using 25 adjacent markers at a time were conducted using GenSel software and BayesB algorithm. In Holstein, Simmental and Brown Swiss we detected at least one significantly associated window for one or more of the three traits analysed. The detected regions are located on different chromosomes indicating complex inheritance. Interestingly a significantly associated window was found in Holstein for hp2, which colocalizes with the known locus for *APOB*-related cholesterol deficiency (CDH). Based on our findings for the trait hp2, a negative and additive effect on heifer rearing success was detected for the identical genomic region on chromosome 11, which supports previous findings that this locus might act in a dominant way with incomplete penetrance. In order to further analyse and understand these observations, we aim to apply a haplotype and linkage disequilibrium based approach at whole-genome sequence data resolution in order to unravel the most likely causal variants.

Genetic evaluation of a novel longevity indicator for North American Angus cattle

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Genetic evaluation of longevity in beef cattle usually relies on culling records reported by the farmers. However, only a small proportion of cows in the American Angus Association pedigree file have culling records available. Defining a longevity indicator that does not rely on culling information might optimize the use of data in the genetic analyses. Thus, this study aimed to propose and compare the use of a novel longevity indicator (NL; which assumes that cows were culled one year after their last reported calving) to the use of the actual culling date (CD) in the genetic analysis of longevity. Longevity was defined as the time period in which the cow was alive and continued calving after its first calving, considering either cows that were culled due to natural reasons (e.g. age) or fertility-related problems. Both NL and CD were coded as binary traits (0 = culled and 1 = calved) for all ages (from 2 to 15 years-old). Moreover, NL and CD were analysed using a Bayesian random regression linear model based on fourth order Legendre polynomials to describe systematic (i.e. year-season of birth) and random (i.e. herd-year-season of birth, additive genetic, and permanent environmental) effects. A total of 150,229 and 154,419 animals were included in the phenotypic files for natural reasons and fertility, respectively. Heritabilities estimated using data from cows that were culled due to natural reasons ranged from 0.01 to 0.26 for CD, and from 0.03 to 0.32 for NL. Heritabilities estimated using the fertility data ranged from 0.01 to 0.14 for CD, and from 0.01 to 0.17 for NL. Rank correlation considering the top 10% animals at 4 years-old was 0.87 and 0.95 when using data from cows that were culled due to natural reasons or fertility, respectively. These results suggest that using either CD or NL to select for longevity might have similar impact in the breeding program. In addition, greater differences in the rank of selected animals are expected for longevity based on natural reasons than longevity based on fertility.

Session 33 Poster 14

Identification of copy number variants (CNVs) associated with somatic cell score in Holstein cattle

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Genomic variants, such as SNP, have been widely used to identify QTL and candidate genes associated with mastitis resistance in dairy cattle. Due to the challenges to identify Copy Number Variants (CNVs), very few studies have explored the possible association of CNV with mastitis-related traits in dairy cattle. Copy number variants can be defined as deletions or duplications of genomic sequences from 50 bp to 5 Mb long. The aim of this study was to perform a CNV association analysis using de-regressed estimated breeding values for somatic cell score (SCS), an indicator trait for mastitis. The association analysis was carried out using PLINK 1.07 implemented by the R package CNVRanger. Functional analyses were performed using GALLO R package and WEB-based GEne SeT AnaLysis Toolkit. A total of 262 CNV segments were identified in 1,077 Holstein cattle. A total of 20 CNV segments were associated with de-regressed EBVs of SCS ($P<0.05$) on BTA3, BTA5, BTA6, BTA7, BTA10, BTA12, BTA18, BTA23, BTA26, and BTA28. Gene and QTLs annotation resulted in 45 and 53 unique genes and QTLs, respectively. Thirty-two of the annotated genes were protein-coding genes. Gene HSP90B1 on BTA5, which is involved in the IL-17 signalling pathway, was reported to be associated with mastitis resulting from *Staphylococcus aureus* infection. Nearly 2% of the annotated QTLs corresponded to 'health' QTL class. These preliminary results uncovered potential CNVs associated with SCC, which will contribute to identifying novel QTL and candidate genes for mastitis resistance in Holstein cattle.

Session 34 Theatre 1

Breeding for the future: technology finds application in new phenotypes

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The application of new technologies has given animal production endless opportunities for innovative strategies to reach the aims set by the Sustainable Development Goals and the European Green Deal and the subsequent Farm to Fork and Biodiversity Strategies. The availability of modern technologies such as (wearable) sensors or cameras, database laboratory access and farm management systems provide us an incredible amount of data, waiting to be put to use in domains like animal breeding. Current research has focused on finding patterns in this vast amount of data, thereby aiming to link these patterns to phenotypic traits or even genomic blueprints. The application of Big Data give us the opportunity to produce proxies for new phenotypes important to a sustainable and responsible livestock production. Can we use proxies to improve not only robustness, efficiency and disease resistance but also to improve welfare related traits or adaptation to heat stress/climate changes? The focus of animal breeding has always been on adapting animals to their environment, thereby only indirectly selecting for behavioural traits. Can we challenge the societal assumptions and (directly) select and breed for better behaviour? Ultimately, can we combine tools such as big data, artificial intelligence, functional annotation, genomic selection and genome editing to unite the benefits of all domains? What would these developments mean for traditional farming systems and how do these new technologies and tools impact the farmers and their farm management? This challenge session is organised by FABRE TP and aims to engage in discussion with research, academia and the private sector on how we can use the available technologies to produce new phenotypes and improve livestock and aquaculture breeding.

Pre-weaning feeding management to support early robustness of piglets and post-weaning health

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Post-weaning health limits sharply the performance of growing pigs, while the use of antimicrobials must be restricted. Research showing the efficacy of single integrations of base diets in the post-weaning are abundant, but often the responses in comparative trails are not sufficiently reliable. The robustness of piglets at weaning can affect the success of dietary solutions in the post-weaning period. The size of suckled litters is currently increasing and there are indications that the quantity and/or the quality of suckled milk is not sufficient to: (1) promote growth, (2) modulate immune status, (3) enhance functional intestinal maturation and integrity, (4) modulate gut microbial composition or function and (5) prevent or limit pathogenic infection of all the piglets of the litter. Substitutive or complementary dietary solutions are coming for this purpose, particularly for interventions at earlier stages than in usual practices; these tools could also reduce competition for teat access. Improved knowledge on ideal composition of these products, on the way of presenting, on their prebiotic and probiotic relevance is requested, as also the ability to activate early maturation of cellular and humoral immunity. Single classes of nutrients need to be considered (amino acids, type of fats, minerals, vitamins), nutraceutical molecules, and way of presenting (liquid vs solid, cups or automatic dispensers). Furthermore there is the need to revise the aims and the modulation of the classical creep feed supply in the pre-weaning, to prepare the best use of the diet in the post-weaning, to accompany the waves of gut microbiota stabilisation and reduce health risks and need of antimicrobials. Finally the best way to link supplemental feeds pre-weaning to post-weaning diets needs to be better explored.

Two ways of milk replacer administration enhances survival rate and faecal microbial profile of pigs

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Large litters are exposed to undernutrition risk due to the competition for the teats, this represents a risk for litter' health. Milk replacers (MR) can support litter growth. The study aims to evaluate the effect of two ways of MR (DanMilk™) administration on the health and gut eubiosis of piglets during the suckling and post-weaning period. 30 litters from DANBRED sows (398 piglets) were included in the trial. Three groups were assessed: (1) control group (CO), sow milk; (2) CO plus MR administered automatically (AA); (3) CO plus MR administered manually (AM). The milk supplement was available from d0 to d14 of life, thereafter only creep feed was provided until the weaning (d21 of the study). At weaning, the same groups were maintained. Piglets were weighed at d0 (3 days of life), d14, d21 and d31 (ten days post-weaning). Faecal samples were collected at d14 and d21 for microbiota analysis. During suckling, AA group increased the survivability of pigs compared to CO and AM groups at d0-d14 and at d0-d21 (AA vs CO, $P<0.0001$; AA vs AM, $P<0.05$). The MR supplement to piglets increased the alpha diversity indices as MR group had a higher Shannon index than CO group at d14 ($P=0.027$) and d21 ($P=0.055$). Moreover, MR supplement increased InvSimpson index ($P=0.021$) in comparison with CO group at d21. The increase of alpha diversity indices in the MR supplement group might indicate a more mature and stable microbial profile in piglets. For the Beta diversity, the group and time influenced the microbial composition ($P=0.001$, $R2=0.042$; $P=0.003$, $R2=0.02$, respectively) without interaction. At d14, AA was discriminated by the genera Oribacterium, AM by the genera Ruminococcaceae_UBA1819 and Peptococcus, CO by genera *Muribaculaceae_CAG-873, Pediococcus, Ralstonia, Acidaminococcus, Bifidobacterium, Kurthia, Pyramidobacter.* The MR contributes to increase the pig survivability in the pre-weaning, with an additional advantage observed with the automatic system, probably due to the continuous availability of the MR. Moreover, MR supplement favours the establishment of a diverse microbiota profile in piglets.

Differences between conventional and artificially reared piglets from the same herd

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The aim of this study was to investigate differences in growth, body composition and activity of digestive enzymes of sow reared piglets (SOW) compared with artificially reared piglets (MILK) given a basic milk replacer. Thirty-six piglets were selected at birth based on their birth weight; half of these were reared under conventional conditions on a commercial farm and the other half were transferred to a university animal facility for artificial rearing from day 3 after birth. Piglets in the MILK group were housed in individual cages, and received a milk replacer diet 8 times daily, where the volume given was adjusted to body weight. Piglets of both the MILK and SOW group were euthanized on day 25 of age to study differences in body weight, daily gain, body composition and activity of disaccharidases and peptidases in the small intestine (SI). Differences were observed in activity of digestive enzymes, with a greater ($P \leq 0.01$) activity of sucrase in the SOW group (9.9, 13.0 and 4.1 U/g) compared with the MILK group (7.5, 6.5 and 2.1) across the SI (proximal, medial and distal part. Sow piglets had a greater absolute amount of fat (1,149 vs 99 g), muscle (5,314 vs 3,779 g) and bone mass (110 vs 49 g) content compared with MILK piglets. Multiple differences ($P \leq 0.05$) with respect to haematology parameters, blood serum metabolites, as well as a calculated difference in dry matter (DM) intake between SOW piglets (187 g/d) and MILK piglets (132 g/d) were found. No differences were observed with regards to immunity related parameters and only a few differences with respect to gut integrity, exemplified by a lower ($P<0.05$) brush border score in MILK (0.94) compared with SOW (1.66) piglets were found. This comparison of piglets from the same herd, but reared under different conditions revealed that body composition and gut integrity can be manipulated through dietary intervention, and that an increase in DM intake during the suckling period is beneficial for gut function. This study warrants further research investigating pre-weaning dietary means for preparing the piglet for transition from suckling to weaning.

Preliminary results on the effect of keeping pigs in home environment post weaning

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Abrupt weaning of 28-day-old pigs is associated with several stressors: Removal from the sow and the transition from milk to solid diet, movement to a novel pen, regrouping and establishment of a social hierarchy. The post-weaning period is associated with reduced weight gain. To reduce the number of stressors, we investigated the effect of weaning pigs in farrowing pens for loose housed sows in a 2×2 factorial design: The litter stayed in the farrowing pen after removing the sow (STAY) or two litters were regrouped in a conventional weaning pen (MOVE), and two sow hybrids: Danbred (DAN) or Topigs Norsvin TN70 (TN). The study included 57 litters from 4 batches (33 litters from 1st parity sows and 24 from 2nd parity sows), with an average age of 26 days at weaning (range 22-30 days). Pigs were weighed at weaning (W0), 7, 14 and 28 days after. The effect of treatment, hybrid, and their interaction on average daily weight gain (ADG) was analysed in a mixed effects model including W0 and batch as covariates, and a random effect of litter before weaning partially nested within pen after weaning. ADG was modelled separately for the first 7 (ADG7), 14 (ADG14) and 28 (ADG28) days post weaning. The preliminary results showed a two-way interaction between treatment and hybrid for ADG28 ($P=0.007$). The highest ADG28 was found for TN STAY (0.392 kg/d) being significantly higher than TN MOVE (0.252 kg/d), DAN STAY (0.307 kg/d) and DAN MOVE (0.275 kg/d). A similar tendency of interaction was found in ADG14 ($P=0.054$), but not for ADG7 ($P=0.23$). For every 1 kg increase in W0, ADG7 decreased by 7 g/d, ADG14 increased by 5 g/d and ADG28 increased by 15 g/d ($P<0.01$). In conclusion, reducing stress at weaning by keeping pigs in their home environment at weaning for 28 days post weaning increased ADG28 significantly in one but not the other hybrid. Thus, differences in growth potential and/or in stress resilience may exist between hybrids. Additional results on feeding behaviour, saliva-cortisol levels and faeces scores collected post weaning will follow.

Effect of dietary resin acid and zinc oxide in weaned piglets subjected to an immune challenge

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Resin acids (RA) derived from coniferous trees are phytochemicals with antimicrobial, antifungal and antiparasitic activities. This study aimed to compare the effect of dietary RA concentrates (RAC) with therapeutic level of ZnO on immune responses of weaned piglets with a systemic *E. Coli* lipopolysaccharide (LPS) challenge. Forty-eight 26-day old piglets entered a completely randomized design with 4 treatments, including a non-challenged control (phosphate-buffered saline, PBS), a challenged control (LPS) and 2 challenged groups with dietary treatments of 2,500 mg/kg ZnO (LPS+ZnO) and 200 mg/kg RAC (LPS+RAC). Piglets were fed *ad libitum* for 21 days (d) and injected with PBS or LPS on d 7 and 21. Blood samples were taken at 1.5 hour (h) and 3 h after each injection. Weekly growth performance and rectal temperature at various times were recorded. Rectal temperature reflected activation of the immune system by LPS. There was no treatment effect on overall growth performance. Serum immune biomarkers including interleukin (IL)-1β, 4, 6, 8, 10, 12, tumour necrosis factor (TNF)-α, interferon (IFN)-α and IFN-γ were measured and analysed by two-way ANOVA (i.e. treatment and time). No treatment effects on IL-1β, IFN- α, IFN-γ and IL-4 were observed. There was an interaction between treatment and time ($P<0.001$) at both challenges (i.e. d 7 and 21) for IL-10 and TNF-α. For IL-10, piglets from PBS showed low level at 1.5 h and 3 h, piglets from LPS and LPS+ZnO showed higher levels at 1.5 h but decreased at 3 h, and piglets from LPS+RAC showed the highest level at 1.5 h and 3 h. For TNF-α, piglets from LPS+ZnO and LPS+RAC showed the highest level at 1.5 h, followed by piglets from LPS and piglets from PBS showed the lowest level. All piglets showed low level of TNF-α at 3 h. Treatment effects were found on IL-6 and IL-8 on d 7 and 21 ($P<0.001$), in which piglets from LPS+RAC showed the highest levels, followed by piglets from LPS+ZnO, and piglets from PBS and LPS showed the lowest levels. These results indicate that RAC compared to ZnO and control, may possibly boost the immune response of piglets without penalizing growth during an LPS challenge.

Effects of live yeast supplementation as a substitute to antibiotics growth promotors in piglets

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Two studies were conducted in China to investigate the effects of live yeast (LY) in replacing antibiotics (ANT) and/or zinc oxide (ZnO) on gut health and performance of piglets. In the 1st study, 192 piglets were assigned into 4 treatments [Control (CON), ANT+ZnO, LY or LY+ZnO] with 8 pens/group, 6 piglets/pen. Results showed piglets fed LY or LY+ZnO had higher ($P<0.05$) ADG while piglets fed LY had decreased ($P<0.05$) FCR compared to CON. CON piglets had higher ($P<0.01$) diarrhoea as compared to all other groups. Superoxide dismutase was increased ($P<0.05$) in LY and LY+ZnO groups, while Malondialdehyde was significantly decreased ($P<0.01$) in these two groups on day 28. Furthermore, piglets in the ANT+ZnO, or LY group had higher ($P<0.01$) levels of short chain fatty acids, including propionic and butyric acid. In the 2nd study, weaned piglets were assigned into 4 treatment groups [unchallenged control (unch-CON), challenged control (Ch-CON), ANT+ZnO, or LY] with 6 pens/group, 6 piglets/pen. All piglets except the unch-CON, were challenged with *E.coli* on day 8. Results showed piglets fed ANT+ZnO diet had the highest weight gain and feed intake ($P<0·05$) in the 1st week (before challenge). In week 2 (after challenge), ADG of piglets fed the ANT+ZnO or LY diet were higher ($P<0.05$) than the Ch-CON group. Challenged piglets in the ANT+ZnO or LY supplemented group had lower diarrhoea ($P<0.05$) than piglets in the Ch-CON during the 24 h after challenge. Compared to the unch-CON piglets, faecal *E. coli* counts, urinary lactulose to mannitol ratio, plasma IL-6 concentration, mRNA abundances of innate immune genes in ileum and mesenteric lymph nodes were increased ($P<0·05$), whereas the villous height of jejunum and relative protein expression of claudin-1 in ileum were decreased ($P<0·05$) in the Ch-CON piglets; however, these parameters did not markedly change in the challenged LY or ANT+ZnO groups. In conclusion, these two studies suggested that probiotic yeast could be used to substitute growth promoters including antibiotics and/or ZnO.

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Effect of plant-derived product supplementation on sow and piglet physiology

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Early and abrupt weaning is a critical period for piglets with a high prevalence of digestive disorders. Plant-derived products are potential alternatives to antibiotics to prevent and treat post-weaning diarrhoea through their antioxidant, anti-inflammatory or anti-bacterial properties. The study aimed to investigate the effects of plant-derived product supplementation on sow and piglet physiology during lactation and around weaning. Sixty-four sows were assigned to control or extract treatment. Extract sows were supplemented with 25 g/d of plant extract supplied in feed from day of gestation (DG) 106 to day of lactation (DL) 28 and received 20 ml of a mixture of essential oils on DG109. Within each sow group, 2 ml of a mixture of essential oils (EO) was orally administrated to all the piglets of 1 litter out of 2 at DL3. Piglets were weaned on DL28. Blood samples were collected from sows on DG94, DG112 and DL26 and from 2 piglets per litter on DL25 and 5 d post-weaning (PW5) for the analyses of blood cell count, plasma metabolites and inflammatory (haptoglobin) and oxidative (oxidative products, dROM, and antioxidant capacity, BAP) status indicators. Colostrum and milk samples were collected at farrowing and at DL6 and 26. Extract treatment had no effect on sow metabolic, inflammatory and oxidative status or nutritional composition of colostrum and milk at DL6 ($P>0.10$). However, it decreased the number of lymphocytes in sow blood at DG112 ($P<0.05$). It also tended to decrease dry matter and gross energy ($P<0.10$) and reduced haptoglobin concentration ($P<0.01$) in milk at DL26. The EO treatment increased the average daily gain of piglets from DL6 to weaning ($P<0.05$), regardless of sow treatment. In piglet blood at DL25, EO tended to increase the lymphocytes proportion ($P<0.10$), decreased the granulocytes proportion ($P<0.05$) and tended to decrease the oxidative stress index (OSI=dROM/BAP, $P=0.06$). Piglet OSI and blood concentrations of haptoglobin and leukocytes were increased by weaning but not affected by sow or piglet treatments. Plant-derived products could thus modify the mammary secretions composition and health status of piglets before weaning. This study was financially supported by the Pays de la Loire region.

Session 35 Theatre 8

Effect of extracted olive oil wastewater in post weaning phase on infected piglets

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Weaning is often connected to impaired health status of piglets. Various environmental stress factors favour colonization of enterotoxigenic *Escherichia coli* (ETEC) in the gastrointestinal tract and cause life-threatening diarrheal disease also known as post weaning diarrhoea (PWD). The aim of this study was to evaluate the effect of olive oil wastewater (OOW), known to contain antimicrobial bioactive compounds, in weaned piglets infected with ETEC F4. A total of 64 Swiss Large White piglets (weaned at 25 d, 26 d or 27 d of age, 7.83±1.35 kg of weight) were randomly assigned to two dietary treatments (control = control diet + 0% OOW; OOW = control diet + 2% OOW) balanced for litters, sex, body weight, age of weaning (AW) and ETEC F4 susceptibility (S/S and S/R genotype). The body weight (BW), average daily gain (ADG), were recorded on day 1, 5, 7 and 12 post weaning, faecal score (fs) and colony forming unit of ETEC (cfu) were observed after infection (5th day from start to study) on 5, 6, 7, 8 and 9 day post weaning. The data were analysed using a model with repeated measure contemplating dietary treatments. The BW, ADG, fs and cfu were not influenced by the OOW inclusion ($P>0.6$). All traits were affected by the AW ($P<0.02$): younger piglets grew slower, were lighter, had greater cfu values but lower fs than older piglets. Regardless of the dietary treatment, the piglets of the S/S had higher cfu values ($P<0.01$) than those of the S/R genotype (10.9 vs 9.4 log cfu). These results highlight the impact of age at weaning and the genotype of piglets on the assessed traits. However, supplementing the starter diet with 2% OOW did not alleviate the ETEC challenge.

Medium-chain fatty acids boost polymorphonuclear cell viability in weaned piglets

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The positive impact of medium-chain fatty acids (MCFA) on microbicidal activity is commonly accepted. In addition, there is increasing evidence that MCFA might have immunomodulating activities in humans and dairy cows. In swine, limited scientific information about these effects is available. A total of 64 weaned piglets were randomly assigned to 2 treatment groups. The control group received a diet not containing MCFA, whereas the trial diet was supplemented with 2 kg/T MCFA-blend (Aromabiotic®). At d28 of the trial, 1 pig of average weight per pen was euthanized for tissue sampling. Ileum samples were fixated in 4% paraformaldehyde solution and further processed for paraffin embedding. Paraffin sections were stained with haematoxylin-eosin for morphometric measurements (villus length, crypt depth, V/C ratio). Blood samples were collected using heparin vacutainer tubes. After isolation of white blood cells and incubation for 18 h at 37 °C and 5% CO_2, polymorphonuclear (PMN) cells were selected via flowcytometry using their forward-scatter and sideward scatter characteristics and viability was determined using annexin V FITC/propidium iodide labelling. There were no significant differences in performance data nor morphometric measurements. However, we could observe a significant increase (P=0.05) in PMN cell viability in the MCFA group compared to the control group (23.52 vs 14.45%). To our knowledge, this is the first study that demonstrated an increased PMN cell viability in swine. Neutrophils are the most abundant PMN leukocytes in blood circulation. Because of their critical role in immune response, increasing their viability might result in a faster response towards attacking pathogens. These findings demonstrated that oral supplementation of MCFA might have, besides the well-known antimicrobial benefits, immune-modulating activities as well in swine.

Supra-nutritional levels of riboflavin on gut health and oxidative stress in post-weaned piglets

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Riboflavin or Vitamin B2 (B2) is a water soluble vitamin that both *in vitro* and in human studies has demonstrated a positive impact on oxidative stress, inflammation, nutrient absorption and microbiota modulation in the hind gut. Dietary recommended dose of B2 in weaned piglets (NRC 2-4 mg/kg) is rapidly absorbed in the foregut making it difficult to reach the hind gut. Thus, supra-nutritional doses of 50 and 100 mg/kg of B2 were added in post- weaning piglet diets to evaluate the effect on gut health and oxidative stress parameters of weaned piglets. A total of 54 male pigs weaned at 28 days old were housed in 9 pens and assigned to either T1: 10 mg/kg B2 (control), T2: 50 mg/kg B2 or T3: 100 mg/kg B2 during 2 weeks. Plasma samples were collected to analyse B2 concentration, antioxidant enzymes and oxidative stress index (OSI); ileal and caecal content to determine concentration of B2 and short chain fatty acids (SCFA); ileal histology and caecal transcriptome were determined. Differences between treatments were statistically analysed by one-way ANOVA using Statgraphics. Results on B2 flow (mg/kg DM) at ileum and caecal levels were higher in T2 and T3 (P<0.001) compared to the control group, indicating B2 reached the hindgut but no differences between treatments were observed on plasmatic B2 concentration. At the ileum, T3 showed higher number of goblet cells (P=0.002) compared to T1 and T2, indicating a higher protection of the gut by mucus synthesis but no differences were observed in SCFA at the caecum level. Moreover, a dose response was also observed on the differentially expressed genes (DEG; T3 vs T1=1,828; T2 vs T1=604) in caecum. A downregulation of the genes related to oxidative stress-related genes (P<0.001) was observed in T2 (HSPH1, HSP90AA1 and HSP70) and even more in T3 (HSPH1, TIMM21, NFE2L2, LEO1 and RAD17) but no differences in plasmatic antioxidant enzymes nor OSI was detected. In conclusion, supplementation of supra-nutritional levels of riboflavin has the potential to protect intestinal epithelial cells of weanling pigs by increasing mucus production and regulating antioxidant genes expression.

Effect of different sources and doses of Cu on ileal microbiota and feed efficiency of weanling pigs

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The addition of high doses (125-250 mg Cu/kg) of copper (Cu) in weaned piglets diet may improve their performance and intestinal microbes. Therefore, this experiment was performed to determine the effects of two sources of Cu as copper sulphate ($CuSO_4$) and dicopper oxide (Cu_2O; CoRouge®, Animine, France) at two levels of inclusion (50 and 150 mg/kg) on ileal microbiota and feed efficiency. A total of 256 twenty-eight-day-old piglets (Topigs × Pietrain) were randomly distributed to 4 dietary treatments: 50 or 150 mg Cu/kg as $CuSO_4$ or Cu_2O, with 8 replicates per treatment and 8 piglets per pen followed for 35 days. Individual body weight (BW) and average daily feed intake were recorded at d 7, 14 and 35. At d 36, one animal per pen was euthanized to obtain samples of blood, liver, bile, bone, and ileum content for the analyses of oxidative status (plasma and liver), mineral concentration, and microbiota, respectively. Data were analysed with ANOVA using the GLM MIXED procedure of SAS. Alpha and beta diversity were analysed using Vegan package and taxa differences with MetagenomeSeq in RStudio v.3.5.1. Pigs fed 150 mg/kg of Cu from Cu_2O had an 8.8% higher ($P=0.082$) BW at d 35 (19.67±1.4 kg) compared with pigs fed 150 mg/kg Cu from $CuSO_4$ (17.93±2.8 kg). Additionally, feed conversion ratio (FCR) was decreased by 12% ($P=0.047$) in pigs fed Cu_2O at 150 mg/kg in comparison with $CuSO_4$ at 150 mg/kg (1.37 vs 1.56). The effects of Cu supplementation for both sources and doses on the microbial characterization of ileum content were determined using 16S rRNA gene sequence. There were no differences in α and β diversities among the treatments. However, pigs fed 150 mg Cu/kg from Cu_2O had greater abundance ($P\leq0.09$) of some beneficial genus of *Pasteurellaceae, Methanobacteriaceae,* and *Lachnospiraceae* families. In conclusion, supplementation of 150 mg/kg of Cu as Cu_2O established a healthy gut microbiota by increasing the abundance of beneficial microbial families in the ileum, which may explain the positive impact on the FCR of pigs.

Effects of two copper sources on oxidative stress, inflammation, and gene abundance in growing pigs

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A trial was performed to test the hypothesis that therapeutic Cu supplementation will affect oxidative stress, inflammation, and gene expression, but these effects will be different depending on the Cu source used. A total of 120 pigs (initial BW:11.5±0.98 kg) were randomly allotted to 3 treatments with 8 pens per treatment during 28 days. Treatments were a negative control diet (NC, 25 mg Cu/kg) and two diets including 250 mg/kg of Cu from $CuSO_4$ or Cu_2O. Pigs' weight and feed intake were recorded at day 1 and 28. On day 28, 8 pigs per treatment were sacrificed to obtain samples of serum, liver tissue, and jejunum tissue to analyse oxidative stress markers, malondialdehyde (MDA), cytokines concentration, and gene expression of 56 intestinal genes related with barrier function (BF), immune response (IR), gut hormones, gut enzymes, and nutrient transport (NT). Pigs fed with the high levels of Cu had greater ($P<0.05$) growth and feed intake than pigs fed the NC diet; and showed increased ($P<0.05$) gene expression related to intestinal BF (CLDN15, MUC2, and TFF3) and NT (SLC39A4, SLC5A1, and SCLC11A2), but reduced ($P<0.05$) abundance of genes related to the IR (CXCL2, IL-6, IL-8, and TGF-β1) compared with pigs fed the NC diet. Among sources, $CuSO_4$ showed greater ($P<0.05$) MDA levels in liver, and serum concentrations of TNF-α ($P<0.05$) and IL-1β ($P<0.10$) than the other treatments. Supplementing therapeutic doses of Cu increases growth and feed intake which could be explained by the increase of genes related to NT and BF, and the reduction of genes related to IR. Also, supplementing pigs with Cu_2O induces less lipid oxidation and inflammation than pigs fed diets containing $CuSO_4$.

Testing natural alternatives to iron injection for organic piglets

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So far, the most common and efficient iron supply to prevent neonatal anaemia in piglets has been the injection of iron dextran or gleptoferron. This treatment is problematic because the use of chemically-synthesized allopathic drugs is strictly limited in organic farms. Based on the observation that piglets raised outdoors seldom develop anaemia, we hypothesized that piglets satisfy their needs in iron by ingesting soil from their environment. We compared the efficiency of a 100-mg iron dextran intramuscular injection (Dex, 8 litters, n=98 piglets) at 5 days of age (d5), with daily *ad libitum* supply of dried soil (Soil, 8 litters, n=101) or dried peat-like river mud (Peat, 8 litters, n=102) from day 5 to weaning on d49. Pigs were raised according to organic pig faming rules. Blood was collected on 3 males and 3 females per litter on d5, 21, 42, 50 and 70. A severe digestive *Escherichia coli* episode affected piglets during the experiment: litter mortality rate between d5 and d70 did not differ between groups (24%, P>0.1). Body weight was similar (P>0.1) in the three groups on d5 (1.89±0.02 kg), d21 (5.19±0.07 kg), d42 (10.0±0.2 kg), d50 (11.8±0.2 kg) and d70 (24.1±0.4 kg). Blood haemoglobin concentration (Hb) was similar in all groups at d5, weaning and d70 (7.8, 10.1 and 10.0±0.1 g/l). However, Hb was greater in Peat and Dex groups (10.4 and 9.9±0.4 g/l) than in the Soil group (7.8±0.4 g/l, P<0.01) on d21, and greater in the Peat group (11.3±0.4 g/l) than in Dex and Soil groups (9.2±and 8.9±0.3 g/l, P<0.01) on d42. Mean globular volume (MCV) of erythrocytes was stable in time in the Peat group (55.0±0.2 fl). In comparison to the Peat group, MCV dropped in the Soil group on d21 and d42 (45 and 45±1 fl, P<0.001), and in the Dex group on d42 (46±1 fl, P<0.01). Soil and Dex groups had returned to values similar to Peat group by d70 (P<0.1). To conclude, the supply of soil was not able to ensure a satisfactory level of iron in the piglets whereas peat-like river mud seems satisfactory. The daily supply of peat-like river mud was more efficient than the 100-mg iron injection beyond 21 d.

Zinc excretion from weaned pigs relates to zinc intake: a meta-analysis

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Several studies have shown that a high dietary zinc (Zn) content in pig diets leads to a high faecal Zn excretion, which is considered an environmental issue. However, there is limited knowledge about the relation between Zn intake and faecal Zn excretion in newly weaned pigs. Therefore, we performed a literature search for peer-reviewed experiments, where different dietary Zn levels were provided to pigs the first three weeks post-weaning and daily Zn intake and faecal excretion measured in mg/d. The meta-analysis included six studies with different dietary Zn sources and levels (49 data points in total). The statistical analysis showed a linear relationship between daily Zn intake and Zn excretion in faeces, uninfluenced by the Zn source. During the first week post-weaning, the linear relationship showed a slope of 0.18 (P<0.01, R^2=0.95), which was significantly lower than the slope for the second and third week post-weaning. The positive linear relationship between the Zn intake and the Zn excretion in faeces in the second and third weeks were similar, with a slope of 0.84 (P<0.01, R^2=0.94). Based on these results, the daily faecal excretion of Zn appears to be lower at a given Zn intake the first week post-weaning, indicating a higher Zn uptake compared with the following two weeks post-weaning. We applied the identified linear relationships between daily Zn intake and faecal Zn excretion during week 1 and 2-3 post-weaning on our own data of daily Zn intake in 180 pigs distributed to six different doses of total Zn in the diet (155, 492, 1,024, 1,604, 2,056, or 2,419 ppm) the first 3 weeks post-weaning. The calculated average daily excretion of Zn during week 1 post-weaning was between 0 and 47 mg/d/pig. The excretion increased during week 2-3 to between 143 and 1,651 mg/d/pig depending on the Zn intake. This shows the calculated daily Zn excretion is up to 35 times higher in week 2-3 compared to week 1 post-weaning. Moreover, the Zn excretion in week 2-3 is about 11 times higher when the diet contains 2,419 compared to 155 ppm Zn. Overall, the results of this meta-analysis can be used to estimate the daily Zn excretion from pigs the first three weeks post-weaning when the daily Zn intake is known.

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A mixture of amino acids and condensed tannins reduces post-weaning inflammation in weaned piglets

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Weaning is a critical period during which piglets are faced with many changes causing stress, with dramatic effects on feed intake. Commonly, weaned piglets are offered high quality proteins and supplemental amino acids (AA) to balance the diet. Functional AA may provide additional benefits. Therefore, 96 weaned piglets were allocated to 16 pens. Two experimental pre-starter diets (d0-14) were offered: a wheat-corn-barley-soy basal diet (10.5 MJ/kg NE, 1.15% SID Lys, and other essential AA according to ideal AA profile) or basal diet supplemented with a 0.2% mixture of valine, isoleucine, leucine, arginine, cystine, and an extract from grape containing condensed tannins. A common starter diet (10.3 MJ/kg NE, 1.10% SID Lys) was fed until d40. At d14, one piglet per pen was euthanised to collect blood and harvest distal small intestinal mucosa. No differences were found in performance in pre-starter and starter period, though numerically growth (+9.5%), feed intake (+6.2%), and feed-to-gain (-7.2%) in pre-starter period were best for the supplemented group. Hence, body weight at d40 was 23.9 and 24.7 kg for the control and supplemented group, respectively ($P>0.05$). Erythrocyte abundance and haematopoiesis were not affected. Leukocyte differentials showed higher percentage of lymphocytes ($P<0.05$) and lower percentage of neutrophils ($P<0.05$) whilst total leukocyte was unaffected; a shift that indicates enhanced cellular immune response capacity and lower acute inflammation by the mixture in the diet. Indeed, higher serum IgG and IgM levels were found in supplemented piglets (both $P<0.10$). mRNA levels in distal small intestinal mucosa of tight junctions proteins, mucins, oxidative stress markers and pro-inflammatory cytokines were not altered. However, *NFE2L2* (Nrf-2), *IL10*, *DEFB1* (β-defensin) and *CASP3* (caspase-3) were all downregulated, suggesting lower upregulation of defensive and apoptotic pathways in intestinal tissue. Collectively, the supplementation with mixture of AA and condensed tannins may reduce post-weaning stress levels in piglets.

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The physiological requirement for zinc in newly weaned piglets: a dose-response trial

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From 2022, the EU bans the use of high zinc (Zn) levels in weaner diets. Hereafter the allowed dietary Zn concentration will be 150 ppm, assuming it covers the physiological requirement. However, the feed intake of newly weaned piglets is often very low, leading to our hypothesis that a diet with 150 ppm Zn is insufficient to cover the physiological requirement. We investigated pigs' Zn requirement three weeks post-weaning in a dose-response experiment including 180 pigs (28 days of age, 7.63±0.98 kg) distributed to one of six dietary Zn concentrations (155, 492, 1,024, 1,604, 2,056, or 2,419 ppm total Zn). The basal diet contained 50 ppm Zn, and the added Zn was high purity zinc oxide (ZnO). During the first week post-weaning, dietary Zn did not affect the average daily feed intake (ADFI) or average daily gain (ADG). Nevertheless, in the second and third week post-weaning Zn levels showed a quadratic effect on ADFI and ADG; 1,024 and 1,604 ppm led to the highest ADFI and ADG ($P<0.5$), respectively. The optimum dietary Zn level to support maximal ADFI and ADG was estimated to be between 1,221 and 1,354 ppm dietary Zn. Even though 2,419 ppm Zn did not result in the highest productivity, it did reduce the probability of a diarrhetic faecal score compared to 155 ppm Zn ($P<0.05$). Dietary Zn levels of 492-1,604 ppm had a similar probability for a diarrhetic faecal score as 155 and 2,056 ppm dietary Zn ($P>0.05$). At weaning, serum Zn level (which can be used as an indicator of the Zn status of the pig) was 767±9 ppb and we assumed maintaining this serum level as a minimum the first weeks post-weaning would fulfil the pigs' physiological requirement. On day 7 post-weaning, it required 1,060 ppm Zn in the diet to maintain the weaning level of Zn in the serum, while the necessary dietary Zn level to maintain had decreased to 710 and 411 ppm serum Zn on day 14 and 21, respectively. To ensure optimal feed intake, growth, and sufficient serum Zn status, a weaner diet should therefore contain 1,060-1,354 ppm total Zn when supplemented as ZnO, indicating 150 ppm dietary Zn is far from sufficient in the first period after weaning.

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Dietary supplementation of standardized citrus extract to pigs shortens their fattening period

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In swine production, feed consumption accounts for the majority of production costs. However, in a large part of the world, cost of raw materials is increasing, directly impacting the profitability of the farms. At the same time, pressure from consumers to limit the environmental impact of farming is increasing. Improving feed conversion and thus limiting the fattening period is a major challenge from an economic and environmental point of view. Feed additives such as plant extracts could be very interesting in this context. In fact, some of them such as citrus extract have already shown beneficial effect on pig performances and health. In this study, we assessed the effect of a standardized citrus extract (SCE) on slaughter pigs. The trial has been performed in a commercial farm in Switzerland. Briefly, 40 pigs were divided into two group. A control group fed with a standard diet and a SCE group fed with the same standard diet but supplemented with 150 ppm of SCE. Each group was composed of 2 replicates of ten pigs. (field conditions didn't allow to have more replicates). Pigs were individually weighted at the beginning and at the end of the trial, just before the slaughtering. Feed intake was monitored by replicate. An intermediate weight measure was performed at day 57. Results show that SCE improves the production parameters of finishing pigs. In fact, the average daily gain was higher in SCE group (877 g/day) compared to the control group (839 g/day). SCE supplementation also improves the FCR of pigs by 2.12% (2,36 vs 2,31). The days to slaughter was also reduce in the SCE group (92 days) compared to the SCE group (94 days). In conclusion, SCE supplementation on pigs increase bodyweight while reducing FCR. These zootechnical performances improvements results in the reduction of the fattening period of pigs fed with SCE by 2 days, compared to the control group. According to these data, SCE supplementation on finishing pigs seems to be a good solution in order to minimize production costs and by reducing raw material consumption.

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Preliminary study of microbial quality of animal drinking water for fattening pigs

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Water is one of the most important feeds for livestock. Despite its immense importance for the animal, the assurance of a high biological water quality is often neglected. The aim of this preliminary study was to investigate the microbiological quality of drinking water for fattening pigs in relation to the type of husbandry and to detect possible weak points. For this purpose, characteristics of the drinking water supply, like the water origin and the type of drinkers were recorded by questionnaire on 4 conventional and 4 organic farms. On the farms, a total of 24 animal drinking water samples (3 per farm) directly from the drinker were collected and analysed for the following parameters: aerobic total viable count (TVC), total coliform count, *Escherichia coli, Campylobacter* spp., *Salmonella* spp., *Staphylococcus aureus*, methicillin-resistant *S. aureus* (MRSA) and extended-spectrum beta-lactamases producing bacteria. All data were descriptively analysed and statistically evaluated by calculation of Spearman rank correlations, in order to determine possible influencing factors. The TVC showed a wide range, depending on the farm and the type of drinker, varying between 2.8 and 8.2 $\log_{10}$ cfu/ml with significant higher counts for bowl drinkers ($P<0.001$). *E. coli* and other coliforms could only be detected in bowl drinkers, whereas the value for nipple drinkers were below the detection limit (<1.0 $\log_{10}$ cfu/ml). In no samples *Salmonella* spp. were present. MRSA was detected on one organic farm and on two conventional farms. Based on the results, the type of drinker (nipple drinker vs bowl drinker) ($P<0.001$) and visually perceptible contamination ($P<0.01$) were found to be important factors influencing microbiological condition of TVC, TCC, *E. coli* and *Campylobacter* spp., which is promising with regard to improvements of the quality. Microbial quality seemed to be independent of the production system. To confirm these preliminary results, to gain more detailed information about risk factors influencing the microbiological drinking water quality and to assess measures for improvement, a larger-scale study should be conducted.

Session 36 Theatre 1

Advancing sustainable livestock systems for resilient sustainable food systems

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The Global Agenda for Sustainable Livestock (GASL) is a multi-stakeholder partnership (MSP) established in 2011. GASL facilitates sharing good practices and policies among diverse livestock stakeholders to make livestock systems more sustainable. While livestock systems are relevant to all SDGs, GASL recognizes nine SDGs and four sustainability domains with particular importance for the livestock sector: SDG 1, 2, 3, 5, 8, 12, 13, 15,17. The domains are Food and Nutrition Security, Livelihoods and Economic Growth, Animal Health and Animal Welfare and Climate and Resource Use. The 2020 virtual Multi-Stakeholder Partnership meeting (MSP) of GASL focussed on: 'Lessons from COVID-19 for building a better future through Sustainable Livestock'. While considerable diversity was evident, there were some key common elements globally: Preparedness, One Health and Planetary Health, Resilience and a Holistic Approach. The key actions identified for a sustainable livestock sector transition were providing scientific evidence, fostering sound policies, enhancing dialogue, improved communication and promotion of trans-disciplinarity. These elements have informed the focus of the 2021 Multi-Stakeholder Partnership meeting of GASL in June 2021 focused on: 'Advancing sustainable livestock systems for resilient sustainable food systems'. This theme will examine dimensions of sustainable livestock sector transformation, both in relation to the recovery from the pandemic as well as to the contribution towards the action tracks of the UN Food Systems Summit 2021. The keynote will offer an overview of the outcomes from the GASL MSP 2021 with reference to regional and global livestock sector issues as affected by the Covid-19 pandemic and a report on multi-stakeholder action towards more sustainable and more resilient livestock production systems that contribute to food systems across the globe. These dimensions will be illustrated by reference to GASL's innovative projects including a new initiative on net zero carbon dairying, the Livestock Environmental Assessment and Performance Partnership (LEAP) and others. The keynote will also formulate a set of key questions for the Livestock Farming System session.

Session 36 Theatre 2

Estimating carbon sequestration in Portuguese sown biodiverse pastures using machine learning

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Carbon (C) sequestration is one of the main ecosystem services provided by grasslands. In the Mediterranean region, sown biodiverse pastures (SBP) rich in legumes are a nature-based, innovative and economically competitive livestock production system. SBP are a mixture of up to 20 species or varieties of high-yield grasses and legumes. As a co-benefit of the increased yield, they contribute to carbon sequestration through soil organic matter (SOM) accumulation. Here, we develop models for estimating SOM concentration by combining remote sensing (RS) and machine learning (ML) approaches. We used field-measured data from six different farms collected during two production years (2018 and 2019). Sentinel-2 data was used, including 12 reflectance bands and five vegetative indexes. We used also 8 covariates (climatic and terrain variables). Multiple linear regression (MLR) and random forests models were used. In order to improve generalization of the models and enable a better estimation of the estimation error, we used a random cross-validation approach (with 10 folds). For random forest models, in order to find the hyperparameters that lead to the best performance, we used a Bayesian optimization approach. The search variables considered were: the number of trees, the minimum number of samples per leaf, the maximum depth, the error function, and the maximum number of features/inputs and using a bootstrap approach. Results showed that the random forest method led to higher estimation accuracy than MLR for all variables, on average with 50% lower root mean squared error (RMSE). The average RMSE among all folds was 2.21 g SOM/kg soil (average SOM content is 26 g SOM/kg soil). The obtained models were applied to the entire farms, leading to SOM content maps for all farms and years. From the year 2018 to the year 2019, the SOM content increased, on average, 0.75 g SOM/kg soil. This work showed that innovative methods combining RS and ML can accurately estimate C sequestration in Portuguese pasture systems as a co-benefit of livestock production.

Session 36 Theatre 3

Technical, economic and environmental performances of two contrasted dairy production systems

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An experiment was implemented in Trevarez experimental farm (Brittany, France) from 2009 to 2017 to assess the technical, economical and environmental consequences of the choice of a dairy production system. The two systems implemented were representative of the ones found in western France: one based on maize silage (S1, 0.15 ha of grazable area per cow), one rather based on grazing (S2, 0.4 ha of grazable area per cow). Both systems were run simultaneously during 8 years after final allocation of fields and dairy cows. Each involved some 60 cows and 60 hectares. In average the dairy cows from system S1 produced 8,155 kg of milk per year, from which 7,594 l were sold, with a use of 180 g concentrate per l and a feeding cost of 75 € per 1000 l sold. In average the dairy cows from system S2 produced 7,500 kg of milk per year, from which 71,590 l were sold, with a use of 127 g concentrate per l and a feeding cost of 52 € per 1000 l sold. No difference in terms of reproduction or health troubles appeared. The Farm Gross Surplus (without social costs) of the S2 system was in average 35€ per 1000 l higher than the one from system S1. Both systems were characterised by N balances and C footprints below the references from Breton dairy farms. Though the surplus of the N balance of S2 was lower by 20 kg per ha AA compared to S1 (89 vs 109 kg ha per ha AA), the net carbon footprint being close but lower for S2 (0.79 vs 0.85 meq. CO$_2$ per 1000 l milk). If they are well managed, both systems may be productive and environment friendly. Though the economic balance appears to be more favourable for the system with the greatest share of milk produced from home grown forages and in particular, from grazed grass. In our case, the difference of profit would have reached +50,000€ in 5 years for a farm producing 400,000 l milk per year. But producing more milk from grazed grass requires a non fragmented grazing platform.

Session 36 Theatre 4

Assessing the sustainability of multiple grass-fed and grain-fed beef systems in the Western USA

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Grass-fed beef sales are expected to increase globally by 40 billion U.S. dollars by the year 2025. Increased demand for grass-fed beef raises many producers' and consumers' concerns regarding product quality, economic viability, and environmental impacts that have gone unanswered. Therefore, using a holistic approach, we investigated the performance, carcass quality, financial outcomes, and environmental impacts of four typical grass-fed and conventional beef systems raised in a Mediterranean climate in the western United States. The treatments included: (1) steers stocked on pasture and feedyard finished for 128 days (CON); (2) steers grass-fed for 20 months (GF20); (3) steers grass-fed for 20 months with a 45-day grain finish (GR45); and (4) steers grass-fed for 25 months (GF25). The data were analysed using a mixed model procedure in R. Using carcass and performance data from these beef production systems, a weaning-to-harvest life cycle assessment (LCA) was developed in the SPARKS LCA model framework, to determine global warming potential (GWP), consumable water usage, energy, smog, and land use footprints. Final body weight varied significantly between treatments ($P<0.001$) with CON finishing at 632 kg, followed by GF25 at 570 kg, GR45 at 551 kg, and GF20 478 kg. Dressing percentage differed significantly between all treatments ($P<0.001$) with CON at 61.8%, followed by GR45 at 57.5%, GF25 at 53.4%, and GF20 at 50.3%. Breakeven costs with harvesting and marketing for the CON, GF20, GR45, and GF25 were $6.01, $8.98, $8.02, and $8.33 per kg hot carcass weight (HCW), respectively. The GWP for the CON, GF20, GR45, and GF25 were 4.79, 6.74, 6.65 and 8.31 CO$_2$e/kg HCW, respectively. Water consumptive use for CON, GF20, GR45, and GF25 were 933, 465, 678 and 1,245 l/kg HCW, respectively. Energy use for CON, GF20, GR45, and GF25 were 18.69, 7.65, 13.84 and 8.85 MJ/kg HCW, respectively. The results from this study indicate that varying grass-fed beef production systems in the western U.S. yield significant differences in animal performance, carcass quality and environmental and economic impacts.

Session 36 Theatre 5

Dairy farming in need of a new approach and paradigm?

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To gain economic viability is of utmost importance for farm management. In general, performance parameters are used across individual enterprises to provide guidance for strategic decisions, while undesired side effects and associated failure costs are often not considered. To deal with the complexity within farms, there is a need for new parameters to harmonize internal conflicting areas. Surveys were conducted on 32 heterogeneous dairy farms to identify the farm specific cost structure. Expenditures and income were assigned to the lifetime of the single culled cows. Cow-individual income over service life cost (iIOLC) emerged as a new parameter, used within a deductive approach to identify the reasons behind this parameter within a farm specific system and to allow recognition of individual contribution to the farm profit. The results of the farm surveys showed that the median iIOLC was negative for 59% of the farms while only 41% managed a herd with more than 50% of the culled cows making a profit. iIOLC constitutes a normative goal (paradigm) and functions as a guideline for actions needed to be taken by individual farms to sustain economic viability. Because an inductive approach based on indicators with poor explanatory power encloses a high risk for misleading interpretation and misguiding decisions, there is need for a systemic approach based on data of individual animals as reference systems. Benchmarking across farms according to iIOLC provides orientation about the current state and the production goals that need to be targeted in the future. For internal purposes, our approach enables a diagnostic procedure to identify farm specific weak points responsible for missing the targets, and farm specific measures required to gain economic viability. Simultaneously, iIOLC serves as a reference to validate efficiency when implementing the identified measures. The results indicate that a strategy to further increase productivity while fading out negative side effects such as animal health and welfare which result in short productive lifespans of dairy cows can lead to negative marginal profits.

Session 36 Theatre 6

Livestock disease resilience: from individual to herd level

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Infectious diseases are a major threat to the sustainable production of high-producing animals. Control efforts, such as veterinary vaccines or breeding approaches often target improvement of animals' resilience to infections, i.e. they strengthen the animals' ability to cope with infections, rather than preventing infection per se. Alerted by increasing evidence for the contribution of asymptomatic individuals (that is, individuals who become infected and are infectious, but don't develop symptoms, i.e. are highly resilient) to the overall health and production of the population, we strongly advocate a shift of focus from increasing the disease resilience of individual animals to herd disease resilience as the appropriate target for sustainable disease control in livestock. Herd resilience not only captures the direct effects of vaccination or host genetics on the health and production performance of individuals, but also the indirect effects on the environmental pathogen load that herd members are exposed to. The latter is mediated both by individuals' susceptibility to infection and by characteristics (magnitude of infectiousness, duration of infectious period) that influence pathogen shedding from infected individuals. We review what is currently known about how vaccination and selective breeding affect herd disease resilience and its underlying components, and outline the changes required to improve herd resilience. To this purpose we also seek to clarify and harmonise the terminology used in the different animal science disciplines to facilitate future collaborative combined approaches to infectious disease control in livestock.

Session 36 Theatre 7

Monitoring emissions of the intensive livestock systems in Northern Italy: the Mitigactions project

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Mitigactions project (Regione Veneto PSR 2014-20 misura 16 DGR n° 736/2018) aims to assess greenhouse gases and ammonia emissions related to the main livestock systems (dairy and beef cattle, pigs, and poultry) of the Veneto region (Northern Italy, responsible for nearly 80% of the national livestock production) and to identify feasible mitigation options. This contribution reports some preliminary results related to the emissions (Life Cycle Assessment) and the conversion ratio of crude protein (CP) of the whole diet (CPCR) or of the potentially human-edible portion of the diet (HeCPCR) into food protein. Data originated from 77 farms, belonging to the following systems: 28 dairy (120±110 cows), 17 young bull (689±291 places/farm), 17 pig (with breeding sows, 1,200±720 animals, and/or fattening heavy pigs, 3,255±1,996 places/farm) and 15 chicken broiler (39,492±26,648 places/cycle) farms. Impact categories were global warming (GWP), acidification (AP), and eutrophication (EP) potentials. Functional unit was 1 kg of CP in the product. Protein conversion ratio was computed as CP in feeds per 1 kg edible CP in the product (CPe). Mean GWP, AP, and EP per 1 kg CP ranged from 15.4 to 135.8 kg CO_2-eq, 0.3 to 1.7 kg SO_2-eq and 0.2 to 0.6 kg PO_4-eq, respectively. The poultry meat showed the lowest values and beef meat the greatest ones in all the impact categories, whereas milk and pork meat had similar results, two-fold greater than poultry. The large variability observed for GWP was due to enteric methane (43 to 60% of whole impact in cattle systems). Feed production was the biggest contributor for all the livestock systems (29 to 76%, 30 to 64% and 64 to 72% of GWP, AP and EP respectively). The production of 1 kg CPe required from 3.3 to 15.1 kg of dietary CP, of which from 25 to 82% was potentially human-edible. Poultry meat showed the best values in terms of CPCR whereas milk in terms of HeCPCR. The different emission values and the rank of the livestock systems in terms of emissions and CP conversion ratios suggest that the emission mitigation options should be specific for each livestock system and oriented to maintain production efficiency.

Session 36 Theatre 8

Modelling economic performance and greenhouse gas emissions in dairy calf to beef production systems

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The abolition of EU milk quota and subsequent expansion of the dairy herd has resulted in a greater proportion of beef derived from the dairy sector. Future beef consumption in the EU is predicted to be greater than indigenous production (EC, 2015) and so beef sourced from dairy production systems are expected to play an important role in meeting future demand within the EU. Subsequently, the economic and environmental performance of dairy calf to beef systems are now under scrutiny. An evaluation of economic and environmental performance from early maturing and Holstein-Friesian origin sired steers on high and low input systems was undertaken. A hybrid modelling approach combining both the Grange Dairy Beef Systems Model (GDBSM) and the Beef Greenhouse Gas Emissions Model (BEEFGEM) was used. Data was entered into the whole farm simulation model GDBSM to determine key system parameters including feed budget, farm structure, area, herd size and beef output per year. Output variables from the GDBSM model were used as the inputs into the BEEFGEM model which consequentially estimates the GHG emissions for the system being simulated. Variation in beef and concentrate price along with the proportion of grazed grass in the animal diet were found to be the key drivers of profitability. Low input systems were found to be most sustainable. Finishing dairy steers at pasture at 20 months of age represents the most profitable system with moderate GHG emissions.

The nitrogen footprint assessment on South Korean intensive dairy farms

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The surplus of on-farm nitrogen (N) inputs is one of the global challenges worldwide which threatens the sustainability of livestock farming systems and the environment. Dairy cattle production is considered one of the major contributors to the N surplus in the livestock sector. The nitrogen footprint (NF) was proposed as a metric of nitrogen pollution. This study aimed to assess the NF of 1 kg of fat and protein-corrected milk (FPCM) in twelve dairy farms (n=12; average animals number = 135±46; average area = 3±1.3 ha) in the most productive province (Gyeonggi-do) in terms of milk production in South Korea. NF was estimated using country-specific N and the life cycle assessment approach. The average NF of the evaluated farms averaged 4.62 kg N/kg of FPCM. The NF of milk was dominated by ammonia (NH_3-N) at 72% of the total NF of milk. The second main contributor was N loss to water at 23% because of the high dependency on a bought feed from off-farms. Losses as N emissions from nitrous oxide (N_2O-N) and NOx-N represents 3 and 0.2%, respectively. Over the whole NF, the dominant contributor from Korean dairy farms was the manure management system at 81% (mostly from housing and treatment stages) of the total NF, followed by feed production at 5%, manure spreading (11%) and energy-related emissions at up to 3%. Dairy farms in South Korea have been changing to large intensive housed systems and this study aligns with other studies, indicating an improvement in productivity through increased per-cow milk production associated with low NF of milk. We suggest that simplified and standardized NF methodology should be used on dairy farms in South Korea to help identify opportunities for improvements in nutrient management decisions especially N surplus.

Grazing systems performance across increasing levels of sward biodiversity in non-fertilised swards

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While permanent grasslands receiving low fertiliser inputs account for the largest area of lowland managed under the agri-environment schemes they currently provide only minimal benefits for biodiversity or ecosystem services and have low value for livestock production. In 2008 a multi-factorial experiment was established on an experimental grassland in Devon to develop simple, low-cost options that both enhance biodiversity and ecosystem services. We established three seed mixtures differing in the key groups of plants they contained ('grass' only (G), 'grass & legume' (GL), and 'grass, legume & forb' (GLF)) under contrasting management practices and establishment options. Paddocks were grazed on the four block and stocked with 12 groups of beef cattle during the grazing seasons from 2009 to 2012. The number of animals and duration over which the swards would support livestock were recorded for each of the separately fenced groups of sub-plots on each sown seed mix. Grazing days was calculated as the accumulated daily stocking rate throughout the grazing period. Cattle were weighed periodically. ANOVA was ran using Genstat® software. Fewer grazing days ($P<0.001$) and lower stocking rates ($P<0.001$) were supported on the unfertilised grass (G) than the GL and GLF plots in 2009 (425 vs 526 d; 3.8 vs 4.7 calle/ha) and 2012 (502 vs 605 d; 3.5 vs 4.3 cattle/ha) and a trend was observed in 2010 ($P=0.09$, 992 vs 1,232 d; $P=0.08$, 5.9 vs 7.2 cattle/ha) (statistical comparisons were not possible in 2011 as there was no error term). In 2011, daily liveweight gains were 1.01, 1.04 and 1.06 kg/d ($P=0.867$), whereas in 2012 were 0.78, 0.81 and 0.74 kg/d ($P=0.480$), for G, GL and GLF, respectively. In general, the inclusion of legumes (GL) or legumes and forbs (GLF) in the swards supported more grazing days and mean daily stocking rates in all four years. Although more expensive (grass only = £100/ha vs £140-230/ha) the addition of legumes and non-legume forbs to seed mixes resulted in swards that could be grazed for longer periods of time and with a greater carrying capacity.

Session 36 Theatre 11

Modelling pathways of slurry from livestock production facilities into the environment

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Antibiotic consumption has been successfully reduced in German livestock production, but as required by the German Animal Welfare Act, diseased animals require treatment. Therefore, the excretion of antibiotic residues as well as the development of antibiotic resistant bacteria in slurry fail to be completely eliminated in livestock production. Pathways gain importance to: (1) interpret antibiotic and resistant bacterial loads found in slurry; and (2) develop realistic, feasible measures to reduce the risk of spreading these undesirable loads when applying slurry. Hence, the aim of the study was to model the common pathways of slurry from livestock production. The study was conducted in a two-step approach: First, possible pathways and classifications were generated by performing structured interviews (n=5). Second, results of these interviews were used to set up an online survey with 22 semi-closed questions. The link was distributed among participants of an online animal fair (EuroTier 2021). The survey was opened by 406 participants, while 160 started to answer and 65 participants finished all questions. Farms were classified as specialized farms when they mentioned only one animal species and production branch (14.5%), while farms with several animal production branches and/or animal species were considered unspecialized (85.5%). Predominantly specialized farms build as a whole had simple and straight pathways (excretion to slurry pit, to storage tank, to application). Besides that, the majority of farms has grown in size and production capacity over decades and were characterized by complex, very unique pathways resulting in pools of organic material from different stables, production branches or even different animal species. Due to technical reasons at least 10% of the slurry cannot be pumped out and remain in the storage tank usually. In conclusion, the remaining proportion might explain findings of persistent antibiotic residues and antibiotic resistant bacteria, particularly when no antibiotic usage has been reported for the previous months.

Session 36 Theatre 12

Livestock practices of health monitoring in organic farms: useful to think agroecological redesign

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Agroecological principles for design of agroecological livestock systems highlight issues of resilience i.e. capacity of animals and livestock systems to absorb different types of perturbations. Health troubles are examples of possible perturbations and health management is an example of risk management. In addition, organic agriculture is a prototype for agroecological systems with: low-input objectives and limitations of medical inputs by organic specifications. Therefore, studying health practices of organic farmers can provide good examples of possible design or redesign of agroecological systems. Our objective was to analyse how do organic farmers deal with sanitarian risk when monitoring health status of animals and herd. Datas of this study come from semi-structured qualitative surveys with farmers from 3 areas of France: Western crop-livestock farms (plains) with cattle, sheep and goat herds, Eastern crop-livestock farms (plains and mountains) with cattle herds, South-East crop-livestock farms (mountains) with sheep herds. Herds were of dairy and meat production. Surveys were focused on farmers' practices and representations of animal monitoring. A focus was made on small tips that facilitate their observations. Results consist in identification of key-periods of their daily work used by farmers to organize their observations. Secondly, different levels of observation were used by farmers: from more individual to more herd-level ones. Finally, three types of tips to facilitate herd observation were identified: some referring to tactical choices, other were more systemic and the last ones to the capacity of communication inside and outside the farm. As a conclusion, these results contribute to a better understanding of farmers' capacity to adapt their practices to local agrobiodiversity and to pilot their system by using resources heterogeneity, instead of applying generic recipes, monitoring of herd health status as an example of animal heterogeneity monitoring.

Blue water productivity in beef farms in two Italian northern regions

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Agriculture is the human activity that consumes most of the fresh water available: 84% of the cultivated land uses rainwater, the remaining 16% uses irrigation water, and 70% of the water withdrawn is destined for irrigation, with a large country-to-country variability (HLPE, 2015). The water consumed to produce1 kg of beef in Italy accounts for about 11,500 litres, of which 13% of this is blue (mainly for irrigation) and grey (polluted), and 87% green (precipitation). Blue water is critical, because it is object of competition with other human activities and with environmental requirements. Crops differ substantially in blue water consumption; for example, Autumn and Winter crops do not require irrigation, unlike Summer crops. Therefore, forage system is one of the main drivers influencing blue water consumption. In the framework of LIFE BEEF CARBON project, the blue water productivity (BWP) of 20 specialized fattening beef farms in Piemonte and Veneto regions was estimated. The blue water consumed embodied direct and indirect water for crop production and water required for animal drinking and care and did not consider the water necessary to grow the replacement calf. The functional unit was the live weight gain (LWG). Blue water productivity (BWP) is the ratio between the blue water consumed (direct + indirect) for 1 kg of LWG; direct consumption refers to the water used on farm and indirect water is that associated to purchased feeds. Direct blue water for irrigation was estimated with CROPWAT software, version 8 by FAO. Production of crop and meat, climatic data, evapotranspiration, and blue water consumption were estimated for 2016 and 2018. Data were analysed with R software version 3.4 (R Development Core Team, 2008). The BWPs (l/kg LWG) were no significant different between 2016 and 2018; on the contrary, analysis showed a significant difference ($P<0.05$) when comparing the following forage systems: Summer crops (S); Summer and Winter crops (SW), and Summer, Winter, and meadow (SWm); the BWPs were 969±444; 960±441; and 980±459 for farms with S, SW, and SWm crops, respectively. The activity was funded by European Commission area Environment, Project LIFE+: BEEF CARBON (LIFE14 CCM/FR/001125).

Milk urea concentrations in different dairy cattle breeds

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With 94% agriculture is the major source of ammonia emissions in Switzerland. Out of these 70% are produced by cattle farming. Various within-breed studies revealed that milk urea concentration is predictive for the quantity of nitrogen excreted in the urine and the resulting ammonia emissions. However, milk urea concentration depends on various factors. Studies investigating the effect of the breed are sparse. Therefore, we investigated milk urea (mg/dl) from routine milk recordings (years 2014-2018) in Braunvieh (BV), Holstein (HO), Jersey (JE), Original Braunvieh (OB) and Swiss Fleckvieh (SF) dairy cows. To control the effect of feed, only data from Braunvieh farms with two or more breeds were considered. Milk urea results from 11,104 dairy cows (BV-HO=9,415; BV-JE=836; BV-OB=724; BV-SF=129) with totally 27,151 standard lactation yields (BV-HO=22,943; BV-JE=2,008; BV-OB=1,827; BV-SF=373) were used. The relationship between the milk urea concentration and breed was investigated using a linear mixed model for each breed-comparison. The model included the fixed effects breed, number of lactation and milk yield, as well as farm and animal as random effects. The mean milk urea concentration within breed was 25.21 (BV-SF) to 26.80 (BV-JE), 22.83 (HO), 24.10 (JE), 24.76 (OB) and 21.39 (SF) mg/dl. In all models, the BV breed exhibited significantly higher milk urea concentrations than the other breeds ($P<0.001$). The estimated fixed effect was 4.21, 1.75, 1.18 and 3.89 mg/dl for BV-HO, BV-JE, BV-OB, BV-SF, respectively. A significant positive effect was found between milk yield and milk urea concentration in all four models (BV-HO=0.44; BV-JE=0.46; BV-OB=0.60; BV-SF=0.87 in mg/dl per 1000 kg milk yield). In addition, in the models BV-HO, BV-JE and BV-OB, significantly higher milk urea concentrations were found for cows in the first lactation in comparison to cows in higher lactations. These results make clear that, in addition to feed, other factors such as breed have an important effect on milk urea concentration. This needs to be considered if urinary nitrogen excretion is calculated based on milk urea concentration.

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The effect of teat disinfectant ingredients on teat skin bacteria associated with mastitis

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Teat disinfection is an important step in the control of mastitis within a dairy herd. The objective of this study was to evaluate the effectiveness of 96 commercially available teat disinfectant products in Ireland against bacterial isolates on teat skin. Teat disinfection products were applied to the teats of seventeen Holstein-Friesian cows, which were housed indoors. A split-udder model was used where one cow received two different teat disinfection products on each day. A composite swab sample was taken of the left teats and the right teats before and after teat disinfectant application. Swab samples were plated onto 3 different selective agars to enumerate bacterial counts of streptococcal, staphylococcal and coliform isolates. Streptococcal isolates were the most prominent bacterial group recovered on teat swabs taken before the application of a teat disinfection product (55.0%), followed by staphylococcal isolates (41.3%) and coliform isolates (3.7%). Products were reclassified by active ingredients (n=9) for analysis. These ingredient groups included; chlorhexidine, chlorine dioxide, diamine, iodine, iodine and lactic acid, lactic acid, lactic acid and chlorhexidine, lactic acid and hydrogen peroxide, and lactic acid and salicylic acid. The ingredient group, chlorine dioxide, resulted in comparable reductions to the iodine group for streptococcal isolates. The ingredient group, iodine combined with lactic acid, resulted in the greatest reduction of staphylococcal isolates. When observing products individually, a product containing 1.6% w/w lactic acid combined with hydrogen peroxide was the most effective at reducing streptococcal isolates on the teat skin, whereas a product containing lactic acid combined with 0.6% w/w chlorhexidine was the most effective against staphylococcal isolates. Minor differences were observed regarding the relationship between effectiveness and active ingredient concentration between products. This study suggests that some teat disinfectant products achieve a higher reduction in bacterial levels against different specific bacterial groups on teat skin than other products. Therefore, when choosing a teat disinfectant product, the bacteria in the dairy herds' environment should be considered.

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Comparison of different methods for the evaluation of teat disinfectant products

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There are many test methods used to determine the effectiveness of teat disinfectants. The objective of this study was to determine the outcome from the use of three different test methods (BS EN 1656 [laboratory], disc diffusion [laboratory] and an experimental challenge teat swabbing method [in-field]) when evaluating the effectiveness of ten disinfectant products containing different active ingredients of varying concentrations. Products were tested against three bacterial strains (*Escherichia coli* [ATCC® 10536™], *Staphylococcus aureus* [ATCC® 6538™] and *Streptococcus uberis* [ATCC® 19436™]). All products satisfied the requirements of the BS EN 1656 method achieving >105 log reductions. Two laboratory methods were consistent in identifying effective products against *S. aureus* and *E. coli*, whereas, all three methods were consistent in identifying effective products against *S. uberis*. Within the two laboratory methods, products containing 2% lactic acid combined with 0.3% chlorhexidine gluconate and 5% lactic acid combined with 0.3% chlorhexidine were within the top 4 most effective products against *S. aureus*. For *S. uberis*, products containing 2.4% lactic acid, 0.29% iodine combined with 0.8% lactic acid and 0.5% iodine (control) were within the top 4 most effective products. This study has established that teat disinfectant products can be screened using different test methods.

Differences in nutrient use efficiency and food-feed competition among Chinese dairy farms

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With rapid intensification and a decoupling of animal and crop production, Chinese dairy farmers are increasingly relying on the import of high-quality feed, while facing difficulties to return the animal manure to the fields. This leads to high environmental losses of nutrients such as nitrogen (N) and food-feed competition. To provide guidance towards a sustainable dairy sector within a circular food system, this study explores differences in N use efficiency (NUE) and food-feed competition between major farm types in North-Central China. From October to December 2020, a farm survey was conducted among 48 dairy farms in Henan. Herd size ranged from 115 to 4,289 heads and 52% of the farms were landless. Data on farm characteristics were collected to calculate the NUE and human-edible protein conversion ratio (HePCR). NUE was defined as the ratio of aggregated N inputs and outputs (i.e. meat and milk) at farm level, while NUEM also considered a fraction of 48% of the N in exported manure as a valuable output, to account for the amount that ended up in crop products when used as fertilizer. HePCR was defined as the amount of protein in feed that is potentially edible for human over that in milk and meat. Three major farm types were identified by cluster analysis, varying in herd size and milk yield. Typologies include (A) large-scale farms (≥1,309 heads) with high yielding cows (9.5 ton/cow/yr); (B) small and medium-scale farms (170-1,022 heads) with medium to high yielding cows (8.6 ton/cow/yr); (C) small-scale farms (≤485 heads) with low yielding cows (5.2 ton/cow/yr). Preliminary results showed that the average NUE was 19% (A), 20% (B) and 14% (C). The average NUEM was 29% (A), 30% (B) and 34% (C). The HePCR varied from 0.9-3.1 for typology A, from 0.4-3.2 for B, and from 1.1-4.5 for C. Overall, farm type B performed better both in terms of NUE and feed-food competition, mainly owing to its moderate concentrate usage and relatively higher milk yield. Differences in NUE and NUEM show the importance of recycling manure by recoupling animal and crop production. With most of the farms having a HePCR>1.0, effort should be made to improve utilization of by-products and human-inedible biomass as dairy cattle feed.

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Estimation of carbon footprint in Iberian out-door pig farms

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The livestock sector contributes significantly to global greenhouse gas (GHG) emissions. In Spain, the pig sector ranks first among livestock productions and its economic importance reaches 39% of the Final Livestock Production. At present, the reduction of GHGs and the ecological footprint of the livestock sector must be a priority objective in order to meet the food needs of the world's population in a context of population growth and climate change. This case study used the Life Cycle Assessment (LCA) methodology to calculate the balance of GHG emissions, both carbon footprint (CF) and carbon sequestration. Four pig out-door production systems were analysed, two organic and two non-organic, all located in Dehesa in the southwestern region of Spain. The calculation of CF was carried out following the IPCC 2006 and 2007 guidelines for national greenhouse gas inventories, adapting it to the characteristics of out-door livestock systems. For the calculation of carbon sequestration in this case, the livestock-manure-grassland system proposed by Petersen in 2013 was adapted, with a time horizon of 100 years. The system boundaries were the so-called cradle-to-gate and the functional unit used was kg CO_2 eq/ha of total area. The results show the existence of differences in emissions between organic and non-organic farms. In organic farms, the results were 249.83 kg CO_2eq/ha for 'organic Iberian pig montanera fattering farm' and 502.35 kg CO_2eq/ha for 'organic Iberian pig closed herd', while in non-organic farms emissions increased, with 743.81 kg CO_2eq/ha for 'non-organic Iberian pigs montanera fattering farm' and 591.67 kg CO_2eq/ha for 'non-organic Iberian pig closed herd'. Likewise, the results for the estimation of carbon sequestration show differences between systems. In 'organic Iberian pig montanera fattering farm' the total kg CO_2eq fixed per ha and year reached 485.43 kg CO_2eq/ha and for 'organic Iberian pig closed herd' 575.43 kg CO_2eq/ha. While for non-organic farms the values decreased, with 353.00 kg CO_2eq/ha for 'non-organic Iberian pig montanera fattering farm' and 287.20 kg CO_2eq/ha for 'non-organic Iberian pig closed herd', which represents a considerable carbon offset with respect to the emissions caused.

Analytical methods to formulate blend of rich-saponin plants for reducing ammonia emission in farms

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Diet supplementation with saponin-rich plants is a well-known solution to reduce ammonia emission, especially *Yucca schidigera*. However, *Y. schidigera* is collected from the wild and its slow development make it an endangered natural resource that tends to be an expensive solution. Alternative solutions, economically and environmentally improved, appear to be necessary to reduce ammonia emission in fattening pigs' farms. In this study, we formulated a commercial blend of saponin-rich plants consisting of four saponin plants (Norponin Opti®, Nor-Feed) by following the analytical properties of saponins: total saponin content and ammonia binding capacity (ABC_{50}). The new formula, constituted of steroidal and triterpenoid saponins, has a total saponin content of 7.9% and an ABC_{50} of 3.0 compared to *Y. schidigera*: respectively 7.9 and 3.4. To evaluate the link between the analytical methods and the reduction of ammonia emission in farms, a trial on fattening pigs was performed. Briefly, 468 pigs (10 weeks old/30 kg) were divided into three groups. A CTL group fed with a standard diet, a Yucca group fed with a standard diet supplemented with 120 ppm of *Y. schidigera* and a OPTI group fed with 120 ppm of the commercial blend of saponin-rich plants. Measurements of ammonia were assayed before and during the 4 weeks of supplementation. Results show that Yucca group and OPTI group limit ammonia production along time by respectively -22 and -41% compared to the CTL group ($P<0.01$). Moreover, the OPTI group appears to be twice more efficient 41 vs 22% ($P<0.0001$) than the Yucca group. The results correlate with the data obtained with the analytical methods. In conclusion, the use of total saponin content and ABC50 methods are interesting tools to estimate the potential efficiency of saponin plants for reducing ammonia emissions in farms. The commercial blend of saponin-rich plants shows better efficiency than *Y. schidigera*. This results could be explained by a higher structural diversity of saponins in the blind than *Y. schidigera*. However, further studies are needed to confirm the observed efficacy and structural diversity of saponins.

Does mixing cattle with broilers on pasture yield any benefits?

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Combining poultry production with grazing of other species might offer several advantages: Farmers could benefit from having another option for using their grassland while challenges like parasitic load and predation might possibly be tackled more efficiently. We aimed to explore potential benefits and challenges arising from the combination of young cattle and broilers in a pasture-based production system, i.e. does keeping young cattle and broilers together affect the ranging behaviour of broilers and does it lower the losses in broilers due to predators? In addition, we hypothesized that cattle will benefit from this combination due to a lower parasitic burden. We used two systems in the following set-up: The first system consisted of one group of broilers (n=55-60, ISA JA 757) assigned to the same pasture as ten young cattle (German Holstein, 10-13 months of age). In the second system the same number of broilers had access to a pasture that had been grazed by ten young cattle two weeks before. Both systems were embedded in a rotational grazing system with access to a new paddock every week. After six weeks each experimental round concluded with the slaughter of the broilers. Behavioural observations were conducted twice per week in both systems parallelly. Faeces of the cattle were sampled every two weeks. In total, seven replicates are going to be conducted from 2018-2021. So far, each round showed lower losses of broilers (median, minimum – maximum) in the system where broilers and cattle had access to the same pasture (1; 0-3) compared to the separated grazing system (4; 2-5). Furthermore, the proportion of broilers ranging the pasture during observation periods was higher in the combined system (mean ± SD: 20.72±13.81 vs 14.87±9.72%/h). However, no broilers were seen to scratch at the cattle dung pats, and parasitic egg counts in faeces of cattle did not differ between the systems. These preliminary results indicate that cattle might provide protection to broilers, while the presence of broilers has no effect on infections by intestine parasites in cattle.

The sheep performances in a grassland-based system is improved when combined with beef cattle

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The specifications and the high price of feed in organic farming require implementing forage fattening. Based on the principles of agroecology, we hypothesized that the livestock farming system performance (animal productivity, environment and level of inputs) would be improved by associating beef cattle and sheep, allowing better valorisation of forages and parasitism control for the sheep. Three suckler farming systems (cattle (CAT), sheep (SH), mixed sheep-cattle (MIX)) were managed for 5 years into separate farmlets in INRAE Herbipole experimental farm, in order to test this hypothesis and the feasibility of producing beef and sheep-meat with only grass in a context of permanent grassland at 1000-1,300 m asl. In order to favour the production of finish grass-fed meat with young animals, we used crossbreeding between hardy breeds (Limousine ewes, Salers cows) and early maturing breeds (Suffolk rams, Angus bulls). Calving and lambing periods were adjusted to grass growth in order to optimize grazing. Young cattle were kept with their dam at pasture, weaned at 8-9 months in October, fattened indoors with hay and haylage-silage and slaughtered between 12 and 15 months. The results showed that MIX sheep took advantage of the association, with a lower use of concentrates and anthelmintics and a better lamb growth rate compared to SH enabling 100% of the lambs to be exclusively fattened on pasture. The complementarities in foraging behaviour and the lower parasitism level are underlying explanations. Despite a satisfactory degree of fatness, the carcass of young cattle were considered too light by the industry (230-280 kg), with poor price. The high need for conserved forages for beef fattening in winter, coupled with droughts, has generated additional costs through the purchase of hay. In the MIX system, the higher sheep performance and the lower concentrate input led to lower GHG emissions and energy use for sheep, compared to SH (-12.6% EqCO_2/kg LW and -16.9%MJ/kg LW); it also resulted in a 15% higher income per sheep livestock unit, in MIX vs SH.

French emission factors of TSP, PM10 and PM2.5: results of field measurements

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Attention paid to indoor air quality in pig housing increases due to human and animal health but also to environmental aspects in relation with ammonia. Recent evolutions of European Regulation tend to increase the PM emissions issue in the restrictions imposed on pig farms. Due to Emission Factors variability observed in literature, a project has been set up in order to establish French emission factors for TSP, PM10 and PM2.5 in relation with different technical practices. The first stage of the project was to develop an adapted protocol to French national conditions of pig breeding. The second stage was to implement it on pig farms during two contrasted climatic periods (hot vs cold) to establish emission factors for total suspended particles (TSP), PM10 and PM2.5. In the 30 selected piggeries two forms of feed presentation (pellets vs liquid feed) were combined with two slurry management modes (pit storage vs scraping). Indoor PM concentrations were measured using an optical spectrometer (Grimm Mini-LAS) during a 24 h period. In parallel, over parameters (ambient and outdoor temperature, ventilation rate, number and weight of pigs, etc.) were recorded for the calculation of emissions. For all fractions, concentrations measured during the warm period were significantly lower than those during the cold period, while emissions were significantly higher. The ratios of PM2.5, PM10 and TSP were not influenced by feed presentation or slurry management modes. However, concentrations and emissions were significantly lower for rooms that combined liquid feeding and slurry scraping than for rooms with other combinations.

The effect of litter type on broiler performance, litter quality and ammonia concentrations

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Since the ban on the use of animal products and antibiotics in animal feed in the EU, poultry farms have been experiencing problems with wet litter. Drier litter leads to lower ammonia (NH_3) emissions and reduces the formation of foot lesions. This trial studied the effect of litter type on performance, litter quality and NH_3 concentrations at litter level. Six litter materials were tested: wood shavings, flax, peat, maize silage, wheat straw and flax pellets. The feeding program consisted of a starter (d0 to d10), a grower (d10 to d28) and a finisher (d28 to d39). 2160 Ross 308 male broilers, divided over six treatments received the same diets with a crude protein content of 20.5, 19.5 and 18.5% during each phase, respectively. Each treatment was replicated six times. For the total period, highest feed intake ($P<0.001$) and body weight ($P=0.011$) was obtained for birds housed on peat. There was a significant treatment effect for growth ($P=0.035$) and feed conversion ($P=0.020$), however, the post-hoc analysis showed no further distinction between the treatments. Peat had a lower moisture content than wood shavings ($P=0.008$) at d38 and neither differed significantly from the other treatments. Peat, maize silage and flax pellets received the best litter quality scores at d38 ($P<0.001$). However, NH_3 concentrations was highest for peat and wheat straw ($P=0.024$). Birds kept on wheat straw and wood shavings had the highest incidence of foot lesions at d38 ($P<0.001$). The higher density of these materials (and therefore thicker litter layer) reduced bathing and scratching by the birds, which may have led to wetter litter and the formation of a crust on top. These conditions are conducive to the formation of foot lesions. The higher NH_3 concentrations for peat may be due to its thinner layer, lower moisture content and the absence of a crust on top of the litter through which NH_3 can volatilize more easily.

Entomoponics: combining *Tenebrio molito*r production and greenhouse vegetable production

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Mealworm production systems are generally monocultures where the insects are kept in an enclosed environment with a stable climate (e.g. 27 °C, 60% RH) to maximize production and prevent an infestation with plague insects. To maintain these conditions, ventilation, heating and cooling are necessary which may result in high operational costs for mealworm producers. High-wire greenhouse cultivation might pose an opportunity. They are heated to a certain extent and most components in high-wire systems are suspended well above the greenhouse floor. This results in plenty of available heated space where mealworms could be reared. Moreover, pruning waste and aesthetically declined fruits could serve as a wet feed for the mealworms. However, the climate in greenhouses still tends to follow a diurnal and seasonal pattern of which the influence on mealworm growth is unknown. To examine the feasibility of combining both production systems, mealworms were reared simultaneous in two locations: in a climate room and below the gutters of a cucumber high-wire cultivation system during the summer of 2020. 4 different wet feeds were compared: whole and mashed cucumber pruning, tomatoes and agar-agar, the latter serving as a control. Growth was monitored and 10 weeks after the start of the experiment the mealworms were harvested and the yield was compared among treatments. The growth in the greenhouse was on average only 8.1% slower than growth in a climate room even though the average ambient temperature in the greenhouse was lower (22.8 instead of 27 °C) with a higher variability (between 18 and 37 °C). This indicates that mealworms require less narrow temperature ranges than is generally assumed. As long as the average inner crate temperature is near optimal, they tolerate deviations (to a certain extend) around this average. Of the wet feeds only whole leaves did significantly worse than the control and tomatoes even outperformed the control significantly. This indicates that the larvae can grow well on greenhouse residues. Combining mealworm production and greenhouse high-wire cultivation can act as a way to valorise greenhouse residues, unused heated space and could be a way to cut on major operational costs of common mealworm production.

Industrial symbiosis in insect production – a sustainable eco-efficient and circular business model

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Insect meal (IM) is a source of high-quality protein for aquafeed while insect oil (IO) is a source of fatty acids used in monogastric feed with identical or better performance than premium fishmeal (FM) or vegetable oils (VOs) respectively. Although insects' ability to feed on agricultural by-products and the entire valorisation of insect products (IM, IO, frass) suggest insect production is sustainable, very few studies have documented its environmental impact using industrial-scale production data. In this context, the attributional life cycle assessment (A-LCA) was conducted based on data from an industrial-scale facility implementing an innovative symbiosis production model. This A-LCA was used to: (1) assess the environmental performance of the symbiosis model vs a no-symbiosis model; and (2) compare the environmental impacts of IM and IO production vs their respective alternatives. The results revealed that the symbiosis model introduces a meaningful change in terms of environmental footprint by reducing CO_2 emissions by 80% and fossil resources depletion by 83% compared to the no-symbiosis model. The higher sustainability of the IM and IO produced using the symbiosis model was also demonstrated, as CO_2 emissions were reduced by at least 55 and 83% when compared to the best FM and VOs alternatives, respectively.

ValuSect: valuable insects

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The global population is growing and expected to reach 9 billion by 2050, while resources are decreasing. The North-West Europe (NWE) area is a very densely populated area characterized by intensive agriculture, so it is necessary to find sustainable alternatives for food resources. The Interreg NWE ValuSect project has a consortium consisting of 10 full (Thomas More Kempen vzw, Inagro vzw, Aberystwyth University, Zürcher Fachhochschule, Stichting Fontys, BB projects, Teagasc the Agriculture and Food Development Authority in Ireland, New Generation Nutrition Pro-Active, AliénorEU and BIC Innovation Limited) and 7 associated partners (Flanders Food, BIIF, Greenport Westholland, Eurasanté, Pole Valorial, Food Processing and the Welsh Government) from 7 countries in the NWE area. ValuSect enhances the innovation performance of enterprises throughout NWE regions. The focus will be the development of a sustainable transnational Accelerator program including all partners with expertise on insects, food production, innovation and commercialisation to support and cooperate with enterprises. Three insect species (e.g. *Tenebrio molitor*, *Acheta domesticus* and *Locusta migratoria*) are considered in this project. The knowledge will be transferred by pilot demonstration and real life testing in close collaboration with end consumers via open/thematic calls for cases (vouchers for SMEs). During the duration of the project 40 cases from companies/SMEs will be selected and performed. The next call for cases will be autumn 2021 and the knowledge platform is under construction. The accelerator program will be sustained after the project. This will increase the innovation levels of enterprises in the food industry in the NWE Area and will accelerate the creation of a consumer market for sustainable insect food applications in the long term.

Bioavailability of zinc, iron and calcium in cereal porridge fortified with cricket powder

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Globally, there is growing interest to integrate nutrient-rich insect-based meal (flour) into food products to combat food and nutrition insecurity. Though, finger-millet-meal porridge is widely used for infant feeding in many sub-Saharan African countries, there is lack of information on its enrichment with readily available edible cricket *Scapsipedus icipe* (Hugel and Tanga). Therefore, the current study evaluates the nutritional, antinutritional and mineral bioavailabilty properties of five traditionally processed nutrient-dense porridge products: fermented FM-A and CM [FFM-ACM]; Roasted FM-A and CM [RFM-ACM]; germinated FM-A and CM [GFM-ACM); Unprocessed FMA and CM [UPFM-ACM] and existing commercial porridge (FAMILA). Finger millet-meal porridge enriched with cricket flour had higher protein (2-folds), fat (4-folds) and energy (1.1-1.2-folds) compared to FAMILA. The stearic, linolenic and palmitoleic acids were higher in finger millet-meal porridge fortified with cricket compared to commercial porridge. Blended formulation increased energy and nutrient densities compared with the control ($P<0.05$). Processing by germination resulted in high phytic acid degradation (66%) followed by fermentation (33%) which led to improved mineral bioavailability. Total flavonoids significantly reduced during germination and roasting but increased during fermentation. Tannin content decreased during germination but increased during roasting and fermentation. Our findings revealed that processing techniques of low-cost fortified porridge can potentially have a significant effect in reducing antinutrients and improving iron, zinc and calcium status in complementary sustainable foods.

Precision insect farming: heatmaps to monitor growth and behaviour of *Tenebrio molitor* larvae

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The Precision Insect Farming (PIF) research project at VIVES focuses in the first place on monitoring the rearing process of the mealworm (*Tenebrio molitor*) using temperature. The monitoring of the temperature which are not always visible at the surface in the rearing boxes can reveal certain behavioural patterns, important for the optimal and sustainable insect production. These patterns can be related to feeding state, mortality, developmental stage, etc. Moreover, the growth of the larvae could be monitored using temperature, given that a higher mass and density of larvae generate more heat. In our experiments the box temperature was measured with penetration thermometers, sensors and infrared (IR) cameras. However, towards automation heat mapping with IR cameras of the complete surface or installing permanent sensors which can regularly collect and send data are preferred. Initially sensors were used because we assumed that a heat camera could not observe what happens deeper into the substrate. However, the sensors were easily damaged by the mealworms and protecting them influenced the behaviour of the mealworms due to the increased surface area. Moreover, most activity is situated not deeper than 1 to 2 cm under the surface and this can be monitored perfectly with the heat camera. Therefore, we connected more sensitive heat cameras to our setup which automatically take pictures at certain timepoints. The preliminary results show that for example the speed of consumption of moisture source, aggregation behaviour in response to the environment, or because of the developmental stage of the mealworms and increase in mealworm biomass can be revealed using heatmaps.

Environmental opportunities and trade-offs of using feedstuffs for laying hens: LCA of 3 pathways

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Feed ingredient production for livestock including poultry has numerous associated environmental impacts including the release of greenhouse gases from fertilizer production and application, the release of pollutants such as nitrates into freshwater ecosystems, the conversion of native habitats for production, and the loss of biodiversity in key ecoregions, such as the Northern Great Plains, from this conversion. This life cycle assessment of US laying hens' nutrition looks at the impacts associated with substituting traditional feed ingredients such as corn and soybean meal with alternative feed ingredients derived from reclaimed bakery by-products, chemically-processed grocery food wastes and black soldier fly larvae (*Hermetia illucens*; BSFL) utilizing nutrients from similar grocery wastes. A total of 9 alternative diets were examined incorporating the 3 alternatives at a 5, 10 or 15% of the hens' diet per weight. All diets are nutritionally equivalent (iso-caloric, iso-nitrogenous) to the conventional baseline diet. The overall results for the 3 alternatives do not distinguish a clear best option across the impact categories including global warming potential, land use, water consumption, and marine eutrophication. Instead, the results demonstrate trade-offs amongst the indicators. Bakery meal showed the lowest impact in the categories investigated with a range of 1-10% lower than the baseline diet, whereas BSFL leads to the largest increase in global warming potential, water consumption, and marine eutrophication impacts, with impacts 2-350% greater than the baseline diet. These findings indicate that the benefits of substituting processed food waste into laying hens feed are modest and potentially carry risks of significantly higher environmental impact. The research reinforced the need for further study into these emerging circular pathways and how key differences in production processes such as selection of energy options can significantly impact the comparative results.

The effect of rearing conditions on the CO_2 output and required air flow in *Tenebrio molitor* rearing

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Food accounts for 15 to 25% of the carbon footprint of an average person. With the growing human population it is becoming more important than ever to improve the sustainability of the food chain. Meats and other protein sources tend to be the largest contributors to the carbon footprint of Western diets. The carbon footprint of plant and animal-based proteins is influenced by the farming and processing methods used. Optimisation is a first step to provide more sustainable products. However, radical innovations are required to provide food and feed with significantly reduced CO_2 output per kilogram of comparable proteins. Various insect species have been shown to be effective replacements for standard protein sources for human and animals. Mealworms (*Tenebrio molitor* larvae) are one such insect species that can be reared and processed into various products for food or feed while also reducing the land usage compared to conventional sources. That is why production capacities for mealworms are increasing on a global level and the overall growing efficiency has already improved. Optimised growth rate provides a sustainable and economically viable insect production, but also linked to higher CO_2 concentrations inside the rearing chambers. It is important that air flow rates through the rearing units are sufficient to ensure that the CO_2 concentration, temperature and general rearing environment remain ideal for larvae growth. Key parameters, such as the effects of rearing feedstock and climate conditions on CO_2 output of mealworm larvae were explored. A custom insulated box was developed and integrated with CO_2 concentration and temperature meters. A known quantity of larvae at a known average weight per larvae were dosed into the box with controlled feedstock at a controlled temperature. Data readings were done periodically, while care was taken to ensure that larvae never experienced stressful or dangerous climate conditions or concentrations of CO_2. Results measured allow for the design of effective climate control units to maintain good growth conditions for larvae throughout their lifecycle while also providing a good model to optimise final CO_2 output per kilogram of product.

Environmental impact of feeds utilized for poultry protein productions: soybean vs insect larvae

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Poultry meat and eggs are among the most consumed foods of animal origin. Feed production is responsible for most of poultry's environmental impacts. New environmentally friendly sources of protein for feed are required to reduce the environmental footprint of poultry production. The use of insects as feed ingredients is a hot topic for at least 10 years. The considerable progress made led to the authorization and use of insect processed animal protein in feed for farmed fish, with feed for other farmed monogastric animals expected to follow soon. As insects are a natural part of a poultry diet, feeding insects to chicken also has a positive impact on animal health and welfare. Additionally, several insect species can convert a wide range of organic side-streams, making them a sustainable alternative to conventional chicken feed and allowing decrease of the overall environmental footprint of chicken rearing. Proteins originating from poultry come in 2 main forms: eggs and meat. Even though results depend on the season and geographical location, eggs have somewhat bigger global warming potential per kg of product (over 5 kg CO_2/kg, which is reached by meat only in winter), if laying hens are not also accounted for meat production. This is even more pronounced when calculated per 1 g of protein, as chicken meat has higher protein content than eggs. The aim of the current research within Poultrynsect project (supported by SUSFOOD2 and CORE ORGANIC) is to decrease these impacts by rearing black soldier fly larvae on organic food side-streams and feed them to slow-growing broiler chickens. Inclusion of about 10% of insects into the feed has shown to have a positive impact on chicken welfare, productivity, and environmental impact.

Rapid authentication of edible insect powders by DART/HRMS coupled to mid-level data fusion

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The use of insects powders in the food and feed sector is of high interest. Different insects species are available for farming purposes but production cost are variable due to species characteristics and feeding requirements. Authenticity of insect based powder is likely to become an important challenge for buyers and health authorities. Direct Analysis Real Time-High Resolution Mass Spectrometry (DART-HRMS), coupled to multivariate statistical analysis, represents an interesting analytical approach due to its high discriminating ability based on metabolic profiles characteristic of each species. We focused on *Tenebrio molitor, Acheta domesticus, Hermetia illucens* and *Bombyx mori*. Two different extraction procedures were applied to the edible insect samples to extract polar and non-polar metabolites. The instrumental analysis was carried out using a DART SVP 100 ion source coupled with an Exactive Orbitrap. The data were statistically analysed using MetaboAnalyst 5.0 web portal and Rstudio 3.6.1 software. The four separate pre-processed datasets were submitted to initial supervised partial least squared discriminant analysis (PLS-DA) with the aim of retrieving the most informative molecular features. The selected informative ions were used to build a support vector machine model able to classify the four groups of samples. The PLS-DA score plot of the four fused datasets showed a good clustering of the four insect powders demonstrating the ability of DART-HRMS to capture the high variability of the four groups based on the differences in their metabolic profiles. The classification model successfully classified a set of authentic and adulterated independent samples with high accuracy (>90). The data fusion we employed allowed to merge different datasets and analyse them as a unique global fingerprint. We could comprehensively explore the whole information content, obtain a good discrimination by PLS-DA and build a powerful classification model able to authenticate genuine samples and to identify adulterated samples. Results suggest the possibility to use DART-HRMS coupled to multivariate statistical analysis to assess the authenticity of edible insects powders.

Session 37 Theatre 10

Strengthening agricultural circularity – the relevance of insect frass

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Insect production generates a multitude of products and by-products that bring added value from a sustainability standpoint – but also commercially. One specific example is the dejecta of insects, commonly referred to as 'insect frass'. The application of frass in agriculture has shown benefits to plant growth, health and development. The excrements of insects contain relevant nutrients in forms that are easily assimilated by plants. Additionally, the inclusion of insect frass into fertilisation strategies could not only provide plants with essential nutrients and micronutrients but also with microorganisms that may inhibit the growth of pathogens. In turn, such properties will reduce the necessity to apply additional agrochemicals, also addressing the needs of European crop farmers and horticulturists In the light of the recently-published EU 'Farm to Fork' strategy, the development of new fertilising products – in line with the concepts of 'circular economy' – was highlighted as a priority by the European institutions. Through this presentation, the IPIFF Secretariat will present the latest regulatory developments of relevance for the subject 'insect frass', as well as possible short-/medium-term opportunities for actors involved in insect farming activities (e.g. insect producers, academic actors, etc.).

Session 37 Poster 11

Inventory and state of the art for sustainable insect production

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Over the recent decades, insect-based feed and food (e.g. black soldier fly larvae, house fly larvae, yellow mealworm and house cricket) have gained attention among Western countries, as they can be less resource intensive than other animal production systems. Moreover, in addition to planetary health concerns, further interest surrounding the nutritional value of insects as feed and food has increased. Therefore, to gain a complete understanding of the insect-based feed market and relevant agents in the insect production chain, we compiled an inventory of current commercial insect producers, in addition to retailers, restaurants and online-shops in Europe. The inventory was structured under the following broad categories: geographic location, insect species produced or sold; application of the product; and the current status of the company. As company data was limited, we supplemented this information with data from the literature and other databases like Ecoinvent, Feedipedia and Global Feed LCA Institute (GFLI) database, because, currently, no holistic and harmonized inventory database for insects exists. We therefore sought to collect nutritional and environmental data on insect feed and insect production for key impact categories including greenhouse gas emissions, water use, land use, and energy consumption. Relatively speaking, the data for feed was more complete than for insect production. Here, we found that significant gaps exist, particularly for footprint areas beyond greenhouse gas emissions. With respect to the nutritional dimension, we found that while the main macronutrients (i.e. carbohydrates, protein, and fat) were often reported there were gaps in terms of micronutrients and amino acids. Both of these areas are key considerations when comparing insect production (inclusive of feed) against traditional livestock systems. The next stage of our project seeks to address some of these shortcomings with a multidimensional model. [*]These authors share first authorship.

Session 37 Poster 12

CELLOW-FeeP Project – circular economic: live larvae recycling organic waste for rural poultry

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Recently, insects, and in particular *Hermetia illucens* larvae (HIL), have received great attention as innovative, alternative and sustainable source of raw materials for animal feed due to their high nutritional value (proteins, essential amino acids, lipids, minerals and vitamins). HIL can be grown on organic waste efficiently, converting low value substrates into high value products, exalting the Circular Economy concept. In a Circular Economy perspective, the insect rearing residues (insect frass) may find use as an organic fertilizer. Insects are therefore considered as highly sustainable. In addition to nutrients, insects are also a source of bioactive compounds (chitin, lauric acid, antimicrobial peptides) with positive effects on health and, in poultry, live HIL enable birds to express their normal behaviour (i.e. foraging activity), with expected positive impacts on animal welfare and product quality. However, data on HIL diets based on organic waste and information on birds performances, health and product quality are still scarce and further research is needed. The CELLOW-FeeP (Circular Economic: Live Larvae recycling Organic Waste for rural Poultry) project, funded by the Cariplo Foundation, aims to produce live HIL reared on diets based on local organic waste and to identify the HIL optimal inclusion level into diets for slow-growing chickens, based on birds growth and slaughtering performances, meat quality, health and welfare parameters by adopting a multidisciplinary approach involving novel, innovative methodologies (i.e. metagenomics). In addition, CELLOW-FeeP aims to valorise the agronomic and energetic potential of the HIL frass and to widely disseminate results.

Session 37 Poster 13

Development of new methodologies to measure digestibility of *Tenebrio molitor*

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The industry of insects for feed and food is becoming a new pillar of the circular economy driven by the ability to bioconvert low-value feedstuffs into high-quality products. The bioconversion principle is indeed a foundation stone for the sector, its mastery could also be a key of success. Like all animal husbandry systems, improvement in feed use efficiency requires a better knowledge of the insect's digestive abilities. Digestibility assessment is particularly primordial for the formulation of competitive and efficient diets in an industrial farming context. Therefore, the present study proposes the establishment of standard and robust methodologies for measuring digestibility, considering the optimal rearing conditions established for industrialization. Based on the current knowledge from both traditional animal farming and academic entomology, we developed a direct (or gravimetric) methodology to estimate the digestibility of cereal by-products in a mealworm population (*Tenebrio molitor*). This methodology was tested on wheat by-products, then adapted to protein-rich feedstuffs gradually incorporated in a nutritionally balanced basal diet to assess digestibility values using a statistical regression approach. In parallel, trials on indirect methodologies to measure mealworms digestive capacities were also conducted and compared to the gravimetric method. Bibliographical research allowed us to select four markers that have a potential to be used in an indirect methodology: chromic oxide, titanium oxide, acid insoluble ashes and lignin. For both direct and indirect methodologies, approximate digestibility, growth and feed conversion performances calculations were made according to Waldbauer indicators. The complex interactions between the different indices illustrate the strong ability of this insect to adapt to its diet and environment. This project describes the very first steps of this new methodology whose validation and deepening are underway in order to optimize of the mealworms nutrition and, by the way, to strengthen the circular economy using insect farming.

Session 37 Poster 14

Susceptibility of *Alphitobius diaperinus* meal to infestations by major stored-product insects

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As insect production for food and feed should sustainably scale up in the years ahead. The quantities of insect meal that will be produced and stored in the insect-producing facilities will drastically increase. However, as with all agricultural commodities, insect meals may be infested by other insects during storage. In the present study, we evaluated the susceptibility of *Alphitobius diaperinus* meal to infestations by major storage insects. In a first series of experiments, *A. diaperinus* meal was artificially infested with adults of eleven stored-product insect species, i.e. *Sitophilus granarius*, *Sitophilus oryzae, Tribolium confusum, Tribolium castaneum, Trogoderma granarium, Rhyzopertha dominica, Oryzaephilus surinamensis, Lasioderma serricorne, Prostephanus truncatus, Tenebrio molitor* and *A. diaperinus*. Briefly, 5 g of insect meal were infested with 20 adults of each insect species using different vials for each species. The vials were opened after 65 d and progeny production was evaluated. In another series of experiments, substrates based on *A. diaperinus* meal and wheat bran at different percentages (0, 10, 50, 75 and 100% *A. diaperinus* meal) were artificially infested with *T. molitor, T. castaneum* and *A. diaperinus* adults and progeny production was determined, as previously described. Based on our results, *A. diaperinus* meal is susceptible to infestations by *T. molitor, L. serricorne, T. confusum, T. castaneum, A. diaperinus* and *T. granarium*. The same counts for all insect meal-based substrates with bran tested, which were highly infested by *T. molitor, T. castaneum* and *A. diaperinus*. These results suggest that insect infestation may cause serious damage to stored insect meal quantities.

Session 37 Poster 15

Awareness of chemical hazards in edible insects: toxicokinetics and toxicodynamics of Hg in mealworms

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The use of insects as a food source could be of great interest as a possible solution for the increasing food demands for the coming years. The need for a comprehensive understanding of the potential hazard of contaminants to edible insects in rearing facilities, alongside the mechanisms of their accumulation in insects, was recently highlighted by European Food Safety Authority as a main priority. This leads to an urgent need to go further and comply with the applicable food safety regulations, promoting an increased number of studies in the last years. Following previous reports that mercury (Hg) can accumulate in the *Tenebrio molitor* mealworm larvae, this study aimed to go forward, investigating the toxicokinetic and toxicodynamic (TK/TD) of Hg in mealworms exposed to Hg contaminated food. This study is a step forward on understanding how mealworms accumulate Hg throughout the larval stage. Here, a complete bioaccumulation design is proposed, where insects' larvae are exposed for 21 days to contaminated Hg oat, followed by the same period with non-contaminated oat. With this, it is possible to follow the uptake and elimination rates, evaluating the TK/TD of Hg, which is extremely important for the knowledge on how Insect larvae respond to Hg contaminated substrate/food and consequent uptake and elimination. Prior to this, a biomass change assay will serve to compare changes in the weigh of different larval stages (20-40 and 60-80 mg) exposed to different conditions (use of soil as medium and presence of food source) on a 21 d test. Data retrieved from these biomass change tests will be crucial for the optimization of bioaccumulation assays using mealworms.

EntoPower: greenhouse gases emissions from *Hermetia illucens* larvae production

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The overall purpose of EntoPower is to develop an intensive, innovative and industrial production concept for black soldier fly larvae (BSFL) production. To achieve high production efficiency, it is important to reduce the manual processes as much as possible while optimizing the growth and quality of the larvae. Previous screenings show a significant efficiency potential in heating and ventilation systems for BSFL production. In EntoPower, an innovative production concept, strongly inspired by industrial concepts for automated processes, is developed. The new heating and ventilation system aims to capitalize on this untapped energy efficiency potential and reduce the total energy consumption in a production. Initially, measurements of CO_2 and NH_3 have been performed in the pilot production laboratory at the Danish Technological Institute. Data was collected on 4 different feed substrates to cover a wide range of possible gas production patterns. It was found that CO_2 production ranged between 6.4 and 9.2 kgCO_2/kg larval biomass produced and peak heat production ranged between 25 and 69 watts/kg of larval biomass produced during the hour of highest biological activity. Ammonia production also showed a wide span across the different substrates, ranging between 0.16 to 0.37 kg/kg biomass produced. These data will be the ground for assessments of the requirements for energy optimized climate system, before development and pilot scale testing.

The shades of gray in model building and its opportunities for precision livestock farming

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For many years, the literature in animal modelling has had the tendency of opposing two apparently irreconcilable approaches, namely the white-box and the black-box modelling approaches. In the white-modelling approach, the modeller is motivated by the goal of providing a mechanistic insight of the system under study. Modelling construction is then supported from the knowledge of the mechanisms that underlie system behaviour. The models resulted from this approach are named with different adjectives such as mechanistic, phenomenological-based, knowledge-based or white-box models. The white-box models fulfil the property of interpretability. That is that the variables and in particular the model parameter have biological meaning. The black-box modelling approach is data-driven. The modeller goal here is to approximate the observed behaviour of a system without the need of incorporating knowledge on the mechanisms responsible of the observed data. The models are derived from data analysis to quantify relationships between variables of interest. Models in this category are called empirical, data-driven, behavioural or black-box models. The black-box models are powerful tools for exploiting high-throughput data and time-series data for predictions and diagnosis applications. However, these models lack of interpretability. The relevance of the dichotomy between white-box and black-box modelling approaches has recently being revisited by new perspectives of the modelling practice in the context of precision livestock farming (PLF) and big data. Between the white and the black, there are shades of gray. Hybrid modelling approaches combining white-box and black-box models result in gray-box models that can handle big data while having some level of interpretability. By this property, the gray-box modelling approach offers great opportunities for PLF. In this work, we elaborate on how mathematical tools from the automatic control domain can contribute to the development of gray-box models adapted to address animal science problems. We illustrate our approach *via* relevant examples addressing the dynamics of methane emissions from cattle and the animal response facing perturbations.

Opportunities for using computer vision and animal tracking in welfare assessment

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The multi-factorial nature of welfare assessment in farm animal species and its complexity requires robust and flexible tools to monitor both individual and group levels at varying conditions. The continuous development of state-of-the-art computer vision (CV) and deep learning (DL) algorithms allows for their more user-friendly counterparts to be adjusted to animal scientists' needs providing the framework for non-invasive monitoring of animal welfare and health. However, the high developmental cost of CV applications requires an optimized workflow to avoid unnecessarily large time and resource investments. By comparing the performance and speed of different CV architectures, the initial size of the required reference dataset used for training the model and the efficient annotation strategy could be estimated and applied across different animal species. The aim is to avoid the typical 'black box' scenario, where the actual welfare assessment becomes secondary due to the complexity of the hyperparameter tuning and model optimization. One possible solution is a generalized cross-disciplinary 'solution zoo,' helping select the optimal CV model and deployment strategy based on a particular problem or a specific animal-based parameter to be monitored. To illustrate the opportunities and potential for different CV and DL algorithms', practical examples from different production animal species and scenarios will be highlighted: (1) pre- and post-calving monitoring in dairy cattle using the custom neural network architectures for activity and pose assessment; (2) multiple object tracking (MOT) in pigs for studying activity and resilience; (3) robust object detection and tracking for individual gait scoring in broilers; (4) use of lightweight object detection frameworks for mobile platforms for estimating size and weight in dairy cattle; These examples will be presented in the manner of so-called building blocks or solutions within three main domains: object detection, single or multiple object tracking and data visualization. Each of these domains will be further discussed from the suitability for an automated animal welfare assessment point of view.

Session 38 Theatre 3

Q&A with invited speakers

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Discussion with the invited speakers.

Validation of a real-time location system for neighbour detection in dairy cow groups

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Interest is growing in practical ways to gain more insights about the social bonds in dairy cow groups and new technologies, such real-time location system (RTLS), are becoming more popular for this purpose. We investigated the influence of RTLS data quality and parameter settings on detecting direct neighbour cows in lying stalls and at the feed bunk. We followed a group during two 3-day periods using two RTLS calibration setup (P1: laser distance meter (15 cows); P2: professional surveying (14 cows)). In addition, we collected data from electronic feed and water bins and performed 1-day continuous video analysis to record the locations of all cows. We applied our developed R-pipeline, including various data filtering and smoothing approaches, to obtain RTLS data for each second. These data were assigned to zones in the pen (e.g. specific lying stall). The prepared data were used to reveal direct neighbours at the stalls and bins applying a distance and a zone approach. We also tested the effect of a time threshold defining the shortest duration that cows had to be direct neighbours. Outcomes were compared with revealed direct neighbour events based on video or electronic bin data using Spearman's rank correlation coefficients (Rs) with cow pairs as units. We found that the RTLS calibration setup had a strong impact on data quality and influenced the direct neighbour detection, especially at the feed bunk ($Rs<0.6$ in P1; $Rs>0.9$ in P2). The distance and zone-based approaches performed well, however the zone-based approach has the practical advantage that no specific threshold has to be found. Generally, using a least duration time threshold did not improve the results. We recommend to perform RTLS calibration using professional surveying to get high quality data for drawing inferences about dairy cow behaviour based on location data. A sufficient tracking accuracy will also lead to improved detection of health problems, welfare, and comfort of cattle at individual and at farm level.

Detecting behavioural patterns of cows using an automated tracking system

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Detecting behavioural patterns is increasingly coming into focus for the assessment of welfare-related issues in farm animals. Precision Livestock Farming systems provide an opportunity to obtain such data automatically. The aim of the present study, conducted during summer 2020, was to investigate whether dairy cows exhibit daily patterns in the use of functional areas in the barn, via a commercial animal indoor tracking system. We hypothesised that patterns of individual cows are more consistent than between cows. The herd consisted of lactating Brown Swiss (n=13) and Swiss Fleckvieh (n=7) cows housed in cubicles. The barn was organized into three functional areas, feeding, lying and activity. We collected the spatial data of cows continuously over a period of 7 days. To compare the similarity between daily data sets of cows, we performed a hierarchal cluster analysis. The frequency of visits to and duration spent in each area and the total number of transitions between areas were used as key variables. A Principle Component Analysis revealed that the variables total number of transitions and the number of visits to the walking area explained 81.7% of the variability in the data set, whereas time spent in the three areas hardly varied between cows and across days (0-0.2%). A difference in the average distance between days within cows (mean ± SD: 2.4±1.0) and days between cows (mean ± SD: 3.5±1.5, $P<0.0001$, T(497.5)=18.9) was found. The Cohen's d estimate was 0.7 (medium size). Although the daily patterns within cows were more similar than between cows, the days of single cows could not be assigned to a respective cluster. A possible explanation for the less distinct individual patterns might be that the cows lack a well-defined activity rhythm or that the activity behaviour is not strictly bound to the functional areas. To assess whether such data can still be used to detect behavioural changes, the next step will be to investigate the constancy of distances within and between cows over time and under different challenges.

Happy cow or thinking pig – facial recognition tool for automated welfare

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Emotions play an indicative and informative role in the investigation of farm animal behaviours. Systems that respond and can measure emotions provide a natural user interface in enabling digitalization of animal welfare platforms. The face of farm animals can be one of the richest channels of emotions expressions. We present WUR Wolf (Wageningen University & Research: Wolf Mascot) – a real-time facial expression recognition platform that can automatically code the emotions of farm animals. Using Python based algorithms, we detect and track the features of faces of cows and pigs, and analyse the appearance, ear postures and eye white regions and correlate with the mental/emotional states of the farm animals. The system is trained on dataset of facial features of images of the farm animals collected in over 8 farms and has been optimized to operate with an average accuracy of 86%. From these, we infer the emotional states of animals in real time. The software detects 13 facial actions and 9 emotional states including whether the animal is aggressive or calm or neutral. Real-time emotion recognition system based on faster convolutional neural networks (CNN) and YOLO-based facial detection platform is presented. Detecting expressions of farm animals simultaneously in real time makes possible for many new interfaces for automated decision-making tools for livestock farmers. Emotions sensing offers a vast amount of potential for improving animal welfare and animal-human interactions.

SESAM sensor technology for milk producers

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Sensor assisted Alpine milk production (SESAM) project is an Interreg Alpine Space project, with the main aim to promote in the Alpine region intelligent, sustainable and integrative growth. The objective was to develop a decision-support and alert system for dairy cows based on an existing system that integrates the data from the milk performance test and the health monitoring systems with the activity data. The sensor system at final stage is monitoring: walking, standing, lying, eating and rumination of dairy cows. The system is reliably working on 25 pilot farms since mid-2020 and in the last 4 months, more than 2 million data records have been transmitted. In early 2021, the system has been delivering data from Alpine region for: 5 farms from Baden Württemberg, 2 farms from Alsace region, 7 farms from Austria and 1 farm from Slovenia. The first step was the data quality check before further evaluation. In the meantime, all events related to animal behaviour and health were systematized so they could be synchronized with activity data in order to identify behavioural (activity) patterns for different health events, with economic or herd management significance. These patterns are the basis for continuous developments by a wider group of participants, gathered from all the partners. Combining the SESAM sensor data with milk recording and health monitoring in the 2nd level algorithms should improve the accuracy of individual alerts. Also feedback from farmers and experts gathered by target working groups will increase the reliability of the information in the decision-support and alert system.

Classification of cow behaviour for sensor assisted alpine milk production

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The SESAM project (sensor assisted alpine milk production) aims at introducing an innovative IT-sensor based framework for innovative decision-support, tailored to small farm needs in the alpine space that enables them to improve competitiveness and animal wellbeing through real-time monitoring. For the analysis of cow behaviour and to understand its well-being, analysis of the activities of the cow is needed. Since manual analysis of several cows on multiple farms is not feasible, an automatic system was developed to classify the activities of cows. Here a machine learning approach was chosen and trained with data that was gathered by manual observation of some of the cows. Currently five different activities are recorded for these cows: 'eating', 'standing', 'lying', 'ruminating' and 'walking'. Multiple cows in different farms were equipped with neck-mounted sensor boxes containing a three-axis accelerometer. The recorded acceleration data was divided into 25-second intervals and different features were calculated. The combination of these features and the manual observations of the cows were used as input for the machine learning model. Here a decision tree ensemble was used to classify the cow activities. This ensemble calculates for a given set of features a probability for each of the five classes. The final output of the model is the class that achieved the highest probability. For validation the model was trained using data of a specific farm and tested using an independent test set gathered from another farm. The recognition accuracies for the different classes range from 54 to 92%. Especially the distinction between the 'Lay' and 'Standing'-classes has proven to be difficult. Both of these classes are characterized by only sparse movements of the cow, so that the acceleration data measured by the sensor has similar properties during these times. In the remaining project time, the model is going to be improved by continuous data labelling in farms in France, Germany and Slovenia.

Convolutional neuronal networks reveal promising performance in non-invasive pig weight monitoring

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The precise knowledge of pig weight is important to draw conclusions about the health state, the daily gain and the exact time point for marketing. Up to date, the most accurate method for general weight determination is manual weighing. However, this procedure is very time consuming and sometimes stressful for animal and stockman. In order to overcome these obstacles, non-invasive weight estimation measures have to be established. In this study, we present our approach of pig weight estimation based on depth images using convolutional neuronal networks (CNNs). Thereby, we use solely the depth images for network training and the corresponding weights without performing any further feature extraction on the images. Our data basis consists of more than 400 individual animals that have been weighted up to four times during fattening period, resulting in a weight interval between 20 kg to 133 kg. For each video, we: (1) select the best quality images; and (2) exclude too similar images to avoid their overestimation in network training. Afterwards, we fine-tuned a pre-trained CNN model on our training data set and reached a coefficient of determination R^2=0.978 on the test set. The generalization of our approach was confirmed by a five-fold cross validation. Our findings reveal that the model is able to extract the most important features for weight estimation on its own. Therefore, our results point out the perspective that CNNs can be used for proper animal weight monitoring in a commercial pig-housing environment.

'It's a long way until they STOP' – development of a software to annotate pecking injuries in turkeys

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Cannibalism in turkey husbandry is a widespread, serious problem in animal welfare. There is a consensus that bloody injuries act as trigger mechanism to induce pecking. Therefore, continuous monitoring of the herd should be provided to implement a timely intervention. Thus, the overarching aim of the present study is to develop a camera-based warning system to detect the signs of injuries due to cannibalism in the flock. In the first step, images taken of turkey hens (BUT 6) on farm were included in a software developed in the project. With this software, several observers annotated the images by marking the injuries of pecking in the pictures. Based on this manual labelled data, a neural network was trained to detect such injuries in newly recorded images. The agreements between observers and the trained network were not acceptable (32%). Thus, a second step followed. Here, the markings that had been conducted in the first step (by humans or by the network) were checked using a 'human-in-the-loop' approach by presenting them a second time to three human observers in the 'new' MEMO-software (MEMO = Many Experts Many Opinions). The observers now had to confirm the presence of a detection ('injury' vs 'no injury'), with being blinded to the initial decision. In total, 24,173 assessments were provided by human observers, with the detection rated as 'no injury' in 4,481 cases (18.5%), and as 'injury' in 19,692 cases (81.5%). Finally, in 81.6% of the determined detections, all opinions were the same, thus, the observers agreed that the injury was 'true'. With the creation of a pseudo-binary system, the agreement between the human observers was improved substantially. In the next step, the neural network will receive the unanimous results from this approach for further training. The project is supported by the 'Animal Welfare Innovation Award' of the 'Initiative Tierwohl'.

Liveweight and condition score trajectories to parametrize self weighing scales in small ruminants

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Monitoring of small ruminants flocks could be enhanced by warnings sent by devices often frequented by animals. The OtoP-3D project aims to develop devices as self-weighing scales or 3D-body condition display. In the action 2 of the project, liveweight of lambs and ewes and ewes body condition score have been described following 3 livestock systems: indoors system, outdoors system, dairy system. Four lambs liveweight trajectories have been separated following the growth rate, the destination (lamb production vs replacement) and the early rearing system (maternal vs artificial suckling). Trajectories were based on median ±1 median absolute deviation forming an envelope wrapping the liveweight points of the group of animals. Liveweight and body condition score trajectories have also been described for ewes by testing 4 methods. Hierarchical ascending classification of similarity indices gave the best result. Those trajectories will be used to parametrize self weighing scales as WoW (walk over weighing) devices in small ruminants. Such devices will provide warnings to the breeder/farmer when a small ruminant will get its auto-weighing deviant from the trajectory it has been assigned following its previous recorded weights and its livestock system/physiological stage/destination/growth rate class.

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DATAMATION: DigitAl Twin AniMAl emoTIONs

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A digital twin can be described as a digital replica of a real-world entity. It simulates the physical state and may be the biological state and behaviour of the real-world entity based on input data. It helps in predicting, optimizing, and improving decision making. It has revolutionized the industrial world, particularly the manufacturing industry, construction and healthcare sector, smart cities, and energy industry. Here, we explore the development and implementation of a digital twin for modern animal farming. Detecting, measuring, and possibly predicting farm animal emotions has applicability in the design of automated animal welfare systems with outcomes in enhancing the quality of farm animal lives and overcoming the ethical and social concerns. Statistical techniques are essential to overcome the subjectivity of farmer/scientist user-defined rules and parameters in the facial feature analysis from the video and images in determining emotions, as well as in manipulating the design of the livestock automated welfare systems through the ability to learn from data and thereby create the digital twins. Here, we propose a 'Digital Twin' system for predicting and classifying farm animal emotions using generative adversarial network classifiers. The deep generative model will allow synthesizing realistic data samples for enabling automated emotion recognition and classification, including cross-modal and uni-modal emotion synthesis applications for livestock animals. We use the data from this real-time biofeedback system (from wearable sensors and images of farm animals collected from cameras) to determine the animals' emotions and provides the farmers with emotional feedback through digital twins. This emotion-aware digital twin system is aimed at increasing the welfare of animals through visual feedback and aims to provide critical insights into realistic implementations in enhancing animal welfare.

Session 38 Poster 13

Feasibility study to characterize calf auricle structure using a laser scanning method

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Ear tagging of cattle is obligatory to ensure traceability along the agri-food chain. However, it impairs animal welfare due to the painful procedure itself, acute or chronical wounds, tissue deformation and loss of the ear tag by entanglements. To optimize the tagging process and the fit of ear tags as well as to analyse risk factors for impairments, simulations on virtual calf ear models could be useful. The aim of this feasibility study was to test laser scanning methods for creating a virtual calf ear model. Two Holstein calves that were born dead on commercial German dairy farms were brought to the research facility immediately after their birth and stored deep-frozen until the day before scanning. The coat of one calf was predominantly black, whereas the other was white. Ears were scanned with a Perceptron ScanWorks V5 triangulation laser scanner attached to a ROMER Measuring Arm 2.0. The exposure time of the laser scanner was increased for black coloured coats compared to white, to deal with less reflecting surfaces. Scans were repeated after coat clipping (3 mm). The resulting point clouds were pre-processed (i.e. outlier removal, data reduction) and meshed in Geomagic® to create a virtual, closed surface model. It was found that stabilizing elements that might impair scanning can be omitted by scanning frozen calves. Scanning unclipped ears challenged the determination of a closed surface model of the ears since the laser scanner then partly measured the hairline instead of the natural surface leading to noisier data. Hence, the pre-processing and the meshing had become more complex and less accurate. Nevertheless, the meshing was feasible for clipped and unclipped ears. Adaption of laser exposure time enabled data collection independent of coat colour. Ear ridges were clearly visible and their positions were measurable in the surface model. These are promising results to investigate laterality in greater sample sizes, animal-individual differences of ears in shape and form, position of the ridges as well as simulating the virtual fit of the ear tags including changes by growth.

An open animal data platform: how open knowledge can serve the livestock and companion animal sector

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The ‘Open Knowledge Foundation’ formulated the ‘open definition’ in 2006. It describes the characteristics and use of open data and open knowledge. Both serve to promote transparency and collaboration and are closely related to initiatives such as ‘open source, open content, open access and open education’. Everyone is certainly familiar with the online encyclopedia Wikipedia, or Swiss citizens with the website ‘corona-data.ch’, or the ever-increasing number of scientific journals with freely accessible articles. Open knowledge sees itself as an active part of civil society. A noble cause in times of uncertainty and ‘alternative facts’. Another goal of open knowledge is to promote business, innovation, and competition. The Federal Statistical Office (FSO) operates ‘opendata.swiss’ for open government data (OGD) as part of an initiative called ‘egovernment Schweiz’. Open data includes data sets in the interest of the general public as well as specialized data collections. Open data may not include personal data or data subject to data protection. In 2020 Identitas AG started its own open data project, a platform giving access to livestock and companion animal information in Switzerland and the Principality of Liechtenstein. The Animal Statistics Platform proposes key data on the sectoral structure, e.g. the development of the various species and breeds over time and geographical regions. This includes number of births and stillbirth, imports, exports, culling, relocations, ownership change, data on livestock holdings and owner demographics. Various performance traits are also presented. Geographic representations show local evolutions and trends. Another vast topic includes epidemiology, risk assessment, disease spread and welfare status. The data from diverse systems also serve to model the needs of the animal sector in terms of spatial accessibility to services. It may also help to model the needs of large-scale infrastructure projects, such as access to alpine pastures and housing for animals and men. The platform Animal Statistics is a rich source about and for the livestock and companion animal sectors, and a suitable form for the sustainable use of animal related data.

Effects of dietary nutrient composition on the response to intramammary LPS challenge in dairy cows

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Effects of the nutritional and metabolic status on the immune response of dairy cows undergoing an intramammary lipopolysaccharide (LPS) challenge in the third week p.p. (DIM=19±3) were investigated. From 3 weeks a.p. until 4 weeks p.p., 38 dairy cows were included. After calving they were fed hay *ad libitum* and increasing amounts of concentrate either rich in glucogenic precursors (glucogenic, n=13), crude protein (aminogenic, n=12) or lipids (lipogenic, n=13). Day -1 and 0 are referred to as mammary immune challenge phase whilst day 1 to 9 are referred to as recovery phase. Daily milk lactose content was greater in cows fed lipogenic diet ($P<0.05$) and higher milk and plasma urea contents were observed in cows fed aminogenic diet ($P<0.05$). During the mammary immune challenge phase higher plasma cholesterol in cows fed lipogenic diet compared to cows fed aminogenic diet at +3 h ($P<0.05$), lower milk yield after challenge phase in cows fed glucogenic or aminogenic diet ($P<0.05$), and decreased plasma NEFA in cows fed glucogenic or lipogenic diet ($P<0.05$) were observed. During the recovery phase a faster decrease in somatic cell count (SCC) in cows fed lipogenic diet (d1 to d2 $P<0.05$) than in cows fed glucogenic or aminogenic diet (d1 to d3 $P<0.05$), and higher plasma cholesterol in cows fed lipogenic diet compared to cows fed aminogenic diet at +47 h and +71 h ($P<0.05$) was observed. Within 24 h after LPS administration daily milk fat content was higher ($P<0.05$), daily milk lactose content was lower ($P<0.05$) and SCC was elevated ($P<0.0001$) in all groups. An increase in plasma glucose and haptoglobin and a decrease in white blood cell count was observed in all groups in response to the LPS challenge ($P<0.05$). During the recovery phase all parameters went back to basal levels in all groups. In conclusion, dietary nutrient composition had only few effects on metabolites and milk composition. However, besides faster recovery of SCC in cows fed lipogenic diet, provided diets did not seem to have substantial effects on the mammary immune response in early lactation based on the measured factors.

Metabolism and performance of dairy cows under different dietary regimens at dry-off

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The cessation of milk production at dry-off causes metabolic changes in dairy cows. In practice, concentrate withdrawal and feed restriction prior to dry-off are used to decrease milk production. The present study investigated metabolic and performance changes in cows fed 3 rations varying in nutrient composition and restricted feed allowance before dry-off. The trial included 43 cows of similar BW and milk yield from d 12 before until d 6 after dry-off (56 d before calving). In 3-day-intervals from d 12 to d 6 before dry-off, cows were supplied with increasing concentrate amounts rich in crude protein (aminogenic, n=14), glucogenic precursors (glucogenic, n=14) or lipids (lipogenic, n=15). Between d 3 before until d 6 after dry-off, half of the animals per feeding group were restricted to access 50% of their diet while the feeding level of control animals was unchanged. Cows were milked twice daily until dry-off. BW and DMI were recorded daily, blood was sampled once daily. Plasma glucose, BHB, NEFA, urea, cholesterol and triglycerides were measured as well as milk gross composition. Dietary composition did not affect milk yield and DMI. In restricted cows, energy balance turned negative and milk yield was reduced compared to non-restricted cows ($P<0.05$). Diet and restriction had no effect on gross milk composition and plasma concentrations of cholesterol, glucose and triglycerides. Increasing amounts of the aminogenic diet resulted in increasing milk and plasma urea concentrations ($P<0.05$). Restriction did not alter urea concentration in milk and plasma. Except for the lipogenic group, plasma glucose was higher after dry-off ($P<0.05$). Feed restriction did not change plasma glucose and BHB concentration, but increased NEFA concentration in all dietary groups, which remained elevated until 3 d after dry-off ($P<0.05$). In all 3 feed restricted groups, plasma triglyceride concentration increased after dry-off ($P<0.05$). In conclusion, feed restriction before dry-off reduced milk yield and increased plasma NEFA concentration. Except for milk and plasma urea, dietary composition did not affect performance and measured metabolic parameters before and after dry-off.

Metabolic adaptation to short-term of dietary nutrient imbalances in mid lactation dairy cows

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Short-term imbalances of dietary nutrients may occur during fluctuations in roughage quality or temporal shortages of supplementary feed components. In contrast to a deficiency, especially dairy cows with a moderate performance, in mid or late lactation, or in extensive farming systems may be exposed to imbalanced rations. The objective of the present study was to investigate the short-term metabolic and endocrine responses to different levels of concentrate formulated to particularly provide one major macronutrient source. Based on parity number, lactational stage, milk yield and BW, 9 mid lactating cows (211±19 days in milk) were grouped into 3 blocks of 3 animals each. Concentrate supplements (aminogenic: rich in crude protein and nitrogen sources; glucogenic: high content of carbohydrates and glucogenic precursors; lipogenic: high lipid concent) were fed in a factorial arrangement at 3 increasing levels for 3 d each. Milk yield, BW and feed intake were recorded daily. Blood and milk were sampled every 3 d at the end of each concentrate level. Milk fat, protein, lactose and urea contents were determined. In blood, concentrations of various metabolites, endocrine factors and enzyme activities (e.g. glucose, non-esterified fatty acids (NEFA), β-hydroxybutyrate, urea, cholesterol, triglycerides, insulin, glucagon, ASAT, GGT, GLDH) were measured. Milk yield, milk composition and BW were not affected by diets and concentrate levels. Feed intake increased in cows receiving the aminogenic and lipogenic diet. Milk und plasma urea concentrations were clearly elevated in the aminogenic and to a lesser extent in the lipogenic treatment. Glucose concentrations in plasma were not affected by treatments, whereas insulin and glucagon increased only in the aminogenic treatment with concomitantly reduced NEFA concentrations. Activities of ASAT, GGT and GLDH as well as the total antioxidant capacity were not affected by diets. We observed immediate metabolic and endocrine responses due to the short-term dietary changes. Particularly a surplus of nitrogen supply via the aminogenic diet affected metabolic responses and stimulated insulin and glucagon secretion.

Session 39 Theatre 4

Increased MDA in early lactating cows with elevated blood levels of NEFA and BHB

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During the transition period from pregnancy to lactation, cows undergo tremendous physiological, metabolic and nutritional changes. The three most notable issues include: metabolic and oxidative stress as well as inflammation. Biomarkers of each of these three were simultaneously monitored during the current experiment. We hypothesized that metabolically imbalanced transition cows also show impaired antioxidant ability. A total of 109 transition dairy cows, following the same nutritional and management transition strategy were involved in this experiment. Blood samples were taken 3, 6, 9 and 21 days after calving and used to cluster cows (k-means clustering) into two metabolic categories based on blood NEFA, glucose and BHB. We assessed the oxidative stress by GSSG/GSH (ratio of oxidized to reduced state of glutathione), GPX (glutathione peroxidase), MDA (malondialdehyde), ORAC (oxygen radical absorbance capacity) and SOD (superoxide dismutase) at 21 days after calving. The two categories, i.e. metabolically imbalanced (MI) vs metabolically balanced (MB) groups included 34 and 75 cows respectively. Average (d3, d6, d9, d21) concentrations of glucose were lower (2.88±0.28 vs 3.19±0.30 mmol/l plasma, respectively) ($P<0.05$) while NEFA and BHB were higher in the MI group compared to the MB group (0.83±0.29 vs 0.56±0.22 and 1.54±0.48 vs 0.81±0.13 mmol/l serum, respectively) ($P<0.05$). No differences in the ratio of GSSG/GSH, GPX, ORAC and SOD were found. However, MDA was higher in the MI compared to MB group (4.87±1.29 vs 4.25±1.22 nmol/g plasma, respectively) ($P<0.05$). This suggested that more severe lipid peroxidation damage has occurred in MI group.

Session 39 Theatre 5

Inflammatory gene expression of bovine peripheral blood mononuclear cells following spirulina intake

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Inflammatory diseases reduce the health status and productivity of livestock and result in financial losses of farmers. The microalga spirulina (*Arthrospira platensis*) has demonstrated anti-inflammatory properties in monogastrics but this interplay has not yet been explored in ruminants. To investigate potential anti-inflammatory effects of spirulina in ruminants, we replaced soybean meal in the diets of dairy cows and fattening bulls by spirulina (both experiments: n=6 per diet group) and collected blood samples to obtain plasma and isolate peripheral blood mononuclear cells (PBMCs). We analysed the plasma concentrations of potential anti-inflammatory bioactive compounds (β-carotene, α-tocopherol, polyphenols) via HPLC and photometric methods. The PBMCs were stimulated *in vitro* with the bacterial endotoxin lipopolysaccharide (LPS) in concentrations of 0, 10 and 100 ng/ml. Subsequently, RNA was isolated, reversely transcribed to cDNA and qPCR was performed to determine the expression of in total 10 inflammation-related genes such as pro- (e.g. interleukin (IL)-1β, tumor necrosis factor α) and anti-inflammatory (e.g. IL-10) cytokines, the prostaglandin-producing enzyme cyclooxygenase-2 and their upstream transcription factor nuclear factor kappa B. The β-carotene plasma concentration was higher in spirulina- compared to soybean-meal fed animals but plasma concentrations of α-tocopherol and total phenols did not differ due to the experimental diet. Stimulation of bovine PBMCs with 10 and 100 ng/ml of LPS significantly increased the expression of most cytokines. Gene expression did however not differ between PBMCs derived from spirulina- compared to soybean-meal fed animals at both unchallenged as well as LPS challenged state. Our results indicate that despite increasing the β-carotene plasma concentration, spirulina might not have anti-inflammatory properties in ruminants similar to those observed in monogastrics. Future studies should evaluate the degradation of spirulina and its bioactive compounds in the rumen to provide further data on potential beneficial health effects in ruminants.

DHA and ω6-DPA modify toll-like receptor 4 signalling in monocytes and neutrophils of dairy goats

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Docosahexaenoic (DHA) and docosapentaenoic acid (DPA) compete with arachidonic acid for the eicosanoid production. These derivatives show less pronounced pro-inflammatory action compared to 2-series prostaglandins resulting in a beneficial balance between pro-inflammatory and anti-inflammatory pathways. The objective of this study was to investigate the impact of dietary inclusion of *Schizochytrium* spp (rich in DPA and DHA), on the expression of several genes involved in the innate immunity of goats. Twenty-four dairy goats were divided into four homogeneous sub-groups (n=6). All goats were fed individually with alfalfa hay and concentrates. The concentrates of the control group (CON) had no microalgae while those of the treated groups were supplemented daily with 20 (ALG20), 40 (ALG40) and 60 (ALG60) g *Schizochytrium* spp. Monocytes and neutrophils were isolated from goats' blood in the 20th, 40th and 60th day from the beginning of the experimental period. The relative transcript levels of *TLR4, MYD88, MAPK, IRF3 and IFNG, IL1B, IL2, IL8, TNF, CCL5,* and *CXCL16* were decreased in monocytes of microalgae treated goats compared to the CON. In contrast, MAPK and *IL1B* relative transcript levels were increased in neutrophils of ALG40 and ALG60 groups. In conclusion, the supplementation of goats' diet with 20 g *Schizochytrium* spp. results in an improvement of the immune function through the downregulation of the pro-inflammatory response in transcript level.

Gut microbiota variations during the lifespan of two high-yielding laying hen strains

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Gut microbiota affects nutrient digestion, pathogen inhibition, gut epithelium nourishment, endocrine activity, and interaction with the gut-associated immune system. Decreasing the amount of energy needed in the gut for those purposes leads to an improvement in the laying hens' productive performance. A balanced microbiota is a benefit to the fowl. In laying hens, previous studies have rather focused their research on one gut section using digesta samples, disregarding the other gut segments or the mucosa. This study aimed to characterize the gut active microbial community in two laying hen strains: Lohmann Brown-Classic (LB) and Lohmann LSL-Classic (LSL) during their productive life span. Birds were kept under the same diet feeding, housing, and management conditions. Samples from digesta and mucosa of the crop, gizzard, duodenum, ileum, and ceca were collected at 10, 16, 24, 30, and 60 weeks of life. RNA was extracted from 1000 samples and analysed by target amplicon sequencing. Phylogenetic analysis of the bacterial sequences was assessed using Mothur, followed by multivariate statistical analysis. A statistical significance was observed for strain, gut section and type of sample (digesta and mucosa) and the week of life, and the combination of all factors ($P<0.05$). The most abundant genera were *Lactobacillus*, *Coprobacter*, *Clostridium*, *Staphylococcus*, unclassified Bacteroidales, and unclassified *Ruminococcus*, which colonized the digesta and mucosa of all animals and contributed to more than 70% of the total community in the crop, gizzard, duodenum, and ileum. Depending on the strain, those genera were present in higher or lower abundances. A significant shift in the active microbiota of the laying hens and between the LB and LSL strain was observed between digesta and mucosa samples and with the transition to laying eggs between weeks 16 and 24, particularly with the presence and absence of specific species of *Lactobacillus*. Thus, the animal's host and age have an impact on gut microbiota dynamics.

Tailoring amino acid-based solutions for improved performance and intestinal function of broilers

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Two experiments were conducted to investigate the effect of L-arginine (Arg), a combination of Arg and L-glutamine (Gln) as well as an amino acid-based product (PRD) containing a combination of Arg, Gln, L-threonine (Thr), and grape seed extract on performance, intestinal permeability and expression of selected mechanistic genes. Using 240 male Ross 308 off-sex broiler chickens, 4 experimental treatments were replicated 6 times with 10 birds per replicate. The experimental treatments were prepared by supplementing 5 g/kg Arg (treatment 2), 2.5 g/kg Arg and 2.5 g/kg Gln (treatment 3) and 1 g/kg PRD to a basal diet as control (treatment 1). The second experiment included a 2×4 factorial arrangement of treatments with each of the 4 dietary treatments given to 24 individually housed-birds either injected with synthetic glucocorticoid, dexamethasone (DEX) or sham injected on d 14, 16 and 20 of age. DEX injection was used as a gut dysfunction model and the passage of fluorescein isothiocyanate-dextran (FITC-d) into blood was used to measure the intestinal permeability (d 21 of age). Feed intake and body weight gain were not affected by dietary treatments. However, feed conversion ratio was improved by all the supplemented treatments when assessed from d 7 to 21 ($P<0.01$) and from d 7 to 35 of age ($P<0.001$). DEX injection increased ($P<0.001$) the FITC-d in all treatments which was partially reversed by the supplementation of Arg or PRD. Supplementation of additional Arg, Arg-Gln and PRD suppressed ($P<0.05$) the overexpression of interleukin 1-β (IL-1β) generated by DEX observed for control group of birds. Independently of the challenge, feeding birds with PRD treatment increased ($P<0.05$) expression of sodium-dependent glucose transporter 1 and glutathione synthetase. In conclusion, tested amino acid-based solutions were effective to improve feed efficiency and restraining intestinal inflammation caused by DEX through IL-1β pathway.

Regulation of host and microbiome interactions in ruminant livestock

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Rumen microbiome research has attracted tremendous interests due to its critical roles in contributing to ruminant's growth and productivity. Especially, rumen microbiome has been linked to many economically and environmentally important traits such as feed efficiency, methane emission, meat quality, resistance to diseases such as ruminal acidosis, and mastitis. In addition to these production and health related traits, it has also been reported to be related to animal's responses to stress such as heat stress. As such, manipulation of rumen microbiome for better performance has been widely aimed as one of the effective strategies to improve animal's performance. However, there has no effective method yet to intervene the rumen microbiome as desired because it is dynamic and complex, and its interactions with host are largely unknown. In addition, unlike the well-controlled germfree or knockout (small) animal models, the causal relationships have not been characterized due to the complex nature of microbiome and host interactions in larger outbred animals that constantly interact with the changing environment. Therefore, it is crucial to understand the modulatory mechanisms of host-microbial interactions in ruminants to develop novel cutting-edge microbiome-based interventions to improve ruminant production and health performances. This presentation will highlight research on host mechanisms that regulating gut microbiome in ruminants, including host genetics and epigenetics, host gene expression and metabolism, and non-coding RNAs. Particular emphasis will be placed on the knowledge generated on host-microbiome interactions in young ruminants, and their impact on gut health and animal performance. Understanding the key mechanisms regulating the host microbiome interactions will provide foundation for the development of microbiome intervention strategies.

Housing hygiene conditions impact adult stem cell populations in adipose and muscle tissues of pigs

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In pigs, the lean mass/fat mass ratio determines production efficiency and is strongly influenced by the number and size of cells in the tissues. During growth, the increase in the number of cells results from the recruitment of different populations of multipotent adult stem cells residing in the tissues. We hypothesized that the impact of a hygiene challenge on the proportions of adult stem cells in adipose tissue and skeletal muscle may differ between pigs with different residual feed intake (RFI), a measure of feed efficiency. At the age of 11 weeks, Large White pigs from two lines divergently selected for low and high RFI were placed in two contrasting housing hygiene conditions (good vs poor). After six weeks of challenge, pigs were slaughtered (n=6/group). Samples of subcutaneous adipose tissue and longissimus muscle were removed, and cells from the stromal vascular fraction (SVF), which includes adult stem cells, were isolated for each tissue. Adipose and muscle cell populations from the SVF were phenotyped by flow cytometry using antibodies that targeted different cell-surface markers (CD45 to identify hematopoietic cells; CD34, CD38, CD56 and CD140a to identify cells with adipogenic and/or myogenic potential). Adipose tissue and muscle shared some common cell populations with differences in their proportions. For example, CD45-CD56+ cells were abundant in both tissues, with an average proportion of 74% in muscle and 40% in adipose tissue. Among the cell populations identified in adipose tissue, the proportion of CD45-CD56-CD34- cells were higher ($P<0.05$) in pigs housed in poor hygiene than in good hygiene conditions whatever the RFI line. In skeletal muscle, preliminary analyses indicate there was an increase ($P<0.05$) in the proportion of CD45+ cells in pigs of both RFI lines housed in poor hygiene conditions compared with those housed in good conditions. Other cell populations are poorly affected by hygiene of housing. This study shows that hygiene of housing conditions has an impact on some populations of adult stem cells in adipose and muscle tissue, without any difference between the two pig lines. The animal experimentation was funded by the EU FP7 PROHEALTH project (no. 613574).

In vivo and organoid experiments reveal beneficial effects of amino acids and polyphenols in piglets

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Nutritional products represent promising alternatives to antibiotics to preserve gut health in piglets. We hypothesized that a mixture of amino acids and polyphenols could have beneficial effects trough the modulation of the gut microbiota and epithelial barrier. We used a combination of experiments *in vivo* in piglets and *in vitro* in jejunum organoids. Piglets received during the first 14-days after weaning a pre-starter feed supplemented or not with 0.1% amino acids (arginine, valine, leucine, isoleucine, cystine) and 100 ppm grape polyphenols (AA+PP). The microbiota composition and activity were analysed in jejunum and caecum content collected at day 14 after weaning. Piglet jejunum organoids were treated for 7 days with amino acids (arginine [0.8 mM], leucine [1.6 mM], isoleucine [1.6 mM], valine [1.6 mM], cystine [0.4 mM]) and 20 µg/ml grape polyphenols (AA+PP). Gene expression was analysed in organoids by qPCR. Body weight gain and gain-to-feed were significantly increased in AA+PP piglets by 22 and 15%, respectively, when compared to the control piglets. In the jejunum content, AA+PP supplementation increased the relative abundance of Lactobacillaceae when compared to the control group while it decreased microbiota richness and the relative abundances of Bacteroidaceae, Lachnospiraceae and Ruminococcaceae. In the caecum content, AA+PP supplementation reduced the relative abundance of Proteobacteria when compared to the control group. AA+PP supplementation also increased the caecal concentration of short chain fatty acids, putrescine and 3-phenylpropionate while it reduced the concentration of lactate. The growth and morphology of piglet jejunum organoids was not modified by the treatments. AA+PP reduced the expression of genes involved in epithelial proliferation (OLFM4, MKI67, PCNA) and inflammation (LBP, NFKB2, RELA) while it strongly enhanced the expression of ALPI, a marker of epithelial differentiation. In summary, our results show that AA+PP supplementation promoted the growth of post-weaning piglets. The modulation of the gut microbiota and epithelial barrier function by AA+PP could be involved in this beneficial effect.

Session 39 Theatre 12

Glutamate and glutamine supply to improve the health and immune competence of post-weaning piglets

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Glutamate (Glu) and glutamine (Gln) could have synergistic effects to promote gut health, but the ideal ratio between them when supplied to post-weaning piglets still needs to be established. This study aimed to test the effect of different dietary combinations of Glu and Gln on growth, health and gut microbiota of post-weaning piglets. A total of 120 weaned piglets (24±2 days of age, d0) were equally assigned to 6 groups according to the following diets: (1) standard diet (CO); (2) CO plus 6 kg/T Glu (100%Glu); (3) CO plus 4.5 kg/T Glu and 1.5 kg/T Gln (75%Glu + 25%Gln); (4) CO plus 3 kg/T Glu and Gln (50%Glu + 50%Gln); (5) CO plus 1.5 kg/T Glu and 4.5 kg/T Gln (25%Glu + 75%Gln); (6) CO plus 6 kg/T Gln (100%Gln). The trial lasted 21 days. Blood and caecal content were collected at d7 for haematological and microbial profile analysis, respectively. Data were fitted by GLM model including batch, litter of origin, sex and diet. From d7 to d14 CO had the lowest average daily gain (ADG) ($P=0.05$). Within AA groups, from d0 to d21, ADG and gain to feed (G:F) ratio decreased linearly with the increased contribution of Glu in the mix ($P=0.05$). During d14-d21 and d7-d21 periods feed intake tended to decrease linearly with the Glu:Gln ratio ($P=0.07$ and $P=0.09$). Faecal score was significantly reduced (drier faeces) in the AA groups vs CO ($P=0.002$) and the Glu:Gln ratio had a quadratic effect ($P=0.005$) from d0-d7. Blood neutrophil percentage (%) and lymphocyte % had a quadratic effect and monocyte % a positive linear effect of the Glu:Gln ratio ($P=0.05$). Lymphocyte % tended to be higher ($P=0.092$) and monocyte % lower ($P=0.07$) in the 100%Gln compared with the other groups. Alpha and Beta diversity of faecal microbiota profile did not differ between groups. Overall, both Gln and Glu were able to improve piglets' growth and the consistency of the faeces 7 days post-weaning. Based on these results, the optimal ratio appears to be 25%Glu + 75%Gln, further research is needed to optimize the dose of supplementation.

Session 39 Theatre 13

Effect of different Zn sources on rumen fermentation, immune, and antioxidant response in dairy cows

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Zinc (Zn) is involved in several catalytic and regulatory functions. Deficiency leads to a reduction in feed intake, growth, abnormalities of skin, udder, bone reproductive, and immune system in dairy cattle. Dietary supplementation of Zn was reported to affect rumen microbiota and to reduce the bioavailability of other minerals (Ca, Fe). This study focused on evaluating the effect of different organic Zn sources: Zn propionate (ZnP), Zn methionate (ZnM), Zn glycinate (ZnG), as well as inorganic Zn sulphate (ZnS) on rumen fermentation and bioavailability, retention, and immune and antioxidant responses in dairy cattle. Two segments of studies were done both *in vitro* and *in vivo*. The *in vitro* study using the rumen simulation technique confirmed that none of the Zn-supplementation at 175 ppm affected dry matter degradation, cellulase production, and fibre degradation during rumen fermentation. An *in vivo* trial with 30 Karan Fries mid-lactating animals (n=5 animals/group) was conducted for 23 weeks. The animals were fed with a total mixed ration containing the respective Zn supplementation at 50 ppm or 100 ppm on different trial periods. No differences were observed in feed intake, milk characteristics, plasma calcium between control and Zn supplemented groups. ZnP (50 ppm) resulted in higher ($P<0.01$) intestinal absorption and highest ($P<0.01$) in plasma Zn, as compared to the other Zn-sources. ZnP and ZnM had higher ($P<0.01$) ZN concentrations in red blood cells than the other groups. All Zn-supplemented animals had higher ($P<0.01$) metallothionein (MET) protein and MET gene expression in blood than control during the entire trial period. At 100 ppm, ZnP had higher ($P<0.01$) MET gene expression than other treatments. ZnP had higher ($P<0.01$) antibodies (Ig) than control. All Zn supplemented groups showed higher ($P<0.01$) total antioxidant potential, plasma alkaline phosphatase, and IgG than control, but no difference among them. This study enlightens the essentiality of Zn supplementation to dairy animals and the superior efficacy of ZnP over other Zn sources.

Dietary administration of oregano essential oil to newborn dairy calves improves feed efficiency

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EOs are rich in bioactive compounds and when included in the milk replacer (MR) may modify the health status and feed efficiency of new born dairy calves. To test this hypothesis 16 new-born Holstein calves received 3 L of colostrum within 2 h after birth, and then they were kept in straw-bedded single hutches during 45 days. Starting at the age of 3 days, animals were assigned to two groups of eight animals each. The first group was supplied daily 0.23 ml of non-encapsulated oregano EO accounting for 200 mg of carvacrol (EO group) diluted in the first 100 ml of milk replacer (MR). The second group (CONTROL) was fed MR (Novilac Turbostart, Schils) with no EO added. Animals were milk-fed using buckets provided with a tip to allow the reflex closure of the oesophageal groove, and MR was gradually increased at the same rate for all the animals until the maximum of 9 L liquid feed (145 g/l MR) was reached. No concentrates were offered and no leftovers of MR remained during this phase, so dry matter intake was similar for all the animals. Animals were blood sampled and weighted at birth and being 45 days-old to estimate average daily gain (ADG). The ADG of the EO group was higher (101 vs 158 g/d; P=0.027), so these animals were heavier (44.1 vs 47.4 kg; P=0.024) and more efficient because they consumed the same amount of dry matter than the control group. These differences were not related to changes in the biochemical profile (albumin, AST/GOT, GGT, beta-hydroxybutyrate, total bilirubin, Ca, Zn, Mg, ceruloplasmin, creatine kinase, creatinine, HDL, LDL, cholesterol, glucose, insulin, NEFA, triglycerides, urea, protein, globulin) parameters (P>0.05 in all of them). Further analyses are in progress to verify if this effect is related to gut microbiome modification and to identify different patterns in the metabolomic profile underlying new EO's mechanisms of action. Also, the long-term effects of EO administration during the milk-fed period on feed efficiency during the replacement phase will be tested.

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Supplementing milk with seaweeds affects liver mineral concentrations in preweaning Holstein calves

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Seaweeds can contain high concentrations of macro- and microminerals and heavy metals. This study aimed to evaluate the effect of feeding milk supplemented with either Ulva sp., Ascophyllum nodosum, or *Saccharina latissima* on mineral accumulation in muscle and liver tissue of preweaning calves. Forty Holstein calves in 10 blocks of four were used (birth BW 41±4 kg and plasma BRIX%≥8.7% d 2 after birth). Calves were fed 4 L of cow's milk twice a day (8 l/day) without (control group; CTRL, n=10) or with either Ulva sp. (SW1, n=10), A. nodosum (SW2, n=10) or *S. latissima* (SW3, n=10). The SW groups received dried and ground seaweeds from d 2 until d 42 of age, mixed into milk at a concentration of 50 g/8 L of milk (i.e. 5% on DM basis). Calves were sacrificed (n=4/treatment) and M. longissimus dorsi and liver samples were collected. The samples were analysed for concentrations of macrominerals (Ca, Mg, P, K, Na), microminerals (Cu, Fe, Mn, Zn, Se, Cr, Co, Mo) and heavy metals (As, Cd, Pb, Sr). Among macrominerals, muscle K and liver Na concentrations were affected (P<0.05), where SW1 (2.90±0.15 g/kg DM) had higher liver Na concentrations than CTRL calves (2.44±0.15 g/kg DM) (P=0.02). Among microminerals, muscle Fe (P=0.08), liver Co (P<0.01) and liver Mo (P=0.07) tended to be affected, where SW2 had higher liver Co concentration than CTRL calves (P<0.01). Among heavy metals, muscle Sr and liver Sr, Pb and Cd concentrations were affected (P≤0.05), where liver Pb concentrations of SW3 (0.14±0.01 mg/kg DM) tended to be higher than CTRL calves (0.10±0.01 mg/kg DM) (P=0.09). Furthermore, liver Cd concentrations were higher in SW2 (0.23±0.01 mg/kg DM) and SW3 (0.12±0.01 mg/kg DM) than CTRL calves (0.01±0.01 mg/kg DM) (P<0.01). In addition, liver Sr concentrations were higher in SW1 (0.26±0.03 mg/kg DM), SW2 (0.28±0.03 mg/kg DM) and SW3 (0.38±0.03 mg/kg DM) than CTRL calves (0.10±0.03 mg/kg DM) (P<0.001). In conclusion, seaweeds supplementation increased several macro-, microminerals and heavy metal concentrations, mainly in the liver tissue of preweaning calves. However, the increased heavy metal concentrations are below harmful limits for health.

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Dietary L-carnitine supplied to early feed-restricted lambs during the fattening period

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Early feed restriction of lambs (suckling period) reduces feed efficiency during the fattening phase due to mitochondrial dysfunction and increased fat accumulation. The administration of L-carnitine in the diet might help to alleviate these effects through different mechanisms (e.g. activation of the transport of fatty acids to the mitochondria and modification of ruminal fermentation and microbiome). Twenty-two newborn Merino lambs early feed restricted during the suckling period were weaned and divided into two groups of 11 lambs each. The control group (CTRL) received a complete pelleted diet (CPD) *ad libitum*, and the carnitine group (CARN) received the same ration formulated with 6 g of Carniking® (50% L-carnitine, 35% silica and 15% water, Lonza) per kg. After 75 days in fattening conditions, the lambs were slaughtered. No differences were observed in residual feed intake (RFI=-25.9 vs 25.9 g/day for CTRL and CARN, respectively; P=0.293). However, the ruminal pH was lower in CARN (5.70 vs. 5.18; P=0.023), whereas the concentration of total VFA (113 vs. 154 mmol/l; P=0.036) and the proportion of propionate (18.5 vs. 22.6 mmol/100 mmol VFA; P=0.079) were increased. On the other hand the proportions of isovalerate (1.50 vs. 0.65 mmol/100 mmol VFA; P=0.045) and isobutyrate (1.20 vs 0.67 mmol/100 mmol VFA; P=0.040) were lower in the CARN group. These differences could be caused by variations in the microbiota promoted by the degradation of L-carnitine, which produced a decrease in the relative abundances of *Fibrobacteraceae, Lachnospiraceae, Rikenellaceae* and *Spirochaetaceae*, whereas *Succinivibrionaceae* (involved in propionic acid production) was increased. Therefore, the administration of 6 g Carniking®/kg CPD modifies ruminal fermentation and microbiota but does not improve the feed efficiency during the fattening period of early feed restricted lambs.

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Bovine miRNA-mRNA dynamics during high-grain feeding and phytogenic supplementation in cattle

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The bovine rumen epithelium has a crucial role in nutrient uptake and maintenance of ruminal health. This study aimed to explore the role of microRNAs (miRNAs) in the epithelial transcriptome during transition from forage to a high-grain diet and potential modulation by supplementation with a phytogenic feed additive (PYT). Rumen biopsies were collected from nine ruminally canulated non-lactating Holstein-Friesian cows fed a baseline forage diet (HF; 0% concentrate) and then transitioned to high-grain feeding (HG; 65% concentrate). The animals were divided in two groups: five were fed the control (CON) diet and four were supplemented with PYT. Total RNA was extracted for miRNA and mRNA sequencing, and analysis of differential expression, pathway enrichment and miRNA-mRNA interaction networks was conducted. We identified 527 miRNAs shared by all samples of the rumen epithelium, from which bta-miR-21-5p, bta-miR-143 and bta-miR-24-3p were the most abundantly expressed. Eight miRNAs were differentially expressed between forage and HG feeding and 6 miRNAs between CON and PHY (FDR<0.05). Transcriptome analysis identified 9,481 differentially expressed genes (DEGs) in regards to the diet, with most of those enriched being associated with tricarboxylic acid (TCA) cycle (GO terms; biological process), sodium channel complex (GO terms; cellular components), sodium:amino acid symporter activity (GO terms; molecular function) and TCA cycle (KEGG) (FDR<0.05). MiRNA target prediction and anti-correlation analysis was used to construct interaction networks and identified 168 DEGs targeted by 8 differentially expressed miRNAs responsive to the dietary change. Supplementation of PYT modulated the expression of AZGP1, CA1, RND3, ENSBTAG00000053508 and ENSBTAG00000048514. Therefore, miRNAs are involved in both the modulation of the host response towards a HG diet and PHY supplementation.

DHA-enriched oil supplementation in MR does not affect blood concentration of DHA metabolites

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Docosahexaenoic acid (DHA) has been proven to exert anti-inflammatory effect, but the mechanism of its action is not yet fully elucidated. During the last two decades numerous DHA metabolites which function as pro-resolving lipid mediators have been discovered, with resolvins (RvD) and neuroprotectin 1 (NPD1) among them. In our study we hypothesized that dietary supplementation of DHA-enriched oil will affect blood concentration of DHA metabolites in calves. Twenty bull calves (8.1±2.0 d-old and 46.6±4.2 kg; mean ± SD) were randomly allocated into two experimental groups of 10 animals each: (1) not supplemented (CTRL); (2) supplemented with 7.5 g of DHA-enriched oil (DHA S40-O400 oil)/d in milk replacer (DHA). After 4 weeks of treatment blood samples were collected and blood concentration of RvD1, RvD2, RvD3, RvD4, NPD1 and DHA was determined using specific ELISA kits (RvD1-4: Shanghai Coon Koon Biotech Co., Shanghai, China; NPD1 and DHA: Bioassay Technology Laboratory, Shanghai, China). Data were analysed using the MIXED procedure of SAS (version 9.4). The statistical model included the fixed effects of treatment (CTRL vs DHA) and the random effect of block of animals (time of introducing to the study). No effect of the DHA-enriched oil supplementation on the concentration of investigated metabolites in the plasma of calves was found ($P \geq 0.60$), although there was a tendency towards higher blood DHA concentration in the DHA group ($P=0.07$). It can be assumed that most probably DHA provided in the diet was not transformed into metabolites but was incorporated directly into the cell membranes of white blood cells. However, this needs further verification. This is also the first study reporting blood level of investigated DHA metabolites in the calves blood. The study was supported by the Ministry of Science and Higher Education in Poland (DS-3217/KŻiDZ/2019).

DHA-enriched oil dietary supplementation does not affect cytokine production in bovine WBC

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Nutritional supplements are one of possible ways of improving health of young calves, which suffer from immunodeficiency caused by underdeveloped immune system. In our study we hypothesized that dietary supplementation of DHA-enriched oil, due to beneficial properties of docosahexaenoic acid (DHA) in resolving inflammatory processes, will decrease pro-inflammatory cytokine production in bovine white blood cells stimulated with mitogens. Twenty bull calves (8.1±2.0 d-old and 46.6±4.2 kg; mean ± SD) were randomly allocated into two experimental groups of 10 animals each: (1) not supplemented (CTRL); (2) supplemented with 7.5 g of DHA-enriched oil (DHA S40-O400 oil)/d in milk replacer (DHA). After 4 weeks of treatment blood samples were collected and stimulated with combination of staphylococcal enterotoxin B (SEB; 1 µg/ml) and LPS (500 ng/ml), for 24 hours (37 °C, Smartcell CO_2 Incubator). For each blood sample (3 ml), another 3 ml was used as a control without mitogens. After incubation, mRNA from white blood cells was isolated and transcribed to cDNA. Subsequently, mRNA expression of selected cytokines (IFNγ, TNFα, IL1β, IL4, IL6, IL10) was analysed using quantitative Real-time PCR. Data were analysed using the MIXED procedure of SAS (version 9.4). The statistical model included the fixed effects of treatment (CTRL vs DHA) and mitogen stimulation (no mitogen stimulation vs SEB + LPS stimulation) and the random effect of block of animals (time of introducing to the study). No effect of the DHA-enriched oil supplementation on the selected cytokines mRNA expression was found. In both experimental groups, the use of mitogens significantly increased cytokine expression in the white blood cells of calves ($P<0.01$). To conclude, DHA-enriched oil dietary supplementation failed to diminish mitogen-induced mRNA expression of investigated cytokines in white blood cells of calves. The study was supported by the Ministry of Science and Higher Education in Poland (DS-3217/KŻiDZ/2019).

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GSH supplementation increases diarrhoea prevalence and increases an inflammatory response

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Weaning stress induces severe gut health problems in piglets. Weaned piglets cope with gut inflammation and increased prevalence of diarrhoea. Recent studies also document that weaning is associated with oxidative stress and redox disturbance in the small intestine. Therefore, the aim of this study was to investigate the effect of supplementing the diet with glutathione (GSH), which is considered the main endogenous redox player of the antioxidant system. The hypothesis is that supplementation of GSH reduces inflammation and diarrhoea in weaned piglets. Single factor random block design was used in this experiment, 48 weaned piglets were allocated to 3 treatments according to body weight, litter origin and gender, The first treatment was basic diet(CON), whereas the other treatments studied a low dose(LGSH, 1 g/kg) or a high dose(HGSH, 10 g/kg) of the addition of feed grade reduced glutathione. The experiment lasted 14 days, and all animals were euthanized and sampled for blood at d5 (n=8) and d14 (n=8) post-weaning. Blood samples were subjected to blood cell counts. Feed intake and faeces score were registered daily. No difference in average daily feed intake and average daily growth was found between treatments. However, the diarrhoea incidence was significantly higher for both LGSH (15.75%) and HGSH (16.0%) as compared to the CON(4.5%) ($P<0.05$) during d0-14 post-weaning. Furthermore, the white blood cell count was increased for both LGSH (29.61 K/µl) and HGSH (27.43 K/µl) as compared to the CON (22.05 K/µl) ($P<0.05$) on d14. Neutrophils were significantly higher in LGSH (14.30 K/µl) vs CON (9.95 K/µl) ($P<0.05$), the same as lymphocytes in HGSH (13.98 K/µl) vs CON (10.50 K/µl) ($P<0.05$). These results indicate that dietary GSH supplementation does not support animal health but rather increases inflammation and diarrhoea problems. This could relate to the luminal GSH was used by bacterial to create an optimal redox environment, which will be detected in the future.

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Effect of a cultivated fungal vs a synthetic enzyme cocktail on *in vitro* NDF digestion of forages

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A fungal strain, termed ABO 374, was isolated from South African soil and cultivated at Stellenbosch University to yield an enzyme cocktail. The primary enzymes in the supernatant included xylanase, endoglucanase, mannanase and amylase. Our previous research showed that ABO 374 significantly increased *in vitro* NDF digestibility (iNDFD) of certain forages. The objective of the current trial was to compare ABO 374 with a mix of synthetic commercial enzymes, mixed in such a way that the different enzyme activities of the two products were similar. Lucerne hay (LH) and wheat straw (WS) were used as substrates and samples of each were treated with: (1) the ABO 374 supernatant (ABO); (2) the commercial enzyme mix with the same primary enzyme activities (SYN); and (3) distilled water (CON). After treatment, a curing time of three hours was allowed before the *in vitro* assay. Samples were incubated for 6 h, 12 h, or 24 h. Rumen fluid of two cannulated dairy cows was used in four separate runs to yield eight replications. Amylase and sodium sulphite were used in the NDF analysis. Data were analysed according to a main effects ANOVA with treatment, substrate and incubation time as main effects. Across incubation times, there was no difference among treatments regarding LH iNDFD, but iNDFD in WS tended ($P<0.07$) to be higher in the ABO treatment than in CON, whereas the SYN treatment did not differ from either. Regarding the three incubation times, there was no difference among treatments in iNDFD at any of the times in LH. In WS, however, treatment had an effect at all three incubation times. After 6 h, iNDFD was higher ($P=0.04$) in the ABO and SYN treatments than in CON (2.8, 6.3 and 4.0%), respectively. After 12 h, ABO resulted in higher ($P<0.03$) iNDFD (11.2%) than CON (3.7%), and ABO values tended ($P=0.08$) to be higher than that of SYN (6.6%). After 24 h, iNDFD tended ($P<0.09$) to be higher in the ABO treatment than in CON, while SYN did not differ from either. Values were 18.2% for ABO, 14.1% for SYN and 13.0% for CON. It was concluded that both enzymes improved iNDFD in a poor quality forage, such as WS. However, the fungal enzyme cocktail appeared to be superior to the commercial enzyme mix.

Session 40 Theatre 1

Evaluation of growth and reproduction performance of replacement dairy heifers

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The objective of the current study was to evaluate the current status of heifer rearing under typical Mediterranean conditions. Data were collected from 8 dairy farms in the region of Thessaly and Macedonia (Greece) during a 1-day visit. Records on reproduction performance for all cows and heifers present in the herd were obtained by the farmer and used to determine time of first service (T1stSer), services/heifer and time of first calving (T1stCal). Depending on heifer grouping of the farms, 15-20% of heifers in all age groups and older cows were taped measured to estimate body weight (BW). This allowed the determination of average daily gain (ADG) in different ages. Moreover, the mature BW of the cows in each herd was estimated and used to evaluate growth targets. Total mixed ration (TMR) offered to heifers was recorded and feed samples (TMR and ingredients) were collected and chemically analysed for nutrient composition. Based on cows mature BW, and 14 and 24 months for T1stSer and T1stCal, respectively, growth targets for heifers were estimated in each farm. The diets were evaluated with the Cornell Net Carbohydrate and Protein System (CNCPS 6.5). It was found that T1stSer and T1stCal was 15.83±1.96 and 25.83±2.53 months, respectively and pregnancy was achieved with 1.55±1.04) services/heifer. Heifer BW at T1stSer and T1stCal was 416 and 668 kg, respectively that was within the targets (404 and 625 for T1stSer and T1stCal, respectively) but achieved with a delay of 1.83 months. Our analysis suggested that T1stSer is the most critical point where heifers need to meet higher ADG. In all farms and heifer growth stages Metabolizable Energy (ME) was the first limiting factor and Metabolizable Protein (MP) was supplied in excess (up to 140% of requirements for older heifers). Overfeeding crude protein increased feeding cost from 116 to 236 €/heifer depending on protein source.

Session 40 Theatre 2

Impact of enhanced compared to restricted milk feeding on performance and welfare of rearing calves

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Within the Core Organic Cofund project ProYoungStock, an experimental multi-site study was performed to investigate the effects of an increased level of milk feeding (10-12 l/d, ≈ 14-16% milk per body weight, enhanced milk feeding, EMF) compared to common milk feeding (6-8 l/d, ≈ 10-12% milk per body weight, restricted milk feeding, RMF). Data from 10 organic dairy farms, originally applying RMF by bucket-feeding in Austria (dual-purpose breed Fleckvieh; 4 farms) or Germany (Holstein Friesian, partly crossed with other dairy breeds; 6 farms) were analysed concerning calf welfare including health. Per farm, 6-14 female rearing calves were randomly allocated to the two feeding treatments RMF and EMF, which were applied over the milk feeding period of 13 weeks. Assessments of welfare were carried out during the milk feeding period in weeks of life 3/4, 7/8, 11/12 (±2 days) as well as after weaning in about week 15/16. Farm visits took place from October 2018 to April 2020. Welfare measures related to behaviour, observed from video recordings (manipulating other calves, manipulating objects, vocalization, duration of feeding solid feed), to health (clinical scoring during farm visits), and to performance (weight gain). Preliminary results showed that EMF led to higher weights and daily weight gains in rearing calves, especially in Austrian Fleckvieh calves. Manipulating objects was not related to milk amounts but to the feeding duration of solid feed. Analyses are still in progress; final results will be presented at the congress.

Plasma vitamin E in calves fed different sources and doses of vitamin E around weaning

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It is a challenge to keep a sufficient vitamin E level (2 mg/l) in calf plasma after weaning, and this seems to be critical for a good immune response. Two experiments were performed in order to find the most efficient source and dose of vitamin E in calf starter concentrates to keep plasma vitamin E level above the critical level after weaning. Experiment 1 (Exp 1) and 2 (Exp 2) included a total of 32 and 40 calves, respectively, from at least 2 weeks before weaning until at least 2 weeks after weaning. In Exp 1, calves were infused through mouth a daily dose of 200 mg of different vitamin E sources including no α-tocopherol in the feed (0 dose; Control), and 200 mg/d of either RRR-α-tocopherol (ALC), RRR-α-tocopheryl acetate (ACT) or *all-rac*-α-tocopheryl acetate (RAC). In Exp 2, a dose response study was carried out with 0, 60, 120, and 200 mg/kg of ALC in pelleted calf concentrates. In both Exp 1 and 2, calves had free access to concentrate, grass hay, and fresh water. In Exp 1 and 2, body weight and average daily gain were not affected by either different sources or doses of α-tocopherol, respectively. In Exp 1, plasma RRR-α-tocopherol level was affected by source of α-tocopherol ($P<0.001$), week ($P<0.001$) and interaction between them ($P<0.001$). At weaning, plasma RRR-α-tocopherol was 4.3, 3.3, 1.5, and 1.0 µg/ml in ALC, ACT, SYN, and control, respectively. In Exp 2, total plasma α-tocopherol level was affected by dose of ALC ($P=0.04$), week ($P<0.001$), and tendency for interaction between dose and week ($P=0.06$). At weaning, 36, 31, 28% reduction in plasma α-tocopherol level was observed compared to beginning of experiment in 0, 60, and 120 mg/kg of ALC, respectively; however, in 200 mg/kg of ALC, more than 9% increase in plasma α-tocopherol level was observed. In addition, 200 mg/kg of ALC was able to keep plasma α-tocopherol high after weaning (3.1 and 2.9 µg/ml in weeks 1 and 2 after weaning, respectively), which is well above the recommended level. In conclusion, the ALC was the most efficient source of α-tocopherol supplementation to be used in a calf starter concentrate. In addition, the 200 mg/kg of ALC in calf starter concentrate was the only effective dose to keep the plasma vitamin E levels higher than the critical level after weaning.

Effect of enhanced early life nutrition on the of the anterior pituitary proteome of heifer calves

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Advancing the age at which puberty is attained is central to the financial and environmental sustainability of cattle production systems. Nutritional control during early maturation in heifers exerts a substantial influence on the timing of puberty. Indeed, metabolic hormones and metabolites are a major component of the complex neural signalling system involved in the maturation of the hypothalamic-pituitary-ovarian (HPO) axis and control of reproductive function. Whilst knowledge of the biochemical interplays that triggers the pubertal process has improved in recent time, much of the intricate mechanistic detail still eludes us. Therefore, the objective of this study was to determine the effect of an enhanced plane of nutrition from 3 to 21 weeks of life in heifer calves on the proteome of the anterior pituitary gland. Angus × Holstein-Friesian heifer calves (19±5 days of age, BW: 51.2±7.8 kg, mean ± SD) were offered either a high (HP, n=14) or moderate plane of nutrition (MP, n=15) from 3 to 21 weeks of age. Target growth rates were 1.2 kg/d and 0.5 kg/d, for HP and MP groups, respectively. At 21 weeks of age all calves were euthanized and anterior pituitary tissue harvested from all calves. Protein fraction was isolated from all anterior pituitary samples and subsequently subjected to label-free quantification analysis. Average daily gain was affected by diet ($P<0.001$) and was 1.18 and 0.50 kg/day, for HP and MP calves, respectively. Proteomic analysis resulted in the identification of 251 differentially abundant proteins (DAP) ($P_{adj}<0.1$; fold change>1.5). Functional biological analysis of DAPs identified, indicated that prevailing dietary management affected reproductive development in the heifer calves through an effect on both folliculogenesis ($P.adj<0.001$) and development of the female reproductive tract ($P.adj.<0.01$) which were both predicted to be affected by prevailing dietary management based on DAPs identified. Results from this study show that enhanced dietary intake during early calfhood affected the anterior pituitary proteome, with DAPs involved in reproductive development specifically affected.

Effects of maize silage (MS) inclusion at different age in Holstein Friesian and Belgian Blue calves

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The provision of forages to calves before weaning is important for animal development and performance. In common practice, preweaned calves are fed with concentrates and hay or straw. However, little is known about the ideal age to start feeding MS. A randomized controlled trial was performed to assess the impact of the age of MS inclusion in Holstein Friesian (HF) and Belgian Blue (BB) calves on dry matter intake (DMI), animal growth and feed efficiency. Sixty heifer and fifteen steer HF calves were at birth randomly assigned to 3 treatments: (1) MS feeding in the preweaning period from the age of 4 wks (MS_h-4W), (2) MS feeding in the weaning period from 10 wks of age (MS_h-10W) and, (3) MS feeding in the postweaning period from 16 wks of age (MS_h-16W). Forty female BB calves were randomly distributed in 2 groups: (1) MS feeding in the preweaning period from the age of 6 wks (MS_b-6W) and (2) MS feeding in the postweaning period from 16 wks of age (MS_b-16W). All calves followed a standardized milk replacer (MR) feeding protocol. Between 4 and 10 wks of age, MS_h-4W calves had a lower concentrate intake ($P<0.01$), but did not differ in DMI and growth compared to MS_h-10W. Between 10 and 16 wks of age, MS_h-10W had a higher DMI (3.23 kg DM/d, $P=0.003$) compared to MS_h-16W (2.93 kg DM/d), but no effect on growth was seen. For both HF and BB calves, the timing of inclusion of MS had no impact on feed efficiency. Feeding MS in the preweaning period of BB calves had a negative impact on hay and DMI (MS_b-6W: 1.66 kg DM/d; MS_b-16W: 1.86 kg DM/d, $P<0.001$) and on the average daily gain (MS_b-6W: 0.83 kg/d; MS_b-16W: 0.98 kg/d; $P=0.0001$). This effect can be explained by the lower feed intake capacity of BB calves resulting in a depression in concentrate intake when MS is given early. In conclusion, feeding MS to preweaned HF calves can improve the DMI in the postweaning stage. However, an early inclusion of MS in BB calves should be avoided because of the lower DMI and growth in the preweaning period.

Grazing behaviour of pregnant dairy heifers depending on pasture growth

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Dairy Heifers are often kept on pasture during summer months not only to improved animal welfare. Demands on condition of heifers and of pasture need to be harmonized for an optimal start into lactation. To investigate the grazing behaviour of eight dairy heifers on an intensive pasture system in lower Saxony the heifers were equipped with GPS trackers ('Vertex Plus Collar' ('Vectronic Aerospace GmbH')) and activity sensors ('IceTag' ('IceRobotics Ltd.')). Heifers were kept consecutively on four pastures (2.4 ha to 5.0 ha) from August 2019 to November 2019. Position, number of steps, lying and standing time were observed. Additionally, grass height was measured two times a week using a rising plate meter. Lameness and body condition score were determined once a week or biweekly, respectively. To analyse the heifers' behaviour the day was divided into nighttime (00:00 – 06:59 h), morning (07:00 – 12:59 h), afternoon (13:00 – 18:59 h) and evening (19:00 – 23:59 h). As main grazing time periods the morning and the evening were identified. On average heifers walked 2.5 to 3.5 km a day with an average step length of 0.65 m. As expected, longest lying times occurred during the night (59.3 and 85.4%, depending on heifer and pasture number). Older heifers as well as heifers late in pregnancy were found to have longer lying periods than the other heifers, which instead had more lying bouts. However, lying time itself did not differ. The grass height showed a mosaic pattern between shorter and higher grass (differences from approx. 8 cm down to approx. 2 cm). Higher grass could be found around main dung places, which the cows avoided for grazing and were located at the high frequented places near water trough and main resting areas. All heifers remained free of lameness and in a good condition during the study. In conclusion rearing on pasture is a well usable system for dairy heifers not only to increase animal welfare but for sustainable land use. Results showed that management options to guide heifers and with that optimize grazing activity do exist.

Heart girth measurements to estimate body weight of growing and mature Holstein-Friesian cattle

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Replacement heifers are required in all dairy herds, but they consume feed and excrete polluting nitrogen products during the first two or more years of growth before becoming productive. The industry standard for Holstein heifers to achieve first breeding is 15 months, to calve at 2 years old. Increasing the time to first calving reduces nutrient use efficiency of dairy farms, so ensuring ideal growth rates and calving ages is important. Even today, many dairy farms still lack access to weigh scales, although knowledge of body weight (BW) is necessary for monitoring growth and optimising nutrition. Weigh tapes are a cheap, widely available alternative. Body weight (kg), recorded using a weigh crush, and heart girth (HG, cm) were recorded in growing Holstein-Friesian heifers, fed different dietary regimes, from ~7 weeks old to the end of 2nd lactation. One hundred heifers started the experiment and 8,948 individual animal BW and HG measurements were recorded. Mean data were calculated for every 2 weeks of age (range 7-247 weeks old) resulting in 233 paired comparisons of BW (range 51-942 kg) and HG measurements (range 88-232 cm). A quadratic equation was fitted to investigate the relationship between HG and BW: $BW = 0.032 \times HG^2 - 3.89 \times HG + 154.4$; $R^2=0.999$. A commercially available weigh tape (ANImeter®, Göbel, Germany) was used to predict BW from known HG, which were compared against known BW measurements. Predicted values were within 20% of the true BW>99% of the time, with a Lin's concordance correlation coefficient (L_c) of 0.999. Two other prediction equations (Heinrichs *et al.*: $BW = 102.71 - 2.876 \times HG + 0.02655 \times HG^2$, and Sastry *et al.*: Schaeffer's formula: $W = (L \times G^2) / 300$, where W=BW in lbs, L=animal back length from withers to tail head in inches, and G=HG in inches) were also used to estimate BW, but resulted in lower L_c values (0.987 and 0.811 respectively) with current data. It is concluded that the correlation between HG and BW is sufficiently accurate to allow the use of BW estimated from HG measurements for the nutritional management of growing dairy heifers in the absence of weigh scales, and that commercially available tapes are appropriate for this.

Body measurement for female Holstein Friesian calves' weight estimation from birth to 15 months old

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The growth monitoring process should represent an important part of dairy heifers from the birth to the first insemination. Body measurements could be used to estimate body weight and growing rate with no need of a scale. The objective of this study was to estimate the heifers' weight at different ages on the basis based on their body dimension characteristics. A total of 25 Holstein heifers were monitored from the birth to 15 months of age (23 time points) at the University of Pisa's experimental dairy farm, representing the study group (SG); 13 different animals were monitored during the same timeframe as a validation group (VG). Immediately after birth, weekly until 2 months of life and monthly until 15 months of age, all the heifers were weighed (BW) and wither height (WH), shin circumference (SC), heart girth circumference (HG), body length (BL), hip width (HW) were measured using a tape measure. Moreover, for each time point and animal, body condition score (BCS) was also assessed. Equations were selected with a stepwise regression in order to estimate the BW at each different time using body measures for the SG. These equations were run with VG data for obtaining the estimated heifers BW, then actual and predicted heifers BW were compared by linear regression, in order to validate the equations. Equations found were all statistically significant ($r^2=0.688$ to 0.894; $P<0.0001$). Residuals were normally and randomly distributed. The best estimation of BW was obtained by using three or less variables at 11/23 time point whereas at 10/23 and 2/23 time points 4 and 5 variables were needed, respectively. In any case, HG was the most informative parameter in order to achieve optimal estimation of BW.

Session 41 Theatre 1

Introduction to FreeWalk housing project

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Aim of project FreeWalk (www.freewalk.eu) was to further develop economic sound free walk cattle faring systems, which improve animal welfare and soil structure, utilize waste products and have public support. As innovative housing systems, the compost bedded-pack barn – 22 case farms – and the permeable artificial floor (‘cow garden’) are applied with a completely free walking and lying area, and compared with 22 cubicle farms for reference, all spread over 6 European countries. The bedded pack barn uses organic waste materials as bedding. Housing as part of intensive and extensive farming has been examined in a holistic context, encompassing the whole farm: bedding material, artificial floor, animal welfare, health, milk quality, manure quality, and CPN-balances. Insight in the composting process plays a crucial role in the success of the bedded-pack system. Innovative techniques, like the Cow Toilet were introduced. Societal appreciation of the freewalk farming systems and products was examined in the eight participating countries. Finally, an integrated assessment of the case farms was undertaken by multi-criteria-analysis. It was done in intensive cooperation with cattle housing companies.

Session 41 Theatre 2

Dairy cattle housing, management and welfare in Switzerland

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Tie-stalls were the traditional housing system for dairy cows in Switzerland. Over the last 50 years, however, loose housing systems became more and more popular, and this trend was associated with an increase in herd size per farm. Nowadays, the majority of cows are kept in cubicle housing systems, and the number of farms equipped with automatic milking systems has risen considerably. The Swiss animal welfare legislation contains detailed regulations on the housing and management of dairy cows. For example, tethered dairy cows must have access to an outdoor exercise area on a regular basis, but at least on 60 days during the vegetation period and on 30 days during the winter feeding period. With regard to loose-housed cows, minimal cubicle dimensions as well as feeding space at the manger are defined in relation to withers height. In addition, farmers may receive direct payments if they increase the number of days with outdoor access or improve the quality of the lying area for the cows. The Centre for Proper Housing of Ruminants and Pigs is part of the Federal Food Safety and Veterinary Office and situated at the research site of Agroscope in Tänikon. It is responsible for the testing of mass-produced equipment and housing systems for dairy cattle and conducts research to improve housing conditions with regard to animal welfare. For example, we showed that both the frequency and duration of outdoor exercise have significant effects on the prevalence of hock lesions in tied dairy cows. Recent studies also investigated how body size in relation to cubicle dimensions affects lying behaviour in dairy cows, and whether agonistic interactions and heart rate variability differ between horned and dehorned cows when exposed to different space allowances of the outdoor exercise area. In the final part of the presentation, animal welfare issues that could be important for the future development of cattle housing in Switzerland are addressed.

Future of dairy cattle housing from a U.S. perspective

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The dairy sector in the U.S. has had numerous changes over the last ~50 years. Annual production per cow was 2,053 kg in 1944 but has increased to over 10,500 kg currently. This demonstrates a large increase in efficiency. The dairy industry in the U.S. contributes about 1% of total greenhouse gasses. One clear trend is consolidation, with a striking reduction in the number of farms. The U.S. has now approximately 35,000 dairy farms, especially after large recent declines (2017 to 2020). However, the number of cows has stayed around 9 million. This means that cows are leaving smaller herds and moving to larger ones. Therefore, another trend has been the reduction in the percent of operations with tie-stalls. Surveys show a reduction from 61.4% to 38.9% of dairy operations. The recent loss of herds since 2017 has most likely further reduced the number of farms using tie-stalls. The majority of large operations (51.5%) in the NAHMS 2014 survey housed lactating cows in a freestall with no outside access. The top three primary bedding types used for lactating cows by the highest percentages of operations were straw and/or hay (29.7%), sand (21.9%), and sawdust/wood products (21.9%). However, the highest percentage of cows were housed on sand (30.1%), followed by dried manure (21.2%). Sand is the bedding of choice in many large operations in the U.S. as it offers excellent cow comfort contributing to improved productivity and better health and welfare. Animal welfare is an important aspect of dairy sector sustainability (social license) along with economics and environmental sustainability. In the future there will most likely be increased consolidation of farms in the U.S. with the majority of cows housed in freestall barns with or without access to open lots. The adoption of automation for milking, feeding, barn management, and cow management will escalate. Labour availability, especially for milking cows, is driving this trend. We expect more stringent environmental regulations, with a constant thrust to continually improve milk production efficiency. Will this be done taking into account animal welfare? Consumers are not only interested in environmental sustainability; animal care is also paramount for the continued existence of the dairy sector in the U.S.

Future of cattle housing

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Increased labour efficiency has been an important driver of the change from tie-stall barns to cubicle barns, also known as freestall barns. In future housing systems, the natural behaviour of cows, climate control, emissions of ammonia and greenhouse gasses, reuse of waste, manure quality, the aesthetics of buildings in the landscape and capital efficiency are becoming increasingly important elements. To address these future requirements, new concepts beyond cubicle barns must be developed. Freewalk housing systems, i.e. loose housing systems without cubicles, would meet some of these future demands. These systems operate with composting bedding material or artificial permeable floors as lying and walking areas. However, these barns are still in development. For example: there is a great variation in m^2 per cow and management of the bedding, as shown at the twenty-two freewalk system in the project www.freewalk.eu. Ammonia emissions in the freewalk barn with wood chips bedding were found to be 31% lower per cow than those in the reference cubicle herd, whereas methane emission was 34% higher. Combinations of cubicle and freewalk housing systems, together with other techniques being developed might become a major future housing system. Because quality of manure is getting more important, systems which deliver a composted bedding material or manure separated into faeces and urine that function both as fertilizer and soil improver are favoured. Other techniques and systems that are being explored according to sustainability criteria include the multi-climate shed with the bedding as a biofilter, capturing and burning methane from the barn and the manure storage, the CowToilet and permeable floors to separate faeces and urine. These buildings and techniques, and also multifunctional buildings, can be part of land based or, less common, city based farming systems, such as floating farms.

Lying behaviour in freewalk vs cubicle barns

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A limitation of space for lying down and getting up is one of the key characteristics of cubicle barns (CB), even when cubicles are well dimensioned. This spatial limitation is absent in freewalk barns (FWB) and at pasture, and therefore it is assumed that it is easier for cows to lie down in FWB than in CB. This corresponds with observations during welfare assessments at farm level for the freewalk project, that showed that cows in FWS took less time to lie down. These observations however also showed substantial variation between cows and barns. This indicates that individual differences in ease of lying down and getting up have to be taken into account when comparing figures of different herds. Moreover, not all cows may experience the same difficulties with limited space for lying down and getting up, e.g. due to differences in body size. Of course, for overall comfort around resting the absence of obstacles is not the only determinant, but also the bedding should provide comfort, barn climate should be adequate and animals should be minimally disturbed when lying down. Regarding bedding preference, it is known that previous experience of animals has its influence, e.g. in relation to preference for sand bedded stalls. In order to obtain more detailed information about the impact of spatial limitation for time needed to lie down and get up, we have carried out an observational experiment at research farm Dairy Campus (Leeuwarden, the Netherlands). Three groups of 16 cows were kept in either a reference CB, an FWB with deep straw bedding or an FWB with artificial floor. The cows were monitored for 3 weeks in a row, and were allocated to another housing weekly. Group composition was kept constant during the observation period. Time budgets and lying down and getting up events were monitored with IceQubes. Video footage is annotated to obtain durations of lying down and getting up events of all cows under the three housing conditions. Moreover, time budgets and lying bout lengths were monitored for three commercial farms from march 2018 until May 2019 (one with CB, one with FWB with wood chip bedding and one with FWB with artificial floor). Results of comparison of lying behaviour between CB and the two types of FWB will be shown and discussed.

Bedding management in compost-bedded pack and cubicle barns for dairy cows in Europe

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Over the last years, compost-bedded pack barns (CBP) have become popular as an alternative housing system for dairy cows. Unlike in cubicle barns (CB), cattle excreta produced in CBP are mixed into the bedding. As a consequence, CBP require larger volumes of bedding materials to maintain adequate hygienic conditions of the resting area. The objective of this study was to assess bedded pack characteristics and bedding management in CBP and CB in Europe. The study was conducted on 40 commercial dairy farms from 6 EU countries with CBP (n=20) or CB (n=20). Each farm was visited 6 times over a period of 20 months (December 2017-July 2019) to monitor bedding management and associated costs. The main dimensions and characteristics of the housing facilities for lactating cows were also recorded. Total pen area was significantly larger in CBP (16.45 m^2/cow) than in FS (8.77 m^2/cow). In CBP, stocking density on the bedded pack was 12.01 m^2/cow while cows in FS were stocked at 1.08 cows/stall. In CBP, bedded pack moisture was affected significantly by season, ranging from 59.5% in summer to 68.1% in spring. Utilization of bedding materials was significantly higher in CBP (9.36 kg/cow/day) than in CB (3.07 kg/cow/day). A significant effect of season was found for bedding utilization in CBP, while the use of bedding remained stable throughout the year in CB. Although the larger amount of bedding required in CBP resulted in higher bedding costs (177.1 vs 53.1 €/cow/year), the time required for bedding management was significantly lower in CBP (1.63 h/cow/year) than in CB (3.69 h/cow/year).

Session 41 Theatre 7

Cowtoilet and bedding cleaner

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The expectations for future dairy housing systems is to develop floor types and other techniques that separate faeces and urine and maintain walkability, because of the decreased ammonia emissions due to separation, the decreased methane emission due to rapid removal from the barn and the possible creation of different manure products for efficient use of N, P, K and organic matter C. The separation of urine and faeces can be performed with a grooved floor that separates in a horizontal direction; by using plates that separate in a vertical manner, such as an permeable artificial floor; or by using a belt underneath a slatted floor. A current innovation is the CowToilet, invented by Hanskamp since 2016 on the basis of research on defecating and urinating behaviours. The CowToilet is an automatic urinal that cows use voluntarily. The innovation has been designed to collect the urine before it hits the floor. When a cow is at a concentrate feeder, she is stimulated to urinate by touching a nerve at the back of her udder in a gentle way by the movable toilet during a maximum of two minutes; then, when giving response, this movable toilet collects the urine. This technique results in better separation of faeces and urine than separation on a floor, but not all urine will be collected by the CowToilet, approximately 20 to 40%. The faeces and the not collected urine can be stored underneath a slatted floor. The CowToilet can be seen as a separate technique to be used as an alternative for low emission floors in cubicle barns or in combination with freewalk (no cubicles) housing systems to decrease bedding moisture content in the bedding area with woodchips, sawdust or straw. Another development to clean the bedding in a freewalk housing system is by using a bedding cleaner, also invented by Hanskamp. Once a day this cleaner can pick up the faeces from a sand bedding or a bedding with fine organic material. The urine in the sand bedding will be drained through pipes and stored outside. The urine in a organic bedding will be absorbed. The cowtoilet and bedding cleaner ensures a cleaner environment for the cow and a reduction of emissions in the stall by separating the faeces and urine.

Session 41 Theatre 8

Health aspects of dairy herds in FreeWalk and cubicle housing systems

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Alternative housing systems for dairy cows, such as compost-bedded pack (CBP) barns has received an increasing interest in Europe. The objective of this study was to assess the animal health in CBP systems compared to health in the more conventional cubicle housing systems (CH). Data from twenty commercial dairy farms with CBP housing from 6 European countries (AT, DE, IT, NL, SE, SI) and twenty matched farms with CH housing systems were collected, although only 16 herd-pairs were included in these analyses. Routinely recorded data within the dairy herd improvement organizations were retrieved and subjected to a common procedure for calculation of variables. Data on individual cows from the calendar years 2017 and 2018 were included in this analysis. Health variables were somatic cell counts, incidence of high fat/protein ratios (risk of ketosis), and incidence of prolonged calving intervals while incidence of first parity heifers was also evaluated. Generalized linear mixed models were used to assess the associations between housing system and outcome variables, simultaneously accounting for fixed effects such as breed, parity, days in milk, year-month of test, etc. where relevant. The models also accounted for the matched design and for the repeated observations within herd and cow (where relevant). The average somatic cell count was significantly higher in the FW systems, while there was no difference in the risk for ketosis. The proportion of first calving heifers was significantly lower in the CBP system, and they also had a lower incidence of prolonged calving intervals. Acknowledgments: ERA-NET SUSAN.

Session 41 Theatre 9

Dairy cattle welfare in two housing systems in Europe

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Cattle welfare assessment in 20 compost barns (CPB) and 20 cubicle housing (CH) systems was carried out with the Welfare Quality (WQ®) protocol. Herds from 6 countries (NL, DE, IT, SE, AT, SI) were visited during winter 2017-18 and summer 2018. Using the WQ® software program, values of 30 measures determine, through aggregation steps, the scores of 12 welfare criteria, four welfare principles (Good feeding, Good housing, Good health, and Appropriate behaviour) and the overall score (excellent, enhanced, acceptable and not acceptable). Scores are given on a scale from 0 (worst) to 100 (best situation). No farm showed excellent level but at least one-third of the herds reached the enhanced level. Herds with enhanced and acceptable level did not differ among systems. There was a large variation within systems, but 'Good housing' was significantly better for CPB. The median of 'Appropriate behaviour' for CH reached the first quartile (<25 scores). Among welfare criteria there were differences in 'comfort around resting' ($P=0.007$, better scores in CPB), 'absence of diseases' ($P=0.051$, better scores at CH), and 'positive emotional state' ($P=0.009$, better scores in CPB). There were differences between countries in welfare principles, i.e. 'good feeding' ($P=0.062$, top scores in AT), and 'appropriate behaviour' ($P=0.06$; top score in SE); as well as in welfare criteria such as 'absence of pain induced by management' ($P<0.001$; better scores in SE, AT), 'expression of other behaviour' ($P=0.033$; better scores in SE, NL), and 'good human-animal relation' ($P=0.050$; better scores in SE, AT). Medians of the two last criteria reached the first quartile. WQ® extracted differences among systems and revealed that 'good housing' in CPB is linked to better physical comfort and social environment for cows. Country differences could be caused by varying handling practices that affect animal behaviour. Acknowledgements: ERA-NET SUSAN.

Session 41 Theatre 10

Animal-centric approach to mitigate heat stress – housing and management practices in Israel

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Open cowsheds, dry climate conditions, compost bedding with high roof naturally ventilated – have shifted the attention in Israel from core barn design to complementary monitor animal response to heat and other stressors. Technological progress enables individual cow's temperatures, activity, production, and physiology status to be measured in real time, using varied sensors such as bolus sensor inserted into the rumen (reticulorumen). The presentation will focus on current cooling systems (often work at a constant schedule, not monitoring the animal itself) vs tailoring the cooling management to the cow's thermal reaction. A total of 30 lactating cows were randomly assigned to one of two treatment groups; the groups received two different evaporative cooling regimes. A control group received cooling sessions on a preset time-based schedule, the method commonly used in farms; and an experimental group, which received the sensor-based (SB) cooling regime. Results: the proposed treatment can mitigate heat stress. The two treatment groups of cows had similar milk yields (44.7 kg/d), but those in the experimental group had higher milk fat (3.65 vs 3.43%), higher milk protein (3.23 vs 3.13%), higher energy corrected milk (ECM, 42.84 vs 41.48 kg/d), higher fat corrected milk 4%; (42.76 vs 41.34 kg/d), and shorter heat stress duration (5.03 vs 9.46 h/day) comparing to the control. Dry matter intake was higher in the experimental group. Daily visits to the feed trough were less frequent, with each visit lasting longer. The sensor-based cooling regime may be an effective tool to detect and ease heat stress in high-producing dairy cows during transitional seasons when heat load can become severe. In the presentation, the same concept will be demonstrated also with other PLF concepts applying different housing and sensors.

Session 41 Poster 11

Potential alternative bedding materials for dairy cattle

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The evaluation of the bedding materials is important to understand the nature of the materials and how their physical characteristics can affect the dairy system. The aim of this paper is to evaluate the physical properties of different alternative and conventional bedding materials for dairy cattle for use in compost bedded pack barn or free stall barn systems. The experiment was carried out in the Department of Agriculture, Food, Environment and Forestry at the University of Florence within the ERA-NET SusAn project 'FreeWalk' (European Union's Horizon 2020 Research and Innovation Program under the grant agreement No. 696231). For these analyses, eight bedding materials obtained from farms were evaluated (bark from pine, barley husk, flax straw, fresh forest, hemp straw, triticale husks, wheat husks and wheat straw). The physical properties analysed included bulk density (BD), water holding capacity (WHC), air-filled porosity (AFP), global density (GD), container capacity (CC), total effective porosity (TEP) and saturated humidity (SH). Based on the results, the conventional materials wheat husks and wheat straw presented the highest values of WHC (3.1 and 4.1 kg/kg, respectively). WHC can be considered as one of the most important physical properties to potential materials to be used as bedding. Fresh forest presented the highest value of BD (453.1 kg/m^3) and GD (153.1 kg/m^3). Higher BD values imply an increase in mass and a decrease in AFP, TEP. Triticale husk and wheat husk presented the smaller values of BD (27.4 and 35.9 kg.m-3, respectively). Triticale husk had a low CC (7.6%), indicating that when this material is under saturation and drainage conditions, it has a low capacity for water retention. The results demonstrate that the physical properties of the bedding materials differed widely among the materials. All of the materials presented good behaviour with regard to their physical properties. From this analysis, it was possible to understand how the physical properties of the bedding materials may influence their behaviour.

Session 42 Theatre 1

How individual sorting behaviour in dairy goats might modify dietary nutritive characteristics?

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Goats are able to adapt to various environments due to their developed sorting behaviour. They are able to select the most nutritive fractions of a forage. This study aimed to test if this sorting behaviour and the resulting nutritive value are part of an animal's personality i.e. if they differ between, but are repeatable within individuals. Eight early-lactating Alpine goats (week 1, 3, 8 and 14 post kidding) were housed in individual pens and fed corn silage, alfalfa hay and concentrate in three separate troughs. Roughages were fed *ad libitum*. Samples of intake and refusals were taken during five consecutive days and analysed for lignocellulose content (ADF) and for morphological fractions (stems and leaves for alfalfa hay and five fractions for corn silage: stalk and fine leaves, stalk and medium leaves, husk, cob and grain). Percentage of refusals was around 25% for each forage. For hay, goats preferred leaves as they sorted against stems (43.0% stem in the offered hay vs 80.7% in the refusals), with a between-goat standard deviation of 7.23 and a significant goat effect. Three animals were very selective with around 90% of refusals constituted by stems, whereas one had only 68% of stems in its refusal. The outcome was that ADF of hay decreased by 49 g/kg DM, between the hay offered and the hay ingested (347 vs 298 g/kg DM) with a significant between-goat effect (decrease per goat ranging from 34 to 62 g/kg DM). According to intra-roughages relationships based on the INRA 2018 tables, the mean energy value of the alfalfa hay was therefore improved by 0.12 UFL (between 0.08 and 0.15 UFL/kg DM), and the fill value decreased by 0.076 (between 0.05 and 0.10 UEL/kg DM). The personality aspect was more pronounced for corn silage, especially for sorting against husks and cobs. Silage ingested was less fibrous than offered (167 vs 182 g ADF/kg DM), with individual values ranging from 143 to 182. Thus, the mean improvement of the energy value was 0.03 UFL/kg DM (maximum 0.07/kg DM for one goat) and the fill value decreased by 0.059 UEL (maximum 0.160/kg DM). Sorting behaviour may be a personality trait in dairy goats which might enhance the nutritive value of roughages fed *ad libitum*.

Nutritive value of cabbage and pelleted complete feed in free-ranged organic growing rabbit

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Free-ranged organic rabbit correspond to the consumer's demand of improved animal welfare conditions, but lacks in technical reference values and nutritive value of plants available at farm. We thus studied the digestibility of cabbage (broccoli, leaf stage) and of commercial pelleted feed for three groups of 5 growing rabbits (74 days old, mean live weight = 1,830 g) housed individually in movable cages on pasture and following organic regulation for rabbit farming. Each cage had a shelter of 0.4 m^2 and a pasturing surface of 1.2 m^2. The group C was fed only with cabbage (500 g fresh on morning and on evening), the group P with pelleted feed only, and the group CP was fed with cabbage + 60 g/d of pelleted feed. After one week of adaptation to movable cage and feeds (74-81 d old), the digestibility was measured for four days, with feed intake and faecal total collection measurement (a board was installed on the cage floor to collect the faeces). During digestibility period, the dry matter (DM) intake of cabbage averaged 95.5 g/d per rabbit of the group C, corresponding to a daily fresh intake of 664 g. Pellet intake of the P group averaged 240 g DM/d, while in CP group the cabbage intake averaged only 54.5 g DM/d for a pellet intake of 53.9 g DM/d. The cabbage contained a high content of ash (19.4%) for a protein content of 18.4% and a fibre content of 23.1% of NDF and 16.6%ADF and 4.5% ADL. Organic matter (OM) digestibility of cabbage was very high (85.0%) as well as for crude protein and energy digestibility (85.4 and 81.7% respectively). Cabbage digestible protein and energy content (direct measure), fed alone, thus averaged 158 g PD/kg and 12.16 MJ DE/kg. The commercial pellet nutritive content (group P) was moderate (9 g PD/kg and 8.76 MJ DE/kg). When the cabbage was fed freely and complemented by 60 g/d of pellets (group CP), the DM digestibility was reduced proportionally to the pellet intake (49.7% of the total intake), and averaged 65.9%, similar to that recalculated from C and P values (67.8%). Thus, mixing cabbage with pelleted feed did not alter the nutritive value of the cabbage.

Effect of feed competition on activity and social behaviour of gestating sows

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Since 2013, in EU, gestating sows are housed in groups. Even with automatic feeders, depending on individuals (i.e. age, hierarchy, weight) or groups (i.e. number of sows per feeder) characteristics, sows spend time and energy accessing feed which might modify their nutrient requirements, due to changes in their physical activity. The aim of this study was to evaluate the impact of an induced feed competition on the activity and social behaviour of gestating sows. A feed competition was set up by closing during 5 days (test period) one of the two feeders available in a pen housing 15 sows on average, doubling the number of sows per feeder. The previous week with the two feeders opened served as control (basal period). A total of 31 sows were included in the experiment. Skin lesions were visually recorded every week. Feeding behaviour was automatically recorded by the feeders. Videos recordings allowed to manually identify different behaviours: position, occupation, location in the room, and social interactions. The influence of the period (basal vs test) and parity (primiparous vs multiparous) on these measurements was analysed on R applying a linear mixed-effects model taking into account the random effect of the sows. The behaviour of the sows was impacted by the feed competition. During the first 36-h of test period, the number of visits to the feeder without receiving feed decreased compared to the basal period (2.3 vs 4.7 visits/d, $P<0.01$) while the number of visits with feed was not affected by period (1.1 visit/d). During the test period, the sows were waiting more in front of the feeder than during basal period (98.5 vs 40.1 min/d, $P<0.01$). Despite the increased number of negative social interactions during the test period (i.e. head shots, bites, $P<0.01$), there were no significant effect on the number of skin lesions. These results show that sows were more active and express more negative social interactions during a feed competition test. It can be calculated that this resulted in about 5% increase of their energy requirement. The number of sows per feeder is thus an important parameter to consider for improving gestating sows' welfare and calculating their energy requirements.

Particle sizes in faeces of sheep, goats, and steers fed on corn silage vary with NDF intake levels

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We ran three 4×4 Latin squares (LS, 21 d; 7 d collection) to predict particle size distribution of NDF in faeces of goats, sheep, and steers. Animals fed on corn, soybean meal, urea, and corn silage at four NDF intake levels (g/d) scaled to live weight (W, kg): t1=4, t2=6, t3=8, and t4=10.5 g/kg/d. We solved the nonlinear diet problems with Microsoft® Excel® Solver®. Goats (LS1), sheep (LS2), and steers (LS3) weighed 85±7.7, 67±1.9, and 486±32.2 kg. Faeces were weighed, sampled, freeze stored and wet sieved, and analysed for NDF. Sieve apertures (x) were 25.4, 9.52, 4.0, 2.0, 1.18, 0.5, and 0.075 mm, with 2.5-3.0 l/min of water flow for 10 min at moderate to high vibration. The general model used for NDF distribution was $y=\mu_0\cdot f(x)$. The NDF retained in the j-th sieve is y (g), μ_0 is a scale parameter (g), and f(x) is a density function, namely exponential, Weibull, or Weibull scaled to W. The last combination was $y=\mu_0\cdot W^{\beta}\cdot\eta\cdot\lambda_s\cdot(\lambda_s\cdot x\cdot W^{\beta})^{\eta-1}\cdot\exp(-(\lambda_s\cdot x\cdot W^{\beta})^{\eta})$. Parameters β and η are dimensionless and λ_s expressed as mm^{-1}. The mean particle size in faeces was $\mu=W^{-\beta}\cdot\Gamma(1+\eta^{-1})/\lambda_s$. The theoretical diameter in which 0.95 particles are retained is $x(0.95)=(-\log(0.05))^{\eta-1}\cdot(W^{-\beta}/\lambda s)$. This model was the best solution and estimates were: $\mu_0=4.2\pm0.33$, $\beta=-0.44\pm0.067$, $\lambda_s(t1)=16.5\pm5.369$, $\lambda_s(t2)=19.3\pm6.265$, $\lambda_s(t3)=22.2\pm7.176$, and $\lambda_s(t4)=21.7\pm7.017$ mm^{-1}, and $\eta=1.03\pm0.006$. The random predictions were analysed using SAS GLIMMIX as a two-step prediction. Mean particle sizes were: 0.42, 0.36, 0.32, and 0.32 for goats, 0.38, 0.33, 0.28, and 0.29 for sheep, and 0.92, 0.78, 0.68, and 0.69 mm for steers (SE=0.016). Confidence intervals revealed no difference between sheep and goat faeces, but steers differed because of W. The x(095) estimates were: 1.24, 1.06, 0.93, and 0.94 for goats, 1.12, 0.96, 0.83, and 0.85 for sheep, and 2.68, 2.30, 2.01, and 2.04 mm for steers (SE=0.047). NDF intake and W affected the particle size distribution for goats, sheep, and steers fed on corn silage and concentrates.

Silage type and *Acacia mearnsii* influence N metabolism of dairy cows and slurry released ammonia

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Dairy cow production systems using herbage based diets are often characterized by an excess of rumen degradable protein (CP). Breakdown of CP in the rumen beyond the de-novo synthesis potential of the rumen microbes increases the excretion of urinary urea and, consequently, the ammonia (NH_3) emission potential of the excreta. Therefore, diets should be formulated to limit ruminal protein degradation and urinary N losses. In this study we investigated the effect of supplementation of condensed tannins (CT) extracted from the bark of *Acacia mearnsii* on N metabolism of dairy cows and NH_3 release from their fresh mixed excreta. Six Holstein cows were assigned randomly to six diets, in a 6×6 Latin Square. The diets contained 75% silage, 5% whole corn plant pellets, and 20% concentrate. The diets were supplemented at 2% of the total diet either with Acacia or with straw meal. The silages used were either ryegrass-rich (R, moderate CP), clover-rich (C, high CP) or sainfoin (S, intermediate CP), the latter being also a tannin source. Feed intake and milk yield were recorded and the entire faeces and urine were collected daily. Mixed excreta were incubated in a dynamic chamber system to analyse headspace gaseous NH_3 concentration over 48 h using cavity ring-down spectroscopy. Silage type affected dietary N content and thus N intake, which was greatest for C, intermediate for S and lowest for R ($P<0.001$). The same pattern was found for total excreted N, milk N, faecal N and urinary N. Acacia supplementation did not affect N intake, but reduced dry matter intake ($P<0.05$). Acacia had no impact on total N losses in the excreta, but rerouted urinary N to faecal N (ratio of 0.30:0.70 vs 0.38:0.62 without Acacia; $P<0.001$). Concentration of gaseous NH_3 released from excreta was greatest for C, intermediate for S and lowest for R ($P<0.001$). Acacia reduced the release of NH_3 across all silage types by on average 37.1% ($P<0.001$). Independent of silage type and therefore dietary N content, Acacia seems promising to reduce ammonia emissions from dairy cows at least in the first 2 days after excretion.

Fresh herbages as forage basis for dairy cow TMR: effects on performance and rumen characteristics

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Alfalfa hay/maize silage mixture is the common forage basis of winter TMR in Mediterranean climate dairy systems. Fresh forages can improve milk quality and systems sustainability. Berseem clover (BC) is a productive winter forage sown with annual ryegrass. Alfalfa's winter growth is poor and, contrary to BC, can cause bloat in cows. The objective of the study was to evaluate the use of fresh alfalfa (ALF), fresh mixed annual ryegrass/BC (MIX) or alfalfa hay/maize silage (CON) as forage basis for TMR during the winter period on dairy cows' milk and rumen characteristics. Dairy cows (n=21) were allocated to each TMR according to a 3×3 Latin Square design. Cows were individually housed and received *ad libitum* TMR and water. TMRs were isoenergetic and formulated as a 50:50 forage-to-concentrate ratio. Each period lasted 21 d and data was collected during the last 7 d of each period. Data were analysed using a linear model including TMR and period as fixed effects. TMR differed ($P<0.01$) in dry mater (ALF: 392; CON: 456; MIX: 402; 8.7 g/kg), crude protein (ALF: 165; CON: 164; MIX: 159; 1.8 g/kg DM) and neutral detergent fibre (ALF: 360; CON: 356; MIX: 391; 7.22 g/kg) contents. Cows had similar intakes (22.9±0.94 kg DM/d), milk yield (31.5±0.82 kg/d) and milk fat (3.63±0.129%) but milk protein content differed (ALF: 3.69; CON: 3.58; MIX: 3.54; 0.044%; $P<0.01$). Cows had similar rumen pH (6.85±0.051), N-NH_3 (4.8±0.26 mmol/l) and most of volatile fatty acids (acetic acid: 64.3±0.82; propionic acid: 22.7±0.64; butyric acid: 10.8±0.30; valeric acid: 0.7±0.04; isovaleric acid: 0.9±0.05 mol/100 mol), but not isobutyric acid (ALF: 0.45; CON: 0.42; MIX: 0.59; 0.038 mol/100 mol; $P<0.01$). Fresh forages as forage basis for TMR can be used during the winter period without negative effects on milk yield or composition and rumen characteristics.

Replacing corn with bakery meal in weanling pig diets: effect on welfare and growth performance

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Bakery meal (BM) consist of bread, breakfast cereals, cookies and other foods that cannot be used for human consumption. BM can be used in animal diets, reducing competition between feed and food industries. This study was conducted to test the hypothesis that replacing corn with BM will not influence pig growth performance if diets are balanced for digestible nutrients. A total of 160 newly weaned pigs were allotted to 1 of 5 dietary treatments. A 2-phase feeding program was used (d1 to 14 phase 1 and d15 to 35 phase 2). There were 4 pigs per pen with 8 replicate pens per treatment. A total of 10 diets were formulated. In each phase, a control diet (CTR) containing corn and soybean meal was formulated. For each phase, 4 additional diets in which BM replaced 0, 25, 50, 75, or 100% of the corn in CTR diet were also formulated. Individual body weights (BW) were recorded on d 1, 14 and 35. Feed addition was recorded daily and the weight of feed left in the feeder was recorded on d 14 and 35. Diarrhoea scores were assessed visually per pen every other day using a score from 1 to 5. At the end of each phase, 1 blood sample was collected from 1 pig per pen via vena puncture. Average daily feed intake (ADFI), average daily gain (ADG), and gain to feed ratio (G:F) within each pen and treatment group were calculated. Results indicated that there was no effect of increasing concentrations of BM on final BW, ADG, ADFI, or G:F from d 1 to 14, but ADG from d 15 to 35 and for the overall experimental period tended to decrease ($P<0.10$) as the concentration of BM increased in the diets. Phase 2 and overall G:F linearly decreased ($P<0.01$) as BM inclusion increased in the diets. No differences among dietary treatments were observed from d 15 to 35 and for the overall experimental period for final BW and ADFI. Increasing concentrations of BM did not affect faecal scores in pigs and no effects on blood urea nitrogen, total protein, or albumin were observed. In conclusion, increasing concentrations of BM reduced the overall ADG and G:F of nursery pigs. However, it appears that increasing concentrations of BM do not affect blood indicators of energy and protein utilization.

Effect of altering broiler diet ingredients and phytase content on nitrogen and phosphorus excretion

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This work evaluated the use of locally sourced rapeseed meal (RSM) and maize distiller dried grains with solubles (DDGS) diets varying in nutrient content and phytase levels as alternatives to traditional wheat/soya based diets containing commercial phytase levels and assessed the effects on phytate degradation and nitrogen (N) and phosphorus (P) excretion. Male Ross 308 chicks (n=64) of similar body weight (40.15 g±0.28 g) were provided with one of four experimental diets (16 birds/treatment); positive control 1 (PC1); a soya based diet + phytase (500 FTU, ®Quantum Blue, AB Vista); positive control 2 (PC2), a RSM and maize DDGS diet + phytase (500 FTU); negative control (NC), a RSM and DDGS diet with reduced nutrient specification without phytase; and the NC diet plus a phytase superdose (NC+, 1,500 FTU). From day 0-28 birds were offered starter formulations of diets (PC1 and PC2: ME=12.6 MJ/kg, CP=21.5%, P=0.74%, Ca=0.96%; NC and NC+: ME=12.2 MJ/kg, CP=20.9%, P=0.52%, Ca=0.72%) and excreta collected from 21-28 d. A second batch of birds (n=32) were kept in group pens and offered finisher diets from 29-42 d (PC1 and PC2: ME=13.4 MJ/kg, CP=20%, P=0.66%, Ca=0.85%; NC and NC+: ME=13.1 MJ/kg, CP=19.4%, P=0.45%, Ca=0.61%). On day 42 birds were euthanized and dissected for samples of ileal digesta. Excreta and digesta were analysed for N, P, inositol and inositol phosphates (IP6-IP2) concentration. The addition of a phytase superdose significantly increased the degradation of IP6 ($P<0.001$) and IP5 ($P<0.001$) resulting in significantly higher inositol ileal concentrations ($P<0.001$) compared to birds offered all other diets. Overall, the total N and P excretion of birds was not significantly different between groups, however the addition of a phytase superdose numerically reduced N and P excretion by 12 and 15% when compared to PC2 and NC birds, respectively.

Toasted faba beans as economical replacement of soybean expeller meal in organic dairy rations

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Triticale as a forage crop is a common choice in the crop rotation of organic dairy farmers in Belgium. The last decade triticale is often grown in combination with faba beans. This not only increases dry matter yield but certainly the crude protein yield per ha and reduces the need for external protein-rich inputs. However, the protein of faba beans is easily degraded in the rumen. In combination with the protein from gras/clover the amount of easily degradable protein in the rumen is often too high with protein losses as a result. To achieve high milk production, additional protein rich concentrates are still necessary usually in the form of soybean expeller meal. In order to reduce the rumen degradable protein three organic dairy farmers toasted their home grown mixture of faba beans and triticale using a mobile toaster. The rumen degradability of the toasted grains was analysed using the in sacco digestibility method and compared with the untoasted material. The percentage of rumen undegradable crude protein increased from 27 to 45% in the faba beans. Hereby the DVE value (DarmVerteerbaar Eiwit = protein digestible in the intestine) increased from 134 to 184 g/kg dry matter (DVE soybean expeller meal = 261 g/kg dry matter). The degradability in the rumen of the starch of triticale also reduced, which increases the possibility to feed higher amounts of toasted triticale without the risk of rumen acidosis. Four 'on farm' feeding trials were conducted. The animal performances during a period with feeding toasted faba beans were compared with a period with less beans and a higher amount of soybean expeller meal. Replacing soybean products with toasted faba beans did not change milk production and milk composition but reduced the feeding cost, resulting in a higher income for the farmers. It was concluded that toasting faba beans is a profitable procedure that can increase the proportion of regional protein sources in organic dairy rations.

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Multispecies grazing, an alternative economic model for small scale farmers

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The 20th-century farm specialization process simplified farm diversity, increasing productivity while deepening the dependency on external inputs. This dependency reduces farm economic resilience (60% of production costs are on animal feed) and is a failure point under climate change's growing impact. Meanwhile, perineal pastures offer multi-layer habitat with several biological niches of grazing animals such as selective (sheep/goat), non-selective (cattle/equines), and partial(poultry). A single area could be used as a feed source for several production chains such as the 'Dehesa'/'Montado' systems in Spain and Portugal. The current work explores possible pasture management practices based on agroecological principles built on these niches on a 3 ha pasture in the Abruzzo region (central Italy). Animal density was measured by animal unit (AU) per square meter per day of staying on the grazing area ($AU/m^2/d$). Poultry (4 $AU/m^2/d$), sheep (1 $AU/m^2/d$), and donkeys (1 AU/15 m^2/d) were rotated in sequence following High-Intensity Short Durational Grazing. Land use cycle for m^2 lasted 60 days, with4 days of pasture and 56 recovery days. Animal welfare assessment was conducted using adapted Welfare Quality® protocols for poultry and AWIN protocols for sheep and equines. A multi-factor sustainability assessment was adapted from S.R Gliessman (9 parameters linear scale), while economic feasibility was calculated by Return On Investment (ROI) per m^2. Welfare assessment had an overall score similar to traditional high-quality outdoor production systems ('Bio'). Sustainability evaluation of the production system highly positive for 6/9 parameters while ROI/ha was reached within the first production season with net revenue of 2,000€/ha. In conclusion, planned multispecies pasture management could provide a low input and low-risk production method suitable for small-medium scale farmers (3-15 ha). The system could be further expanded with other species (productive equines, pigs, pollinators, etc.), and ROI further increased by coupling the production with short-chain distribution systems (farm to table services).

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Lowering crude protein in diets for late finishing pigs

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Two trials were conducted at a research institute and on a commercial farm to identify the optimum dietary crude protein (CP) level for boars and gilts from 60-140 kg liveweight. The trial at the research institute was ran as 2×3 factorial with boars vs gilts and three feeding regimes; 13% CP from 60-140 kg, 15% CP from 60-140 kg and 15% CP from 60-100 kg followed by 13% CP from 100-140 kg. Diets were balanced for ideal protein down to tryptophan. The trial on the commercial farm used the same feeding regimes but offered to mixed sex groups of pigs. Performance parameters and nitrogen (N) excretion values were calculated. Boars significantly outperformed gilts and excreted less N. Gilts performed equally well in terms of growth rate whether offered a 15% CP diet or a 13% CP (15% CP=1,218 g/d; 13% CP=1,250 g/d). Whereas boars grew significantly better when offered a 15% CP diet throughout the period (15% CP=1,347 g/d; 13% CP=1,248 g/d, $P<0.05$). In terms of N excretion, boars on 15% CP had the highest N retention at 32.1 g/d. This work shows that, to optimise performance and minimise nutrient excretion then boars and gilts should be kept separately in finishing and offered diets which meet their potential. In the trial at the research institute, changing the diet at 100 kg from 15% CP to 13% CP was reflected in a significantly poorer growth rate than offering a 15% CP diet throughout, with performance on the 13% CP being intermediate. This was similar to the performance on the commercial unit where again the 15% CP resulted in the highest growth rate, the 13% CP resulted in significantly lower growth rate and the phase 15:13 treatment being intermediate. This work shows no benefit in terms of N excretion by phasing for either boars or gilts. Numerically, the optimum scenario in terms of performance and N excretion is to offer single diets throughout the late finishing period specified at 15% CP for boars and 13% CP for gilts.

Alternative energy and protein components in broiler diets

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Swiss commercial broiler diets are mainly composed of cereals and soybean meal. Following the concept of 'feed no food' and for environmental concerns in soybean production, alternative energy and protein sources are sought. A broiler diet composed of alternative energy and protein components and with less soy is expected to be of lesser quality and therefore could reduce birds' performance. Thus, the aim of the study was to test the suitability of the alternative feed components barley, triticale, rapeseed cake and protein peas in poultry fattening diets. Growth performance, foot pad dermatitis and hock burn of the standard broiler hybrid Ross 308 was assessed, fed two different diets. A control diet (C) and an experimental diet (E), where soybean meal and broken rice were reduced. Compensations were done with local cereals, rapeseed cake and protein peas. Both diets contained the same amount of energy but the crude protein content was reduced in E. The 2×2,700 birds were kept in 20 m^2 compartments of 270 birds each and they were fed *ad libitum*. Data were subjected to ANOVA using SAS 9.4 and were analysed considering diet as fixed effect. For multiple comparisons of the Least Square means the Tukey-Kramer option was used. Statistical significance was set to $P<0.05$. Body weights differed from day 21 to the end ($P<0.05$) with final body weights of 2.29 kg with C and 2.19 kg with E, thus the average daily gain differed by 2.7 g/animal/day ($P<0.05$). The feed efficiency was unfavourable with E compared to C (1.63 vs 1.54, $P<0.05$). The European broiler index was greater with C than with E (385 vs 350, $P<0.05$). The mortality was low and similar for both diets (1.7%). Likewise, the footpad dermatitis and hock burns were similar with both dietary treatments. In conclusion, the crude protein reduction was too far reaching to prevent a growth depression. Nevertheless, it can be concluded that the alternative protein and energy components had no negative impact on animal welfare.

The use of alternative feed ingredients plus phytase on the nitrogen and phosphorus balance of pigs

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This work compared the nitrogen (N) and phosphorus (P) balance of finisher pigs offered rapeseed meal (RSM) and maize distillers dried grains with solubles (DDGS) diets varying in nutrient and phytase content or traditional cereal/soya based diets containing a commercial phytase dose. Dan Duroc boars (n=40) of 12wks of age were placed in metabolism crates for two 14 d periods with a 7 d rest period in-between during which pigs were offered one of the four experimental diets (10 pigs/diet); Positive control 1 (PC1), a cereal/soya based diet + phytase (250 FTU, ®Quantum Blue, AB Vista); positive control 2 (PC2), a RSM, maize DDGS diet + phytase (250 FTU); negative control (NC), RSM and DDGS diet with no phytase and NC plus a phytase superdose (NC+, 1000 FTU). PC diets were formulated to requirements whereas, NC and NC+ were reduced in DE, CP, P and Ca. Finisher 1 formulations were offered in the first period and finisher 2 formulations were offered in the second period. Samples of urine and faeces from each pig were collected daily in each period. Total tract digestibility of N and P was determined. N retention and excretion were calculated using data from a corresponding performance trial and P excretion levels were calculated using digestibility data for the early, late and overall production period (~40-110 kg). Pigs offered PC diets had significantly greater N digestibility than those offered NC diets ($P<0.001$) in the late finishing period. P digestibility was greater in NC+ pigs than pigs offered all other diets in the early finishing phase ($P<0.001$) and NC pigs in late finishing phase ($P<0.05$). N excretion of late finishing pigs was significantly reduced (by 10%) in pigs offered NC and NC+ diets compared to those offered PC2. While total P excretion to 110 kg did not statistically differ between groups, P excretion was reduced by 8% in pigs offered NC diets. This work highlights the need for balance in formulation to maximise performance and reduce N and P excretion from finishing pigs.

Rumen fatty acids in pasture cattle supplemented with safflower or soybean, and chitosan addition

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Safflower and soybean grain are rich in linoleic acid (75 and 51% of fatty acids; FA) and can improve polyunsaturated fatty acid (PUFA) in cattle meat. Also, chitosan can reduce the biohydrogenation of PUFA in the rumen because of its antimicrobial activity. To evaluate the effect of dietary inclusion of soybean or safflower grain, associated with chitosan in rumen FA. We used five rumen cannulated cattle, remained in the pasture of *Urochloa brizantha* cv. Marandu. We use a Latin square design, with five periods (23 days each) and five treatments, in a factorial arrangement with additional treatment (2×2+1). The treatments were proteic supplementation of 2% of body weight as control (additional treatment); factor 1: inclusion of grains soybeans (10.2%) or safflower (13.8%) in the supplement; factor 2: with or without chitosan addition in the rumen (900 mg/day). The rumen was collected on day 22 of each period. FA methyl esters from rumen were extracted and quantified with gas chromatography. We evaluate the intermediaries of linoleic acid biohydrogenation and the sum of main groups of FA. The treatments or time of collection did not affect the total FA (51.74 mg/g of DM). The inclusion of grains with or without chitosan (control vs treatments) reduced the percentage of rumenic acid (CLA c9t11), improve the percentage of C18:0 and the sum of others FA. The safflower inclusion increased the percentage of CLA c9t11 and trans monounsaturated fatty acids (t-MUFA). The chitosan addition increased the percentage of t-MUFA. The chitosan reduced the percentage of vaccenic acid (C18:1 t11) in safflower supplementation, with no effect in soybean supplementation. The use of grains in proteic supplementation increases the rumen biohydrogenation, and the safflower increases the intermediaries of linoleic acid biohydrogenation in pasture cattle. This work was supported by FCT – Fundação para a Ciência e Tecnologia IP, grant UIDB/00276/2020, CAPES, and CNPq.

Inclusion of whole plant green tomato on corn silage-based diets in sheep

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This study determined productive performance, nutrient digestibility and *in vitro* fermentation kinetics in sheep supplemented with whole plant green tomato (GT; *Physalis philadelphica*) on corn silage (CS) based diets. In a completely randomized design, 18 Suffolk lambs (38±4 kg of live weight) were grouped into three experimental diets for 21 days. Animals were fed on a control diet consisting of alfalfa hay, sorghum grain, soybean meal, rapeseed meal, wheat bran and corn silage. Then, CS was replaced by GT at 180 and 360 g/kg DM (GT18 and GT36, respectively). *In vitro* fermentation kinetics (40±1 kg of body weight) were determined using three rumen cannulated Suffolk sheep. Ether extract intake was decreased with GT36 and lignin intake linearly increase with the inclusion of dietary GT. Compared to control, GT18 and GT36 did not affect intake and digestibility of NDF and ADF, while digestibility of dry matter, organic matter, NDF and ADF was lower for GT18. Nitrogen intake was not affected by treatments but N excretion in faeces was increased in GT18 while GT36 increased N excretion in urine. *In vitro* gas production was reduced by G18 and G36, at 3, 6, 9, 12, 24, 48, 72 and 96 h of incubation. Dry matter disappearance at 96 h, was reduced by G18 while relative gas yield was decreased by G18 and G36. Overall, inclusion of whole plant green tomato on corn silage-based diets in sheep resulted to be deleterious for nutrients intake, N balance and *in vitro* fermentation kinetics.

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Impact of ergot alkaloid and steroid implant on protein turnover and muscle mTOR pathway of cattle

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Consumption of ergot alkaloids by cattle grazing endophyte-infected fescue negatively affects growth rate. However, this reduction is mitigated by steroid implants. We hypothesized that ergot alkaloids reduce muscle protein synthesis and implants reverse this inhibition. Accordingly, 32 steers (332±6 kg) were used in a 35 d experiment with a 2×2 factorial arrangement of treatments: i.m. injection of bromocriptine (BROMO; 0.1 mg/kg BW/3 d) or vehicle and with or without a steroid implant (IMP; Revalor®-S; 120 mg of trenbolone acetate, 24 mg of oestradiol 17β). A corn silage-based diet was fed at 1.5-times NEm. On d 27-32, whole-body protein turnover was determined using an i.v. pulse dose of [15N] glycine. Muscle samples were collected on d 35 from the musculus obliquus externus abdominis before (basal) and 60 min after (stimulated) an i.v. glucose challenge (0.25 g/kg BW). Blood samples were measured for concentrations of glucose and insulin. Implant increased ($P<0.0001$) and BROMO decreased ($P=0.01$) ADG. Implant tended ($P=0.10$) to decrease whole-body protein turnover, but had no effect on protein synthesis. Bromocriptine did not influence rate of protein turnover or synthesis. Area under the glucose response curve tended ($P=0.09$) to be greater for BROMO. Insulin area under the curve ($P=0.04$) and time to peak ($P=0.09$) was greater for BROMO, whereas IMP had no effect on glucose or insulin concentrations. Total abundance ($P=0.05$) of mTOR protein in the basal state and phosphorylation ($P=0.04$) of mTOR following stimulation were greater for BROMO, but this did not translate to greater downstream activation of S6K and 4EBP1 proteins. Although, IMP had no effect mTOR and S6K proteins, it did decrease ($P=0.02$) 4EBP1 activation following glucose stimulation. These data show that BROMO alters glucose metabolism, but does not inhibit activation of the mTOR pathway or whole-body protein synthesis. Although IMP mitigates the negative effect of BROMO on body weight gain, it does not appear to be through the muscle mTOR pathway.

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Nutritive value of *Opuntia ficus-indica* and its use in ruminant feed

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A possible strategy for an eco-sustainable animal feed involves taking advantage of invasive plants. The *Opuntia ficus-indica* is a perennial shrub of the Cactaceae family, made up of a root, stem (cladodes), where its flowers can be found, and fruits, which are used in human diet as well as traditional medicine. To assess the possibility of using *O. ficus-indica* in the diet of cattle, we evaluated its nutritive properties. Samples were harvested and dried at 65 °C in an oven with controlled air circulation. The chemical composition, *in vitro* digestibility, and gas production were determined in triplicate. Opuntia´s chemical composition shows low dry matter (7.12%) and crude protein (4.15 DM%) values. Fibre values are also low (NDF 20.88 DM%), with it being mostly comprised of cellulose (16.61 DM%). The *in -vitro* dry matter digestibility is high (83.13%), which can be explained by the plant's low fibre contents. The gas production is characterized by having a 2.83 lag time, which is evidence of the ease and speed at which this plant is digested by the rumen's microorganisms. In conclusion, the *O. ficus-indica* can be used in the ruminant feed as an alternative water source in association to a dry forage and a compound feeding stuff with a high crude protein content.

Study nutritional value the *Eucalyptus globulus* and *Cryptomeria japonica* in ruminant feed

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The forest is a striking and structuring element of the Azorean landscape, occupying about one third of the territory of the Azores archipelago. In the regional production forest, cryptomeria (*Cryptomeria japonica*) occupying about 56% of the forest production area while eucalyptus (*Eucalyptus globulus*) occupies about 16% of this area. The aim this work is evaluate the nutritional value of these species in the ruminant's diet, as well as the potential for *in vitro* gas production to value forest exploitation residues. Three samples each plant foliage were collected and dried at 65 °C. Subsequently, the leaves were ground into 1 mm particles to perform the analyses determining their chemical composition, digestibility and *in vitro* gas production. Chemical analyses showed dry matter contents of 48.88±0.21 and 36.76%±0.02 in eucalyptus and cryptomeria, respectively. The crude protein content observed in eucalyptus was 8.16±0.30% DM and 6.58±0.24% DM cryptomeria. The NDF (46.84±0.77% DM) and ADF (32.84±0.71% DM) values of cryptomeria were lower than those observed in eucalyptus 30.69±0.4% DM for NDF and 28.71±0.37% DM for ADF. The eucalyptus presented *in vitro* dry matter digestibility (DMD) of 22.13±1.78% lower than those verified in cryptomeria 31.15±0.15%. The low digestibility content of these unconventional forages is mainly due to the lignin content they present 18.94±0.65% DM in eucalyptus and 18.40±0.49% DM in cryptomeria. Both plants are characterized by presenting values below 10 ml/200 mg DM in gas production *in vitro*. The lag time is zero hours in the eucalyptus while in the cryptomeria it is 3.2 hours, that is, the digestion of cryptomeria by the ruminal microflora is slower. Concluded both unconventional forages used are poor and with low digestibility but can be used as roughage foods in periods of food shortages. The low production of gas in *vitro* may be associated with the high content of lignin or the presence of secondary compounds able of inhibiting protozoan and proteolytic bacteria.

Effect of short-term supplementation of marine n-3 PUFA on the acute phase response of calves

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As nutritional factors can modulate the calf immune system's functions, using marine n-3 fatty acids sources as feed supplements in dairy calves' diets has become popular recently. Fish oil (FO), one of the main marine n-3 fatty acids sources, contains eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), both of which have shown to improve the performance of the immune system in humans and animals. Although most calf mortality occurs at the first 30 days of age, most studies so far evaluated the effect of long-term supplementation of marine n-3 fatty acids on calves' health. This study is the first to evaluate the effect of short-term supplementation of FO in response to an LPS inflammation model in Holstein calves. A total of 24 male Holstein calves were randomized into four groups: (1) negative control group (CONT); (2) positive control group (LPS); (3) tallow 350 mg/kg BW group (TA, isocaloric control); (4) fish oil 350 mg/kg BW group (FO). Except for the CONT group, others were intravenously challenged with 0.5 µg/kg BW ultrapure LPS. For 11 days TA and FO were mixed with whole milk and supplemented to calves from a week before the LPS challenge to the end of the experiment. Blood collection and clinical scoring were conducted at regular time points until three days post LPS challenge. Data were analysed by analysis of variance with repeated measures using JMP (13.2) software. There was no significant difference between LPS and FO in clinical signs; however, the TA represented highest level of the area under the curve of rectal temperature during post LPS challenge ($P<0.01$). No difference in cytokines, acute-phase proteins, and sickness behaviour was observed between LPS, FO, and TA. In conclusion, short-term supplementation with FO did not exert any significant effects on acute phase response in Holstein calves.

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Effects of corn stover on growth performance of lambs

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Due to the shortage in pastures and high feed prices, livestock producers resort to alternative feeds to overcome this problem. These alternative feeds can be obtained from plant residues and/or agro-industrial by-products. The use of such products benefits both livestock producers (by having a cheap source of nutrients) and the environment (through reducing the potential hazards from the accumulation of these by-products). Therefore, the current study was conducted to evaluate the effects of replacing wheat straw with corn stover (CS) in the diets of finishing lambs. Thirty-two male lambs were distributed equally into two treatment diets in 8 adjacent open-sided pens (4×4 m; 4 pens per treatment diet; 4 lambs per pen). Lambs received diets containing either 0% CS (CS0) or 10% CS (CS10) for 50 days, following a 10-day adaptation period. The two diets were designed to be isonitrogenous and provide 16% crude protein (CP) to meet the requirements of finishing lambs. The growth performance parameters that were evaluated in the study included feed intake, nutrient digestibility, N balance, total gain, average daily gain as well as the cost of production. Intakes of dry matter (DM) and CP increased ($P \leq 0.05$) for lambs fed the CS10 diet compared with lambs fed the CS0 diet. Neutral detergent fibre (NDF) and acid detergent fibre (ADF) intakes were similar ($P \geq 0.30$) between the two diets. Lambs fed the CS10 diet showed greater ($P \leq 0.05$) digestibility of DM, CP, NDF, and ADF than those in the CS0 group. N intake, N lost in faeces, N retained, and N retention tended to be greater ($P \leq 0.08$) in lambs fed the CS10 diet compared with lambs fed the CS0 diet. Initial and final body weight and feed efficiency did not differ ($P \geq 0.25$) between the two diets. Average daily gain was greater ($P=0.03$) and cost was gain was lower ($P=0.001$) in lambs fed the CS10 diet compared with lambs fed the CS0. Results of the current study indicate that feeding lambs diets containing corn stover at 10% of the dietary dry matter is efficient and would increase profitability. In conclusion, feeding corn stover have had a significant positive impact on feed intake, digestibility, and weight gain, thus it is recommended to be in the diets of finishing lambs.

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Betaine supplementation mitigates hyperthermia in heat-stressed, hypohydrated Awassi ram lambs

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Fattening lambs in desert areas often experience disturbances in thermoregulatory abilities during the summer season, mainly manifested as hyperthermia. Betaine is an osmolyte with promising heat stress-alleviating potential. This trial aimed to examine effects of daily betaine at 8 g/head in modulating thermoregulation in fattening Awassi lambs, followed by heat stress and hypohydration protocols. Thirty six 60-days old Awassi ram lambs were allotted to 4 treatment groups. Following a fattening period of 56 days, all animals underwent minor surgical procedures to implant thermologgers at various body pockents to measure temperatures of the intraperitoneum (T_{ip}), subcutaneous (T_{sq}), intra-scrotal cavity (T_{sc}) and reticulo-rumen (T_{rum}) sites. Half of the lambs ingested daily dietary betaine (BET; top spread) at 8 g/head throughout the fattening and trial period (total of 77 days), while the remaining half received no BET (CON). Animals were initially kept in shaded (SHD) housing for 7 days. Then, 9 lambs from each treatment group were moved to unshaded (SUN) housing, and exposed to high summer solar radiation. Shifting from SHD to SUN housing resulted in rises of Tsq and Tip in both treatments, with BET being responsible for lower degree of displayed hyperthermia than CON. The upsurges in T_{ip} and T_{sq} for CON over BET counterparts suggest improved osmoregulation by BET, and hence enhanced thermotolerance. Regardless of BET and/or SUN treatments, all lambs similarly ($P>0.70$) maintained a pooled mean daily gradient of T_{sc} below Tc by 3.71-4.93±0.02 °C. Interestingly, Trum did not vary among treatment groups. Consistently, based upon mean daily values, Trum exceeded T_{ip} by 0.33 and 0.50±0.05 °C, in SHD and SUN exposure, respectively, suggesting cardiovascular and peripheral systemic adjustment, distinct from the metabolic pathways within the GIT. Betaine inclusion in lamb diets is a promising tool in mitigating eminent hyperthermia.

Metabolic risk factors for impaired growth performance in intensively-fed light lambs

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The light lambs' growth may be predicted on the basis of their earlier metabolic status. Forty-six composite Spanish local lambs, half by sex (females and males) and season (winter and summer seasons), were weaned at 41±5.4 days of age (14.6±1.3 kg of body-weight) and fed with concentrate and straw *ad libitum* until slaughter at 85 days of age (24.6±3.7 kg of body-weight). The average daily gain (ADG) was calculated by regression of body-weights against time on finishing. Blood samples were collected at day 50 of age to analyse protein catabolite markers (urea, creatinine), energy markers (triglycerides, cholesterol, glucose) and oxidative status markers (free, protein-bonded and total malonaldehyde-MDA, and total antioxidant capacity through ABTS method). Partition tree data predictive modelling through JMP software was used as data mining to predict ADG on the basis of early-status plasma metabolites. The overall ADG was affected by season (248 g in winter vs 185±17 g in summer, $P<0.05$) but not by sex (210 g in females vs 222±17 g in males, $P>0.05$). At the start of the finishing period in winter, the worst performant lambs (ADG 164±61 g, n=8) had glucose level below 88 mg/dl and concomitant total MDA above 6.78 µM (R2=0.62). In summer, the worst performance (ADG 137±66 g, n=10) was observed in lambs that had early plasma creatinine above 0.83 mg/dl (R2=0.56). The performance was optimum in winter when plasma glucose was above 88 mg/dl (ADG 308±48 g, n=10) and in summer when creatinine was below 0.83 mg/dl concomitantly with protein-bonded MDA above 6.93 µM (ADG 280±40 g, n=5). These target thresholds may be used at the start of the intensive finishing as metabolic markers to trace a successful subsequent growth response.

Metabolic effects from severe protein reductions adjusting the amino acids supply in finishing pigs

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Several European regions must seek precise dietary strategies to reduce N excretion aroused by high pig density challenge. A total of 80 Pietrain × (Landrace × Large White) pigs, 50% males and 50% gilts (110 days of age; 93±3.82 kg) were used to study the effects of reducing dietary crude protein (CP), supplementing with crystalline amino acids, on key blood metabolites markers and faecal odour-active compounds. Two diets were tested in both sexes; control (15% CP) vs low CP (13% CP), until the slaughter (145 days of age; 117.8±4.86 kg). Feeds were isoenergetic (10.3 MJ net energy/kg) and meet ideal protein profile (0.90% Lys, 0.59% Thr, 0.35% Met, 0.59% Val and 0.50% Ile). Jugular blood samples were collected from 3 pigs/pen at days 115, 130 and 145 to analyse blood metabolites. Pooled rectal faecal samples (3 pigs/pen) were collected at days 110 and 145 of age to analyse dry matter (DM), pH and volatile fatty acids (VFA). Data were analysed by mixed models with repeated measures including the diet and age as fixed effects as well as their single interactions, and the animal (blood) or pen (faeces) as a random effect. Albumin level did not differ between diets, sexes or age ($P>0.05$). Creatinine level nor differed between diets or sexes at day 115 but, at day 145, it resulted higher in gilts and with control diet ($P<0.05$). Urea level was similar in both sexes with control diet but lower in males with low CP diet ($P<0.05$) and similar with both diets at day 115 but higher with control diet at day 145 ($P>0.05$). Total protein, alkaline phosphatase and gamma-glutamyl transferase were not affected by diet or sex ($P>0.05$). Faecal DM was lower in control than in low CP diet ($P<0.05$) and it increased with age ($P<0.05$), whereas faecal pH and VFA was not influenced by diet or age ($P>0.05$), although less total VFA and branched chain fatty acids were observed numerically with low CP diet. It can be concluded that serum urea level was the best metabolite to reflect differences in available dietary N, but reduced levels were more remarkable in intact males than in gilts. Low dietary CP also improved faecal consistency.

Crude protein and amino acids in finishing pig diets

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A trial was designed using 180 finishing pigs to establish the effect of finisher diets containing 13% CP with either standard or low levels of essential amino acids (aa), and also to establish the effect of finisher diets containing 13 or 15% CP on performance of, and nitrogen (N) excretion from, finishing pigs. There were six experimental treatments: (1) 13% CP with low essential aa, boars; (2) 13% CP with low essential aa, gilts; (3) 13% CP with standard essential aa, boars; (4) 13% CP with standard essential aa, gilts; (5) 15% CP standard essential aa, boars; and (6) 15% CP with standard essential aa, gilts. Crossbred (LW × LR × Duroc) pigs (n=90 boars and 90 gilts, 31.1±6 kg) were assigned to single-sex pens (10 pigs/pen) at 10 weeks of age. Each pen was equipped with an MLP FIRE feeder which recorded individual feed intake and bodyweight daily. Pigs were offered a commercial grower diet (CP 18.5%, lysine 1.3%, 15.0 MJ/kg DE) until 12 weeks of age and then a pre-finisher diet (CP 16.5%, lysine 1.25%, 14.3 MJ/kg DE) until the pen average weight was 65 kg. At 65 kg pen average, the pens were assigned to one of the six treatments. Feed intake and bodyweight data from the feeders were used to calculate average daily feed intake (DFI), linear-regressed average daily gain (LWG) and feed conversion ratio (FCR). The performance of boars was adversely affected by lower essential aa in the 13% CP diet but gilt performance was not affected. The response was most pronounced for LWG and FCR and was reflected in more pig days required to reach 115 kg for boars offered the diet lower in essential aa (45 days vs 41 days). Boars offered the 13% CP diet containing the higher level of essential aa performed similar to those offered the 15% CP diet, highlighting the potential to reduce dietary CP and N excretion through maintaining performance with diets balanced with essential aa.

Effect of genotype and diet on growth performances of broiler chickens and environmental parameters

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The effects of two different dietary protein sources, pea bean (*Pisum sativum* L.; P) vs soybean (*Glicine max* L.; S), were investigated on *in vivo* performances of two genotypes broiler chickens and on environmental impact. A total of 120 male birds 20-day-old, 60 Kabir Rosso Plus (KP) and 60 New Red (NR), were randomly divided into four homogeneous groups (P-KP, S-KP, P-NR, S-NR) housed in 20 floor pens (n=6). From d 47 to d 83 of age, each group was fed an average of 1.18 kg dry matter (DM)/group including a mixture of wheat bran, durum wheat, corn meal and faba bean, mixed either to soybean (3.39%) in S group, or to pea bean (6.78%) in P group. Diets were isoenergetic and isonitrogenous. Birds had free access to water during the trial. Group DM intake was daily recorded. At d 36 of the trial, group body weight (BW) was recorded, and both group average daily gain (ADG) and feed conversion rate (FCR) were calculated. Data on DM intake, BW, ADG and FCR were processed by ANOVA, in a 2×2 factorial design. Greenhouse gas (GHG) emissions were evaluated in one-year period by Global Livestock Environmental Assessment Model – Interactive (GLEAM-i, ver. 1.8). Diet did not affect ($P>0.05$) group DM intake, BW, ADG and FCR. Compared to KP, NR showed higher group DM intake ($P<0.001$) and BW ($P<0.01$) while ADG and FCR ($P>0.05$) were similar between the two genotypes. Regarding the global warming potential, P diet was found less impactful, showing -7.0% of total GHG emissions and -24.1% of total CO_2 than S diet. Taken together results suggest that pea bean can successfully substitute soybean in broiler chicken diet so that the dietary substitution could represent a possible management strategy to reach a trade-off between good farming practices and environmental protection on small scale.

Bench-testing future systems: combining genetics, nutrition and farm types to predict outcomes

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Sheep and goats production systems have to tackle the challenge of being economically efficient and sustainable in uncertain and constraining environment. They should build up both efficiency and resilience to adapt to changes while maintaining the performance to subsist. Finding out strategies to achieve such balance between efficiency and resilience is a major research issue. As trade-offs exist between resilience and efficiency, there is a need to understand drivers and relationships between these properties, rather than finding a one-size-fits-all solution. Further, many levers, relying on different processes, can be combined to improve resilience (e.g. genetic improvement, changing herd size). Finally, sheep and goats systems are located in very different environments, with contrasted levels of intensification. In light of these aspects, modelling is a useful approach to explore relationships between metrics of efficiency and resilience in a diversity of situations. This report illustrates how a simulation model combining genetics, animal nutrition and farm type can be a concrete way to integrate knowledge from different disciplines and bench test strategies to improve resilience and efficiency of production systems. The first part will present the key features of the modelling approach: a dynamic and mechanistic animal model, simulating lifetime trajectory and adaptive capacities, which is used as a building-block in an individual-based herd model, representing farmer's practices and genetic changes across generations, integrated in a simulation tool. Based on examples from 2 European projects (H2020 SMARTER n° 772787 and PRIMA Adapt-Herd), the second part will focus on 3 potentialities of the simulation tool: studying interactions between elementary components (e.g. feeding and reproduction), scaling-up effects from animal to farm level (e.g. impacts of adaptive capacities on herd performance and resilience) and evaluating long-term effects of scenarios related to changes in the environment (e.g. impacts of climate change on feed resource, change in genetic selection). Finally the third part will discuss perspectives on how recent research developments (e.g. precision livestock, genomics) can be integrated to deepen our knowledge on resilient production systems.

Sheep farming systems in Italian Eastern Alps and their relationships with agroecosystems

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Traditional pasture-based livestock farming systems play an important role in the management and maintenance of mountain agroecosystems (pasture and meadows), and they can generate a series of ecosystem services (ES) and public goods. This study presents preliminary results of the project Sheep Up (Rural Development Plan, Veneto Region). The aims of this project are: (1) to recognize and promote the link between local sheep breeds and mountain agroecosystems and landscape; (2) to valorise the ES guaranteed by animals' grazing and the management of pasture and meadows; (3) to develop new territorial marketing strategies, considering the sustainability which characterizes this kind of livestock systems. The project Sheep Up considers 4 local breeds of Veneto region: Alpagota, Lamon, Foza and Brogna. Data were collected through surveys and involved 45 farms (14 for Alpagota, 14 for Lamon, 4 for Foza and 13 for Brogna). Data regarded general information about farms (herds' size and management, land use, farm surface, etc.). Moreover, the project provided focus groups, which involved the local stakeholders, to analyse the strengths and the threats in managing local sheep breeds in mountain areas. The total surface managed by the farms surveyed was 1,220 ha (average farms' surface of 24.9 ha) of which 812 ha was covered by pastures and meadows (73% of the total). Half of farmers included the direct sale of their products; 30 and 20% of the farms which involved Alpagota and Brogna breeds provided, respectively, recreational services and agritourism. The project would like also to encourage and support the conversion to organic farming (currently only 8 farms were organic due to the high costs). According to the Farm to Fork strategy, the European Commission support the conversion to organic farming. The result of this project can support consumers' choice through a sustainability food labelling framework which allow to develop new marketing strategies for farmers and which recognize their important role to manage mountain agroecosystems and their link with the landscape.

Session 43 Theatre 3

Needs of the sheep industry regarding health and nutrition management in Europe, UK and Turkey

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Within the EuroSheep project, an online survey was launched in 2020 to assess the opinion of sheep farmers and stakeholders in 6 European sheep producing countries (FR, GR, HU, IR, IT, SP), Turkey and UK. The objective was to identify the main challenges and needs to enhance the sustainability of sheep farming through the nutrition and health of adult sheep, yearlings and lambs, as well as potential best management practices and the most frequent sources of information used. A total of 1,163 valid surveys were accepted to assess the needs on nutrition, 1,182 for health, 1,246 for management practices and 1,223 for information sources. There was a significant contribution of farmers and shepherds, who represented 57% of the participants, followed by advisors or consultants (12%), veterinaries (9%) and researchers (5%). The main topics identified from the surveys were discussed through multi-actor participatory approaches in national and transnational online workshops. Across the countries, a lot of the issues identified were similar. The knowledge of the nutrition requirement was the main nutrition issue for ewes, replacement or lambs. Grassland and grazing management was also a major need identified for ewes. For lamb nutrition, lamb performances targets before weaning was the main issue, whilst, for replacement, it was growth target for first lambing at 1 year. For health of the ewes and the replacement, the most important needs were related to internal parasitism, lameness and mastitis. The main issues identified for lamb health were neonatal diseases, coccidiosis and acute death. Finally, the main issues for management were flock health plans and outdoor welfare management. The EuroSheep network will try to provide solutions and tips and tricks to respond to these challenges within each region and production system.

Session 43 Theatre 4

Assessing heat tolerance in dairy sheep – production vs physiology

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Assessing heat tolerance (HT) of animals is a key issue for the inclusion of climate resilience in breeding schemes. Changes in productive traits (PrT) or physiological traits (PhT) with increased temperatures as HT indicators have been previously analysed independently, but no studies have quantified both on the same set of individuals. The objective of our study was to estimate the magnitude of the relationship of HT measurements. Data of PhT (rectal temperature, RT and respiration rate, RR) and PrT (milk yield and fat and protein contents) were recorded biweekly from lactating ewes of Manchega breed located in three flocks. Meteorological data were recorded hourly from June to September, 2018. After edits, 2,398 records from 413 ewes were used. Statistical models used to describe the phenotypes included known environmental effects, an animal effect (intercept) and a regression coefficient (slope) on the temperature deviated from the comfort threshold (set to 25 °C) as random effects (relationships not considered). Two trait models including the two PhT and combinations of one PhT and one PrT were analysed to obtain estimates of the correlations between intercepts (basal level of the trait) and slopes of change (measure of HT) within and across traits. Correlation between the intercepts of the two PhT was weak (0.24), but a very high estimate of the correlation between the slopes of response (0.85) was observed, indicating that RR and RT are closely associated. A moderate positive correlation (0.40) between basal levels and HT was found, implying that animals with higher/lower RR and RT tend to be less/more HT. The estimated correlations between intercepts of PhT and PrTs were close to zero but between slopes were around -0.4, which indicates that animals with low HT from a productive perspective tend to be also less HT from a physiological perspective. Estimates of correlations between intercept of PrT and slopes of both PrT and PhT showed that animals with higher productive levels tend to be less HT. These results suggest that selecting HT animals using productive or physiological criteria may yield different correlated response. Funding from the Spanish grant RTA2015-0035 is acknowledged.

Exploring the effects of innovations on the sustainability of sheep farming systems in Spain

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Small ruminant farming systems are among the primary sources of rural populations' livelihood and employment in arid and semiarid environments. They are relevant for regional food security, human wellbeing, and the provision of ecosystem services. In Aragón (Spain), sheep farming systems are of great importance for rural areas' economic, environmental, and social sustainability. However, they are facing many challenges such as climate change and low generational turnover, among others, which threaten their persistence in the long term. Innovative and adaptive capacities are needed to face these challenges. Here, we used a multi-method and a multi-actor approach to identify the most valued innovations by stakeholders and analyse their multi-dimensional effects on the sustainability of the extensive sheep farming systems in this region. We adapted, parameterized, and validated the model PASTOR to simulate a representative sheep farm using 1-year own monitoring data. We assessed the effects on key sustainability indicators under four management scenarios that include: (1) a business-as-usual scenario (without innovations); (2) implementing a PES scheme; (3) increasing the added value of farm products through a quality label (i.e. hormone-free); and (4) the combination of both innovations. Implementing the PES scheme improves the farm's sustainability performance, achieving optimal values for 6 out of 7 sustainability indicators. We identified a trade-off between the effects of both innovations on the economic balance of the farm. The PES innovation counteracts the negative effects of the hormone-free label in the economic balance. There is a slight synergy between the effects of both innovations reducing the indicator referring to the excess of protein. Among the trade-offs across the sustainability dimensions, we found that PES can enhance the farm's economic balance but slightly worsens the CO_2 emissions per unit of production and protein excess. These preliminary findings can guide the stakeholders' decision-making processes and facilitate and reinforce their innovation capacity.

Effects of lactation stage, parity and season on goat milk quality in alpine area

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Milk production with dairy goats has become increasingly important over the last decades and reflects the increasing demand for milk and dairy products from goats farmed in Alpine region of northern Italy. However, little is known about production relevant effects like season or animal related parameters such as parity or stage of lactation for dairy goats reared in this area. Therefore, side specific information in order to evaluate management and production systems with the finality to improve milk production and quality is required. The aim of our study was to investigate the effects of parity, lactation stage, season of sampling and goat breed on milk composition over a long period. Overall, Saanen goats were more productive in terms of daily milk yield, but Chamois coloured goats showed greater fat and protein contents in milk. Milk production increased significantly from primiparous to multiparous goats highlighting the importance of considering longevity in breeding. Somatic Cell Score was higher in Chamois coloured goats and increased almost linearly with increasing number of parities and days in milk. Seasonal variation in productivity and milk quality was mostly related to seasonal kidding practice except for SCS, which was significantly greater in summer probably due to more unfavourable farming conditions in that season. In conclusion, the present comprehensive insights on alpine dairy goat production systems provide important information for optimizing breeding and farm management that could allow local breeders and farmers to further improve productivity and more importantly product quality. Future studies are planned to complete the findings of the following study.

Session 43 Theatre 7

Socio-economic drivers of farmer participation in livestock breeding for heat tolerance

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Despite the potential of breeding to improve livestock adaptation to CC, farmers' willingness to select for heat tolerance (HT), and the socioeconomic factors influencing it, are largely unknown. This study aims to identify the socioeconomic drivers of dairy farmer's attitude towards breeding for HT in Spain so that such breeding scheme is enhanced by high farmer participation. We performed a survey-based choice experiment in which farmers were asked to choose one among four potential breeding schemes (stressing the trade-off between milk production and HT): (1) remain breeding as currently; (2) moderate HT focus; (3) intensive HT focus; (4) HT prioritization. The survey also gathered information about farmer and farm profiles and about attitudes towards CC, breeding tools and HS on-farm impact. 38 Holstein-Friesian cattle, 43 Manchega sheep and 41 Florida goat farmers were interviewed. Answers were analysed using multinomial logistic regression models. Most farmers believe that CC is happening although half of them think that it is due to both natural and human factors. Nevertheless, most farmers show concern about its impact on their farms. HS on-farm impact is perceived as more severe by cattle farmers than sheep and goat farmers but all agree that more should be done to adapt farms to reduce animals' HS. However, 50 and 25% of the dairy cattle and small ruminant farmers, respectively, would not include HT in their breeding goal. The rest mostly favoured a moderate selection on HT. Farmer and farm profiles (except for farmer's age) and general attitudes toward CC did not show significant effects on willingness to select for HT. Only perception about on-farm impact of CC and HS, and positive attitudes towards breeding tools influenced positively farmers' willingness to select for HT. Finally, our study shows how farmers' attitudes towards HT selection many times stem from biased knowledge of HS impact on animal performance and a biased view of the utility of breeding tools.

Session 43 Theatre 8

Effect of farm typology on the sustainability of goat farms in Spain

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This study aimed to investigate the effect of different goat farm typologies on their sustainability in south Spain. A total of 20 goat farms were selected to cover the most representative farm typologies: the farm size (small n=5 vs medium n=10 vs big n=5), the type of product (dairy n=16 vs meat n=4), the intensification level (intensive n=8 vs semi-extensive n=12), the implementation of precision livestock farming (PLF n=10 vs no-PLF n=10) and product processing (cheese making n=4 vs no-milk-transformation n=16). Sustainability assessment was conducted using the Public Goods Tool adapted for small ruminant farms within H2020 iSAGE project which covered 13 dimensions. Results indicated that farm size had no impact on the overall farm sustainability or on the economic indicators. The intensification level did not affect the overall farm sustainability but meat farms reached higher scores in environmental aspects, whereas dairy farms performed better for animal health, farm business resilience and governance. Dairy farms had 2.2 times higher feeding cost per goat than meat farms but this was compensated by 3.7 times higher income per goat and lower subsidy dependency. Semi-extensive farms had improved scores in animal welfare, landscape conservation and circular economy, whereas intensive farms had higher income per goat (+41%) resulting on similar overall sustainability. The implementation of PLF did not impact the overall farm sustainability but allowed improving farm business resilience as a result of improved business strategy along with an increased income per goat (+19%). On farm cheese production tended to improve the overall farm sustainability as a result of higher farm business resilience, product diversification, circular economy and community engagement. Despite the limited number of farms analysed, this study suggested that the farm typology can partially modulate but is not key in determining the farm sustainability. The large variation observed in this study across farms independently of the farm typology suggests that more effort should be focused on promoting farmers professionalization to improve farm sustainability.

Session 43 Theatre 9

Exploring the impact of within flock variability on hormone-free dairy sheep farm performances

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Hormone-free artificial insemination (AI) in dairy sheep breeding has been made possible through the development of automated heat detection. However, in order to facilitate its implementation and success, it is necessary to control the synchronisation of heat entry into the flock. It is therefore necessary to understand how to manage the individual variability in heat entry within the flock, inherent in management without synchronising hormones. This variability is related to individual responses to heat induction and synchronisation, which result from the diversity of individual characteristics. To understand how individual characteristics such as age, body condition score (BCS) or milk yield impact the individual responses, as well as the overall flock productive and reproductive performance, we built a dynamic agent-based model. The model integrates ewe's individual reproduction and lactation processes. We simulated the weekly number of lambings and milk yield. We tested different flock scenarios in which we have increased or decreased the age, BCS and milk yield values, alone or simultaneously, compared to a reference scenario from a hormone-free organic sheep flock (µAge=2.5, µBCS=2.5, µTMY=195 L). The results showed that improving simultaneously age, BCS and milk-yield in the flock (µAge=+0.5, µBCS=+0.5 and µTMY=+50 L) seems to significantly increase flock's number of lambing (+3%), concentration of lambing over the first lambing period (i.e. the success of synchronisation method) and total milk yield (+4%), whereas decreasing simultaneously the values of those factors (µAge=-0.5, µBCS=-0.5 and µTMY=-50 L) seems to provide the opposite effect ($P<0.05$). Preliminary simulation results suggest that the model seems adequate to predict how the structure of the ewe population influences the performances of the flock in a hormone-free context. It thus gives us the opportunity to link flock structure with its management strategies in order to design management strategies that would facilitate the implementation and the success of hormone-free AI in dairy sheep farm.

Session 43 Theatre 10

Effect of production system on quality of lamb meat from eight European breeds

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The production of high and consistent lamb meat quality can contribute to ensure the sustainability of the European sheep farming sector. The objectives of this study were: (1) to evaluate quality attributes of lamb meat during cold storage originating from eight European breeds raised under their normal production systems; and (2) to elucidate the influence of storage and intrinsic properties on the aforementioned quality attributes measured on the 3rd, 9th, and 15th-day post-slaughter. All meat quality properties displayed a high variability due to the breed production systems, although, as a whole, L*, a*, b*, TBARs, and cooking loss increased during storage ($P<0.001$), whereas the tenderisation process was of non-linear decay nature ($P=0.001$). On the 15th day of storage, TBARs from intensive production systems were higher ($P<0.001$) than those of extensively raised lambs (0.839 mg MDA/kg vs 0.299 mg MDA/kg, respectively). The extensive and semi-extensive production systems produced less tender meat ($P<0.05$) than intensive production systems, on the 15th day of storage, meat from extensive (23.4 N/cm^2) and semi-extensive systems (24.4 N/cm^2) presented as good tenderness quality ($P<0.10$) as that of the intensive systems (22.1 N/cm^2). Within breed × production systems, higher CCW or intramuscular fat was associated to darker meat ($P<0.001$ and $P=0.014$), higher redness ($P<0.001$ for both), greater lipid oxidation ($P=0.016$ and $P<0.001$), and shear force ($P=0.027$ and $P=0.001$). These results can be used by breeders to enhance current flock management, feeding strategies and pre-slaughter in order to improve meat quality as demanded by the consumers'.

Effects of grass silage quality and sheep breed on enteric methane emissions from ewes

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Globally, sheep account for approx. 3.5% of the GHG emissions from the livestock sector, mainly through enteric methane (CH_4) emissions. Improved feed digestibility is one of several potential mitigation options. The aim of this study was to investigate effects of grass silage quality and sheep breed on CH_4 emissions. Grass silage qualities investigated were: (1) early; cut in the boot stage; and (2) average; cut in the heading stage. The two breeds were the modern Norwegian White Sheep (NWS), selected for production and maternal traits and the Old Norwegian Spæl (ONS), a lighter, rural breed. Mature ewes in early gestation, 20 of each breed, were individually fed the two different silage qualities *ad libitum* in three weeks periods in a 2×2 crossover design. The ewes were divided in four groups of 10, five NWS and five ONS. CH_4 emission from each group was measured in 10 portable accumulation chambers (PAC) at different times of the day during the last two weeks of each period, each ewe being measured 15 times per feed. An Eagle 2 instrument was used to capture the 50 min accumulated CH_4 emission. CH_4 emission was converted to gram per hour, obtaining a mean emission of 1.65 for NWS and 0.95 for ONS. Ewes were weighed just prior to entering the PAC chamber. Mean weights of NWS and ONS were 91 and 60 kg, respectively. Feed intake was continuously logged throughout the experiment. Mean daily DM intake of early and average silage was 2,479 and 1,835 grams for NWS, and 1,337 and 1,143 grams for ONS indicating a relatively lower decrease in DM intake for ONS compared to NWS. CH_4 emissions were analysed using a mixed linear model, including breed and silage DM intake and their interaction, with individual ewe and day as random effects. Preliminary results (corrected means) showed ONS had lower enteric CH_4 emissions than NWS (1.07 vs 1.52 grams CH_4/hour), whilst feeding early cut silage led to slightly lower emissions than average cut (1.28 vs 1.32 grams CH_4/hour). There may be a mitigation potential in improving grass silage quality and in utilising the difference in nutritional requirements between breeds. However, the difference in emission between NWS and ONS should be validated against meat production.

Selfmedicative behaviour: alteration in feed preferences of GIN-infected Boer goats

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Nematode infections are a common thread in ruminant livestock and excessive usage of conventional anthelmintics led to emergence of resistant nematode populations. This underlines the relevance of paradigm shift towards a sustainable control approach of nematode infections. In this study, possible changes in taste perception and in feed preferences of goats were scrutinized to prove ascertained higher feed intake of tanniferous plants by goats in case of nematode-infection. Feed preferences of 18 Boer goats were analysed via cafeteria-trial (12 weeks) regarding influence of changes in health status from non-infected to infected. Goats were divided in different groups: I) Non-infected + feeding-trial II) Infected + feeding-trial III) Infected without feeding-trial. The cafeteria-trial was conceptualized with pellets of tanniferous plants (leaves of sainfoin, willow, walnut, blackberry) of various tannin-contents and tannin-free hay pellets. After four weeks a mixed nematode-infection was administered to group II) and III). Besides feed intake and selection procedure, blood parameters, saliva composition and faeces were analysed on weekly basis in order to make assessments of the course of infection and potential shifts in feed preferences due to changes of taste perception. Analysis of trial data revealed an alteration from tannin-free (hay) and low tannin-containing feed (sainfoin) to higher tannin-contents (walnut, blackberry) for the infected group II) in the course of infection.

First estimations of methane emissions using Sheep GreenFeed in the Romane breed

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Greenhouse gas (GHG) emissions by livestock largely contribute to global warming. In ruminants, eructed CH_4 and CO_2 are mainly the results of the ruminal microbiota activity. In small ruminants, GHG emissions have already been measured with different technologies such as SF6 gas tracers and both fixed and portable accumulation chambers. The INRAE P3R Experimental unit has been equipped with two sheep Greenfeed. The objective of this study was to define a phenotyping protocol and to analyse the recorded GHG emissions. We analysed a group of 34 Romane male lambs (with average body weight of 67.43±8.87 kg) bred in one pen equipped with one sheep Greenfeed. At each visit, GHG emissions are measured, but to be retained as a confident value, the sheep must stay at least two minutes with the nose placed close to the nose position sensor. Therefore, over 6 weeks of control, about 25% of the visits were confident enough and values were automatically retained in the dataset. This corresponded to an average of 3.92 visits with GHG values per animal per day. Visits were not equally distributed along the day: they depended on the frequency of distribution of concentrate and on the availability of device (dominance relationship among animals). Similarly to what had been observed in cattle, GHG emissions vary along the day with less emissions occurring before the meal. Therefore, because of the distribution of the visits and because of the variability of the GHG emissions along the day we had to correct raw emissions with these effects in a linear model: animal LS means were finally retrieved and considered as the animal emissions. In this group of 34 individuals, average CH_4 and CO_2 emissions were of 41.22±4.52 and 1254.92±100.53 g/d respectively. This fitted an average of 0.67±0.08 g CH_4 per day per kg of body weight. The correlation between CH_4 and CO_2 was high (0.80). GHG emissions were also positively correlated with the body weight and average daily gain, in agreement with the literature. This work is supported by the GrassToGas project (ERA-GAS no. 39413).

Greensheep LIFE: finding common methods to estimate environmental impact from sheep farms

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Common estimation methods allows comparable carbon footprint (CFP) across production systems and countries. This work aimed disseminating activities of the LIFE Green Sheep (LIFE19 CCM/FR/001245) targeting a common CFP assessment methodology at European level. It is based on CFP studies and tools already existing in France (CAP'2ER/DEO), Ireland (Carbon Navigator), Spain (ArdiCarbon) and Italy (Carbonsheep). A qualitative comparison was firstly assessed to map the impact areas covered by the existing tools. These tools were inspired by IPCC (2006) for animal and farm emissions. Modified algorithms and locally developed equations were adopted in each tool to better fit production systems within country. All tools, except Carbon Navigator, covered the 'from cradle to farm gate' boundaries, are based on Life Cycle Assessment principles and uses farm input to estimate environmental farm impacts. Carbonsheep only estimates carbon footprint whereas CAP'2ER/DEO and ArdiCarbon also estimate other impact categories such as water, energy, ammonia emissions, nutrient and energy balance, acidification, eutrophication, particulate matter, biodiversity and additional estimation of economic and social impacts. ArdiCarbon also estimates carbon sequestration from soils. Carbon Navigator does not quantify CFP of farms and has not a LCA module for sheep. It uses territorial databases of inputs and provides greater focus on cutting emissions instead of counting footprint. It has a more oriented policy focus allowing comparison with peers and potential to reduce emissions. The tools estimation will be evaluated with a simultaneous test in France, Spain, Ireland, Romania and Italy with next project actions. Specific modules from different tools have to be integrated in a common method targeting broad use and flexibility to different farming systems for accurate estimates of CFP at European level.

Carbonsheep: a GIS tool to benchmark and spatialize the carbon footprint of sheep farms

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Decision tools to benchmark farm performances are relevant to simplify and extended to many farms the quantification of the mitigation potential of greenhouses gas emissions (GHG) from livestock. Fitting the objectives of the EU LIFE project Forage4Climate (F4C; LIFE15CCM/00039) and the sustainable development goals of UN #12 and 13, this work presents a decision support tool based on a simplified method to estimate GHG of dairy sheep farms and implemented in a web GIS platform to spatialize the farms. Twenty essential inputs from dairy sheep farms were selected to estimate the carbon footprint (CF; kg of CO_2eq./kg of fat and protein corrected milk). Essential inputs included sheep categories, housing, produced milk and meat, purchased cereals and protein grains and forages, owned land use, consumed fertilizers, energy and fuel. A simplified LCA was developed in a Excel® spreadsheet to perform CF calculations with a Tier 2 approach of IPCC (2018), modified with local developed equations. Simplified LCA vs complete LCA estimations performed with the software SIMAPRO® of 12 farms from the F4C project showed precise CF estimations of the simplified approach (R2=90; P<0.01). The simplified LCA model was included in the Carbonsheep tool, then implemented in the ARCGIS Online platform, a mapping and analytic platform by Esri Italia s.p.a. (Rome, Italy), accessible from smartphone and allowing to: (1) gather essential farm inputs; (2) show outputs from simplified LCA; (3) spatialize descriptive statistics of recorded farms; (4) compare farm CF with benchmark values. CF benchmarks of the Sardinian dairy sheep sector implemented in Carbonsheep were also obtained using data from the Forage4Climate project, analysed with a detailed LCA with the software SIMAPRO. Average CF was proportional to milk production level ($CF = 53.524^{-0.663} \times$ Mcal of delivered milk/yr per present ewe). Thus, Carbonsheep can be considered an effective tool to target dairy farms with high carbon footprint potential candidate of mitigation plans.

Effects of some climatic factors on water consumption in Boer goats

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The research was performed on nine Boer breed he-goats in the conditions of semiarid climate from the South of Texas (USA). The effects of a few climatic factors were studied, such as: temperature, wind and rainfalls on the water consumption in the conditions of feeding with a granulated fodder in quantity of 2.42 kg/animal/day, which ensured a daily ingestion of 2.1 kg dry matter. The average body weight of he-goats was 130.0±2.01 kg. The water consumption was daily recorded through difference between administered and remained quantity in the water trough. The temperature was recorded three times on day at 08:00, 13:30 and 19:00 o'clock. The experiment was done in January, on a period of 21 days. The water consumption may be influenced by a single climatic factor or by their combined action. For this period of the year the temperature was ranging from 2 and 18 °C. On sunlit periods, without wind, the water consumption had the highest values, even if it was not associated with high temperatures. Thus, on average, the he-goats ingested between 7.03-7.63 litres/animal of water at 12, 13 and 16 °C temperatures, the correlation between water consumption and temperature being very high and significant (r=0.72±0.13). The fog and humid atmosphere, associated with high temperature 18 °C had a negative influence on water consumption, which decreased at values between 5.63 litres and 6.22 litres/animal. When temperature decreased between 9 and 2 °C a reduction of water ingestion to 5.47-4.43 litres/animal was observed. Similar values of water consumption of 5.07-5.93 litres/animal were recorded in the case of cloudy sky and an average intensity of wind, as well. The lowest quantity of water, 4.23-4.83 litres/animal, was ingested by he-goats in the days with rain and wind, even when the average air temperature was between 8 and 17 °C (r=0.2±0.39).

Session 44 Theatre 1

Automated animal-based measures of poultry welfare for precision livestock farming

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On farm poultry welfare assessment typically occurs during one, or a few, short visits made by an auditor from an assurance scheme. In contrast, automated measures of poultry welfare can provide real-time and continuous information to better support welfare monitoring and improvements. Candidates for automated measures of animal welfare can be found from digitising manual welfare measures or innovating novel methods made possible with technology. Here I present an example of each of these approaches. Firstly, the automation of a validated indicator of stress in chicks, the distress call. Secondly, the use of patterns in individual behaviour as a potential novel welfare measure. Distress calls have been validated as measures of acute stress in chicks using cognitive, physiological and pharmacological methods. Through a series of studies, we were able to demonstrate that distress calls not only indicated stress for one individual but could also induce stress in conspecifics. In a commercial trial of 12 broiler chicken flocks, we developed a simple automated method for monitoring the calls. More distress calls (as measured using the automated method) were associated with increased mortality and decreased weight in the days immediately following call measurement and towards the end of the flock cycle. Monitoring welfare using vocalisations has the advantage that it can be low cost and easy to install. Patterns in behaviour have been traditionally difficult to study because they require longitudinal data collection, but automated methods present a new range of possibilities. The recent finding that poultry have highly individual behaviour provides support for welfare monitoring via sentinel animals within larger flocks. Patterns in consistency of behaviour could be used to indicate changes in welfare state. I present some preliminary data in support of such an approach. Much like manually collected welfare indicators, it is likely that that combinations of automated animal-based measures of poultry welfare will be most useful in notifying farmers or stockpersons of welfare compromise. The methods presented here offer new avenues for research of animal-based measures of poultry welfare which could support the future of poultry farming.

Session 44 Theatre 2

Automated assessment of broiler resource-use via computer vision

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Broilers are produced in large flocks making it difficult to monitor welfare. Novel technologies with automated assessment of behaviour would be valuable as they create a basis for better management decisions and ultimately will improve broiler welfare. The aim of this study was to develop an automated system to monitor resource-use of broilers via computer vision. Here we present first steps in the system development. The complete dataset contained 336 hours of recordings collected from cameras installed above two experimental pens with 25 Ross 308 broilers each. An algorithm for semantic segmentation based on U-Net fully convolutional neural network (NN) was developed to automatically segment and detect individual chickens. For this, a subset of 494 randomly chosen frames from day 14 to 37 of age were annotated and contained 12.350 chickens in total. A second algorithm, which used the detections produced by NN, then automatically monitored the number of chickens that were in close proximity of feeders, drinkers and perch at 31 days of age. The F1 score for individual chicken detection was 0.96. The average number of chicks using feeders, drinkers and perch per 15 min intervals was 1.75, 1.12 and 0.44, respectively (7, 4.5 and 1.8%, respectively). Overall, these findings show that computer algorithms could help in continuous monitoring of broiler resource-use, ultimately resulting in a new efficient way of managing the large-scale production system.

Feet control: reliability of an automatic system to assess foot pad dermatitis at the slaughterhouse

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Foot pad dermatitis (FPD) is an accepted indicator for animal welfare in turkeys, giving evidence of the animal's health and providing information on the management of animal husbandry. Automatic systems for the assessment of FPD at slaughter can provide a useful tool for an objective data collection. However, using automatic systems require them to assess the incidence reliably and, in the case of a system breakdown and in consequence the need to rely on manual assessment, presupposes a comparability to the human observer. In the present study 1.993 feet of turkeys were scored for FPD by an automatic camera system and a human observer, using a five scale score. Observer reliability was calculated (Krippendorff's alpha, SAS, V.9.4). With an agreement coefficient between both methods of 0.43 results were not acceptable. Therefore, pictures of 3,000 feet, scored by the automatic system were evaluated systematically to detect deficiencies. Accurate detections were found in 26.8% of the analysed feet. In 58.5% of the feet, the reference area (metatarsal foot pad) was not detected correctly. In 28.3% of the feet, false detections of the alteration on the foot pad (FPD) were found. In 21.4% of the feet reference area and alteration on the foot pad, were detected wrong. In 44.7% of the feet the foot was not presented straight to the camera (multiple findings were possible). According to these results, the algorithm of the automatic system was modified. The pictures of the feet, now scored by the modified algorithm, were evaluated again. Observer reliability was improved considerably (Krippendorff's alpha=0.62). However, the detection of the reference area remained to be a core issue with 46.6% incorrect detections. The automatic detection system therefore can be considered as promising approach to assess FPD at the slaughterhouse reliably. However, there is plenty of upward scope to improve the existing method. The project was supported by funds from the Federal Ministry of Food and Agriculture (BMEL) based on a decision of the Parliament of the Federal Republic of Germany via the Federal Office for Agriculture and Food (BLE).

Effect of farm on milk yield characteristics during mastitis at farms with automatic milking systems

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Milk losses caused by mastitis have a detrimental effect on farm profitability and sustainability. Information on characteristics of perturbations in milk production during mastitis can be used for several purposes, e.g. precision phenotyping. To this end, farm effect on the perturbation characteristics should be taken into account. By identifying these effects, the milk perturbation data can be used for individual and herd level monitoring applications, for example, to identify cows that are more resistant to mastitis or to identify management practices that limit the impact of mastitis. A selection of 25 commercial robot farms in Belgium and the Netherlands was made, based on the installation of an automatic milking system (AMS) at least 4 years back in time. To estimate the theoretical unperturbed lactation curve and calculate the milk losses, an iterative procedure using the Wood model and a variance-dependent threshold on the milk yield residuals was implemented. Next, the day of maximum perturbation (DMP) was determined as the first quarter level measurement to reach a smoothed milk loss of 80% of the maximum milk loss within the perturbation. Presumed cases of mastitis and the infected quarter were identified by leveraging quarter level milk losses and electrical conductivity measurements within a fixed window of day -5 to day 30 around the DMP. For each case, perturbation characteristics, such as perturbation depth, perturbation length, overall milk losses, etc., were determined. This data-based method allows to select mastitis cases without depending on the quality of the farms' treatment records. The mastitis perturbation characteristics were compared over the 25 farms. To investigate the effect of farm size and production level, farm characteristics were extracted from the AMS and milk recording results. Preliminary results indicate that the number of mastitis cases, their length and depth vary greatly among farms.

Use of infrared thermography and rectal thermometer to measure body temperature of gestating sows

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Body core temperature is generally used as a proxy to detect health or thermoregulations problems. In summer, sows can face thermal stress, with negative impacts on their welfare and performances. In practice, body core temperature is evaluated by using rectal thermometer but this method may be stressful for the animal, with possible subsequent effects on the measurement quality. Thermal imaging camera represents a non-invasive alternative for measuring animal temperature. The purpose of this study was to compare gestating sows' body temperatures measured with a rectal thermometer and with a thermal imaging camera in different ambient situations (14 to 32 °C). Individual temperatures were taken on 40 sows (8 primiparous), housed in two rooms, every morning (8am) during 12 days with a rectal thermometer and with a thermal imaging camera (model FLIR, E60; extracting software FLIRTOOL) on a shaved dorsal cutaneous area (2×12 cm^2). The fixed effects of parity (primiparous vs multiparous), room (1 or 2), sow's posture during measurement (lying vs standing) on body temperatures (rectal and cutaneous) were analysed on R applying a linear mixed-effects model taking into account the random effect of the sows. Correlation coefficients between the sows temperatures from one side and the room temperature, live body weight, backfat thickness on the other side were calculated using Pearson correlation tests. Primiparous sows had higher rectal temperature than multiparous sows (38.3 vs 37.9±0.61 °C, $P=0.007$). Rectal temperature was also correlated positively with room temperature ($R^2=0.34$, $P<0.001$) and negatively with sow body weight ($R^2=0.38$, $P<0.001$). The sows lying down had higher cutaneous temperature than the sows standing (35.3 vs 31.8±3.19 °C, $P<0.001$). Cutaneous temperature was positively correlated with the room temperature ($R^2=0.77$, $P<0.001$) and rectal temperatures ($R^2=0.48$, $P<0.001$). To conclude, there is a relationship to explore between sow's rectal and cutaneous body temperatures. Few factors seem to influence these temperatures and therefore, they should be taken into account in the next step of this work aiming to develop a model predicting rectal temperature with infrared temperature and other non-invasive measurements.

Analysis of daily rumination time under heat stress of lactating dairy cows

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In the course of the predicted climate change, the heat stress of dairy cows has become increasingly important. The temperature-humidity index (THI) is the most common approach to identify heat stress, but the index does not include animal based indicators and the stress condition cannot be determined individual for each animal. The object of the study was to determine a heat stress threshold of the average daily THI that results in changes in the daily rumination time (RT) of lactating, high-yielding cows. The data set was composed of a high sample size of 183 cows and long-duration measurements over two years (June 2015 – May 2017), collected in a naturally ventilated barn in Großβ Kreutz, Germany. The THI was calculated in 5-min intervals in different positions inside the barn. Additionally, every cow from the herd of an average herd size of 53 cows was wearing a neck collar with a Lely Qwes HR system that provided the RT 24 h/d. The study showed that heat stress also negatively influenced RT in moderate climates. The heat stress threshold of 52 THI was determined by broken-stick regression. Below the threshold, the RT already decreased slightly with increasing THI, but above the threshold, RT decreased more strongly from 535 min/d down to 485 min/d at 77 THI. During the experimental period, the determined threshold of 52 THI was reached from April to September for up to 720 h/month. The changes in RT in reaction to heat stress will be affected by cows' characteristics. Therefore, we considered several cow-related factors, such as milk yield, lactation number, lactation stage and pregnancy stage to better understand cows' individual reactions. High-yielding cows showed high RT at approximately 9 h/d during environmental conditions with THI units under 52, but they strongly reduced their RT to 8 h/d under conditions above THI 52. Multiparous, high-yielding cows in later lactation stages are potentially more strongly affected than other cows.

Estimating sickness-probabilities for identifying dairy cows at risk of disease

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Developing algorithms for sickness detection is a major field in precision dairy farming. Such approaches are often characterised by high false positive rates that reduce the practicability on farm. Monitoring sickness behaviour as an early indicator could enhance early disease detection in dairy herds. Hence, this study aimed at the estimation of sickness-probabilities from different behavioural patterns of dairy cows to identify animals at risk of disease. Therefore, behavioural data of 618 cows with 791 lactations were collected by two accelerometer sensors delivering information about activity, resting and feeding behaviour. The behavioural observations were used to build a logistic regression for each of the observed behavioural patterns in a stepwise manner, where the variables were included with $P<0.2$. All behavioural variables were included except for the number of 'standing-ups' ($P=0.38$) in the resting model. Further, the lactation stadium nested in the lactation number was added ($P<0.0001$) to each model. For health monitoring, the models were applied to data of 173 cows to estimate daily sickness-probabilities for each cow from all behavioural patterns. Resulting sickness-probabilities were used as input for multivariate control charts to identify an increase in the sickness-probabilities simultaneously within five days prior to a diagnosis documented by a vet. Health monitoring resulted in a sickness detection rate of at least 70% that corresponds to a suggested minimum sensitivity for automated sickness detection systems and reached a precision of 61%. Furthermore, the specificity showed a reasonable level of 94%, thus the false positive rate was set at 6%. The derived receiver operating characteristics (ROC) curve and the precision recall (PRC) plot additionally showed an overall acceptable classification as the areas under the curves were 0.9 (ROC) and 0.7 (PRC). In summary, the present approach shows an acceptable level of sickness detection with showing a comparably low number of false positives.

Measurement of the dielectric constant for hyperkeratosis detection in the teat of dairy cows

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The teat of a dairy cow is exposed to a large load during milking because it is the interface between the udder and the milking technique. A milking system that works improperly can damage the teat resulting in the formation of teat end hyperkeratosis (HK). Currently, the severity of HK can only be assessed visually, and there is no way to detect the severity of hyperkeratosis on the bovine teat automatically. Thus, the aim of the present study was to test and evaluate the measurement of the dielectric constant (DC) of the teat skin as a method of automatic detection of hyperkeratosis. The study focused on surveying the occurrence of hyperkeratosis in a total of 241 teats of lactating dairy cows. A scoring system consisting of four categories was used to macroscopically assess the severity of HK, whereby score 1 indicated a healthy teat without a ring at the teat end and score 4 implied a severely roughened keratin ring, extending more than 4 mm with clearly observable old keratin. Additionally, the DC of all milkable teats was measured in a double iteration with the MoistureMeterD (Delfin Technologies, Kuopio, Finland) on four different days before the teat was prepared for milking. The Spearman rank correlation coefficient revealed a negative correlation between the DC value and HK score ($r_s=-0.55$ to -0.36). The results of the regression analysis showed that the DC values differed significantly between healthy teat ends (score≤2) and teat ends with HK (score≥3). Thus, the non-invasive measurement of DC provides a promising method of objectively assessing the occurrence and severity of HK. However, further studies using more animals and repeated measurements are required to validate this method and to define exact DC value ranges for the different HK scores.

Session 44 Theatre 9

Multivariate analysis of milk parameter dynamics for healthy dairy cows and during clinical mastitis

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With increasing demands on high yielding and sustainable production and animal health and welfare, clinical mastitis poses one of the biggest challenges on modern dairy farms. On farms with automatic milking systems (AMS), mastitis is often detected later and followed up in less detail due to the absence of visual controls during milking and the lack of a conclusive system for automatic detection. As milk reflects the physiology and health status well, better use of on-farm milk parameters can offer solutions for better monitoring and recovery during mastitis. In this study, a variety of high-frequent milk parameters were analysed to gain a better understanding of milk yield and content dynamics for healthy dairy cows and during clinical mastitis. Milk parameter data were collected for 3 years at 3 AMS farms on which every mastitis case was followed up in detail. Milk yield, conductivity and milk flow were available at quarter level for every milking. Measuring at quarter level allows the separation of systemic (effect of mastitis on all quarters) and local (effect of mastitis on infected quarter) impact. Every second day, online SCC and, via a Herd Navigator™ sensor LDH were measured on-farm. Lactose, fat, protein and SCC were measured weekly and, during clinical mastitis, every milking via milk samples analysed in the lab. The data were analysed to determine baseline and perturbation behaviour and the milk parameters' covariates (lactation stage, parity, health history), both for healthy dairy cows and in particular during mastitis. For each identified clinical mastitis case, a detailed analysis of the milk parameters was conducted separately. Hereby, the focus was set particularly on deviation patterns for single and between multiple milk parameters for future identification and classification of mastitis cases.

Session 44 Theatre 10

External validation of PLF technologies with application for animal-based dairy welfare assessment

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Precision livestock farming (PLF) technologies have the potential to add value to the farm management by improving decision making. However, sensor technologies can require substantial investments, which may not always be offset by obtained profits. Therefore, for successful technology uptake, the merits of each sensor system should be described and verified by independent validations. The aim of this study is to provide a systematic overview of technologies for animal-based indicators of welfare that have been externally validated. Peer-reviewed studies on the validation of sensor technologies used in dairy management published between 2000-2020 were systematically reviewed. According to obtained results, 12 different sensor technologies were externally validated for monitoring activity (e.g. lying, walking), 11 on feeding (e.g. presence at a feeder), 8 on reproduction (e.g. oestrus detection), 5 on milk quality (e.g. somatic cell count), and 3 on gait assessment. We have also identified several tools for monitoring rumen pH, body temperature, and drinking (2 examples for each monitored trait), as well as body condition score (1 example). Reliable technologies for monitoring welfare-related traits exist, however there are areas concerning sensors and algorithms which require further developments. According to our results, several validated technologies had application for good health (e.g. milk quality sensors), good feeding (e.g. load cells, accelerometers) and good housing (e.g. accelerometers), but only two tolls were tested for evaluation of appropriate behaviour (grazing monitoring). Furthermore, the performance (accuracy and precision) of monitoring systems was most often tested on adult animals and only a small number of tools were validated for heifers or calves. The validation process increases transparency of PLF systems and bring more trust to data generated by sensors. This study was conducted within the ClearFarm project (H2020, grant agreement No. 862919).

Session 44 Theatre 11

Influence of environmental risk factors on coughing frequency on a respiratory disease-free pig farm

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Coughing in response to environmental irritants can detrimentally affect pig health and performance even in the absence of disease. This study aimed to characterize the influence of temperature, relative humidity, and ammonia concentrations ([NH_3]) on the frequency of coughing on a respiratory disease-free farm. This was an observational study where pigs (n=460) were monitored from 34±0.9 kg to slaughter 110 kg. Two consecutive batches of pigs were housed in two rooms (A and B) at the Moorepark Pig Research Facility. A SOMO box (SoundTalks®) and a NH_3 sensor (DOL61) were installed in both rooms, issuing a daily respiratory distress index (RDI; average number of coughs/pig/24 h), average temperature and relative humidity values; and [NH_3] every 30s for a total of 156 days per room. Autoregressive distributed lag models were used to capture delayed effects on coughing frequency by the exposure to daily indoor temperature, relative humidity and [NH_3] (daily averages). Both RDI and environmental factors were lagged by up to 5 days. The model built for room A showed that both [NH_3] and RDI in the previous day had a significant impact on RDI on any given day ($P=0.002$ and $P<0.001$, respectively; model Adj. R^2=52%). Results were less clear for room B, where [NH_3] in the current day and RDI in the previous day had an impact on RDI on each given day ($P=0.090$ and $P<0.001$; model Adj. R^2=16%). Understanding the effects of environmental risk factors on pigs' respiratory health can help determine when to implement appropriate preventive measures.

Session 44 Poster 12

Circadian rhythm of temperature, heart rate and activity of cattle grazing under different densities

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Precision livestock farming is supported by devices to help farmers to track animal movements with GPS and accelerometers, or collect physiological data, such as temperature and heart rate. Based on data collection and algorithms, these devices can determine signals of oestrous, pregnancy or illness. The aim of this work was to determine circadian changes in temperature (T), heart rate (HR) and activity (ACT) of cattle managed under different density grazing, using an implantable bio-logger. Six cows managed under extensive grazing conditions at a density of 1.5 livestock units per hectare (LSUs/ha) (low density, LD), were moved to a smaller grazing area, to a final density of 128 LSUs/ha (high density, HD). Animals were subcutaneously implanted with a T, HR and ACT bio-logger (DST centi-HRT ACT, Star Oddi, Iceland), and were programmed with data logging every 5 minutes. Cosinor rhythmometry was applied to the data recorded for each animal, during 5 days in each density, determining mean Mesor (the average value around which the variable oscillates), Amplitude (difference between the peak and the mean value of a wave), and acrophase (time of peak activity) of the three parameters. Paired t-test for related samples were used to determine differences in the cosinor curves between the two different densities. All cows presented a cosinor curve which fitted a 24-h rhythm ($P<0.0001$) for T, HR and ACT during both grazing periods. No differences between Mesor (T: 37.98 vs 38.02 °C; HR: 69.12 vs 65.91 bpm; ACT: 49.39 vs 40.41 mg, for LD and HD, resp.) and Amplitude (T: 0.28 vs 0.28 °C; HR: 4.12 vs 3.14 bpm; ACT: 18.14 vs 11.28 mg, resp.) were observed. However, significant differences between densities ($P<0.05$) were detected for the acrophase of the three parameters, so that the time of the peak of T was delayed two hours under the high density (LD: 20:45; HD: 22:45 h), and was advanced three hours for HR (LD: 19:51; HD: 16:49 h) and two hours for ACT (LD: 14:47; HD: 12:49). In conclusion, circadian rhythmicity can be a useful tool to detect changes in the welfare of cattle when is managed under a high animal density, so that cows experiment changes in the acrophase of their cosinor curves, which could represent some signals of distress.

Session 44 Poster 13

Using an accelerometer based on inertial measurement unit to evaluate the sexual activity of the ram

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The objective of this study was to evaluate the suitability of a system based on an Inertial Measurement Unit (IMU) accelerometer to assess rams' sexual behaviour (RSB). 12 adult males (~3.5 years old;), equipped with IMU accelerometers placed between the two shoulder blades, were used in 2 parallel pen tests. The tests were replicated 6 times during the same week and consisted in 30-min. visual observations for each ram interacting with Merinos d'Arles ewes (i.e. ratio= 1 ram per 6 ewes). The RSB was then recorded according to two categories: pre-copulatory (PRE-COP: i.e. number of Anogenital Sniffs AS, Leg Kicks LK, Emission of Sounds ES, Revolve Around the Ewes RAE, Head on the Rump HR and Flehmen Fl) and copulatory (COP: number of mounts MO) behaviours. Raw accelerometer data were analysed by an algorithm developed with the Scilab software (5.4.1,2013). Overall, our results demonstrate the ability of this device to detect some, but not all, of the evaluated PRE-COP (LK, RAE and Fl) and COP behaviours (MO). In total, for the 12 rams used during the 6 tests, 80.5% of the 1,388 COP behaviours visually recorded were automatically detected by the device. Concerning the PRE-COP behaviour, from a total of 18,232 records visually recorded, the best sensitivity was performed with the LK activity (i.e. 66%, 3,002/4,484), whereas for Fl and RAE activities, the values were 57% (32/56) and 32% (67/212), respectively. The technical performance of the device evaluated here was affected both at the inter- and intra-tests levels (depending on the ram and on the behaviour parameter to be interpreted). The absence of effectiveness in interpreting other parameters was likely related to the quality of the algorithm built rather than to the device by itself. In conclusion, our preliminary results demonstrate that some of RSB can be automatically estimated by using this kind of technology. Nevertheless, more research is required to test its effectiveness regarding other RSB and with a more significant number of rams, in other seasons, etc.

Session 44 Poster 14

Early mastitis detection in cows using 3D motions sensors

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Mastitis is still a serious problem in dairy herds. Hence, early detection of this disease improves an animal welfare and increase an economic efficiency. The aim of the research was to evaluate a suitability the electronic 3D motion detectors to detect the early symptoms of mastitis. Such system would allow to introduce effective prevention of this illness. The experiment was carried out on 118 randomly chosen cows (64 and 54 cows of Holstein Friesian and Brown Swiss, respectively). The animals were housed in loose housing barn with access to the pasture. The occurrence of mastitis cases and microbiological culture in milk (as measurement of the development of infection) were registered. Data, which were defined as a time spent by animals on feed intake, ruminating, physical activity and rest came from motion sensors and were subject to the analysis. An information about feeding group, breed type and lactation number are included as well. Two approaches were used to analyse the data: artificial neural networks and logistic regression. The area under curve rate exceed 0.8, and the receiver operating characteristics, which were the basis for its determination, were similar for total and sub data sets (using both methods). The proposed methodology based on 3D motions sensors can be useful in the process of automating detection of mastitis. This investigations was supported by the NCBiR [CoreOrganicPlus/2-ORGCOWS/125/IGHZ/2015] as part of the European research program Core Organic Plus.

Session 44 Poster 15

Identifying and characterizing disturbances from high-throughput phenotyping data

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With the development of automatic self-feeders and electronic identification, it is possible to record, on a large number of animals, individual feed intake and body weight whenever an animal accesses its feeder. In addition to allowing for more accurate estimations of the aforementioned traits for genetic selection, these longitudinal data could be used as a tool for detecting and characterizing the disturbances faced by the animals in a context where unknown disturbances may be of varying intensity and duration and may not affect all animals at the same time. We propose a two-steps approach that uses individual and group information to identify and characterize disturbance. The first step is to identify the animals that have faced a disturbance. An animal is considered as having undergone a disturbance if its residual variance (from the model fitted to the longitudinal data) is abnormally high or if it belongs to a group with a high number of animals detected as disturbed. Such an approach avoids wrongly considering very robust animals as undisturbed. The second step consists in characterizing the disturbances (starting date, duration and intensity) by studying the changes in the 'speed' of the phenotype (second derivative) and its maximal variation for animals identified in the first step. Sensitivity, specificity and quality of disturbance characterization of the proposed method has been evaluated using simulated data of various disturbance types (disturbance that occur at the individual or different group levels). Such a characterization of the disturbances faced by the animals would allow a better assessment of their robustness.

Session 44 Poster 16

Automated area of interest and feature extraction from thermal images for cattle lameness monitoring

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Infrared thermal images are used frequently to measure temperature remotely. This temperature can be used as an indicator for detecting diseases and inflammation. In cattle, the temperature of claws may be useful for identifying inflammation, and thus, lameness. Analysing infrared thermal images requires the selection of the area of interest (AoI) in each image manually to obtain temperature-based features of the selected area. These manual methods are time-consuming and error-prone, especially when dealing with large groups of animals and large data sets, and requires the skill of trained persons during image analysis. This study reports on an automated system for selecting and analysing AoI on the claws of cattle from infrared thermal images. Thermographic images of claws were collected using a digital infrared camera (Testo 882). Template-matching with OpenCV in python was used to identify the AoI, which was, the coronary band of the claw, and compute two basic features, the average and maximum temperature, from that AoI. In the template-matching algorithm, a small part of the image is searched with a template image. Images analysed using template-matching (n=635) were highly correlated with manually analysed images for maximum (R^2=0.94) and average temperature (R^2=0.99). These findings demonstrate that the developed template-matching algorithm is a suitable method for automated detection and analysis of AoI of claws from thermal infrared images This algorithm could be applied in the development of automated method for monitoring lameness.

Session 45 Theatre 1

A genetic study of temperament in American Angus heifers and cows

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The variability in temperament traits is built upon genetic and environmental factors and recent studies suggest that animals become more docile with positive experiences. The goal of this study was to evaluate the temperament in American Angus females across their productive lifetime. Measurements of temperament score (1-6 scale) were recorded at yearling (365 days old; T_h), and when the female weaned a calf (up to 10 years-old; $T_{c:X}$, where X represents the year-age interval). In total, 139,506 animals recorded for T_c were used in this study. Additionally, 24,359 animals with estimated breeding values (EBV) for T_h were available for this analysis, which represents animals overlapping with T_c. Animals measured for T_c had from one to eight repeated records, totalizing 267,871 records clustered in 1-year intervals. Single-trait Bayesian animal threshold models were used to estimate the variance components for all T_c year-interval independently. The statistical model included the systematic effects of embryo transfer, age of dam, birth season-year, and age of the animal in days as a covariable, and contemporary group and additive genetic effect as random. The heritability estimated for T_c ranged from 0.10 (0.01) to 0.17 (0.02) over the years. The approximate genetic correlation between pairs of $T_{c:X}$ ranged from 0.39 to 0.68, and was equal to 0.52 between T_h and $T_{c:2\text{-}3}$. Furthermore, the re-ranking among selected animals was evaluated (i.e. 10% docile animals with EBV accuracy ≥ 0.25). The proportion of commonly selected animals between pairs of $T_{c:X}$ ranged from 0 to 35%, and it was 0% between T_h and $T_{c:2\text{-}3}$. These results suggest that temperament as a yearling heifer and temperament as a breeding cow are different traits. In conclusion, temperament scores measured at an earlier age (e.g. heifers) should be treated as a different trait to later-parity cow's temperament. Further studies will be performed to better understand the genetic correlation of temperament across animal lifetime (e.g. multi-trait analysis) and the environmental mechanisms affecting these traits.

Session 45 Theatre 2

Genomic loci for uterine diseases in Holstein cows and impact on milk production and fertility

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Uterine diseases (UD) imply high economic losses in dairy herds due to impaired production and fertility. According to clinical severity, several forms of UD exist, which may differ in their genetic background and causal physiological mechanisms. This study aimed on: (1) the effect of UD on milk production and fertility; (2) the estimation of heritabilities for UD using pedigree- and SNP-based relationships; and (3) genome-wide association studies (GWAS) to infer candidate genes for UD. The dataset contained producer-recorded UD of 14,810 first-lactating genotyped Holstein cows from 63 herds. Binary defined UD traits according to clinical manifestations were *endometritis catarrhalis* (EM stage I), *endometritis mucopurulenta* (EM stage II), *endometritis purulenta* (EM stage III), *pyometra* (EM stage IV), *endometritis* (EM; no clinical severity defined by farmers) and *metritis* (MET). The overall prevalence for UD was 26.7%. All stages of EM significantly influenced the calving-to-first service period, e.g. plus 7.4 days for cows with EM. EM stage I, II and IV unfavourably influenced the non-return rate after 90 days, with 6.4% higher pregnancy rates in healthy cows. 305-day protein and fat yield were significantly lower in EM stage IV cows compared to healthy cows. Genetic correlations between EM with milk, protein and fat yield ranged from 0.20 to 0.31. Heritabilities for EM traits ranged from 0.00 to 0.06 for pedigree relationships, and from 0.001 to 0.02 for SNP-based relationships. For MET, pedigree- and SNP-based heritabilities were 0.05 and 0.01, respectively. GWAS revealed five SNP on BTA26 for EM stage I, one SNP on BTA19 for EM stage II, four SNP on BTA2, 18, 20, 25 for EM stage III, and four SNP on BTA4, 16, 20 for EM stage IV above the significance threshold ($P=1\times10^{-4}$). For EM, we identified 15 significantly associated SNP on four chromosomes, and four significant SNP on BTA3, 20, 22, 28 for MET. Marker associations for UD were annotated to 17 candidate genes using the ENSEMBL database. *ADAMTS20* and *RXFP1* were identified as most important candidate genes for bovine UD. Different SNP significantly influenced different EM stages, explaining the inter-individual variations in clinical severity of UD.

The impact of sub-clinical mastitis infections on technological traits in Holstein cattle

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Mastitis adversely affects animal health and welfare, with significant economic damage for the dairy industry. Herein, we investigated the associations between sub-clinical mastitis infection from *Streptococcus agalactiae*, *Staphylococcus aureus*, and *Prototheca* spp. and milk technological traits, assessed on single quarters, in a cohort of 450 Holstein cows belonging to three dairy herds. After an initial bacteriological screening, 85 positive cows were identified and followed over time to assess disease progression. A total of 613 single-quarter records were available at two sampling times (T): T0 and T1, one month after T0. Target traits were analysed with a hierarchical linear mixed model with the following fixed effects: days in milk (DIM), parity, herd, bacteriological status (positive and negative), T, and bacteriological status × T interaction. The random effect of the individual cow nested within herd, DIM and parity was used as error term for the latter effects. Mastitis decreased casein index ($P<0.05$) and lactose ($P<0.01$) and increased milk conductivity ($P<0.01$), somatic cell score ($P<0.001$), polymorphonuclear neutrophils plus lymphocytes count and macrophages count ($P<0.001$). Mastitis was also associated with a deterioration of milk coagulation properties, as it decreased curd firmness measures by ~14% ($P<0.01$). Regarding cheese-making traits, mastitis decreased the curd nutrients recovery by ~8% ($P<0.01$) and cheese yield measures by ~5% ($P<0.05$). Further, bacteriological status × T interaction was significant for 17 traits, including udder health indicators and milk technological traits. This study provides new insights at the mammary gland quarter level into the impact of mastitis on dairy cattle's milk technological characteristics.

Genetic association between direct and indirect measures of IgG concentration in bovine colostrum

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In cow colostrum, immunoglobulins G (IgG) are important for the passive transfer of antibodies from dam to calf. Direct determination of such trait in colostrum through the gold standard, i.e. radial immunodiffusion, is highly demanding in terms of time and costs. For this reason, the optical refractometer is commonly used at farm level for the indirect determination of total solids, which include also IgG. In fact, it has been showed that density of colostrum is positively associated with IgG concentration. In the present study, 672 colostrum samples (1 per cow) were collected within 6 h after calving in 9 Holstein farms located in north-east of Italy. The refractometric index (RI, on the BRIX scale) was assessed using an optical portable refractometer and in the same samples IgG (g/l) was determined in laboratory through radial immunodiffusion kit specific for bovines, according to the manufacturer instructions. A bivariate analysis was carried to estimate (co)variance components and genetic parameters for IgG (g/l) and RI. Parity (1, 2, 3, 4, and ≥5), calving season (4 classes), calving year (2019 and 2020), and herd were the fixed effects; the additive genetic animal and the residual were the random effects. The pedigree file (6,714 individuals) consisted of all sampled cows and 6 generations of their ancestors. The IgG and RI averaged 93.24±36.44 and 23.24±4.28 and were heritable (0.18±0.13 and 0.23±0.14), showing an additive genetic variance of 200.60±151.97 and 3.56±2.20. The phenotypic correlation (0.78±0.02) estimated between the two traits mirrored the genetic correlation (0.74±0.21). Findings support the use of the refractometer device for screening and management purposes, e.g. assessment and appropriate administration of the farm colostrum bank. As cheap and easy to be used, this tool could facilitate the collection of phenotypes on a large scale in a near future for definition and improvement of potential breeding strategies to enhance colostrum quality in the major Italian dairy cattle breed.

Studying relationships between production traits in Holstein Friesian using structural equations

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The main goal of the project was to investigate the relationships among production traits in Holstein-Frisian cattle, based on a data set of 5200 Polish Holstein-Friesian cows. Production traits compromised: milk yield (MY), fat yield (FY), and protein yield (PY). Phenotypes were expressed as measured yields. Graphical and statistical models that account for the recursiveness among traits were used to describe the relationship between traits. In the first step, we create a graphical model showing the dependencies between traits by using Bayesian network (BN). BN belong to a class of graphical models, which allows representation of the probabilistic structure of multivariate data using graphs. In the case of BNs, model selection and estimation are known as learning. Learning usually consists of two stages. The first of them is structure learning, in which the structure of a direct acyclic graph (DAG) is learned. In the second stage, which is called parameter learning, the local distributions implied by the structure of DAG from the previous step are learned. To infer the structure of the residual phenotypic network, we used the hill – climbing (HC) algorithm. HC algorithm represents a family of greedy search algorithms, to which a network score is assigned. The network score reflects the goodness of fit, which the algorithm attempts to maximize. Results from BN were used as structural coefficients in structural equation models (SEM). SEM took account for structural coefficients as well as for fixed and mixed effects. In the last step, multitrait genome-wide association study (MTM-GWAS) for production traits was done. Results obtained from SEM and MTM-GWAS were compared.

Stability of breeding values in the genomic era

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With the adoption of genomic evaluations and the accumulation of genotypes, changes in GEBV in subsequent evaluations are being frequently reported; this is true even for animals with no added phenotypes from one evaluation to the next. Based on traditional BLUP, EBV for animals with no new data would be more stable. Here we investigated the changes in EBV and GEBV with new phenotypes added into the evaluation system. A dataset from the American Angus Association was used that contained 8.9M records for weaning weight and 4.4M for post-weaning gain. A total of 11M animals had pedigree information, of which 500k were genotyped. Changes in EBV and GEBV were computed after the addition of six months of phenotypes. In a second scenario, changes were computed monthly after the addition of phenotypes, pedigree, and genotypes based on date of birth. Changes in GEBV when updating the core animals in the algorithm for proven and young (APY) were also investigated. In the first scenario, we observed an average change in GEBV for genotyped animals without added phenotypes of 2.4% of one additive genetic standard deviation (SDa) and in EBV was 2.0%. Although average changes were greater for GEBV, changes were up to 2 SDa for EBV and up to 0.8 SDa for GEBV. For genotyped animals with phenotypes added, the maximum change was 2.8 SDa for EBV and 0.9 SDa for GEBV. In the second scenario, the largest changes were observed for the months with more data added, with greater average changes for GEBV and more extreme values for EBV. As more information is used to compute GEBV than EBV, their accuracy is higher and possible changes are less extreme. Updating the core group in APY generated similar changes in GEBV as the addition of two months of data. Those changes can be minimized by keeping the same core group for a year and updating it when adding a large amount of data. Changes in GEBV for more animals when new data are added is an inherent factor of the genomic evaluation system because genomic information connects more animals through identity-by-state relationships. Therefore, the ranking of top animals can change rapidly even though the average merit of top animals is stable. A way to minimize the impact of these changes is to market groups of sires with high average accuracy instead of individual sires.

Design of breeding programs for Butana cattle using a stochastic simulation approach

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Butana cattle is one of the local dairy cattle breeds of Sudan commonly kept by smallholder producers. Due to its relatively high milk production performance and good adaptation to smallholder production conditions, the breed has been strongly promoted to improve the dairy production sector in the country. The main problem, however, is the lack of a systematic breeding program that involves smallholder producers. The aim of the current study was to simulate and identify the most promising design of a breeding program that would be able to improve the milk yield performance of Butana cattle under the smallholder production conditions. In total, three breeding scenarios including a farm bull, a village bull, and a rotational use of village bulls within village groups were simulated using the stochastic simulation program AlphaSimR. For each breeding scenario, three selection methods for bulls were considered, namely random, phenotypic and selection based on estimated breeding value (EBV). The effect of selection and breeding for 10 generations on the genetic gain and variance of the breeding goal trait (daily milk yield) was evaluated. The results showed that genetic gains with random selection across all breeding scenarios were approximately zero. With farm bull breeding scenario, genetic gain ranged from 0.03 to 0.96 under phenotypic selection and from 0.07 to 1.96 with EBV selection. Under village bull breeding scenarios, the genetic gain ranged from 0.04 to 1.05 and 0.07 to 2.26 with phenotypic and EBV selection, respectively. The lowest levels of genetic gain were found in rotational use of village bulls among villages within groups, and ranged from zero to 0.27 with phenotypic selection, and from 0.01 to 0.48 with EBV selection. Through the rotational use of village bulls among villages within groups, however, a relatively higher genetic variance was maintained. We concluded that a village bull with EBV selection is the most promising breeding program in achieving the breeding goal. Further studies are needed to assess the organizational feasibility of such a breeding program to ensure its sustainability.

A strategy for maximizing genetic diversity in the first generations of a pig synthetic line

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Our study focuses on the development of a strategy for the generation of a new pig line from a three-way crossbreeding scheme, as crossbreeding of two or more breeds with complementary traits typically will lead to higher genetic diversity. For this, a strategy based on genomic information was developed to enhance genetic diversity during the first three generations. Reproducers were chosen according to a genomic originality index and the founder breeds' composition. Although selection was not intended, animals with very low performances were discarded. Mating plans were designed to minimize the occurrence of runs of homozygosity (ROH) in the offspring. By simulation, we compared the genomic diversity between this strategy and two alternative scenarios over ten generations. The first scenario was based on a random choice of reproducers and random mating. In the second scenario, we applied selection on performances and generated a mating plan based on the minimization of pedigree relationships. Simulations were performed with the MoBPS R package. We performed genomic diversity analyses with ROH detection and molecular inbreeding and molecular coancestry based on identity by state. The strategy we developed results in higher genomic diversity compared to the two other scenarios with a lower increase of inbreeding based on ROH, molecular inbreeding and molecular coancestry. As expected, the selection on performance traits induced the highest inbreeding increase based on ROH over 10 generations (0.17). The random strategy induced a slight inbreeding increase (0.05), whereas inbreeding when using our suggested mating plan basically stayed constant over time (0.02). Complementary simulation analyses will help to determine at which generation selection should start to provide a sustainable line. The following step will be the development of a genomic breeding scheme that will maximize genetic progress and preserve genomic diversity.

Session 45 Theatre 9

Genetic parameters for growth rate in Danish organic pigs

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The organic pig sector in Denmark is highly dependent on genetics from conventional pig breeding programs. However, housing, management, and feeding characteristics are very different for organic pigs compared to pigs kept in conventional systems. Before weaning around week 10, piglets are reared in an outdoor production system, whereas after weaning organic pigs have access to both indoor and outdoor facilities. They are fed an organic diet without amino acid supplementation, and they have free access to roughages. Little is known about genetic parameters for growth rate in organically reared pigs. Yet, the differences between the conventional and the organic production system indicate that they might differ. Therefore, this study aimed at estimating genetic parameters for growth rate in Danish organic pigs. The analysed dataset included body weight records of 6,089 pigs from 1,147 litters of 653 sows. Body weight was recorded every time the pig entered the feeding area. Records were collected after weaning from about 10 weeks of age to slaughter. Not all pigs had records for the entire period. A total of ~1.3 million records was available, with on average 57 days of body weight recordings per pig, and 3.8 records per day. A random regression model with 3rd order Legendre polynomials for both genetic and permanent environmental effects was applied. Preliminary analyses resulted in heritability estimates ranging from 0.60 to 0.85 for body weight and 0.49 to 0.83 for growth rate. The corresponding repeatabilities were 0.95 to 0.98 for body weight and 0.70 to 0.91 for growth rate. In conclusion, the preliminary analyses indicate high heritabilities and a large genetic variation for body weight and growth rate, which shows that selection for a higher growth rate is possible in Danish organic pigs.

Session 45 Theatre 10

Selection for environmental variance of litter size modified the cecum metabolomic profile

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Metabolites from cecum could modulate the host health and the immune response. Likewise, environmental variance (VE) has been related to the immune system and the animal resilience. Animals with a low VE cope better with environmental disturbances, being more resilient. The aim of this study was to identify metabolites with different concentration levels on the gut microbiota of rabbits selected for high and low VE of litter size (LS). For that, 725 metabolites were identified in 28 does of both rabbit lines. We used partial-least square-discriminant analysis (PLS-DA) to identify the metabolites showing the largest contribution to the classification of the rabbit lines. The classification performance of the PLS-DA model was always higher than 80% for both lines, with five metabolites making the highest contribution to the model. Moreover, two-sided Mann-Whitney test and the fold change were used to find metabolites with differences in means concentration levels. From all relevant metabolites identified, we highlighted the behenoylcarnitine and the equol metabolites because were consistent across all statistical methods used. These metabolites are involved in the lipid and xenobiotic metabolism, respectively. These two metabolites could be relevant to modulate the VE and the animal resilience. However, further studies are needed to understand the effect of this metabolites in these rabbits.

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Impact of genetic and environmental covariance between dam and offspring on predictive ability

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Improper modelling of maternal effects can cause inflation of predicted breeding values. Adding maternal genetic effect and permanent environmental maternal effect into prediction model in broilers reduced already the inflation. Nevertheless, we hypothesize that including a correlation between the additive genetic effect (a) and the maternal genetic effect (m) but also between the permanent environmental maternal (pe) and residual (e) belonging to the dam into evaluation models could reduce inflation. In our study, we estimated those correlations in broilers and investigated their impact on accuracy and inflation of breeding values. Body weight was recorded in both males and females in order to estimate the environmental covariance between dam and offspring in males, variance components and breeding values were estimated with bivariate models, where BW in males and BW females were considered as two different traits. Four models were tested: a basic model without covariance (Basic), a model including the genetic covariance (Coram), a model including the environmental covariance (Corpee) and a model including both covariance (Corampee). The correlation between a and m was found to be negative (from -0.27 to -0.37) whereas the correlation between pee and e was positive (from 0.26 to 0.32). Adding genetic and/or environmental covariance reduced the inflation of breeding values. Using simulation of similar models, we show that the models accounting for genetic and environmental correlations between dam and offspring increased prediction accuracy. However, standard cross-validation strategies lead to wrong choice of models. When accuracy was computed as the correlation between true simulated breeding value and estimated breeding value a strong gain in accuracy were observed, whereas no gain in accuracy was observed when the accuracy was computed using Legarra and Reverter regression methods due to biases in both predicted breeding values and corrected phenotypes. So adding the genetic and environmental covariance might improve the realized genetic gain while controlling inflation of breeding value in broilers.

Session 45 Poster 12

Age of cows, as a factor shaping the level of immunostimulating properties of colostrum

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A characteristic feature of the organic system of production is the lower productivity of cows. The aim of the study was to determine the effect of cow age and collecting number on the level of immunostimulating colostrum components in an organic farm. From the basic herd specializing in organic milk production, 40 cows of the Polish Holstein-Friesian Black and White breed were selected: 10 primiparous, 10 cows in 2nd lactation, 10 cows in 4th lactation and 10 cows in 5th lactation. The colostrum samples were collected according to the following scheme: the first one maximum of two hours after calving, the second on the same day and the third and fourth on the following day. For three consecutive days samples were taken once a day (7 colostrum samples from each cow). Statistically significant differences in the level of bioactive components of colostrum with immunostimulating properties has been shown due to the time of intake from calving and significant differences in the level of these components due to the age of cows. Multiparous cows synthetized colostrum with a higher content of total protein, casein and non-fat dry matter than the primiparous. Variability of the immunoglobulin content of colostrum obtained in 1st and in the 2nd collecting after calving was higher in multiparous cows than in primiparous cows. Furthermore, it has been shown that there was a clear correlation between the quality of colostrum and the age of cows. In conclusion, a high impact of the interaction of age of cows x collecting number on the development of colostrum stimulating ingredients in the organic production system has been demonstrated.

Estimation of effects of pathways on dairy cattle stature using GWAS summary statistics

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The aim of this study was to estimate effects of GO terms and metabolic pathways on height in three Nordic dairy cattle breeds, based on effects of single nucleotide polymorphisms (SNPs). The analysed data comprised SNP summary statistics from genome-wide association study (GWAS) performed on 5,062 Nordic Holstein bulls, 924 Danish Red Dairy bulls and 2,122 Finnish Red Dairy bulls, performed for 25.4 million imputed variants. Animals were genotyped with different versions of Illumina BovineSNP50 BeadChips. The summary statistics (effect, standard error and P-value) resulted from single SNP linear mixed model GWAS using de-regressed breeding values for stature, implemented in the GCTA software. Furthermore, the BioMart tool was used to select SNPs from the ARS-UCD1.2 reference genome which mapped to genes. The annotation of those SNPs to GO terms and metabolic pathways was performed using the DAVID software. The final step was to estimate the effects of GO terms and metabolic pathways using a linear mixed model fitting SNPs' z-values from GWAS as dependent variables and a random effect of GO terms / metabolic pathways as an independent variable. Their similarity between particular GO terms or metabolic pathways was expressed by a non-diagonal covariance matrix constructed based on the percentage of genes common between GO terms or metabolic pathways.

Genetic parameters of maternal traits recorded in beef cattle progeny test stations

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Maternal performance traits are of major interest for sustainability of beef farms. Until now, 251 Blonde d'Aquitaine (BLA) sires and 213 Limousine (LIM) sires were evaluated on maternal traits using progeny test stations. Between 20 to 30 females per sire born in commercial farms entered the stations after weaning, were inseminated between 16 and 18 months, calved around 26 months and finished the test after their calves' weaning. Several traits were recorded such as weight at 18 months (W18), age at puberty (month classes; CP), birth weight of the calf (CW), calving ease score (CS), milk yield using weight-suckle-weight technique (MY) and calf weight at 4 months (W4). Genetic parameters were estimated using WOMBAT software with an animal model containing the contemporary group for all traits; month and region of birth and calving rank of the dam for W18, CP, and MY; calf sex and age at calving for CW, CS, MY and W4; calving ease and interval (in days) between calving and milk yield record for MY. For W18, BLA females were heavier than LIM (501±55 vs 460±59 kg) and had a higher heritability coefficient (0.52±0.05 vs 0.41±0.05). Limousine females were cycled later (41% at 15 months vs 65%) and had lower heritability coefficient (0.12±0.03 vs 0.21±0.03). For BLA breed, CP was negatively correlated with W18 (-0.20±0.10). Similar to W18, CW was more heritable in BLA (0.34±0.05) than in LIM (0.24±0.05) as well as CS (0.20±0.04 for BLA; 0.14±0.04 for LIM). Calf weight was positively correlated with CS (0.81±0.06 for BLA; 0.48±0.14 for LIM). Heritability coefficients for MY and W4 were higher for LIM (0.61±0.07 vs 0.42±0.06 for MY; 0.24±0.05 vs 0.17±0.04 for W4) with stronger genetic correlation between them (0.77±0.07 for BLA; 0.82±0.05 for LIM). Maternal progeny test stations are a valuable tool to estimate maternal traits and single step evaluation will be tested in the hope of improving the accuracy of breeding values.

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Influence of intensity of selection on the accuracy of genomic breeding values – a simulation study

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One requirement for unbiased genetic estimation is the unselected population, which is not the case in livestock breeding programs. With genomics available, many approaches in breeding values (BV) estimation can be applied. The aim was to test modalities in genomic BV estimations in the simulated population under no selection, moderate selection, and intensive selection. Three dairy cattle populations were simulated in 10 replicates, consisting of 3,000 males and 25,000 females for 20 generations. The trait was milk yield with h2=0.30. For the last 5 generations for 29 chromosomes, 45,000 SNPs were simulated. It was assumed that the trait is affected by 450 QTLs equally distributed over the genome. Accuracy of predicted breeding values for young animals in the last generation were calculated using BLUP, single-step genomic BLUP (ssGBLUP), and SNP-BLUP. In the evaluations, the pedigree and SNPs for animals – parents of the young animals were restricted to the last 3 generations. In case of no selection in population, the predicted breeding values for young animals were unbiased with accuracy from 0.64 (BLUP) to 0.77 (ssGBLUP). In the scenario with moderate selection compared to the scenario without selection, the bias was slightly increased while the accuracy of predicting young animals' performance was decreased. The accuracies of prediction with BLUP, SNP-BLUP, and ssGBLUP were 0.50, 0.65, and 0.70, respectively. In the scenario with intensive selection, estimates of predicted BV of young animals were even more biased than for the scenario with moderate selection. The accuracy of BV predictions with BLUP was low (0.16), while ssGBLUP and SNP-BLUP were remarkably higher (0.61). The ssGBLUP and SNP-BLUP performed similarly only in the scenario with intensive selection.

Session 45 Poster 16

Efficiency of genomic selection for fibre traits in alpacas

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Alpaca breeding is of great economic importance in the Peruvian High Andes. To satisfy the textile industry's demand, the improvement of fibre quality is the main breeding goal. A recently developed SNP chip for alpacas could potentially be used to implement genomic selection and accelerate genetic progress. Therefore, this study aimed to compare the increase in prediction accuracy of three important fibre characteristics: fibre diameter (FD), its standard deviation (SD), and percentage of medullation (PM) in Huacaya type alpacas. Pedigree information and phenotypic data were obtained from the PacoPro v5.10 software from the Pacomarca Scientific Research Experimental Station. Genotyping data from the SNP chip for alpacas with 69,685 SNPs were used. The reference population size was 431 alpacas. Prediction accuracy of breeding values was compared between a classical BLUP prediction and a single-step Genomic BLUP (ss-GBLUP) prediction. Deregressed phenotypes were predicted with both methodologies. The accuracy of the genetic and genomic values was obtained by the correlation coefficient between the predicted breeding values and the deregressed value of 100 random animals for the cross-validation process under both methods. Ten replicates were done. The BLUPF90 family programs were used. The heritability estimates were 0.243, 0.357, and 0.155 for FD, SD, and PM, respectively. Accuracies with ss-GBLUP improved by 9.8±3.5, 29.8±10.8 and 29.1±6.2%, on average, for FD, SD, and PM respectively compared to the BLUP method. The increase in accuracy was relevant even when the number of genotyped animals was limited, suggesting that adding genomic data in prediction models could be beneficial for alpaca breeding programs.

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Genetic diversity analysis at LALBA gene promoters in domestic camelids

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α-lactalbumin (α-LA) is a milk whey protein whose main role is played in lactose synthesis. The α-LA is encoded by *LALBA* gene. The importance of the promoter regions lies into binding sequences for transcription factors (TFs), necessary elements to activate gene expression. Therefore, variations in the promoters affecting TFs may alter transcription rate and, consequently, proteins translation. *LALBA* gene has been well studied in many species like cattle, sheep, buffalo, etc., but no information is available at the genomic level for the domestic camelids. The aim of this study was to characterize the promoter of *LALBA* gene in the four domestic camelids (*C. dromedarius, C. bactrianus, V. pacos* and *L. glama*) and to search for genetic diversity. About 800 bp were amplified using two pairs of primers (5′- TGCACACTTTTCCAGTTCTCTGT-3′ and 5′-GAGAGGCATCCACTCTGGCTGAATGT3′) in 12 animals (3 for each species) and sequenced in both directions by Sanger technology for SNP discovery. To determine the effect of each SNP on the TF binding sites, *in-silico* prediction was performed by Ali Baba 2.1 online tool. Further, a PCR- RFLP method was developed for the genotyping of the most interesting SNP in additionally 45 llamas. Alleles frequency and Hardy -Weinberg equilibrium were calculated. The complete sequence of the promoter regions plus exon 1 was obtained. In total 8 SNPs were found in the promoters (1 SNP in dromedaries, 4 in alpacas, 3 in llamas, whereas no SNP were found in bactrian camels). Moreover, 3 of them were common between llamas and alpacas, specifically the SNPs complementary to JEMW01005898: g.82991G>A; g.82862C>T; g.82475G>A. In silico prediction showed that the SNP g.82991G>A affected a binding site for the transcription factor EGR-1 (early growth response protein1). The genotyping data revealed that allele A had a minor frequency (0.44). Chi-square analysis evidenced equilibrium for Hardy-Weinberg equilibrium. Studying the genetic diversity of promoter in 4 species confirmed that the promoter region of alpacas and llamas are more polymorphic compared to dromedary and bactrian camels. Future studies will clarify the functional relationship between this SNP and gene expression.

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Analysis of chromosome abnormalities in Murrah buffalo secondary oocytes by tri-colour FISH

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In the present study we report a preliminary analysis of aneuploidy incidence on *in vitro*-matured Metaphase II (MII) oocytes with corresponding first polar bodies (PB) in Chinese Murrah buffaloes (*Bubalus bubalis*, 2n=50, XX) by using fluorescence *in situ* hybridization (FISH). Sample collection has been achieved at Buffalo Research Institute (Nanning, China). A total of 60 cumulus-oocyte complexes (COCs) were collected from ovaries of slaughtered buffaloes. After examination, COCs were washed in PBS and transferred in 50 µl maturation droplets made of TCM199 supplemented with 10% FBS, 0.5 µg/ml FSH and 5 µg/ml LH for 24 h at 38.5 °C and 5% CO_2. After maturation, COCs were washed in a hyaluronidase solution (1 mg/ml) and exposed to hypotonic solutions (Na-Citrate 0.8% and KCl 0.5%). Oocytes were individually fixed on microscope slides by methanol/acetic acid fixative (1:1). Aneuploidy was detected by tri-colour FISH experiments using chromosome-specific painting probes (1q, 5q and Xcen). Out of 60 oocytes, 10 did not reach the MII stage, therefore the efficiency of the *in vitro* maturation process was 83%, whereas in 5 of them chromatin of the first PB was not found and they were excluded from the analysis. Finally, the number of slides successfully analysed was 45. One oocyte was disomic for the Xcen (2.22%). This incidence is almost 10-fold higher than the average level of disomy for the X-chromosome observed in domestic cattle (0.25%) but in agreement with the total aneuploidy level (2.25%) of *Bos taurus*. An unreduced diploid set of chromosomes was detected only in one oocyte (2.22%). Mammals fertility is strongly affected by chromosomal abnormalities and it represents one of the main causes of foetal and embryonic mortality. Estimate the baseline level of aneuploidy in buffalo breeds could be an important step to assess the impact on productive and reproductive efficiency. In addition, aneuploidy knowledge could be useful for improving the *in vitro* production of embryos destined to transfer.

Session 45 Poster 19

CLARITY: an R-shiny app to explore the combined physical-genetic map in Holstein cattle

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The arrangement of markers on the genome can be defined either in physical or linkage terms. While a physical map represents the inter-marker distances in base pairs, a linkage map pictures the recombination frequency between pairs of markers. The rate by which two measures are correlated varies along the chromosomes and provides insights into the nature of a genomic region. We developed an R-shiny app that allows users to interactively explore the genetic and physical map of the German Holstein cattle genome. Currently, the app provides access to the linkage map built on the 50K genotypes of a large pedigree of Holstein cattle (i.e. 876 half-sib families which comprise more than 367,000 genotyped animals) and based on estimates of recombination rate between intra-chromosomal marker pairs. Markers were ordered according to the physical coordinates of the most recent bovine reference genome assembly (ARS-UCD1.2). The user is able to interconnect the physical and linkage map for a whole chromosome or a specific chromosome region. Moreover, the user can investigate which genetic map function (e.g. Haldane, Rao and Felsenstein) locally fits best. The CLARITY app also offer functions for detecting putative recombination hotspots through an adjustable threshold. We further provide auxiliary information of markers being putatively misplaced in the ARS-UCD1.2 release. The corresponding outputs such as tables and figures can be downloaded for being locally stored. We are working on to facilitate data integration from different breeds (datasets are also welcome) to enable comparison of different genome features.

Session 45 Poster 20

A new candidate region associated with the appearance of wattless in Mangalitsa pig

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Wattles are congenital thumb-shaped appendages on the ventral throat which appear typically in goats, however similar structures can be also occasionally found in other species including sheep, pigs and humans. They consist of epidermis, dermis, muscles, nerves, blood vessels and cartilage tissue. In pigs, the appearance of wattles was described in several breeds and it has been proposed that the appearance of wattles could be, similar as in goats, controlled by a single autosomal locus, with a dominant mode of inheritance. However, the genomic analysis in Casertana pig breed revealed two candidate regions at SSC11 and SSC1 containing seven and 35 annotated genes, respectively. In our study we analysed 40 pigs belonging to the swallow bellied Mangalitsa breed from which 12 animals (30%) had one or two wattles. The genotyping was performed using PorcineSNP60 BeadChip array (Illumina) and 57,935 SNP markers were included in the basic allele tests. In GWAS analysis, the strongest signal ($P=1.54E^{-07}$) for presence of wattles was found for SNP ALGA0006347 on SSC1 (rs81348874). The closest potential candidate in this region is Galanin Receptor 1 gene (*GALR1*). Galanin receptor 1 is a transmembrane protein activating a variety of intracellular second-messenger pathways. It is widely expressed in the brain and spinal cord, but also in the small intestine and heart. In the mouse model the GALR1 deficiency alters food intake and glucose homeostasis.

The long non-coding RNAs recognition and diversity analysis across different pigs breed and tissue

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Long non-coding RNAs (lncRNAs) are defined as RNAs longer than 200 nucleotides that are not translated into functional proteins. The lncRNAs are involved in chromatin remodelling and transcriptional and post-transcriptional regulation functions as decoys, scaffolds, and enhancer RNAs. The microRNA (miRNA) are both play significant regulatory roles in many life processes. Moreover, non-coding fractions of the transcriptome in gene expression regulation and translation control have not been established, especially for the quantitative and functional traits in pigs (muscling and fattening). The study aimed to recognise and analyse existing and novel long non-coding RNAs in the pig genome across different breeds, tissues and tools. Additionally, the lncRNA-miRNA interaction based on the nucleotide sequences will be predicted. As a material of this study, we used pig transcriptome acquired from the GEO NCBI repository, mainly submitted by the National Research Institute of Animal Production. The final set covered more than 100 samples belonging to the different breeds (Landrace, Large White, Pietrain, Duroc, native Puławska and Złotnicka breed) AND across other tissues (muscle, fat, pituitary). The analysis of the next-generation sequencing tuns was based on STAR alignment and Cufflinks transcript reconstruction models. The FEELnc software was used to recognise lncRNAs. The identified long non-coding RNAs were filtered, functionally enriched and finally analysed across the breeds and tissue. The most exciting form the breeding point of view lncRNAs will be verified according to the lncRNA-miRNA interaction using the Freiburg IntaRNAv2 tool.

In vivo estimation of body composition of crossbreed beef cattle using ultrasound measurements

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Accurate *in vivo* estimation of body composition of beef cattle is of great interest in terms of recording nutritional status or determination of optimal time for slaughter. The aim was to estimate the body and carcass anatomical compositions of crossbreed beef bulls (n=36, 477 to 530 kg body weight (BW), mothers Brown Swiss and sires Angus, Limousin and Simmental, the most widespread in Switzerland) using ultrasound (US) measurements. Subcutaneous adipose tissue (AT) and muscle (MU) thickness were measured by US (9L-RS Linear-Sonde (4.0 MHz); GE Healthcare) at 4 specific body locations (ischium-sacrum, lumbar, dorsal, flank) 3 to 7 days before slaughter. After slaughter, empty body weight was determined after removal of digestive content, the weight of visceral AT (omental, mesenteric, perirenal and pericardiac) was recorded, as well as the weights of carcass AT and MU after anatomical dissection of one half-carcass. Relationships between BW and US measurements as predictive variates, and empty body and carcass AT and MU proportions were analysed by linear regressions using GLM and PHREG procedures (SAS 9.4). Empty body (456.8±20.5 kg) contained 13.9±2.7 (9.5 to 21.9)% AT, hot carcass (288.6±16.0 kg) contained 14.1±3.0 (9.0 to 23.6)% AT, and 64.3±3.0 (58.2 to 70.8)% MU. The best single estimate was AT dorsal thickness, including crossbreed type as a fixed effect, for empty body AT% (R^2=0.72, residual standard deviation (rSD)=1.5%, $P<0.05$), carcass AT% (R^2=0.70, rSD=1.7%, $P<0.05$) and carcass MU% (R^2=0.58, rSD=2.0%, $P<0.10$). BW combined with crossbreed type did not explained empty body AT% nor carcass AT% or MU% ($P>0.20$). Neither dorsal MU thickness, nor US measurements at the ischium-sacrum, lumbar or flank ($R^2\leq0.58$) locations were good estimators of empty body or carcass composition. In conclusion, the dorsal AT thickness measurement by US gave an accurate estimator of empty body and carcass compositions and seems therefore suited for the *in vivo* follow-up of their dynamics.

Similarities between Mendelian sampling values in a large commercial cattle population

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Mendelian sampling variance (MSV) of additive genetic values is increasingly being used in breeding decisions due to a growing interest in future offspring variability. A similarity matrix, which has MSVs of individuals on its diagonals and pair-wise similarities as off-diagonals, was introduced recently to visualize haplotype diversity and optimize breeding decisions. However, no such matrix has been computed for a large commercial population, and it is unclear how important factors affect the values of this matrix. Herein, we present findings from similarity matrices derived from a German Holstein-Friesian cattle population comprising 71,719 individuals genotyped with 50K SNP chip, four traits (longevity, DLG; milk fat, FKG; milk urea nitrogen, MUN; and milk protein, PKG), two sexes (male and female), 2,829 half-sib families, and 14 birth years (2007-2020). The MSVs of all individuals ranged from 5.3-30.4 for DLG, 30.9-215.1 for FKG, 13.8-85.9 for MUN, and 17.8-95.5 for PKG. The contributions to the total MSV varied across chromosomes. In FKG, chromosome 14 contributed nearly 57% of the total MSV, whereas chromosome 4 contributed just nearly 12%. As expected, females had significantly ($P<0.05$) higher MSVs than males for all traits except MUN. Unselected individuals had higher ($P<0.05$) MSVs than the selected individuals except for PKG. The MSVs for all traits except MUN were unaffected by the year of birth. The observed standardized pair-wise similarities for all traits ranged from 0.1 to 1 with a mean of about 0.5. Further investigations of individuals with a perfect similarity showed no definite trend regarding pedigree, birth year, and heterozygosity, except for same-sex and selection groups. Therefore, a high similarity between individuals can be interpreted as many shared chromosome segments with markers of large additive effects in the same linkage phase. The novel information on MSV and similarities between Mendelian sampling values will help improve genetic gain and manage genetic diversity in breeding programs.

Chicken model to study the epigenetic impact and inheritance thereof

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Epigenetic modifications are essential mechanisms that fine-tune the gene expression in response to extracellular signals and environmental changes. Carefully managed, the epigenetics may perhaps carry the greatest potential for animal health and welfare. Epigenetic impact may be posed by environmental factors, nutrition and bioactive epigenetic stimulators like choline or a synbiotic. The changes in gene function that result from an epigenetic impact can be mitotically or meiotically heritable through the cell lineage and tissue development, and influence the adult phenotype. The chicken not only has a fast generation turnover, but since the embryo develops externally to mother's organism, it can be easily accessible for *in vitro* and *in vivo* manipulations. *In ovo* treatment proved to be effective in modulating gene expression in a range of tissues, some of those changes showing epigenetic characteristics. Recently, we have shown changes in the level of global methylation on ED 8, in gonadal PGCs (primordial germ cells) isolated from Green legged Partridge (GP) and White Leghorn (WL) breeds. Those changes appeared to be influenced by sex and breed, suggesting a faster development of GP embryos. We propose a study model, in which the prenatal epigenetic impact is initiated *in ovo* and followed in the cellular germline and in the adult phenotypes: from a juvenile to a mature female (hen), through several consecutive generations. Such a model can be relevant to study epigenetics in a poultry breeding context, leading to understanding as to how epigenetic marks can be inherited through the germ line and contribute to improvement of accuracy in breeding.

Session 45 Poster 25

Genomic PCA – a proxy for monitoring genetic connectedness between flocks (case study on sheep)

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Fair comparison of predicted breeding values of animals belonging to different flocks is crucial for exploiting available genetic pool of breed. Low connectedness between flocks, as commonly present in many sheep breeds, represents unfavourable population structure for joint, i.e. across-flock, genetic evaluation. The issue is mainly present in populations with no or limited: (1) usage of artificial insemination; and (2) exchange of sires between flocks. Genomic selection (GS) is supposed to alleviate this issue in many animal breeding programs. In addition to its benefit in term of accuracy and earlier provision of breeding values for some animals, genomic information can be also beneficial in monitoring connectedness between flocks. The main idea of the study was to examine if results of principal component analysis (PCA) conducted on whole genome SNP markers (Illumina ovine 54K SNP chip) can be used as an easy-to-implement method to examine connectedness. The PCA was conducted on 719 genotyped Istrian sheep coming from 14 flocks. Cleaning of the SNP marker file and PCA were conducted with PreGSF90 program. Genetic variance explained by PC1 and PC2 were 5.8 and 3.5%, respectively. Pedigree based connectedness was estimated by Gene flow method (GF) and Genetic drift variance (GDV) on 5,090 phenotyped ewes and their known ancestors (a total of 6,477 animals in the pedigree). Comparison of PCA, GF, and GDV results revealed substantial matching, implicating that genomic PCA can be used as fast and effective method for this purpose. The genomic PCA has not yet been proposed in this context, despite its simplicity in contrast to other computationally very complex methods, so we hereby advocate its widespread usage.

Session 45 Poster 26

Negative impact of single and double stranded sperm DNA damage in *in vitro* fertilization in pig

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Sperm quality is known to be a key determinant of the fertility status of production animals. In this regard, pig sperm quality has been widely analysed from different variables, including motility, viability, acrosome integrity or mitochondrial activity, amongst others. The search of more powerful biomarkers related to fertility brought researchers to the analysis of sperm DNA integrity, and recent studies pointed out that it may add important information regarding sperm fertilizing capacity. The aim of the present study was to elucidate the relationship between this parameter and *in vitro* fertilization outcomes. Sperm samples from eight post-pubertal boars were obtained from local farms, which provided standard doses (90 ml) intended to artificial insemination. These sperm samples were processed to analyse single- and double-stranded DNA fragmentation using alkaline and neutral Comet assay, respectively, after 0 h and 5 h of incubation at 37 °C in fertilization media. *In vitro* fertilization was performed following the standard method previously described in porcine. Mean olive tail moment for alkaline Comet assay at 0 and 5 h were 17.83±1.89 and 18.85±2.06, respectively. Mean olive tail moment for neutral Comet assay at 0 and 5 h was 2.64±1.05 and 2.71±0.53, respectively. Fertilization rate was 28.75%±6.27, mean percentage of blastocysts was 8.98%±2.90 and mean percentage of morula and blastocysts was 23.39%±9.01%. Percentage of morula and blastocysts were found to be negatively correlated not only with alkaline but also with neutral Comet Olive tail after 5 h of incubation (Rs=-0.743, P=0.035; and Rs=-0.707, P=0.05, respectively). Both single- and double-stranded sperm DNA breaks may have a negative influence on embryo preimplantational development in pig.

Session 45 Poster 27

Seeking for the inner potential: integration of geographic differentiation in domestication programs

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To promote the aquaculture sector sustainability, one way relies on the production diversification, notably through the domestication of new species. However, domesticating new species is challenging because of technical issues, socio-economic limitations, or biological features of species. Yet, these latter can vary at the intraspecific level. Indeed, geographic differentiation (i.e. differentiation between allopatric conspecific populations) can shape genetic, phenotypic, and ecologic specificities, affecting the domestication predisposition or the socio-economic attractiveness of a particular population. Therefore, geographic differentiation could facilitate domestication by choosing wild populations exhibiting a high potential for aquaculture (i.e. presenting interesting expression of key traits). We aimed at (1) developing a standardized and pragmatic approach to integrate geographic differentiation in domestication programs and (2) applying this methodology on a species of interest for inland aquaculture diversification, the European perch (*Perca fluviatilis*). The methodology includes three steps: (1) classification of wild populations into prospective units (i.e. groups of populations genetically differentiated), which are likely divergent for key traits; (2) performance evaluation of the different prospective units in standardized aquaculture conditions through a multi-function and multi-trait approach; and (3) identification of the prospective unit with the highest potential for aquaculture. When applied to *P. fluviatilis*, geographic differentiation for key traits and divergent aquaculture potentials were shown between prospective units. Overall, this three-step approach could allow facilitating domestication of new species or species at incipient stages of domestication which face major bottlenecks. However, translocations of individuals within the species distribution range and potential escapees from aquaculture systems can lead to many conservation concerns such as biological invasion, pathogen spill-over, and genetic homogenisation/outbreeding. Therefore, the choice of the prospective unit for domestication must be made based on a trade-off between aquaculture potential and potential risks for wild conspecific populations.

Session 45 Poster 28

A CRISPR/Cas9 approach to confer viral resistance in pigs

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The domesticated pig is invaluable as a global protein source. However, pork production frequently faces health challenges induced by viral diseases causing production losses and animal welfare concerns. For instance, the viral disease; African Swine Fever (ASF) has been spreading in eastern Europe and reached Germany in 2020. Since no approved vaccines or treatments are available, different approaches to confer ASF resistance may be of great value. The discovery of the bacterial adaptive immune system CRISPR/Cas and its genome editing capability provides great opportunities to generate disease resistant animals. By integrating a Cas9 transgene into the porcine genome, Cas9 can be programmed to target viral DNA upon infection and disrupt viral replication *in vivo*. Cas9 expressing cells were generated by transposon-based (TB) or random integration (RI) of a Cas9 transgene and were used as donor cells in somatic cell nuclear transfer. Out of 8 embryo transfers, 6 gilts carried to term and delivered a total of 38 piglets, of which 15 carried a TB Cas9 integration and 2 a RI. Expression analysis of piglets' fibroblasts by reverse-transcription qPCR revealed Cas9 transcription and an up to 27-fold expression change in TB integration compared to RI. Higher Cas9 expression of TB integration was also found in a variety of tissues such as spleen, kidney, lung, and liver. To verify Cas9 activity, fibroblasts of five animals were subjected to *in vitro* genome editing. The cells were transfected with guide RNAs targeting different porcine loci (GGTA1, B4GalNT2, CMAH, B2M). Analysis showed that editing occurred at varying frequencies (2-85%) between the different target genes and animals, which could be attributed to a different amount of copy numbers of integrated Cas9 transgenes in the animals. A targeted single integration of Cas9 may address the variation between animals and enhance expression further. In addition, experiments must be conducted to investigate whether the *in vivo* expressed Cas9 can be directed towards viral DNA and thereby inhibit viral replication.

Session 45 Poster 29

Effect of HOXA10 and HOXA11 genes polymorphisms on the size of reproductive organs in pigs

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Studies with mice have shown that *HOXA9*, *HOXA10*, *HOXA11* and *HOXA13* genes are expressed along the Müllerian duct axis. The *HOXA10* gene plays a crucial role in uterine development, and *HOXA11* in uterine and cervix development. The aim of the study was to determine the effect of the polymorphism of some genes associated with the structure and function of the reproductive system on morphometric parameters of the reproductive system in pigs and on the measurement of vagina-cervix length (VCL). A total of 115 Polish Large White (PLW), Polish Landrace (PL) and PLW ´ PL sows were studied. After slaughter, their reproductive systems were collected to make VCL and morphometric measurements: VCL measured with a spiral catheter (cm), weight of uterus with vagina, ligament, oviduct and ovaries (kg), uterine length (kg), vaginal length (cm), uterine body length (cm), right uterine horn length (cm), left uterine horn length (cm), uterine capacity (ml). To identify polymorphic changes, Sanger sequencing was used for the *HOXA10* gene and PCR-RFLP with HpaII restriction enzyme for the *HOXA11* gene. The analysis accounted for the polymorphisms of *HOXA11 g.3786A>G*, *HOXA10 g.45398502C>A*, and *HOXA10 g.45398714C>G* genes. Statistical analysis and the estimated coefficients of correlation showed that the polymorphism of the studied mutations had no effect on VCL length and morphometric measurements of the reproductive organs except for the *HOXA11 g.3786A>G* mutation. Sows with the AA genotype of this gene had a longer left uterine horn compared to those with the GG genotype ($P \leq 0.05$). Based on the results obtained, the studied mutations are not recommended as genetic markers for predicting traits related to the size of reproductive organs in sows.

Session 45 Poster 30

Multibreed genetic evaluation for Finnish beef cattle population

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Beef cattle breeding scheme in Finland has been developed for each breed independently and with data excluding F1-animals, building mostly on purebred recording herd animals. In the recent years there has been an increasing demand in including data from F1 animals and also publish their estimated breeding values (EBV). In 2013, Finnish evaluations were upgraded to include the carcass weight, and EUROP and fat classifications for slaughtered animals originating from both recording and production herds. The single-breed multi-trait slaughter evaluations consisted of birth, weaning, and yearling weights from beef recording herds, and carcass weight, EUROP and fat classifications from abattoirs. Slaughter traits are available from all herds, increasing number of observations for cross-breeds. The current study included F1 animals in the data that now covers most of the beef cattle population in Finland. Pedigree information was obtained from the EU bovine register. Full population level breeding scheme makes possible to achieve higher environmental impacts through breeding. The interactions between breed and sex and calving age effects were added to model but maintaining one of the fixed effect classes same across all breeds. Breed of an animal was defined using breed proportions rounded in quarters. Individual animal variance components were derived from the original purebred variance components according to breed proportions. Heterosis was modelled with total and breed interactions among the 10 most common breed crosses. Total recombination loss was also included in the model. Within breeds the correlations of EBV between multi-breed and pure-breed models were high. Also the multi-breed model was able to rank the EBVs of animals with different breeds logically. In the next phase, the models will be upgraded in the single-step genomic evaluation models utilizing a reference population compiled by genotyping animals of 5 main breeds, both from the beef recording and production herds.

Session 46 Theatre 1

Genomic inbreeding and highly homozygous regions in Colombian Creole cattle populations

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The traditional local *Bos taurus* Creole cattle are a notable part of the South American animal genetic resources, widespread in multiple countries. In Colombia we find Creole breeds with well-defined characteristics, such as the Blanco Orejinegro (BON), Costeño con cuernos (CCC), Romosinuano (ROM) and Sanmartinero (SAM). In general, the genomic analysis of the Creole cattle breeds is of interest to strengthen their breed management efforts, as well as to identify production and adaptation-related traits. In our study, we analyse single nucleotide polymorphism (SNP) data from a total of 300 animals (75 from each breed). They were genotyped with an SNP chip consisting of 138,974 SNPs located throughout the genome. The data were subjected to quality control, removing SNPs and animals with more than 10% missingness, and breed identity. Finally, 132,163 SNPs and 288 animals remained for the analysis. The genomic inbreeding coefficients were assessed using runs of homozygosity (ROH). The minimal length of the considered ROH segments ranged from 1 Mb to 16 Mb, to account for inbreeding of historical, but also of recent origin. The genomic inbreeding coefficient was around 2-5% when considering ROH>8 Mb, which is equivalent to a pedigree depth of 6 generations in the past. The historical inbreeding from all ROH>1 Mb considering common ancestors up to 50 generations in the past was between 9-13%. The highest inbreeding levels were observed in the ROM breed. There were several well-defined, highly homozygous regions in all breeds. For our purposes, we defined ROH islands as overlapping ROH regions with the top 1% most homozygous SNPs within each population. The regions were further analysed using the cattle QTLdb. The ROH islands contained QTLs relevant to the beef production characteristics of the breeds. Interestingly, one of the strongest ROH islands was at 0.5-2.5 Mb on BTA21, with a strong connection to reproductive traits and calving ease.

Session 46 Theatre 2

Preliminary analysis of ROH in two divergent lines of mouse selected for birth weight variability

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Runs of homozygosity (ROH) are defined as long continuous homozygous stretches in the genome which are assumed to arise from a common ancestor. It has been demonstrated that divergent selection for variability in mice is possible, and that low variability in birth weight is associated to robustness. The objective of this study was to analyse ROH patterns in the genome of two mice lines selected for high (H) and low variability (L) for birth weight during twelve generations. Up to 172 H and 211 L female mice were genotyped using the Affimetrix Mouse Diversity Genotyping Array. After quality filtering, 586,123 SNPs were retained for analyses. ROH were computed using the R package detectRUNS under the consecutive-runs method. Two inbreeding coefficients were calculated for each individual and generation for either selection line: inbreeding based on pedigree (FPED) and inbreeding based on ROH (FROH; the sum across the genome of ROH length divided by the autosomal genome length covered by SNP data). Consensus ROH were identified in the three last generations of either selection line using PLINK v1.7. The consensus ROH that were not shared between lines were identified using Bedtools. Mean FROH was 0.56 for both lines. In the first generation average FROH was 0.59 for both lines. In the 7th generation, FROH was 0.58 and 0.60 in the H and the L lines, respectively. Average FPED in the first generation was 0.03 and 0.02 in the H and the L lines, respectively; in the 7th generation of selection FPED was 0.10 and 0.11, respectively. Most ROH were 1-2 Mb long (36.8% in H and 37.0% in L). ROH>10 Mb were 10.3% in H and 10.1% in L. Up to 60 and 59 were exclusive for the H and the L lines, respectively. Most exclusive ROH in the H line were located on chromosome (CHR) 3 (8.23%) and CHR 1(8.23%). In the L line most exclusive ROH were located on CHR 11 (7.61%) and CHR 3 (7,07%). These results suggest that differences in ROH distribution between lines might be caused by divergent selection. However, differences between lines were not high and further analyses should confirm that new homozygous stretches are caused by selection.

High-density genome-wide association for variability of litter size in pigs

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Litter size is a trait of high economic relevance for pig breeding. However, due to existence of genetic correlation (~0.5) with selection for increased litter size also its variability is increasing, which is not desired from economic and welfare stand point. Thus the aim of this study is the genome-wide association (GWAS) for variability in litter size (TNB) in Large White population in order to evaluate opportunities to increased uniformity of TNB. We used 607,553 litter records from 121,088 sows coming from Topigs Norsvin multiplication farms. Variability of TNB was studied as the residual variance of TNB and its variance components were estimated in ASReml either with Double Hierarchical GLM (varTNB) or with log-transformed variance of residuals from a classical animal model (LnVarTNB). The obtained estimated breeding values (EBVs) of variability traits were deregressed, which allowed using phenotypes of both sows and boars. In GWAS performed with GCTA a total of 12,550 animals genotyped with 660k SNPchip were used. The correlation between EBVs of varTNB and LnVarTNB was 0.72 indicating that the two methods yield rather different results. Genetic coefficient of variation on standard deviation level was estimated as 0.096 for varTNB and as 0.087 for LnVarTNB, suggesting good opportunities for improvement of uniformity by selection. For varTNB only one significant association was found on chromosome 7 with two possible candidate genes *ADGRF1* (adhesion G protein-coupled receptor F1) and *ADGRF5* (adhesion G protein-coupled receptor F5) within 50k bp range from significant SNP. For LnVarTNB on chromosome 17 a total of 8 significant association was detected within a close distance from each other located in three candidate genes: *CABLES2* (Cdk5 and Abl enzyme substrate), *TAF4* (TATA-box binding protein associated factor 4) and *CDH4* (cadherin 4). Also one significant SNP for LnVarTNB was found on chromosome 16 located in gene *FABP6* (fatty acid binding protein). The results of this study indicate that the two methods provided essentially two genetically different traits. Research funded by National Science Center grant no. 2016/23/D/NZ9/00029.

Quantitative variation of male fertility in Brown Swiss cattle is shaped by autosomal recessive loci

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We take advantage of the large amount of genotype and phenotype data that are available for artificial insemination bulls to investigate the genetic underpinnings of male fertility. Genome-wide association analyses were performed between non-return rate, semen quality and genotypes at 683,609 SNPs for 3,881 Brown Swiss bulls. Using haplotype-based association testing, we discover five recessive QTL on BTA1, 6, 18, 25, and 26 that affect bull non-return rate. Using whole-genome sequence and transcriptome data, we characterize the QTL at nucleotide resolution and prioritize candidate causal variants. The recessive QTL on BTA6 ($P=9.34\times10^{-44}$) reduces sperm motility and increases the proportion of anomalous sperm, particularly defects of the mitochondrial sheath. The QTL on BTA1 ($P=2.76\times10^{-17}$) increases the proportion of sperm with head shape anomalies. Three QTL on BTA18, 25, and 26 are associated with non-return rate, but not semen quality. Through whole-genome sequence variant filtration, we reveal coding variants in *SPATA16*, *WDR19*, *VWA3A*, *ENSBTAG00000006717* and *ENSBTAG00000019919* that are in linkage disequilibrium with the QTL affecting non-return rate. These genes are expressed in bovine testis. However, in an association study with imputed sequence variants, non-coding variants exhibit stronger association with non-return rate than the coding variants at all QTL. To detect additional QTL for sperm morphology, we performed a principle component analysis on 21 sperm morphology characteristics and used the eigenvectors that cumulatively explained >90% of the phenotypic variation as response variables in the haplotype-based association testing. We discover 14 recessive, 2 additive, and 1 dominant QTL, including two previously detected QTL for non-return rate on BTA1 and BTA6. Our findings in a large mapping cohort of Brown Swiss bulls provide compelling evidence for non-additive effects to contribute substantially to quantitative variation in male fertility.

Session 46 Theatre 5

Dimensionality of genomic information and its impact on GWA and variant selection

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Genome-wide association (GWA) is used to identify causative variants for the traits of interest. The ability to identify true-positive variants increases as the number of genotyped individuals increases. Although millions of individuals can be genotyped, the dimensionality of the genomic information is limited. This means that there is a certain number of individuals that represent all chromosome segments (*Me*) segregating in the population. The *Me* can be derived from the eigenvalue decomposition of the genomic relationship matrix (G). Therefore, the limited dimensionality may give some insights on the optimal number of individuals to be used in GWA. The first objective of this study was to test different discovery set sizes in GWA, with set sizes based on the number of largest eigenvalues explaining a certain proportion of variance in G. Besides, we investigated the impact of adding variants selected based on different set sizes to regular SNP chips used for genomic prediction. Sequence data were simulated that contained 500k SNP and 2k QTL, and all the genetic variance was explained by QTL. The effective population size was 200. The GWA was performed with the number of genotyped animals equal to the number of largest eigenvalues of G (EIG) explaining 50, 60, 70, 80, 90, 95, 98, and 99 percent of the variance in G, so the number of genotyped animals for each set was 530, 920, 1,540, 2,650, 5,300, 8,800, 15,200, and 22,000. A set of 30k discovery animals was used as a benchmark. Significant SNP had a P-value lower than 0.05 with Bonferroni correction. Besides, SNP with the greatest effect size were also selected to be added to the 50k regular chip. The top 10, 100, 500, 1k, 2k, and 4k were added. Genomic evaluations using the 50k chip and the selected SNP were performed using single-step GBLUP (ssGBLUP). Using the number of animals corresponding to at least EIG98 enabled the identification of the largest effect size QTL. The greatest accuracy of prediction was obtained with the top 2k SNP added to the 50k chip. The rate of increase in accuracy was the largest when using EIG98 number of genotyped animals. The dimensionality of genomic information should be taken into account for variant selection in GWA.

Session 46 Theatre 6

Gradient boosting is a competitive method for genomic prediction of complex traits in outbred mice

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Machine learning methods can capture gene interactions, possibly outperforming linear models for genomic prediction for traits with relevant epistatic effects. Our objective was to compare the performance of GBLUP and BayesB methods to a non-parametric tree-based ensemble (gradient boosting machine – GBM) method for genomic prediction of complex phenotypes in mice. The dataset contained phenotypic and genotypic information for 835 mice from 6 non-overlapping generations. Traits analysed were chosen by the assumption of distinct genetic architecture groups. The final set contained bone mineral density at 12 weeks (BMD), body weight at 10, 15 and 20 weeks (BW10, BW15 and BW20), circulating cholesterol at 19 weeks (CHL), circulating glucose at 19 weeks (GLU), fat percentage at 12 weeks (FAT%), insulin at 8 weeks (INS) and circulating triglycerides at 19 weeks (TGL). The mice were genotyped for 50,112 SNPs. Animals in the latest generation (n=192) were used as the validation subset. For each trait, the best GBM model was obtained by hyperparameter tuning through an extensive grid search scheme. Accuracy was measured as the correlation between adjusted phenotypes and the predictions for the validation subset. Estimated heritabilities (GBLUP) for the analysed traits ranged from 0.22 to 0.42. Higher prediction accuracy for INS (23.4%) and TGL (11.5%) was obtained by GBLUP, for BW10 (32.3%), BW15 (23.7%), BW20 (27.4%) and FAT% (35.8%) by BayesB; and for BMD (20.8%), CHL (30.2%) and GLU (16.3%) by GBM. Accuracies from GBLUP and BayesB models were proportional to the trait heritabilities, which was not the case for GBM. Interestingly, for those three traits there is strong evidence in literature of a relevant portion of variance being explained by epistatic effects. Our results indicate that GBM is a competitive method to predict complex traits in an outbred mice population, especially for traits affected by epistatic effects.

Session 46 Theatre 7

A 200K SNP-chip for endangered German Black Pied cattle (DSN)

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German Black Pied cattle (DSN, *Deutsches Schwarzbuntes Niederungsrind*) is an endangered dual-purpose breed kept as a genetic resource in Germany. The study of DSN genetics on the populational level is crucial for the conservation of its genetic diversity and provides avenues for genetic improvement. Whole-genome sequencing and customized SNP chips have emerged as a solution to improving the detection of causative variants, controlling diversity, as well as genomic prediction. Inclusion of variants previously associated with traits of interest and/or with high predicted impact on transcription or protein structure is expected to contribute to achieving this central goal. We designed a customized Axiom® myDesign™ TG Array for DSN harbouring 200k probe sets (DSN200k SNP-chip). We used a pool of 20,587,181 sequence variants detected from whole-genome sequencing data of 304 DSN animals for selecting variants to include in the new chip. The chip contains important variants derived from distinct selection categories: previous GWAS results, parentage panels, high or moderate impact, DSN unique. Additional 36k DSN informative variants from the Illuminia®BovineSNP50 chip were incorporated. Ultimately, 175,537 SNPs and 8,618 InDels targeting 103,801 haplotype blocks in DSN were included. The DSN200k SNP-chip was first applied to 1,547 DSN and 277 animals of 9 other breeds. In the initial data analysis, a total of 167,518 variants (92%) were functional considering all breeds. This result demonstrates the utility of the customized chip not only for DSN, but also for other local breeds such as Rotbunt-Doppelnutzung, Angler, Rotes Hoehenvieh, Pinzgauer, Hinterwaelder, Original Braunvieh, and Gelbvieh. The segregating variants for each breed could be easily employed to produce cheap low-density customized SNP chips for other local breeds.

Session 46 Theatre 8

Estimation of dam line composition of 3-way crossbred broilers using genomic information

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Commercial pigs and poultry are typically 3-way or 4-way crossbred animals. Many recent studies looked into using crossbred data to routinely estimate genomic breeding values for purebred selection candidates. Line composition is usually fitted as a regression on expected breed fractions, being 0.5, 0.25 and 0.25 for a 3-way crossbred. Actual breed fractions for the dam lines, however, can vary between ~0.1 and 0.4, and ignoring this variation may affect genomic estimated breeding values of purebred selection candidates. Our aim was to evaluate different approaches for estimating breed fractions in a 3-way crossbred broiler dataset. Genotype and pedigree data included A(BC) crossbreds (n=10,120), and purebred line A (n=8,205), B (n=372), and C (n=720) animals, including all line B maternal grandsires and line C maternal granddams of the A(BC) crossbreds. We used breed fractions estimated from assigning breed-origin-of-alleles (BOA) for the crossbreds as gold standard, but considered the BOA method itself to be too computationally demanding to estimate line composition routinely. Methods considered were: linear regression of crossbred genotypes on mean genotypes of each of the three purebred lines, maximum likelihood estimation using the program ADMIXTURE, and the genomic relationship of crossbred animals with their maternal grandsires and granddams. Estimated breed fractions of the dam lines were rescaled for each individual to sum to 0.5, while in the linear regression method the sire line contribution was fixed to be 0.5. Breed fractions estimated with the linear regression method and ADMIXTURE all had a correlation of 0.92 to the BOA results, while the relationships with maternal grandparents had correlations of 0.86 to 0.89. Estimated breed fractions from all three methods were somewhat biased towards the intermediate value of 0.25, compared to BOA results. The results will be verified using simulated data for which the true breed-of-origin of all crossbred animals is known and BOA results have been obtained previously.

Session 46 Theatre 9

Optimised core subset construction for the APY model

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We are entering the era of mega-scale genomics, which is causing computational issues for standard genomic evaluation models due to their cubic computational complexity. A number of scalable genomic evaluation models have been proposed, like the APY model, where genotyped animals are randomly partitioned into core and noncore subsets. While the APY model is a good approximation of the full standard model, the random partitioning can make results unstable, possibly affecting accuracy or even reranking individuals. In this contribution, we present alternative optimised constructions of the core subset and show how to use them to update the core subset with the arrival of new data. We compared constructions that were either (1) random; (2) optimised based on the value of diagonals of genomic relationship matrix; (3) optimised via random sampling with weights from (2); and (4) optimised using conditional sequential sampling algorithm. We have compared proposed constructions with the GBLUP setting and assessed their effect on accuracy and continuous rank probability scores (CRPS) of predictions. To understand the different constructions we have visualised the core subsets using non-linear dimension reduction technique UMAP – uniform manifold approximation and projection for dimension reduction. While the accuracy and CRPS of the proposed core subset constructions were mainly governed by the size of the core subset, the optimisation reduced variation compared to the standard random sampling construction. In addition to addressing the challenges caused by random sampling, sequential sampling algorithm was equally accurate when applied to the reduced-rank genotype matrix instead of the full one, and was easily expandable with the arrival of new data. Furthermore, there is an indication that the sequential sampling strategy is capturing the fine-scale population structure (e.g. paternal half-sib families in our study) as visualised by UMAP, spreading the core individuals across the given genotype space. We are further exploring the benefits of the proposed core subset constructions in non-homogeneous populations or populations with the unbalanced structure.

Session 46 Theatre 10

Using a priori biological information to evaluate the ability of BayesRC model in genomic prediction

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The increase in technology and decrease in SNP genotyping chip costs have led to identifying the candidate variants of complex traits, analysing genetic architecture, and estimating genomic breeding values. The newest Bayesian genomic evaluation methods have the property to deal with all three aspects. This is the case of the BayesR which models different class of genetic variance for SNPs, where SNP effect is assumed to be distributed as β_i~π_1 (β_i=0)+π_2 N(0,0.0001σ_g^2)+π_3 N(0,0.001σ_g^2)+π_3 N(0,0.01σ_g^2), but it cannot account for the prior biological knowledge. However, BayesRC, an extension of BayesR, accounts for this property and offers to divide SNPs into functional annotation categories as β_i |annotation(i)=a~π_(1,a) N(0,σ_g^2)+π_(2,a) N(0.0001,σ_g^2)+π_(3,a) N(0.001,σ_g^2)+π_(4,a) N(0.01,σ_g^2). Also, a SNP can only belong to one category of annotation. Therefore, we propose to test, on a real dataset of 7,483 Holstein bulls, the ability of a panel of annotation categories to improve the quality of prediction. This study used daughter yield deviation (DYD) phenotypic records for milk, fat and protein yield, clinical mastitis, and somatic cell count. We categorized SNPs into different annotation categories based on their association with a complex trait and verified them by literature and genomic databases (i.e. AnimalQTLdb: https://www.animalgenome.org/cgi-bin/QTLdb/index, and cGTEX: https://cgtex.roslin.ed.ac.uk/). Results indicate that the same level of prediction accuracy is obtained for BayesR and BayesRC. However, BayesRC has a better ability to arrange SNPs in the effect classes because of the prior knowledge. The results also indicate that the top 20 SNPs for milk yield based on their inclusion probability in the fourth effect class ($0.01\sigma^2g$) correspond to chromosomes 1, 5, 6, 11, 14, 15, 20, and 26. These SNPs were then mapped and found to be associated with known milk production QTLs spanning genes like *DGAT1, GHR, ABCG2, PAEP, ABCG2*, etc. BayesRC is quite a promising approach given its prediction accuracy and repeatability.

Session 46 Theatre 11

Extension of Bayesian genomic prediction models for the integration of functional annotations

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The primary objective of genomic prediction is to use genomic variation, usually single nucleotide polymorphisms (SNPs), to predict complex phenotypes. In particular, genomic prediction models are widely used as an evaluation tool for genomic selection in plant and animal breeding. However, the prediction accuracies of many complex quantitative traits still have room for further improvement, due to factors such as marker density, the underlying genetic architecture, and population structure. Alongside this, there is an increasing accumulation of knowledge about the genome, including improved functional annotation and more widely available high-throughput molecular assays (i.e. omics data), providing a bridge from genome variation to phenotypes. Integrating this information into genomic prediction models could potentially lead to improved prediction accuracy and a better understanding of the underlying architecture of complex traits. Bayesian models provide a straightforward way to introduce known functional information into genomic prediction models through the use of prior distributions. In particular, BayesRC divides SNPs into disjoint annotation categories, allowing the proportion of QTLs to vary in each. Although BayesRC has shown promising results, it is limited by the non-overlapping nature of the annotations, which prevents SNPs from belonging to more than one functional list. As the number of potential annotation categories increases, this constraint will become a key limitation. To address this issue, we present two novel extensions of BayesRC to handle potentially overlapping annotations through either a cumulative or stochastic approach. Our approaches allow SNPs with multiple annotations to be respectively upweighted or preferentially assigned to the annotation that best characterizes them. We compare and evaluate these two proposed models with state-of-the-art Bayesian genomic prediction models on simulated and real data, with a simultaneous focus on prediction quality and QTL mapping accuracy.

Session 46 Theatre 12

Long-term changes in the genome due to genomic selection

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Allele frequencies in a population change over time as a result of drift and selection. The introduction of genomic selection has likely accelerated those changes. Our aim was to investigate the changes in allele frequencies of existing causal variants and new mutations under different methods of selection. We simulated a population for 50 generations with selection for a single trait affected by 2,000 segregating loci at the start of selection. Each individual had on average 0.6 new causal mutations, which resulted in a mutational variance of $0.001\sigma_e^2$. The trait was controlled by either only additive effects, or additionally also dominance and epistatic effects. Our results show that the average absolute change in allele frequency across all causal loci with genomic selection was 5% higher than with pedigree selection, 23% higher than with phenotypic selection, and 60% higher than with random selection. The average change in allele frequency was approximately 15% smaller when epistasis was present than when only additive effects were present. Genomic and pedigree selection both resulted in fixing roughly three times more loci than phenotypic selection and 130 times more loci than random selection. When epistasis was present, a lower number of loci became fixed for all selection methods. With genomic and pedigree selection, roughly six times more loci became fixed for the unfavourable allele than with phenotypic selection. The number of newly generated mutations that were segregating in generation 50 was 82% higher with random selection, 61% higher with phenotypic selection, and 20% lower with pedigree selection compared to genomic selection. The average minor allele frequency of segregating mutations followed the opposite trend. Altogether, those results confirm that the introduction of genomic selection accelerated the change in allele frequency, but also shows that genomic selection maintained more mutations than pedigree selection when using own performance in both selection methods.

Session 46 Poster 13

The impact of parent-of-origin effects on the variation in milk production traits

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Genes are genomically imprinted when their expression is limited to one of the two inherited gametes depending on their parental origin. Effects of imprinted genes are part of the wider class of parent-of-origin effects (POEs). POEs were shown to be important for the genetic variation of growth-related traits in agriculturally important species (cattle, pigs) and model organisms (mice). There are, however, only two previous studies on POEs for milk traits in dairy cattle. The objective of this study, therefore, was to fill this gap by estimating genetic parameters and POEs by applying fixed regression test day models to several milk production traits. These models employed a recently developed generalized relationship matrix applied. Thereby, parental transmitting abilities (TA) are predicted for animals without phenotypes and parental gametic effects for animals with phenotypes. This results in a reduced number of equations compared to a classical gametic model. A dataset of 730,437 milk samples were analysed. The traits of interest were milk yield, protein- and fat- yield and percentage, milk urea content and -yield, and somatic cell score. Samples were derived from 88,678 German Holsteins from Mecklenburg Western Pomerania with 298,591 individuals in the pedigree. The analyses resulted in significant variances due to parent of origin effects (POEs) for all eight milk production traits. The ratio between imprinting variance and additive genetic variance ranged between 5 and 15%. This suggests a role of POEs for milk production traits. Further investigations on the importance of POEs, for the genetic variability and possible consequences for breeding, are needed.

Session 46 Poster 14

Links between homozygosity and autozygosity in the major histocompatibility complex in dogs

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The major histocompatibility complex in *Canis lupus familiaris* (dog) harbours a crucial subset of immunity-related genes. The high heterozygosity and the increased diversity have been demonstrated as advantageous in outbred populations. The strong artificial selection and small population size in dogs cause an increase in their homozygosity levels, which might unfavourably affect the diversity parameters of the MHC region. This study was to explore part of the dog genome covered by MHC class II genes DLA-DRB1, DLA-DQA1, and DLA-DQB1. The location of these genes is between 2.15 and 2.31 Mb of chromosome 12, according to the CanFam3.1 assembly. The homozygosity status was determined with specialized genetic tests in 120 Leonberger, 33 Boxer, 42 Standard Poodle, and 51 Rhodesian Ridgeback dogs. In addition, all dogs were genotyped with Illumina CanineHD BeadChip (CanFam3.0 assembly) as an additional method to determine their homozygosity status. The cgaTOH software was used to detect ROH regions with a minimal length of 0.5 Mb. In particular, we were interested if the region containing the DLA genes is part of a run of homozygosity (ROH). The homozygosity status of the within breed DLA tests was on average between 23 and 65%, with the highest in the Standard Poodle population. The strength of the correlation between the DLA test and ROH homozygosity status influenced by breed identity. The highest correlation was found in the Boxer population with average r=0.94 for the three DLA genes. The lowest was the correlation in the Standard Poodle population with average r=0.52. When comparing correlations of DLA and ROH-based homozygosity levels the DLA-DRB1 seemed to be more consistent with r=0.75 on average, compared to the r=0.60 for the other two genes. The discrepancies causing a non-uniform result for both methods were the coming from homozygous results for DLA test in dogs that were not in ROH for this region. These results indicate that the DLA homozygosity status for certain animals might be determined by even shorter ROH segments (below 0.5 Mb) or results of other events different from autozygosity.

Commercial crossbred data could be used for selection in pure lines with the help of DNA pooling

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Large quantities of health data is collected in slaughterhouses on commercial broilers. This data is valuable for breeding programs as these animals are raised with challenges not faced by the pure lines. It is not viable to individually identify or genotype large groups of commercial animals. Therefore we explored DNA pooling as a solution. We extracted DNA from chicken feet and amplified it to a known concentration, mixed DNA samples of multiple individuals with a similar phenotype, and genotyped these pools. The pools showed to be good representations of the underlying individuals. Our aim was to determine if pooled DNA can be used to estimate SNP effects for selection in pure lines. We simulated a typical 4-way cross broiler population, where each line had divergent selection for footpad dermatitis, and the final cross consisted of 50,000 commercial broilers. Here we present the results of two pooling strategies and compare them to the gold standard of 50,000 individually genotyped crossbreds. For pooling, 1000 individuals were randomly selected from each of the top and bottom 20% performing animals. We created either 200 low and 200 high pools with five individuals each, or 100 low and 100 high pools with 10 individuals each. The genotypes for the pools were estimated as allele frequencies and fitted as imputed genotypes. A SNPBLUP model was used to predict genomic breeding values (GBV) of the sires in each pure line and of the 2,000 underlying individuals used in the pools. For the 2,000 crossbreds, the correlation between GBVs estimated with 50,000 individuals or either pooling strategy were high (0.92-0.93). For the sire GBVs of crossbred performance, estimated with 50,000 genotypes or the pooling strategy of five, the correlations were lower and ranged between 0.12-0.42 (depending on line). Further validation with real data is ongoing, but these results are encouraging as they suggest DNA pooling is a viable method to include commercial traits that would otherwise have no means of selection.

Decay of accuracy of genomic predictions over time in broilers

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The objective of this research was to investigate trends for accuracy of genomic predictions over time in a broiler population accumulating phenotypes, genotypes, and pedigrees. Additionally, we tested if data from distant generations are useful in maintaining the accuracy of genomic predictions in selection candidates. The data contained 820k phenotypes for a growth trait (GROW), 200k for two feed efficiency traits (FE1 and FE2), and 42k for a dissection trait (DT). The pedigree included 1.2M animals across 7 years, of which over 100k from the last 4 years were genotyped. Accuracy was calculated by the linear regression method. Before genotypes became available for training populations, accuracy was nearly stable even with the accumulation of phenotypes and pedigrees. The inclusion of genotypes in the training populations led to an increase in accuracy of 56, 77, 39, and 111% for GROW, FE1, FE2, and DT, respectively. With genomic information, the accuracies increased every year except the last one, when they declined for GROW and FE2. The decay of accuracy over time was evaluated in 3 generations after the training populations, i.e. progeny, grand-progeny, and great-grand-progeny. Without genotypes, the average decline in accuracy across traits was 41% from the first to the second generation, and 19% from the second to the third generation. When genotypes were available, the average decline across traits was 14% from the first to the second generation, and it was 2% from the second to the third generation. The accuracies in the last 3 generations were the same when the training population included 5 or 2 years of data, and a marginal decrease was observed when the training population included only 1 year of data. In this broiler population, training sets including genomic information provided an increased accuracy and persistence of genomic predictions compared to training sets without genomic data. The two most recent years of data were enough to maintain the accuracy of predictions in selection candidates.

Impact of integrating genomic data into fertility genetic evaluation in the Florida goat breed

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Genomic evaluation is a method for improving the accuracy of breeding value estimation. In genomic prediction, the single-step genomic best linear unbiased prediction (ssGBLUP) method allows the inclusion of information from genotyped and ungenotyped relatives in the analysis, along with phenotypes. The purpose of this study was to compare estimates of genetic parameters and reliability of breeding values for female fertility in Florida goats between classical approach with pedigree-based REML (Restricted Maximum Likelihood) and ssGREML. Female fertility was defined as the reproductive efficiency (RE) calculated as the deviation between optimal and real parity number of age of each goat. A total of 17,642 reproductive records of the Florida goat breed and 26,170 animals in the pedigree were included in the analyses. With the Illumina 55 K Goat Bead-Chip, a total of 621 animals were genotyped (53,347 SNP). Genetic parameters, genetic (EBVs), and genomic (GEBVs) breeding values were estimated using, respectively, pedigree and genomic relationship matrices. Analyses were performed using the AIREMLF90 software of BLUPF90 family programs. Estimates of variance components and genetic parameters were equivalent in both strategies and heritability of RE was 0.18±0.015 indicating that this trait is heritable and can be improved by selection. Estimated EBVs and GEBVs were similar too, and no significant gains in reliabilities for genomic over conventional evaluations have been observed. The results showed that RE can be used as a selection criterion to improve female fertility. Nevertheless, further work on optimizing the genomic reference population will be required for the genomic selection for reproductive efficiency in this breed.

Classifying aneuploidy in genotype intensity data using deep learning

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Aneuploidy is the loss or gain of one or more chromosomes. Although a rare phenomenon in living diploid animals, cases have been reported. Such cases should be identified to avoid breeding and to be able to study the potential causes and heritable factors of these anomalies. In livestock, breeding individuals are genotyped on a routine bases with SNP arrays. In addition to genotypes, these SNP arrays provide genotype intensity information, which can be exploited for the detection of aneuploidy. This allows for the routine screening of large numbers of animals. Instead of using the intensity values, we tested the use of images of the X and Y intensity data, plotted on the X and Y axis respectively. Such images are often used to confirm cases of aneuploidy. Based on the X and Y intensity plots, chromosomes can be classified into three classes: normal (3 genotype clusters (AA, AB, BB)), loss of a chromosome (monosomy; 2 genotype clusters (A, B)), or gain of a chromosome (trisomy; 4 genotype clusters (AAA, AAB, ABB, BBB)). We used a deep-learning framework using convolutional neural networks with 5 layers to predict aneuploidy from images of XY intensity plots on the chromosome level. By looking at chromosome level images the classification model is generic across species. The model was trained on a multispecies dataset consisting of 3,321 diploid chromosomes, 1,759 monosomic chromosomes, and 164 trisomic chromosomes annotated by experienced researchers. On an unseen test dataset this model achieved a specificity of 0.997, 1, 0.995 and a sensitivity of 1, 1, 0.91 for normal, loss and gain of chromosome respectively. This indicates that the model has some trouble discriminating between trisomy and normal, which are also difficult to discriminate for human experts. The model will be further tested on genotype intensity data from routine genotyping at breeding companies.

EuroGenomics SNP MACE – projecting national full reference SNP estimates to a common marker set

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EuroGenomics countries are in the process of developing a new multitrait across country genomic evaluation method for the Holstein breed. The method will use information, not raw data, from full reference populations of EuroGenomics members. Similar to the work of Interbull for the Brown Swiss breed, the method is a meta-analysis combining information from national genomic evaluations in the form of an MME left-hand side (LHS) matrix along with the corresponding right-hand side (RHS). The SNP solutions with across country information are then obtained combining the national LHS and RHS in a SNP MACE framework. One of the largest challenges in implementing the method concerns the handling of different SNP sets and different evaluation methods used by the participants. Our approach includes establishing a common EuroGenomics marker set satisfying the needs of all participating countries, imputing the full national genomic data to the common EuroGenomics marker set and projecting the national SNP solutions onto the common marker set space. The feasibility and performance of the method is tested in two phases. First the shared EuroGenomics bull data, comprising about 46,000 marker genotypes and 35,000 observations, is used to verify whether the method produces approximately the same results as when using raw geno- and phenotype data. Then the method compared with existing evaluations for national full reference data for protein yield, somatic cell score and female fertility.

How can participatory approaches help stakeholders to progress in monogastric productions?

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Livestock production is facing many issues, with an increasing pressure from consumers and citizens. To help the livestock sector progress, research is now switching from 'research for' to 'research with' thanks to participatory research involving various stakeholders in the research process. We illustrate how participatory approaches can help stakeholders to progress into the transition of the monogastric livestock sector. In the poultry sector, the OVALI® method was developed to assess sustainability of chicken supply chains. The conceptual framework was co-constructed and validated by a participatory group composed of farmers, private operators from upstream to downstream, researchers, and NGOs. This resulted in a shared and consensual vision of sustainability, and the method can be used to evaluate existing supply chains or *ex-ante* scenarios. In the rabbit sector, the Living Lab project aims to evaluate rabbit husbandry/housing strategies to improve animal welfare and health, meat price, and labour conditions of farmers. Various stakeholders (upstream and downstream of the sector, NGOs, consumers) discussed on targeted behaviours and possible solutions (housing size and enrichment, floor type, group size, etc. The most promising were selected and tested in experimental then field conditions. For instance, housing enrichments allow rabbits to express specific behaviours (gnawing, hiding, jumping, isolating) but certain practices can impair welfare (e.g. females grouping lead to severe injuries). Finally, the EBENE® method was co-constructed with the French poultry/rabbit sectors with the participation of researchers and animal welfare NGOs to provide a practical tool to assess welfare in farms. The participatory approach ensured to select relevant criteria and indicators pertinent and easy to measure. This tool is now available for farmers in a free application. These three examples illustrate the potential of participatory approaches to share knowledge among stakeholders and provide them with tools to progress into the livestock transition. They also strengthen the science-society link to produce actionable knowledge.

Session 47 Theatre 2

Consumer perceptions of a multi-level animal welfare label in Germany

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The German government announced to implement a national animal welfare label during the current legislative period. It is planned as an optional label on end-consumer products with three levels. Thereby, the requirements are lowest for level one and highest for level three. Market introduction is planned for pig production but other species and are planned to follow. All production levels, from piglet production to slaughter will be included in the requirements of the label. The announcement of implementing the state-run national label has been largely debated and criticized from different stakeholders such as NGO's or the Green Party. Level one criteria of the label are accused as being not strict enough and not increasing animal welfare levels on farm. It is claimed that consumers are fooled through the low standards of the label. We took up this criticism and analysed consumer perceptions of the label. We focused on level one requirements in order to see how consumers perceive the criteria and how trustworthy they perceive the label. In April 2020, we questioned 529 German consumers online using a standardized questionnaire. Recruitment was facilitated by an online access panel provider. The questionnaire included questions about meat consumption, attitudes towards farm animal welfare, and involvement in animal production. Respondents received an information treatment about level one requirements followed by questions about perceived trustworthiness and implications for animal welfare levels on farm. We found that only 23.8% of respondents perceived the level one-criteria as sufficient but nearly half of the sample perceived the label as such as trustworthy. Using principal component analyses and multiple linear regression, we analysed what influences the perceived trustworthiness of the label. Comprehension of the comparably low level one standards as a motivator for farmers to participate has shown the largest influence on overall trustworthiness (standardized $\beta=0.44$, $P\leq 0.05$), whereas attitudes towards animal welfare in farming revealed as not being a predictor (standardized $\beta=0.05$, $P\geq 0.05$). Mistrust on the aims of the label is hindering trustworthiness (standardized $\beta=-0.22$, $P\leq 0.05$).

Session 47 Theatre 3

Serious game: a cutting-edge tool for stakeholders to redesign livestock systems towards agroecology

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The agroecological transition of livestock farming systems is a complex process with undefined paths. Current changes in agriculture need to improve the adaptive capacities of farms under uncertainty. The participation of farmers and other stakeholders might be a requirement for designing relevant systems to meet these challenges. Recently, scientists have suggested improving agricultural sustainability by using together with farmers and other stakeholders, serious games as powerful tool to encourage the redesign of livestock systems while promoting collective intelligence. We illustrate this through different games used in different case studies implemented at different scales. In the first type, 'Lauracle', a card game permits farmers and fodder management advisors to identify new levers for adapting the livestock farming system face to climate change. The second type, as board games, crop and livestock farmers co-design crop-livestock integration. With 'ICLS challenge' players renew at farm level land-use, budget allocation, forage balance along with the planning of the crop-rotations in space and time. With 'Dynamix', beyond the farm level, players create collective maps of their region to represent the flows of cereals, fodder and manure exchanged and to design logistical means. The third type using 'Lego® serious game' to rethink agricultural management processes, involving players from the agricultural and other sectors, at different levels (animals: genetics, farms: feed self-sufficiency, territory: manure management). In all these case studies, we highlight that manipulating game pieces and describing their uses to the other stakeholders engaged them in action with other players. Serious games functioned as boundary objects, known to promote fruitful and realistic discussions between participants. Serious games are a learning experience promising for redesigning livestock systems toward agroecology as they allow designing disruptive scenarios while being easily out-scalable and pedagogical tools.

Identifying levers to transition to prudent antimicrobial use through multi-stakeholder Living Labs

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Living Labs (LLs) are built on multi-stakeholder participation with a negotiated and mutually agreed aim of reaching a common goal. LLs enable the development and testing of solutions that are: (1) adapted to the local ecological, cultural, economic and sociological factors connected to the AMU; (2) accepted by the involved actors and end-users; and (3) tailored to each given context. LLs constitute a vital and important part of the ROADMAP project with the overarching aim of fostering prudent use of antimicrobials in animal farming and reduce the risk of antimicrobial resistance (AMR). 12 LLs in 7 countries involve multiple stakeholders and actors, who play a direct or indirect role in antimicrobial use (AMU) in the agricultural sector. Involved stakeholders contribute to the process with their expertise, experience, creativity and knowledge. A common, but adaptive, framework for LLs has been developed, including common indicators for assessment of LL-processes. Experience between LL-coordinators is exchanged through a platform and meetings. Each LL is unique and choose their focus given the context. The ROADMAP-LLs operate at different levels, either in parallel or in consecutive steps, to address crosscutting issues related to AMR and fostering transitions towards prudent AMU. Innovations and implementations are on technical, social or institutional / structural levels. Research activities explore and analyse how LLs address levers for intervening in animal sectors to change structures and practices around AMU, and transform conflicts of interest to commonalities of interest within multi-stakeholder LLs. This presentation is about findings across LLs regarding enabling environments, LL processes and levers, which facilitate the LLs to play potential important roles in transition towards prudent use of antimicrobials. We focus on critical points of change and action at four levels: (1) regulatory / governance level; (2) industry (agriculture or food chain); (3) agricultural local actor level; and (4) societal level.

Green assets of equines in Europe: possible overview through the creation of a data collection tool

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An overview of green assets of equine sectors in Europe is carried out by the French Institute for Horse and Riding, the French National Research Institute for Agriculture, Food and Environment, and the European Horse Network. Given the absence of central database on this topic and regarding the Green Deal challenges, the aim is to gather and share knowledge and data on environmental impacts of equines at the European level. Through an initial bibliographical review, five major green assets have been identified: land use, grazing, domestic biodiversity, tourism and equine work. A data collection tool was created to gather information on these five themes. It consists of 58 indicators (qualitative and quantitative) aimed to be filled in by state agencies or federations. The tool was improved in 2020 after initial tests (France FR, Sweden SW, Poland PL, Croatia CR, and Belgium BE) and then presented to five new countries: the Netherlands, Spain, Ireland, Slovenia SL and Germany GE (some of which are still under study). Main results show that: 1-Most countries report the presence of equines in suburban areas (in SW, 75% of equines are in these areas). In all the countries surveyed, sensitive areas are grazed by semi-wild local equine breeds. 2-It has been generally difficult for countries to inform the area valued by equines, but some gave estimates: 329,600 ha in SW and more than 750,000 ha in GER. 3-In majority, FR, BE, GE and SW have saddle equines intended for sport or leisure, whereas PL, CR and SL have draft equines for meat market. 4-Equestrian tourism is a practice whose dynamism is stable in PL and is increasing in FR, GE, and SW. 5-The herds of working equines are low in all countries but FR, GE and SW show an increasing interest in these activities, unlike PL, CR and SL. This tool provides first insights for a global view of green assets of European equine sectors in their different contexts, allows further exchanges of knowledge, creates a network between various European bodies, and serves as a support for lobbying activities in order to better involve equines into ecological transition of agriculture.

Exploration of health practices in dairy farms: links with the farming system and work organization

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One pillar to reach the agroecological transition refers to the 'integrated management of animal health', an approach through more preventive practices. Innovations exist to improve this management: new technologies (robotics, sensors), the use of alternative medicine (homeopathic, essential oils) and the reduction of the farming system intensity. Our goal is to explore the links between the combination of these three types of health management practices, Livestock Farming System (LFS) consistency and various parameters of work. We surveyed nine dairy farms in Western France selected for their diversity: milk production, workcell size, number of cows/labour unit. Four farms are using a milking robot to decrease their routine work duration but two of them spend specific time to analyse and detect health issues at an early stage. Six farms use sensors to improve their results and to increase their free time (automatic heifers monitoring). The ones who do not use sensors (n=3) are smaller farms and have less cows per labour unit. They find sensors useless and in opposition with the breeders' role of animal caretaker. The use of alternative medicine is frequent in our sample: Six followed trainings on the use of alternative medicine but four use it as a preventive medicine and three as a curative one. There is no evident link with the cows productivity or the number of cows per labour unit. It appears more as a personal approach, notably from spouses who have often the role of animal nurse in the workforce. The ones using it as a curative medicine tested the products step by step to be confident: they followed trainings years ago. The others followed their training only a few months ago (n=3) and still test it on their herd only in a preventive way. Several work dimensions are evoked in this exploration: workload and robotization, professional identity (meaning of work, digitalization of the data-decision system, gender), labour organization (division between workers, workforce communication). These first results lead us to deepen our understanding on the interactions between the LFS components (health management, work, LFS operation and performances).

Carbon neutral Brazilian beef: from the scientific concept to the world's market

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Agriculture copes with increasing demand for food, timber, fibres and bioenergy while reducing emissions from the production process. This scenario offers additional opportunity for the Brazilian beef chain. To add value on exports, some aspects must be addressed, like animal welfare, water and soil conservation, while mitigating greenhouse gases emissions (GHG). Today, Brazil is already able to commercially supply such services through cattle farming, neutralizing or minimizing GHG emissions by MRV certified cattle systems. Today, discussions about innovation are on top of development agendas. However, innovation relates not only to technology, but also to trade arrangements within the value chain. In this context, supported by the Brazilian Ministry of Agriculture and Food Supply through the Low Carbon Agriculture Plan 'Plano ABC', the Brazilian Agricultural Research Corporation-EMBRAPA has developed trademarks based MRV certification protocols for beef production. They are able to fully neutralize emissions from cattle raised under silvopastoral system or to substantially improve carbon balance through improved pastures. Parameters and indexes adopted for the protocol are rigorously science based. These trademarks, already on the market, are the Carbon Neutral Brazilian Beef® and the Low Carbon Brazilian Beef®. As a result, there is already a formal partnership with a global player meat processor, who is trading beef under these labels. Another important feature of these labels is that they are suitable for ranches of all sizes, from smallholders to large operations, all contributing to tackle global warming. It was a long path to reach this stage of having a scientific concept put into market, but lessons learned are supporting faster development of the same concept for other major produces from Brazil, like soybeans, coffee and cocoa. With the launching of the next phase of the ABC Plan, there is a national trend for upgrading in the next 10 years the national agriculture, increasing food production while providing a major environmental service to the world.

Ecological transition in dairy territories: the role of collective brands sustainability standards

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Face to the end of quota systems and new societal demands dairy farmers started to organize collectively at a territorial level to creating new brands. These emerging brands promotes their 'ecological territorial' image and seems to push farmers to adopt sustainable standards (SS). While there is abundant literature about SS in globalized agri-food chains, little attention has been paid to these emerging local farmers' initiatives. The objective of this study is, therefore, to analyse the main SS adopted, and how they implemented and ensure compliance. We first identified 5 brands owned by collectives of dairy cow breeders in Occitanie region (France). Second, we collected data through semi-directive interviews with brand managers/coordinators and 15 breeders between April and June 2020. Our work has produced several insights. We shed light that the brands adopt different SS with a variability of formalization and practices requirement. Some standards are predefined by third-party organizations (e.g. organic agriculture). Others are specific to each breeders' collective. Some brands also combine both types of standards. The choice and implementation of the SS itself is not the result of a strictly internal process. It is based on interactions with various actors of the agricultural world such as professional organizations. Concerning the control systems, we note that they can be internal (self-declarations in the case of smaller groups of farmers) or external (third party certification). Trust-based systems involve the issuing of oral and written warnings in the case of non-compliance. Repeated non-compliance can lead to suspension or exclusion. Finally, the ecological transition seems to be operating in dairy territories trough the adoption of sustainable standards pushed by farmer's new collective brands. In this respect, these brands follow the general tendency of the agri-food sector, a sector characterized by the emergence of alternative standards that bring to the fore environmental virtues of certain production systems but also to certain territories.

The role of certification in the transition towards a more sustainable livestock sector

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The production and consumption of food is linked to many environmental, economic and social challenges of our society today. Sustainability certification is widely seen as a strategy to address these challenges and encourage sustainable food consumption and production. Producers can potentially receive an added value for their certified products, it can help consumers to select sustainable produce. In recent years, the number of certification schemes in crop, livestock and aquaculture production has expanded rapidly. Insight in the effectiveness and validity of certification schemes is, however, limited. This research compared approaches used in global sustainability certification schemes in crop production and Dutch certification in egg and dairy production. The analysis shows the myriad of approaches (e.g. for auditing, scoring and reporting), sustainability indicators and organisation structures underlying sustainability certification. Each certification scheme presents its own perspective on sustainability and selects different sets of sustainability issues and indicators. This raises questions with regard to the credibility of certification systems. Moreover, the transparency of certification schemes, the accessibility for farmers to participate and actual sustainability improvement on farm, are issues that require attention. The study shows the influence that sustainability certification has on food consumption and production practices. Sustainability certification inherently requires dealing with trade-offs between sustainability issues. How these trade-offs are addressed and prioritizations between sustainability issues are set by private certification organisations can have a large impact on food production. This raises the question whether a more hybrid form of governance combining public and private institutions is needed to support the transition towards a more sustainable livestock sector.

Income over service life cost – a concept to calculate cost-benefit relationships of dairy cows

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The single cow is both the basic biologic and economic unit of a dairy farm. Because her lifetime-performance depends on a variety of largely unpredictable bio-economic interactions, we developed a cow-individual full cost concept on the base of a retrospective approach to identify the main levers behind the economic result of individual cows. To evaluate the new methodological framework, lifetime data of 4,962 culled cows from 32 German dairy farms were combined with farm-specific accounting data to estimate individual income over service life cost (iIOLC). To achieve an individual attribution of costs at animal level, (1) farm-specific costs were calculated 'per cow, per day', (2) farm-specific feed costs were adapted to estimated individual dry matter intake during the productive life and (3) farm-specific rearing costs were adapted to individual age at first calving (AFC). Farm-specific univariate linear regression models and two-sided tests for equality between high- and low-profit cows (HP and LP) grouped according to the farm-specific median iIOLC were performed to evaluate the effects of cow variables. Variables include lactation number (iLact), days in milk at death (iDIM), average daily milk yield during the last lactation (iMYLL), AFC, type of death as well as farm-specific costs and revenues. Median (IQR) iIOLC of culled cows was -479 € (1,047 €). Across farms, only 42.9% of the cows were culled in 4th or higher lactation, while farm-specific maximum iIOLC was not reached before 5th lactation. Farm-specific ranking of effect sizes (ωp^2) showed that iLact had the strongest impact (0.29+-0.19) followed by iMYLL (0.16+-0.07) and iDIM (0.08+. 0.06). iLact, iMYLL and iDIM were significantly greater in HP cows compared to LP cows for 19, 19 and 14 herds, respectively. The share of cows that died on farm was significantly lower in HP cows for 11 herds. Based on our data, the duration of the productive life as well as the timepoint and circumstances of culling of dairy cows are major factors of individual economic profitability.

Animal farming acceptability: corporate sustainability projects must embrace a feed-land-fork vision

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The image of animal farming is being worsened at high speed. Moreover, there is a need to improve the sustainability of the sector. The way to play a sound role in both aspects (public perception of animal farming and increase the sustainability of the sector) will be only achieved successfully by following an integral approach of the whole value chain. This means that, although there is a growing interest and publication of sustainability/CSR reports by Feed and Food companies, it is needed to integrate in CSR projects and reports information about different practices related carried out by the different links of the agri-value chain. As a consequence, such projects will have a greater influence on sustainability and their report will be more informative and compelling for citizens, then increasing animal farming acceptance. The objectives of the present work are: (1) to review the image of animal farming, (2) to analyse and discuss Corporate Sustainability reports related to the feed industry, and (3) to give examples on how to improve such reports with the integrative approach abovementioned in order to increase animal farming acceptability/image.

Session 47 Poster 12

Ensiling agro-industrial byproducts to use as feedstuffs for ruminants

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Agro-industrial by-products are a good source of nutrients and bioactive compounds but perish easily because of their high moisture content. The conservation of these products as silage in mixtures is an option to overcome this limitation. The aim of this study was to preserve tomato pomace, carrot, sweet potato and common potato as silage in mixtures nutritionally balanced. Tomato has 45% dry matter (DM), moderate organic matter digestibility (OMD 54%), high CP content (20% DM) and 58% DM of neutral detergent fibre (NDF). Carrots, sweet potatoes and common potatoes have 10, 17 e 22% of DM, respectively, low CP (6% in DM) and NDF (13 -18% DM), high sugar content (carrots 39% and sweet potato 23% in DM) and starch (sweet potato 39% and potato 34% in DM). The OMD is high (75-90%). Three mixtures with 35% of tomato pomace and 30% of either carrot, sweet potato or common potato, were ensiled mixed with 20% wheat bran and 15% hay, in plastic bags. The carrot, sweet potato and common potato were previously crushed. Wheat bran and hay were used as absorbents to increase DM of mixtures. The silages obtained were well-preserved, with pH 4, high lactic acid content averaging 113 g/kg DM, low levels of NH_3-N and soluble N (averaging 6 and 43% in total N, respectively). The CP was higher in sweet potato silage (16% DM) than in carrot and potato silages (14% DM). Organic Matter Digestibility was similar among silages averaging 55%. By-products In mixtures can be ensiled producing well preserved silages, with high potential to use as feedstuffs for ruminants. This work is funded by PRD2020 through the FEADER, project SubProMais (PDR2020-101-030988) and by National Funds through FCT – Foundation for Science and Technology, projects UIDB/05183/2020 (MED) and UIDP/CVT/00276/2020 (CIISA).

Session 47 Poster 13

Process-based 'dynamic energy budget' metabolic theory for managing livestock growth and emissions

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Livestock production is one of the main contributors to global greenhouse gas (GHG) emissions. The tools provided by the Intergovernmental Panel on Climate Change to estimate GHG emissions (Tier 1 and Tier 2) cannot be used as a tool for advising farmers or national policy on how to minimize GHG emissions because these estimates are not explicitly related to any metabolic properties. For that, we need a metabolic model that obeys mass and energy balances and that models the trade-offs between different allocations of energy in the organism, throughout the life-cycle, as a function of environmental variables such as temperature and the amount and type of food. The dynamic energy budget (DEB) Theory ensures mass conservation and models the transformations of state variable and product formation, allowing for explicit GHG emissions estimates. Therefore, we used this theory to build models for different bovine breeds, including autochtonous breeds from Portugal, such as Alentejana, and more international breeds such as Angus. The goodness-of-fit of DEB models to data is good for all breeds. Differences in DEB breed specific parameters, such as the maximum reserve density and the maximum specific assimilation rate, explain differences in GHG emissions, ultimate lengths and weights and von Bertalanffy growth rates. Accumulated methane and nitrogen production ranges between 0.2 and 2, and 0.1 and 0.7 kg per kg of body weight, respectively. In terms of accumulated direct GHG emissions, there is a strong dependence on breed and age.

Session 47 Poster 14

Biogas production from Azorean animal manure

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Energy consumption has been increasing significantly in society, which, associated with the present efforts for reducing the greenhouse gases emission and guarantee a lower fossil fuels dependency, led to an increase of the renewable energy demand. Although this technology is not relevant in Portugal, several studies are being carried out concerning its cost effectiveness and associated environmental issues, hopping to reduce pollution, and contributing to the energy problem. Manure can be an alternative energy source for livestock farmers. An anaerobic digester can partially convert manure to energy in the form of biogas which contains methane. Anaerobic digestion consists of a process in which organic waste is degraded, by the action of anaerobic microorganisms, in the total absence of oxygen. The process occurs naturally when the surrounding conditions provide it. The choice of studying biogas production on dairy farms was because manure is easily collected on dairy farms where cows are routinely confined. Thus, an assessment was made, to know how many farms existed in Azores archipelago, who would have the necessary qualities to data collection. Despite the existence of 250,000 animals in Azores, we only collected data on what could be likely to be used in biogas production. Therefore, preliminary results indicate us a total manure production 86.477 tons per year. This study aimed to understand the implementation of a circular economic business where animal manure can be used to produce biogas and an alternative fertilizer in a regional network of manure suppliers and biogas producers.

Session 47 Poster 15

Farming methods information as food claim to counteract the negative image of foods of animal origin

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The public image of animal farming is being damaged. This constitutes a risk for the sustainability of the agri-food system and the survival of animal farming. Livestock farmers must take action to counteract consumers' perceptions by means of effective communication of the positive effects of their farming methods. The present work is aimed at reviewing consumers preferences towards food attributes and recommending effective claims about livestock farming methods in order to be helpful to livestock farmers. Methodology in based on literature review, preliminary results of author's studies, and author experience in both Academy and private sector. Consumers associate the concept of sustainability with environment and barely with the socio-economic dimension (e.g. health). 'Organic' is perceived as environmentally sustainable. 'Carbon footprint' is interesting scientifically, however, many consumers do not know this logo and do not have value references for this parameter and different products so that benchmarking during purchasing is not possible. As consequence, the usefulness of this claim is low. Moreover, many customers do not know the logo. 'Food miles', 'local' or 'flags' are better understood by consumers and thus will have a higher impact commercially. Moreover, health-related information is key for consumer's attitudes towards food, so that specific claims in this sense such as 'antibiotic-free', are very important, although they are technically redundant or unnecessary (e.g. 'gluten-free', 'lactose free', 'zero-pesticides'). Regarding production methods, 'traditional', 'manufactured since …' and claims conveying uniqueness, authenticity and/or culture are also valued by consumers. In this sense, claims about the production system such as the feed management ('feed on acorns') or 'free-range' have utility for consumers. The adequate combination of the above mentioned claims to every single segment of consumers and market niche will help farmers to (1) improve consumer's knowledge about the livestock sector sustainability and (2) adapt to new market trends, and therefore, increase the sustainability of their operations (especially, in the time scale).

Black soldier fly larvae: standardized feed protocol development and evaluation

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There is a growing interest in the capacity of black soldier fly larvae (*Hermetia illucens*; BSFL) to convert low-value organic resources into high-value products. This resulted in a significant increase of publications with conversion data for various organic resources, but considerable variations in methodologies hinder advancements in BSFL research and use of results in practice. The need for a standard methodology to determine conversion efficiencies was discussed at the EAAP conference 2019, which led to an international coalition of BSFL researchers consisting of members of the EAAP working group on standardisation, H2020 SUSINCHAIN and independent researchers to develop a standardized protocol and evaluate it via a ring test. The project entailed three phases: (1) setting up a protocol by combining existing ones and expertise from the researchers; (2) a ring test yielding insights into the protocol quality and variation among institutes; (3) obtaining insights to optimise the standardized protocol. The protocol described procedures starting with eggs until the harvest, how to sample larvae and a standardised list of parameters to log during the experiment. Two diets (chicken feed, 'Gainesville house fly diet') both made as a single batch were distributed to all participants to exclude feed variation. Four institutes also evaluated a Gainesville diet based on local-sourced ingredients. Each diet was evaluated with 6 replicates. Variations within and between institutes were quantified. Results of the ring test are shared and discussed.

Session 48 Theatre 2

Standardisation of yellow mealworm feed experiments: first tests and optimizing the protocol

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Due to the upcoming insect sector, mealworms gained increased interest of researchers. This has led to numerous studies and publications on mealworm production. However, differences in the experimental designs, rearing techniques and conditions hamper comparison among them. Furthermore, clear instructions on performing mealworm feed experiments are lacking often resulting in the absence of important information within the publications. To ensure comparability of the results, a clear rearing protocol for conducting feed experiments which takes into account practical terms applicable in different organizations is needed. Within the ValuSect project (Interreg NWE), in collaboration with Susinchain, an initial standard protocol for feed experiments with yellow mealworms (*Tenebrio molitor*) was constructed. This protocol will be tested on three different sites (Thomas More University of Applied Sciences, Inagro and Aberystwyth University) in order to be able to assess the variables and to further optimize the protocol. The rearing conditions, the control diet and preparation of the feed, the rearing densities, the minimum scale, the performance of different actions during the experiment and the measurements to be done are taken into account. The experiment on the standardisation of the protocol will be performed using the control diet, which was send to the other partners. Results include the feed conversion ratio, larval growth, mortality and chemical composition. After screening these results the protocol will be further optimized in a larger group consisting of members of the ValuSect project, Susinchain, the EAAP working group on standardisation and independent researchers. Finally a full ring test will be performed to assess the final protocol.

Periconceptional diet and epigenetic programming of offspring health and productivity

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The concept that offspring development and health is influenced by maternal nutrition during pregnancy is firmly established in a number of species including farm animals. Although nutrient requirements of the conceptus increase during pregnancy, it is the rapidly proliferating pluripotent cells of the pre-elongation embryo that are most sensitive to maternal metabolic state, as sweeping epigenetic changes occur during this early stage of development. One-carbon (1C) metabolism is highly active in embryonic cells and critical in this regard. It consists of an integrated series of pathways involved in the competing synthesis and methylation of DNA, biogenic amines, phospholipids and proteins. Disturbances can have a profound effect on foetal and offspring development. In sheep, physiological reductions in the dietary supply of cobalamin and methionine around the time of conception can epigenetically-alter DNA in progeny leading to sex-biased insulin resistant and hypertensive offspring. Epigenetically modified genes involved in insulin signalling and endoplasmic reticulum stress have since been identified. Confirmative parallel studies in rats fed folate, choline and methionine deficient diets reported similar sex-biased effects in offspring. Specific 1C metabolism enzymes (e.g. BHMT) are not expressed in somatic lineages of the ovary, oocyte and preimplantation embryo; and differences in expression of other 1C enzymes exist between species. This sensitized bovine embryos to the provision of 1C substrates/cofactors such as methionine, leading to genome-wide alterations in DNA methylation in >1,600 genes, including a cohort of imprinted genes linked to the Large Offspring Syndrome in farm animals. Consequences for polygenic traits of commercial importance such as milk yield, however, remain to be fully determined. Whilst perhaps subtle, effects are likely to accumulate across generations; especially following continued exposure. In this regard, the trend towards gamete collection from prepubertal donors merits consideration. Research supported by BBSRC (BB/K017810/1 and BB/R007985/1).

Effects of cow milk protein genotypes on health, growth and β-casomorphin-7 level in the offspring

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Bovine milk generally contains two types of the milk protein β-casein (β-CN), termed A1 and A2. Enzymatic digestion of the A1 variant yields the opioid peptide β-casomorphin-7 (β-CM7), which is suggested to adversely affect human and animal health (i.e. gastrointestinal motility). In the case of the A2 variant, the cleavage of β-CM7 does not occur or occurs at a very low rate. Based on these prior findings, the aim of the study was to compare the effects of cow milk containing either homozygote A1 or A2 β-CN on health and growth parameters of dairy calves. Forty-seven neonatal calves (24 females, 23 males) of the breeds German Holstein (GH, n=9), German Simmental (GS, n=33) and their crosses (GH × GS, n=5) were used in a 21-day feeding study. Faecal score (FS), respiratory frequency (RF), and rectal body temperature (BT) were recorded daily, whereas body weight was measured at birth and at day 21 for the calculation of the average daily weight gain (ADG). Blood was collected from calves three times during the experimental period and, for the first time, the respective plasma samples were analysed for intact β-CM7. Consumption of pure A2-milk led to a lower daily milk intake (dMI) ($P<0.05$). Faecal consistency was softer for calves fed A2-milk ($P<0.05$). While 44% of A2-calves had diarrhoea or revealed a tendency towards it ($FS\geq3$), A1-calves had a prevalence of 21%. Nevertheless, similar ADG and end weights (EW) of calves fed A1- or A2-milk ($P>0.05$) indicate that A2-milk may compensate higher diarrhoea rates and lower dMI due to the associated higher protein content. Intact β-CM7 was detected in plasma after A1- and also A2-milk consumption, but was almost 5-times higher for A1-calves. Evidence from this study suggests that, due to the change in the amino acid sequence, A2-milk consumption minimizes the cleavage of β-CM7, and therefore contributes to an improved development of pre-weaned dairy calves. These findings will also have an impact on human health by contributing new prospects to the field of functional food, infant formulas or other pharmaceutical products.

Session 49 Theatre 3

Can we alter the microbial composition in bovine colostrum through the dry period ration?

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When colostrum management is discussed with farmers, a recurring question is how to improve IgG levels in colostrum. Low quality colostrum leads to insufficient IgG transfer to calves. Which, on its turn, leads to lower survival of calves and economic losses. Besides IgG, colostrum contains other factors that could shed some light on the situation. In our previous study we discovered a link between the colostral microbiota and the IgG level in colostrum and in the calf's serum. In the present study, we aimed to improve the colostral IgG level and microflora of dairy cows by implementing different dry period feeding strategies. Specifically, we tested the effect of two crude protein (CP) levels of the dry period diet on the colostral IgG level and microflora and the subsequent transfer of passive immunity to the neonatal calf. We included 75 multiparous HF cows, fed a high (11% in far off, 17% in close up; n=40) or low (8% in far off, 14% in close up; n=35) CP diet. Colostrum IgG level was quantified with radial immunodiffusion (RID) and was lower in cows fed the low CP diet (P=0.05). Colostrum from only 49 out of the 75 cows (n=26 high CP,n=23 low CP) met the quality threshold of 50 g IgG/l, was stored at -20 °C and subsequently administered to 49 newborn calves in a 2×2 factorial design study, in order to account for prenatal effects of the maternal dietary CP level. Dietary CP level only affected the serum IgG level of calves born out of low CP cows: calves receiving colostrum from high CP fed cows had significantly lower serum IgG levels (mean=14.2 g IgG/l) than calves receiving colostrum from low CP fed cows (mean=20.9 g IgG/l; P<0.01). Microbial DNA was extracted from colostrum with the Powerfood microbial kit (Qiagen, Germany). Library preparation and amplicon sequencing of the bacterial V3-V4 region of the 16S rRNA gene is currently being performed using the Illumina MiSeq V3-technology (Macrogen, South-Korea) and results will be presented at the conference. Acknowledgement: I. Van Hese is an SB PhD fellow at FWO, Research Foundation – Flanders, project number 1S20220N.

Session 49 Theatre 4

Effects of improved early life nutrition on miRNA and mRNA expression in testes of the bull calf

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Enhanced nutrition is known to progress sexual development in the bull calf through neuroendocrine signalling in the hypothalamic-pituitary-testicular signalling axis. Enhanced nutrition may result in advanced hypothalamic derived gonadotropin releasing hormone (GnRH) release, follicle stimulating and luteinizing hormone production in the anterior pituitary and accelerate testes development. Molecular mechanisms leading to this effect are not yet expounded. This study assessed the impact of contrasting feeding regimes, for bull claves in the first 12 weeks of life, on differentially expressed (DE) genes in the testes. Holstein bull calves with a mean (SEM) bodyweight of 48.8(5.3) kg and age of 17.5(2.8) days, were assigned to either high (H; n=15) or moderate (M; n=15) dietary groups, with diets designed to elicit growth rates of 1.0 and 0.5 kg/day. At 12 weeks of age all calves were euthanized and testes parenchyma harvested. RNA was extracted from the testes and used for miRNAseq and mRNAseq analyses. Subsequent bioinformatic analyses identified DE miRNA and mRNA genes, and DE-mRNA directly affected by DE-miRNA determined. H calves were heavier at slaughter (112 v 88 kg, P<0.001), and had greater ADG (0.88 v 0.58 kg, P<0.001). Seven DE-miRNA and 20 DE-mRNA were identified between the H and M groups. Two miRNA, miR-2419-5p and miR-11995 were up-regulated in H with corresponding target mRNA, CDH13 and TNFSF18 down-regulated in H, indicating a direct relationship between the miRNA and corresponding mRNA. miR-2419-5p is involved in IGF-1 and AMPK signalling. Its target, CDH13 is a cadherin gene, with functions in testes development. TNFSF18 gene is a member of the tumour necrosis factor family of cytokines which have important roles in normal testicular homeostasis. These results provide evidence that testes development due to enhanced dietary intake is mediated by miR2419-5p and miR-11995 and their target mRNA genes. This study was funded by Science Foundation Ireland (16/IA/4474).

Milk composition and productive performance from dairy goats fed with sunflower and rapeseed oils

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The objective of this study was to determine milk composition and productive performance from dairy goats supplemented with sunflower and rapeseed oils. Additionally, since goats were housed with their kids, body measurements from their respective kid were determined. Nine Danish Landrace dairy goats (42±5 days in milk at the beginning of the study) were allocated to three treatment groups. For 42 days, all animals received a basal diet based on (% of dry matter) 21% lucerne and clover, 23% clover, 42% lucerne and grass and 14% straw. Concentrate (300 g/d) was supplied during milking. Dietary treatments contained 85:15 forage to concentrate ratio. The control concentrate consisted (% of dry matter) of 93% of a grain mix, 6% of molasses and 1% of a premix of vitamins and minerals. Oils-supplemented concentrates were composed by 89% of a grain mix, 6% of molasses and 1% of a premix of vitamins and minerals and 4% of either sunflower oil or rapeseed oil. Performance data was recorded on days 14, 21 and 42. Compared with control and sunflower oil, rapeseed oil increased milk yield (1.03 and 0.92 vs 1.26 kg/d). Milk fat (2.18±0.17 g/100 g), protein (2.61±0.16 g/100 g), lactose (4.49±0.25 g/100 g), casein (2.05±0.19 g/100 g), total solids (10.0±0.45 g/100 g), solids-non fat (7.98±0.39 g/100 g) and density (1,026.01±1.56 kg/l) were similar among treatments. Compared with control, sunflower and rapeseed oils increased body condition score (2.4 vs 2.6 and 2.7). Body weight was similar among treatments (43.6±4.05 kg). Compared with control and sunflower oil, rapeseed oil increased kid´s body weight (13 and 14 vs 16 kg), body length (47 and 47 vs 48 vs 51 cm), hip height (50 and 50 vs 53 cm) and heart girth (54 and 55 vs 59 cm). Overall, rapeseed oil at 4% of DM inclusion in forage-based diets, improves milk production, kid performance with no negative effects on milk composition.

Effect of the dietary calcium level on the performances and bones' resorption in of lactating sows

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Meeting the calcium (Ca) requirement of lactating sows is crucial while feeding it in excess should be avoided. Few data that describe the Ca requirement of modern sows are available in the literature. The objective of this trial was thus to assess the effect of the dietary Ca level on the performances and bone' resorption of lactating sows. To this end, three diets that contained 0.7, 0.9 or 1.1% of total Ca were compared in a single factorial design. The three diets contained no exogenous phytase and had the same level of digestible phosphorus, i.e. 0.3%. Diets were offered to three groups of 26 sows from the entrance into the farrowing room (day 107 of gestation) until weaning (21 days). Regardless the criterion considered, no Parity × Diet interaction was observed. No effect of the Ca level was detected on performances of sows or piglets ($P>0.10$). The CrossLaps® plasma levels, a biomarker of bone' resorption, did not differ significantly among the three groups of sows, either at the beginning of the trial or at weaning. However, the CrossLaps® level increased during the trial, suggesting that bone' reserves were mobilized, regardless the dietary Ca level. Finally, these data suggest that, in the context of this study (litter size, weaning age, etc.), the lowest Ca level was high enough to optimize performances in lactating sows. The intensity of bone' resorption during lactation, evaluated using a biomarker, seems not related to the dietary Ca level.

Sex-specific transgenerational effects of maternal diet type and spirulina supplementation in pigs

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Maternal intake of a Western diet high in fat and sugar during pregnancy and lactation can predispose the offspring for metabolic disorders. *Arthrospira platensis* (spirulina, Sp), a microalga rich in bioactive compounds may mediate metabolic effects. To study if maternal Sp supplementation can ameliorate the detrimental transgenerational effects of a Western diet, gilts (Landrace×Large White) were assigned to two groups, receiving either a diet high in fat and sugar (WES, n=9) or a standard pig feed (CTR, n=10) *ad libitum*. After two months of pre-feeding, Sp supplementation was started for part of each group, resulting in four experimental groups, CTR, CTR+Sp, WES, WES+Sp (n=4-5), and gilts were artificially inseminated with sperm from the same boar. Piglets' birth weight was registered, and their body weight (BW) was recorded weekly. At weaning, 3-4 piglets per sow (male and female) with a birth weight closest to the average litter birth weight were slaughtered to collect liver and blood samples (n=14-20 per group). Preliminary analyses on IGF-1 plasma levels and RNA sequencing from liver samples were performed on half of the collected samples. Following differential gene expression analysis, ingenuity pathway analyses was performed. At birth, males were heavier than females ($P<0.01$) regardless of the maternal diet. During the suckling period, the male offspring remained heavier than their female counterpart ($P<0.05$). Interestingly, the males from CTR+Sp mothers were lighter than the males from CTR ($P<0.05$) and WES ($P<0.05$) mothers. An interaction of diet and sex ($P<0.05$) was observed for IGF-1. Pathway analyses performed on RNA sequencing data showed an activation of lipid metabolic pathways (e.g. accumulation of triacylglycerol) in the male but not in the female WES offspring. Our results indicate that maternal diet and Sp supplementation in pigs might have a transgenerational sex-specific impact on offspring growth and metabolism. We expect to gain more detailed insights into these metabolic programming effects by completing the analysis of the second half of samples and thus increasing statistical power.

Dry grape extract supplementation in sows improves colostrum quality and piglets' performances

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Lactation is a critical aspect of swine production for both the sow, which mobilises different body reserves to produce milk, and for the piglets, which depend on efficient transmission of essential nutrients and biological substances through the colostrum and milk. The aim of this experiment was to study effects of supplementation with a commercial standardised grape extract (SGE, Nor-Grape® Fertility, Nor-Feed, France) on colostrum and sow and piglet performances. A total of 305 sows were randomly divided into two groups when entering the maternity ward: a control group (CTL, 146 sows) and a supplemented group (NG, 159 sows, CTL feed + 50 g/T of SGE). The parameters studied were the Brix value of colostrum; piglets' individual birth weights and weaning weights; the total number of piglets born, live born, stillborn and mummified per litter, average daily gain of piglets and litters, pre-weaning mortality and loss of sow backfat. Individual daily feed intake was also recorded for the sows during lactation. At farrowing, no significant difference was observed between groups in the number (total, live born, stillborn or mummified) or weight of piglets. However, the Brix values of colostrum from supplemented sows were significantly higher than those of CTL sows (27.8 vs 26.4, respectively, $P<0.05$). Whilst no difference was observed between groups of sows in terms of lactation feed consumption or backfat loss, NG piglets were significantly heavier than CTL ones at weaning (5.62 vs 5.33 kg, respectively, $P<0.001$), resulting in a significantly heavier weaned litter weight (66.5 vs 63.6 kg, respectively, $P<0.05$). Supplementing sows with a commercial SGE during lactation thus improves colostrum quality and piglet performances. Furthermore, the absence of difference in feed intake for the sows or back fat loss indicate that these improvement in piglets' performances may be linked to a better transformation of the diet in milk by the supplemented sows, resulting in a better conversion of the lactation feed into piglet gain.

Maternal subnutrition increases antioxidant defences during peri-implantation period in beef cattle

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Successful implantation requires a physiological balance between reactive oxygen species (ROS) and antioxidant activity. However, nutritional deficiencies could impair pregnancy recognition mechanisms and ROS homeostasis in cattle. This study sought to determine whether maternal subnutrition during early pregnancy affects antioxidant enzyme gene expression in peripheral blood mononuclear cells during the peri-implantation period in two beef cattle breeds. Parda de Montaña (n=32, PA) and Pirenaica (n=16, PI) multiparous cows were synchronized to oestrus and artificially inseminated (AI). Dams were randomly allocated to a control (CONTROL, n=19) or subnutrition (SUBNUT, n=29) group, and were fed at 100 or 65% of their estimated energy requirements during the first 82 days of pregnancy. Dams were weighed fortnightly. The average daily gain (ADG) was calculated by linear regression. Dam BCS was registered monthly. Pregnancy diagnosis was performed by ultrasonography on Day 37 post-AI. Blood samples were drawn on Day 21 post-AI. Gene expression of CAT, SOD1 and SOD2 was analysed by qPCR. A general linear model including fixed effects (nutrition, pregnancy status, and breed) and their interactions was used to compare relative CAT, SOD1 and SOD2 gene expression with JMPro software. When significant differences were detected, the TUKEY test was used to examine all possible pairwise comparisons. Pregnancy was confirmed in 31 dams. No significant effects were observed for reduced nutrient intake on pregnancy status. Treatment affected significantly cow ADG ($P<0.001$) and BCS change ($P<0.05$) from AI to Day 82 post-AI. No effect of pregnancy status on CAT, SOD1 and SOD2 mRNA was observed on Day 21 post-AI. SOD1 mRNA was higher in SUBNUT ($P<0.01$) than in CONTROL group, suggesting an oxidative damage response to dietary deficiency in these two cattle breeds during the early pregnancy. A trend to higher SOD2 mRNA was observed in PA cows respect PI cows ($P=0.09$). To sum up, maternal nutrient restriction during early pregnancy increased antioxidant defences during peri-implantation period in suckler beef cattle.

In utero undernutrition affects heifer growth, follicle and metabolic traits but not age at puberty

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Undernutrition in early stages of pregnancy can have detrimental effects on the developing foetus. We studied the effects of peri-implantation subnutrition of beef cows on the performance of their female offspring. Fifty-three Parda and 32 Pirenaica multiparous cows were artificially inseminated (AI) and distributed into two nutritional treatments during 82 days post-AI: CONTROL (100% energy requirements) vs SUBNUT (65%); thereafter they were fed 100%. Newborns were fed on maternal milk and were weaned at 120 days. During rearing, heifer weights were recorded and blood samples were taken to study their metabolic status. On days 1 and 10 every 28-day period blood samples were collected to assess the age at puberty. Ovarian follicular dynamic was recorded at 10, 13 and 16 months of age by ultrasonography. Heifers born from SUBNUT cows were lighter at weaning (4 months old) than heifers born from CONTROL cows (133 vs 152 kg, $P<0.05$), but this difference disappeared at AI (16 months old, 400 vs 415 kg, $P>0.05$). Consequently, SUBNUT heifers had increased the requirements for growth in the rearing period, compromising their metabolic status around the onset of puberty (higher concentrations of NEFA, urea and cholesterol). Maternal undernutrition impaired their ovarian development, with lower counts of large follicles (>10 mm) at 13 months (0.4 vs 0.9, $P<0.05$) and small follicles (<5 mm) at 16 months (11 vs 16, $P<0.05$). However, the age (12 months), weight (339 kg) and percentage of mature live weight (59%) at the onset of puberty were similar in all heifers. Regarding the breed, Parda heifers had higher gains during rearing than Pirenaica ones (0.82 vs 0.71 kg, $P<0.01$), higher weight at AI (420 vs 395 kg, $P<0.05$), more large follicles at 10 months (0.8 vs 0.4, $P<0.05$) and larger dominant follicles at 16 months (12.4 vs 9.5 mm diameter, $P<0.05$). In conclusion, undernutrition of beef cows in early gestation had long-term effects on postnatal growth, follicular and metabolic parameters of their female offspring, but no effect on the age or weight at puberty. Further research is needed to determine the possible consequences of intrauterine energy restriction on heifer adult weight and reproductive lifespan.

Vitamin A regulates myoblast hyperplasia and myogenesis in bovine stromal vascular cell

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The hyperplasia of foetal myoblast and subsequent myogenesis are crucial for meat production in beef cattle. Vitamin A is an essential nutrient for beef cattle, which plays the important role in the formation and maintenance of numerous tissues such as muscle. The objective of this study was to investigate the effect of all-*trans* retinoic acid (RA, the active form of vitamin A in the body) on myoblast hyperplasia and myogenic differentiation in isolated stromal vascular (SV) cells from Korean native cattle. The SV cells were isolated from muscle tissue in the slaughtered Korean native cattle by collagenase digestion method. After 2 serial passages, the SV cells were cultured in 10% foetal bovine serum (FBS/DMEM) until 90% confluence and then changed to differentiation medium containing 2% horse serum (HS/DMEM) afterwards. The different dosage of RA (0.01, 0.1 and 1 μM) and vehicle were treated only in the growing or differentiation phase of SV cell. As a result, mRNA expression results showed that both concentration of 0.1 and 1 μM of RA treatment significantly up-regulated myogenic factor 5 (Myf5) ($P<0.05$) during the growing phase. Moreover, 1 μM RA treatment in growing phase showed the significantly increased of mRNA expression of myogenesis markers ($P<0.05$) including myogenic differentiation 1 (MyoD), myogenin (MyoG) and myogenic factor 6 (Myf6) on later myogenic differentiation day 6. In addition, when treated RA only in the differentiation phase, 0.1 μM RA treatment significantly elevated ($P<0.05$) mRNA expression of myogenesis markers (MyoD, MyoG and Myf6) compared with control group. In conclusion, we found the RA treatment in the growing phase of SV cells that indirectly promoted hyperplasia of myoblast as well as myogenic differentiation afterwards. Similarly, RA treatment during the differentiation phase also promoted myogenesis of SV cells. It will provide a reference for the vitamin A has the potential to promote muscle development from the foetal to the postnatal period of beef cattle.

Energy level and milking frequency at dry-off do not affect colostrogenesis in dairy cows

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This study aimed to evaluate whether the different management strategies used at dry-off based on energy level and milking frequency can affect colostrogenesis prepartum, influencing therefore colostrum yield and composition in dairy cows. In this study, 40 Holstein cows dried-off from 1st, 2nd and 3rd lactation with milk yield ≥15 kg/d were used in a randomized block (parity groups 1st or 2nd+3rd) design with 4 treatments in a 2×2 factorial arrangement. Cows were continuously enrolled every second week in batches of 2-6 cows. Treatments were based on feeding either 100% (HEn) or 50% (LEn) energy requirements for late lactation cows and milked either twice (HMi) or once (LMi) daily in the last week before dry-off. Therefore, these resulted in the following experimental groups: (1) HEn-HMi (n=10); (2) HEn-LMi (n=9); (3) LEn-HMi (n=13); and (4) LEn-LMi (n=11). After dry-off, cows were fed with a ration formulated to fulfil all nutritional requirements for dry cows. Within 6 h postpartum, cows were milked and the colostrum yield and BRIX% were recorded. In addition, colostrum samples were used to determine fat, protein, somatic cell count (SCC), immunoglobulin G (IgG) and calcium concentrations as well as lactate dehydrogenase activity (LDH). A one-way ANOVA model was used including fixed effects of energy level (100 vs 50% energy requirements), milking frequency (twice vs once daily), and the interaction between both. Significance was set at $P<0.05$. None of the effects tested in this experiment (i.e. energy level and milking frequency) affected either colostrum yield (10.2±0.89 l) or BRIX% (25.5±0.79%) ($P>0.05$). Similarly, treatments affected neither contents of fat (4.53±0.66%), protein (15.3±0.99%), IgG (79.0±9.84 mg/ml), calcium (60.0±3.81 mM), SCC (2.91±0.13 $\log_{10}$/ml) nor LDH (1.85±0.07 $\log_{10}$/ml) activity ($P>0.05$) in colostrum. In conclusion, the dry-off strategies based on either energy level or milking frequency did not seem to affect colostrum yield and composition.

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Does dam nutrition during prepubertal phase affect muscle gene expression in male foetal progeny?

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There is some evidence for transgenerational effects on progeny of fluctuations in nutrient intake of their dams. However, there is no information on whether such effects can be carried over to the next generation, when the original dietary fluctuations were incurred during the pre-pubertal period. The objective of this study was to determine if maternal diet during the prepubertal phase would affect the expression of key genes related to muscle development and growth in male foetuses. Eighty Angus × Holstein-Friesian heifer (141±8 days of age and 119±23 kg of BW, mean ± SD) were offered either a high (H) or moderate (M) plane of nutrition from 4.5 to 8.5 months of age. Target growth rates were 1.2 kg/d and 0.5 kg/d, for H and M groups, respectively. Following this, all heifers were offered a conventional moderate level of nutrition. At breeding, heifers were bred to a synchronised oestrus using semen from a single AI sire (mean age and BW: 502±8 days and 360±46 kg). A transrectal uterine ultrasound was performed 63 days after AI to determine pregnancy and foetal sex. On day 100 of gestation a cohort of heifers (H: n=11; M: n=12) reflecting the average BW and age of the group and carrying male foetuses were slaughtered. Skeletal muscle tissue was sampled from each foetus, with RNA subsequently isolated from each sample. RNA was reverse transcribed into cDNA and then used for qPCR evaluation of key genes (GHR, IGF2, IGF2R, MSTN, MYF5, MYF6, MYOD, MYOG, PAX7) related to skeletal muscle development and growth. Differences in gene expression between H and M groups were determined using mixed model ANOVA (PROC MIXED, SAS). No treatment differences ($P>0.05$) were detected in expression profiles of the candidate genes notably associated with skeletal muscle cell differentiation and fibre development. In conclusion, this study shows no transgenerational effect on key genes related to muscle development and growth in male foetuses, when dam nutrition fluctuated during prepubertal phase.

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Effect of maternal *Solanum glaucophyllum* intake on suckling piglet growth and muscle gene expression

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Supplementing vitamin D to sows is suggested to facilitate parturition and support neonatal piglet survival. It is however unknown, if this may affect offspring growth as well as muscle gene expression with potential consequences for postnatal development of the offspring. *Solanum glaucophyllum* (waxy-leaf nightshade) contains high proportions of calcitriol, the active form of vitamin D, and increases circulating calcium concentrations after oral intake. At about 107 days of gestation, sows were randomly divided into two experimental groups (n=6 per group). All animals received a commercial lactation feed. The feed of one group (SG) was supplemented daily with a top-dressing containing 600 mg Panbonis 10 (Herbonis, Augst, Switzerland), providing 10 mg/kg calcitriol-glycosides (equivalent to 6 µg/sow/day of calcitriol). The second group (control) received the same top-dressing without Panbonis 10. At farrowing, the piglets' body weight (BW) was determined. Cross fostering took place within feeding groups. At about four weeks of age, one male and one female piglet with a birth weight closest to the respective average litter birth weight were selected from each sow. The BW of these selected piglets was determined before slaughtering by electrical stunning and exsanguination. The weights of liver, kidneys, perirenal adipose tissue, lungs, heart, and spleen were measured. Muscle tissue was collected for gene expression analysis via qPCR. Birth weight of SG piglets (1.58±0.27 kg (mean ± SD)) tended to be higher ($P=0.085$) than that of control piglets (1.39±0.22 kg) while litter size tended to be smaller in SG (13±3) than in control (16±3) sows ($P=0.088$). At slaughter, SG piglets (7.77±0.96 kg) were heavier ($P=0.050$) than control piglets (6.99±0.71 kg). No significant difference was observed for any of the organ weights. Gene expression of myogenic regulatory factors (myogenin and myogenic differentiation 1) in muscle did not differ between groups. Diet group and sex did not show any interaction for any parameter. Gene expression analyses are ongoing to evaluate the potential underlying reasons why maternal intake of *S. glaucophyllum* providing the active form of vitamin D resulted in heaver piglets (+11%) at four weeks of age.

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Influence of *in utero* undernutrition on beef heifer performance up to their first lactation

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The productivity of suckler cows, considering their ability to conceive, calve and rear a calf, can be impaired under malnutrition. We studied the consequences of early maternal nutrition on the performance of female offspring along with their first breeding, gestation and lactation. Sixteen-month-old heifers (n=36) born from CONTROL (100% energy requirements during the first third of gestation) and SUBNUT (65%) cows were artificially inseminated (AI). After calving, heifers reared their calves until weaning on day 105. Heifer diet was calculated to supply 100% of energy requirements during the experiment. Heifers and calves were weighed fortnightly, and heifer BCS was assessed at calving. Calving ease was classified as unassisted or assisted. Data were analysed with a generalized or mixed linear model with heifer maternal nutrition and sex, in the case of calves, as fixed effects. Fertility rate, calving ease and male/female ratio were assessed using the F-test. Heifer daily gains during rearing (0.77 kg/d), fertility rate to a single AI (80%) and BCS at calving (3.0) were similar in all heifers. No difference in calving assistance was found between CONTROL and SUBNUT heifers (26.7 vs 16.7%, $P>0.05$). The male/female calf ratio was higher in CONTROL heifers, but not significantly (8/7 vs 3/9, $P>0.05$). During lactation, all heifers lost weight (-0.52 vs -0.35 kg/d, for CONTROL and SUBNUT heifers, $P>0.05$), probably due to their high metabolic rates. Heifer prenatal nutrition had no effect on their calf weight at birth (35 vs 34 kg, $P>0.05$, for CONTROL and SUBNUT calves) nor on calf ADG during lactation (0.72 vs 0.68 kg/d, $P>0.05$), neither of which were affected by calf sex. Calf ADG during lactation was correlated with their dams' weight at calving ($r=0.65$, $P<0.001$). Calf weaning weight was correlated with their dams' weight when they were weaned ($r=0.61$, $P=0.001$). In conclusion, undernutrition during early gestation had no long-term effects on heifer postnatal performance during their first breeding, gestation and lactation. However, as heifers grow until their fifth year, further research is needed to study the impact of prenatal nutrition on maturity and performance during their productive lifespan.

Session 50 Theatre 1

Back to the future: re-introduction of cow-calf contact into dairy farming requires new knowledge

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For more than one hundred years, dairy production has separated calves from dams. More milk and reducing the risk of infectious diseases for calves and cows were sensible goals. This practice is being questioned not only by a critical public but also by farmers who now are again allowing calves to have contact with their dams and to suckle. In addition to aspects concerning the management of these cow-calf contact (CCC) systems, however, questions are increasingly arising concerning fundamental relationships between dairy cows' physiology, genetics and behaviour, which, due to the exclusion of contact with their own offspring, were not any longer in focus of research. Especially milk secretion and machine milking were investigated without any interference of the calf's role in these processes. Answers to the questions that arise in the context of CCC could also help to address existing challenges of intensive dairy farming where calves are raised separately, e.g. the causes of milk ejection problems in heifers. We still do not know what processes underlie the – compared to machine milking – higher oxytocin secretion in cows that have established a bond with their calf when suckled by it; we do not know if and how the more and repeatedly oxytocin releases due to multiple suckling, that result from unlimited cow-calf contact, affect the cows' well-being including their performance, and we do not know the cause of the differences in the nursing cows' response to machine milking, which range from complete blockage to (presumably) complete milk let-down as can be deduced from the large variation in yield and milk composition. It is well known that intensive suckling by more than one calf in addition to machine milking increases the frequency of udder emptying und thus induces an increase in productivity that is maintained even after returning to a lower milking frequency. However, this does not seem to apply to all CCC systems. In conclusion, cow-calf management on dairy farms was not in the centre of research for many years. This topic is now back on the scientific agenda because of the obviously successful re-introduction of suckling into European dairy farms.

Strategies for keeping dairy cows and calves together on European farms

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The purpose of this study was to identify and describe strategies used by European dairy farmers with cow-calf contact (CCC) systems. Farms allowing at least 7 days CCC in Austria, France, Germany, Italy, Poland, Sweden and Switzerland were interviewed between September 2018 and January 2019 following a standardised questionnaire. In total, 104 interviews were included in the analysis; no CCC farms were identified in Poland. Average herd size was 53±59 (mean ± SD) cows. Dam rearing was practiced on 34% of farms, while 12% used foster cows, 28% used a mix of dam and foster cows and 23% first let the calves suckle their dam and then manually milk fed them. On 46% of farms, the calves had full day contact (except at milking), while 5% practiced half day CCC and 36% let the calves suckle at milking. Farmers perceived several benefits with keeping cow and calf together, including improved overall health (79% of farms) and weight gains (84%) in calves. Udder health in suckled cows was most often perceived as the same (40% of farms) or improved (38%) as in systems with early separation. Common drivers for using CCC systems were improved calf health, more natural farming system and increased labour efficiency. The most commonly stated barrier for implementing CCC was barn construction, while stress responses when separating cows and calves were observed by many farmers (87% of farms). The study suggests that there is a range of different strategies allowing CCC used on European farms, and identifies research regarding suitable housing during the suckling period and routines for cow-calf separation as key.

Calf or grass if the cow gets to choose?

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There is a growing interest for keeping dams with their calves for an extended period, and a need to develop applicable systems supporting this management. Twenty cow-calf pairs housed in an AMS with free access to outdoor areas in Uppsala, Sweden were included in our study, with the aim to investigate cows' preference for access to their calves over high-quality pasture. Scan sampling of cow location and behaviours was conducted every 10 min on June 03 (D1), June 23 (D2) and July 03 (D3). The area with the calves (age 73±10 days at D1; calves confined on pasture) and the area with fresh grass were located opposite to each other, at the same distance from the barn. Cows received concentrate, *ad lib*. roughage and water indoors and had access to a water trough in the lane between the areas. Data was summarized per observation day and divided into Out of sight (cows in driveway or in barn) and In sight. In sight was subdivided into locations: pasture area with calf and pasture area without calf, and proportion of behaviours performed (grazing, standing, and lying) was calculated. Preliminary analyses of data was performed in generalized linear mixed models with observation day (D1, D2 and D3), parity (primiparous and multiparous), and breed [Swedish Red (SRB) and Swedish Holstein (SH)] included as fixed effects. Cow within observation day was included as random effect, with a compound symmetry covariance structure. Temperature humidity index was lowest on D1, highest on D2 and intermediate on D3 ($P<0.001$), but never exceeded the suggested comfort zone threshold of 72. Indoor feed intake was lower on D1 compared to D3 ($P<0.05$) but did not differ from D2. Cows were less likely to choose calves over fresh grass on D1 compared to D2 (OR 0.189; CL 0.053-0.671; $P<0.05$). Proportion of observations In sight was higher on D1 compared to D2 ($P=0.05$), and for SRB compared to SH ($P<0.05$), but SH tended to spend more time on pasture with calves compared to SRB ($P=0.06$). Proportion of observations of lying down was higher on D1 compared to D2 ($P<0.001$), and there was a tendency for day to affect proportion of grazing ($P=0.08$). Other motivations than access to calves seem to influence the cows' choice, which should be considered when keeping calves confined on pasture.

Evaluating bonding and maternal behaviour of Swedish dairy cows in a cow-calf-contact system

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We investigated the maternal behaviours of modern dairy cows and assessed bonding behaviours between cow and calf. A total of 10 Swedish Red (SR) and 9 Swedish Holstein (SH) cows with either a female or male calf were included in the study. The animals were housed in a cow-calf contact system from 48 hours after birth. Maternal-filial interactions were observed at the maternity pen when calves were 1 day old. A preference test was used at 4 weeks of age to assess the time that calves spent in proximity of the mother cow in comparison to an unfamiliar cow (CaT) and to assess the preference of the mother of her calf over an unfamiliar calf (CoT). A neophobia test (NT) was also performed at 5 weeks of age. Data were analysed in R using GLM (count data) and mixed models. The results from the maternity pen showed that SR cows sniffed their calves more than SH (Dev. 54.9, $P<0.01$), and multiparous cows (MC) vocalized more (Dev. 138.1, $P<0.05$) than primiparous cows (PC). In CaT, calves of PC sniffed the unfamiliar cow more often than calves of MC (Dev. 3.9, $P<0.05$), SR and male calves vocalized more than SH (Dev. 21, $P<0.01$) and female calves (Dev. 22.7, $P<0.01$), respectively. SR calves were closer to their mothers for a longer period ($F_{1,14}=5.4$, $P<0.05$). In the CoT, the unfamiliar calf spent more time sniffing when the cow was an SR (Dev. 8.2, $P<0.01$). Calves of an MC cow sniffed the mother more often than calves of PC (Deviance= 5, $P<0.05$). Male calves were also more vocal during the cow test (Dev. 26.9, $P<0.05$). Mothers of female calves were faster to leave the startbox ($F_{1,12}=5.4$, $P<0.05$). During NT, mothers of a male calf spent more time in the buckets ($F_{1,14}=8.1$, $P<0.05$), ate more concentrate ($F_{1,13}=6.2$, $P<0.05$) and were in longer proximity of their calves, compared to female calves ($F_{1,14}=8.4$, $P<0.05$). Besides, calves of PC spent more time in the buckets ($F_{1,14}=5.7$, $P<0.05$). This study showed that maternal and bonding behaviours varied between cows, with some indications that SR, MC and mothers of male calves were potentially more bonded in the preference test, which did not follow the same pattern in NT. Further studies on bonding behaviour development in cow-calf systems associating it with later social behaviours in the group are encouraged.

Effects of two different separation strategies on AMS milk production in a cow-calf contact system

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Public acceptance is pivotal for a sustainable dairy production, and concerns about the quality of life for cows and calves are being raised. A specific management practice, which recently has been questioned, is the early separation of cow and calf. This tradition has become a highly contentious issue. New rearing systems allowing cow-calf contact (CCC) should both fulfil natural behavioural needs of the cow-calf dyad and be economically viable for the farmer. However, short and long-term effects on health, fertility and production needs to be investigated. The aim of this study is to compare the effect of two types of separation strategies on milk yield in a novel cow-directed CCC system with automatic milking (AMS). Machine milk yield may be affected by both timing of separation and separation strategy. Cows (n=16) have free access to their calves during the first weeks followed by a gradual decrease in the cows' access. Milking permission is granted after 5.5 h and cows are milked minimum twice daily. Daily AMS milk yields between two separation strategies are compared: early but slow separation (gradual separation from 4 to 8 weeks of age, n=8 cow-calf pairs) and late but rapid separation (gradual separation from 6.5 to 8 weeks of age, n=8 cow-calf pairs). None of the cows have previous experience with taking care of a calf. AMS milk yield is automatically registered for the first 9 weeks in lactation. Descriptive statistics will be used to show how AMS milk yield can vary with separation strategy. The experiment is ongoing and results will be presented at the conference. However, preliminary data suggests that milk yield increased from the first day of separation in both groups. The first days of gradual separation the daily milk yield among cows separated early ranged from 8.3 to 33.0 l, among cows separated late it ranged from 3.0 to 42.7 l.

Effects of two weaning and separation methods on milk yield of dams nursing their calf

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The reduced amount of saleable milk often prevents introduction of cow-calf contact under current farming conditions. Another shortcoming is the stress caused in animals by weaning and separation. We investigated the effect on milk yield of the dams (German Holstein, 86±7 DIM) during a two-step weaning using a nose flap (NF, full-time contact>2 weeks access to dam with nose flap>1 week fence-line contact, n=18 cow-calf-pairs) or by gradual reduction of contact time with the dam (GR, full-time contact>1 week half-day contact>1 week morning contact>1 week fence-line contact, n=18 cow-calf-pairs). Cows were milked twice daily in a tandem parlour (GEA Group AG: IQ cluster, 38 kPa, vibration stimulation, automatic stripping device and cluster removal). Milking routine started with manual stripping followed by cleaning of the teats and attachment of cluster. Milk yield was recorded using the management system DP21. Statistical analyses were conducted in R 4.0.3 using linear mixed models including the cow nested in classes of lactation numbers as random effect. Average milk yield per day was calculated for five weeks (before, first and second week of weaning (NF or GR), fence-line separation and the week after the calves were moved to another barn). The course of milk yield over the weeks differed between weaning methods (interaction: method×week, $P<0.001$). As expected, the NF cows had higher milk yields than GR cows during the two weeks when the calves still had access to the cows but could not suckle in NF and only for a limited time in GR. Even during the period of fence-line contact the GR cows had a lower milk yield than NF cows (LSM: 19.7 vs 23.8±2.1 kg/day, $P<0.05$). Only when the calves were moved to the other barn, the milk yield of the GR cows increased to the level of the NF group (LSM: 25.1 vs 24.1±2.1 kg/day) indicating that up to a week without suckling is needed till milk yield of dams achieves a consistent, higher level.

End-of-milking fat content in a cow-calf contact system with automatic milking

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The interest for cow-calf contact (CCC) systems is increasing among farmers and consumers worldwide. However, research is lacking, particularly in automatic milking systems, as well as practical solutions for management of cows and calves. During the suckling period, cows in CCC systems deliver less saleable milk. Previous research has indicated that CCC can lead to decreased fat content, which may partly be explained by the suckling itself, but may also be a consequence of low udder emptying due to less efficient milk ejection. Milk yield alone provides insufficient information about the milk ejection in a CCC system, as milk yield varies not only with the milking interval but also with the suckling interval. The aim of this study was to evaluate udder emptying in a novel cow-driven CCC system with AMS, using end-of-milking fat content as a proxy for milk ejection, together with milk yield and milking interval. A total of 244 quarter level strip milk samples were taken directly after milking in 15 cows in early lactation (DIM range 12-31) and analysed for fat content. Quarter milk yields ranged from 0.0 to 5.7 kg milk per milking. End-of-milking fat content ranged between 1.1 and 24.4%, with great variation between cows, quarters and milkings. Preliminary descriptive data suggests that different cows have different patterns for the relationship between milk yield and end-of-milking fat content. Some cows consistently produce high yields with high end-of-milking fat content, while others consistently produce low yields, with predominantly low end-of-milking fat content. Some cows also display a large variation, ranging in both milk yield and end-of-milking fat content, likely due to variations in time since last milking and suckling. Further data analysis will investigate the variation between udder quarters and whether recently suckled quarters can be identified using this method.

Session 50 Theatre 8

The cow and the machine – developments, interactions and test methods

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The basic principle of machine milking has not changed for the last 100 years and is still based on milking with a two-chambered teat cup and applying vacuum to the teat. The influence of machine milking on milk yield, milking performance, teat condition, milk quality, and udder health has been the subject for many analyses, collection of field experience, experiments, and basic research leading to further development of the milking machine and understanding of the interaction between the cow and the machine. The milk yield of the modern dairy cow has increased considerable and especially within the last 20 years. We have seen a parallel increase in the risk of poor udder health. This sets further requirements to the milking equipment and technique, but it also allows for a challenge of the guidelines for conventional milking. Cows may be milked fully automatically today and by a quarter rather than an udder based system. A quarter based system offers the opportunity for individual settings of attachment, pulsation, vacuum and detachment without causing overmilking. A cow-based system compromise the optimal milking technique for the individual quarter. The basic principles of conventional milking is quite different from methods applied during calf suckling. The calf applies pressure and vacuum and allows for resting periods when changing suckled teat. The opportunities of extracting milk are much wider than what we apply today and there are possibilities for newer and extreme use of vacuum and pulsation as long as there is milk to damp the forces on the teats. The paper will focus on the basic principles of conventional milking, the interaction between the cow and the machine with special attention to teat condition, and look further into the playground of pulsation, vacuum and milking technique. Pros and cons of quarter and udder milking will be discussed. A short guideline will be given of how to observe and test the milking process.

Session 50 Theatre 9

Teat end hyperkeratosis and milking induced change in teat wall thickness in a cow-calf system

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With the increased interest of keeping cow and calf together on dairy farms there has also been an increased concern on how the combination of machine milking and suckling affects teat health and teat integrity. In an ongoing project where dairy cows are kept with their calves in a voluntary milking system, teat end hyperkeratosis (TEH) was assessed in 30 cows of the Swedish Red breed and Swedish Holstein. A control group of 26 non-nursing cows was also included. TEH was assessed by visual inspection on four occasions over six months; 2 weeks before calving, at peak lactation (8 w p.p.), at weaning (16 w p.p.) and at post weaning (24 w p.p.). On a subset of cows (14 nursing and 13 non-nursing cows) the teat wall response to milking was assessed by ultrasound measurement of the teat wall before and within 2 min after milking at one occasion (7-13 w p.p.). Level of TEH increased significantly from before calving to after calving and teat wall thickness increased during milking, but there were no differences between nursing cows and control cows in these two parameters. In conclusion, we found no evidence that suckling has a detrimental effect on teat health or teat integrity.

Prestimulation or milk flow adjusted vacuum and pulsation settings to optimize milking efficiency?

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Efficient machine milking requires either prestimulation before teat cup attachment or a reduced milking intensity by reduced vacuum and/or reduced liner-open phase of pulsation until milk ejection occurs. Bimodal milk flow curves (BIMO) indicate a transient limitation of milk available for removal before milk ejection. Milk flow was recorded in 10 Holstein dairy cows (72-119 DIM). Three milking routines (MR) were applied each at 2 morning and 2 afternoon milkings (14 h and 10 h milking intervals). MR1 consisted of a 15 s forestripping, teat cleaning and stimulation followed by 1 min latency period before cluster attachment at normal vacuum and pulsation (claw vacuum 44 kPa, pulsation rate 60 cycles/min; pulsation ratio 65/35). In MR2 and MR3 the cluster was attached immediately after a 5 s forestripping and cleaning at a transiently inverted pulsation ratio (30/70), reduced pulsation rate (50 cycles/min; MR2) and additionally reduced system vacuum (34 kPa; MR3) until milk flow exceeded 400 g/min. Cluster detachment was set at 400 g/min in all MR. BIMO was more frequent at 10 h than 14 h milking intervals with the highest rate in MR2 (35 and 10% in MR1, 90 and 75% in MR2, and 55 and 55% in MR3, respectively). Severe BIMO (milk flow nadir <1 kg/min) was more frequent at 10 h than 14 h milking intervals in all MR (10 and 0%, 60 and 10%, and 35 and 0% in MR1, 2, and 3, respectively). Total milk yield did not differ among MR. Machine-on time was longer at 14 h than 10 h milking intervals and was longer in MR3 than MR1 ($P<0.05$). However, the occupancy time (time from first touch of udder until cluster detachment) was longer in MR1 than in MR2 and MR3 ($P<0.05$; 8:03, 6:59, and 7:19 min at 14 h milking intervals, and 6:34, 5:50, and 5:49 min at 10 h milking intervals in MR1, MR2, and MR3, respectively). In conclusion, both MR2 and MR3 shorten the occupancy time compared to the more work intensive MR1, and consequently increase the capacity of the milking system. In addition, the effective avoidance of severe BIMO combined with a low mechanical load on the teat tissue by the gentle vacuum and pulsation settings of MR3 even if BIMO occur prevents a harmful impact on the teat during the period before milk ejection.

Physiological considerations for optimal control of vacuum level during milking

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Research and field experience indicate that the low flow period at the end of milking presents the highest risk period for teat tissue congestion. This is due to the negative correlation between teat vacuum and milk flow rate in a conventional milking machine. As a result of these limitations the vacuum during the peak flow period of milking are less than optimal so that teat tissues are protected during the low flow period. Flow-Responsive Milking (FRM) technology developed by DeLaval allows for selecting teat vacuum level independent of milk flow rate. A series of laboratory experiments confirmed that management of teat vacuum during the low flow period was protective of teat tissues and allowed for increased teat vacuum during the peak flow period. In a field trial of the FRM technology applied at the cluster level on a low-level milkline system peak milk flowrate was increased by 12%, average milk flowrate increased by 4% and milking duration of individual cows decreased by 4%, with the decrease in milking duration proportional to milk yield per milking. Changes in post-milking visual assessment of teat condition showed a slight reduction in teat end roughness and no meaningful difference in the occurrence of teats with blue colour, palpable rings or petechia. In a second field trial where FRM was applied in an automatic milking system the peak milk flowrate of quarters increased by 6%, average milk flowrate increased by 5% and milking duration was reduced by 4% at the udder level. The greatest effects were observed on slow milking and low yielding quarters, resulting in a 6% reduction in the difference in quarter milking duration within the same udder. The FRM also resulted in more complete milking of quarters with 5% higher milk yield per milking. These results indicate that the milking vacuum level in the peak milk flow period can be increased above current 42 kPa ISO recommendations if teat cups are removed to eliminate the low flow period of milking or teat-end vacuum is maintained below 40 kPa and low flow period at the end of milking.

Teat cup detachment affects milking characteristics in AMS with cleaning and milking in the same cup

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Milking in automatic milking systems (AMS) allows quarter individual teat cup detachment, and overmilking of individual quarters is avoided. The goal of the present study was to determine the optimal quarter detachment level depending on lactation stage and milking interval to optimize the milking process. Milking characteristics and post-milking teat condition were recorded in an AMS with all actions from teat cleaning to post-milking teat dipping in the same teat cup and liner (GEA DairyRobot R9500). In 24 dairy cows, 12 in early (<80 DIM) and 12 in late lactation (>180 DIM) 297 milkings were recorded during 12 consecutive days. Teat cup detachment was set at a quarter milk flow of 50, 87.5 or 125 g/min. Voluntary milking intervals varied similarly in cows in early (6.1-14.8 h) and late lactation (6.3-15.7 h). Based on the herd median, milking intervals were divided into ≤10 and >10 h, respectively. Total milk yield, milk production per h, and average milk flow were higher in early than in late lactation cows. Total milk yield per milking did not differ between detachment levels. At milking intervals ≤10 h the total milking time was reduced by up to 1.5 min with between the lowest and highest detachment level both in early and late lactation cows ($P<0.05$). Average milk flow was higher at milking intervals >10 h than ≤10 h ($P<0.05$). Only in early lactation cows the average milk flow increased with higher detachment levels ($P<0.05$). Delayed milk ejection (shown as bimodalities of milk flow) occurred only in 7 out of 297 recorded milkings and only at milking intervals ≤10 h in early lactation, indicating that the automated prestimulation before the start of milking is suitable for timely induction of milk ejection. The teat condition did not differ among the investigated detachment settings or milking intervals. In conclusion, high teat cup detachment up to 125 g/min reduces milking time in both early and late lactation, without a loss of milk yield or affecting the teat condition. By shortening the milking time without affecting the milk yield and avoiding too short milking intervals, leading to higher average milk flow, the milking efficiency can be optimized.

Milking permission and performance on a pasture based automatic milking system

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This study assessed how milking permissions (MPs) affect milking performance in a pasture-based seasonal calving automatic milking (AM) herd. A key objective of such a system is to optimize milk output. Possible approaches are to milk relatively high cow numbers (over 80) per AM system with low milking frequency (MF) per cow or alternatively, to milk lower cow numbers (60-70) with higher MF, which may have associated benefits of reduced labour and improved cow welfare. Therefore, the objective of this study was to establish the effect of increasing MP on MF and milk yield. Three groups of 24 cows each were milked in 1 AM unit. Cows were blocked based on calving date, milk yield and MF in the previous lactation and randomly assigned to one of three MP: 1.9, 2.2 or 2.5 per day. Cows were grazed in a 3-way system and received 546 kg of concentrate/cow over the lactation. Cows with a MP of 1.9, 2.2 and 2.5 had a MF of 1.4, 1.6 and 1.7 milkings/day, respectively. Milk yield/cow per day was 22.8 kg and 21.9 kg for cows with a MP of 2.5 and 1.9, respectively ($P<0.001$), while milk yield/milking was 13.7 kg and 15.2 kg for the same MPs ($P<0.01$) over 4 main milk producing months of a spring milk production system (May to August). Increasing MP from 1.9 to 2.2 increased milk yield by 0.7 kg/cow per day. Milking time/milking and Box time/milking were each numerically longer ($P>0.05$) for cows with a MP of 1.9 compared to 2.5. These data may be used to calculate potential cow numbers that may be managed in an AM system and its associated performance. Milking frequency did not match milking permission, therefore other strategies to incentivize cows to achieve greater MF in a pasture based AM system are required.

Relationship between cows' daily milk yield and daily rumination time in an automatic milking system

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Automatic milking systems allow us to study behaviour data in correlation to production data, and use results to improve the rearing technologies in dairy cows. For this study, data was collected from an AMS used for milking 64 Romanian Spotted cows, during 15 days in April 2019. Correlation of daily rumination time with daily milk yield and stage of lactation was studied, and gamma incomplete function was used to study the evolution of rumination time during lactation. Effect of number of milkings on daily milk yield was studied using ANOVA. All statistical inferences were made using STATISTICA software. Daily rumination time varied from 275 to 535 minutes, while daily milk production varied between 5.2 and 52.6 kg. Generally, rumination time decreased with DIM, but this was not consistent and not statistically significant (r=-0.049; P=0.122). A significant correlation between daily rumination time and daily milk yield was found, r=0.147 (P<0.001). Using the gamma incomplete function, it was found that both daily rumination time and milk yield had a similar evolution during lactation. On average, milk production was 27.96±0.269 kg and the average number of milkings was 2.70±0.03. The correlation between daily rumination time and milk yield was 0.72 (P<0.05). Generally, cows choose to be milked twice (38.5%) or three times (37.5%) a day, with an average production of 23.65±0.265 kg and 30.47±0.348 kg milk, respectively. Daily milk yield increased with number of milkings. Cows milked once a day produced on average 14.76±0.403 kg milk, while those that were milked 5 times a day produced 40.55±0.891 kg milk. We could state that daily milk yield and daily rumination time had similar evolution during lactation, but rumination time was not consistent. Cows preferred to use the AMS twice or three times a day.

Flow-adjusted automatic stimulation versus standard pre-milking routines

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Standard pre-milking is based on consistent and homogeneous working routines. Consequently, it does not account for differences between cows. Fact is, however, that the time elapsed between first tactile stimuli to cow´s milk ejection is very individual and mainly determined by degree of udder fill. A milk flow based system that both stimulated cows teats and synchronized the onset of milk extraction to the actual time of milk ejection was compared to traditional pre-milking routines. At a 1000 cow dairy farm in Germany (Brandenburg) the standard pre-milking routines (SPR) was used for one month, followed and compared to one month of automatic flow adjusted stimulation routines (FASR). Data were collected in early 2020 using DeLaval DelPro™ FarmManager. Two milkers milked 3x per day in a parallel parlour (D25) with an almost constant milking-interval of 8 hours. The standard pre-milking routines (SPR) consisted of premilking and cleaning of 6 cows (7-9 seconds per cow), then walking back and attaching same 6 cows (7-9 seconds per cow). The flow-adjusted stimulation routine (FASR) consisted of premilking & cleaning (7 seconds per cow) followed by immediately attaching the cluster, (7 seconds per cow) all in one step followed immediately by next cow. Comparing the parlour performance data, FASR reduced the average cups-on-time (including the time used for automated stimulation) from 271 to 254 seconds (-6,1%). FASR resulted in a higher peak milk flow (mean 4,34 vs 4,15 kg/min) and reduced the number of bimodal milk-flow curves from 42 to 24%. For the milkers, the FASR resulted in a 66% reduction in walking distance compared to SPR. A detailed analysis of data based on lactation stage shows that in particular cows in mid and late lactation (>DIM 150) benefit from automated and individualized stimulation. Among these cows 4 to 5% more milk was recorded whilst the milk yield per cow and session for total herd increased by 2,3% (10,2 to 10,4 kg). This data confirm that automated flow-adjusted stimulation reduces milking-point occupation time and thus improve parlour efficiency, as well as improve working condition for milkers.

Current use of contentious inputs in European organic animal production

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Contentious inputs are used by organic farmers to maintain production sustainability and high standards in animal health and welfare. Their implementation has detrimental impact on the environment and present a risk for the development of the organic sector. Information regarding the application of contentious inputs is not available at the European scale and it is a prerequisite to devise and implement roadmaps to phase them out. This work aimed to determine the extent to which antibiotics, anthelmintics and synthetic vitamins are currently used in organic livestock and to highlight differences in their controlled use. A multi-dimensional approach, incorporating: (1) literature reviews; (2) Europe-wide surveys or interviews of organic experts; (3) analysis of publicly available research data; and (4) national and international authorities (veterinary and agricultural organisations) was followed to quantify the current use and need of contentious inputs in European organic animal production. Overall, there was considerable variation in the use of anthelmintics and antibiotics reflecting country-specific differences in the level of pathogen threat and control. Estimates suggest that overall anthelmintic treatments do not exceed one treatment per animal per year in organic farms, although exceptions were noted. Extrapolated calculations indicate that on average 0.7 antibiotic treatments per animal per year are input in organic livestock systems. Dosages of synthetic vitamins are highly standardized, and decisions are made in the premix producing industry rather than on the farms. With few exceptions there are no differences between conventional and organic practice regarding dosage. Research-based background for organic-specific recommendations is therefore needed. The results provide the first benchmark on patterns of contentious input uses in organic livestock farming across Europe. Funded by EU H2020 No 773431 – RELACS.

Perception of organic livestock farmers about the organic farming situation in Europe

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An online survey to gather information about organic livestock production was conducted (Nov 2018- Feb 2019) among organic livestock farmers in 13 European countries. A total of 426 responses were analysed, of which 59% were French and Spanish farmers. Characteristics of the participants and farms were representatives of the European organic livestock sector. Farmers express their difficulty to obtain information on alternatives to antiparasitics (4.4/7) and antibiotics (4.3/7) compared to bedding (3.1/7). Feeding/nutrition (6.6/7), animal health (6.5/7) and welfare (6.5/7) were the most relevant issues in their farms. Yet, for farmers transitioning to organic farming systems, production costs (5.5/7), access to organic market (5.1/7) and animal health (5.1/7) were important factors. Although farmers report that the use of alternative therapies depends on the health issue and the usage of conventional treatments when necessary, in the last year, 64% of the organic farmers did not treat ≥90% of their animals. The three main source of information on alternative treatments were veterinarians (51%), other farmers (46%) and the internet (38%). In conclusion, organic farmers indicated more difficulty in retrieving information on alternative treatments than on bedding. Although production costs were decisive to become an organic farmer, animal health and welfare were the most relevant issues for those already farming organically. Despite rising popularity of phytotherapy, conventional treatment is still the predominant form of therapy. Funded by EU H2020 No 774340 – Organic-PLUS.

Session 51 Theatre 3

Mineral concentrations in milk from organic and conventional dairy herds in the South England

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Milk is a good source of major minerals and trace elements, which are essential for optimum health. However, milk mineral concentrations can be affected by animal diet (pasture characteristics, use of conserved forage and concentrate feeds) which also differ in organic and conventional dairy herds. This study aimed to: (1) investigate milk mineral concentrations in organic and conventional dairy herds; and (2) assess the relative influence of individual feeds. 809 milk samples were collected monthly between January-December 2019 from 43 conventional and 27 organic farms. Breeding and feeding practices were collected simultaneously via questionnaires. Analysis of variance by linear mixed effects models used production system, month, and their interaction as fixed factors. Farm ID was used as a random factor. A multivariate redundancy analysis assessed the relative impact of breed and feeds on milk mineral concentrations. Organic milk contained significantly more Ca (+53.7 mg/kg), K (+21.2 mg/kg), P (+14.0 mg/kg) and Mo (+12.7 mg/kg) but significantly less Cu (-8.2 µg/kg), Fe (-1.37 mg/kg), Mn (-16.2 µg/kg), Zn (-0.49 µg/kg) and Al (-0.82 µg/kg) compared with conventional milk. Significant seasonal variation was observed in the concentrations of all determined minerals, potentially reflecting dietary changes between indoor and grazing seasons. Milk I concentration, which is usually lower in organic milk, was not significantly affected by production system for most of the year, although organic milk still contained significantly less I in June-July. Dietary factors that contributed to the differences between production systems included intakes of maize silage, dry-straights and oils (higher in conventional diets), and pasture, clover and wholecrop (higher in organic diets).

Session 51 Theatre 4

NMR spectroscopy as a tool to discriminate between organic and conventional cow milk

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The organic farming system involves higher production costs and economic risks which must be converted into an adequate market price. The premium price of organic products increases the risk of frauds. Nuclear magnetic resonance (NMR) spectroscopy is a robust analytical technique with high reproducibility, minimal and cost-effective sample preparation. NMR technique is commonly used in metabolomics, allowing to obtain information on the state of the organism originating the sample. NMR spectroscopy and metabolomics have been applied in the dairy sector for the identification of the geographical area and frauds, the determination of nutritional and technological traits, and the authentication of production systems. The present study evaluated the sensitivity of NMR spectroscopy for the authentication of organic bovine milk from conventional milk. Bulk milks of 24 herds (12 conventional and 12 organic) were sampled between September 2019 and May 2020 in the North of Italy. Three bulk milk samples per farm were collected in different months for a total of 72 samples. Milk samples were analysed for traditional composition traits using mid-infrared spectroscopy and for metabolites using NMR. Among quantified metabolites, the acetate was significantly greater in organic than conventional milk. Multivariate and univariate approaches were used to assess the capability of NMR spectroscopy to discriminate between the farming systems. Models built using the Random Forest learning method exhibited an accuracy between 63.9 and 76.4% using the entire NMR spectra and the areas of the identified metabolites, respectively. The present research was funded with the support of EU Horizon 2020 under grant agreement N° 774340 (OrganicPlus project).

Session 51 Theatre 5

Plant feed additives as natural alternatives to synthetic antioxidant vitamins in organic livestock

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The restrictive regulation on synthetic molecules use, the development of organic livestock production and the consumers' demand for more 'natural', 'eco-friendly' and 'sustainable' products is rising the interest for the use of plant feed additives (PFAs) as natural sources of antioxidant in animal nutrition. The PFAs include aromatic plants essential oils, extracts and residues and by-products from plant processing. After a systematic review of the studies comparing PFAs antioxidant effects with synthetic antioxidant vitamins, a total of 46 peer-reviewed articles published in the last 20 years (2000-2020), were discussed. The literature on the use of plant feed additives denotes a general variability of the experimental protocols used to test their activity as antioxidant or in general their impact on animal performance and metabolism. The PFAs have been proven as an effective nutritional tool to counteract animals' oxidative stress with positive impact also on animal productivity and efficiency. The PFAs can partially or completely substitute antioxidant synthetic vitamins (the latter administered at doses between 150 mg/kg and 500 mg/kg) in animal rations, sometimes modifying important physiological functions. In some cases, they express a synergistic effect with the synthetic antioxidants. It is crucial to take in consideration the issues related to the absorption and metabolism of these additives, as well as their interaction with gut microbiota. Some form- and dose-dependent negative effect on growth performances have being observed, so further studies are needed to clarify these side effects for practical application. This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 774340.

Session 51 Theatre 6

Revision of vitamin E recommendations for ruminants in organic agriculture

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Vitamin E is essential, and supplementation on top of the native tocopherol contents in feed components is often needed to meet the animal's requirement. The recommended supplementation for ruminants may, however, be overestimated, especially in forage-based feeding systems where grazing or grass-clover silages are the basal feed with low to moderate concentrate levels in the diet (<40% of the DM intake). In such feeding regimes, which are frequent in organic animal husbandry, transfer rates for tocopherol may be higher than assumed in standard literature. We have done a systematic literature review, and critically evaluated the relationship between basal feed type and quality, forage to concentrate ratio, stage in animal production cycle, indicators of animal health and animal vitamin E status. Based on this information, we reassess dietary vitamin E requirements for cattle in organic systems. Subsequently, the revised recommendations are related to typical diet compositions of organically managed ruminants across Europe, and necessary additive supplementation levels will be estimated. The results will be presented as the basis for specific recommendations for the vitamin E supply in organic forage-based cattle husbandry. Funded by EU H2020 No 773431 – RELACS.

Scutellaria baiacalensis extract: effects on intake, health status and performances of beef cattle

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The present study evaluated the effect of a commercial product containing *Scutellaria baiacalensis* (SB) extract administered as a top dressing in the feedbunk during 160 d (beginning of the fattening period to slaughter) as supplemental antioxidant on intake, health, vitamin E status and growth performances of Charolaise beef cattle. A total of 143 male cattle were allotted into 12 pens of 11-12 animals each. The pens were assigned to one of 2 dietary treatments: control (463.9±21.48 kg body weight; BW) and treated (469.8±17.91 kg BW) in which 20 g SB/animal/d was added. Each thesis was replicated in 2 subgroups of 3 pens. Feed intake was measured monthly on a pen base by difference between the amount of feed delivered and orts of 2 consecutive d. The total mixed ration (1 kg) was sampled for each pen and pooled by subgroup for chemical analysis. The correspondent pooled faecal samples were collected and analysed to monitor digestibility. Blood samples were collected at the beginning of the trial and at slaughter for individual α-Tocopherol and metabolic profile determination (total protein, albumin, globulin, urea, NEFA, glucose, total cholesterol, triglycerides, AST-GOT, GGT, bilirubin, creatine kinase, Ca, P, Mg, BHBA). Final BW at slaughter (BWS) and carcass weight were measured individually to calculate carcass yield, average daily gain (ADG) and feed conversion ratio (FCR). Overall, no differences in digestibility were observed during the whole experiment. The dietary SB inclusion did not affect feed intake, growth performances, BWS, ADG, FCR, blood α-Tocopherol or metabolic profile. These findings suggest that the supplemental doses of SB do not exert a negative impact on diet palatability and animal health. The present research was funded with the support of EU Horizon 2020 under grant agreement N° 774340 (OrganicPlus project).

Organic pig and poultry farmers' attitudes towards ration planning

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Organic farmers are obliged to provide monogastric animals at all stages of their development with the nutrients they require. The specific requirements of young pigs and poultry for essential amino acids pose a challenge in organic feeding, where synthetic amino acids are not permitted. In addition, the use of conventional protein sources is restricted and organic purchase is limited by low availability and high costs. Due to highly heterogeneous nutrient contents in organic protein components, the formulation of appropriate feeding rations is therefore of major importance. A survey with 56 organic pig and poultry farmers was carried out in Germany to explore their attitudes towards ration planning. Factor-cluster analyses revealed four groups: Farmers in Group 1 (n=20) were characterised by uncertainty towards organic animals' nutrient requirements. At the same time, they accepted the importance of need-based feeding as a basis for health and performance and deemed regular ration adjustments necessary. Farmers in Group 2 (n=8) showed the same uncertainty, but without recognising the importance of need-based feeding and of regular ration adjustments. They believed that animals respond to nutrient shortages by reducing their performance and thereby modulating their demand. Need-based feeding and regular ration adjustment were most important to farmers in Group 3 (n=11), who rejected the idea of animals downregulating their demand. They were characterised by professional feeding regimes on their farms. Farmers in Group 4 (n=17) were least uncertain towards nutrient requirements and did not believe animals would lower their demand when undersupplied. Although the importance of need-based feeding was recognised, regular ration adjustment was of little significance for this group. In order to comply with the organic regulation, farmers must meet their animals' nutritional demands. This study identified a need for: (1) clarification of these demands to eliminate farmers' uncertainties; (2) monitoring tools that pinpoint the links between need-based feeding, performance and health; and (3) advisors and other professionals making an effort to understand their clients' attitudes.

Amino acid supply of slow- and fast-growing female turkeys in organic production

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Specific recommendations for the amino acid supply of turkeys selected under conventional conditions and raised under organic conditions do not exist. In especially, demands of slow- and fast-growing genotypes are unknown. Most critical are the first weeks of age where demands in amino acids are greatest. Given that synthetic amino acids are not allowed in organic production, alternative feed stuffs rich in essential amino acids especially lysine and methionine may be necessary when diets are formulated for 100% organic feeding. In a 4×2 study design four diets (complete feeding mixtures) were tested in a slow- (Auburn) and fast-growing (B.U.T. 6) turkey genotype. At two research facilities 960 one-day-old females were raised in groups of 20 animals. The study period of 8 weeks was divided into two 4-week feeding phases (P1 and P2). The G1 received 100% the recommended levels for conventional fast-growing genotypes of lysine (1.67 and 1.38 g/kg in P1 and P2) and methionine (0.59 and 0.51 g/kg in P1 and P2). The G2 received 90% the recommended levels in both phases, G3 80% in P1 and 90% in P2 and G4 received 80% in both phases. As protein components diets contained sunflower cake, pea protein concentrate and rapeseed kernel cake. At 4 and 8 weeks, animals were weighed, and feed consumption was measured at group level. In both phases, the group×genotype interaction affected body weight. In the slow-growing genotype body weight ranged between 2,993 g (G1) and 2,705 g (G4) where G1 and G2 differed to G3 and G4 ($P<0.05$). In the other genotype it ranged between 4,099 g (G1) and 3,471 g (G4) with differences ($P<0.05$) between all groups at 8 weeks. Feed intake but not feed conversion was affected by the interaction. In conclusion, diets with 90 and 100% the recommended lysine and methionine levels exceeded the demand of the slow-growing genotype, while the growth performance of the fast-growing genotype corresponded with the amino acid level in the diets.

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Effect of 100% organic feeding on performance, carcass composition and fat quality of fattening pigs

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In organic pig production, the aim is to achieve 100% organic feeding. According to the EU organic regulation (EC) No 889/2008 and the implementing regulation (EU) 2021/181, 100% organic feeding is mandatory for monogastric animals from 2022 onwards. This generates the challenge of achieving adequate protein quality in pig feed, which is currently still achieved by using 5% conventional components mostly in the form of potato protein. To investigate the effects of 100% organic feeding on growth performance, meat and fat quality in pigs, a total of 700 fattening pigs were studied in an on-farm feeding trial on three farms (A, B, C). The 95% organic diet fed on each farm was served as control diet (CON) and a 100% organic diet with higher soybean press cake and legume content was used as experimental diet (ORG). CON-fed animals achieved with 867 g higher average daily weight gains than ORG-fed animals with 825 g. Carcass weight and meat area were also reduced under diet ORG. A change in fatty acid composition in back fat was induced by diet ORG. Thus, iodine value (71.7 vs 73.7) and PUFA content (17.2 vs 19.2%) were significantly higher under this diet. 100% organic feeding seems to be associated with reduced performance and altered meat and fat quality. An adjustment of the requirements for performance, meat and fat quality of organic pigs at markets should therefore accompany the introduction of 100% organic feeding.

Session 51 Theatre 11

From the lab to the farm: using a multi-actor approach to foster sustainable parasite control

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While anthelmintics can be effective to control parasitic disease, due to their synthetic nature they are contentious inputs for organic systems. Development of alternatives is a prerequisite to reduce the environmental footprint of organic agriculture, whilst maintaining livestock health and welfare. To develop implementable and bespoke alternatives for parasite control, in RELACS we have followed a multi-actor approach which entails engagement with multiple stakeholders, throughout the project. At the first stage, information on current management strategies, farmers perceptions and potential use of alternatives was collected. Expert opinion (advisors and scientists), farmer interviews and surveys, and data analysis from previous projects, were the sources of information. This enabled us to identify two alternatives: heather, as a model bioactive plant, and nematophagous fungi, as a model biological control method. Farmers associations were interviewed to report on the grazing patterns of upland sheep and potential access to heather in different countries; they then orchestrated heather sample collection, which were sent to researchers for *in vitro* analysis. The optimisation of fungi's anthelmintic efficacy was achieved through a series of *in vitro* testing. Animal experimentation followed on, at the research facilities of RELACS partners. This step was vital to identify undesirable/complementary effects of heather and fungi supplementation on the health, productivity and welfare of the animals, prior to their roll out on farm. At the final stages of the project, scientists, farmers and farmer associations interacted in focus groups, to discuss the scientific evidence generated and to debate management practices and cost-benefit analysis of the alternatives. Some farmers carried out on-farm validations, to corroborate the experimental outcomes and identify constraints during the practical implementation of lab-developed tools. This multi-actor approach is expected to maximise impact and facilitate implementation of the alternatives for sustainable parasite control.

Session 51 Theatre 12

Anthelmintic efficacy of heather extracts against ovine gastro-intestinal nematodes

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The use of anthelmintics in organic farming is regulated; however its limited use is permitted due to GI nematodes threatening the health and welfare of animals worldwide. There is a requirement for a reduction of contentious inputs in organic farming, such as anthelmintics, and the H2020 project RELACS focuses on developing alternatives for sustainable parasite control. One of such alternatives is the use of bioactive plants; this study focuses on the perennial shrub heather (*Ericaceae* family) as it is a plant rich in condensed tannins, which have been shown to have anthelmintic properties, and the plant is highly abundant across Europe. The objective was to compare the anthelmintic efficacy of heather acetone extracts from five European countries, across two seasons, against two of the most common GIN species in small ruminants: *Teladorsagia circumcincta* and *Trichostrongylus colubriformis*. A total of 11 heather samples were analysed to determine their polyphenol content, including type and amount of CTs, and tested *in vitro* with the aim of quantifying their impact on egg hatching and larval motility. Results showed the heather extracts reduced egg hatching in a dose dependent manner ($P<0.001$) and reduced larval motility at the highest concentration of 200µg/ml ($P<0.05$). The anthelmintic efficacy of the heather varied by season, country of origin and the species of heather tested on both egg hatching and larval motility, with variation also seen on the susceptibility of the two GIN species egg hatching rate. These variations in extract activity were not always consistent between the two *in vitro* tests indicating that the active compounds may target different parasite life stages. Results demonstrate heather does show anthelmintic ability when tested *in vitro*, so has promise as a sustainable parasite control method and should be investigated further.

Duddingtonia flagrans: a promising biocontrol agent for gastrointestinal nematodes

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Controlling gastrointestinal nematodes (GIN) challenges owners of small ruminants with access to pastures. Biocontrol using the nematophagous fungus *Duddingtonia flagrans* is expected to complement existing alternatives for controlling GIN in grazing animals in the future. Animals receive spores of *D. flagrans*, which pass through the gastrointestinal tract. Fungal mycelium grows out of the surviving spores, spreads through the deposited faeces and forms structures with which it traps, colonizes and destroys the GIN larvae. This leads to reduced pasture contamination with GIN larvae and lower infection of grazing animals. An experiment with lactating goats, which were naturally infected with GIN, was carried out on an organic farm. Ten animals each received either a feed additive without *D. flagrans* spores (control), a feed additive with *D. flagrans* spores at recommended sheep/cattle dosage (normal) or a feed additive with *D. flagrans* spores at a10 times higher concentration than the normal dosage (high). Spores were administered daily to each animal individually during three days. Faecal samples were taken from all animals on the day before and on the last day of feeding spores. For each sample, faecal egg counts were determined by a modified McMaster technique. Samples were cultured for 14 days and larvae were subsequently obtained. Biocontrol efficacy of *D. flagrans* was calculated for each animal individually as a percentage reduction of developed larvae after treatment compared to the number obtained before treatment. As compared to the control, in the group with the normal *D. flagrans* dose infective larvae were reduced by about 20%, whereas reduction was almost 70% in the high *D. flagrans* dose group. In the H2020 project RELACS a similar setup was carried out with artificially infected sheep, using the normal and a 10 times lower dosage of *D. flagrans* spores. Infective GIN larvae were reduced by over 95% in faeces of both *D. flagrans* groups as compared to the control. Compared to goats, lower doses were required to substantially reduce GIN larval development in faecal cultures of sheep. Funded by EU H2020 No 773431 – RELACS.

Requirement recommendations for riboflavin in organic broilers

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GMO-free riboflavin is required for organic broiler diets. Currently it is produced by only one German manufacturer at high price. The recommended dosages are based on outdated studies, and current studies are rare. Due to high costs of GMO-free produced riboflavin, an accurate assessment of the requirement in poultry is of economic relevance. Furthermore, specific requirement recommendations for organic poultry do not exist. In the organic sector, slow-growing genotypes are used and diet composition partly differs from conventional systems. Both may influence riboflavin requirements. Four trials with riboflavin supplementations between 3.3 and 9.6 mg/kg feed DM in one- to three-phase feeding were conducted to identify the essential minimum amount of riboflavin supplements. The riboflavin source used was a GMO-free yeast strain (*Ashbya gossypii*). Across all experiments, the lowest dosages (2.5; 3.3; 3.5 mg/kg) resulted in lower performances and partly deficiency symptoms. Supplementation of 4.5 mg/kg feed DM was found to be a safe lower threshold. It was shown that an adequate supply is particularly important in the first phase of life, as certain early performance deficits cannot be compensated subsequently. A three-phase dosage gradation of 6.37; 5.28; 4.22 mg/kg for starter, grower and finisher diets, respectively, proved to be particularly suitable for generating best performance (feed conversion and efficiency). In conclusion, a general minimal supplementation of 4.5 mg/kg feed DM (as compared to 8-10 mg/kg starter feed and 6-8 mg/kg finisher feed currently used in the EU) is recommended for slow-growing broiler chicken in organic agriculture. Phase grading can improve efficient riboflavin use. Furthermore, the use of riboflavin-rich feed components such as yeast, whey and milk powder, alfalfa meal and grass meal or silages is recommended for on-farm feed mixtures.

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Animal health and welfare planning for European organic dairy herds – development and expansion

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Animal Health and Welfare Planning (AHWP) is a long-term continuous approach, as initially developed in the Core Organic ANIPLAN-Project. Within the framework of organic principles it is based on regular data collection on animal health and welfare and therewith farm specific. It identifies not only problematic areas but also farm specific successes. The approach is based on the inclusion of external knowledge and persons in the process to reach, finally, farmer ownership of the envisaged improvement measures. A team of farming advisors, veterinary practitioners and on-farm researchers from Spain, France, United Kingdom and Switzerland developed an improved RELACS AHWP protocol and introduced the AHWP approach for the first time in France and Spain. The improvement entails the introduction of the Farmer Field School approach (FFS), a specific form of facilitated farmer inter-collegial advisory in groups representing 5-7 farms. A facilitator is responsible for organization and moderation of the meeting as well as writing up and distributing the minutes to all participants afterwards. Each meeting comprises a farm walk (including the 'success case'), a structured discussion about two problematic areas pointed out by the host farmer and subsequent inputs from each individual participant on how to solve it. Discussion on each problematic area is closed by a conclusive statement from the host farmer about the next steps to guarantee a high level of farmer ownership within the process. In RELACS, 35 farms (4 from Spain, 11 from United Kingdom and 20 from France) are involved in 6 FFS groups. The impact of the advisory action will be determined based on a comparison with another 31 monitored farms (5 from Spain, 9 from United Kingdom and 17 from France) that did not undertake the FFS approach.

Session 51 Poster 16

In vitro testing of plant extracts and essential oils as antimicrobials in organic farming

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The aim of the present work was to test *in vitro* the antiparasitic and antimicrobial activity of some selected plant extracts (PE) and essential oils (EO) to explore their possible use in organic farming. Three PEs (*Malva sylvestris*, *Chamomilla recutita* and *Althaea officinalis*) were assessed for their antiparasitic properties against gastrointestinal worms through a traditional microscopic detection method (larval development assay, LDA) using a laboratory strain of *Haemonchus contortus* as target organism. Moreover, the Minimal Inhibitory Concentration (MIC) assay was measured on 8 EO following the CLSI guidelines (CLSI, 2018b) protocol, with minor modifications, on 4 main bacterial strains (*Escherichia coli* ATCC 25922, *Salmonella* Typhimurium ATCC 14028, *Staphylococcus aureus* ATCC 25923 and MRSA ATCC 43300). All the tested PE demonstrated antiparasitic effect up to 20 mg/ml concentration, but a stronger activity of *M. sylvestris* and *A. officinalis* than *C. recutita* was observed, even at lower concentrations. The average EO's MIC was 1.02% for *E. coli*, 1.5% for *S.* Typhimurium, 1.2% for *S. aureus* and 1.2% for MRSA. The lowest average MIC among all the tested strains was found for oregano oil (0.09%), followed by thymus oil (0.19%), tea tree oil (0.29%) and rosemary oil (0.8%). The highest average MIC were found for Lavender oil and Clove oil (2.57% each) followed by cinnamon oil (2.4%) and mentha oil (1.75%). Some of the tested PE and EO appear as viable alternatives to antimicrobials in organic farming based on the *in vitro* anthelmintic and antibacterial activity we observed. The setup of *in vivo* administration protocols (i.e. dosage, frequency of treatment) and the evaluation of potential side-effects deserve further studies. This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 774340.

Session 51 Poster 17

Evaluating the impact of essential oils on bovine mammary alveolar tissue cells *in vitro*

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The increasing resistance of bacteria to antibiotics and their regulated use in organic production systems has prompted research into the identification of new nutraceuticals with broad activity to reduce the use of contentious inputs in organic systems of production. Essential oils (EO) contain a wide variety of metabolites that are capable of inhibiting or slowing the growth of bacteria and offer a promising alternative for the control of mastitis; one of the most common infections requiring antibiotic treatments in both organic and conventional livestock farming. There is anecdotal evidence that French organic farmers use EO to minimise mastitis by applying it topically onto the bovine mammary gland, however scientific quantification of their efficacy and evaluation of their potential toxicity is lacking. The focus in H2020 RELACS project is to identify EO which can safely be used to reduce antibiotic input in organic systems of dairy production. This study aimed to test *in vitro* the toxicity of 12 EO, reportedly used by French organic farmers. The impact of each of the EO on the growth and proliferation of bovine mammary alveolar epithelial cells (MAC-T) was monitored using the xCELLigence® real time cell analyser (RTCA) system over 48 hours and compared against a control (sunflower oil). All 12 EO were screened for cytotoxic effects at a concentration of 0.1%. The results showed that 10/12 EO had an impact on cell growth. Of these, two EOs had slightly higher cell indices than the controls, indicating that they may promote cell growth. The eight remaining EO had lower cell indices compared to the controls, indicating they may promote cell death. In conclusion, the results identify potential cytotoxic risks associated with the use of EO when tested *in vitro* on a monolayer of MAC-T cells. Cytotoxicity results may vary if used on the complete mammary organ *in vivo*, however our *in vitro* data provide clear indication for potential cytotoxic risks of some EO. *In vivo* toxicity tests and/or close monitoring of the application site should always accompany the use of EO for mastitis control in organic dairy animals to ensure their welfare.

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Selecting botanicals for mastitis control: efficacy and safety characterization of essential oils

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Organic Farming principles for health care and welfare give preference to preventive methods. When prevention is not enough, the EC Organic Regulation states that organic livestock should be treated preferably using phytotherapeutics. However, there is a lack of methodology to assess the assumed effects and the safety for human consumption of these non-antibiotic alternatives. In France, farmers' groups have been using essential oils to control mastitis on dairy cows to explore alternatives to the use of antibiotics. A RELACS multi-actor panel developed an innovative approach to evaluate essential oil's effectiveness, safety, main effects on animal health, and final product quality. Based on a selection of 10 essential oils reported to be efficient by farmers to control mastitis, their effects in several major mastitis pathogens were quantified using aromatograms. Cytotoxicity tests using mammalian epithelial cells were performed. Chromatograms provided a chemical characterization of the active molecules thus linking them to presumed effects according to their composition. Bibliographic reference research on each essential oil for mastitis control and/or presenting antibacterial activity and bibliography about its potential risk for humans and animals completed this work. These results were compared with the regulatory status and availability of the essential oils. As a result, two essential oils were selected to be further evaluated on commercial farms. The second round of tests analysed the effects on milk sensory and technical quality, using milk samples of animals treated with essential oils. Tests included: detection of markers of the essential oils in the milk, their potential impacts on acidification and sensory properties of milk, and verifying the absence of reaction in a test to detect antibiotic residues. Funded by EU H2020 No 773431 – RELACS.

Session 52 Theatre 1

Climate-Care-Cattle farming systems: introduction and first results of the CCC farming project

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At the European level the EU is committed to ambitious polices to educe GHG-emissions from agriculture by 30% in 2030 compared to 2005. Agriculture belongs to the sectors which lie outside of the European Emission Trading system (ETS). Agriculture has thus to follow the so-called Effort Sharing Regulation. Livestock systems need both to adapt and mitigate in the face of climate change. For the cattle sector, almost two-thirds of all GHG-emissions take place within the farm gate (in particular rumen fermentation, manure handling and land cultivation) and almost one-third by the production of inputs (especially fertilizers and concentrates). This study focusses on the processes within the farm itself, addressing two-thirds of the GHG-emissions. But it will also include the interaction with the input and output flows of the chain when relevant. The objective of the CCC farming project is to develop climate smart cattle farming systems which reduce GHG and ammonia emissions while maintaining sustainable social-economic farm businesses. Emission reductions will be achieved by the use of innovative housing and manure handling systems, (such as use of composted bedding material and separation of faeces and urine), artificial floor constructions, manure cleaning robots, cow toilets, novel cropping systems, precision crop management, virtual fencing and ITC data collection techniques like using tools and drones. Promising feeding, breeding and grassland mitigation practices will also be examined. The CCC farming project will deliver an assessment of the environmental and socio-economic performance of the given range of mitigation measures applied on a network of study field farms in eight EU-countries on basis of NPC balance tools and emission measurements. Progress and experiences of the field work and first results will be presented.

Session 52 Theatre 2

Consumer acceptance and willingness to pay for cow housing systems in eight EU countries

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Consumers are concerned about the well-being of livestock. Freewalk housing systems (compost bedded and artificial floor) aim at increasing cow welfare; however, little work has evaluated consumer preferences for housing systems and their products. The purpose of the study was to estimate consumer acceptance and willingness to pay of products from tie-stall, cubicle, compost bedded and artificial floor housing, and attitudes towards grazing and re-using compost. Focus groups were held in in Germany, Austria and Slovenia, to identify concepts for a quantitative survey. Locations were chosen to represent different willingness to pay (WTP) for and attitudes towards animal welfare products. Two 90-minute sessions of six to 10 people were held in each country. Amount of available space, slaughter conditions, and type of transport were associated with animal welfare. Purchasing local foods was also felt to be important and associated with better quality. While the groups in Austria and Germany discussed needing to lower meat consumption and increasing government regulations, Slovenian groups did not bring up these topics. The quantitative survey was conducted online in Austria, Germany, Italy, the Netherlands, Norway, Slovakia, Slovenia, and Sweden in 2019 with nationally representative samples of 400 to 600 participants per country. The majority of consumers believe animal welfare can be improved. The compost bedded system is liked best in all countries and has an association with better animal welfare. Moreover, there is an overall positive attitude towards re-using the compost from the bedded pack housing. The choice experiments show further evidence of consumer heterogeneity for milk product preferences with almost half of consumers preferring organic milk and a tie-stall housing system, 40% preferring organic milk and grazing, and 10% preferring only grazing. Although consumers like the free walk systems, it does not translate into a higher WTP across all consumer groups. Consumers appear to prefer milk produced organically and/or with grazing over conventional production with the freewalk systems.

The future of dairy calf housing: group housing systems and the use of precision technologies

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Dairy calf raising is a short but critical phase for appropriate physical, behavioural, and cognitive development that lasts into adulthood. Group housing systems and enhanced milk feeding programs are becoming common; these practices have been deeply studied in the last decades. Conventional raising may lead to short-term and long-term effects when compared to calves raised with peers. There are many practices associated with group housing of calves, here we will discuss problems and suggested solutions, especially related to cross-sucking, competition, aggression, and disease control. In a recent review we found that social housing improves solid feed intakes and calf weight gains before and after calves are weaned from milk to solid feed, especially when calves are fed high allowances of milk. Another change that is happening in the dairy industry is the use of precision technologies to monitor individual calves continuously. It is rapidly becoming an important management tool on-farm for calves; automated monitoring of feed intake and feeding behaviour is particularly common. We present some of the technologies available, and two key uses of technology that show promise to refine calf management through individual monitoring. First, changes in feed intake and behaviour using data from automated feeders can identify pre-clinical signs of illness in calves. Second, feeding behaviour from automated feeders may also reveal which calves respond to antibiotic treatment (success), and which will may not recover. Third, data from technologies can be used to support nutritional management decisions and culling of calves from an early age. Automated feeders can be used to wean calves automatically based on their intakes of grain; we found a large variability in grain intakes and weaning ages when weaned using this method, and how individual characteristics may be used to identify those calves that will successfully wean early and cull animals. Changes in the calf raising systems are happening and the potential of group housing and precision technology tools are likely to become more common on farms, and consequently improve calf management strategies that affect the success of future dairy replacement animals.

How to reduce GHG emission – latrine training of dairy calves

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Due to high ammonia and other greenhouse gas (GHG) emissions from livestock farming, both technical and behavioural solutions for reducing discharges need to be developed. Ammonia is formed when faeces and urine come into contact. A behavioural approach to facilitate separation of excreta would be to train cattle to use a latrine. Reliable latrine use would require cattle, like other species, to learn to control a range of voluntary and reflex responses associated with toileting. So far, this has not yet been reliably demonstrated. In a proof-of-concept approach, we aimed to determine if it is possible to train calves to use a latrine for urination. 16 calves across two replicates were trained individually every other day in 45 min sessions on a step-wise learning procedure including: entering and leaving the latrine via one-way gates and accessing food rewards in the designated latrine area (habituation and magazine training); associating the latrine with urination (training phase1); and moving into the latrine from an adjacent laneway (training phase 2 and 3). Animals were free to enter and leave the latrine at any time. After entry to the latrine, urination events occurring within 90 s were rewarded. Urination events occurring outside of the latrine were discouraged by the administration of water from an overhead sprinkler. The learning curve for the calves that successfully passed phase 1 was steeper (0.73±0.08) than for the calves that did not (0.27±0.03; W=60, P=0.001). Eleven calves were successfully trained to use the latrine for urination in phase 2. Calves with steeper learning curves in phase 1 were more successful in phase 2 (P=0.036). In phase 3, nearly three-quarters of all urinations were self-initiated in the latrine. These results provide convincing evidence that cattle can be trained to use a latrine for urination. This innovative behavioural management allows for effective mitigation of GHG emission.

Session 52 Theatre 5

Phenotypic and genomic analyses of microscopic differential cell counts in compost bedded pack barns

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New free walk housing systems such as compost bedded pack barns (CBPB) might positively influence udder health. However, for in depth-analyses, it is imperative to consider better udder health indicators than somatic cell count. Furthermore, genomic mechanisms with regard to udder disease resistance in CBPB are unclear. In this study, milk samples from 2,198 udder quarters of 537 cows housed in CBPB or conventional cubicle barns (CCB) were collected. Microscopic somatic cell differentiation focused on the fractions lymphocytes (LYM), macrophages (MAC), banded (bN) and segmented neutrophils (sN), with the latter two grouped together as polymorphonuclear neutrophils (PMN). Furthermore, specific mastitis pathogens such as *Aerobe bacteria* (AER) and *Aesculin* hydrolysing streptococci (AESC) were determined bacteriologically und analysed individually or used to define bacteria groups like four most important pathogens (4MI) and major pathogens (MAJOR). Production system comparisons revealed higher prevalences of mastitis pathogens and proportions of cell fraction bN in CCB compared to CBPB. Heritabilities of cell fractions ranged from 0 to 0.138 (PMN), and those of specific mastitis pathogens from 0 to 0.091 (AESC). Genome-wide association studies (GWAS) considering 43,095 SNP marker genotypes were performed. Datasets were split into CCB and CBPB, but also a new GWAS approach with interaction between housing system and SNP effect was applied. We identified nine SNPs significantly associated with bacteria AER, AESC and bacteria groups 4MI and MAJOR in CCB. In contrast, only one significant SNP for the cell fraction bN was found in the CBPB dataset. Furthermore, we identified six significant interactions between SNP and housing system for PMN, MAJOR, MINOR, AER, AESC and for a culture negative result. Some of the potential candidate genes could have an influence on inflammatory processes. Differences of significance values for SNPs and traits between systems CCB and CBPB as well as significant SNP × housing system interactions indicate the impact of environmental particularities on genetic mechanisms, suggesting production system specific breeding approaches.

Session 52 Theatre 6

Characteristics of solid manure from freewalk housing compared to slurry, and appreciation by farmer

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In the EU-project 'FreeWalk' new housing systems for dairy cattle are investigated in eight countries. The freewalk housing system is compared with reference cubicle housing systems. These systems represent two floor types: a slatted floor, resulting in slurry, and a composting bedded pack or artificial floor, resulting in solid manure, respectively. The two types of manure have different characteristics. Slurry has a relatively high water and mineral content and low organic matter. The solid manure from the bedded pack is mixed with bedding material like straw or woodchips and has a relatively high content of organic matter. On four farms from different countries these two types of manure are sampled and analysed: at the beginning when new bedding arrived, in the middle and at removal of the bedding just before application. The analyses show large differences between the two types of manure. It is of interest if farmers' appreciation of the manure types corresponds with these assumptions. This was evaluated with a questionnaire. Freewalk farmers applied solid manure types on both grassland and arable land. Reference cubicle farmers applied solid manure types mainly on arable land. Two-third of the farmers appreciated liquid manure types solely as fertiliser and one-third as both fertiliser and soil improver. More than half of the farmers appreciated the solid types of manure solely as soil improver and one quarter as both fertiliser and soil improver. The cubicle farmers appreciated liquid manure types more frequent as soil improver next to fertiliser than the freewalk farmers; the freewalk farmers appreciated the solid manure types more as fertiliser next to soil improver than the cubicle farmers.

Individual cow methane emissions in freewalk farming systems and associations with breeding traits

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62% of the greenhouse gas (GHG) emissions in Germany are attributed to the agriculture sector. Regarding livestock species, dairy cows are the main producer of methane (CH_4). The aim of the present study was to assess individual CH_4 emissions from dairy cows, which were mostly kept in alternative farming systems (e.g. in compost bedded pack barn), and to associate derived CH_4 parameters with production and functional traits. In this regard, we measured individual CH_4 emissions using a mobile laser detector, considering 1,200 cows kept on 9 dairy farms. Aiming on a longitudinal data structure for CH_4, the total number comprised 2,000 CH_4 measurements. In a first step, we focused on CH_4 trait derivations (eructation and respiration traits) considering different approaches, based on either a double normal distribution-, a boxplot-, a SD- and a quantile-method. The quantile method generated most reliable results with regard to physiological background information, and was used in ongoing analyses. Via mixed model applications, we inferred the impact of fixed and random effects on the total (allsum) and mean (allmean) CH_4 output, on the total (respsum), mean (respmean) and maximal (respmax) exhaled air CH_4, and on the total (erucsum), mean (erucmean) and maximal (erucmax) CH_4 output during eructation. Most significant environmental impact on all CH_4 traits was due to humidity, wind speed, farm type (organic or conventional, compost or cubicle), and observer. In a second mixed model analysis, we inferred the impact of the CH_4 traits allmean and allsum on production, female fertility, energy efficiency and disease traits. Both CH_4 traits were significantly associated with protein percentage ($P<0.001$), and with fat percentage, milk yield and somatic cell count ($P<0.05$). Least squares means for milk yield increased with increasing CH_4 output, and vice versa, fat and protein percentage decreased. Least square means for somatic cell count decreased with increasing CH_4 output. In a final step, we will develop an overall breeding index for freewalk farming systems, including the CH_4 traits allmean and allsum, and considering all the trait associations from the present study.

Innovations in bedded pack barns

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In Israel, the FW housing system with dried manure as bedding is predominant. In other countries in Europe and America different bedding material is used like wood chips or saw dust. The solutions to keep the bedding dry may differ between countries because of differences in temperature, humidity and solar radiation. Also regulations about environmental issues may differ like emissions and reuse of wastewater. Points for further development of bedded pack barns will be illustrated. In a dry climate, an entire building area could be dedicated to walking and lying areas for cows and young stock. Such barns have no feed alley for tractors and no slatted or solid floor. The feeding is done with simple mobile feed troughs spread around the bedding area, which are moved daily to prevent the bedding areas around the feeders from becoming excessively dirty. The capital expenditure for this type of barn is approximately 40% less than that of traditional bedded pack barns. Also in a dry climate, milking parlour wastewater can be spread into the bedding. Consequently, the farm no longer discharges large amounts of wastewater into the sewer system. To keep the bedding dry in areas with high humidity, it is important to have sufficient m^2 per cow, bedding height and dry bedding material. To reduce emissions, a new housing system, the multi-climate shed, will be illustrated. The multi-climate is achieved with a 'tent' type additional roofing above the feeding area. This tent creates a microclimate within the entire building with respect to temperature, humidity and fresh air, and it provides the possibility of removing gasses (ammonia and methane) and odours. The V shape of the tent structure enables the collection and removal of air. The 'in house biofilter' (bedding with wood chips) filters the emission of ammonia and odour by transporting the air on top of the building through pipes toward the floor beneath the bedding into the bedding material. The methane gas will be burned when the air is released from the barn by passing through a burner or flame outlet. Capturing of air to burn methane can also be done above a mobile feeder or in cubicles.

Session 52 Theatre 9

Towards dairy housings providing animal welfare and reduced ammonia emissions

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Ammonia (NH_3) reduction measures in animal housings are often attributed as trade-offs with animal welfare. Two structural measures were investigated in a dairy housing for emissions measurements in three seasons (summer, autumn, winter) with regard to their NH_3 reduction potential as well as animal welfare aspects. The housing consists of two experimental compartments which enable comparable measurement conditions (e.g. climate) on a practical scale. To determine emissions under natural ventilation, a dual tracer-ratio method was used. Relevant accompanying parameters were recorded (e.g. climate, feed, milk data, area's soiling) to describe each measurement situation. With regard to the effects on animal welfare, depending on the individual measures, specific behaviours such as slipping, displacements and eating were investigated. Firstly, the solid floors in one compartment were designed with a 3% transverse slope and a urine-collection gutter to enable a rapid urine drainage. Initial results showed a NH_3 emission reduction of around 20% in the compartment with floors with slope and urine-collecting gutter compared to the reference without slope. The number of slipping events differed only slightly between the two floor variants. In case of the second measure 'feeding stalls' the raised platform with partitions guided the cows at the feeding place in a way, that very little excrements accumulated on the platform, and thus led to reduced heavily soiled area. First results showed a NH_3 emission reduction for feeding stalls of around 8% in summer, 19% in autumn and 16% in winter compared to the reference without feeding stalls. The feeding stalls had no influence on the feeding duration per day and the number of displacements at the feeding barrier. However, they resulted in a lower number and therefore longer undisturbed feeding periods per cow and day. In conclusion, the applied NH_3 reduction measures did not show disadvantages with regard to animal welfare. The feeding stalls improve the conditions for dairy cows in loose housings. For instance, the manure scraper in the aisle behind feeding stalls can run frequently without disturbing the animals while they are eating.

Session 52 Theatre 10

RCT shows living space impacts the reproductive and yield performance of housed dairy cows

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Current literature does not provide a clear understanding of the relationship between cow health, welfare productivity, and the housed environment. Of particular note, is the dearth of evidence on living space requirements. Objective To evaluate the impact of living space on cow production and reproduction, including underlying physiological changes. A randomised controlled, long term (364 d) study was designed to evaluate a spatial intervention. Cows were matched based on parity and days in milk and randomly allocated to a control group (total: 9 m^2 per cow, GB median) or 'treatment' group (total: 14 m^2 per cow, GB upper 95%ile). All other aspects of the environment were matched between groups and both groups experienced identical management routines. Data collection included all key reproductive events, ultrasound scanning to quantify follicular development, blood sampling to assay anti-Mullerian hormone and milk sampling to assay progesterone. Production data included daily yield per cow, rumination time and bodyweight. Data analysis comprised time to event and mixed effects modelling, conducted using R statistical software. The group with more living space produced significantly more milk; an increase of 366 litres per 305 d lactation. Rumination times were 15 minutes longer per day in cows given more space. There was a reduced time to conception in the control compared to the treatment group (hazard ratio 0.60, P=0.03). Commencement of luteal activity showed no significant difference between groups and no differences were found in time to first service, AMH concentrations, endometritis scores or antral follicle counts. Housed cows produced substantially more milk when provided with the same feed but greater living space. However, the cows with greater living space took significantly longer to conceive than cows in the control group. This is the first study to identify alterations in reproductive and production performance solely attributable to a change in living space allowance in dairy cows.

Nematode infections in chickens: impact and challenges

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This paper reviews the impact of nematode infections on chicken performance and health, and presents challenges for diagnosis and a sustainable control of infections. Nematode infections of chickens in European farms have been re-emerging during the last decades. This is mainly related to the EU-wide ban on conventional cage systems in 2012. Recent data indicate that even indoor housing systems are susceptible to nematodes with short life cycles. The most prevalent species are *Ascaridia galli, Heterakis gallinarum* and *Capillaria* spp. High worm burden with multi-parasite species are common. Along with impairment in host performance, particularly in those chickens of high-performing genotypes, nematodes can act as vector for transmission of other pathogens, e.g. *Salmonella enterica* and *Histomonas meleagridis*, and have the potential to modulate vaccine-induced humoral immunity. So far no resistance to anthelminthics has been reported for the most commonly used drugs for chickens in the EU. Heavy reliance on anthelmintic drugs nevertheless produces a continuous selection pressure on nematodes, particularly when proportion of worms in refugia is low. This results inevitably in the development of drug-resistance in nematode populations, implying the necessity of non-pharmaceutical solutions to control the infections. Currently there is no vaccine available against chicken nematodes, leaving the most powerful prevention tool out as an option. The most important prerequisite for any nematode control strategy is an accurate and precise quantification of the infections. There has been considerable effort towards development of diagnostic tools, including coproscopic (e.g. FLOTACs), serologic (ELISAs) and molecular techniques (PCRs). There are however naturally occurring patterns (e.g. circadian rhythms) and parasite-parasite interactions (e.g. density dependency) influencing nematode egg excretion that eventually interfere with estimations of infection intensity through coproscopy. The most promising future strategies to control nematode infections include nutritional tools, genetic selection of parasite resistant individuals, biological control of parasitic stages in and outside the host animal, and targeted-selective treatments.

In vitro evaluation of the anthelmintic potential of plant extracts against *Ascaridia galli*

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The present study aims at finding efficient alternatives among ethno-veterinary herbs. *Ascaridia galli* eggs isolated from the worm uterus were exposed *in vitro* to 9 methanolic extracts (ME) in dimethyl sulfoxide (1%) from the cultivated plant species *Achillea millefolium* (AM), *Artemisia absinthium* (AA), *Artemisia vulgaris* (AV), *Cicerbita alpina* (CA), *Cichorium intybus* (CI), *Inula helenium* (IH), *Origanum vulgare* (OV), *Tanacetum vulgare* (TV), *Tanacetum parthenium* (TP), positive (flubendazole) and negative controls. The ability of different ME concentrations (0.5, 0.325, 0.2 mg/ml) to affect the embryonation rate of *A. galli* eggs was assessed in duplicate/ME/concentration. Eggs' embryonic development (ED) was evaluated (560 eggs/replicate) from the day of egg isolation until day 28, resulting in the examination of 40,320 eggs. Analysis performed using generalized linear mixed model, stating a negative binomial distribution, having plant species and concentration as fixed effect and day as repeated measure. The highest ME concentration of the species CA and TV showed significant lower ($P<0.05$) ED estimated at 35±3.17 and 38±3.29% followed by TP and IH with 40±4.02 and 44±4.47% consecutively. Lower tested concentrations had similar patterns with CA and TV having the best performance, showing a dose dependent effect. ME extracts obtained from mentioned plant species have promising results in inhibiting ED, contributing to the identification of alternative anthelmintic treatments against *A. galli*. The present study is part of the 'HERBAL' project that has received funding from the GECT 'Euregio Tirolo-Alto Adige-Trentino', 3rd call.

Immunomodulatory and performance-enhancing effects of dietary resin acids in broiler chickens

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In-feed coniferous resin acids have been reported to improve the performance of broiler chickens and to reduce the inflammation-associated collagen degradation in intestinal epithelium. Here we studied the effect of a resin acid concentrate (RAC) on the growth performance of broiler chickens and on the expression of ten immune response -related genes in their intestine. On day 1, 280 male Ross 308 hatchlings were allocated into 20 pens, 14 chicks/pen. The two dietary treatments, both with 10 replicates, included a wheat-soy -based control diet (CON) and the same diet amended with 176 g/ton of RAC (40% resin acids + 60% wheat flour; Hankkija Ltd, Finland). The birds were weighed on days 1, 21 and 35. Feed intake and feed conversion ratio (FCR) were determined for periods d1-21, d21-35, and d1-35. On d21, gut mucosal tissue samples were collected from 2 birds/pen. The samples were stored in RNA-preserving solution and analysed for the expression of nuclear factor (NF)-κB subunits 1 and 2, NF-κB inhibitor α, matrix metalloprotein (MMP)-2, MMP-7, MMP-9, interleukin (IL)-6, IL-10, tumour necrosis factor (TNF)-α and peroxisome proliferator activated receptor-ϒ, relative to two housekeeping genes: RNA polymerase II subunit B and Tatabox binding protein. Independent sample's T-test and Pearson's correlation analysis were used for data analysis. Compared with CON, the body weight of RAC-fed birds was higher by 8.6% on d21 and by 5.7% on d35 ($P<0.05$ for both). Feed intake was increased by RAC for d1-35 (+7%, $P<0.05$). RAC improved FCR (-6 points, $P<0.05$) for d1-21, and tended to improve FCR also for d21-35 and 0-35 ($P<0.1$ for both). The expression of the immune modulator NF-κB subunit 2 was higher in the RAC than CON group ($P<0.05$), but the other genes showed a similar level of expression in CON and RAC treatments. Bird body weight correlated negatively with the expression of the three inflammation-associated MMP-genes. The results support earlier findings on the performance-enhancing effects of in-feed resin acids in broiler chickens, and suggest a beneficial immunomodulatory activity through the NF-κB subunit 2 gene.

Assessment of the capacity of sepiolitic clay (E 563) to adsorb aflatoxin B1 in poultry diets

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Aflatoxins are mycotoxins that are produced by two major *Aspergillus* fungi, *A. flavus and A. parasiticus.* The main aflatoxins are B1, B2, G1 and G2, being B1 the most toxic. The consumption of aflatoxin contaminated diets has a negative effect in zootechnical parameters such as feed conversion and body weight gain. In order to reduce the risk of aflatoxin contaminated diets, different strategies can be developed. Aflatoxin binders as clay minerals, because of their adsorption capacity, may sequestrate aflatoxins and avoid their absorption in the gastrointestinal tract of the animal. From previous *in vitro* study, it has been shown that sepiolitic clay can adsorb aflatoxin B1. For this reason, the main aim of the present study was to determine the ability of sepiolitic clay to absorb aflatoxin B1 in *in vivo* conditions. A total of 60 Ross 308 broilers, from one-day old to 28 days were used. Five treatments were tested: T1 (diet without adding neither aflatoxin B1 nor sepiolitic clay), T2 (diet was contaminated with 400 µg/kg of aflatoxin B1, no sepiolitic clay added), T3, T4, and T5 (diets were contaminated with 400 µg/kg of aflatoxin B1 and were supplemented with 0.5, 1 and 2% of sepiolitic clay, respectively). During the global study period (1-28 days of trial), significant differences were observed between groups ($P<0.05$). Growth was significantly higher in group T4, and lower in group T2 at 28 days of trial (1,736±49 vs 1,562±30 g, $P<0.05$). In addition, feed efficiency improved in groups T4 and T3 with respect to the group T2 (1.30 kg/kg and 1.34 vs 1.43 kg/kg, respectively). Regarding the liver lesion, the histological analyses showed a proliferation increase of bile ducts (chronic lesion) with T2. No significant differences were observed on haematology and biochemistry analysis for most parameters measured. The results showed that sepiolitic clay (E 563) is capable to adsorb aflatoxin B1 with high affinity and stability supporting those results observed in the *in vitro* study. Furthermore, it was observed that the supplementation of sepiolitic clay improved the conversion index and the body weight gain in poultry.

Impact of black soldier fly larvae oil in slow growth broiler diet on breeding and meat parameters

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Black Soldier Fly is considered as a promising source of proteins and fats for animal nutrition. In particular, Black Soldier Fly larvae (BSFL) oil can be used to replace plant-based oils in broiler chicken diets. However, to date, only a limited number of small scale studies were conducted on this topic. The aim of the present study was therefore to evaluate the impact of the replacement of soybean oil (SO) by BSFL oil in the diets of slow-growing broiler chickens reared according to the French 'Label Rouge' specifications across performance, health, animal welfare and meat quality. 8,800 chickens were randomly allocated to either a control group (CTRL, 100% soybean oil) or a test group (TEST, 100% BSFL oil) and reared for a total of 84 days in commercial conditions. Health and welfare indicators, as well as feed intake and waste were monitored all along breeding. Upon slaughtering, growth performance indicators (daily weight gain; DWG, feed conversion ratio; FCR) were calculated. Physico-chemical parameters, ultimate pH (pHu) and colorimetric analysis were measured on meat samples, and meat sensory traits were evaluated by a qualified jury panel. No adverse effect of the replacement of SO by BSFL oil was shown on meat quality and sensory traits, and a positive impact of this replacement was evidenced on growth performance, health and welfare parameters. A superior total feed consumption (+0.66%) and DWG (+1.93%) were measured in TEST group. The FCR was also improved in TEST group (-1.08% compared to CTRL). An improvement of faeces quality and animal welfare indicators was also noted. Results therefore suggest that replacement of soybean oil with BSFL oil in slow-growth broiler chickens can generate benefits on breeding and slaughtering parameters, with no major changes in meat quality nor organoleptic perception by the consumer.

In ovo modulation of 'muscle health' with synbiotics – effect on vascularization and myopathies

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The effect of *in ovo* application of synbiotics on the occurrence of histopathological changes in the superficial pectoral muscle was analysed. The *in ovo* techniques allowed the introduction of the beneficial factor during embryogenesis, when the muscle fibres start to develop. On 12ED, the embryonated Cobb 500FF eggs (n=5,850) were injected with 0.2 ml of 1/physiological saline, 2/SYN1 composed of GOS (Clasado Ltd., UK) and *Lactobacillus salivarius IBB3154* and 3/SYN2 composed of RFO (lupin oligosaccharides) and *Lactobacillus plantarum - IBB 3036*. The roosters (n=2,040) subject to rearing were fed the standard feed mixtures *ad libitum*. The superficial pectoral muscles were sampled (n=15/per group) directly after the slaughter, on d 42. The histological analyses included quantitative determination of fibre necrosis, giant fibres and splitting, fibre diameters and the blood supply (H+E and Brown 1976 staining). The biochemical analyses of protein levels, cholesterol, liver enzymes, and mineral levels in serum was included. Injecting synbiotics *in ovo* significantly increased the blood supply to superficial breast muscle. The highest density of capillaries, both within the analysed muscle area and per muscle fibre were found in SYN1 group. Also, the SYN1 group showed a higher no. of normal fibres and the least necrosis and splitting, compared with the control. The results suggest a relation between the observed blood supply and the occurrence of pathological changes. A significant increase in Ca and P in the blood of SYN1 chickens was found. Ca affects proliferation of endothelial cells, and it's levels are increasing in response to pro-angiogenic factors. P affects genes with pro-angiogenetic activity. The *in ovo* applications of natural prebiotics and probiotics require further attention, as they can be potentially used to improve the health of muscles and reduce occurrence of myopathies in broilers. The funding: Polish Ministry of Higher Education statutory research BN- 51 and 52/2019, and the Polish National Agency for Academic Exchange GA No. PPI/APM/2019/1/00003.

Panel discussion

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Panel discussion

Impact of feeding oregano, sage and lavender essential oils on broiler gut microbiota community

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This trial was performed to evaluate the impact of feeding essential oils on broiler chicken gut microbiota populations. 360 one-day-old male broiler chicks (Ross-308) were randomly allocated to 4 treatments with 6 replicates (of 15 chicks), housed in floor pens. Over a period of 42 days, control treatment (A) was fed with commercial maize-soybean meal diet. In the other treatments (B, C, D), these diets were further supplemented with essential oils of oregano, sage and lavender; B: 50, 25 and 25 mg/kg, respectively; C: 100, 50 and 50 mg/kg, respectively; D: 200, 100 and 100 mg/kg, respectively. After slaughter, ileum and caecum samples were collected, DNA was extracted and 16S rRNA amplification, Illumina sequencing and bioinformatic sequence analysis was performed. In the cecum, Firmicutes, Bacteroidetes, Actinobacteria, Proteobacteria and Cyanobacteria were the most representative phyla in all treatments (A: 50.5, 42.8, 2.9, 2.1 and 1.6%; B: 72.6, 10.3, 15.4, 1.1 and 0.3%; C: 82.2, 9.6, 5.7, 1.5 and 0.4%; D: 74.2, 20.6, 0.6, 3.8 and 0.8%, of the sequences respectively). In the ileum, Firmicutes, Actinobacteria, Proteobacteria and Cyanobacteria were the most abundant phyla in all treatments (A: 84.1, 14.4, 1.3 and 0.1%; B: 90.3, 0.9, 1.3 and 1.2%; C: 92.2, 2.1, 0.1 and 0.01%; D: 99.8, 0.06, 0.1 and 0.01% of the sequences respectively). Based on this analysis, the control treatment A had distinct profile from the supplemented treatments. Acknowledgments: This research has been co-financed by Greece and the European Union (European Regional Development Fund) in context ‘Research-Create-Innovate’ within the Operational Program (Competitiveness, Entrepreneurship and Innovation (EPAnEK) of the NSRF 2014-2020. Project Code: T1EΔK-03856. Acronym ‘Green Poultry Meat Antifree’.

Probiotics potential and microencapsulation of lactic acid bacteria as potential source for poultry

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The presented study aimed to isolate, identify, characterize and assess the benefits of lactic acid bacteria (LABs) from the poultry gastrointestinal tract as potential lyophilized probiotics products. The cell morphology of 15 isolates was screened by macroscopic and microscopic examination. Probiotic properties including survivability in simulated gastric juice (pH 2 and 3), bile salts (0.3% ox gall), haemolysis activity, and antibiotic susceptibility were evaluated. The LABs were identified by API 50 CHL, ABIS online soft, and 16S rRNA sequencing. The microencapsulation cultures of selected strains with high probiotic potential were prepared by the spray-drying method. Maltodextrin and glucose were used as encapsulating agents. The microencapsulated strains were evaluated for their viability by dilution method. From all isolates, only 7 strains were confirmed as *L. acidophilus* (IBNA 26, IBNA 64), *L. fermentum* (IBNA 37), *L. brevis* (IBNA 50), and *L. salivarius* (IBNA 29, IBNA 33, IBNA 41). The fresh bacterial cultures showed significant ($P<0.05$) pH (73.74 to 98.20%) and bile salts resistance (77.89 to 99.49%). The antibiotic susceptibility test presents 100.00% resistance of LABs to oxacillin, with multiple antibiotic resistance indices above 0.5. The microencapsulated process was applied for IBNA 64, IBNA 33, and IBNA 41 where viability registered 69.35, 79.15 and 79.76% (10^6-10^8 cfu/g) after spray drying. In conclusion, only *L. salivarius* (IBNA 33 and IBNA 41) could be used as probiotics for poultry fields with satisfactory viability for enhancing the performance and pathogens control by equilibrating the intestinal microflora. The present study received financial support UEFISCDI through the project 8PCCDI /2018- PC 2, and part of activity received technical support from Romanian Ministry of Education and Research by Project No. PN 19.09.01.04.

The impact of tributyrin and essential oils on broiler chicken cecum microbiota populations

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The aim of this study was to evaluate the impact of feeding phytobiotics and butyrates on broiler chicken gut microflora populations. 360 one-day-old male broiler chicks (Ross-308) were randomly allocated to 4 treatments with 6 replicates (15 chicks), housed in floor pens for 37 days. Treatment A (Control) was fed a basal diet; B diet was supplemented with a phytobiotic mixture (oregano oil 50 mg/kg; garlic oil 5 mg/kg; dried sage 2 g/kg; Crithmum 1 g/kg); C diet was supplemented with tributyrin (1 g/kg); D diet was supplemented with both the phytobiotic mixture and tributyrin. At day 37, cecum digesta samples were collected, DNA was extracted, and 16S rRNA amplification, Illumina sequencing and bioinformatic sequence analysis was performed. Firmicutes, Bacteroidetes, Actinobacteria, Proteobacteria, Cyanobacteria, Euryarchaeota, Synergistetes, Spirochaetes and Verrucomicrobia were the most representative phyla in all treatments: A showed 59.0, 36.0, 1.0, 2.3, 0.46, 0.30, 0.39, 0.20 and 0.21% of the sequences respectively; B 61.0, 35.2, 1.85, 1.57, 0.2, 0.03, 0.08, 0.07 and 0.004%; C 56.6, 36.2, 0.8, 4, 0.8, 0.6, 0.4, 0.3 and 0.01%; D 62, 32.4, 0.9, 2.7, 0.6, 0.5, 0.6, 0.2 and 0.01%. Proteobacteria, Cyanobacteria, Euryarchaeota, Synergistetes and Spirochaetes were more abundant in treatments C and D. Treatment B had the highest Actinobacteria and the lowest Proteobacteria, Cyanobacteria, Euryarchaeota, Synergistetes and Spirochaetes percentages. Acknowledgments: This research has been co-financed by Greece and the European Union (European Regional Development Fund) in context 'Research-Create-Innovate' within the Operational Program 'Competitiveness, Entrepreneurship and Innovation (EPAnEK) of the NSRF 2014-2020'. Project Code T1EΔK-03856. Acronym 'Green Poultry Meat Antifree'

Session 53 Poster 11

The effect of hatching system on serum diamine oxidase levels in chicks

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In the poultry industry, a new concept is being explored to overcome some of the drawbacks of conventional hatcheries: *on-farm hatching*. In on-farm hatching systems, broiler hatchery eggs are transported to the broiler house on day 18 of incubation (ED18). Chicks hatch on ED19 and ED20, and have immediate access to light, nutrients and water. Previous studies showed that these systems provide chicks with an improved intestinal health, a lower feed conversion rate and a reduced use of antibiotics. However, the underlying mechanisms resulting in these differences have not been extensively studied yet. To assess the effect of hatching system on intestinal development, the level of diamine oxidase (DAO), an intestinal epithelial cell-specific protein that is used as an indicator for intestinal permeability (IP), was measured in serum samples. An *in vivo* experiment was conducted to analyse the effect of hatching system on serum DAO levels. A full factorial experiment was designed, taking into account commercial age (minus two days (D-2), D-1, D1 and D2) and hatching condition (on-farm hatched and NestBorn) as factors. Fifty serum samples per sampling day and per group were taken, resulting in a total of 400 Ross 308 chicks used during the experiment. Chicks that hatched in the hatchery did not receive water, nor feed until arrival at the farm on D1. DAO activity in the serum samples was analysed by spectrophotometry. Analysis of the serum samples showed that serum DAO levels in NestBorn chicks were significantly lower ($P \leq 0.01$) compared to their hatchery-born counterparts on all four days. An extensive literature study showed that DAO is frequently used as an indicator for intestinal development; it is suggested that higher serum DAO levels correlate with increased IP and thus with lower intestinal development. This could indicate that intestinal development in NestBorn chicks is better than in their hatchery-born counterparts. However, although showing promising results, more research is needed to establish DAO as a serum immune biomarker for IP.

Session 53 Poster 12

Evaluation of the current model for environmental risk assessment of antibiotics in the EU

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Antibiotics applied to livestock represent a risk for the environment and non-target organisms including humans. As part of the approval process of antibiotics, an environmental risk assessment (ERA) has been carried out for the last 15 years in the European Union. During this time, European and national legal requirements as well as livestock production systems and animal health management changed. The aim of the study was to critically review the ERA for antibiotics considering recent husbandry systems and monitoring data of antibiotic consumption. In a systematic approach, all 19 questions of the ERA decision process were compared to the relevant environmental risks determined by a risk analysis for frequently used antibiotics in livestock production. For this purpose, the risks were first described, structured and analysed. The results were presented in a risk matrix, which demonstrates the effects and the probability of occurrence. We found that, from an environmental and one-health perspective, it was doubtful why the ERA and the monitoring applies to selected animals only, especially considering the rededication for minor animal species. Due to the national monitoring of antibiotic consumption, except for poultry, herd treatments were widely replaced by small group or individual treatments leading to major reductions in the total consumption of antibiotics. The current model excluded all veterinary medical products used to treat a small number of animals from further assessment, which was quite understandable at the time of the model initiation, but will likely not lead to further improvements. Another important aspect was the categorization of the husbandry system as either reared on pasture or housed in (warm) stables. Nowadays, a broad variety of husbandry systems with hourly access to pasture or free-range production systems are used. The lack of valid data on animal numbers in these husbandry systems is one factor promoting that modelling entry pathways to the environment turns out to be challenging. Based on our data, we propose an update of the ERA and a standardized, systematical animal census including information about their husbandry systems.

Session 53 Poster 13

Standardized natural essential oils prove their repellent effect on chewing lice

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Wing lice (*Lipeurus caponis*) and body lice (*Menacanthus stramineus*) are common ectoparasites in worldwide poultry production, responsible for feather shearing and skin scrapping then imbibing the host's blood. Infestations not only impact human and animal welfare but also the economy due to stress, anaemia, weight loss, decreased laying rate and death. To face it in a sustainable and environmentally friendly way, natural repellents like essential oils (EOs) are viewed as a promising solution. This study assessed the repellent effect of a standardized EO mixture (SEOM), composed mainly of EOs from *Cymbopogon nardus* and *Eugenia caryophyllus*, on these lice in layers. 22,000 layers, about 100-weeks old, were divided into 11 sections. Hens were infested with wing and body lice for about 16 months. The hens were fed a standard diet supplemented with SEOM at 750 ppm for 7 consecutive weeks. The intensity of lice was estimated with traps through 3 sections (4 traps/section). This evaluation was performed 3 times: 2 days before starting the trial; 6 weeks after supplementation; 3 weeks after stopping supplementation. Statistical analysis was performed by using ANOVA. The first assessment was done with the average of 72 lice/trap, in which wing lice represented about 36% and body lice 64%. After 6 weeks giving SEOM feed, the average lice population reduced significantly to 44 lice/trap ($P<0.05$). More precisely, the body lice decreased dramatically by 83% and the wing lice went down 17% compared to the initial infestation. Moreover, the repellent effect of SEOM was observed up to 3 weeks after stopping the SEOM supplementation. The total lice density continuously dropped by 52% which differs significantly ($P<0.05$) compared to the intensity before treatment. In conclusion, SEOM supplementation in feed for 6 weeks resulted in significantly restricting the proliferation of wing and body lice within such experimental condition. This effect lasted until 3 weeks after stopping. The protocol was adapted to be as close to practical conditions as possible, since a real control group was not possible to set up. Further studies with controlled conditions are recommended to reinforce the results.

Session 53 Poster 14

Saponins and citroflavonoids reduced growth of *Histomonas meleagridis in vitro*

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Histomonas meleagridis is a flagellate protozoan parasite living in the caecum of the digestive system of birds. It is the causative agent of histomonosis or commonly known as 'Blackhead disease' in turkey. While chicken are asymptomatic carriers of the parasite, the effect on turkey is more dramatic. Indeed, the mortality due to this infestation can reach 80 to 100% leading to important economic losses. Feed supplementation with plant-based solutions may help. This study was performed in collaboration with North Carolina University, USA in order to assess the *in vitro* the effect of active compounds of Citronin XO (CXO), Nor Feed SAS; France, on the growth of *H. meleagridis* and compare their effects to metronidazole. 100,000 *H. meleagridis* cells grown in log phase were added to Dwyer's media with the compound to be tested and incubated at 40 °C for 48 hours. Metronidazole a 12.5 ppm was used as a positive control, while media was used as a negative control. saponin/citroflavonoids solution was applied at 500 ppm. Counts was performed using a Neubauer hemocytometer. 3 replications per treatment and each replicate was counted 4 times. Results show that Both metronidazole and CXO reduced the growth of *H. meleagridis in vitro*. As expected, incubation with metronidazole reduced the growth by 99.65%.Active compounds of CXO, namely saponins and citroflavonoids, were able to reduce the growth by 70% after incubation with the parasite. Results from this *in vitro* study showed that saponins and citroflavonoids active compounds of CXO reduced the growth of the causative agent of blackhead disease by 70% in these experimental conditions. These results support the fact that feed supplementation with CXO could contribute to improve turkeys' gut health and reduce antimicrobial use in livestock. *In vivo* studies with and/or without parasite challenge are needed to confirm this *in vitro* results.

SheepNet: the technical and practical knowledge reservoir to increase EU sheep productivity

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The EU is the second largest sheep meat importer in the world. An increase in ewe productivity of 0.1 lambs reared per ewe joined would increase EU meat supply by 64 thousand tonnes and self-sufficiency to 92%. Ewe productivity (number of lambs reared per ewe joined) has not improved in many countries in the past 30 years. SheepNet, an EU funded thematic network, with the objective of increasing sheep productivity, ended in 2019. SheepNet involved the 6 main sheep producing countries in the EU (Ireland, France, United Kingdom, Romania, Spain and Italy) and Turkey. SheepNet was about practice-driven innovation and practical knowledge among stakeholders and used multi-actor and codesign approaches to promote and establish durable exchange of scientific and practical information among researchers, farmers and advisors across Europe. The SheepNet network stimulated knowledge exchange and promoted the implementation and dissemination of innovative and best technologies and practices for the improvement of sheep productivity. A total of 976 and 337 stakeholders participated in 34 national and 5 EU multi-actor meetings, respectively. SheepNet produced 218 technical articles and videos including 42 solutions and 73 'tips and tricks' on factors impacting ewe productivity; undertook on-farm evaluations of many of the solutions; and described 19 sheep rearing systems. Most of the communications are translated into 6 languages and are available on the SheepNet website (www.sheepnet.network). The SheepNet reservoir provides the technical and practical knowledge required to improve sheep productivity and profitability across the EU.

Response to male effect in two Greek indigenous sheep breeds, Karagouniko and Florina

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Greece has a long history of ovine dairy products, many of which are an integral part of cultural heritage. However, ovine milk production in the Mediterranean presents a pronounced seasonal character, wherefrom many sheep breeds exhibit seasonal oestrus behaviour and ovulation. This seasonal pattern induces variations in milk production, inconvenient for both producers and industry, causing income losses. Keeping this in mind, the control of the animal's reproductive season is an essential management tool. Among the various methods for controlling ovine reproduction, such as the administration of exogenous hormones, prostaglandins and photoperiodic treatments, the implementation of male effect is the most eco-friendly method. Nevertheless not all breeds response equally to this treatment. Here, we evaluate the response to male effect methodology in two indigenous Greek sheep breeds, Florina and Karagouniko. Fifty ewes from each breed were examined, isolated from ram contact for more than 90 days. Ram introduction took place on June 2020 at a ration of 1 ram per 10 ewes. All animals were kept under the same feeding and management conditions. The percentage of oestrus was 87% in Florina ewes and 100% in Karagouniko ewes post male introduction. Specifically, Florina ewes expressed oestrus at a percentage 19% on Days 16-17 and 68% on D20-25 and gave birth in November and December, respectively. Karagouniko ewes expressed oestrus at a percentage of 21% on D16 and 79% on D21-25 and gave birth in December. The fecundity was 80% for Florina and 94% for Karagouniko ewes. The prolificacy was 1.46 and 2.22% in Florina ewes and in Karagouniko, respectively. In conclusion, both breeds indicated a positive response to male effect, which was more intense in Karagouniko. The research work was supported by the Hellenic Foundation for Research and Innovation (H.F.R.I.) under the 'First Call for H.F.R.I. Research Projects to support Faculty members and Researchers and the procurement of high-cost research equipment grant' (Project Number: HFRI-FM17-2987)

Session 54 Theatre 3

Factors influencing the short term ovarian response of milking Lacaune ewes to the male effect

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During a 5-year longitudinal study, we assessed the main factors impacting the short-term ovarian response of ewes to the male effect (ME) in a commercial organic dairy sheep farm. Each year the cyclicity before ME and the ovarian response following ME was monitored within a flock varying from 228 to 269 adult Lacaune dairy ewes. Mature rams (from 3 to 12 each year), equipped with an apron that prevented mating, were introduced at D0 (day of the ME) for 14 days. Blood samples were collected at D-10, D0 and D+11 to measure plasma progesterone concentrations and determine the occurrence of cyclicity before the ME as well as the proportion of ewes responding to the ME. We collected for each ewe five continuous and two categorical explanatory variables: age, lambing to ME (LME) interval, total milk production, third milk control, last litter size and body condition score (BCS). The BCS was classified into three categories: High ($BCS>3$), Medium ($2<BCS\leq3$) and Low ($BCS\leq2$). Binary logistic regressions were performed within-year analysis while a mixed-effect binary logistic regression model was used for the between-year analysis. Cyclicity before the ME and the response of ewes to the ME were highly impacted by the year of the study (from 29 to 61% and 31 to 85%, respectively). The ewes that combined relative high BCS, low milk production at the third milk control and long LME interval were most likely to be cyclical before ME ($P<0.05$; $P<0.001$; $P<0.001$, respectively). On this same parameter, age also had a positive linear effect on the probability of being cyclical ($P<0.05$). Regarding the response to the ME, ewes with a relative high BCS, low milk production at the third milk control and high age were most likely to respond ($P<0.001$; $P<0.001$; $P<0.001$, respectively). In conclusion, we show that the ME can disrupt the inhibitory effects of the photoperiodic signal depending on the nutritional status of milking ewes. Since this study was conducted in a commercial farm, we established several practical recommendations to maximize the performances of the ME.

Session 54 Theatre 4

Oestrus and fertility responses in ewes treated with modified Ovsynch and progesterone therapy

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The development of economical interventions to enhance reproductive performance during out of season breeding in ewes is still warranted in India to drive their utilization. Aim of the study was to evaluate reproductive efficacy of short, medium or long-term modified Ovsynch plus progesterone (P4) therapy protocols in ewes. The crossbred (Nali×Rambuillet) ewes were enrolled into 4 groups for out of season breeding (May-July; summer season). The Ovsynch protocol (GnRH, 4 µg buserelin acetate-PGF2α, 125 µg cloprostenol-GnRH, 4 µg buserelin acetate) with modifications was employed in 3 treatment groups sequentially on days 0, 5, 7 (Group-I, short-term); on days 0, 8, 9 (Group-II, medium term) and on days 0, 12, 13 (Group-III, long-term). This was combined with P4 therapy (with AVIKESIL-S® intravaginal P4 sponges, each having 350 mg P4) for 5 (0-5), 8 (0-8) and 12 (0-12) days in Group-I, -II and -III, respectively. One group (Group-IV) of ewes was kept untreated as control group. Each group comprised of randomly selected 50 ewes. Five proven breeding rams per group were used for mating with the ewes exhibiting oestrus. Data on oestrus activity and fertility variables were recorded for each group. The oestrus induction rates in ewes of group-II (52%, $P<0.01$) and group-III (38%, $P<0.05$) were significantly greater than in Group-IV (20%, control) ewes. The oestrus duration was significantly longer in ewes of group-II than in group-IV (35.6±3.3 vs 26.5±2.3 hours, respectively). The pregnancy and lambing rates in ewes of group-II (48%, 46%; respectively) were significantly greater than in group-IV (16%, 16%; respectively). However, prolificacy and fecundity was similar among the groups. Serum P4 levels at sponge removal were lower ($P<0.01$) in group-III ewes compared to others. Serum P4 (day 28 post-mating) and estradiol-17β (at oestrus) profiles were also higher ($P<0.05$) with medium term protocol compared to others. In conclusion, the modified protocol with P4 therapy (of 8 days duration as in Group-II compared to short and long-term) resulted in better augmentation of fertility in ewes during out of season breeding in the subtropical climate.

Session 54 Theatre 5

Influence of heat synchronization and feeding programs on reproductive performances of Awassi sheep

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Use of prostaglandins is an alternative method for controlling reproduction by inducing luteolysis of the corpus luteum and inducing a subsequent follicular phase with ovulation, The objective of this experiment is to study two possible hormonal programs, to detect the best protocol for the synchronization of heat cycles in a herd, taking into consideration dietary effects. The experimental design includes 86 Awassi ewes divided into 4 groups; FAPA, receiving a hormonal treatment using an intravaginal sponge impregnated with 40 mg of FGA (fluorogeston acetate) in combination with PGF2α injection 2 days prior to sponge removal, and a normal feed diet; FAPB, receiving a protocol consisting of double injection of PGF2α 12 days apart, as well as a normal feed diet; FBPA, receiving a feed diet with flushing, alongside the sponge and prostaglandin protocol; and finally FBPB, receiving feed with flushing, with double injection of prostaglandin. Onset of oestrus, oestrus duration and oestrus response were registered, with other reproductive parameters such as copulation, mounting, and preference. F-test, t-test and ANOVA in SPSS 20.0 and excel were used. Results showed no difference between groups for the onset of oestrus ($P<0.05$); treatment with PGF2α alone showed an average of 38.59±14.32, while treatment with FGA combined with PGF2α showed an average of 39.37±13.21. Duration of heat was longer in ewes receiving an intravaginal sponge with PGF2α injection (30.14±20.03 h) than in those receiving prostaglandin treatment alone (18.23±16.31 h). Oestrus response showed no difference between groups with a total average of 84%. And a marked increase in the preference behaviour was seen in ewes receiving progestagen pessaries and the flushing effect than those receiving prostaglandin and a normal feed diet.

Session 54 Theatre 6

Development of diagnostic tests for subclinical mastitis in sheep at the point of care

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Subclinical mastitis is an issue of moderate significance for animal health, but of great importance for the sustainability of dairy sheep farms. Since ovine milk is used mainly for the production of premium-quality cheeses and yogurts in Mediterranean countries, the microbiological quality of milk should be high in order to avoid milk-borne illnesses which might challenge public health. Therefore, according to European legislation, the control of *Staphylococcus aureus* is mandatory to mitigate the risk from the occurrence of strains with a zoonotic potential (Directive 92/46ECC Council, 1992). In the early stages of intramammary infections, pathogens invade the mammary epithelium of the animal, directly infecting mammary epithelial cells. The aim of our study was to establish a novel *in vitro* mastitis model using 2D and 3D cell culture and proteomic technologies, in order to identify early biomarkers, occurring during subclinical mastitis in ewes. Subsequently, these biomarkers were used to develop novel point-of-care (POC) diagnostic tests, allowing for the early and accurate detection of subclinical mastitis. We have successfully isolated and established primary 2D and 3D cell lines of mammalian epithelial cells from mammary tissue of ewes. Using two mastitis associated bacterial strains, namely *S. aureus* (NCIMB 701499) and *Escherichia coli* (NCIMB 702070) we determined the appropriate multiplicity of infection that reproduces the conditions of subclinical mastitis. Proteomic analysis identified inflammation factors expressed during the first stages of infected mammary epithelial cells. Using the identified biomarkers, two novel POC diagnostic immunoassays have been developed for the detection of subclinical mastitis in ewes, which are currently under validation. These tests are simple, quick and non-invasive, whereas, they have the potential to serve as tools for the active surveillance of mastitis and the on-site assessment of milk hygiene. This research is co-financed by Greece and the European Union (European Social Fund- ESF) through the Operational Programme 'Human Resources Development, Education and Lifelong Learning 2014-2020', project code MIS5048811.

Session 54 Theatre 7

Evaluation of a multi-actor farm team in prudent use of antibiotics in French and Greek dairy sheep

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The prudent use of antibiotics in animal production can be quite challenging, as each farm faces specific health problems. To evaluate a farm-specific approach, 10 dairy sheep farms were selected in the Roquefort cheese area of France (n=5), and in Peloponnese and Thessaly in Greece (n=5), as part of 42 case studies by EU H2020 network DISARM in pig, broiler, dairy cow or dairy sheep sectors. A Multi-Actor Farm Health Planning (MAFHP) was prepared by facilitators using questionnaires for farmer, veterinarian and farm advisor about the challenges of each farm, potential actions and perspectives on antibiotic use. The Biocheck® questionnaire was adapted to evaluate the biosecurity status at dairy sheep farms. During Multi-Actor Farm Health Team (MAFHT) meeting, health challenges were prioritised by the different team members. Smart action points were defined and documented in a farm action plan. This plan has then been monitored, evaluated and adapted in the follow-up meetings of the MAFHT. In some dairy sheep farms, lambing biosecurity appeared to be a weak point, as there is no physical separation from the rest of the flock. During milking, the farms did not practise udder disinfection before or after milking, except two in Greece. On the other hand, external biosecurity was high in all farms. In both countries, mastitis and lamb mortality were the main concerns. Alternatively, abortions and even parasitism were of higher concern in France than in Greece. In some farms, the use of antibiotics was more as routine administration than occasional necessary use. The Greek health groups decided to reinforce internal biosecurity at lambing, whereas this was a bottleneck in France, with sheepfolds being too small and the workload too heavy. A common decision for all groups was to make systematic analyses to search for causes of abortion. Almost all French and Greek groups tried to improve early detection of mastitis using cell counts and California Mastitis Test. This study discusses the first progress in animal health and results in antibiotic use. These experiences show the value of working as a team for the improvement of animal health.

Session 54 Theatre 8

The use of olive cake by-product in ruminant diets

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The Mediterranean countries produce the 98% of olive oil worldwide and generate large quantities of olive by-products that could be considered as alternative feedstuffs with the intention to reduce both the feeding costs and the environmental impacts associated with waste accumulation. Among these by-products, olive cake (OC) is the most abundant containing nutrients and bioactive compounds that vary according to the cultivation conditions (geographic origin, year, season), the process of oil extraction (three or two phase centrifugation), or form and preservation method (crude, partly-destoned, dried or ensiled). With regards to nutrition, OC has a high fibre content, an attribute that makes its inclusion in ruminant diets as a replacement of forages, particularly useful in areas with roughage scarcity. However, its low protein and energy content, or the seasonal availability, possible rancidity and the need for processing may hinder its regular application. In the present review, data from the literature will be presented from earlier and recent studies conducted in ruminants evaluating the effects of various forms of processed OC on milk yield and composition as well as on the lipid profile of meat, milk and dairy products. Then, the proposed underline mechanisms of OC effects on intake, ruminal fermentation, metabolism and milk and dairy products quality will be discussed, suggesting possible ways of this by-product utilisation in ruminant diets.

Influence of synchronisation protocols and feeding programs on vaginal flora of Awassi sheep

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Intravaginal synchronisation devices constitute a predisposing factor for the vaginitis caused by opportunistic micro-organisms leading to a high incidence of unfertilized ova, as well as, negatively affecting ewes' sexual attractiveness. The objective of this experiment is to study the influences of synchronisation and feed protocols on vaginal flora, of Awassi ewes in Lebanon. The experimental design included 86 Awassi ewes, divided into 4 groups: FAPA (Feed A and Protocol A), received an intravaginal sponge impregnated with FGA (fluorogestone acetate) and 1 ml of 10% oxytetracycline solution, in combination with PGF2α injection 2 days prior to sponge removal, and a control ration; FAPB (Feed A and Protocol B), received a double injection of PGF2α 12 days apart, as well as a the control ration; FBPA, received a flushing feed protocol, that consists of adding 400 g more to each individual ewe of the control ration, alongside the sponge and prostaglandin protocol; and finally FBPB, receiving the flushing protocol, with double injection of prostaglandin. Vaginal swabs using eNAT, were taken, before sponge insertion and after sponge removal, from the 4 groups. Swabs were cultured on 5 different media agars, in search for, *S. aureus*, *S. epidermidis*, *P. merabilis*, *E. coli*, *Salmonella* spp., *Enterobacter* spp., *P. vulgaris* and *Lactobacillus* spp., and to assess total bacterial count. For every measure, Z-Tests (F-Test) were carried out using SPSS 20.0 and Excel. Results showed no difference between groups for the onset of oestrus, oestrus response with a total average of 84%. Duration of heat was longer in PA ewes (30.14±20.03 h) than PB (18.23±16.31 h). *S. aureus* recorded a significant increase after sponge removal, in both protocols ($P<0,05$) (150% PA, 166% PB). Also, PA recorded (1,98) a higher preference than PB (0,35), and FAPA (2,64) registered the highest preference average next to FBPA (1,29) ($P<0,05$).

Effect of the presence of adult companion animals on rumen microbial colonization in goat kids

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Newborn ruminants are usually separated from the dam and fed on milk replacer in intensive production systems. The lack of contact with adult animals until weaning may hinder rumen development at microbiological and physiological levels. In this study, we aim to assess the effects of rearing newborn goat kids in contact with adult companions. 32 newborn goat kids were randomly allocated to either a experimental group isolated from adult animals (CTL) or sharing the same pen with two non-lactating adult goats (CMP). Goat kids had *ad libitum* access to milk replacer, starter concentrate and oat hay. Weaning was performed abruptly at 7 weeks of age. Blood and rumen samples were taken at 5, 7 and 9 weeks of age to evaluate blood metabolites and rumen microbial fermentation. Next generation sequencing was carried out in rumen samples at 7 weeks of age. CMP animals presented higher rumen pH, iso-acids and butyrate molar proportion, with the significant differences of the latter being greater as time went by. Blood β-hydroxybutyrate concentration was higher in CMP goat kids than in CTL across all sampling times, and the ratio BHB/glucose tended to be higher in CMP kids. The methanogens abundance was greater in CMP goat kids and no protozoa were detected in CTL animals based on optical counting. The rumen bacterial community in CMP kids was significantly more biodiverse and had a distinct structure compared to the bacterial community in CTL kids. The core rumen bacterial community in CMP animals had 53 more OTUs than that of CTL animals. Furthermore, the number of overlapping OTUs with the two adult companions was >4 times higher in CMP kids than in CTL kids. Higher levels of early rumen colonizers Proteobacteria and Spirochaetes were found in CTL kids, while Bacteroidetes tended to be more abundant in CMP kids. The prevalent rumen methanogens species were different in the two experimental groups. An important rumen microbial transfer was observed from the adult goat companions to the CMP kids, which accelerated the rumen microbial development and increased the complexity of the rumen microbial community.

Ultrasound measurements of eye muscle depth in rams of two sheep breeds in Slovenia

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The aim of the study was to introduce eye muscle depth measurement with ultrasound as selection trait in breeding programmes of two Slovenian sheep breeds, the autochthonous Jezersko-Solčava (JS) sheep and Improved Jezersko-Solčava (JSR) sheep. Eye muscle depth was measured with ultrasound equipment on 274 rams on two test stations at the end of performance test in year 2020. On the alternative test station Jezersko were 28 rams of JS sheep, while on the test station Logatec 148 rams of JS sheep and 98 rams of JSR sheep were tested. The average eye muscle depth was 2.23 cm at the average age of 265 days. We used the GLM and MIXED procedures in SAS to analyse the collected eye muscle depth data. The fixed part of the model included breed-test station interaction (JS-Jezersko, JS-Logatec, JSR-Logatec), litter size (1, 2+) and dam parity (1-2, 3-4, 5-6, 7-8, 9+). Age at scoring was included as a linear covariate. Random part of the model included random effect of flock of origin. Analysis of variance showed that breed-test station interaction, dam parity and age at scoring had a significant effect on the eye muscle depth, while the effect of the litter size was not significant. JS rams on the alternative test station Jezersko had significantly thinner eye muscle (1.94 cm) compared to JS and JSR rams on the test station Logatec (2.24 cm and 2.31 cm, respectively). The difference between breeds on the test station Logatec was not significant. Rams born from the seventh and eighth parities had significantly higher eye muscle depth than rams born from other parities, but this could be a random association. The effect of flock of origin explained 17% of phenotypic variance. In the future, we will continue with eye muscle depth recording and develop breeding value estimation.

Inclusion of sainfoin in the fattening concentrate: performance and carcass traits of light lambs

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The aim of this study was to assess the effect of the inclusion of sainfoin (*Onobrychis viciifolia*) at different rates (0, 20 and 40%) in the concentrate on productive parameters and carcass characteristics of fattening lambs. After weaning (BW: 14 kg; age: 30 d), 26 male lambs of Rasa Aragonesa were distributed in 3 isoproteic and isoenergetic feeding treatments: one group was fed with a commercial concentrate with 0% of sainfoin (Control), a second group was fed with a concentrate with 20% of sainfoin (20 SF) and the third group was fed with 40% of sainfoin (40 SF). The lambs were allocated in individual pens and fed *ad libitum* during all the fattening period. The individual dry matter intake (DMI) was recorded daily. The lambs were weighed once a week and the average daily gain (ADG) was calculated. At day 40 of trial, all the lambs were slaughtered and the carcass characteristics were registered (hot and cold carcass weights, fatness, fat and *Rectus abdominis* colour). The DMI was similar among treatments until the 5th week when the inclusion of 40% of sainfoin increased DMI compared to the other treatments. Weight gains of the lambs were affected by the inclusion of sainfoin ($P<0.05$), increasing with the inclusion of 40% sainfoin compared to their counterparts (290, 281 and 333 g/d for Control, 20 SF and 40 SF, respectively). The final BW tended to be greater for the lambs fed the 40% sainfoin concentrate than for the other lambs (24.5, 24.1 and 26.3 kg, for Control, 20 SF and 40 SF, respectively; $P=0.10$). The carcass weights and dressing percentages were similar among treatments ($P>0.05$), but the weight of the perirenal fat tended to increase with the inclusion of sainfoin (91, 115 and 139 g for Control, 20 SF and 40 SF, respectively; $P<0.10$). The inclusion of sainfoin did not produce changes in the colour of *R. abdominis* and caudal fat deposits either ($P>0.05$). An inclusion of 40% of sainfoin in the fattening concentrate would be advisable as it increased weight gains and had no negative effects on carcass characteristics.

How omega 3 enriched diets of sheep affect the microbiota of produced kefalograviera cheese

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This trial examined the possible effect of omega-3 enriched sheep diets on the microbiota of traditionally produced kefalograviera, a well-known Greek hard cheese. Forty ewes (Frizarta and Lesvos crossbreed) were randomly allocated to either a control dietary treatment (fed alfalfa hay, straw and concentrates) or a treatment that had 20% of soybean meal in the concentrates replaced by flaxseed and lupin seeds. After one month of feeding, milk was collected to produce Kefalograviera cheeses at the same cheese factory by using a traditional recipe and identical preparation conditions (pasteurization of milk, salt, rennet, culture). After three months ripening, cheese analysis was performed by 16S RNA genome sequencing with the Illumina MiSeq chemistry. *Firmicutes* was the dominant phylum in all Kefalograviera samples (Control 99.92%; Omega-3 enriched 99.62%). On genus level, Streptococci were the most abundant (49.81%; 46.89%), followed by Lactobacilli (37.89%; 30.18%), Lactococci (10.90%; 13.19%) and Enterococci (1.21%; 4.07%). *Leuconostoc* (1.44%) and Pediococci (2.11%) were only identified in the omega-3 enriched treatment. The results demonstrate that kefalograviera cheeses produced by milk from sheep fed omega-3 enriched diet can potentially be differentiated by the phylogenetic analysis of their microbiological communities. Acknowledgments: This research has been co-financed by Greece and the European Union (European Regional Development Fund) in context 'Research – Create – Innovate' within the Operational Program (Competitiveness, Entrepreneurship and Innovation (ΕΠΑΝΕΚ) of the NSRF 2014-2020. Project Code: T1ΕΔΚ-04727. Acronym 'GreenFeeds'

Seasonal variation of Selenium, manganese, zinc and copper concentration in milk of Sarda dairy ewes

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Selenium (Se) is an essential trace element involved in many physiological processes of mammals. In sheep, Se deficiency in feeds is related with WMD (white muscle disease), attributable to a probable deficiency of this micro-element the suckled milk. However, clinical manifestations of WMD, which is widely spread in Sardinia dairy sheep farms, can be found also in suckling lambs from ewes supplemented with Se, suggesting a complex interaction with other minerals and heavy metals. The objective of this study was to determine the concentrations of Se and others selected minerals in the milk of Sarda dairy ewes. A total of 403 milk samples were collected from 80 ewes selected in 10 farms (10 animals/farm) in Sardinia. Sampling were carried out every 45 days from the begin to the end of lactation and analysed with inductively coupled plasma mass spectrometry (ICP-MS) for Se and other microminerals (Cu, Mn, Zn and Ni), and heavy metals (Cd and Pb). Data were analysed using a linear mixed model that included the farm, sampling and their interaction as fixed effects and animal as random. The results showed that Se and Zn peaked at the begin of lactation as a possible consequence of common mineral supplementation in this stage, to reduce both WMD in lambs and mammary infection of mothers. Their concentration decreased gradually until the sheep dry-off. Similar pattern has been shown for Pb, whereas the lactation curve of Cd evidenced an opposite trend. Not defined pattern during lactation has been observed for Ni. The knowledge of mineral concentration in milk and a better understanding of their interaction, that could affect bioavailability and metabolism, will be important in establish more experienced recommendations for Se supplementation in dairy ewes. Acknowledgements: Research supported by FSC regional fund LR72017 'SEL-OVIN'.

Session 54 Poster 15

The effect of concentrate supplementation on grazing or housed lamb carcass and meat quality

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In the present study, the effect of *ad libitum* concentrate supplementation (18% CP) during the post-weaning phase of lambs in two different production systems (grazing vs housed) were evaluated for lamb carcass and meat quality traits. Fifty-eight lambs (29 Improved Jezersko-Solčava lambs (JSR) and 29 crossbreed lambs JSR × Texel), with an average weaning weight of 22.0±3.4 kg and an average age of 81.7±17.2 days, were randomly assigned to the following treatments: GC: grazing native pasture and *ad libitum* supplementation with concentrate (8 male + 8 female JSR lambs and 8 male + 8 female JSR × Texel crossbreeds); HC: hay-fed housed lambs supplemented *ad libitum* with concentrate (9 male + 6 female JSR lambs and 8 male + 5 female JSR × Texel crossbreeds). Thereafter, lambs were managed separately in the GC and HC groups until the end of the fattening period, which was 56 days long, on average. The data was analysed using a GLM of the SAS statistical package, where feeding treatment, genotype, and sex were considered fixed effects and the weaning weight as linear regression. The feeding treatment in lambs of the HC group resulted in a higher daily gain from birth to slaughter (278.9±4.89 g/day vs 214.17±4.67 g/day), a higher cold carcass weight (18.6±0.30 kg vs 15.9±0.29 kg), and a better dressing percentage (48.12±0.46% vs 45.82±0.44%) ($P<0.001$) compared to the GC group. On the other hand, the intramuscular fat of the GC group's longissimus dorsi had a higher concentration ($P<0.01$) of PUFA (16.26±0.31% vs 14.43±0.33%) and n-6 PUFA (12.92±0.31% vs 11.35±0.33%) compared to HC lambs. Differences in C18:3 n-3, C20:5 n-3, C22:6 n-3, and n-3 PUFA concentrations were not significant between the feeding treatments. No differences ($P>0.05$) between the lamb meat n-6/n-3 PUFA ratios could be found between the GC (4.42±0.16) and HC (4.30±0.17) groups. In conclusion, *ad libitum* supplementation of housed lambs should be a feeding strategy to improve lamb growth rate and carcass traits. On the other hand, *ad libitum* concentrate supplementation of grazing lambs could eliminate the positive effect of green grazing on the fatty acid composition of lamb meat.

Session 54 Poster 16

Evaluation of Avocado (*Persea americana*, Laureceae) by-products for Ruminants Feeding

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The exponential increase in avocado production in south Spain over the last years and the large amounts of by-products (ABP) generated requires the implementation of a circular economy system through their use in the agri-food sector (discarded-fruits-DF, peels-AP, seeds-AS and mixture of both last). As an initial stage, the objective of this study was to evaluate *in vitro* the nutritional value of ABP using the batch culture system and rumen fluid from goats as inoculum. The high moisture content in ABP is the main challenge for their proper handling, so two industrial drying systems were assayed: air drying (AD) and oven drying at 70 °C (70D). In addition, DF were also used after industrial oil extraction (DDF). Except for the AS the highest DM content was obtained with the AD than 70D procedure, being this last a system that resulted in products with an inadequate degree of moisture (under 85% of DM except for AP). The highest fat content was observed in DF (41.1 and 37.9%, respectively for AD and 70D), while the lowest corresponded to AS (1.69 and 2.94%, respectively for AD and 70D). In general, the protein content was low, with higher values for DDF (11.5 and 12.1%, respectively for AD and 70D) and the lower again for AS (5.61 and 5.00%, respectively for AD and 70D). Despite the low protein content, there was a relevant starch content in AS (up to 60%). The *in vitro* fermentation study showed no significant effect of the drying method on the fermentation characteristics of the different ABP. The lower total gas production (GP), metabolizable energy (ME), total volatile fatty acids (VFA) production and organic matter digestibility (OMD) was observed for AP, whereas the highest values were obtained with AS. There were no significant differences in the ammonia N content and methane emissions among different ABP. The fermentation of AS after 72 h produced values of total GP similar to those obtained with a commercial diet (CD) for milk production, but at a slower rate of GP than AP (0.052 and 0.118 s^{-1}, respectively for AS and CD). Results show that AS could be the most promising among the different ABP to be used in *in vivo* experiments for replacing high starch content feeds (i.e. cereals).

Does carob inclusion in lambs diet affect rumen parakeratosis and faecal dry matter digestibility?

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Carob pulp (which contains condensed tannins 'CT') could prevent ruminal acidosis generated by intensive feeding, but high CT levels could be negative for apparent nutrient digestibility. This study evaluated the effect of carob pulp (*Ceratonia siliqua*) inclusion in lambs' feed on rumen parakeratosis and apparent dry matter (DM) digestibility. A total of 144 weaned male and female crossbreed lambs with 15.0 kg of body-weight were used. The lambs were raised in two batches and were allocated in 12 pens in each batch (mixed sex per pen). They were randomly submitted to three isoenergetic and isoproteic concentrates: C0 (without carob), C15 (15% of carob) and C30 (30% of carob). At slaughter, dorso-caudal rumen tissues were sampled and ruminal epithelium colour was scored for parakeratosis (absence, mid or presence) by visual appraisal (5 samples/treatment). To assess the apparent DM digestibility, pooled faeces samples were collected by rectal stimulation in all lambs of each pen (8 replicates/treatment) at 50, 65 and 80 days of age. Rumen parakeratosis score and apparent DM digestibility data were analysed through mixed models with repeated measurements and contingency analyses were performed to evaluate the association between highest degree of parakeratosis and dietary treatment. DM digestibility was not affected by any interactions ($P>0.05$). The C0 and C15 treatments had similar DM digestibility but C30 was lowest (85.9 and 82.5 vs 76.8±1.3%; respectively, $P<0.05$). There was an increased DM digestibility with increased age and differences were observed at day 50 respect to days 65 and 80 (78.7 vs 83.1 and 83.4±0.82%; respectively, $P<0.05$). Overall, the highest rumen parakeratosis score was observed in lambs from C0 (87.5 vs 12.0 vs 0.0% in C0, C15 and C30, respectively, $P<0.05$). Carob pulp inclusion reduced linearly parakeratosis score in females (3 vs 2.2 vs 1.4±0.18, on a 1 to 3 scale in C0, C15 and C30, respectively, $P<0.05$), but these differences were not reported in males ($P>0.05$). In conclusion, results suggested that a 15% carob pulp inclusion would not impact on the apparent pen DM digestibility and would reduce parakeratosis in female lambs.

Barriers and risks for large-scale commercialization of insect proteins for food and feed in Europe

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Insect production has great potential to contribute in the protein transition. The value chain needs to be upscaled to become more sustainable and competitive. The impact of challenges for upscaling has not yet been investigated. A lack of risk profiles hampers credit provision and insurance granting. Additionally, consumer acceptance of edible insects has become increasingly important, but has been low in Europe. The objective of this work is to identify approaches for overcoming supply- and demand-side barriers to large-scale commercialization of insect proteins. It includes analysis of past experienced barriers and future perceived risks by operators. Furthermore, it covers strategies for the promotion of edible insects for consumers. The barriers and risks were assessed through a structured survey focusing on four areas: operations, finances, product safety, and regulations. The survey was conducted among insect rearers (n=25), processors (n=10), and insect-based feed (n=16) and food producers (n=15). Additionally, in-depth interviews with selected operators were conducted. In the analysis, barriers and risks were prioritized and characterized according to the impact on business performance. Furthermore, applied risk management strategies and risk attitude were evaluated. Regarding consumers, an online consumer survey and discrete choice experiments are conducted in Germany, Italy and Portugal to test consumers' acceptance and preference for insect-based products. It is aimed to determine which form and presentation of insect-based products and priming strategy best overcome the demand barriers identified.

Upscaling insect rearing: key results and future prospects

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Efficient, large scale insect rearing is one of links needed in a successful insect chain. Within the SUSINCHAIN project several aspects are assessed for *Hermetia illucens*, *Tenebrio molitor* and *Acheta domesticus*. In a first stage, the transportation of eggs or neonates within or between companies was optimized. Different temperatures, humidities and exposure times were assessed to determine the optimal/extreme climate conditions in which the insects can survive. In a next stage, the feed is optimized with a focus on both nutritional and physical characteristics (e.g. particle size, pH, etc.). To ensure comparability, standardized experimental protocols were constructed. In parallel, a list of pre-treatments was made based on literature data focussing on improving digestibility, storage or safety. The most promising pre-treatments will be tested in feeding trials. Besides quantity, it is important to produce high quality insects in a healthy environment for both workers and insects. A literature study was made on insect diseases, further research will be done on how to identify and reduce the likelihood of an infection. Besides diseases, pest species can be a major problem in insect rearing. Two literature studies were conducted on the most prevalent pest species: the house fly and pyralid moths. Further studies will be conducted to determine how to control these species in an insect farm. Finally, an assessment will be made on how to deal with the frass of insects and the possible valorisations.

Insect processing technologies investigated in the H2020 project SUSINCHAIN

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One work package (WP) in the SUSINCHAIN project, WP3, is dedicated to post-harvest processing technologies for edible insects. The aim is in the first place to investigate and implement a selection of technologies that are (relatively) new to the insect sector and that can be applied at industrial scale. For the technologies, the aim is to fine-tune processing conditions for large scale application, to provide a cost analysis, and to study microbiological safety (in connection with WP6) and sustainability (with WP7). Several industrial and academic partners throughout Europe each focus on a specific technology. Insects considered are the black soldier fly (BSF) larvae and for some techniques also other species, such as the yellow mealworm. In this way, the potential of Controlled Atmosphere Packaging and Storage is studied to extend the shelf life for both living and dead larvae. Microwave drying is applied at industrial scale on the insects to assess the drying efficiency of a number of microwave drying installations. For radio frequency drying, a prototype instrument designed to treat insects is currently built. The potential of low energy e-beam as a decontamination strategy for whole dried insects is being evaluated at pilot scale. High moisture extrusion is applied on fresh or frozen insects mixed with a soy and pea matrix, and the extrudates are being evaluated for texture and microbiological quality. The protein recovery from fresh BSF larvae is validated at industrial scale by using an enzymatic treatment combined with continuous tricanter centrifugation. In addition to demonstrating the feasibility of post-harvesting techniques, the aim of WP3 is in the second place to provide insect meals produced using a few of the aforementioned technologies to be used in animal trials performed in WP4. More specifically, three types of insect meals will be produced, based on fresh BSF larvae heated without enzymes, fresh larvae treated with enzymes and microwaved dried and milled larvae.

Session 55 Theatre 4

'Insects in animal feed': structure, aims and preliminary results of digestibility trials

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The objective of WP4 'Insects in animal feed' is to validate the inclusion of insect derived proteins in animal diets, ensuring optimal growth, quality and animal health of fish, poultry and piglets on commercially relevant scale. The WP is divided into three different tasks. In the first task digestibility trials are carried out to assess commercial black soldier fly (BSF) meals produced within one year. The aim is to highlight fluctuation on the meals composition as this could also influence digestibility parameters. Trials are performed both *in vivo*, and *in vitro*. Digestibility trials are also performed assessing three BSF meals produced under different methodologies in WP3 'Insect processing'. The second task aims to evaluate, under farm conditions, the effects of the inclusion of BSF meal in commercial feed formulae for fish (rainbow trout, sea bass and Atlantic salmon), poultry (broiler chickens and laying hens), and weaning piglets. Finally, the third task aims to validate the impact of diets containing insect derived proteins on product quality and animal health. Physical, chemical and organoleptic quality of animal-derived products (fish meat, broiler meat, and laying hen eggs) are assessed on samples following the large-scale trial. Moreover, physiological effects are evaluated by hematic, biochemical, histological and immuno-histochemical analyses and by metagenomics approach. The final objective is to validate on commercially relevant scale (TRL 6) research the inclusion of insect-derived proteins in animal diets and to provide the market with commercial feed formulae able to guarantee optimal animal growth and health, and product quality. After a brief overview of the WP4 structure and aims, the presentation reports the first results obtained in the *in vivo* and *in vitro* digestibility trials on rainbow trout, seabass, Atlantic salmon, broiler chickens and weaning piglets.

Session 55 Theatre 5

Insects for food: can insect-based food products be part of a regular dinner in Europe?

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Edible insect species can be produced efficiently in farming systems. To reveal the potential of edible insects in more sustainable diets, these must be attractive alternatives in regular meals. A barrier to the introduction of insect-based foods in daily diet is the lack of familiarity in regular meals. To overcome the barriers to incorporating insects in regular meals, SUSINCHAIN expose families in Portugal and Denmark to menus of insect-based dinner products, replacing three weekly dinner servings over a period of six weeks. We assess on protein intake, dietary diversity and perception of insects. Six insect-based dinner products have been developed. These are based either on mealworm or cricket, fresh or powdered, and offer a variety of novel and traditional dinner products. The insect menus are tested in families with young children in Denmark, and in households of young couples without children in Portugal. In total, 80 households in each country will be invited. Over 6 weeks, 3 weekly dinner meals will be replaced. Half of the households in each country will be offered the insect menu, while the other half will be offered a plant-based menu, to control for the effects of exposure to a novel menu. The impact on protein intake and dietary patterns will be measured by dietary assessment methods. The evolution of families' perceptions of the products will be tracked by questionnaires and interviews. We expect to identify actions to overcome barriers for the inclusion of insects in regular diets in Europe.

Session 55 Theatre 6

Safety of insect for feed and food investigated in the H2020 project SUSINCHAIN

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Work package (WP) 6 of the SUSINCHAIN project is dedicated to investigating the safety of insects for feed and food use. Insect species considered include: black soldier fly (*Hermetia illucens*), common housefly (*Musca domestica*), yellow mealworm (*Tenebrio molitor*), lesser mealworm (*Alphitobius diaperinus*), and house cricket (*Acheta domesticus*). The aim of WP6 is in the first place to identify any potential food safety hazard in larvae of these insect species larvae and, second, to identify practices to control the presence of such hazards. First, a literature review has been performed on the potential chemical and microbiological hazards that can be present in insect larvae. Second, for the identified possible hazards, a series of in-depth studies has been set up aimed to investigate the possible transfer of the food safety hazard from the substrate into the insect larvae. Some of these transfer studies have already been performed. Each experiment is performed in controlled environments using substrate spiked with the particular hazard. More particularly, for chemicals, such transfer experiments are being performed with the mycotoxin aflatoxin B1 in BSF and in lesser mealworms, and with plant toxins in lesser mealworms. Plant toxins were chosen because of the possible accumulation of this group of chemicals in insect larvae and the lack of data on this topic. Aflatoxin B1 is known not to accumulate, from previous studies, in BSF and lesser mealworm larvae. This toxin is investigated for its potential breakdown by these insects. In-depth analyses are done into the breakdown compounds, and their possible toxicity. For pathogens, such transfer experiments have been performed with *Salmonella* spp., *Staphylococcus aureus* and *Bacillus cereus* in BSF. Results show that an occasional contamination of feeding substrate with these organisms may cause transfer of pathogen to the insects and involve microbiological safety risks. Similar transfer experiments with yellow mealworms will be performed as well.

Session 55 Theatre 7

Building economic and environmental optimization model of sustainable insect chains

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Food production is facing a challenging task to assure sustainable and secure food production within the planet's boundaries in the next few decades. Insect production demonstrates the potential to deliver local and sustainable protein sources, however in Western countries it is still facing economic and environmental difficulties, calling for the establishment of a sustainable insect production chains. SUSINCHAIN, a H2020 EU-funded project aims to define such optimal production models. There are multiple challenges of data availability, contradicting character of sustainability aspects and a wide variability of seasons and species. Optimal model of sustainable insect chains is based on modular approaches as it allows them to rely on an already defined set of tasks and logics which saves time and implies flexibility. Moreover, modelling complexity behind the entire insect system is divided into smaller solvable and rather simple tasks (models). Current procedure follows three stages: (1) determination of system boundaries of insect production chains via graphical mapping; (2) the modularization of insect production chain according to the modularization scheme and life cycle impact assessment approaches; (3) the consideration of a functional unit and a production scale which may affect the results and alter the final outcomes and conclusions. Various models will be presented from methodological (feed conversion model, price-value model, energetic, mass flow and environmental fate models) and from chains representation (feed production, insect growing, reproduction, processing, product development) perspectives. The outcomes of separate models are then further analysed through multi-objective optimization for the identification of the optimal chains.

Genomic predictions of crossbred dairy cows based on breed of origin of alleles

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Genomic selection has been successfully implemented in many purebred dairy cattle populations and genotyping of cows has become an important management tool for dairy farmers. Increased interest in crossbreeding among dairy farmers has imposed a need for genomic evaluation of crossbred cows and heifers. It is not straight-forward to establish a reference population for evaluation of crossbred animals and therefore it is important to investigate how information from purebred populations can be used for genomic prediction of crossbred cows. We tested two models for estimation of genomic breeding values on 4,576 genotyped crosses of Holstein(H), Jersey(J) and Nordic Red Cattle(R). Estimated effects of SNP markers from the three breeds were combined either with a breed of origin model(BOM), where marker effects at each locus were taken from the pure breed according to the estimated breed of origins of alleles, or a breed proportion model(BPM), where all marker effects were weighted with estimated proportion of the pure breeds in the genome of the crossbred cow. Because estimated marker effects came from separate genomic evaluations within each breed, the different levels of the breeds had to be accounted for. This was done by including intercepts in the models, weighted with breed proportions. The intercepts were calculated from the phenotypic averages of the breeds, corrected for mean direct genetic values of purebred animals of the relative breeds. Assignment of the marker alleles of the crossbred to breed of origin was carried out using a new method. For 99.4% of the marker alleles of the crossbred cows, breed of origin was detected. Across all animals, reliability of genomic prediction for protein yield was estimated to be 0.61 and 0.49 based on BOM and BPM, respectively. For three-way crosses and backcrosses, BOM resulted in more accurate predictions than BPM. For 197 F1 crosses of J and H, the reliabilities were 0.33, 0.34 and 0.33 for BOM, BPM and parent average, respectively, but for 1,117 crosses of R and H the reliabilities were 0.62, 0.61 and 0.28.

Predicting of the purebred-crossbred genetic correlation using marker data in parental lines

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The genetic correlation between purebred and crossbred performance (r_{pc}) is an important parameter in crossbreeding programs. Estimation of r_{pc} requires phenotype (and optionally genotype) data of both purebred and crossbred animals, which can be difficult and costly to collect. In earlier theoretical work, we derived expressions to predict upper and lower bounds of r_{pc} based only on genetic variance components in the parental lines, alleviating the need for crossbred data. Validations in simulations showed that the expressions provided accurate bounds of r_{pc} when genotypes and effects of causal loci are known. However, genotypes and effects of causal loci are unknown in practice, and variance components are usually estimated using marker genotypes. Furthermore, the variance components used in our expressions of r_{pc} are computed from breeding values in the focal parental line, for the trait expressed in all parental lines. Hence, these variance components are fundamentally different from the variance components typically estimated in empirical data. This study therefore aims to approximate the required variance components for the prediction of r_{pc} using marker genotypes, and to validate this method in simulated and real data. In simulations, the results showed that the approximations lead to unbiased estimates of the upper bound of r_{pc}, and a slightly underestimated lower bound of r_{pc}. Across the four genetic models that were simulated, the true value of r_{pc} was between the estimated lower and upper bound in 86% of the replicates. These results suggest that bounds of r_{pc} can be accurately estimated based only on phenotypes and marker genotypes in the parental lines. In addition to simulations, the method will be validated in an empirical dataset of pigs.

Session 56 Theatre 3

Comparing growth and feed intake in pure- and crossbred heifers fed different energy concentrations

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Red dairy cattle (RDC) heifers, raised alongside Holstein (DH) heifers, often end up with higher body condition at first parturition compared to their DH pen mates. This difference has been hypothesized to be due to a better feed efficiency, but is yet to be tested. The aim of this study was to describe differences in growth, condition score, and feed efficiency due to breed and energy concentration of the diet in growing heifers. Three breed groups of 21 DH, 20 RDC and 21 DHxRDC heifers were included in the study. Within breed group, half the heifers were fed a standard heifer diet (STD) and the other half a diet with 15% lower net energy concentration (LOW). The experimental period started at 5 months of age and ended at start of insemination at 14 months. Body condition score and live weight were measured monthly and daily feed intake was recorded using Insentec feed bins. The data was analysed using least squares means from a mixed model analysis. Across the whole period DH and DHxRDC heifers gained more body weight on the STD diet compared to the LOW diet ($P<0.001$). No significant difference was seen between STD and LOW diet in RDC ($P=0.11$). On the STD diet DH and DHxRDC grew better than RDC ($P=0.001$), whereas DH grew less than RDC and DHxRDC ($P<0.001$) on LOW. No significant difference was seen between RDC and DHxRDC with respect to growth ($P=0.20$). Higher BCS on the STD diet compared to LOW diet was seen in DH ($P<0.001$) and DHxRDC ($P=0.03$). No difference in BCS was seen between diets in RDC. RDC had the highest BCS among the breeds. DHxRDC had higher daily dry matter intake (DMI) ($P<0.001$) on the STD diet compared to DH and RDC. On the LOW diet, RDC had the highest DMI, with a small difference to DHxRDC ($P=0.05$). DH had the lowest DMI on the LOW diet. Comparing within breed RDC had similar DMI across diets, whereas DH and DHxRDC had lower DMI on the LOW diet. Results indicate that RDC and DHxRDC heifers perform better on a lower energy diet, compared to DH.

Session 56 Theatre 4

Simulation study on rotational crossbreeding and advanced OCS methods in dairy cattle

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The genetics of many local and numerical small breeds have been replaced by high performance breeds due to economic reasons. In addition, the performance gap between local breeds and high-yielding breeds is increasing, as genomic selection is mainly implemented in high-yielding breeds with large population sizes. One solution can be to introgress high-yielding breeds in local breeds, which was frequently done in the past. This, however, might lead to the genetic extinction of the local breed in the long run. The goal of this study was to recover the native contribution of local breeds while at the same time achieving selection response. It was investigated whether a local breed (i.e. the Angler red dairy cattle breed) can be made economically competitive by two-way rotational crossing with a high yielding breed (i.e. German Holstein) with the aim to utilize heterosis. At the same time, the genetic uniqueness of Angler should be recovered by applying advanced Optimum Contribution Selection (aOCS) methods. A single trait with additive genetic variance of 0.3 and dominance variation of 0.05 was considered. Data used as an input for the simulation consisted of pedigree and 50k genotypes of Angler and Holstein individuals. The simulation started with 2 generations of purebreeding Angler and Holstein followed by 10 generations of the actual rotational crossbreeding. The analysis considered different types of classical and advanced Optimum Contribution Selection strategies for Angler, which either maximized genomic estimated breeding values for purebred performance (GEBVp) or for crossbred performance (GEBVc). Additionally, constraints were defined for kinships, native kinships and native contributions. First results show that selection based on GEBVp while constraining the kinship caused the highest genetic gain in Angler, but reduced the native diversity. When constraining native contribution it slowed down the genetic gain, whereas the original genetic contribution increased.

Simulating common breeding schemes for local and mainstream Red dairy cattle breeds across Europe

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There are various Red dairy cattle breeds in Europe, ranging from local breeds that are numerically small, to larger mainstream breeds mainly used for production. Local breeds might not be economically competitive due to lower milk production than mainstream breeds, but conservation of these breeds is important for maintaining genetic diversity. In order for local Red breeds to keep existing, some form of economic benefit should be obtained. One way to achieve this is to collaborate with mainstream Red breeds by e.g. joint genetic evaluation or the usage of breeding bulls across breeds. The aim of this study was to explore possibilities for collaboration between local and mainstream Red cattle breeds. A stochastic simulation was set up with a mainstream Red breed with low linkage disequilibrium (LD) and two local Red breeds, one with moderate LD and one with high LD. Genome data of the base populations with the real LD structure were simulated for each breed. After this, progeny-testing schemes were simulated for 20 years to build up genomic reference populations, followed by a genomic selection scheme for 10 years in ADAM. Different scenarios were simulated, in which the mainstream breed together with one local breed were included in each simulation. The breeding scheme for the local breeds was approximately 10 times smaller than the breeding scheme for the mainstream breed. Scenarios differed in the number of bulls selected for breeding in the local breed but originating from the mainstream breed. This ranged from 0 out of 10 to 10 out of 10 selected bulls originating from the mainstream breed, with a gradual increase in between. Consequences of the different degrees of collaboration will be compared for rates of genetic gain and rates of inbreeding. The outcome of this study is expected to provide advice on suitable breeding strategies to keep local breeds competitive and maintain genetic diversity.

Impact of kinship matrix in genomic optimum contribution selection on allelic diversity in cattle

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Genomic selection in cattle increases genetic gain considerably by shortening generation intervals, but substantially increases the rate of inbreeding per year. A sustainable breeding program needs to preserve allelic diversity to ensure long-term genetic gain. Optimum contribution selection (OCS) reduces the increase in coancestry by penalizing increases in relationships among selected candidates, while maximizing genetic gain. Different approaches to build kinship matrices provide different measures of relationship among individuals. Using simulations, we examine the impact on allelic diversity of using marker-based, haplotype-based, or pedigree-based relationship matrices. We focus on loci with low minor allele frequency (MAF): effective population size (N_e) varies across the genome, and we test the possibility of increasing N_e at these sites to reduce loss of rare alleles by drift. We will simulate a small cattle population undergoing genomic optimum contribution selection for milk yield using the R package Modular Breeding Program Simulator (MoBPS). The relationship between allele frequency and allele effect is especially important for a scheme that aims at conserving rare alleles because deleterious alleles are more prevalent among rare alleles. Three scenarios of the joint distribution of allelic effects and frequencies will be tested to reflect different assumptions about the true distribution. Also, three types of relationship matrices for OCS will be tested: (1) pedigree-based relationship matrix; (2) marker-based; and (3) haplotype-based. The effects of using these matrices on allelic diversity, inbreeding and relationships in the long term will be compared across different joint distributions of effects and frequency. The results will be useful for breeding programs aiming to preserve genetic diversity and genetic gain in the long term.

Performance of genomic selection conducted from different base populations

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The success of any breeding program depends on the way the base population is constructed. The amount of genetic diversity captured on it will determine the response to selection. Additionally, the phenotypic level for the targeted quantitative trait at the base population may affect the general profitability of the program. Provided there is a large enough number of SNPs genotyped, genetic diversity can be measured as the expected heterozygosity (strategies H) or the allelic diversity (A). In the latter case decisions can be taken on haplotypes of different size to allow for a larger number of alleles to be detected. Another alternative is aiming at maximizing the additive variance (V_A) for the quantitative trait (H'). When phenotype is being maximized (strategies P) still the genetic diversity maintained can be controlled. Finally, differences must be explored between using individual values (I) or mean values of the available strains (G). The aim of this study was to explore the consequences of using different strategies for the creation of base populations in the evolution (especially in terms of gain) of a breeding program under genomic selection. Using computer simulations, we evaluated different scenarios that differ in the type of strains, the level of available information and the type of measure used to characterize the genetic variation and perform the optimization (either expected heterozygosity or allelic diversity at SNP haplotypes). Results show that A strategies are not useful when dealing with SNPs independently. Moreover, the haplotype size is irrelevant except for strategies PA. When using P strategies the higher phenotypic values hold at least for 10 generations. Strategy GH' only yields higher V_A in the base population for scenarios with very divergent strains. However, this superiority disappears in the second generation of selection. Therefore, it seems that the most sensible option is using expected heterozygosity as the diversity criterion when constructing the base populations.

Using high-density SNP data to unravel the origin of the Franches-Montagnes horse breed

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The Franches-Montagnes (FM) is the last native horse breed of Switzerland, established at the end of the 19th century by cross-breeding local mares with Anglo-Norman stallions. In the current FM pedigree, horses born before 1950 are considered to be purebred. From 1950, sporadic introgression with other breeds were permitted until 1997. One purebred Arabian stallion was introgressed in the 1960's, two Swedish Warmblood stallions in the 1970's and two Swiss Warmblood stallions in 1990. Based on these last five introgressions, pedigree-based admixture (PA) proportions are computed for FM horses. In this study, we derived genome-based admixture proportions using the Axiom™ 670K Equine genotyping array to evaluate the pedigree-based admixture proportions and to screen the FM genome for admixture hotspots. To date, we collected high-density SNP genotype data from 407 FM horses (including 35 purebred FM), 134 Swiss Warmblood horses (WB) (including a stallion used for cross-breeding in 1990) and 120 purebred Arabians (AR). We used a supervised model as implemented in Admixture 1.23 to compute genome-based admixture proportions of the FM horses, with purebred FM, WB and AR as the reference population. The majority of FM horses (204) showed 0% AR admixture proportion, and only nine FM horses had an AR admixture proportion >5%. The highest observed AR and WB admixture proportion was 7.5 and 55% (F1 sire), respectively. The average pedigree- and genome-based admixture proportion differed by less than 0.1%, while we noticed high differences from up to 25% for individual horses. To improve the accuracy of genome-based admixture proportions, we will include direct descendants of the stallions used for crossbreeding (e.g. Swedish Warmblood) in the reference population. FM horses will then be separated into subgroups to screen the FM genome for breed-specific admixture hotspots using runs of homozygosity; morphological differences between subgroups will be visualized using the horse shape space model.

Genome wide association studies of growth traits in grazing Rasa Aragonesa ewes

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Rasa Aragonesa sheep is an autochthonous meat breed from the northeast of Spain, mainly reared in extensive or semi-extensive farming systems and characterized by a marked seasonality of breeding activity from March to June, being the longest seasonal anoestrous in ewes with low body condition (BC) and body weight (BW). This study aimed to perform a genome-wide association study (GWAS) to identify SNPs and genomic regions associated with BC, BW and growth rate (GR) in 215 Rasa Aragonesa ewes raised on pasture, of which 110 were genotyped using the 50k and 115 with 680k *Illumina Ovine Beadchips.* BC and BW were collected every three weeks during 2 years from January to August and were estimated and adjusted by BLUPF90 software, and GR was estimated by a linear regression in R. The pseudo-phenotypes obtained were utilized as input for the GWAS. The Beagle 4.0 program was used to impute missing SNPs from the 50k chip to 680k. GWAS was performed using the GCTA program running a mixed linear model association (MLMA) and the leaving-one-chromosome-out approach (LOCO). Only one SNP associated to GR in chromosome 9 overcame the genome-wise significance level (FDR 10%). This SNP was located approximately at 83 kb from the *CYP7B1* gene, which is involved in the metabolism of endogenous oxysterols, which are key mediators of cholesterol and lipid homeostasis. We also identified 1, 3 and 9 SNPs for BC, BW and GR, respectively, reaching the chromosome-wise level of significance (FDR 10%). *NPC2* gene located in chromosome 7 is close to a chromosome-wise significant SNP for GR, and it is involved in the intracellular trafficking of cholesterol and other lipids. No candidate genes were found for BC. However, several genes were annotated near the significant SNPs for the BW trait, namely, *MARK1* located in chromosome 12 and related to gastrointestinal parasite resistance traits in Djallonké sheep, and *ZEB1* gene in chromosome 13 associated with obesity in human and adipogenesis in mice. Future studies characterizing these candidate genes, may uncover the genetic architecture underlying the bodyweight and growth rate traits in Rasa Aragonesa.

Polymorphisms at leptin and pituitary transcription factor genes in four beef cattle crossbreeds

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Growth rates and carcass quality are under the control of multiple genes and have major implications when beef cattle are concerned. Selection of animals with higher growth rates and better carcass composition is of great importance to both breeders and consumers. In this study the genetic variability of the polymorphisms for leptin (LEP, intron 2 and exon 3) and pituitary-specific transcription factor (POU1F1/Pit-1, exon 6) were analysed in four beef cattle crossbreeds. Researches were carried out at the Research and Development Institute for Bovine Balotesti, on four F1 crossbred genotypes: Limousin × Romanian Black Spotted, Charolais × Romanian Black Spotted, Belgian Blue × Romanian Black Spotted and Aberdeen Angus × Romanian Black Spotted. The most frequent allele at the LEP locus was A for the Limousine crossbred animals, with an average of 0.58, compared to Charolais, Belgian Blue and Angus sired animals, which had an average frequency of 0.50 for both A and G alleles. A similar pattern was observed for the LEP genotypes, when the Limousine crossbreds had a frequency of 0.83 for AG and 0.17 for AA, while the other crossbreds were exclusively AG genotypes. For PIT-1 the average frequency of alleles was of 0.42, 0.66, 0.58 and 0.66 for A and 0.58, 0.34, 0.42 and 0.34 for B in Limousin, Charolais, Belgian Blue and Angus crossbreds, respectively. Genotype frequency for PIT-1 was on average of 0.84 for AB and 0.16 for BB in Limousin crossbreds, 0.66 for AB and 0.34 for BB in Charolais sired calves, 0.33 for AA, 0.50 for AB and 0.17 for BB in Belgian Blue dual-breeds, while for the Aberdeen Angus sired animals the AA genotype was most dominant, with an average frequency of 0.66 and of 0.34 for the BB genotype. The studied polymorphisms represent potential markers for beef production in the investigated crossbreeds and the results could be useful for future efforts to create composite beef breeds in dairy farms which are transitioning to beef production.

Use of crossbreeding with Angus and Belgian Blue bulls in dairy herds

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Recent developments in the beef production sector have been influenced by the coupled support in some EU Member States. With the last livestock surveys showing a decline of beef cattle numbers in the main producing EU Member States, except for Poland, Spain and Romania. According to the European Commission predictions for 2030, the average milk yields are expected to grow from 7,300 kg/cow in 2019 to 8,340 kg/cow. Given these efficiency gains, the number of dairy cows could be further reduced by up to 1.4 million until 2030. Moreover, the use of sexed semen has become widely practiced under commercial dairy cattle production, leading to an increase of available replacement heifers, while milk prices have become volatile. Therefore, some farmers could cross their dairy cows with beef sires, in an attempt to transition to beef production or in order to improve overall farm-returns from increasing the marketing value of fattened crossbred calves. The aim of the current research was to evaluate the use of Aberdeen Angus and Belgian Blue beef breeds as sires for crossbreeding with the Romanian Black and White HF dairy breed (n=20). At the age of 14 months the F_1 Aberdeen Angus × Romanian Black and White Spotted heifers had on average 378.0±45.29 kg, while the F_1 Belgian Blue × Romanian Black and White Spotted heifers had an average body weight of 401.7±17.64 kg, with differences between the two genotypes being significant ($P \leq 0.05$). Age at first insemination was on average of 16.7±2.02 months for the Angus sired heifers and of 16.2±1.72 months for the Belgian Blue crossbred females, differences between genotypes being not significant ($P > 0.05$). When crossbreeding Holstein-Friesian derived dairy breeds, the Belgian Blue sires are recommended in order to obtain dual-breeds meant for further beef production. However, the Belgian Blue sired heifers should be crossed with sire breeds that give calves with low birth weights, such as Piemontese breed, for calving ease and not to be introduced at mating before reaching 380 kg body weight. Crossbreeding could represent a viable alternative for dairy farmers who choose to convert for beef production, taking advantage of the hybrid vigour and breed complementarity.

Strengthening the added value of rare breeds in Poland

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Genetic resources conservation programs in Poland are realized from 2000. They covered 83 local breeds of farms animals. At the end of 2020 the total numbers of animals was 108,700 (kept in 3,350 herds) and it was about 10 times more than in 2000. Currently the number of local breeds have increased to 87 (2 more local breeds of sheep: Polish Mountain Sheep and White-Headed-Meat Sheep and 2 breeds of goats: kazimierzowska and sandomierska). In sum, *in situ* conservation brings expected results, but now implementation of more actions covered by The National Strategy for sustainable utilisation and conservation of animal genetic resources is needed. The first step was to strengthened *ex situ* conservation of rare breeds. Last year the collection of genetic material from cattle has been enriched by semen of different breeds and embryos of Polish Red-and-White and Polish Red cattle. Now the long-term strategy of collecting materials is being prepared. According to recent changes in UE breeding legislation there were some activities undertaken to emphasis the role of National Genebank in polish breeding legislation. The certification system of products from native breeds was established and further activities connected with the promotion of brand 'Native Breed' has been taken. From the beginning of 2021, first farms were certificated. This breeders can use the logo not only to label farms but also for products they make. Moreover, the website and database of native breeds and producers was launched (http://ksb.izoo.krakow.pl). Further actions in using rare breeds of herbivorous farm animals in natural service has been taken. There has been growing awareness in this regard among not only farmers but also entities responsible for landscape management in Poland. The information gathered in recent studies shows that in the area of many National Parks and other environmentally valuable areas, grazing was carried out using the growing number of native breeds of animals, for example: Polish-Red, Polish-Red-and-White cattle, Hucul, Polish konik horses and most of sheep breeds. Elaborated as a part of task 03-18-21-09.

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Possibilities of meat use of autochthonous breed beef the Polish Red

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In Poland, we have four cattle breeds in the program for the Conservation of Genetic Recourses of Farm Animal. The oldest of them is the Polish Red cattle. Cattle this breed provide not only milk but also excellent quality meat. Due to their native characteristics, cattle of this breed have become a good alternative for breeders keeping other beef cattle breeds. It is undeniable that the meat of this breed is characterized by tenderness, appropriate marbling and juiciness. Therefore they can be used to develop a new direction of use, which is the beef direction. The lack of previous research on the development of a beef performance model for cattle of this breed is an incentive to start cooperation with the Polish Association of Breeders and Producers of Beef Cattle and with the Institute of Animal Sciences the Warsaw University of Life Sciences to implement a joint project. This project financed by the Ministry of Science and Higher Education as part of the implementation doctorate program. Thanks to its implementation, 72 new herds were created in Poland last year, keeping cattle of this breed in the beef type, and the number of cows increased by 1,027 heads. Also, numerous farms in Poland began to replace the existing breeds of cattle with the Polish Red breed, increasing its number at the same time. The project's overriding aim is to develop a beef performance model and compare it with Polish and French limousine cattle. The research has covered 942 limousine bulls – 492 of Polish origin and 450 of French origin, and 115 heads of Polish Red cattle. The following parameters were determined: body weight at birth (kg), body weight at 210 days of age (kg), bodyweight of bulls at 420 days of age (kg), daily gain (g), bodyweight standardized for 210 and 420 days of life (kg). Additionally, an assessment of the habit was performed for cattle with bodyweight measurement. The animal's conformation was determined with 19 linear features characterizing its muscles, skeleton, functional components, and additional features. In total, 2,033 measurements assessment for Polish Red cattle and 27,318 for Limousine cattle were performed. The existing database consists of 128,592 records, which constitute the described features and pedigree of the father-bulls.

Session 57 Theatre 1

131 deeply sequenced cattle trios reveal parent-of-origin effects in de novo structural variations

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De novo mutations (dnm's) are an important source of genetic diversity and hold paramount significance in understanding evolution. Whole-genome sequencing (WGS) data of parents and an offspring (referred to as trio), at an affordable cost, enabled screening of hundreds of trios, unravelling mutagenesis of de novo single nucleotide variants (SNVs). Despite not as abundant as SNVs, structural variations (SVs) affect more base-pairs (bp) of the genome than SNVs, due to their larger event size (minimum of 50 bp to chromosomal events). Nevertheless, the rate of de novo SV (dnSV) has not been investigated in dairy cattle. Thus, we attempted to screen dnSVs in a healthy Dutch Holstein Friesian cattle cohort, consisting of 131 deeply sequenced trios and their descendants (743 genomes in total). Overall, we detected 21 dnSVs (16 deletions, 5 duplications, 1 inversion) in a size range of 56 bp to 1.2 Mb. The estimated dnSV rate was 0.13/generation (95% CI 0.07-0.19), meaning 1 dnSV per 7.7 births. Strikingly, our dnSVs showed an extremely strong paternal bias of 16:1. This degree of bias is far beyond what is reported in human (dnSV paternal bias of 3.3:1). We speculate that this difference could arise from the different DNA source used: male animals in Damona was sequenced from semen, whereas human studies are based on blood-derived DNA. Furthermore, we found a 50-kb de novo deletion, spanning over the upstream and exons of the centromere protein C gene (CENPC). A mouse knock-out study showed that heterozygous mice are healthy and fertile, however homozygous resulted in embryonic lethality. As such, we suggest performing a large-scale population-wide screening to monitor this variant and to prevent potential carrier matings.

CNV impact on gene expression

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Most of genetic diversity in mammals is caused by large-scaled duplications and deletions called Copy Number Variation (CNV) changes. Their length ranges from 50 bp to 1 Mbp what allows them to cover many functional elements of the genome. In this study, we aimed to define the impact of CNVs on gene expression level. Whole genomic and transcriptomic sequences of six Polish Landrace boars were assessed for quality control. Poor-quality reads were trimmed. Genomic reads were aligned against the 11.1 (GCA_000003025.6) reference genome using the BWA software. The identification of CNV was done using Pindel and CNVnator programs. To minimize the detection of false positive CNV, the overlapping output from both programmes was considered as the final result. Furthermore, polymorphisms of length <50 bp or >1 Mbp and located in ambiguous or homopolymeric regions of the reference assembly were removed. Genomic annotation of CNVs was done using Variant Effect Predictor in order to select polymorphisms located in genes. For trimmed transcriptomic reads the level of gene expression quantification was performed using Kallisto software. As a final step, correlation coefficients were calculated and regression was used to model the impact of CNVs on gene expression in selected muscle tissue. CNVs are important modulators of gene expression. We observed a correlation between CNV in genes and gene expression and between the size of CNV and gene expression level. This study is a part of a project 'Modulation of gene expression by Copy Number Variation in pigs' (N060/0035/20) and was also supported by Poznan Supercomputing and Networking Centre.

Bisulfite vs nanopore long-reads to sequence the dairy cattle epigenome

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Epigenetic information is important in animal breeding. The epigenome can help us to understand gene expression, regulation, prenatal programming, the effect of stressors on the phenotype and even to improve the accuracy of genetic evaluations. However, obtaining this information is still expensive and difficult to obtain in commercial populations. The objective of this work is to understand the differences between obtaining this information using nanopore technology and bisulphite sequencing. We sequenced the genome of a Holstein cow using the MinION device and the Illumina Bisulphite method. The MinION detects the ionic disruptive signal when a DNA strand crosses the nanopore, on the other hand, the bisulphite treatment converts DNA unmethylated cytosines (C) to thymines (T), but methylated Cs remain unchanged by the treatment. We compared the detection of both techniques using the softwares Bismark, Megalodon and Nanopolish. A total of 584,135,436 sequences from bisulphite sequencing were analysed with Bismark. 6,600,558,962 C's were found, with 79.53% of the C's analysed being in a methylated state.195,395,143 of those C's being in CpG sites, Using nanopolish to analyse the MinION sequencing, 8,105,413 sequences were obtained, finding 3,408,574 methylated sites with a different number of C's in each one of them. Nanopore sequencing allows us to detect methylated regions with a lower coverage. This technology could be used to add the epigenetic information of the animal to breeding programmes. Further studies are being conducted to understand and point the exact differences between bisulphite and nanopore sequencing.

Session 57 Theatre 4

Benchmarking phasing software with whole-genome sequenced cattle trios

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Accurate haplotype reconstruction is required in many applications in animal genomics. In this study, we used deeply sequenced cattle genomes from a pedigree of 264 Holstein-Friesian individuals (mean coverage: 25.8X), mapped on the reference genome assembly ARS-UCD1.2. We selected 11,030,905 SNPs after the application of the Variant Quality Score Recalibration procedure with GATK, setting a threshold to keep 97.5% of the Truth Sites. We started by phasing the data based on familial information using LINKPHASE3 and observed on average more than 250 crossovers (CO) per gamete. This value was 10 times larger than expected, indicating that the sequence data required further cleaning. Selection of a subset of 6,402,394 variants, segregating in agreement with Mendelian segregation rules, with genotype frequencies close to Hardy-Weinberg proportions, and with MAF>0.01, decreased the number of CO by more than half, to 105.5 on average, still four times above expectations. Refining genotype calls using Beagle v4.1 clearly improved the genotype calls (45.7 CO on average). Nevertheless, errors in the genome assembly still accounted for 17 spurious CO per gamete. This cleaned dataset was then used to compare accuracy of LD-based phasing algorithms including Beagle (versions 3.3, 4.0, 4.1, 5.0, and 5.1), Shapeit (versions 2, and 4.2) and Eagle v2.4. To that end we used haplotypes from 98 children from trios phased with Mendelian rules as golden standard. These individuals were then phased without pedigree information with the different software. Preliminary results obtained on a similar dataset prepared on the former bovine genome assembly indicated that Shapeit v4.2 performed best (6 times fewer switches than the worst method), followed by Beagle v5.1. New versions of different software performed better than earlier versions. Eagle v2.4 was among the less accurate approaches on our dataset. Results are currently updated on our new dataset.

Session 57 Theatre 5

Effects of adding large scale custom genotypes on genomic prediction accuracy

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Increasing genomic prediction accuracy is a major goal and a challenge within genomic selection (GS). Routine GS schemes in Switzerland are running for five different cattle populations and various population-specific traits. In the past, marker sets between 50 and 110 thousands of SNPs were applied for genomic prediction. Nevertheless, a previous approach has already shown an increased prediction accuracy by increasing the marker density. Archive reference genotype data has always been generated through publicly available genotyping arrays. However, in 2019 a custom Axiom genotyping array encompassing 310K variants including 110K markers from the previously established GS routine was designed and implemented on large-scale by genotyping all routine samples. WGS data of key animals was generated and enabled variant selection from specific genomic regions. Furthermore, genome-wide selected protein-changing variants with predicted low, moderate or high effect were considered during the array design. Since more than 12 thousands of routine samples were genotyped successfully, the entire genotype archive data was imputed to 310K-density. For validation, GWAS runs based on an improved resolution were repeated and compared with previously obtained routine results. Interestingly, 310K-based GWAS results outperformed the results from previously known QTL regions by pinpointing several protein-changing variants as most significant associated markers. For example, the detection of the DGAT1 p.Lys232Ala variant for fat yield, or the APOB loss-of-function variant for heifer rearing success illustrates a proof-of-concept. The aim of the present project is to evaluate the impact of incorporating subsets from newly genotyped variants in genomic evaluation systems and to evaluate effects on prediction accuracy. Individual SNP effects were estimated using the BayesC algorithm and a classical two-step approach is applied by using the 5% youngest animals for prediction accuracy evaluation. A full comparison of GS prediction accuracy is currently ongoing, but the first 310K-based GWAS results suggest a significant improvement.

Bos taurus **introgressed segments in the UOA** ***Bos indicus*** **assembly**

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Reference genome sequences are essential in the analysis of genomic data. As the costs of sequencing decreases, multiple reference genomes are produced for a single species in order to cover nuances that are not captured by previous reference sequences. The latest reference sequence adopted by the scientific community for the analysis of cattle data is ARS_UCD1.2, built from DNA of a Hereford cow (*Bos taurus*). Complementary, the UOA_Brahman1 was recently built over a Brahman cow (*Bos indicus*) haplotype to further support the analysis of *B. indicus* data. In this study, we aligned sequence data of fifteen cattle breeds from *B. taurus* and *B. indicus* to both references. We confirmed that cross-alignments of individuals from one bovine subspecies to reference sequences of another leads to the discovery of three times more single-nucleotide variants (SNVs) with nearly fixed alternative alleles. Interestingly, the alignment of *B. taurus* cattle to UOA_Brahman_1 revealed regions with lower than expected SNV counts with nearly fixed alternative alleles. Since Brahman cattle carry 10% of *B. taurus* introgression on average, we suggest that these regions comprise taurine DNA as opposed to indicine DNA in this particular genome. These taurine loci have been previously reported as being under positive selection in Brahman cattle, and include functional candidate genes implicated in feed efficiency, development and immunity. Overall, the taurine segments amounted to 13.7% of the UOA_Brahman1 assembly. The flagged regions are to be consulted as these regions might produce variant calling bias in forthcoming re-sequencing analysis of indicine genomes aligned to this assembly.

Evidence of rare misassemblies in the bovine reference genome revealed by population genetic metrics

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Creation of the bovine reference assembly paved the way to develop the high-throughput genotyping arrays of the single nucleotide polymorphisms (SNPs) based on the available map coordinates that facilitated major advances in gene mapping and selection programs. The assembly flaws, however, may cause false positive results in the downstream gene mapping studies. The most recent bovine reference genome (ARS-UCD1.2) was built on long-read sequences that ensure an improved quality and continuity. By applying population genetic metrics in this study, we aimed to evaluate the map coordinates to which SNP markers were assigned. We employed a three-step approach by combining the recombination and LD analyses to test if the markers fit into the assigned map coordinates based on the physical distance to the neighbouring loci. We applied the method to the bovine 50K array in a large pedigree of Holstein cattle and revealed a panel of 51 misplaced markers, most of which were re-located either on a different chromosome or re-mapped as far as several millions of base pairs away on the same chromosome. In total, 34 out of 51 identified misplaced markers were located within already known problematic chromosome fragments. This list of misplaced markers accounts for 0.1% of the SNPs in the widely used 50K genotyping array and we foresee a reasonably larger set of markers being misplaced in the bovine 700K array. We suggest pre-removal of the misplaced markers to hinder false positive signals in association mapping studies.

En route to routine genotyping by low-pass sequencing in Swiss pig breeds

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The Swiss Large White pig population was split into maternal and paternal lines roughly 10 generations ago. Since then, the lines have been selected divergently for fertility and fattening traits. About 70% of the genes of 2.5 million fattening pigs slaughtered every year in Switzerland originate from both lines, demonstrating their importance. Using pedigree records, we estimated the effective population size of the dam and sire line to 72 and 44, respectively. In order to assess sequence variation in both lines, we sequenced the genomes of 32 and 38 boars from the dam and sire line at an average coverage of 16.7-fold. The 70 key ancestor animals explained more than 90% of the genetic diversity of both populations. Variant discovery using the GATK revealed 26,862,369 polymorphic sites. Although the separation occurred relatively recent, considerable genetic differentiation was evident from principal components, admixture and FST analyses. Using two complementary approaches (CLR and iHS), we detected signatures of selection at 51 genomic regions. However, only six signatures of selection overlapped between both lines. Using the sequenced haplotypes of the 70 key ancestors as a reference panel, we called genotypes at 22.6 million biallelic autosomal variants in 175 pigs that had been sequenced at shallow coverage (1.11-fold) with Gencove's low-pass sequencing platform. Genotypes from the low-pass sequenced samples were called using the GLIMPSE approach. The genotype concordance, non-reference sensitivity and non-reference discrepancy between GLIMPSE-imputed and array-called genotypes were 97.60, 98.73 and 3.24%, respectively. Diagonal and off-diagonal elements of genomic relationship matrices constructed from array- and GLIMPSE-called genotypes were strongly correlated (0.97 and 0.99). Our results suggest that a reference panel of only 70 animals facilitates genotyping by low-pass sequencing at high accuracy in the Swiss pig breeds. Genotyping by low-pass sequencing increases the variant density over current array-based genotyping solutions by more than 400-fold for the same cost.

Preliminary genomic analysis of robustness in a mice population

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Genetic control of the environmental variability of a trait is possible. This selection criterion has implications in animal robustness and in animal welfare. A divergent selection experiment for birth weight environmental variability in mice has successfully performed during 25 generations. Selection for low variability (line L) was beneficial for traits related to robustness such as birth weight homogeneity, litter size, survival and growth compared with the high variability line (line H). The objective of this study was to identify genomic regions associated to animal robustness in mice. A total of 1,248 animals genotyped using the Affymetrix Mouse Diversity Genotyping Array were used (621 H and 627 L). After quality control (MAF=0.05 and Call rate=0.95) 191,679 SNPs were kept. Observed values for variance (V), standard deviation (SD) and coefficient of variation (CV) of birth weight, litter size (LS), mean birth weight (MBW), individual pup birth weight (BW) and total litter weight (LW) corrected for the main effects affecting the traits were used for analyses. The model included the generation, the number of parturition and LS (except when it was used as trait) as fixed effects. The Misztal's software suite *postGS* was used to estimate the marker effects and the significance level. Gene-annotation enrichment and functional annotation analyses were performed with BioMart Software by Ensembl Genes103 and DAVID v6.8 bioinformatics tools. A total of 83, 91, 92 and 26 SNPs were considered significant ($-\log(p)\geq 4$) for V and LS and ≥ 7 for BW and LW respectively. Enrichment analyses allowed to identify 8 functional gene clusters on the mice genome (chromosomes 2 and 18) for LS and 1 located on chromosome 10 for BW. Genes forming these functional clusters were associated to physiological processes related with response to stimulus, homeostasis and reproduction. This preliminary analysis suggests that our experimental populations can be informative on the genomic basis global robustness and BW homogeneity.

Session 57 Poster 10

De novo genome assembly of the red-legged partridge (*Alectoris rufa*)

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Listed among the most popular small game species of western Europe, the red-legged partridge (*Alectoris rufa*, Galliformes) is also a species of major zootechnical interest. In this study, we generated a high-quality genome assembly (96× coverage; estimated genome size 1.19 Gb; N50 11,577 Mb; overall genome heterozygosity 0.0023; GC content 41.44%; no runs of homozigosity) – the first complete genome for the genus *Alectoris* – by utilising novel Linked-Reads 10X Chromium technology. We flagged 94.9% of single copy avian orthologs (7,913 single copy orthologs out of 8,338 proteins) relying on BUSCO (Benchmarking Universal Single-Copy Orthologs) analysis, while genome annotation allowed us to identify 21,589 protein coding genes which were assigned to 16,772 orthologs. A comparative approach based on publicly available and fully annotated avian genomes (i.e. domestic chicken *Gallus gallus*, Japanese quail *Coturnix japonica* and zebra finch *Taeniopygia guttata*), indicated that 201 gene families were unique to *Alectoris*. Enrichment analyses revealed their involvement in processes such as positive regulation of epithelial cell migration, viral genome integration and maturation. This novel genome will offer the opportunity to improve the poultry husbandry practices for this and related species by bettering our understanding of the physiological processes associated with disease resistance as well as egg and meat production.

Session 58 Theatre 1

New methodological approaches in ethology: social information in equine movement gestalt

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Domestic horses are known for their highly developed perceptual skills relating to processing patterns of movements. This ability allows horses to draw conclusions about the intentions and the motivational state of conspecifics. In this study, the highly developed perceptual skills of domestic horses were the object of the interest. The first aim of the study was to examine what kind of intrinsic information was comprehended in the behaviour of freely moving horses. In order to assess and analyse this behavioural information, we collected inertial movement sensing data recorded from multiple locations in free-moving horses (n=26) (Equus caballus). These recordings were synchronized for all the sensors- and segments of one second were randomly chosen. Out of these segments, statistical characteristics were extracted ('feature extraction'), which were then subjected to a supervised machine learning algorithm in order to extract kinematic gestalt profiles. This yielded rich and multi-layered sets of information. In particular, breed, identity, sex and some personality traits were discriminated from the overall movement patterns of freely moving subjects. Our study suggests that, by attending to movement gestalt, domestic horses, and probably many other group-living animals, have access to rich social information passively but reliably made available by conspecifics. This study opens an interesting direction for further research in the field of ethology by applying new methodological approaches by coding behaviours, towards a highly objective, exhaustive, sensitive and autonomous monitoring procedure.

Do horses perform social referencing with their owner?

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Social referencing is a phenomenon which was first observed in infants and their primary caregiver. In uncertain situations, infants use the emotional expression of their caregiver towards an object, person or situation to orientate their behaviour. This process allows to precociously recognize dangerous situations. Social referencing has also been observed to happen between animals and humans. A study, published in 2020, found that horses use social referencing with humans. The emotional expression of an unknown experimenter influenced vigilance behaviour and positioning with regard to an unknown object in horses. In this study, we tested 60 horse-human dyads in three uncertain situations. In contrast to the above mentioned study, horses participated with their owner who was considered to act as an equivalent to a primary caregiver. Dyads were confronted in a randomized order with three unknown objects. In every test run, the owner had to express either a positive (happy), a negative emotion (fear) or a neutral emotion towards the object. The criteria required for social referencing to be observed were the display of referential looks (RL), i.e. a look towards the object briefly followed by a look towards the owner or vice versa, gaze alternation (GA), i.e. a series of three looks between owner and object, and behavioural adaption according to the displayed emotion. RL could be observed in 96% of horses and GA in 62%. Furthermore, RL and GA had an significant effect on the time horses spent interacting with the object, as well as on latency to approach the object. Results concerning behavioural adaptation with respect to the exhibited emotions are still pending. Nonetheless, the outcomes of this study allow the interpretation that horses actively look at their owner to gather additional information when confronted with an unknown object. Findings on this subject matter are relevant because they allow us to gain a deeper knowledge on the cognitive abilities of equines and the complex relationships they form with humans.

Do rider-horse pairs have similar personality traits?

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The personality of the horse and that of its owner influence the quality of the horse-human relationship. In this study, we created our own questionnaire to assess the personality of horses working with 55 items. 2,392 horse-owners filled it out for their own horse along with an existing questionnaire to determine their own personality. They also indicated their level of satisfaction with their horse. Out of this sample, 40 horses were additionally tested in personality tests carried out by 2 experimenters, in order to monitor the owners' responses to the questionnaires. We then compared the results of the equine questionnaire to the results of the personality tests and then investigated which components of the equine personality and of the owner, contribute to the quality of the relationship. Personality scores obtained from the questionnaire showed, first, that riders with a higher neuroticism score perceived their horses to be also easily stressed and described themselves as satisfied with this relationship. Second, we were able to show that conscientious riders described their horses as conscientious as well. Tests carried out on the 40 horses confirmed the personality scores obtained by questionnaire for the neuroticism trait. Indeed, horses described as being easily stressed were much more active during personality tests. A better horse-rider match would increase owner satisfaction and indirectly horse well-being by limiting conflicts due to misunderstandings. However, these results do not allow us to exclude the effect of the rider's personality on his horse in the long term.

The relationship between upper body motion and vertical ground reaction forces in trotting horses

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Differences between contralateral peak vertical ground reaction forces (dFz_{peak}) can be considered the gold standard for the objective quantification of weight-bearing lameness. In practice however, measurements of vertical motion asymmetries (VMA) are more common and convenient. VMAs, typically quantified as min/max differences between steps, have previously been correlated to both subjectively evaluated lameness and dFz_{peak}. Head and withers VMA have been associated with forelimb lameness, and pelvis VMA with hindlimb lameness. However, the interrelationship between the combined effect of the three VMA and the two dFz_{peak} has yet to be investigated. Horses (n=103) with a wide range of fore- and hindlimb dFz_{peak} had been measured at trot on a force measuring treadmill synchronized with an optical motion capture system. Different linear combinations of the head, withers and pelvis VMA, grouped by individual and speed, were used to model the dFz_{peak}. Matlab 2020b was used for the analysis and models were created using the *fitlme* function. The aim was to analyse the model responses to different VMA inputs in order to evaluate their impact on dFz_{peak}. We hypothesized that all included VMA would be relevant for predicting both fore and hind dFz_{peak}. Results showed a complex relationship between dFz_{peak} and VMA. All included VMA were significantly ($P<0.05$) correlated to both fore and hind dFz_{peak}. On average, head VMA contributed with 66/34%, withers with 76/24% and pelvis with 33/67% to the forelimb/hindlimb modelled dFz_{peak}. These results might help determine the clinical relevance of different combinations of head/withers/pelvis VMA as well as distinguishing between primary fore- or hindlimb, ipsilateral or contralateral weight-bearing lameness.

Head and neck position and behaviour in ridden elite dressage horses in warm-up and test situation

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The welfare of the ridden horse is frequently debated in the practical as well as in the scientific horse world. Nearly all rulebooks demand the horse as an ‘happy athlete’, but which are the most important factors influencing the horses’ mental and physical health? The head and neck position (HNP) has been identified in the literature as important influence on wellbeing. In combination with behavioural indicators as marker for the physiological and psychological state of the horses, this may provide an insight into key factors in horse riding. Therefore, in this study it was investigated in ridden elite dressage horses whether there is a relation between the head and neck position, ethological indicators and the grading in the warm-up area and in the test. For the study, 49 starters (83%) of a Grand-Prix Special (CDIO5*) as part of the CHIO in Aachen 2018 and 2019 were examined. For each horse-rider pair, the head and neck positions used were analysed as well as the conflict behaviour for 3 minutes each in the warm-up area and in the test situation. A total of 5,085 individual frames were used for the test and 1,486 single frames for the warm-up. The noseline was carried significantly less behind the vertical in the test than in the warm-up area (5.43±4.19 vs 11.01±4.54 ° behind the vertical; T=34.0; $P<0.05$). The horses showed significantly less conflict behaviour in the test than in the warm-up area (123±54 vs 160±75) (T=76.00; $P<0.01$). In the latter, a smaller head and neck angle and more defensive behaviour of the horses could be observed compared to the test situation. This relationship brings the angle of the noseline and the vertical into the focus as an objectively measurable animal welfare indicator in the ridden horse. As a further result a correlation between the grading of the test and the head and neck position was found (R=0.38; $P<0.05$). The further the noseline was behind the vertical, the higher was the chance of a good rating. This is from the point of view of animal welfare a problematic result.

Session 58 Theatre 6

Mixing feed in compost increases use but not cleanliness of rooting areas for growing-finishing pigs

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Providing pigs with a rooting area filled with an appropriate material enables exploratory behaviour and is therefore considered to improve animal welfare. Mixing corn pellets into the rooting material could increase use and exploration while reducing elimination behaviour in these areas. To investigate this hypothesis, we compared two experimental pens (E) with rooting areas filled with compost produced from garden waste, in which 2 kg of corn pellets were mixed into every morning, with two control pens (C, rooting areas filled with compost only) on a commercial farm. Groups were repeated to obtain six replicates per treatment. We registered behaviour once a week through direct observations of the complete outdoor area and additional video recordings of the rooting area. Behavioural variables were general activity, rooting, agonistic and play behaviour. Cleanliness of the rooting material was assessed via visual scoring and chemical analysis of compost samples. The latter included tests on dry matter content, conductivity, and ammonium concentration. Data were analysed with linear mixed-effects models. Results showed that there was a tendency for a higher total number of pigs in the rooting area in E than in C ($P=0.06$). In E, more pigs were lying in the rooting area than in C ($P=0.04$). There was no difference in activity and rooting behaviour between treatments. Daytime influenced all recorded behaviours in the rooting area ($P<0.001$). Conductivity and ammonium concentration in the compost increased the longer the compost remained in the rooting area ($P<0.001$), but there was no difference between the two treatments. We conclude that mixing corn pellets into rooting material increases the overall presence of pigs, but not the rooting behaviour and cleanliness.

Session 58 Theatre 7

Effect of sudden noises on gestating sows' behaviour

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Previous results on fattening pigs have shown that noise induced behavioural changes and an increase in stress hormone levels. Based on EU Directive (2008), a threshold of 85 dB is required for pig farming in the EU and sudden noises should be avoided. The aim of this study is to determine if sudden noises below 85 dB affect gestating sows' behaviour. The experiment involved a random sample of 11 group-housed gestating sows observed from 13.00 to 18.00 (PM, resting period) and from 23.00 to 0.00 (night, feeding period), of two different days (basal vs test). During test day, for each period studied, various short noises (on average 27 s) below 85 dB were emitted during 4 hours every 10 min. There was no noise emission during the basal day. Individual behaviour was analysed manually via video recording to identify continuously the sow's location in the room (5 areas), its posture (sitting, lying, standing, walking), and its occupation (sleeping, investigating, eating, observing). The influence of the day (basal vs test) and the period (PM vs night) on the time spent for each behaviour was analysed on R applying a linear mixed-effects model taking into account the random effect of the sows. The number of changes of area, posture or occupation was compared between the days and periods with a student t-test. The time spent in different areas and the number of changes of areas were not affected by the day, only by the period. Sows spent more time sitting and less time standing during test day compared to basal day ($P=0.04$). During PM, the number of changes of posture increased during test day compared to basal day (26 vs 15 changes, $P=0.02$). The time spent investigating decreased during test day compared to basal day (-20 min during PM and night), while observation time increased (+16 min during PM and night). The number of changes of occupation was not affected by the day but was higher during the night compared to PM. To conclude, emissions of sudden short noises below 85 dB can affect gestating sows' posture and occupation, which might further impact their nutrient requirements. More investigation should be done to quantify this potential effect.

Is a short observation period sufficient for behavioural studies?

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There are many animal studies, which rely on behavioural observations, but the observation periods (OP) are often limited to a few hours per day because of feasibility. However, does a short time period represent the reality sufficiently? To answer this question, this study analysed video footage of 6 pens with 24 piglets per pen for four days. The number of initiated or received tail-biting or ear-biting and the number of contacts with the pen enrichment material was recorded for 12 h per day. These observations formed the reference point. To create commonly used OP, the first 10 min of every 30 min during the 3 h of activity in the morning and afternoon were combined to two OP (2×6×10 min). Furthermore, these OP were delayed for 10 min and 20 min to cover the whole activity period. The 6 OP were then compared A: among each other, B: to the continuous 3 h OP of activity in the morning or afternoon respectively and C: to the complete 12 h OP. As a measurement, the percentage of agreement (±0 and ±50% tolerance range) of the number of performed behaviour was calculated. Additionally, the robustness of the ranking order of the pigs within a pen was estimated. The overall percentage agreement (±0% tolerance range) for comparison A was around 0.4, for comparison B around 0.3 and for comparison C around 0.1. These scores were 0.1 points higher for the percentage agreement within ±50% tolerance range. For the robustness of the ranking order, agreement of comparison A was around 0.2, for comparison B around 0.45 and for comparison C around 0.25. The results show that the agreement of the investigated behaviours and OP can be very low. Even the agreement among the OP (comparison A) was low, although they were only time shifted for 10 or 20 min. Thus, this should be investigated closer and for other behaviours as well, to determine OP, which are sufficient but still feasible.

Health and behaviour of dairy calves reared with milk from bucket versus mother during four months

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Early separation of cow and calf is still common practice on dairy farms. In recent years, interest in mother-based rearing practices has increased. They are seen as more in line with natural behaviour, labour-saving and health-promoting for the calf, but also pose challenges, e.g. in terms of the cow's willingness to let down milk or the pain of separation after established social bonding. We tested the hypothesis that calves allowed dam suckling (DS) twice a day would benefit with regard to weight gain, health related traits, and show less oral manipulations compared to bucket fed (BF) group mates. Therefore, we conducted two on-farm trials with local German Friesian Cattle (farm 1: n=18 DS vs 17 BF) and Swiss Fleckvieh calves (farm 2: n=12 DS vs 11 BF) until the age of 122 days between autumn 2018 and summer 2020 within the CORE Organic Cofund project ProYoungStock. On farm 1 average daily weight gain (g/d) did not significantly differ between feeding groups (DS: 815±46 vs BF: 807±42 SE g/d, P=0.90). By contrast, calves of primiparous cows on farm 2 benefited from mother bound rearing (DS: 1,133±73 SE vs BF: 714±88 SE g/d, P=0.002), but no statistical difference was found in calves of multiparous cows. Feeding groups did not differ on neither of the farms in terms of clinical findings regarding vitality, body condition traits, indicators for diarrhoea and respiratory disorders, although levels differed between farms. This was also true for number of medical treatment cycles (farm1: DS: 3.06±0.42 SE vs BF: 2.72±0.39, P=0.56; farm 2: DS: 1.00±0.32 SE vs BF: 1.08±0.30 SE, P=0.85). Number of oral manipulations of pen mates was consistently higher in bucket fed calves across both farms (farm 1: BF: 1.34±0.15 SE vs DS: 0.92±0.12, P=0.02; farm 2: BF: 0.24±0.06 SE vs DS: 0.13±0.03 SE, P=0.02), while objects were not manipulated with different frequencies between feeding groups. We conclude that restricted access to the mother alone does only have very limited effect on the traits investigated, while differing overall management conditions between farms show high impact on calf health and behaviour.

Differences in pecking behaviour between conventional layer hybrids and dual-purpose hens

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Dual-purpose hybrids are suggested as an alternative approach to avoid the killing of surplus male layer chickens, but moreover, these strains may also show a lower tendency to develop injurious pecking behaviour. Therefore, in this study, we comparatively assessed the behaviour, with special emphasis on pecking behaviour, of conventional layer hybrids (Lohmann Brown plus, LB+) and dual-purpose hens (Lohmann Dual, LD). Video-based behavioural observations were carried out, both with general behaviour scans and with continuous observations, between 25 and 69 weeks of life. About 1,845 hens per strain with intact beaks were housed in four stable compartments in aviary systems. Behavioural differences between LD and LB+ were revealed and hybrid × time during the laying period affected all of the observed general behaviours ($F_{2,89}$=3.92-10.81, $P<0.001$ – 0.05), except for dustbathing and scratching. General pecking and comfort behaviour did not change over time in the LD hens, whereas inactive behaviours increased with age. In contrast, the LB+ hens performed more general pecking, more locomotion and less comfort and sitting behaviour with increasing age. A significant hybrid × period interaction was found for all forms of pecking behaviour ($F_{2,89}$=4.55-14.80, $P<0.001$ – 0.05) during continuous observations. LB+ hens showed particularly more severe feather pecking (SFP), which increased with age, while in LD hens SFP interactions remained exceptionally low. Therefore, to avoid the development of injurious pecking in laying hen production, dual-purpose hybrids should be considered as an alternative approach as these strains may experience a higher level of welfare under the given conditions in loose housing systems. This study was part of the joint research project 'Integhof'. It was supported under grant number 313-06.01-28-RZ-3-72 by funds of the German Government's Special Purpose Fund held at Landwirtschaftliche Rentenbank.

Analysis of biological pathways related to behaviour in Czechoslovakian Wolfdog

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Even if several studies suggested the inheritance of breed-specific temperament traits in dogs irrespective of the owner's demography or living environment, the polymorphisms controlling the breed-specific behaviour, especially obedience and aggression, are still under research. This study looked for the biological and genetic pathway cascades affecting the behavioural traits in Czechoslovakian Wolfdog (CWD) that is recognized as one of the most popular wolf-like phenotype dog breeds worldwide. The initial literature survey revealed 11 genes (*CLINT1*, *GTF2I*, *GTF2IRD1*, *AMPD1*, *TH*, *SEZ6L*, *TANGO2*, *ARVCF*, *COMT*, *TXNRD2*, *PAPSS2*) significantly acting in the genetic control of behaviour in dogs and humans. Genomic data for 30 animals (high-density canine SNP platform) was then used to find the most important genetic variants responsible for CWD behaviour. After standard quality control of SNP data (minimum call rate set to 90%), up to 50 SNPs inside the sequences of selected protein-coding genes were retained. The biological pathways were identified by the gene ontology (GO) term enrichment analysis, whereas genetic pathway cascades were tested based on the inter-marker linkage disequilibrium. The six most significant enriched GO terms ($P<0.001$) correspond to dopamine metabolic process (GO:0042417), catecholamine metabolic process (GO:0006584), catechol-containing compound metabolic process (GO:0009712), phenol-containing compound metabolic process (GO:0018958), neurotransmitter metabolic process (GO:0042133) and ammonium ion metabolic process (GO:0097164). The genetic pathway analysis revealed the formation of two genes clusters. The first one was composed of the *ARVCF*, *COMT* and *TANGO2* genes, each represented by one SNP. The second cluster consisted of several genetic variants located inside *GTF2I* and *GTF2IRD1* genes. It can be assumed that, because of the selection, there was an increase in the linkage disequilibrium in identified genomic regions; thus, individual genetic variants are inherited from parents to offspring together as haplotypes.

Session 58 Poster 12

The use of elevated platforms and perches in different heights by pullets during rearing

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The rearing of pullets gains importance in the discussion about animal welfare and animal-friendly husbandry systems. Although in Germany no specific legal regulation for pullet rearing exists, this period in the life of a laying hen is decisive for the further development of the animal. Rearing hen husbandry is required to match the husbandry system during laying. In aviaries, hens used to access the structures in all three dimensions are essential. In the 'Model and Demonstration Project LayerHACCP', two herds of pullets from different genetics (Dekalb white and Bovans brown) were monitored during rearing in an alternative system, in accordance with organic standards, with perches and height-adjustable elevated platforms. Observations on the usage of two platforms in different heights and ten perches in three different heights were performed via photos taken by wildlife cameras in three time periods during rearing. In the pullets' active hours from 5:00 to 20:00 o'clock, pictures were taken every 15 minutes. On 2,174 wildlife camera photos in total, the number of birds in each observed area was counted. First results showed that the white and brown genetic differed in the way they used the structures. White hens mostly used perches and platforms in greater heights, while the brown ones preferred those in middle height. At the end of rearing, one meter of the high positioned perches was used on average by 0.51 brown or 6.60 white hens. In addition, time-of-day preferences resulted in more perched pullets during the dark-period. An increased use of the higher structures was observed with increasing age (1.09 white or 0.06 brown hens/m^2 on the highest platform in the early rearing phase compared to 4.52 white or 3.13 brown hens/m^2 at the middle). In conclusion, this on-farm study provided useful information about the space use of pullets in an alternative rearing system. This work is funded by the German Federal Ministry of Food and Agriculture (BMEL) based on a decision of the Parliament of the Federal Republic of Germany, granted by the Federal Office for Agriculture and Food (BLE; grant number 2817MDT200/201).

Session 58 Poster 13

A pain assessment method for goat kids based on the Grimace scale-

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According to the European Directive 2010/63/EU, pain recognition assessment is mandatory in animals used in experimental procedures. A suitable method for pain assessment in goat kids does not exist. As the analysis of facial expressions through the Grimace scale has become an increasingly developed tool to determine the degree of pain in animals, this study aimed to investigate differences between the facial expressions of healthy and sick goat kids by observing the most common facial action units, and to create a suitable method for pain assessment in goat kids based on the Grimace scale. Sixty Majorera goat kids from three different livestock farms were used in this study. All animals were within 20 days of age. Animals were classified by an expert veterinarian into three experimental groups: (1) no pain (n=20); (2) moderate pain (n=20); and (3) severe pain (n=20) and then two photographs of each animal were taken, one of them showed the face profile from the front perspective and the other one from the lateral (i.e. ride side) perspective. Three facial action units were analysed: (1) the orbital tightening; (2) the nose features; and (3) the mouth features. Based on these measurements the following indexes were created: (1) the eye index; (2) the front nose index; (3) the mouth angle; and (4) the profile nose angle. The statistical analysis was performed using RStudio. The eye index as well as the front nose angle were not normal distributed (P=0.024 and P=0.001, respectively), while the mouth angle and the profile nose angle were normal distributed (P=0.559 and P=0.258, respectively). Pearson correlation analysis showed a medium negative correlation between pain degree and the eye index (r=-0.50). In addition, pain degree affected the mouth angle, the eye index and the front nose angle (P=0.013, P=0.001, and P=0.009, respectively), but the effect of pain degree on the profile nose angle was not affected by pain degree (P=0.133). The results of this preliminary study corroborate the hypothesis that goat kids' facial features change according to pain degree. However, further studies are still necessary to increase the number of animals recorded and refine this methodology.

CYBELE: high performance computing for precision livestock farming

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CYBELE generates innovation and create value in the domain of agri-food, and its verticals in the sub-domains of PA and PLF in specific, as demonstrated by the real-life industrial cases to be supported, empowering capacity building within the industrial and research community. Since agriculture is a high volume business with low operational efficiency, CYBELE aspires at demonstrating how the convergence of HPC, Big Data, Cloud Computing and the IoT can revolutionize farming, reduce scarcity and increase food supply, bringing social, economic, and environmental benefits. CYBELE intends to safeguard that stakeholders have integrated, unmediated access to a vast amount of large scale datasets of diverse types from a variety of sources, and they are capable of generating value and extracting insights, by providing secure and unmediated access to large-scale HPC infrastructures supporting data discovery, processing, combination and visualization services, solving challenges modelled as mathematical algorithms requiring high computing power. CYBELE develops large scale HPC-enabled test beds and delivers a distributed big data management architecture and a data management strategy providing: (1) integrated, unmediated access to large scale datasets of diverse types from a multitude of distributed data sources; (2) a data and service driven virtual HPC-enabled environment supporting the execution of multi-parametric agrifood related impact model experiments, optimizing the features of processing large scale datasets; and (3) a bouquet of domain specific and generic services on top of the virtual research environment facilitating the elicitation of knowledge from big agri-food related data, addressing the issue of increasing responsiveness and empowering automation-assisted decision making, empowering the stakeholders to use resources in a more environmentally responsible manner, improve sourcing decisions, and implement circular-economy solutions in the food chain.

A two-step deep learning model for pen-level estimation of slaughter pig live weight distribution

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When pigs are sold for slaughter, the price per kg depends on what weight range, the individual carcasses fall into. Pigs that are either too small or too heavy will thus yield a smaller profit for the farmer, compared to pigs in the optimal weight range. Since carcass weight is closely connected with live weight, accurate automatic monitoring of the pigs' live weight has the potential to be of significant economic importance for pig farmers. Some commercial systems for video based weight monitoring already exist, but these all estimate the weight of one pig at the time while eating or drinking, which is likely to lead to biased data. In the CYBELE project (Horizon 2020, grant no. 825355) we are developing a two-stage deep learning model for estimating the distribution (mean, standard deviation) of live weights at pen level, using images of the whole pen. Our approach will use a pre-trained convolutional combined with a secondary model with regression output. The pre-trained convolutional neural network will be used to extract abstract features from the input images. These, in combination with relevant meta data (e.g. time of the day and the number of pigs in the pen), will be used as inputs for the secondary model, which will produce the live weight distribution as its output. We will systematically compare various combinations of input image dimensions, network architectures, and training strategies. The performance will be measured in terms of the coefficient of determination with 95% confidence intervals.

Video-based classification of agonistic behaviour in pigs using a combination of CNN and RNN

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The majority of commercial pigs in the EU are raised under intensive conditions that are likely to increase the development of agonistic behaviour. Because of its association with stress, pain and an increased risk of infection of inflicted wounds, agonistic behaviour is a major welfare and economic challenge in pig production. Timely detection of the behaviour within groups of pigs could aid farmers in their decision making on prevention and intervention strategies. Hence, this project aims to develop a computer vision based tool for automated continuous monitoring and recognition of agonistic behaviour in groups of pigs, based on video images recorded from above the pen, with the intention to be applied to the farms' own video surveillance. As the input data is visually complex with added temporal features, we intend to achieve this by implementing a deep learning approach, consisting of a convolutional neural network (CNN) combined with a recurrent neural network (RNN). A pre-trained CNN will be used to extract relevant spatial features from images, followed by a RNN trained to take time series of extracted features as input and to provide a behaviour classification as output. We will systematically compare performance of models where spatial features are extracted at different layers of the CNN, before being input to the RNN. The performance will be measured in terms of sensitivity with respect to detecting undesired behaviour, and the number of false alerts per 1000 frames (FAR1000). This research was done with support from the EU's Horizon 2020 programme (CYBELE, Horizon 2020, grant no. 825355).

Individual pig health monitoring using multivariate analysis on feeding, drinking and weight data

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Automated monitoring of individual pigs to assess their performance, health and welfare is being researched and getting into practice in the fattening pig sector. Electronic ear tags are a key technology in this. They can be used to track pigs from birth to slaughter and for individual pig monitoring during the growing phase. Sensors and antennas can be placed in the pen to monitor the pigs' feeding behaviour, drinking behaviour or weight. The data can be used to design warning systems that alert pig farmers in case of problems. In previous research, warning systems based on a single variable have proven successful in the detection of some health problems. However, other problems are missed and false alerts do occur as well. Warning systems based on multivariate analysis could provide more information on several aspects of the pig in order to improve the performance of the problem detection. Data of 10 fattening rounds (2013 to 2020) were collected on the ILVO farm in one high-tech compartment. Individual level data was obtained for 1,365 fattening pigs using a variety of sensors. For 945 pigs feeding behavioural data was gathered with RFID antennas at the feeder to register feeding visits. For 839 pigs, drinking behaviour data was collected using the same RFID antennas at the drinker. The registration of drinking visits was combined with a flow sensor to obtain the water usage per pig. For 420 pigs feed intake, feeding visits and current weight data was collected with an individual feeding station. Health of all pigs was monitored on a daily basis by the caretakers in the barn, while researchers performed a more thorough evaluation of problems three times a week during 6 fattening rounds. Climate data (temperature and humidity) was obtained in 7 fattening rounds. With this data, a total of 44 individual pig features were calculated, combined with 66 group variables. Using the CYBELE framework (H2020 project no. 825355) that enables the use of high performance computing (HPC) for precision agriculture and precision livestock farming, several multivariate models will be trained and compared. Practical insights, results and performance evaluation will be presented for each of these models.

Evaluating machine learning techniques applied to the problem of boar taint

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The main goal of data gathering in industry is to deliver better quality of products while maintaining or increasing production rate. While this holds true, real world data are often dirty with redundancy, noise, diversity and heterogeneity making it difficult to make decisions. A real-life scenario that can showcase the added value of data is that of the unwanted smell/taste of boar meat, also known as 'boar taint'. Stakeholders need actionable information to reduce this occurrence. The European pork sector has committed to phasing out surgical castration of male piglets, traditionally performed to prevent boar taint since 2010 and several countries have made steps towards this goal. When choosing for the 'entire male' alternative preventing tainted meat from reaching the consumer is an important condition. Although statistics, with its hypothesis testing and the systematic study of variable importance, is well established, the same does not apply for the explainability of techniques in machine and deep learning. Even though multiple measures and metrics of performance have been applied, some can be very misleading as they don't convey the 'why' of decisions. This is a research area known as Explainable AI (XAI). Multiple methods for introducing model explainability such as shapley values (SHAP) and LIME, the results and the impact of evaluation metrics, were studied trying to conclude to actionable insights and recommendations for European pork sector stakeholders regarding the reduction of boar taint. Specifically, the use-case was modelled as a binary classification task (i.e. tainted=1, not tainted=0) concluding with a highly imbalanced dataset (i.e. imbalance ratio=1/50). Consequently the development of a robust Classifier that can classify accurately the unseen data is an open question. Some attributes have emerged, both pen and slaughterhouse conditions such as the behaviour, cleaning, antibiotics consumption, the lean meat percentage, and skin lesions.

Enhancing in-line meat processing traceability using deep learning

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In meat production, traceability of the individual animal often stops at slaughter or at carcass cutting. However, quality assessment becomes highly valuable later in the processing chain, after carcass cutting in order to differentiate and valorise parts based on meat quality and purpose. Expanding the traceability from carcass to carcass cut level would allow to link meat quality to the origin of the animal. This linkage may lead to more insights into the effects of diet, breed or other management practices and would accelerate the possibility to steer the rearing conditions in order to get the right quality for the current market demands. However, a practical solution faces the following challenges: (1) it should be food grade and (2) it must track different primals once the carcass has been cut. Therefore, we propose a solution where in-line tracking is deployed using industrial cameras. Non-contact tracking allows flexible, food-grade integration and expansion to other processing lines. The building blocks from this proof-of-concept consists out of (1) four industrial 20 MP Triton cameras (Lucid Vision, Richmond B.C., Canada), (2) a RetinaNet object detector and (3) a DeepSORT tracking module. As the training process of the deep learning models in the solution will quickly increase during deployment, the CYBELE framework (H2020 project no. 825355) will enable the use of high-performance computing (HPC) for precision agriculture and precision livestock farming, which will allow fast retraining when needed. The focus of this presentation will be on model building blocks, data analysis flow and image processing. Practical insights, preliminary results and performance evaluation will be presented for this system.

Session 59 Theatre 7

Prediction of pork meat quality parameters with existing hyperspectral devices

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In this study, the use of two commercially available hyperspectral devices for measuring fresh pork quality traits was investigated as a faster measurement for different fresh meat quality reference parameters. One VNIR linescan hyperspectral camera (CAM) Specim FX10e™, 400-1000 nm and one hyperspectral spectrometer with a probe optimized for meat measurements (SPEC) ASD Labspec 4™ with Bonzai Advanced Meat ProbeTM, 350-2,500 nm, were tested. On 5 days, each time 6 batches of carcasses with different genetic background were selected in the slaughter house. Per batch, four carcasses (two male, two female) were chosen with high and low lean meat percentages, resulting in 120 animals in total of which the *Longissimums thoracis et lumborum* (LTL) was collected and cut 24 hours after slaughter. Spectra from the region of interest on a transverse cut surface were obtained from the hypercubes of the VNIR camera with image processing for three slices per LTL. The spectra were linked to the reference data (colour, pH, shear force, drip loss, IMF, sensory panel) with partial least squares regression. Based on Ratio of Performance to Deviation (RPD) for the prediction (Pred), the models showed promising performances for CIELAB colour for CAM, with (RPD Pred L*=2.19, a*=2.08, b*=1.72) and SPEC (RPD Pred L*=1.60, a*=1.61, b*=1.76) at individual sample level. For CAM, ultimate pH and intramuscular fat models also had RPD promising models with RPD Pred 2.13 and 1.82 respectively. For SPEC, it was necessary to average the spectra and reference measurements on animal level (RPD 1.84 and 1.65) in order to have good predictions. Drip loss and shear force showed clear correlations, but were too unstable to have good predictions. Models for cooking loss and sensory panels results showed low correlations (RPD Pred <1.3). Overall, hyperspectral measurements show potential as in- or at-line (sample based) implementation, although differences between the sensors were found and optimization remains necessary.

Session 59 Theatre 8

Towards in-line fish species classification in beam-trawl fisheries for real time stock assessment

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The sustainable exploitation of marine fish stocks requires an efficient, controllable and enforceable fisheries management. In general, TACs (Total Allowable Catches) and quota are an indispensable management measure to keep fish stocks within safe biological limits. To determine these TACs and quota, stock assessments are carried out based on a broad and intensive data collection, often with a low coverage in space and time. Innovative methods to increase the efficiency of the data collection are needed to improve the quality of stock assessment and to make steps towards the ecosystem approach of fisheries management. An integrated camera above the sorting conveyor belt on board of commercial as well as research vessels could allow automatic species classification and enhance the sampling frequency and area. The improved data collection could also serve business intelligence tools for fishermen which can lead to a more efficient identification of the most valuable fishing grounds for certain species and deepen our understanding of the marine ecosystem by extending the number of monitored bycatch species. As a basis for a trial to analyse images of fish, a dataset was captured using a 20MP industrial camera on a static setup during a scientific fisheries mission. Over 6,600 images were collected of more than 23 fish species. An in-line tracking system to detect fish on the sorting conveyor belt inside beam-trawlers would encompass two phases: (1) an onshore training phase where a larger amount of fish catch images will allow a deep learning object detector to correctly identify fish species and (2) an inference phase where a model detects and classifies fish in real-time. As the training time quickly increases during scale-up, the CYBELE framework (H2020 project no. 825355) enables the use of high-performance computing (HPC) for precision agriculture and precision livestock farming, which will allow fast retraining when needed. The main model building blocks, data analysis flow and image processing will be presented as well as practical insights, preliminary results and performance evaluation of the system.

Applying machine learning to production data from aquaculture to predict fish growth

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Aquaculture is the fastest growing food-producing sector and now accounts for more than 50% of the world's fish that is used for food. With the world population expected to reach nine billion by 2050, the aquaculture sector will play a key role in ensuring food and nutrition security. However, this growth is not without challenges; in order to satisfy the demand and minimize the impact on the environment, the sector has to use new technologies to intensify, diversify and produce in a more efficient, sustainable and environmental friendly way. One of the main issues in commercial aquaculture is the loss of food when the fish are fed. This not only increases the cost of the produced fish (feed cost is a major cost component that accounts for approximately 70% of the OPEX of the farm) but furthermore, this wasted food is deposited in the seabed and generates an environmental impact on the surrounding area. It also causes failures to comply with EU legislation. Another challenge is maintaining the farm in a good condition. If the cages are not in the correct positions, have deformations, anti-bird nets not placed correctly, etc. this usually leads to damages, financial losses and uncontrolled escapes to the environment. This paper outlines how the use of drones, image processing and data mining can be used to optimize feeding, evaluate impact on the environment and evaluate the status of the infrastructure in open sea aquaculture.

Sustainable pig production systems: the SusPigSys system approach

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Society's demand for more sustainable pig production requires methods for measuring the sustainability of a pig farm across all pillars, i.e. economy, environment, society and animal welfare. Also, farmers need facts from their own and other farms for optimising the balance between all sustainability pillars. The ERA-Net SusAn project SusPigSys aimed to fill this gap with evidence from farms and integrative assessment methods. We developed a draft assessment protocol with indicators for all four pillars, which we discussed in stakeholder workshops in each partner country (AT, DE, FI, IT, NL, PL, UK) to ensure completeness and relevance. We then applied the resulting detailed protocol on 68 farms across partner countries. Afterwards, we condensed the protocol based on data quality, feasibility and reliability aspects as well as additional stakeholder workshops. The condensed protocol was applied on 157 farms in the same countries. In order to allow comparison of different farms, we developed formulas for summarising the indicators in sustainability scores at subtheme and theme level. Formulas include relative impact values allocated by experts in a survey. The resulting 'integrative system analysis toolbox' is available as a report and in the form of a free app for on-farm assessment and feedback. SusPigSys thus produced a hitherto unavailable assessment system for all four sustainability pillars on pig farms. Its development required to not just bring together but really consolidate different pillars. This was a challenge due to different terminologies, data formats, typical data sources, etc. We solved most issues within the project. However, successful future sustainability assessment requires more consolidation at large scale, starting with making currently available data more comparable within and across countries.

Session 60 Theatre 2

Improving pig production: a whole system approach – insights and proposals from PigSys

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Suboptimal resource utilization due to outdated building standards, control systems and barn management approaches results in high emissions and losses within EU pig production. Additionally, with increasing concern for animal welfare, pig farmers are struggling to maintain competitiveness. The PigSys project addresses these issues by considering a whole system improvement towards a future sustainable and animal friendly pig production. The developed data warehouse platform is accessible to end-users to collect information on building characteristics and data from on-farm equipment, to connect them with a decision support system (DSS based on a bioclimatic model called ThermiPig). The DSS simulates the thermal balance and climatic conditions of a fattening room depending on a farmer's decisions. A system using deep learning and machine vision techniques was developed to monitor pig behaviour, providing a tool for early warning of problems related to pig welfare and health. New sensor data was used to evaluate level of noxious gases that impact pig performance and welfare. Pig cooling with showers over the slatted area and increasing air velocity at pig lying area were cost-effective options improving hygiene and mitigating ammonia emissions, both being crucial for improved environmental and social acceptability. Models to assess the trade-offs between environmental and bio-economic impacts of different pig housing and manure management were developed.

Session 60 Theatre 3

PEGaSus: Phosphorus efficiency in pig and chicken – An animal-centred view on the phosphorus cycle

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Phosphorus (P) is essential for life, but global commercial resources of mineral P are limited. P emissions from intensive pig and poultry farming raise environmental concerns. Approaches are needed to improve P bioavailability in feed and body P utilisation and retention as well as measures to regulate P release into the environment, taking into account economy, society, and ecology. The strategic objective of the ERA-NET funded project PEGaSus is to provide and evaluate solutions to sustainable agricultural systems with the focus on P management. To this end, the fate of P in feed, pigs and poultry, microbiota, manure, slaughterhouse wastes, and soil was tracked. Different feeding strategies and diets were tested which showed for example that supplementation of 250 FTU/kg diet (DM) phytase levels resulted in manure release with increased plant P use efficiency and decreased soil P availability, thus making the plant-soil system more P conservative. The endocrine and transcriptomic response of pigs and poultry to variable P supply offer perspectives for reduced mineral P input and P-efficient animals employing genetic and epigenetic mechanisms. A bio-economic farm optimisation model was developed to assess the farm level economic and environmental impact of alternative P management strategies. In view of the current P loading of soils and water bodies, the assessment has derived that current European regulations on manure spreading and fertiliser use should take much more account of P surpluses and losses. The project contributes to providing novel sustainable P management approaches to reconcile the economic, social, and environmental sustainability of European pig and poultry production.

Sustainability in pork production with immunocastration

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The ERA-NET project SuSI deals with immunocastration (IC) as a sustainable alternative to pork production with surgical castrates and boars with the aim to optimize pork production on a social, environmental and economic level. Most male piglets in the EU are surgically castrated, which is painful and violates their physical integrity. Raising entire males is not the hoped-for alternative, mainly due to boar taint, while the use of anaesthesia/analgesia during castration also encounters challenges at the level of animal welfare, environment and user safety. The key findings of SuSI are: (1) IC works reliably under different housing conditions and reduces behavioural as well as animal welfare problems; (2) IC effectively reduces boar taint, with differences between animal trials. The chance of boar taint was not completely absent in all trials; (3) IC produces meat of high quality comparable to surgical castrates and is thus an alternative for high-quality traditional products and outdoor systems; (4) IC is ecologically efficient, when feed is adapted to dynamic requirements; (5) IC has a high level of consumer acceptance as an ethical concept as well as due to the good sensorial quality; (6) IC has economic and productivity benefits – if it gains market acceptance. Stakeholders at EU level are generally ready to abandon castration without anaesthesia/analgesia, while the preference for alternatives depends on external factors, e.g. the production system and the market. SuSI offers optimized knowledge of IC to support the stakeholders in their decision-making processes for consumer-acceptable pork production across the EU. IC can make the EU pig industry more sustainable and for some countries more competitive.

PIGWEB: an infrastructure for experimental research for sustainable pig production

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The European Commission considers research infrastructures as key elements for the advancement of knowledge, structure the scientific community, facilitate open, interconnected, and data-driven science, and attract young people to science. PIGWEB is a Horizon 2020 project that started on March 1st, 2021. The aim of the project is to strengthen the pig research community by providing and facilitating access to research infrastructures, reinforce a culture of cooperation between the research community and industrial and societal stakeholders, and improve and integrate the services provided by the research infrastructures. The project gathers 16 partners from nine countries. Twenty-eight installations provide transnational access, allowing external parties to carry out experiments in the PIGWEB installations, funded by the project for a total budget of 1.5 million euro. Transnational access is given to various housing facilities and production systems, slaughterhouses and associated equipment, experimental feed mills, and laboratories to carry out studies on pig nutrition, metabolism, physiology, behaviour, and emissions. The project also focusses on best practices for protocols, standards, and ethics in experimental pig research and on ontologies and Open Data. Joint research activities are carried out to identify and test non- and minimally invasive proxies of efficiency, health, stress, and environmental impact. Also, new methods, tools, and technologies will be developed to measure traits that are currently difficult to measure, related to welfare, behaviour, and body composition. Newly developed and available research methods and tools will be tested to carry out integrated phenotyping of pigs.

Session 61 Theatre 1

Principles and practices of meta-analysis applied to animal science

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Methods of systematic narrative review, based on the concept of statistical significance, are often accused of lacking transparency and repeatability. In fact, how can we interpret or even combine the results of studies whose effect has proved to be significant with others whose effect has been shown to be nonsignificant? Meta-analysis is a statistical technique that allows us to combine the results of two or more independent studies on the same research topic. This focuses on the magnitude and direction of the effect under consideration, which makes it possible to explore the patterns and causes of variation between studies, called heterogeneity, of the published studies on a given theme. On the other hand, the meta-analysis gives us an average effect value, evaluates the consistency of the effect between studies, as well as establishes cause-effect relationships between the effects of the study and the conditions that characterize them. In this lecture, we will present and discuss the principles and practices associated with conducting meta-analytical studies applied to animal science.

Session 61 Theatre 2

Meta-analysis and mechanistic modelling

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One of the main challenge of meta-analysis concerns its combination with mechanistic modelling. First it is possible to adjust a non linear model with a dataset treated through a meta-analytic process. It is the case of non linear models which can be treated as a compartimental one. For larger mechanistic models Sauvant and Martin have suggested to apply meta-analysis at the two different phases of construction and evaluation of the development. At the underlying level, meta-analysis of a first database allows one to obtain realistic values for the key basic parameters and relationships used to build the mechanistic model. Secondly. At the most integrated level, meta-analyses of a second database, obtained at a level of the whole the system, allows to assess the global validity of the model. Otherwise it is possible to build mechanistic models using a top-down approach by relying on intra-experiment regressions Yi=f(Xi) used as 'structural equations' to adjust values of the underlying parameters related to flows and compartments. These structural equations are established from meta-analyses of databases of inputs and outputs flows. Such an approach has already been used to construct (1) a mechanistic model of fibre digestion in the rumen and (2) a mechanistic model of the liver. In this context, the domain of validity of such models is directly linked to the range and representativity of data included in the meta-analysis, and thus, used for the calibration of the structural equations integrated into the mechanistic model. The recent INRA 2018 feed unit system is another example of synergy between meta-analyses and mechanistic modelling. Indeed, to update the system, specific regressions were obtained from meta-analyses of sub-databases focused on various feeding factors. The consistency of these equations was checked on a digestive mechanistic model that was calibrated on structural equations of fluxes derived from meta-analysis of the literature. Moreover, if the data and meta-analysis are representative of the feeding practices encountered in field conditions, this model is capable of application to a wide range of practical situations.

Session 61 Poster 3

Environmental enrichment reduces feather damage in laying hens: a meta-analysis

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Preventing feather damage (FD) is an important challenge in the egg production sector as it impacts laying hens' health, wellbeing, locomotion, and thermoregulation. Modifications to the environment to stimulate animals' biological functioning and psychological well-being, also termed environmental enrichment, are often suggested as a management strategy. However, questions remain regarding the effectiveness of this approach with different studies finding contradictory results. A systematic review and meta-analysis was performed to investigate the effect of environmental enrichment on FD in laying hens. A total of 23 studies met the inclusion and exclusion criteria. Differences in scoring scales and methodology required that FD outcomes were standardized which was done on a 1 to 4 scale where a higher score indicated more intact feather cover. The presence of enrichment, production period when the enrichment started, housing type, beak trimming, bird strain, and age of the birds were also extracted. Linear mixed models were created treating the experiment as a random effect. Variables with $P<0.30$ in univariable analysis were retained for multivariable analysis. Variables with $P<0.05$ were kept in the final models and model selection and evaluation based on corrected Akaike information criteria, the root mean square prediction errors, and concordance correlation coefficients. More severe FD was observed in flocks lacking enrichment ($P=0.018$), older flocks ($P<0.001$), flocks with intact beaks ($P=0.001$) and flocks kept in caged housing ($P=0.042$). These results showed a reduction in FD when environmental enrichment was provided; however, this reduction was relatively small. Environmental enrichment should be considered with other management strategies to effectively manage FD.

Session 62 Theatre 1

Dairy cattle housing and management for warm climates

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Warm climates and weather lead to heat stress in dairy cattle if they not properly managed. Heat stress negatively affects the performance, health and welfare of dairy cattle. The rise of global temperatures exacerbate the problem. Experience and research has shown that targeted housing and management are necessary to alleviate the effects of heat stress on dairy cattle. For example, dietary interventions such as feeding chromium supplementation and melatonin and may reduce the detrimental effects of heat stress on fertility. An effective strategy is altering the timing of feeding to take advantage of the lower nighttime temperatures. Some cattle are more heat tolerant than others, so breeding values for thermo-tolerance are being calculated and used. Inclusion of the slick-hair gene into high producing Holstein cattle is another approach of making dairy cattle genetically more tolerant of high temperatures. *In vitro* produced embryo transfer improves conception rates during heat stress, but cost may still be prohibitive. Housing design, including heat abatement systems, is a primary way to reduce heat stress. Fans and water sprinklers or misters increase evaporative cooling. In the USA, there is continuous development in size, number, and position of fans, as well as position and schedules for sprinklers. Newer systems are designed to be more energy and water efficient. Tunnel barns and cross-ventilated barns are constructed to help direct airflow to cool cows. Heat abatement for heifers and dry cows has received more attention in the last decade. Recent research has shown that cows provided relief from thermal stress during the dry period give birth to heavier calves, produce more milk during the next lactation and have a better immune response during the transition period. Late gestation heat stress of dairy cows also depresses milk production of their daughters and possibly granddaughters. In the USA, economic losses are estimated to be $87 per cow per year if dry cows are not cooled. We observe that heat abatement strategies including management of feeding, reproduction, genetic selection and housing design are varied and continue to receive greater emphasis in dairy production across the USA.

Performances of mechanical ventilation in dairy farms

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High-producing dairy cows are particularly sensitive to heat stress. When the THI (Temperature Humidity Index) threshold is above 68, cows suffer from heat stress. To improve their comfort, it is necessary to reduce solar radiation with shade inside the barn and to increase air speed by opening the side walls and sometimes by using mechanical ventilation. The aim of the study was to measure performances of mechanical ventilation in dairy farms. We measured air speed inside eighteen barns during summers 2018, 2019 and 2020 every two meters in length and width. The air speed averaged 0.63±0.51 m/s in the farms. Without fans (seven farms), we recorded 0.27±0.21 m/s and with fans (eleven farms), we recorded 0.86±0.70 m/s. In seven fan-equipped farms we visited, the air speed inside the barn is below the threshold of the expected 1 m/s. The levels of equipment and/or performance of the fans were insufficient to provide good ambient conditions. Concerning horizontal flow fans, we observed big differences between them. Depending on type of fan, to obtain 1 m/s, the ventilation goes from 2.50 meters to 7 meters wide and from 10 to 15 m long. Sometimes, farmers have invested in fans first to improve air renewing inside the barn. They installed fans with vertical flow which move a large air volume but with low air speed. These fans are usually used all along the year, but the air speed is not sufficient during hot periods. So, the heterogeneity of comfort can be observed resulting in gatherings of animals in the standing positions in the most favourable areas. We have also to consider where the fans are installed. If you only install fans in the alleys, the cows will be standing even more. Fans must be installed in the holding area, above the cubicles and at the feed alley to improve cow welfare during hot periods. To conclude, heat stress management becomes an important topic in France. It's essential to improve the knowledge about fans by measuring their performances and then to give better advice to farmers.

Comfort assessment through different ear positions in Murrah buffalo calves

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Body language is a reflection of internal state which pictures outward expression of behaviour in an animal. A response to situation is a combination of physiological and behavioural changes that prepares animal to handle the state it is in. For investigating the comfortable state of an animal different ear positions (backward, forward, axial and asymmetrical) were considered for assessment where backward position was for state of satisfaction; forward: state of excitement; axial: state of alertness and asymmetrical: state of confusion. The experiment included 24 Murrah buffalo calves from Livestock Research Center of ICAR-NDRI, Karnal, India. Animals were grouped in 3 types of contact system of 8 calves each, i.e. Full Mother Contact (FMC), Restricted Mother Contact (RMC) and No Mother Contact (NMC). FMC calves were kept along with dams for 0-5 days with free choice colostrum intake, post day 5 they were grouped housed with 24 hrs contact through fenceline with their mother and were allowed morning and evening suckling. Calves of RMC were fed colostrum and milk during morning and evening with separate housing from their mother. All NMC calves were separated immediately after birth and were bottle fed colostrum and milk throughout the experiment. Ear postures were noted for different treatments while sitting, standing and during their daily activities for 4 h/day and means were compared in SPSS software among groups. The results displayed time (min) spent in backward ear position to be (110.69±1.50, 78.23±0.60 and 68.39±1.00); forward ear position for (82.46±0.6 vs 73.15±0.8 vs 64.43±0.9) min; axial ear position for (44.53±1.2, 50.36±0.7 and 73.83±1.2) min and asymmetrical ear position for (20.6±0.7, 28.95±0.4 and 24.65±0.4) min for a day with ($P<0.05$) difference among FMC, RMC and NMC groups. Based on the findings it was concluded that mother bonded calves were in a more comfortable space with positive emotional state in comparison to restricted and calves without any maternal bonding.

The effect of floor type on the performance and behaviour of housed finishing beef steers

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The objective was to determine effect of concrete slats (CS) with or without rubber mat (RM) on performance and behaviour of housed finishing beef cattle. There were 140 late-maturing 'continental' breed steers [initial weight 597 (SD 3.5) kg] weighed on two consecutive days, blocked by age and weight and, from within block, randomly assigned to one of 2 treatments: (1) CS; and (2) RM [Durapak Agri Ltd., Co. Cork] for a 120 d 'finishing' period. Animals were penned in groups of 4 at a space allowance of 3.0 m^2 per animal (17 CS and 18 RM). Animals had free access to a total mixed ration (TMR) diet of 60:40 grass silage: concentrate on a dry matter basis. Feed was weighed into each pen daily and refusals were measured twice weekly. Weighing took place at 14 d intervals; ADG was determined by linear regression. Duration and frequency of lying, standing, eating and drinking behaviour over a 24 h period on d 6 and d 105 were analysed. On d 120 animals were slaughtered in a commercial abattoir; carcass weight, conformation score and fat score were determined. Pen was the experimental unit. Data were statistically analysed using the PROC MIXED procedure of SAS 9.4 (SAS Institute, Inc.); the model included the fixed effects of treatment. Animal ADG [1.15 (SD 0.09) vs 0.98 (SD 0.08) kg], feed conversion ratio [10.6 (SD 0.73) vs 12.4 (SD 0.71) kg DM/kg ADG], slaughter weight [735 (SD 12.14) vs 717 (SD 7.81) kg] and carcass weight [414 (SD 7.6) vs 403 (SD 5.86) kg] were superior ($P<0.001$) for RM compared to CS. However, there was no difference ($P>0.05$) in daily feed dry matter intake, kill-out proportion, carcass conformation score and fat score among treatments. Lying duration [14.48 (SD 0.89) vs 13.44 (SD 1.05) h/24 h] ($P<0.024$) and frequency of lying [10 (SD 1.26) vs 7.8 (SD 1.20)] ($P<0.0015$) were greater on RM compared to CS. Under the conditions of this experiment, performance, feed efficiency, lying duration and frequency of lying for finishing steers were enhanced on RM compared to CS.

Antibiotic use in cubicle and freewalk housing systems from 2005 to 2017 in the Netherlands

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Antibiotic use in animals is part of societal discussion. Therefore this topic was included in the www.freewalk.eu project studying different housing systems. Use in cubicle and freewalk (bedded pack) housing systems was examined over the period 2005 to 2017. The antibiotic use in the cubicle herds (92 farms), expressed in no. of daily dosages – ADDD, was rising in period 2005-2007, followed by a period of growing societal interest in animal antibiotic use, resulting in a reduction in use in 2011-2014, where after use stabilized in 2014-2017. ADDD dropped in this period on average from 6.42 in 2007 (top year in use) to 3.00 in 2017 (a 53% reduction). Antibiotic use and treatment classes on these farms are compared to 24 freewalk farms and, additionally, 10 ecological farms, which were included to have a lowest level of use as reference. In total 50 freewalk farms are in operation in the Netherlands, which did move from cubicles to freewalk in years 2009 to 2014. Average herd size for the three housing systems developed similarly from 72 to 87 cows in 2005 to 121 to 132 cows in 2017. Average milk production levels of the cubicle and freewalk herds were also similar over the period (from 8,550 to 8,780 kg in 2005 to 9,480 kg in 2017). In the period 2011- 2017, the group of freewalk farms used significantly less antibiotics than the cubicle farms (ADDD – 30%). In 2005-2010, no differences in use were seen, except in year 2009. In those years the present freewalk farms had almost all still cubicle housing. Antibiotic use in the organic herds in ADDD was half to one-fourth of the use in freewalk herds. ADDD for other than mastitis use (dry cow therapy, claws, calves, etc.) was higher in cubicle housing than in freewalk. Organic herds had, relatively, a high ADDD for calves. Besides housing system, also herd characteristics were studied to help explain the difference in use. The number of culled cows over the years was significantly lower in freewalk than in cubicle housing, while the cell count was higher. Organic farms had quite different herd characteristics: lower milk yield, lower % of heifers and culled cows, and higher age and cell count.

Session 62 Theatre 6

Compost bedding pack barns compared to conventional cubicle housing – a multi-criteria analysis

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A farmer's choice of housing system for livestock is an important and complex decision which requires the farmer to simultaneously evaluate quite different aspects of livestock production. In this presentation, we explore the choice of housing system for dairy production and we compare the novel compost bedding pack barn (CBP) system to conventional cubicle housing (CH). The method used in this study is a type of multi-criteria analysis referred to as the Analytical Hierarchy Process (AHP). The analysis included six different criteria: (1) animal welfare; (2) consumer opinion; (3) ease of handling the herd; (4) herd profit; (5) milk hygiene; and (6) nitrogen efficiency. In a survey send to dairy farmers in seven European countries, the farmers were asked to indicate the priority they would give each of these criteria in the choice of housing system for dairy production. The results indicate that animal welfare and milk hygiene have the highest priority among the farmers. The criteria related to herd profit and ease of handling the cows have a middle priority whereas nitrogen efficiency and consumer opinion have the least priority. Data from the ERA-NET SusAn project Freewalk are used to compare the relative performance of the housing systems with respect to the six criteria included in the analysis. In the finals step of the multi-criteria analysis, the relative weights assigned to the criteria are combined empirical data on the relative performance of the housing systems. The compost bedding and cubicle housing systems both have their positive and negative aspects. The preliminary results presented here indicate that neither of the systems are superior to the other system. The main challenge for the compost bedding pack barn systems seems to be costs related to a larger building, bedding material costs and to some extent also keeping the cows clean. On the other hand, consumers clearly favoured the compost bedding pack barn when asked in a survey to give their opinion. Being the more recently developed housing system, it is likely some potential for improvement in the design and management of bedding pack barns that can make this system even more competitive compared to conventional systems.

Session 62 Theatre 7

Project FreeWalk highlights and policy implications

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In the context of FreeWalk project: (1) 22 freewalk (FW) systems with bedded pack and artificial floor and 22 cubicle (CB) systems (paired) have been studied. Lying and walking area in the CB and FW housing systems range from 5.5 to 10 m^2 (avg. 8.6) and from 10 to 30 m^2 (avg. 16) per dairy cow, respectively. The bedding consumption in FW was 5× higher than in CB (on average 8.6 vs 1.7 kg/cow day). Behavioural research showed a significant higher activity for cows in FW herds and a slightly shorter lying time, and less antagonistic behaviour. DHIA data indicated a significant higher SCC and a lower ketosis risk in FW than CB herds, and antibiotic use was a bit lower. The hygiene of legs and udder was less positive in FW systems. (2) 80 farmers in 6 European countries, knowledgeable about both CB and FW systems, expressed their perceptions on 7-point Likert scales. The FW system was seen as more sustainable with regards to longevity, fertility and welfare and offering more market opportunities than the CB housing system. The composted bedding material from FW was judged as a better soil improver than slurry; however, the cost of the material is mentioned as a big disadvantage of the FW system. Cow and udder hygiene was seen as an attention point for the FW system, and the bacteria level of the bedding as a risk factor by farmers in some countries. (3) A sample of 3,693 consumers, spread over the same countries, did not distinguish clearly between housing systems in their food choice; they focus more on organic production or not, and on grazing or not. Opportunities as seen towards the future are to combine better animal welfare, less emissions, better manure quality, good economics and social acceptance in an integrated way. In general, choose for combinations of CB and BPB.

Session 62 Theatre 8

Effect of the temporal evolution of compost-bedded pack performance and microbial counts in dairy cows

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The objective of this study was to assess the temporal evolution of compost-bedded pack (CBP) performance and microbial counts in dairy cows using sawdust or forest biomass as bedding material. Eight dry non pregnant Holstein cows were used in a cross-over experiment performed in 2 11-week periods (P1 and P2). Treatments were: (1) CBP with sawdust (CBP-S); and (2) CBP with forest biomass (CBP-FB). The CBP temperature (T) was measured on the pack in weeks (W) 2, 7 and 11. Pack samples were took in W 0, 2, 7 and 11 for CBP moisture (M) determination, and in W 0.7 and 11 for CBP microbial content determination. The CBP management was based on twice-daily tilling at a 30 cm depth, and the addition of 0.8 kg/m^2/d of new bedding material. Average ambient temperatures were 14.7±3.63 °C in P1 and 11.2±2.49 °C in P2, and average environmental humidity were 81.5±4.21 in P1 and 74.5±7.18% in P2. Data were statistically analysed using the MIXED procedure of SAS. The model contained the fixed effects of week, treatment, and week × treatment interaction, and the random effect of cow. In P1, CBP T was higher in W2 than in W7 and W11 in both treatments ($P<0.001$). In P2, CBP-S T was higher in W2 than in W7 and W11, but W7 was lower than W11, whereas there were no differences among weeks in CBP-FB T ($P<0.001$). The CBP T was higher in CBP-S than in CBP-FB in all weeks. In both periods, CBP M increased from W0 to W7, and were similar between W7 and W11 in both treatments ($P<0.001$). The CBP M was higher in CBP-FB than in CBP-S in W0 and W2. Regarding CBP microbial counts, total coliforms, *Klebsiella* spp. and yeast and fungi in both periods remained steady from W0 to W11 only in CBP-FB. Aside from these microorganisms, CBP microbial counts increased during the composting period but remain steady between W7 and W11, except total coliforms ($P<0.001$) and *Streptococcus* spp. ($P<0.003$) in CBP-S, with different increases during P1, and *Staphylococcus aureus* ($P<0.010$) in CBP-S, and *Bacillus* spp. ($P<0.033$) in CBP-FB, in P2. In conclusion, it seems that temporal evolution of CBP performance, except CBP-S T, match with temporal evolution of most CBP microbial counts, which were stabilized from week 7 onwards. Forest biomass can better control the temporal evolution of some CBP microbial counts despite its worse CBP performance.

Session 62 Theatre 9

Developments in cattle housing in Brazil

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Growing population, changing lifestyles, expanding urbanization and accelerated climate changes are creating new challenges in cattle breeding systems. Movement of livestock production within a country or region has implications for genetics, adaptation, well-being, nutrition, production, and facilities, particularly in wide countries, such as Brazil. Thus, the objective of this study was to analyse the level of housing of dairy cows in Brazil. Data on cattle housing and production in Brazil were obtained from the website of the Brazilian Institute for Geography and Statistics from 2000 to 2018. The data set included type of barn (free-stall = FS; compost barn= CB), location (city), number of milking cows housing capacity, and production of animals. Descriptive statistics were used to analyse the information. Maps were generated using the number of barn, type of barn, and production for each year, which was then converted into a raster format (GRID) and processed using R statistical software. Cattle numbers grew in all regions over the period studied. The most expressive growth was initially observed in the Northeast and Midwest regions, which sustained this growth over the period; however, this growth has been slower in recent years. The mean production point has moved from the centre of Minas Gerais State (in the southeast region) to the North of Goiás State (in the Midwest region). This reflects changes in environmental factors, such as feed type, temperature and humidity. Acceleration in production in the northern region of Brazil has remained strong over the years. The CB is the most choosing system by dairy producers, but there are more cows housing capacity in FS. Currently has more than 1.000 CB system in Brazil. These maps showed that more housing systems in southeast and south with a trend towards an increase in the west and north of Brazil.

Session 63 Theatre 1

Rumen bolus containing 1,25(OH)2D3-glycosides for prevention of hypocalcaemia in dairy cows

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Clinical and subclinical cases of periparturient hypocalcaemia occur primarily in multiparous (Multi) cows, but subclinical cases have also been reported in primiparous (Primi) cows. A preventive feeding strategy was investigated by providing the physiologically active vitamin D3 metabolite, 1,25-dihydroxycholecalciferol (1,25-$(OH)_2D_3$). A rumen bolus containing tablets of 1,25-$(OH)_2D_3$-glycoside extract from *Solanum glaucophyllum* (SGE) which releases SGE over several days was developed. The aim was to determine whether Primi and Multi cows fed an herbage based diet are susceptible to periparturient hypocalcaemia; whether a bolus containing 0 (C) or 500 µg (SGE) of 1,25-$(OH)_2D_3$ administered 3-4 days prior to expected calving affects periparturient Ca status of Primi and Multi cows until three weeks into lactation. One C or SGE bolus was applied to 12 Primi and 12 Multi cows in a two-by-two factorial design. Blood and urine were regularly sampled and selected a posteriori from actual calving day (d0), at bolus application, d-2, d0.5, d1, d1.5, d2, d4, d8, d11, d15, d18 and d22 and at bolus application, d0.5 and d2, respectively. Blood serum 1,25$(OH)_2D_3$ increased between d0.5 and d2 in Primi-SGE, but remained unchanged in Primi-C, as did parathyroid hormone (PTH) and Ca in all Primi. Urinary Ca of Primi-SGE increased on d2, indicating regulation of Ca excess. Three Multi-C cows with clinical signs of hypocalcaemia needed treatment and thus were excluded from the dataset and replaced. Blood serum 1,25$(OH)_2D_3$ and PTH increased and Ca dropped by 40% in Multi-C between d0.5 and d2, whereas 1,25$(OH)_2D_3$ and PTH remained unchanged in Multi-SGE. Blood serum carboxyterminal telopeptide of type I collagen was higher in Primi than in Multi and increased with time, except in Primi-C. Finally, in contrast to Multi, Primi did not show any sign of subclinical hypocalcaemia. Prevention of hypocalcaemia with one SGE bolus applied 3-4 days prior to expected calving was successful in maintaining blood Ca within normal range in Multi over three lactation weeks.

Session 63 Theatre 2

Characterization of immune and redox system crosstalk in the intestinal tract of suckling lambs

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Suckling lamb meat is a traditionally consumed food in Mediterranean regions. During the suckling period, progressive intestinal maturation is modulated by environmental challenges. However, the pathways preserving gut homeostasis during the oesophageal groove to forestomach function are poorly understood. The aim of this study was to assess the relationship between enzymes involved in the antioxidant defence system and proinflammatory and regulatory immune genes in intestinal tissues of suckling lambs. Twelve lambs were raised on maternal milk and slaughtered at 30.2±3.5 days old and 11.3±0.8 kg of body weight. Immediately after slaughter, jejunum (je) and ileum (il) tissue samples were incubated in RNA later. Messenger RNA expression of proinflammatory (TNFα, IFNγ, NF-κB) and regulatory (TFGβ, IL10) immune genes and antioxidant enzymes (CAT, SOD1, SOD2, GPX1, GPX2, GPX4) was analysed by qPCR. Spearman's rho (r) test was used to identify possible relationships between gene expression levels of immune and antioxidant markers using the computer package JMPro 15. Positive correlations were observed between je-TNFα and je-NF-κB with je-TFGβ (r=+0.9 and +0.6) and il-IL10 (r=+0.5 and +0.7; $P<0.05$), and between il-NF-κB with il-TNFα and il-TFGβ (r=+0.7 and r=+0.6, $P<0.05$). Positive correlations were also observed between GPX1 and GPX4 with TNFα, NF-κB, IFNγ, and IL10 in jejunum and ileum (r=+0.58 to +0.83, $P<0.05$). However, il-CAT was negatively correlated with TNFα, NF-κB, TFGβ, GPX1, and GPX4 in je, and IL10 in il (r=-0.6 to -0.7, $P<0.05$). TNFα may exert apoptotic and anti-apoptotic actions. Cell survival pathways involve NF-κB activation that is stimulated in presence of H_2O_2. Accordingly, our results suggest that activation of NF-κB-dependent survival pathways in je and il downregulates H_2O_2 degrading capacity of catalase in il tissues. To sum up, homeostasis and homeorhetic mechanisms of the intestinal tract in suckling lambs promote cell survival pathways that likely involve NF-κB activation by TNFα, GPX1, and GPX4 transcription and catalase downregulation.

MiRNAs in milk exosomes are differentially expressed based on the health status of the mammary gland

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The aim of the study was to investigate the expression of miRNAs in milk exosomes in cows with less than 200,000 Somatic Cell Count (SCC)/ml and less than 69.3% of Differential SCC (DSCC) (healthy, G), with less than 200,000 SCC/ml but more than 69.3% of DSCC (at risk of mastitis, Y) and with a level of SCC higher than 200,000 SCC/ml and more than 69.3% of DSCC (with mastitis, R). Bovine milk contains signalling molecules as short non-coding RNA, present inside and outside exosomes. The milk-derived exosomes' cargo can vary depending on several factors, especially those regarding the health status of the mammary gland. A total of 34, 13 and 13 milk samples from cows of the G, Y and R groups, respectively, were collected during the same milking from a dairy herd. After RNA extraction, half of the samples were sequenced and annotated to *Bos taurus*. After the DESeq2 normalization, 178 miRNAs were differentially expressed ($P<0.05$) between the three groups; 28 miRNAs between G and R groups, 36 miRNAs between R and Y groups and 114 miRNAs between G and Y groups. The validation of results obtained with sequencing was performed with qRT-PCR on the other half of the samples for bta-miR-30a-5p, bta-miR-29c and bta-miR-223. The target genes of miRNAs were then explored in silico with the bioinformatic tool miRNet 2.0, and gene enrichments were analysed via FunRich software, to search for the pathways significantly affected by relevant miRNAs. Among the others, we found a relevant number of pathways that resulted to be significantly affected and involved in the inflammatory process and immune modulation.

Scutellaria baicalensis extract reduced mammary epithelial cell apoptosis in milk of dairy cows

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Antioxidant supplementation, especially with the plant *Scutellaria baicalensis* (SB), could limit the oxidative stress that is prone to develop during the beginning of lactation in dairy cows. Recent studies suggested that SB extract could increase milk production and reduce the somatic cell count (SCC) and that SB's flavonoids exert, *in vitro*, protective antioxidant effects on bovine mammary cells. These effects could be linked to a change in the milk concentration of mammary epithelial cells (MEC) being a consequence of a change in MEC activity and a better integrity of the mammary epithelium. To study these potential effects, cows received a diet supplemented (n=8) or not (n=11) with 1 g/d of SB from calving until 150 d of lactation. Milk samples were collected at d30, d50, d130 and d150 after calving to purify the MEC from milk by an immunomagnetic method and to determine total and mammary epithelial cell milk concentrations, and the types of MEC death using flow cytometry. Data were analysed using an ANOVA taking into account the day, SB treatment and the interaction of both as fixed effects, and cow as a random effect. SB reduced the total milk cell concentration ($P=0.02$), especially at d130 ($P=0.01$) as compared with the control treatment. At d130, SB also decreased the MEC concentration in milk ($P=0.03$) and tended to decrease the exfoliation of MEC into milk ($P=0.08$). SB decreased the percentage of MEC in apoptosis at d150 ($P=0.03$). The percentage of MEC undergoing through apoptosis progress tended to decrease at d50 ($P=0.08$) as well as the percentage of MEC in necrosis at d130 ($P=0.10$) and the total percentage of dead MEC at d150 ($P=0.10$). In addition, no change in milk quality was observed in terms of fat, protein or lactose content. $Na+$ or $K+$ milk concentrations did not vary with SB suggesting no variation in mammary epithelium integrity. In conclusion, SB had no significant effect on markers of mammary epithelial integrity, but induced positive effects on MEC milk concentration and survival.

Session 63 Theatre 5

Pegbovigrastim injections altered the immunometabolic state of cows when administered at dry off

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The early dry period is a critical juncture in determining mammary gland health and milk production for the subsequent lactation. Mammary involution in the early dry period is characterized by dramatic alterations in metabolism, highly orchestrated immune responses, and changes to oxidant status. One of the initial immune responses to involution include recruitment of neutrophils to the mammary gland. We hypothesized that pegbovigrastim would optimize mammary involution due to its capacity to increase circulating neutrophils. However, it is not known how Pegbovigrastim affects metabolic, immunologic, and redox changes that occur during the early dry period. The objective of this study was to evaluate physiological changes in healthy early dry off cows. Late lactation cows (n=20) were matched by parity, milk production, BLV status, and SCC and randomly assigned to receive either 15 mg Pegbovigrastim or saline 1 week prior to and on the day of dry off. Blood samples were taken -7, -2, -1, 0, +1, +2, +4, +7, and +14 days relative to dry off as well as +5, +10, and +14 days post-parturition. Samples were analysed for number of neutrophils, mononucleocytes, eosinophils, total calcium, BHB, NEFA, albumin, glucose, haptoglobin, reactive oxygen species (ROS), and antioxidant potential. Repeated measures models using PROC MIXED were used to assess the effects of treatment and means were separated using Bonferroni correction (SAS ver.9.4). Pegbovigrastim increased serum concentrations of neutrophils and mononucleocytes compared to control cows ($P<0.001$). There was a significant treatment and time by treatment effect of pegbovigrastim depressing serum glucose concentrations the 4 days post dry off ($P<0.001$). Pegbovigrastim tended to increase serum ROS concentrations while reducing serum calcium and haptoglobin concentrations ($P<0.10$) during the early dry period. Control cows had elevated BHB 14 days post-parturition ($P<0.01$). This study demonstrated pegbovigrastim injection at dry off had broad ranging effects on early dry cows which could influence health and production in the subsequent lactation.

Session 63 Theatre 6

Associations among fertility, mRNA expressions and profiles of cytokines in endometritic buffaloes

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Association between expression of inflammatory markers and fertility in bovines with subclinical endometritis (SCE) is still elusive. Aim of the study was to evaluate inter-relationships of fertility, concentrations and gene expression of cytokines in postpartum (pp) buffaloes with SCE. Endometrial (by cytobrush technique) and low volume uterine lavage samples were collected on day 21 and 28 pp. Based on endometrial cytology on day 21 pp, buffaloes (n=20) were diagnosed as with SCE (≥18% PMNs i.e. polymorphonuclear cells PMNs, SE group, n=11) or without SCE (<18% PMNs, healthy control, C group, n=11). The relative expression (in terms of gene-specific mRNA abundances) of cytokines/chemokine in the endometrial cells (taken by cytobrush) was measured on day 21 and 28 pp by real time-qPCR using TaqMan chemistry. The relative fold change in mRNA expression of each target gene was calibrated in SE group against average $^{\Delta}$Ct value of C group. Relative expression of IL-1β was significantly greater on day 21 pp (6.73 fold, $P=0.001$) and day 28 pp (3.92 fold) in buffaloes of SE compared to C group. Similarly, relative TNF-α mRNA expression in SE group was significantly higher on day 21 pp (13.79 fold) as well as day 28 pp (9.21 fold). Significantly higher mRNA expression of IL-8 (10.06 fold) and IL-6 (1.8 fold) was recorded on day 21 pp in SE compared to C group. The upregulated relative gene expression of IL-1β, TNF-α, IL-8 and IL-6 in the endometrium was corroborated by their greater concentrations in the uterus and serum of SE group than in healthy counterparts, particularly on day 21 pp and in uterine samples. Postpartum interval to first overt oestrus was positively correlated ($P<0.05$) with the endometrial mRNA profiles (at 28 pp) of IL-1β and IL-8. Number of days open was positively correlated with the relative mRNA abundances of TNF-α ($r=0.750$, $P=0.029$). In conclusion, pro-inflammatory cytokines are upregulated in the uterus of postpartum buffaloes with SCE, possibly in a time-related manner and are correlated with postpartum fertility.

Session 63 Theatre 7

Sperm motility and plasma membrane stability rely on aquaporins function in boar sperm capacitation

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Capacitation, which is a key process that sperm undergo to achieve fertilizing ability, is triggered by changes in pH and in different ion concentrations that are present in the female reproductive tract. Aquaporins (AQPs) are a family of ubiquitous transmembrane proteins that allow the transport of water and small solutes across the cell plasma membrane, and which are both cell- and species-specifically distributed in cells. Some AQPs, including AQP3, AQP7 and AQP11, have been identified in mammalian sperm, and are involved in osmoadaptation and motility activation. Given the relevance of AQPs upon post-ejaculatory events and their strict relationship with pH, the aim of this study was to elucidate the potential role of different AQPs during boar sperm capacitation through their inhibition with different transition metal compounds. The effects of AQPs inhibition on sperm function parameters were assessed through a CASA system and flow cytometry. When AQP3 was inhibited through $CuSO_4$, a drastic increase in peroxide intracellular levels was observed compared to the control. All AQPs except AQP7 were inhibited by $HgCl_2$, which caused an increase in membrane lipid disorder and led to a decrease in mitochondrial membrane potential after inducing the acrosome reaction with progesterone. Finally, when Ag sulfadiazine was used as an unspecific inhibitor of all AQPs, the same effects than in the presence of mercury chloride were observed in addition to a decrease in sperm motility and kinetics parameters. In the light of the aforementioned: (1) sperm capacitation and acrosome reaction events are apparently independent from AQP3 permeability to peroxides; (2) motility maintenance during capacitation and acrosome reaction seem to be strictly related to AQP7 function; and (3) the maintenance of lipid membrane stability during capacitation and mitochondria response upon acrosome reaction seem to rely on AQPs as a whole. Hence, further research aimed to elucidate the mechanisms underlying the AQPs involvement in mammalian sperm capacitation and acrosome reaction is warranted.

Session 63 Theatre 8

The role of oxidative stress and autophagy in meat quality defects

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In cattle, pre-slaughter stress causes the appearance of defective meat (DFD), which is identified by an abnormal *post mortem* muscle pH decline ($pH_{24\geq}6$) that compromises the final meat quality. In this study, meat quality traits (pH_{24}, colour, water holding capacity, instrumental tenderness and microbial contamination) were analysed along ageing in normal quality meat (control) vs DFD beef from 'Asturiana de los Valles'. Differences in the oxidative stress processes and cell survival mechanism (autophagy) at 24 h *post mortem* were also analysed in order to understand the changes in the early *post mortem* muscle metabolism related to DFD occurrence. The results indicate significant differences being DFD beef darker and more susceptible of greater microbial growth (vs<0.05) as maturation time increases. Likewise, DFD beef showed lower antioxidant activity (vs<0.001) at 24 h *post mortem*, as well as greater lipid oxidation (vs<0.001). The analysis of the expression of *heat shock protein* 70 (HSP70) involved in stress response and autophagy biomarkers (Beclin-1 and LC3) revealed significant higher expression (vs<0.05) at 24 h *post mortem* in DFD samples. Our data seem to suggest that differences found in quality parameters between both types of beef may be due, at least in part, to different cellular responses to oxidative stress triggered in the early *post mortem* as a consequence of pre-slaughter stress and affecting to the normal process of muscle to meat conversion. These differences could be used as early biomarkers of meat quality.

Effects of different levels of milk replacer for 14 weeks on mammary gland development in heifers

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The ultrasound (US) technique allows for a non-invasive *in vivo* assessment of mammary gland (MG) development that determines a dairy cow´s performance. The tissues´ different ability to reflect or to absorb ultrasonic waves forms the basis for their differentiation via US: The more the ultrasonic waves are reflected, the brighter the structures appear and thus show higher pixel values and vice versa. In total, 1027 US images were obtained from the MG of 37 Holstein dairy heifers, which were fed either on a high plane of milk replacer (MR; 14% solids) at 10 l/d (1.4 kg MR/d; n=18) or on a restrictive plane of 5.7 l/d (0.8 kg MR/d; n=19) until linear weaning from wk 13 to 14 of life. The MG were US-scanned in wk 3, fortnightly from wk 8 to 16, and in wk 20 of life, in standing position, of each quarter, using a B-mode US-device (MyLab Five Vet, Esaote Biomedica GmbH, Germany) equipped with a linear probe (18 MHz) in a standardized position (45° inclination to the teat). The brightness of the tissues visible in the US images (8-bit) was evaluated by assessing the pixel value of mammary parenchyma (PAR) and its surrounding tissue (SURR) with Fiji (ImageJ, Bethesda, USA). Normed squares of 0.25 cm^2 = 900 pixels/squares were positioned on PAR and SURR structures. The difference in mean pixel values of both was calculated as delta pixel value (SURR-PAR). Time and group as well as the interactions thereof were tested as fixed effects and calf was set as a random effect using a linear mixed model (SPSS). Significance was declared at $P<0.05$. The PAR pixel values changed with time, but were neither affected by group nor by the group × time interaction. The assessment of the SURR and delta pixel values were influenced by time, but not by group and without interactions thereof. With progressing PAR development, its pixel brightness increased from wk 10 to 20 of life, i.e. PAR became more hyperechogenic since it spread into its SURR, showing the increasing growth of PAR, independently of MR feeding regimen.

The effect of inclusion rate of concentrates and exogenous butyrate on stomach measurements in sheep

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Increased intake of concentrates is known to stimulate ruminal epithelium growth. This impact is related mostly to the increased ruminal butyrate concentration when concentrates are consumed by ruminants, and thus butyrate supplementation can be used to enhance rumen epithelium development. However, effect of butyrate supplementation on rumen as well as other stomach compartments may depend on concentrate inclusion in the diet. The aim of this study was to determine the effect of inclusion rate of concentrates in the diet and exogenous butyrate supplementation on whole stomach (WS), reticulorumen (RR), abomasum (AB) and omasum (OM) measurements in sheep. Forty two rams (27.8±7.3 kg; 9 to 14 months of age) were allocated into 6 groups and fed a diet with low (22.5% of dry matter (DM) of a diet; L) or high (60% DM of the diet; H) inclusion of concentrates in combination with high (3.2% of DM; SBH), low (1.6% of DM; SBL) or none (SBN) sodium butyrate supplementation. Hay was used in the diet as a main source of forage whereas barley grain as a main source of concentrates. Dry matter intake was limited to 3% of body weight of animals. After 21 days, animals were slaughtered and weights of each stomach compartment (full and empty) were taken and RR fluid was collected for short-chain fatty acids analysis. Reticuloruminal butyrate concentration (mmol/l) was greater for H than L groups ($P<0.01$) and linearly increased with increasing dose of supplemental butyrate ($P<0.01$). Full WS, RR and OM weights were higher for L than H groups ($P<0.01$). The WS and RR tissue weight were greater for L than H groups ($P<0.01$) and it was the highest when L was combined in the diet with SBH (concentrate inclusion × butyrate supplementation interaction, $P<0.05$). There was no effect of investigated factors on full and empty AB weight and OM tissue weight. The results show that the impact of exogenous butyrate on RR tissue weight is more apparent when concentrate inclusion in the diet is low.

Session 63 Poster 11

Bioavailability of three different rumen undegradable methionine sources in dairy cows *in vivo*

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Most diets for high producing dairy cows are deficient in Met. Industry has developed different methods to increase the supply of bioavailable AA to dairy cows. The 2-hydroxy-4-(methylthio)butanoic acid is a Met analogue that it is converted into Met after absorption. Its esterification with an isopropyl group (HMBi) has been reported to enhance its bioavailability. The objective of this study was to determine the bioavailability of two HMBi products produced by different processes and compare them with an encapsulated rumen-protected Methionine using the area under the curve (AUC) method. The new HMBi product (KES, KESSENT MF Liquid® Kemin Animal Nutrition and Health, Belgium) was compared to an already existing HMBi product (MtS, Metasmart® Adisseo SAS, Antony, France) and a pH-sensitive coated Met (SmT, Smartamine® Adisseo SAS, Antony, France). Nine multiparous lactating cows (30 kg/d of milk and 227 DIM) fed a 45:55 forage to concentrate diet were randomly assigned within square to a triplicate 3×3 Latin square design. Each period consisted of 3-d sampling and 3-d wash-out. Treatments were dosed on day 1 of each period and tail blood samples were collected at 0, 1, 2, 3, 4, 6, 9, 12, 24, 30, 48 h thereafter. The daily dose was 50 g of Met equivalent of each treatment. The HMBi treatments were administered directly into cow's mouth, whereas SmT was fed mixed with 0.5 kg of concentrate and fully consumed within 15 min. Non-linear models were fitted to raw data and the basal concentration at time 0 h, time at peak (Tmax), concentration at peak (Cmax) and AUC of plasma Met were determined. Differences among treatments were declared at $P<0.05$. The Met Cbasal (26.7±7.67 µM) and Cmax (210±22.2 µM) were similar among treatments, but the Tmax (11.3 vs 1.4 h) was delayed and the AUC was 1.8-fold larger (3,457 vs 1,868 arbitrary units) in SmT compared with HMBi. Results of this study indicate that the two HMBi products have a similar plasma kinetics and bioavailability. Smartamine had a different kinetic compared with HMBi, products with delayed Tmax, and larger AUC and relative bioavailability.

Session 63 Poster 12

Mitochondrial activity during satellite cell differentiation in piglets

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Myogenesis requires energy production for the execution of a number of regulatory and biosynthesis events. Recent studies in human have shown that mitochondria play a role in the regulation of myogenesis. Indeed, the abundance, morphology, and functional properties of mitochondria contribute to satellite stem cell fate decision during quiescence, proliferation or differentiation. Nevertheless, little attention has been paid to this process in farm animals. Understanding how mitochondria are involved in myogenesis would provide a valuable insight into the underlying mechanisms that regulate the maintenance of cellular homeostasis. The objective of the current study was to evaluate mitochondrial activity including autophagy in satellite cells (SC) during differentiation. Cells were isolated from samples of *longissimus* and *semimembranosus* muscle obtained from 4-day-old piglets. Part of the isolated cells was used to separate slow and fast proliferating SC subpopulations by Percoll gradient centrifugation. Both subpopulations were plated in growth medium, allowed to proliferate up to 80% confluence and then placed in an appropriate culture medium to differentiate into myotubes. Mitochondrial function was explored during proliferation and differentiation using Mitotracker dye in flow cytometry. Within the two subpopulations of cells, we observed two different Mitotracker staining intensity suggesting a difference in the mitochondrial membrane potential. According to two distinct peaks of fluorescence within each subpopulation, cells will be separated by cell sorting. Mitochondrial activity will be studied in myogenic cells as well their potential of differentiation. Mitochondria-related proteins expression (TOM20, MTFN1, COXIV) will be also quantified by Western blotting. This study combined with our previous results suggests that autophagy signalling pathways and mitochondria would drive muscle stem cell fate.

Session 63 Poster 13

Expression of selected miRNAs and their common target genes in dairy cows' mammary gland parenchyma

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The aim of the study was to determine the expression of targeted genes common for 10 miRNAs which were found to be differentially expressed in dairy cattle mammary gland parenchyma during chronic staphylococcal infection in our previous study using the microarray method. The experiment was carried out on Polish Holstein-Friesian dairy cows of the black-and-white variety; all were between their 1st and 4th lactation. Finally, based on the microbiological analysis results, the 36 quarter samples were assigned to 3 groups with n=12 in each: samples collected from cows infected with coagulase-positive staphylococci (CoPS), or coagulase-negative staphylococci (CoNS) and collected from cows without pathogenic bacteria in milk (H). miRNAs selected to the study were as follow: bta_miR-155, bta_miR-146a, bta_miR-29a, bta_miR-99b, bta_miR-223, bta_miR-199b, bta_miR-181a, bta_miR-296, bta_miR-143, bta_let-7b. The common target of those miRNAs are *MAPK1, FOS, MEF2A, MEF2C* and *TLR4* genes which participate in the immune response during mastitis. There were no differences between groups in *MEF2C* gene expression probably because of high intra-group variability (high SE). Despite the use of three different pairs of primers designed for the FOS gene, it was impossible to perform the correct real-time PCR reaction. The highest expression of *MAPK1*, *MEF2A* and *TLR4* genes was stated in CoPS compared to other groups. Expressions of six miRNAs were found, however, expressions of bta-miR-143, 29a, 296 and bta-let-7b was similar in all groups. A lower expression of miR-99b in CoPS than CoNS was found while a higher expression of miR-223 in CoPS than in CoNS and H was stated. Financing the National Science Center, Poland No. 2015/17/B/NZ9/01561.

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In vivo bioavailability estimates of different rumen-protected lysine products for dairy cattle

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The objective of this study was to determine plasma kinetics and relative bioavailability of different sources of rumen protected Lys and its interaction with different sources of rumen-protected Met supplements in dairy cows. Six multiparous Holstein cows (32 kg/d of milk) were used in a 6×6 Latin square design. Treatments were administered in pairs of Lys+Met products. Lys treatments were: Lys-hydrochloride (CTR); LysiGEM™ and Ajipro®. Met treatments were DL-Met, and 5 different rumen -protected products (Met1, Met2, Met3, Met4 and Met5). Treatments were dosed the first day at 40 and 120 g for Met and Lys equivalents, respectively. The control treatments were infused into the abomasum via the infusion line of the canula. Oral treatments were dosed directly into the rumen close to the oesophageal orifice. Blood samples were collected from the jugular vein at 0, 1, 2, 3, 4, 6, 9, 12, 24, 30 and 48 h after treatments administration. Results were used to determine the basal concentration (0 h), the maximum concentration (Cmax), the time to reach the Cmax (Tmax), and the area under the curve (AUC) of Lys. Results were analysed with the PROC MIXED of SAS and differences declared significant at $P<0.05$. The Lys basal concentration was similar among treatments (58.0 µM). The average Cmax was numerically higher (140.5 vs 97.3 µM) and the Tmax numerically lower (5.8 h vs 11.8) in LysiGEM vs AjiPro, but differences were not significant. The AUC was higher in LysiGEM+M1 (1,204 units) and lower in AjiPro treatments (average 276 units). The plasma Lys concentration within the same type of Lys was different depending on the Met supplemented. The AUC of LysiGEM was 1,204 or 790 units, a 35% reduction, when mixed with M1 or M2, respectively. Similarly, the AUC of Lys from AjiPro ranged from 399 to 110, a 73% reduction, when mixed with M4 or M5, respectively. Results suggest that relative Lys bioavailability was higher in LysiGEM compared with AjiPro, and that bioavailability may be affected by the type of rumen protected methionine supplement used.

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In vivo bioavailability of rumen-protected methionine supplement in lactating dairy cows

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The objective of this study was to determine the bioavailability of a rumen-protected Methionine supplement in dairy cows using the plasma free amino acid dose-response technique. Five multiparous Holstein-Friesian cows (640 kg BW; 32 kg/d of milk) fitted with rumen cannulas were housed individually in a tie-stall barn. Cows were fed a 42:58 forage:concentrate total mixed ration formulated to meet or exceed requirements. Cows were milked twice daily. In Experiment 1, 40 g of Met were dosed orally at the am feeding and blood samples were collected from the jugular vein at 0, 1, 2, 3, 4, 6, 9, 12, 24, 30, and 48 h after Met supply to determine plasma Met concentration. In Experiment 2, cows were used in a 5×5 Latin square. One week before the start of the experiment, blood samples were taken 8 h after feeding to measure the plasma AA concentration used as covariate. Each experimental period consisted of 4 d for adaptation, 3 d for sample collection, and 3 d for washout. Treatments were control (CTR): post-ruminal infusion of 15 g/d (InfLow) or 30 g/d (InfHigh) of Met: and 15 g/d (RPMLow) or 30 g/d of Met-equivalent of a rumen-protected Met (RPM, Kessent™, Kemin Animal Nutrition and Health, Belgium) fed orally. Oral doses were fed in two equal portions before a.m. and p.m. feeding. Blood samples were collected at peak concentration determined in Experiment 1 (8 h after the Met supply) during the 3 sampling days following the same protocol described previously. Bioavailability of Met was estimated using the slope ratio method using the NLMIXED procedure of SAS. Results from Experiment 1 indicated that Met plasma concentration reached the peak around 8 h after feeding, and that time was selected for the sampling in Experiment 2. In Experiment 2, the lower slope of the oral treatment compared with the omasal infusion supplementation reflected the lower bioavailability due to rumen degradation and/or intestinal digestibility. The estimated bioavailability (ratio of slopes) of the RPM was 74.6±5.2%.

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Ketotic hepatocytes are less resistant to effect of zearalenone on expression of glucose metabolism

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At the onset of lactation, the liver of cows undergoes several adaptations. Due to the popularity of corn in diets for transition period, cows are often exposed to mycotoxins such as zearalenone (ZEA). We hypothesized that ZEA may affect these adaptations and can be particularly dangerous for cows with ketosis. The aim of this study was to compare the hepatocytes' response to ZEA between healthy (HC) and ketotic cows (KC) on the mRNA and protein abundance of enzymes involved in glucose metabolism. Liver explants were isolated from 0.5 g liver biopsies taken from healthy cows (n=3) and from ketotic cows (ketone bodies >3 mmol/l; n=3). Explants were exposed to ZEA in doses 10-100 µM for 1 to 12 h. Expression of genes involved in glucose metabolism (ENO1, TPI1, PDHB, PGK1, PGAM and PCK1) was determined by the qPCR method and their protein expression by Western blot. Data were analysed by two-way ANOVA with ketotic state and ZEA dose as fixed effects. After 1 h ZEA in doses 20-100 µM ZEA increased PDHB mRNA expression ($P<0.05$) in HC samples, while in KC samples it had no effect. Also, after 1 h ZEA in doses 20-100 µM decreased PGAM1 mRNA expression in HC samples ($P<0.05$), however it had no effect in KC samples. After 6 h, ZEA decreased PGK1 gene expression in doses 50 and 100 µM in HC samples ($P<0.05$) and in dose of 100 µM in KC samples ($P<0.001$). We also observed a decrease of PCK1 expression both at the gene and protein level after 3 and 6 h of in ZEA doses 20-100 µM ($P<0.05$) in KC, while it had no effect on HC samples. There was no observable effect on ENO1 and TPI1 mRNA expression. Our data showed different responses to ZEA between HC and KC. Because of the fact that the primary objective of a liver in transition period is to adjust expression of enzymes involved in glucose metabolism in order to start lactation, negative symptoms of energy imbalance among KC may make them less resistant to mycotoxins such as ZEA. Funding: National Science Centre, Poland, grant number: DEC-2016/21/D/NZ9/01301

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Single nucleotide polymorphisms density in bovine genomes reveals their role in the immune response

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Single Nucleotide Polymorphisms (SNPs) are valuable for identifying and localizing disease susceptibility genes, some of them are associated with multiple traits. The aim of this study was to analyse the density of SNPs in the Bos *taurus* genome. The dataset consisted of whole-genome DNA sequences of 48 bulls representing Brown Swiss breed sequenced by the Illumina HiSeq 2000 Next Generation Sequencing platform. Across the in the analysed genomes 15,107,727 SNPs were identified. The analysis began with calculating distances between neighbouring SNPs to compare distributions of such inter-SNP distances across chromosomes. As a result, significant differences between chromosomes were observed and therefore chromosomes were clustered into groups representing similar inter-SNP distance distributions. In the second step, was checked whether the distribution of polymorphisms in the established regions of the genome was uniform. From the most dense regions genes containing at least 200 SNPs in coding regions accompanied by an inter-SNP distance below 11 bp where subjected to the GO Enrichment analysis. Finally, the longest intergenic regions (>3 kb) without SNPs were analysed. 73% of SNPs was located in intergenic regions, 162,628 SNPs were found in exons. 16% of protein coding genes did not contain SNP in exons – most of them located on BTA7. There were 20 genes (six on BTA12) with more than 200 SNPs in coding regions and 54 genes (17 on BTA4) with average density of SNPs in exons less than 11 bp. GO Enrichment analysis showed that biological processes related to immune response were overrepresented among SNP-dense genes. In particular, the overrepresented terms comprised: antigen processing and presentation of endogenous peptide antigen via MHC class I via ER pathway, TAP-independent (GO:0002486), antigen processing and presentation of endogenous peptide antigen via MHC class Ib (GO:0002476), positive regulation of T cell mediated cytotoxicity (GO:0001916) and immune response (GO:0006955).

Session 64 Theatre 1

Barriers, risks, and opportunities for scaling up the European insect value chain

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Insect production has great potential to contribute to the protein transition. The chain needs upscaling to become competitive. The challenges faced in upscaling have not yet been fully investigated. In addition, risk profiles have not been established, which hampers credit provision and insurance granting. This study aimed to investigate key barriers and risks for efficient upscaling as well as applied risk management strategies. Barriers, risks, and risk management strategies were assessed through a structured survey which included the rating of frequency and impact for experienced barriers in the past and perceived future risks. Barriers and risks were categorized into four areas: operations, finance, product safety, and regulations. The survey was conducted among European insect rearers (n=25), processors (n=10), and insect-based feed (n=16) or food producers (n=15). Data were analysed using descriptive statistics and multivariate methods with the goal to prioritize and characterize barriers and risks for business operators. Additionally, the relationships between perceived risks and applied risk management strategies were evaluated, following Van Winsen *et al*. Insights into key barriers help to develop efficient strategies to overcome these hurdles and increase the chain's economic viability. Furthermore, risk profiles aid in the decision making of banks, investors and insurers. The results contribute to the prospected development of the chain.

The pH of wetfeed: does it influence the growth of *Tenebrio molitor* larvae?

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For optimal growth the mealworm larvae (*Tenebrio molitor*) need both a dry feed (e.g. wheat bran) and a wet feed (e.g. carrots, apples, potatoes, etc.). Storing dry feed is not a problem, but storing wet feed over a prolonged period is more challenging. Exposed to air at room temperature, it will spoil rapidly. It could be stored in a refrigerated room for a long time, but this process is energy consuming and therefore increases the price of production. Another option is to ferment the feed as is done regularly in other branches of agriculture. No energy is needed, and the feed remains stable due to the low pH. However, the effect of low pH feed on the growth of mealworm larvae is unknown. In this study we assessed the growth of mealworm larvae fed with wheat bran and agar-agar (25 g/l), both *ad libitum* at 27 °C and 60% RH. The pH of the agar was changed between 3 and 9 in steps of 1 pH unit resulting in 7 assessed pH values. The average weight of the larvae was determined every week until maximum weight was achieved. At harvest the live mealworm yield and mortality were determined. Preliminary data suggests that there are no significant differences between pH 4 to 9, and pH 3 only results in a minor decrease of growth. No significant differences in mortality were observed between pH 3 and 9. This would indicate that mealworm farmers can ferment their wet feed to store the feed for a prolonged period of time. Yet, further experiments are needed as there are other changes to the feed during fermentation besides a change in the pH.

Transporting mealworm (*Tenebrio molitor*) eggs: the effect of temperature and relative humidity

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The insect industry is booming. It is expected that, similar to the poultry industry, in the future more specialized breeders and fatteners will emerge. This would inevitably result in the transportation of insect eggs or young larvae. In this study we focused on the effect of temperature, relative humidity (RH) and exposure time on the hatch rate and time of mealworm eggs. The assessed ranges were 5-40 °C, 40-80% RH for 1 to 6 days. The latter was to assess differences in express transport and more delayed transport. The results indicate that between 15 and 30 °C there was a similar hatch rate as the control (27 °C, 60% RH) at any RH and time. However, at 15 °C the hatch time did increase with roughly an equal amount of days as the exposure time. Indicating that the development of the embryo is slowed down significantly. Even lower temperatures (5 and 10 °C) resulted in adverse effects when exposed for more than 1 (5 °C) or 2 (10 °C) days?. However, even exposure at 5 °C for 6 days still resulted in a 20% hatch rate. Temperatures of 35 °C adversely impacted the hatch rate when the exposure exceeded 2 days and zero eggs hatched at 40 °C at any exposure time. In conclusion: transportation can occur between 20-30 °C without compromising hatch rate or time, 15 °C is possible with a delayed hatch time. At 5, 10 or 35 °C express transport should be considered to avoid detrimental effects. Finally, in this study we focused on the lethal effects, yet sublethal effects on growth or future fertility of short term exposure to low or high temperatures may occur but are currently unknown and should be further investigated.

Optimising transport conditions for *A. domesticus* nymphs and eggs

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The transportation of live insects is becoming increasingly common within the food and feed industry. However, very little information is available as to its effects on the insects themselves. The overall aim of this study was to monitor how variation in transport conditions, such as duration of transport and external temperature, directly and indirectly affect the health of nymphs of the house cricket, *Acheta domesticus*. In addition, we investigated the effect of external temperature on the hatch rate of eggs, upon completion of a transport event. We investigated how four different temperatures (5, 15, 25 and 35 °C) and four different possible transport durations (1, 2, 3, 6 days) affected survival and growth of the cricket nymphs. To gain insight into the potential transportation of eggs, its effect on egg hatch-rate was studied by storing eggs at three different temperatures (6, 18 and 30 °C) before moving the eggs to optimal hatching conditions. We found that temperature had a significant effect on nymph survival and growth. The extreme temperatures negatively affected nymph survival and increased temperature was correlated with increased growth. The development of eggs was also sensitive to temperature and transport of eggs at suboptimal temperatures prolonged incubation time considerably. Finally, based on the results of this study, we discuss temperature optimization as the most critical factor to consider during transport of live house cricket nymphs and eggs.

Vacuum packaging as storage and transport strategy for living or killed black soldier fly larvae

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As the insect value chain is evolving towards a large-scale industry, storage and transport of insects as steps between multiple production and processing stages gain importance. In practice, storage and transport strategies should be able to maximally preserve survival of living insects, and (microbiological) quality for killed insects. This study evaluated the potential of vacuum packaging as a storage and transport strategy for either living or killed black soldier fly larvae (BSFL, *Hermetia illucens*). For living larvae, storage took place either in absence (high vacuum) or presence (no vacuum, control) of air in high-barrier vacuum bags kept at refrigerated (4 °C), cool (15 °C) or ambient temperature. During 10 days of storage, survival of the larvae and gas composition in the package were evaluated daily for each bag. For killed larvae, identical vacuum packaging and storage conditions were applied on BSFL that were slaughtered either by freezing (and thawing) or blanching (and cooling). Next, microbiological quality of the larvae and gas composition in the packages were analysed after two and six days of storage. It is clear that a large fraction of living BSFL is able to survive at least a few days under vacuum conditions and under high CO_2 concentrations, as was observed already after a few hours of storage. The best survival, however, was observed for BSFL stored in bags without vacuum. The storage temperature showed to be an important influencing factor, with a lower storage temperature resulting in better survival. For killed BSFL, vacuum packaging also did not positively influence the microbiological quality. The initial microbiological quality after killing (which was better after blanching compared to only freezing) greatly determined the microbiological quality and gas composition dynamics in the package. Lower temperature also slightly improved the quality of the insects during storage.

Session 64 Theatre 6

Horizontal transfer of food pathogens from substrate to insects during rearing

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Microbiological food safety of insects as food and feed is a key parameter in order to produce high quality and safe insects and derived products. From a recent literature review in the context of the SUSINCHAIN project, a number of food pathogens were found to be relevant to be studied with respect to their potential of contaminating insects when they are present in the substrate. Depending on factors such as insect species, initial concentration of the microorganism, substrate type, etc., these microorganisms may or may not be able to colonise the insect and pose a risk in following steps in the insect chain. This study aimed to investigate the potential of food pathogens such as *Salmonella* spp., *Staphylococcus aureus*, *Bacillus cereus* and others to horizontally transfer from substrate to insects (including the black soldier fly (*Hermetia illucens*) and the yellow mealworm (*Tenebrio molitor*)). A series of challenge tests involving a specific pathogen and a specific insect species were performed by inoculating rearing substrates with a known level of the pathogen. High and low inoculation levels were included. Here, we will zoom in on *Salmonella* spp. in black soldier fly larvae rearing. It was observed in the challenge tests that the pathogen was able to grow in the substrate and to colonise the gut of the insect, even at low inoculation levels. This means that an occasional contamination of insect feeding substrate with *Salmonella* spp., and likely with others as well, may involve microbiological safety risks and requires proper mitigation.

Session 64 Theatre 7

Texturization potential and microbial safety of fresh-frozen insects within high-moisture extrusion

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High-moisture extrusion (HME) is already widely used to produce plant-based meat analogues (HMMA) holding a fibrous, meat-like structure. After thermo-mechanical treatment of protein-water mixtures within an intermeshing twin-screw extruder texturization takes place within a long cooling die where protein molecules are aligned in flow direction. Next to a broad variety of plant-based proteins, like soy, pea, sunflower and pumpkin, also insects containing remarkable amounts of high value protein can be used to substitute plant-based proteins and complement food and feed products. In order to open markets for insect farming in Europe evaluation of the potential of different insect species for HME can have a significant impact to unlock food and feed applications. The aim of this work (Susinchain work package T3.4) was to investigate texturization potential of different insect species for HME. Extrusion experiments were carried out with soy and pea protein representing a reference base for insect inclusion. After defining optimised extrusion parameters, texture of the soy- and pea-based HMMA was investigated with and without incorporation of 30 g/100 g living or frozen Mealworm (*Tenebrio molitor*) and Black Soldier Fly Larvae (*Hermetia illucens*). Here, cutting and deformation forces of the products were analysed within the scope of a suitable food or feed meat replacement. Additionally, microbiological analyses were carried out to evaluate microbial safety of HMMA products holding incorporated fresh and frozen insects with a high initial microbial load. Previous experiments demonstrated a 4-5 log reduction of the total plate count. Further work will include experimental work on biomass sources from other insect species.

Pretreatment strategies to improve insect processing for use as protein rich animal feed ingredient

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Animal farming currently uses large quantities of high-protein feed ingredients deemed unsustainable (e.g. fishmeal and soybean meal) due to their negative environmental impact. This is expected to worsen with the growing global population as the demand for animal-based products will also rise. Black soldier fly larvae (BSFL), yellow mealworms and housefly larvae have the potential to be a more sustainable replacement, especially when grown on organic side or waste streams (e.g. millings/brewery side streams and vegetable/fruit waste). However, current insect production efficiency and product composition are frequently unreliable with these substrates because of varying compositions. For example, high-fibrous substrates are poorly digested by BSFL, leading to poor growth. Therefore, to increase insect production efficiency by making nutrients more digestible and bioaccessible to BFSL, substrate pretreatment (e.g. physical, chemical, and biological) could be a promising strategy. However, this method currently largely remains untapped. Therefore, our literature study identified the most promising technologies and research directions for BSFL, based on the intensive use of pretreatment for biogas and bioethanol production. These industries have used pretreatment for decades and have focused on substrate optimisation using similar substrates typically used for BSFL production. For example, chemical pretreatment, such as sodium hydroxide, has proven to be an effective method, increasing biogas production by more than 40% on a range of substrates such as rice straw, wheat plant, and organic fraction of municipal solid waste. Mechanical pretreatment has also increased methane yield for biogas production by 9-35%, and thermal pretreatment reported increasing biomethane production by 20-52%. While these pretreatments have enhanced efficiency for biogas and bioethanol production, it's also important to consider the additional costs, and energy demand needed for these methods. Therefore, future research should investigate the applicability to insect production and its potential in improving insect processing on more fibrous wastes.

Session 64 Theatre 9

Apparent digestibility of different batch of *Hermetia illucens* meals for rainbow trout

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One of the aims of the H2020 SUSINCHAIN project is to evaluate the apparent digestibility coefficient (ADC) of four commercial black soldier fly (BSF) meals produced within one year, to highlight how fluctuation on the BSF meals composition could influence digestibility parameters in rainbow trout (*Oncorhynchus mykiss*). *In vivo* digestibility trial has been conducted following the procedure described by Bureau *et al*. Briefly, a high quality reference diet (REF) has been prepared, and four experimental diets were obtained by mixing REF and one of the BSF meals in a 70:30 ratio (as is basis) to produce: BSF1, BSF2, BSF3 and BSF4 diet. Celite® as inert marker (acid insoluble ash) has been included in REF diet to serve as inert digestion indicator. Fish were introduced in 250 l cylindroconical tanks (18 fish/tank), supplied by artesian well water (13±1 °C) in flow-through open system. Diets were randomly assigned to three replicate tank/diet. Fish were fed by hand, seven days/week, and faeces were collected twice a day form each tank for four consecutive weeks, using a continuous automatic device, as reported by Choubert *et al*. Faeces were collected, frozen (-20 °C) and then freeze-dried prior to subsequent chemical analyses. The ADC for the nutrients and energy of the experimental and REF diets were calculated as follows: $ADC = 1- (F/D \times Di/Fi)$ Where: F = % nutrient (or KJ/g gross energy) of faeces; D = % nutrient (or KJ/g gross energy) of reference or experimental diet; Di = % marker indicator of diet; Fi = % marker indicator of faeces. The ADCs of nutrients and energy for each of the tested insect meal (ingredient) were calculated as follows: $ADCing= ADCtest + [(ADCtest- ADCref) \times ((0.7 \times Dref) / (0.3 \times Ding))]$ where: ADC test = ADC of the experimental diet; ADC ref = ADC of the reference diet; D ref = g/100 g nutrient (or MJ/kg gross energy) of the reference diet (DM basis); Ding = g/100 g nutrient (or MJ/kg gross energy) of the test ingredient (DM basis).

Session 64 Theatre 10

In vivo **and** ***in vitro*** **digestibility of black soldier fly larvae meal in Atlantic salmon**

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The inclusion of insects in fish diet are increasing as insects are sustainable alternative to mainstream feed ingredients of marine and terrestrial origin. These are highly nutritious, consisting around 50% protein, essential amino acids (AA), lipids and minerals. Digestibility is a significant indicator of the nutritional quality of feed ingredient. Factors such as feeding substrate and processing methods can affect digestibility of the insect meal. The *in vivo* and *in vitro* digestion method can be useful in obtaining information on the effects of production factors on the digestibility of different insect meals. This study investigated an *in vivo* digestibility method for black soldier fly larvae meal (BSF) in Atlantic salmon and attempted to make a comparison of different batches of BSF meal with *in vitro* digestion. For the *in vivo* digestibility study, BSF meal was used as the test ingredient. A reference diet was mixed with test ingredient containing BSF meals in 80:20 ratio. Yttrium oxide was used in diets to serve as digestion marker. The reference and test diets were fed to Atlantic salmon reared in triplicate tanks for 4 weeks. At the end of *in vivo* digestibility trial, faeces were collected by stripping. UHPLC was used for AA measurement. Minerals and yttrium concentrations in feed and faeces were analysed by ICPMS. The results of the *in vivo* digestibility trial will be presented. For the *in vitro* digestion, acidic and alkaline hydrolysis were applied to evaluate the digestibility of 4 batches of BSF meals, produced by same company within one year of production. After the *in vitro* digestion process, soluble and non-soluble fraction were collected and analysed for AA and mineral solubility. The solubility values of the 4 batches ranged between 55 and 75% for AA; 41-66% for P; 67-88% for Mg; 40-60% for Cu; 15-20% for Zn and 18-38% for Mn in BSF meal. Overall, the *in vivo* data gives information on nutrient digestibility while the *in vitro* data showed that solubility of AA and minerals were significantly different among 4 BSF batches. Complementary *in vivo* and *in vitro* methodologies can be a useful approach for monitoring consistency of nutrient digestibility of insect meal.

Session 64 Theatre 11

The nutritional composition of insect-based products for dinners in Europe

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In order to consider insects a viable alternative source of protein for European families, comparable amount of protein must be incorporated into sensorial attractive products suited for inclusion in ordinary diets. Consumers are more likely to consume insects if incorporated into familiar foods, rather than presented whole. In SUSINCHAIN we develop insect-based dinner products to meet the target of replacing 20% of the meat-derived animal protein in weekly menus. For this target, the products need to incorporate 10.4 g and 13.5 g of insect protein per serving to align with the general consumption patterns in Portugal and Denmark, respectively. The insect protein content is aimed to be reached while upholding realistic serving sizes European families. Six unique insect-based food products were developed by the SUSINCHAIN partners involved. The products were based on either mealworm (*Tenebrio molitor* or *Alphitobius diaperinus*) or cricket (*Acheta domesticus*), prepared from fresh or dehydrated states. Three products (flatbread, spice mix, umami pesto) are based on crickets, and three products (sausage, minced meat, and falafel) are based on mealworms. In addition to considering the energy, fat, carbohydrates, and other attributes of the insect-based foods, the preparation and supplemental ingredients of the final servings as dinner meals are considered in the final nutritional composition in order to present the products as nutritionally realistic replacements of meat-based dishes in regular dinner meals. Nutritional composition of the products are determined through nutritional analysis combined with the use of food composition databases and primary literature. Prior to the family tests in Portugal and Denmark, the products are pretested for sensory quality and consumer acceptance.

LCA modelling strategies and approaches to insect chains

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The inclusion of insect protein into the food system has been suggested as a promising solution to ensure future food security and mitigate adverse environmental impacts related to food production. The market volume for edible insects in Europe is still small. Producers require a decision-support system to ensure a sustainable upscaling of the sector. To this end, insect production's environmental and economic impacts were analysed to identify the most eco-efficient production scenario. A modular eco-efficiency assessment approach was developed and applied to dried black soldier fly larvae production. A hypothetical industrial-scale insect production system was disaggregated into 29 modules which can be recombined to represent 4,608 distinct production scenarios, characterized by different feeds, energy efficiencies, and processing technologies. Environmental LCA and cost analysis were carried out in parallel for each module, and eco-efficiency assessment was used to assess these two sustainability dimensions. The influence of the insect feed on the performance and thus impact of the production system were investigated by employing feed-specific scaling factors to the aggregation of module results to a production scenario. The most eco-efficient production scenarios include highly energy-efficient rearing facilities and use blanching and microwave drying for processing. The insect feed has the largest share of the environmental impact and cost, but from an eco-efficiency standpoint, the choice of feed is, to no small extent, not crucial. Waste-type feeds (manure; fruit and vegetable waste) have low environmental impacts and costs, but the production systems based on these feeds are less efficient. The low impact of the feed is offset by the higher impact of the rearing and processing stages. Conversely, systems based on higher-quality feeds (by-products like wheat middlings or distiller grains) require fewer resources, but the feeds' impact and cost are higher. Only highly processed feeds, such as compound chicken feed, should be avoided for insect rearing.

Multi-objective optimization for sustainable insect chains

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When considering an optimized insect production chain for food and feed, there is no single best solution: multiple conflicting objectives need to be balanced and assessed. Such objectives include economical assessments (costs for energy and feed, capital expenditure, annual production, etc.), sustainability (consumption of water, use of chemicals, production of wastewater, GHG emissions, etc.) and societal impacts (number and quality of new jobs created, safety measures for employees, societal acceptance of insect farming, etc.). Rather than aggregating all optimization objectives into one, for example by using a classical weighted sum, a more unbiased approach is multi-objective optimization (MOO). MOO algorithms are able to deliver, instead of a single solution, a set of different compromises, each one favouring some objectives against others. By showing the possible optimal trade-offs to human experts, MOO makes it possible to explore different scenarios and ultimately making informed choices, being aware of what can be gained and lost by choosing one particular solution over others. In order to apply MOO to sustainable insect chains, we identified several objectives for which either computer/mathematical models are readily available, or for which machine learning models can be inferred from data collected by partners in the project. The long-term objective is to provide both private and public stakeholders with different possibilities for the configuration of modern insect production chains.

Effects of abiotic factors on viability of BSF eggs: preliminary results for improving egg transport

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Insect farming is a fast-growing sector expected to reach a market value of 8 billion $ by 2030, with the black soldier fly (BSF, *Hermetia illucens*) as a key player reaching 2.57 billion $ alone. Storage and transportation of specific BSF life stages are key factors for the industry success both to guarantee a stable production process and for proper transport and shipping conditions. In the framework of the SUSINCHAIN H2020 project, experiments were conducted to determine the impact of short-term exposure to different relative humidities (RH) and temperatures on the incubation of BSF eggs. Moreover, a test with a recorded delivery was conducted. Firstly, the impact of short-term exposure (1 or 3 days) was tested under laboratory conditions: With different temperatures (6, 12, 15, 18, 20, 30 °C) at constant RH (65%) and with different RH (35, 50, 65, 80%) at constant temperatures. Secondly, a tracked shipment was conducted between partners to verify the previously obtained results. Additionally, the impact of a heat source in the packages was tested. Regarding RH, the highest hatching rates were observed in eggs incubated for 3 days at 80% RH, gradually decreasing with lower RH. Meanwhile, the highest hatching rates were observed in eggs incubated for 3 days at 15 °C. The shipment showed a significant impact on the hatching rate, which was reduced by 23 (with heat source) and 16% (without heat source), respectively. Furthermore, the use of a heat source was found to be significantly harmful due to the high temperatures generated. Storing BSF eggs for 3 days at 15 °C showed no significant difference in the survival rate compared to the control group (30 °C), indicating the optimal temperature to be maintained together with high RH (~80%) to avoid the hatching of eggs during storage and transportation.

Session 65 Theatre 1

How animal breeding can help solving societal challenges

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This Challenge session will be in the form of a workshop with short introductory presentations about social issues in animal production followed by discussions in groups. As stated in Animal Task Force's vision paper (2019) 'Livestock provide a diversity of agroecological, social, cultural and economic services linked to the vitality of a variety of territories, employment and activity in rural areas, preservation of diverse landscapes …, biodiversity and cultural heritage.' There are, however, several social issues associated with animal production; e.g. issues related to health and work satisfaction of animal caretakers, animal welfare, and impact of animal production on the local community and the society. Some examples of areas where animal breeding could potentially contribute to solving societal challenges are: Fewer work accidents and higher work satisfaction – Selection for changed animal temperament; Public acceptance of modern animal production systems – Selection for increased feed efficiency, especially feed not suitable for human food; Consumers' willingness to pay for animal products and consequences for producers' economy – Selection for changed product quality; Transparency and fairness – Management of breeding companies and their relations to staff and customers. These and other societal challenges, raised by the participants, will be discussed from an animal breeding perspective in the Challenge session. We will also discuss drivers and obstacles for animal breeders' contribution to solving societal challenges.

Animal breeding to solve societal pig production challenges?

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Sustainability in animal food systems is increasingly important to society as a whole. Although much focus has been on the environment; improvements in economic and social sustainability are also needed. Social sustainability is the least researched, partly due to the challenge of lack of data. Animal food systems present positive and negative social impacts for workers, farmers, wholesalers and retailers, consumers, local community, society, and animals. It can be discussed if animals should be studied as stakeholders along with people, but that is how we did it in a social life cycle assessment. We compared conventional and organic pork production systems and identified social issues for pig production and pork consumption from experts and a literature search. Relevant for animal breeders are issues of feed food competition i.e. the amount and ratio of human edible components fed to pigs, the issues of work accidents due to pig temperament, citizens' desire to see the pigs on pasture in the local community and the farmers' profitability. How can animal breeding as a tool be used to answer these issues? Can pigs with an easy-to-handle temperament be bred? How much genetic gain in feed efficiency can be achieved without a compromise on other traits? Are the crossbred pigs we have today suitable for outdoor production bearing in mind parasitic diseases, leg problems and ability to use roughage fodder?

Social sustainability in aquaculture

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To achieve a more sustainable aquaculture is complex, since the sector spans from small-scale production to high-tech industrial scale production in countries with divergent social and legislative infrastructures, and >500 species with different needs of management and nutrition are farmed. Aquaculture in all scales provide occupation and can contribute to poverty alleviation and food security in low-income countries. Some species, like molluscs, grow without feeding and can improve the environment where they are farmed. However, aquaculture must address numerous social sustainability challenges. Shrimp farming is associated with labour inequities and other social inequities. Antibiotic overuse in salmon production may threaten public health. Seafood is one of the most traded commodities and international unsustainable socio-ecological practices must be changed. The principles of One Health (i.e. collaborative, multisectoral and transdisciplinary approach to achieving beneficial health and well-being outcomes for people, non-human organisms and their shared environment) can be used as a framework to achieve a more sustainable aquaculture. In our One-Health framework we work with success metrics sorted under three headings: People, Organism and Environment. Success metrics describing social aspects include nutritious and safe food, equitable income generation, gender equalization, quality employment and knowledge and skills generation. Safe farms can also be regarded as a social aspect including zoonosis and food safety. The focus of this Challenge session is 'social', but interactions and dependencies between different success metrics must be taken into account when production systems are evaluated. Data collection for monitoring success metrics requires interaction across a broad range of stakeholders.

Session 66 Theatre 1

Interplay between host genetics and the dynamics of the gut microbiota in pigs

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Pig gut microbiota displays high inter-individual variability and it remains an open question to determine to what extent its taxonomic composition relies on host genetic determinism and not only on environmental conditions. We will illustrate links between host genetics and gut microbiota composition by reporting results from different experiments carried out on Large White pig cohorts. Under similar controlled feeding and environmental conditions, we have shown that the gut microbiota after weaning can be consistently classified into two enterotypes by sequencing the V3-V4 variable region of the 16S rRNA gene from faecal DNA. The two enterotypes are characterized by either an overabundance of the genera *Prevotella* and *Mitsuokella* or *Ruminococcus* and *Treponema*. We explored the contribution of host genetics to the gut microbiota composition by estimating genetic parameters and by launching a directional selection based on the enterotype assignment of 60-day-old pigs. Calculated heritabilities of the relative abundance of faecal microbiota bacteria genera and the response to selection confirmed a significant contribution of host genetics to microbiota variability. The results on this ongoing selection experiment should help to investigate coevolution of the gut microbiota with its host.

Session 66 Theatre 2

Gut microbiome information enables additional discovery in genome-wide association studies in pigs

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Several studies have highlighted the relevance of gut microbiome composition in shaping fat deposition in mammals. In contrast, other studies have highlighted how the host genome can control the abundance of individual species in the gut microbiota's make-up. In addition, new technologies allow the recording of a large number of phenotypes, but the effective dimensionality of these measures is seldom studied. There is the need to incorporate the different '-omics' data (host genome, gut microbiome, high-throughput phenotyping) in a model that allows to extract information beyond the simple sum of each component's contribution. We propose a systematic approach to detect host genomic variants that control the gut microbiome, which in turn contributes to the host fat deposition, when this latter is based on multiple phenotypic measures. Using a dataset that included 1,180 longitudinal records of fat deposition, we implemented a mediation test to describe how fat deposition in swine (*Sus scrofa*) is affected by the host genotype and the gut microbiome. The phenotypic outcome was described both by measured and latent variables, taking advantage of structural equation modelling. We also implemented a 'traditional' genome-wide association analysis, testing the (total) effect of host genomic variants on the phenotype. Results for all models were validated using both bootstrapping and permutation tests. The models identified several host genomic features having microbiome-mediated effects on fat deposition. Our work demonstrates how the host genome can affect the phenotypic trait by inducing a change in gut microbiome composition that leads to a change in the phenotype. The host genomic features identified through the mediation analysis do not entirely overlap the group of features identified by traditional GWAS. Microbiome-mediated analyses can help understand the genetic determination of complex phenotypes. The host genomic features that exert a mediated effect could not be identified by traditional genome-wide association analysis. These can contribute to filling the missing heritability gap and provide further insights into the host genome – gut microbiome interplay.

The microbial metabolome and its role in intramuscular fat deposition

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Intramuscular fat content (IMF) is a relevant trait in animal production due to its high importance in meat quality. A divergent selection experiment for IMF in rabbits was developed at the Universitat Politècninca de València, to study the mechanisms involved in the deposition of the trait. In this study, metabolomic analysis of the microbiome was performed to provide direct insight into the microbial activity influencing the host's fat deposition. Cecum content samples were collected from 27 animals of the high-IMF (H) and 25 of the low-IMF line (L), both males and females, at 9 weeks of age, after 4 h of fastening period. The metabolomic analysis identified 711 metabolites. The metabolites absent in more than 40% of the samples were removed. The remaining zeros were imputed with the minimum value detected in the corresponding metabolite. The data was transformed by logarithm transformation, and each metabolite was scaled by autoscaling. Partial least square-discriminant analysis was used to find the most relevant metabolites differentiating between the lines. Only metabolites with variable importance in projection value ≥1.2 and Jack Knife confidence interval that did not include the 0 were kept. The final model included 179 metabolites and had a good prediction ability of 68% after cross-validation. Several metabolic routes of carbohydrate and energy metabolism showed evidence of differential activity between lines. Metabolites from the TCA cycle and mevalonate metabolism were more abundant in the L line, while metabolites from the pentose phosphate pathway, and related metabolisms like nicotinamide and nucleotides, were more abundant in the H line. The former indicates a differentiation in glucose utilization and energy metabolism between the lines. Also, several phospholipids and glycerophospholipids were more abundant in the L line, supporting differences found in previous studies performed in these lines. This analysis highlights the importance of microbial metabolism and gives important evidence of the mechanisms involved in the intramuscular fat deposition of the host.

The rumen microbiota is modified in lambs divergently selected for residual feed intake

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The ruminal microbiota plays a central role in the nutrition of its host. Significant links between the rumen bacteria abundances and host performances (growth or feed efficiency) have been demonstrated in cattle, but few is known in sheep. Residual feed intake (RFI) is commonly used as a criterion to improve feed efficiency. At the INRAE P3R, divergent lines on RFI are being developed in the Romane meat sheep breed: individuals belong either to the RFI+ (inefficient) or RFI- (efficient) line. We proposed to investigate the ruminal population (bacteria, archaea, protozoa and fungi) of 277 lambs belonging to the 2nd (in 2018) or 3rd (in 2019 and 2020) generations of RFI selection. A first rumen fluid sample (C-sample) was taken at 4-5 months of age, after 6 weeks of feed efficiency control under a concentrate diet. Then, 167 out of these lambs were tested during 6 weeks under a forage-based diet (2/3 forage + 1/3 concentrate), after which a second sample of rumen fluid was taken (F-sample). During each of the 2 periods of control, feed intake, body weights and body composition traits were recorded. After DNA amplification, the ruminal microbiota was analysed by sequencing the 16s rRNA gene for bacteria and archaea, and the 18s rRNA gene for protozoa and fungi. Sequences were analysed with the FROGS pipeline to obtain relative abundances of OTUs (in proportion of total sequences per sample) which were then labelled according to phylum, family and genus levels. Due to the huge impact of diet type, C-samples and F-samples were considered separately in the statistical analyses. After a CLR transformation of the OTUs abundances to consider their compositional nature, ANOVAs were performed with a covariate, the age at sampling and the fixed effects of the year, the pen and the sequencing depth. Finally, discriminant analyses such as sPLS-DA (with MixOmics R package) were performed with the divergent line as the discriminant factor. As expected, more OTUs were identified in F-samples than in C-samples. Differences in the composition of the ruminal microbiota were highlighted between divergent lines under the different diets.

Breed differences in microbial composition associated with growth and carcass composition in pigs

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The role of the microbiome in livestock production has been highlighted in recent research. Currently, little is known about the microbiome's impact across different systems of production in swine, particularly between selection nucleus and commercial populations. In this work, we investigated faecal microbial composition in nucleus versus commercial systems at different time points. The data spanned two connected populations: a Duroc nucleus purebred population and a commercial crossbred population, both sired by 28 Duroc founding boars. The nucleus population consisted of 819 Duroc individuals. The terminal population consisted of 1,257 individuals generated by crossing the Duroc sires with two commercial sow lines. Microbiome composition was measured for all individuals at three time points during growth trial. We identified microbial features associated with growth and carcass composition, in each of the two populations and in common between the two. The two systems were represented by a heterogeneous population of individuals and sizeable microbial diversity across the two systems at weaning. At later times microbial composition varied between commercial and nucleus, with spp. of genus *Lactobacillus* more prominent in the nucleus population. In the commercial populations, OTUs of the genus *Lactobacillus* and *Peptococcus* were associated with increased growth rate and fatness. In the nucleus population, members of the genus *Succinivibrio* negatively impacted all traits, while OTUs of the genus *Roseburia* had a positive association with growth parameters. *Lactobacillus* and *Peptococcus* OTUs showed consistent effects for fat deposition and daily gain in both nucleus and commercial populations. Similarly, OTUs of the *Blautia* genus were positively associated with daily gain and fat deposition. In contrast, an increase in the presence of members of the *Bacteroides* genus was negatively associated with growth performance parameters. The current study provides the first characterization of microbial communities' importance throughout the pork production systems. Further studies should focus on further characterizing different production settings and explicitly incorporating microbial composition into the selection process in the quest for affordable and sustainable protein production in swine.

Bovine genomes influenced ruminal microbiome composition related to conjugated linoleic acid in beef

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Food products derived from ruminants are the major source of conjugated linoleic acid (CLA) in human diets, a highly valuable molecule associated with a large range of positive effects on health. The CLA content in meat is partially derived from incomplete microbial biohydrogenation of linoleic acid in the rumen. The rumen bacterial enzymes involved have an optimal activity depending on the rumen environment, which could be affected by the host genome. If demonstrated, this would open up the opportunity to use the abundances of rumen microbial genes (RMG) as a selection criterion for beef-quality, whilst avoiding high measurement costs. This study aimed to investigate whether there is a host genomic influence on the abundances of RMG associated with CLA content in beef. Genomic (36,580 SNPs after filtering and imputing missing positions), metagenomic (1,917 RMG present in all animals), and CLA data from *Longissimus* muscle (mg/100 g muscle) were available from 245 beef steers with a balanced design across several breeds (Aberdeen Angus, Limousin, Charolais, and Luing) and basal diets (forage or concentrate). Univariate and bivariate GBLUP models were fitted to estimate animal genomic parameters of CLA and abundances of RMG after adjustment for breed, diet, trial, and total amount of fat, and solved by Bayesian procedures. A substantial part of variation between animals in CLA content (mean ± sd = 14.8±7.6 mg/100 g muscle, CV=51%) was due to host genomic effects (h^2 [$HPD_{95\%}$] = 0.38[0.09, 0.70] with a Prob ($h^2>0.20$) = 0.89). These animal genomic effects on CLA content were highly host-genomically correlated to the abundances of 54 RMG (median of $r_g>|0.79|$ and Prob ($r_g>$ or <0) >0.95); which are involved in e.g. purine metabolism (*purF, purN, ade*), folate biosynthesis (*moaA, moaC, E3.5.4.16*) or cysteine and methionine metabolism (*metA, metY*). These results demonstrate the abundances of RMG have a great potential to be integrated into future breeding programs as selection criteria to improve CLA content in beef.

Session 66 Theatre 7

Microbiome and genetic contribution to the phenotypic variation of digestive efficiency in pig

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Breeding pigs that can efficiently digest alternative diets with increased fibre content is a viable strategy to mitigate the feed cost volatility in pig production. This study aimed at determining the relative contribution of microbiome and genetics to the phenotypic variability of three digestibility coefficients (DC). Faecal samples were collected at sixteen weeks of age to sequence the V3-V4 regions of the 16S RNA gene and to predict DC with near-infrared spectrometry for energy, organic matter, and nitrogen. Data were available for 1,564 Large White pigs, and 14,366 identified Operational Taxonomic Units (OTUs) were identified. The proportions of phenotypic variance explained by the microbiome, or 'microbiability', and by additive genetic effects were first estimated independently and then jointly using a Bayesian approach. The microbiability was estimated under three scenarios: without filtering the OTUs, by filtering the OTUs presented in more than five samples and with an average abundance higher than 0.001% (2,399 OTUs) or greater than 0.01% (803 OTUs). Estimates of microbiability for the traits were reduced with increasingly stringent filtering, from 54±2 to 66±2% in the first scenario and from 25±1 to 35±1% for 803 OTUs. With intermediate filtering, the estimated microbiability ranged from 37±1 to 46±2% and was higher than heritability (from 26±1 to 29±1%) when estimated independently from genetics effects. The joint estimation of microbiability and heritability will point out how the two random effects overlap in explaining the trait variability. To conclude, our results show that the filtering of OTUs is important and influences microbiability. The microbiome explained a significant proportion of the phenotypic variance of digestive efficiency, which was even larger than heritability estimates.

Session 66 Theatre 8

Microbiability and microbiome-wide associations with feed efficiency and performance traits in pigs

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The present study aimed at investigating in pigs the contribution of faecal microbial composition (microbiability) to feed efficiency and other performance traits including average daily gain (ADG), back fat thickness (BFT), daily feed intake (DFI), feed conversion ratio (FCR), and residual feed (RFI). The operational taxonomic units (OTU) abundances were obtained from 16S rRNA sequencing of faecal samples from about 550 pigs from two lines divergently selected for RFI. The microbiabilities (m^2) were obtained from mixed linear animal models accounting for the additive genetic background of the pigs using a Bayesian approach. Microbiome-wide association studies (MWAS) were run using single-OTU regressions or back solving the solutions of best linear unbiased predictions from the microbiome relationship matrix. The heritability posterior means (h^2) were moderate for all traits, ranging from 0.31±0.13 for FCR to 0.51±0.10 for BFT. The m^2 posterior means were 0.11±0.09 for RFI, 0.20±0.11 for FCR, 0.04±0.03 for DFI, 0.03±0.03 for ADG and 0.02±0.03 for BFT. All traits showed lower m^2 than h^2 values. Omitting the additive genetic effect resulted in higher residual variances, and higher m^2 for BFT only (0.11±0.06). The two approaches used for MWAS showed similar results, but the single-regression method had higher detection power. With this approach, three suggestive OTUs were found for RFI, one significant OTU was found for FCR and BFT. For DFI two significant and one suggestive OTU were found. For ADG, no association was found. These 8 OTUs belonged to the *Streptococcaceae*, *Prevotellaceae*, *Ruminococcaceae*, and *Lachnospiraceae* families, mainly involved in producing short-chain fatty acids and digestive enzymes. Therefore, our results showed a substantial contribution of the microbial effects to the variability of feed efficiency traits and negligible effects for other performance traits. However, associations between microbial community and complex phenotypes could be identified for almost all traits. These could be considered as future biomarkers for genetic improvement of feed efficiency in pigs.

Effect of heat stress on faecal microbiota composition of gestating sows and of the female offspring

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The objective of this study was to evaluate the impact of chronic heat stress (HS) on microbiota composition of gestating sows and of their female offspring. There were two environmental conditions: thermoneutral (TN; cyclic 18 to 24 °C) and HS (cyclic 28 to 34 °C). Pregnant sows were exposed to either TN (PTN; n=12) or HS (PHS; n=12) conditions from d 9 to 109 of gestation. During the finishing period, two female offspring per sow were allotted to either a growing TN (GTN; n=24) or HS (GHS; n=24) environment. All pigs were fed the same diet all throughout the experiment. From 75 to 140 d of age, GTN pigs (n=24) were in TN conditions, while GHS pigs (n=24) were in TN conditions from 75 to 81 d of age and in GHS conditions from 82 to 140 d of age. Spot faecal samples were collected on d 4 and d 105 of gestation in the sows, and on 78 and 136 d of age in the growing pigs for 16S rRNA analyses of microbiota composition. In both sows and offspring, exposure to HS increased rectal (+0.16 and +0.30 °C, respectively; $P<0.01$) and skin (+2.44 and +1.61 °C respectively; $P<0.01$) temperatures. *Firmicutes* and *Bacteroidetes* were the most dominant phyla (~98%) in the samples collected pre-challenge regardless of physiological stage but sows had higher percentage of *Firmicutes* (+20.4%; $P<0.01$) and lower percentage of *Bacteroidetes* (-20.2%; $P<0.01$) than the growing pigs. Using a permutational multivariate analysis of variance on the environment effect on microbiota, chronic HS exposure did not significantly affect sow microbiota at d 105 ($P=0.120$). Microbiota from the pigs born from PHS sows was resistant to a further heat stress during the finishing ($P=0.167$), while microbiota of pigs born from the PTN sows were strongly affected ($P=0.027$). Therefore, the prenatal environment of the growing pigs affect how their microbial composition change upon exposure to a thermal stress during their postnatal life. The difference in the effect of heat exposure on gut microbiota of sows and of growing pigs may be related to their microbial composition or to the duration of their thermal stress exposure.

Multi-ome analysis to predict feed efficiency in pigs

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This study aimed to use relationships between the faecal microbiome, the systemic metabolome, and animal genome to predict feed efficiency related traits in pigs (i.e. feed intake, body weight gain, and feed conversion ratio). Data were collected from 530 three-way crossbred male grower-finisher pigs, all genotyped at 50k SNPs. Pigs were offered feed *ad libitum* in a three-phase feeding program with a commercial diet based on wheat, barley and by-products. On the day before slaughter (mean body weight 125 kg), individual faecal and blood samples were collected. Faecal samples were sequenced for the 16S hypervariable region of bacteria (V3/V4) to profile the faecal microbiome. Blood serum samples were analysed with untargeted LC-MS for metabolites. Using these data, we calculated the proportion of variance in feed efficiency related traits that was explained by variation in the faecal microbiome (m^2), blood metabolites (b^2), and genetic background (h^2). The m^2 values ranged from 34 to 52%, b^2 values ranged from 50 to 63%, and h^2 values from 23 to 28%. Using cross-validation, we estimated the accuracy of prediction based on the microbiome, metabolome, and genome profile of the pigs. Prediction accuracies were highest based on the metabolic profile ($r=0.56$-0.63), followed by the faecal microbiome profile ($r=0.30$-0.48), and the genome profile ($r=0.21$-0.29). Modelling all three profiles simultaneously resulted in the highest prediction accuracy ($r=0.59$-0.66). In conclusion, combining information on the genotype of the pig and its faecal microbiota and blood metabolite profiles improves the accuracy of prediction of phenotypes for feed efficiency related traits, but almost the same prediction accuracy could be achieved using blood metabolite profiles only. This study was part of the Feed-a-Gene Project, funded from the European Union's H2020 Programme under grant agreement no 633531.

Session 66 Theatre 11

Swine growth and carcass traits predicted in nucleus purebred using microbial information collected

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The use of microbial information in selecting for more efficient livestock has received increasing interest in recent years. Nonetheless, research on using microbiome information across different populations and systems of production remains scarce. Notably, the relevance of microbial information collected in terminal crosses as a way to inform selection at the nucleus population has not been explored. The current work's objective was to evaluate the use of faecal microbial profiles collected in a terminal cross to predict growth and carcass traits in nucleus pigs. One thousand one hundred forty terminal pigs born from 28 founding Duroc sires had microbiome collected at three time points (weaning, mid test, end of trial). The same 28 sires were bred in purity, and microbial information was collected on 820 individuals at the same time points during growth. Nine growth and carcass were collected in both populations. The effect of each of 3,001 OTU were obtained in the crossbreds using three models of the Bayesian alphabet (Bayes-C, Bayesian Lasso, Bayesian Ridge Regression). Solutions were used to calculate microbial predictions in the purebreds akin to a genomic selection exercise and employed to predict corrected phenotypes in the testing population. Correlation between predicted and measured phenotype was used to measure the prediction performance. Predictions ranged from ~5% for loin area at weaning to more than 30% for average daily gain and fat deposition at the end of trial. In general, weaning microbiome had poor predicting ability while mid, and end trials performed better. Model choice showed mixed results, with Bayes-C performing better for daily gain and fat deposition and ridge regression for most of other traits. When partitioning variance explained by OTU based on taxonomy, Unassigned OTU contributed most to microbiability (ranging from 30 to 50%), followed by Prevotella and Lactobacillus (ranging from 5 to 15%). From these results, it appears that microbial information collected in commercial settings can be used to inform selection decisions at the nucleus, possibly partially bridging the GxE gap in swine productions systems.

Session 66 Poster 12

Estimation of genetic parameters for ruminal bacteria in Lacaune dairy ewes

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Among livestock species, ruminants such as sheep have the ability to digest the plant fibre, thanks to the main action of symbiotic ruminal bacteria. Some authors studied the link between the microbiota and different complex traits in cattle, and more recently in sheep. But what is the level of host impact on ruminal bacterial composition? The aim of this study is to estimate the heritability of Operational Taxonomic Units (OTUs) and bacterial genera in a population of Lacaune dairy ewes. The data corresponds to 800 dairy Lacaune ewes raised at the INRAE Experimental Farm of La Fage (France). These ewes were adult animals, mostly in their second lactation, feeding the same diet (TMR), and the rumen sampling was done between 2015 and 2019. Ruminal metagenome was sequenced using metabarcoding (V3-V4 of gene of 16S rRNA) with Illumina Miseq technology. The microbiota sequences were analysed with FROGS pipeline to obtain the relative abundances of 2,059 OTUs, grouped in 112 genera. In the abundance table the zero values were replaced with the GBM method, to allow to centred log-ratio (CLR) transform all values. The additive host genetic effects on the rumen bacteria abundance was estimated applying a linear mixed model. The general equation included the OTUs and genera abundance as response variable; fixed effects, such as stage of lactation (45 to 133 DIM), lactation number (2 up to 7 lactations), genetic line (SCS+, SCS-, PERS+, PERS- and SOCS genotype), run sequencing effect, date and year of the rumen sampling; and the individual animal random additive genetic effects ~N(0,Aσ2a), where σ2a is the additive genetic variance and A is the pedigree relationship matrix. With these methodologies, we will estimate the heritability of OTUs and bacterial genera in a population of Lacaune dairy ewes.

Ruminal metabolism study of dairy ewes to link the ruminal microbiota and milk quality

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In ruminants, the symbiotic relationship with the rumen microbiota and its host is crucial for forage digestion. The fermentation of fibres enables the production of volatile fatty acids (VFA), which will be a source of energy and precursors of fat synthesis for milk production. The association of ruminal microbiota and milk composition of cows have been reported in the literature, and more recently in dairy ewes. The objective is to explore ruminal fatty acids (FA) metabolism and to link the rumen bacteria with the FA profile of ewe's milk. The available database is composed of 120 dairy Lacaune ewes raised at the INRAE Experimental Farm of La Fage. These adult ewes were reared indoors, fed a total mixed ration, and belonged in equal numbers to 2×2 divergent lines selected for somatic cells score (SCS) or milk production persistency during lactation (PERS). Rumen fluid and milk were sampled twice for each animal at one-week interval. Ruminal bacteria metagenome was sequenced using 16S rRNA gene with Illumina MiSeq technology and sequences were analysed with FROGS pipeline obtaining 2,079 Operational Taxonomic Units (OTUs). The conventional milk recording traits (milk quantity, fat and protein contents, and somatic cell count) have been supplemented with Mid Infra-Red (MIR) spectra prediction of protein fractions and the main fatty acids in milk. Rumen and milk FA were identified and quantified by gas chromatography. Considering the compositional nature of the OTU abundance, the FA percentage, and predictions by MIR, were Centered-LogRatio (CLR) transformed, after replacement of zeros. Repeatabilities of microbial abundances were compared with those of rumen VFA and FA data and of fine milk composition data. The impact of SCS or milk persistence selections on ruminal FA metabolism and OTUs abundances were studied with a discriminant analysis (sPLS-DA) on divergent lines. Further, the links between the 3 compartments of the rumen bacterial abundances, the ruminal FA metabolism and the milk fine composition were established using DIABLO framework, implemented by the mixOmics R-package.

Assessing livestock systems – how indicators, system boundaries and methods may influence results

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Animal source food and livestock systems play a central role in the debate on sustainable food systems. When referring to climate change mitigation or land use, for example, it is often argued that we should focus on plant-based diets. For animals, we should predominantly reduce ruminant meat and milk, as they have larger impacts than monogastrics' products. However, ruminants play a particular role in food systems, as they can contribute to convert grassland biomass into food, which would otherwise not be usable for humans. Similar for monogastric animals, that can be fed on by-products and waste. When digging deeper into these topics, we see that recommendations for increased sustainability in livestock production and in food systems in general can strongly differ, depending on the indicators, system boundaries and methods chosen. Optimising on footprint indicators (e.g. cropland use per kg meat) gives different results than optimising on total cropland use; reducing nitrogen surplus per kg milk may still result in transgressing critical nitrogen loads, if ecosystem boundaries are not adequately accounted for; recommendations based on life-cycle analysis can strongly differ from recommendations based on full food-system model calculations. In this contribution, I focus on and clarify some of the challenges and apparent inconsistencies that arise from choices of indicators, system boundaries and methods, and I draw conclusions on what this means for sustainable livestock production and for policy making for sustainable food systems.

Session 67 Theatre 2

Combining farm-level modelling and environmentally-extended input-output analysis in beef production

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Establishing the environmental effects of meat production requires an extended analytical approach that encompasses the entire production chain. The goal of this study is to carry out an environmental and economic assessment of the beef production sector in Portugal through a hybrid life cycle assessment (LCA) approach using two tiers of analysis: (1) a farm-level modelling and economic accounting approach that includes ecosystem services (ES) provided by grasslands; and (2) environmentally extended input-output analysis (EEIOA) for indirect effects. The first-tier approach was based on farm processes using farm level data. Data collected from 40 case study beef farms was combined with data from the national Cabinet for Planning and Politics (GPP) to assess provision of ES locally. The second tier linked farm level data with corresponding sectors and regions using a multi-regional input-output database (Exiobase). The use of Exiobase allows to go beyond economic valuation as it also includes a physical input-output model, which accounts for example for N and P flows and water consumption and enables the estimation of effects on ES directly. Characterization models for ES that have been developed recently in the context of LCA are then applied to EEIOA as well. An example of this application is biodiversity, as the same model can be applied both at LCA and physical EEIOA level due to the similar structures. A selection of models for ES are adapted and applied to EEIOA in order to increase the number of services covered and end up with a similar list at foreground and background level. ES are then monetized using the same methods applied at foreground-level before. The use of the two-tier approach enables the exploration of different scenarios of local land use and farm management and to estimate economy-wide global effects.

Session 67 Theatre 3

Environmental, economic and social metrics to enhance the sustainability of livestock systems

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The Agri-Food Quest 'Food Futures' project aims to develop a reporting tool to capture, credit and drive positive behavioural change among livestock farmers and their stakeholders, in order to enhance the sustainability of Northern Ireland agri-food production. Building upon systematic literature and legislative reviews, as well as consultations with scientists and stakeholders from the food supply chain, more than 30 metrics were developed under the pillars of Economic, Environmental and Social sustainability. This multi-dimensional tool was applied on more than 30 dairy, sheep and beef farms to characterise their overall sustainability. Each metric is comprised of up to seven separate indicators, and each indicator is scored on a scale of 0 to 10, with 10 representing: (1) the optimum status for the indicator (e.g. all fields on mineral soils are at optimum pH levels of 6 to 7); (2) best practice (e.g. in terms of slurry and manure management, business plans being developed and reviewed); or (3) most efficient farms (e.g. in terms of carbon sequestration potential, energy usage or GHG emissions per unit of output). The approach taken to score, prioritise and combine each indicator within each metric depends on the nature of each indicator (representing a driver or a state/impact). As a result, the development and uptake of multi-dimensional tools such as the Food Future farm tool is particularly challenging, especially for social metrics which cannot always be 'scored'. Instead, subjective assessments can be used successfully to raise the awareness of potential well being issues. Another key aspect of such tools is to provide useful feedback to the farmers and other stakeholders from the supply chain, in order to drive positive behavioural change. To do so, the Food Future tool is building a digital smart platform with: (1) multiple layers of complexity (from overall scores to raw data); (2) automated data flows to enable a cost efficient use of existing and new database; and (3) a knowledge exchange hub to inform management strategies.

Session 67 Theatre 4

Emergy analysis of the sustainability of mountain grazing livestock systems

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In the context of increasing social concern about livestock systems environmental impact, the development of methodologies for assessing biophysical sustainability is crucial. Livestock sustainability assessments commonly focus on greenhouse gases fluxes which, although are good for assessing livestock interaction with the climate system, generate controversy related to the need to differentiate between carbon released in human activities and carbon belonging to natural cycles. Emergy Analysis (EA) deals with sustainability in terms of energy fluxes, allowing to evaluate separately renewable and non-renewable energy sources. EA computes all the energy sources involved in the production process, expressed in Jules of equivalent solar energy (seJ). We implemented an EA on 50 cattle farms in the Spanish Pyrenees aiming to: (1) evaluate and compare the energy composition (renewable vs non-renewable) across farms using different accounting methods; and (2) determine the main variables enhancing the environmental sustainability of farms. Our results showed high heterogeneity between farms energy requirements despite all of them having similar management and productive orientation. This should raise concerns about using individual farms as representatives of farming systems in comparative sustainability assessments. Between 31 and 50% of the total Emergy used across farms came from local natural renewable sources, and 12 to 23% from purchased feeds. Human labour and services (i.e. emergy required to support the whole socioeconomic network, directly related to its economic cost) represented between 24 and 34% of the total Emergy. The high contribution of services suggests that mountain livestock systems have a reduced capacity to affect their own sustainability, due to their large dependence on the broad socioeconomic system (i.e. reduced autonomy). The contribution of natural pastures to farm Emergy showed huge differences (between 1 and 32% of the total farm Emergy) depending on how it is accounted, which requires deeper discussion.

Session 67 Theatre 5

A refined method for calculating ruminant Livestock Units to improve farm performance analysis

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The notion of livestock unit (LU), established more than 50 years ago, aims to quantify the importance of herds as a proxy of feed resource requirements but also to calculate various indicators such as the stocking rate or the productivity per LU (kg of milk or meat). It thus allows comparisons between farms at the scale of regions or countries, even if the activity of the farms may be based on various types of animals or production. Indeed, one of the expected advantages of LU concept is its transversality between different livestock species and types of production, using coefficients for specific categories of animals. However, these coefficients don't take into account the variability of animal live-weight or productive performance in a given category. At the moment, one of the stakes for livestock farming is the capacity of the animals to use resources and, therefore, their efficiency to produce milk and meat. Thus, this historical concept of LU seems not precise enough to deal with this issue. Therefore, we propose to refine the calculation of the LU values of ruminants by considering their net energy requirements for maintenance, activity and production. For this, we use the IPPC equations. This work consisted in identifying the most determining variables of the net energy requirements for bovine, ovine and caprine species, for meat or milk production and to propose two levels of equations i.e.: (1) equations that are complete enough to give a precise result; but also (2) simplified equations allowing for calculations that are certainly less precise but that can be carried out in studies for which only a few less accurate data concerning farming management and animals are available. We justify, through a sensitivity analysis, the choice of parameters that have been selected for each species and each of the two levels of equation. We illustrate the method and its potential by showing the variability of the LU coefficients thus calculated for some contrasting types of livestock farming system.

Challenges and novel metrics of multi-dimensional tools at farm/regional level

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Developing tools at farm and regional levels faces a double methodological challenge: how to up-scale biological knowledge from the animal/plant level to higher levels of organization and how to avoid the pitfall of labyrinthine models, not easy to use and disseminate? In other words, how can we find the appropriate level of simplification when developing models and tools to understand and evaluate complex systems? Part of the answer implies to identify the key drivers and effects that should be conserved when changing the level of organization. It is also important to clarify input/output ports that connect levels and therefore ensure that information is transmitted across scales with relevant accuracy. Considering these aspects is also crucial when communicating the complexity of livestock systems and transferring knowledge to end-users. Based on concrete examples of models and tools, this session illustrates how it is possible to get the right level of simplification when working in more and more aggregated levels of organization and how to communicate complex relationships. A short introduction with two examples of models combining genetics, nutrition and economics will start the session, followed by four communications. Based on these various examples, the session will end with a general discussion of challenges and future research needs on tools/models for LFS research.

Livestock production within planetary boundaries: the need to look beyond the farm

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Dominant discourse discuss livestock as a major environmental threat. Yet, livestock plays a central role in agricultural landscape in closing environmental cycles and in the efficient transformation of biomass that cannot directly be used for human consumption such as by-products and grass to high value food protein. Livestock is central to foods systems within the safe operating space when crop-livestock interactions unlock synergies key to the circular economy. This requires balancing livestock-related nutrient flows in a context-specific manner matching with local ecosystem processes and boundaries. In addition, environmental impact from livestock is not the mere unrelated sum of the impacts of individual farms: the optimal combination of farm types or land uses and their related outputs within adequate spatial and temporal boundaries allow to exploit niches in which synergies mitigate potential negative impacts from livestock production. Consequently, only through a landscape approach, sustainability of livestock can be assessed adequately and in a systemic manner. This session will discuss why linking livestock to land is a critical feature to assess livestock beyond the farm boundaries and explore modelling approaches to assess sustainability of livestock production at landscape scale.

Session 67 Theatre 8

Sustainability assessment of farms using SALCAsustain methodology

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In recent decades, many sustainability indicators and methods have been developed at farm level, but a validated set of quantitative and scientifically-sound indicators covering all three dimensions of sustain- ability is still needed. For this reason, the sustainability method SALCAsustain was developed in order to estimate the environmental impact and economic and social situation of farms using a manageable number of indicators. The primary aim of this study was to assess the feasibility, explanatory power, and acceptability to farmers of the SALCAsustain methodical framework. To achieve this goal, SALCAsustain was applied for the first time to selected Swiss farms. In-depth personal feedback interviews were conducted to gain more insights into the feasibility and farmers' acceptance of the method. The results showed that SALCAsustain is a feasible, acceptable and robust method for assessing farm sustainability based on a set of indicators. Correlation analysis demonstrated that the number of environmental indicators can be reduced due to high correlation, but that the correlation between environmental impact and socioeconomic indicators was generally low. Evaluation of responses to questionnaires and semi-structured interviews with farmers revealed that the majority would adjust their medium and long-term planning to achieve higher sustainability scores. Additional effort s are needed to speed up data collection and to refine plausibility checks, through exploiting the increasing digitalisation in agriculture. Recommendations and instructions on actions for more sustainable farm management are also needed.

Session 67 Theatre 9

EcoCliCk – a web-based tool to transfer knowledge of GHG mitigation costs

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Numerous tools to assess GHG emissions and mitigation options from dairy farms have been developed using LCA approaches. The main challenge is to create a tool that is on the one hand user-friendly for farmers but also sensitive to their regional and individual conditions. Furthermore, it is important to aware the user of uncertainties and the impact of the choice of system boundaries. In this contribution we present an open -source-web-based tool called 'EcoCliCk'. The tool is based on a partly mechanistic model. It integrates both a full economic and GHG emissions assessment based on a LCA approach for dairy cow production systems. Due to its ease of use, the comprehensible explanation of the calculations and the combination of both assessment approaches to identify farm individual GHG mitigation options with low costs there is scope of its broad use by farmers and advisers. Different methods and new indicators were developed and included into the tool to address the challenge of system boundaries which will be subject of discussion.

Social-media friendly dashboards for communicating trade-offs related to animal production systems

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Sustainability of animal production systems is a complex concept involving multiple dimensions, often with trade-offs involved among them. Management actions and innovative practices applied to animal production systems can surely bring benefits on some targeted sustainability dimensions but, at the same time, might bring unintended consequences on other dimensions. It is therefore important to communicate the concept of 'trade-off' (i.e. it is not possible to obtain simultaneous improvements in all the sustainability dimensions) to a non-academic audience, as well as visualizing the trade-offs. The AnimalFuture project (funded ERA-NET SusAn from 2017 to 2020) was aimed at: (1) assessing sustainability of animal production systems via indicators; (2) making an inventory of innovations applied in some European case studies for improving sustainability; and (3) assessing, via modelling and participatory approaches, the impact of innovations on sustainability dimensions. In order to communicate and visualize trade-offs related to animal production system sustainability, at the end of the project a series of interactive dashboards were produced to present the results of the project. Challenges related to trade-off communications were (1) visualize the information in an easy-to-understand way and (2) reach a wide audience. For addressing these two challenges, these dashboards were thought to be designed a clear and attractive way, and meant to be sharable on social-media (e.g. twitter) in order to gain feedbacks and measure the outreach. For example, a dashboard presents the innovation applied in Bavaria of feeding pigs with fermented proteins in order to use domestic-produced protein crops and to reduce greenhouse gas emissions from pig production and feed transportation. Another dashboard presents an interactive table in which the user can visualise for different innovations, the benefits and the costs on different sustainability dimensions. We will discuss the format of these dashboards as a tool to visualize trade-offs and synergies of innovation in livestock farming systems. We will also discuss feedbacks received on social-media that will be obtained from the launch of these dashboards (after abstract submission).

Session 67 Theatre 11

Discussion on challenges and future research needs of models and tools applied to LFS

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The different presentations of this session showed methodological challenges and concrete examples of tools/models applied to Livestock Farming Systems. This session will end with a general discussion of challenges and future research needs on tools/models for integrated and holistic assessments of Livestock Farming Systems.

Session 67 Poster 12

Assessment of the non-return rate using a codification of the insemination suitability

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The evaluation of the data of the codification of insemination suitability at Swissgenetics, which was introduced in 2019, should show whether a prognosis of the non-return rate (NRR) or the probability of success of the insemination is possible. Further, it should also be possible to draw conclusions about the fertility of the bulls used. Approximately 270 Swissgenetics technicians systematically applied the codification introduced in June 2019 at each insemination throughout Switzerland. Six parameters were assessed at each insemination: position of the vulva, quantity of mucus, uterine tone and size, cervical passage and time of insemination. Each parameter could be selected by icon on a tablet in 3 levels (-/n/+). The + stands for quite good, n for neutral and - for moderate or quite bad. The selection and combination of parameters was based on a sound literature study and should reflect the gynaecological status of the cow at insemination and thus may provide a prognosis for her NRR. In addition, the technician must select an overall score of 1 (poor) to 6 (very good) according to how he/she judges the cow's insemination fitness overall. The collected data were combined with the statistical analysis of the insemination result using the NRR at 56 days (NRR56). From June 2019 to December 2020, approximately 650,000 artificial inseminations (AIs) were assessed with this codification. It was found that the more + selected in the combination, the higher the NRR. The same was true for the overall impression. Cows that had been assessed with a score of 5 or 6 were more likely to get pregnant than those that had been assessed with a score of 1-4. Using suitable gynaecological parameters and an overall assessment score, a prognosis of the insemination suitability of the cow, and thus her NRR, is possible under condition that technicians get adequate training to do the scoring attentively. The estimation of the insemination ability of sires/semen batches could be improved by adding the gynaecological state of the inseminated cow to the statistical model.

Session 68 Theatre 1

HappyMoo project – using milk mid-infrared spectrum to predict dairy cows' welfare status

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Dairy farming in Europe encounters profound changes with less farms but of bigger size, leading to less time for the farmer to monitor health, reproduction and welfare of their animals. In particular welfare monitoring is challenging as it is multidimensional. Currently, the protocols existing to assess welfare on-farm are time consuming and occasional (one-shot picture). As monthly milk analysis and particularly the mid-infrared (MIR) spectrum of milk contains very interesting information, it is now possible to predict several components related to health (ketone bodies, fatty acids, etc.). In a management perspective, numerous applications can be released to the farmer. This is why partners from different countries, using standardization of their analysers gathered in a new project called HappyMoo. The objective is to develop indicators for the absence of hunger (energy balance), disease (mastitis and lameness) and stress for dairy cows using MIR spectral data from milk recording samples. These indicators will produce warnings for the farmer and extension workers. To achieve this, data are gathered and transferred to a common transnational database. The energy balance, bodyweight changes and BCS predictions will be investigated together with prediction of the energy status and biomarkers of ketosis. For stress, an animal experiment highlighted two possible chronic stress biomarkers. Early lameness of cows will be predict thanks to innovative machine learning algorithms after harmonization of lameness scales. Finally, a global model to predict early mastitis will be build based on the MIR spectra and prediction of new biomarkers (Haptoglobin, NAGase and LDH).

Session 68 Theatre 2

Detecting welfare status in a milk sample: effects of housing modifications on milk spectra

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Animal welfare status is assessed through visual evaluations requiring an on-farm visit. A convenient alternative would be to detect cow welfare status directly in milk samples, already routinely collected for milk recording. The goal of this study was to apply a novel approach of spectral analysis that combines principal component analysis and mixed modelling to isolate spectral fingerprints that reflect the effects of housing treatments of dairy cattle on milk composition in the context of controlled design animal trials. This approach was used in a series of trials looking at housing modifications including detecting the effect of 4 tie-rail configurations (TR) and 2 chain lengths (CL) on bovine milk composition. Principal components were extracted from averages of spectra collected on weeks 8-10 and revealed a significant housing treatment effect, corroborating animal welfare responses such as behavioural findings. In terms of assessing negative welfare status, the spectral analysis revealed that milk from cows assigned to the most restrictive TR had higher levels of biomarkers related to body fat mobilization, which suggested that cow's access to feed might have been obstructed. These findings were corroborated by animal observations revealing that these cows had increased injuries in two locations on their necks while reaching the manger. In terms of assessing positive welfare status, milk of cows assigned to the longer CL had lower levels of biomarkers related to acidotic insults in the rumen. Animal behavioural data showed that these cows spent more time with the head in the manger assuming that they might have been chewing more; hence, they were producing more saliva to balance the ruminal pH. The new methodology was able to reveal housing modifications that had a significant effect on milk spectra with differences observed between the treatments with the most and least restricted movement, translating into negative or positive animal welfare status. Spectral analysis provides a new angle to evaluate cow welfare and enables remote detection of cows or herds with welfare issues without requiring an on-farm visit.

Session 68 Theatre 3

Identification of dairy cows chronic stress biomarkers

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Stress in dairy herds can occur from multiples sources. When stress becomes chronic because of a long duration and inability of animals to adapt, it is likely to affect emotional state, health, immunity, fertility and milk production of cows. Therefore, it has a negative impact on welfare, economics and social acceptability of dairy farms. Measuring and assessing chronic stress in herds would be beneficial, however, no real consensus emerge from the literature regarding the biomarkers of interest. The goal of this study is to compare and evaluate potential chronic stress biomarkers after inducing 4 weeks stress, with the final objective to predict the highlighted ones using milk MIR spectra. During this period, 15 cows constituting a stress group were housed under overstocking condition, with 4.6 m^2 per cow. Only 7 feed bunks were available to generate competition for feed access. Once weekly, an additional stress was induced by moving cows to an unfamiliar barn and diffusion of stressing noises. Meanwhile, the 15 cows of the control group stayed in the original barn, with more than 10 m^2 per cow and more feed bunks than cow number. On a weekly basis, following variables were recorded: general production variables, behaviour, heart rate variability, biochemical biomarkers and leucocyte profile. The results were analysed using linear mixed models. No differences were observed regarding milk yield, bodyweight or body condition score but the milk loss was more pronounced in the stress group. Rumination was lower and a higher number of chasing/head-butt was observed in the stress group. The heart rate was lower in the stress group and shown more heterogeneity. No differences were observed regarding salivary cortisol, blood glucose, β-endorphin, thyroxine and leucocyte profile while a higher level of hair cortisol and blood fructosamine were observed in the stress group. Among the variables highlighted, the hair cortisol content seems to be the most promising indicator to practically assess chronic stress at a large scale.

Session 68 Theatre 4

Heat stress assessments in dairy cattle based on milk MIR spectral data

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The effect of heat stress (HS) on milk production traits is well documented. However, HS effect on the detailed milk composition has been much less studied. First findings in different environments (e.g. Belgium, Tunisia) reported responses in MIR spectra to HS in dairy cows. This study investigated the effect of HS on milk production traits and MIR spectrum which reflects the detailed composition of milk samples in the German federal state of Baden-Württemberg (BW). Milk test-day records for the period 2012 to 2019 were merged with temperature and humidity data provided by public weather stations in BW. Values for the daily average temperature-humidity index (THI) were calculated. Statistical analysis was made in R using 'glmnet' and spectral data was first standardized, then pre-processed by first derivative. Models relying in THI index were used to compare between HS effect on milk traits (milk yield, fat, protein contents and fatty acids) and 212 points of standardized spectra. Fixed effects were breeds, parity, milking moment, DIM classes, age at calving. The average 3-day lag average THI value was used as a covariate. In this study, the monthly average THI values ranged between 36±7.3 and 65±5.2 from January until August, respectively. The average daily THI was 50±8.6, 49±8.1, 64±5.4, and 37±7.5 during the autumn, spring, summer and winter periods, respectively. The average THI values indicated that dairy cows are exposed to HS during the summer period in BW. The results showed that there are differences in MIR spectra recorded during HS and thermoneutral conditions; specific wavenumbers of the MIR spectrum were reacting differently. Further analyses will be required to identify potential MIR milk-based phenotypes that could be used in this context for herd management and breeding for resilience to HS.

Session 68 Theatre 5

'MastiMIR' an early warning system based on MIR spectra

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Nowadays a wide range of milk characteristics can be measured due to the mid infrared (MIR) spectrometry. Furthermore, mastitis is associated with milk components, therefore it was possible to develop an early warning system based on veterinary diagnosis from a health monitoring project (GMON Rind BW) and MIR spectra in between ±14 days. Our objective was to test the MastiMIR tool for the determination of the animal health status depending on the milk quality in the field, with the aim to evaluate the diagnosis usability and MIR indicators for the improvement of early mastitis prediction. To identify animal variables that were positively or negatively associated with mastitis determination, the spectral data set was first pre-processed by Savitzky-Golay first derivative to remove the offset differences between samples for baseline correction. Fix effects were selected: the sampling moment, lactation stage and breeds usage such as meat, milk and dual proportion breeds, also spectral data treated with Legendre polynomial based on DIM and 212 EMR/OptiMIR spectra wavenumbers. MastiMIR calibration model showed a good accuracy (0.89) and medium prediction accuracy (0.83). It is needed to underline that, before MastiMIR in 2017 was developed, there existed no information in the literature of successful and direct use of spectral data to predict the mastitis treat. Because the model provides four classes of warning such as not, moderately, significantly and severely endangered, reports based on these warning predictions were created together with Agricultural Chamber of Alsace. The moderately endangered class is a signal for the farmer. In that case the farmer would contact the veterinary and a control would be made in order to prevent the mastitis diseases. The MastiMIR reports are tested since February 2021 in Baden Württemberg as a complementary tool for the SCC model where farmers can control the herd in order to prevent the mastitis diseases.

Predicting mastitis with somatic cell count, differential somatic cell count and milk MIR spectra

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Udder health management and mastitis control are essential in dairy farms, since mastitis is one of the most prevalent diseases. Somatic cell count (SCC) is the commonly used indicator to detect mastitis on individual cow level and to monitor udder health on herd level. Milk mid-infrared (MIR) spectra analysis may be an additional tool for the prediction of mastitis. MIR spectroscopy is the method of choice to determine contents of fat, protein, urea and lactose in official milk recording schemes. Furthermore, there are studies on the prediction of various health traits. Differential somatic cell count (DSCC) seems to be a further promising indicator to detect cows having an infected udder mammary gland. DSCC represents the percentage of polymorphonuclear leukocytes (PMN) and lymphocytes in relation to the total SCC. Recently, we developed a mastitis prediction model using somatic cell score (SCS), which is a log transform of SCC, and MIR spectra data from routine milk recording. This study aimed to expand this model by DSCC and to compare prediction accuracies of the models. Data of Fleckvieh, Brown Swiss and Holstein Friesian cows were collected within the routine Austrian milk recording scheme and its health monitoring system. Test day data including SCC, DSCC and MIR spectra were merged with mastitis diagnosis data. Test day records within 21 days before/after diagnosis were defined as mastitis cases. Test day records outside this time period and records of cows without mastitis diagnoses were considered as healthy cases. First derivatives of selected parts of MIR spectra, corrected for days in milk, were used for modelling. The complete data set with 109,084 records was randomly split by farm into 60% train and 40% test, enabling external validation. Prediction models were built with Partial Least Squares Discriminant Analysis. The model MIR+SCS showed a sensitivity of 0.63, a specificity of 0.76 and the area under the receiver operating characteristic curve was 0.76; respective values for the model MIR+SCS+DSCC were 0.62, 0.76 and 0.75. Hence, adding DSCC to the existing model (MIR+SCS) did not improve prediction accuracy.

Session 68 Theatre 7

#Freenotypes: extracting added value from routine milk recording using deep learning

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Mid-infrared (MIR) spectral analysis of milk samples is used routinely to predict fat and protein content of dairy cow milk and has been shown as an effective non-invasive predictor of several other economically important (and often hard to record) phenotypes due to the chemical imprint left by biological processes in the milk. Recently we developed a new method of predicting phenotypes from individual MIR profiles using deep learning, an artificial intelligence technique that uses artificial neural networks to simulate the way a brain works in order to process data and determine patterns for use in decision making. Bovine tuberculosis (bTB) is a chronic zoonotic disease of cattle which is of great economic, welfare and societal importance. In the UK the main screening test for bTB is the single intradermal comparative cervical tuberculin test (SICCT). The SICCT test is highly specific, however, a lower sensitivity means false negative cows remain in the herd. Our objective was to develop bTB prediction tool for dairy cows to be applied routinely and at a relatively low cost. We trained a convolutional neural network using MIR spectra-matched bTB records to predict the bTB status of individual animals. Cows were from national herds involved in routine milk recording with National Milk Records, distributed across the UK and predominantly Holstein-Friesian (81%). Bovine TB data were available from the UK national bTB testing program and included information from over 40,500 bTB breakdowns. Data from SICCT skin tests, culture, slaughter status, and lesions were used to create a binary bTB phenotype labelled 0 and 1 to represent bTB negative and bTB positive cows, respectively. Spectra were matched to bTB data on sample date. Accuracy of prediction was high (95%) with a sensitivity and specificity of 96 and 94%, respectively highlighting prediction of bTB status from milk MIR spectra and deep learning is promising and could be deployed as part of routine milk recording enabling the early identification of potentially affected cows.

Prediction of dry matter intake of dairy cows across multiple countries and production systems

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We predicted Dry Matter Intake of dairy cows using parity (PRT), weeks of lactation (WOL), milk yield (MY), milk mid-infrared (MIR) spectra, and MIR based predictions of bodyweight (BW) and fat, protein, lactose, and fatty acids contents in milk. The dataset comprised 10,711 samples of 534 dairy cows with a geographical diversity (Australia, Canada, Denmark, and Ireland), from first to third+ parity covering 44 weeks of lactation. When they were used individually, we found that the most contributing predictors were MIR spectra, bodyweight, and milk yield with cross-validation coefficient of determination (R^2_{CV}) >20%, followed by fatty acids and parity with R^2_{CV} around 18%, while the weeks of lactation and the combination of fat, protein, and lactose were associated with R^2_{CV} of 5%. Using Partial Least Square regression (PLS) with performances evaluated by cow-independent 10-fold cross-validation (CV) repeated ten times, we achieved the best Root Mean Square Error of cross-validation ($RMSE_{CV}$) of 3.24±0.08 kg with equations MY + MIR + PRT, and MY + MIR + BW. Because the Australian cows' milk production and composition were significantly different and the milk yield was the second most important predictor in the PLS model, we did a second calibration procedure by grouping the data by providers. The $RMSE_{CV}$ varied from 2.73±0.05 kg to 3.33±0.08 kg, which is in line with the literature. The performances with out-of-sample RMSE employing the data outside the calibration set ranged from 3.49 to 7.68 kg, confirming the interest in combining the datasets across countries.

HappyMoo – dairy cows lameness prediction through milk mid-infrared spectral analysis

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Lameness is one of the most severe health issues in dairy cows' farming systems. Thus, the project HappyMoo, aiming at predicting welfare related problems using milk mid-infrared (MIR) spectroscopy, is focusing on lameness prediction. Concretely, lameness was considered through two perspectives: direct lameness scoring (ICAR scale 1-5) and indirectly through the presence of lesions. Available data consisted in the lameness phenotype and trimming data, animal data and, associated milk analysis and spectra. These data are stored in a transnational database filled in by the project partners. Several machine learning approaches were tested for hoof trimming data (n=11,582) associated to close spectral data (±15 days), especially dealing with deep learning. Data were split in two groups: healthy (without lesions) and unhealthy (with at least one lesion) cows. The imbalance problem between these two categories was taken into account by applying Synthetic Minority Over-Sampling technique (SMOTE). First results were very promising (high accuracy, specificity and sensitivity). Additional tests will be performed by splitting data in 3 groups: healthy/unhealthy-metabolic lesions/unhealthy-infectious lesions. For lameness scoring, decision trees and random forest algorithms were tested on the data (n=5,871). Data were also split in two groups: healthy (score 1) and unhealthy (scores 2-5) cows and associated to close spectral data (±15 days) but the results are not very conclusive. Other models should be tested, as PLS-DA with other grouping of data (score 1 vs score 4 and 5 for example). In conclusion, first results indicate that lesions predictions seem to be promising (but has to be confirmed), but lameness scores prediction needs deeper analysis.

Multiple breeds and countries predictions of mineral contents from milk mid-infrared spectrometrie

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Measuring the mineral composition of milk is of major interest in the dairy sector. Minerals can be considered as biomarkers for udder health and used to manage the environmental impact of dairy herds relative to water eutrophication. The minerals in milk play an important role for human health and for the cheese-making process. The first objective of this study is to develop and validate robust multi-breed and multi-country models predicting the major mineral through milk mid-infrared spectrometry. The second objective is to apply the models on the milk recording large-scale spectral database. A total of 1,281 samples coming from five countries were analysed on spectrometers to obtain the mid-infrared spectra and in ICP-AES for mineral references. Models were performed using partial least square regressions. Two external validation related to Austrian samples were applied: the first without any Austrian samples included in the calibration model and the second with 30 Austrian samples included. The second external validation model provided RMSE of 54.56, 63.6, 7.30, 59.87 and 152.89 mg/kg for Na, Ca, Mg, P and K respectively, and associated RPD were 1.04, 1.94, 1.69, 2.00 and 0.87. Developed models were applied to the Walloon spectral database including 3,510,077 spectra of 235,355 cows recorded between 2012 and 2020. Large-scale predictions on the real database provide new insight regarding content of minerals in the population, as well as the effect of parity, days in milk, stage of lactation, breeds and seasons among others.

Developing quantitative traits related to animal health status using a holistic big data approach

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Among the dairy sector's current concerns, the early detection of animal health disorders is a complex challenge as it includes different diseases. This multidimensionality explains why disease detection is often studied separately and, due to financial and ethical issues, using small-scale datasets. Several studies were conducted in the past using the milk mid-infrared (MIR) spectra, for instance, to detect mastitis, lameness or to quantify the contents of citrate, β-hydroxybutyrate (BHB) or acetone in milk. To solve this issue and the small scale data size, we considered a holistic approach using traits obtained from milk recording to detect animal health disorders: milk yield, the somatic cell count and 27 MIR predictions related to the milk composition and animal health status. From 740,054 records collected from first parity Holstein cows in the Southern part of Belgium, we performed repeated unsupervised learnings. The obtained clustering divided the records into five groups. Significant differences of feature means were found between groups, suggesting that one group was related to mastitis and a second group to metabolic disorders. A validation from 87 milk and blood reference records obtained through the Interreg European project GplusE confirmed this interpretation. Moreover, after using a principal components analysis performed on the used features, it appeared that the first and fourth principal components (PC) were strongly related to the two discovered groups of sick animals. From reference values, the first PC had correlations of -0.68 with blood BHB, -0.70 with blood non-esterified fatty acids, 0.61 with blood Glucose and -0.46 with milk isocitrate. On the other hand, the fourth PC had correlations of 0.51 with milk N-acetyl-β-D-glucosaminidase and 0.55 with milk lactate dehydrogenase. Those results suggest that the obtained PCs reflect directly main health disorders and could be used to monitor dairy farms on large scale data.

Genetic parameters for milk urea in dual-purpose Belgian Blue cows

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Milk urea (MU) is a common tool used for evaluation of diet composition and feeding disorders and is related as well to reproductive performances, longevity, health and product quality (e.g. cheese making ability). In addition, the apparent relationships of MU with N excretion suggest that decreased MU will decrease environmental pollution with N. The Belgian Blue (BB) breed, originated in central and upper Belgium in the 19th century, is composed of two strains: beef Belgian Blue and dual-purpose Belgian Blue (DPBB). The DPBB cattle has been only moderately selected for milk production but can also be popular in organic and direct marketing farms in Belgium. Currently, efforts are undertaken to better describe MU, and other relevant traits, in this breed. The aim of this study was to estimate genetic parameters for MU concentration and its relationship with milk yield and compositions in DPBB cows. The data used were 16,935 test-day records of milk yield and compositions collected from 2014 to 2020 on 3,220 primiparous DPBB cows in 49 herds. A random regression test-day model was used to estimate genetic parameters. (Co)variance components were estimated with the Bayesian Gibbs sampling method using a single chain of 100,000 iterates. The first 20,000 iterates of each chain were regarded as a burn-in period. Mean (SD) of MU was 22.9 (10.1) mg/dl. Heritability estimates for daily MU was 0.08 (SD<0.01) and ranged from 0.07 to 0.11. The mean (range) of genetic correlations between MU and milk, fat and protein yields, fat and protein percentages were, respectively, -0.10 (-0.26 to 0.01), -017, (-0.33 to -0.10), -0.16 (-0.31 to -0.07), -0.07 (-0.16 to 0.07), and -0.02 (-0.26 to 0.00). These results indicate that selection on MU is possible with no effect on milk yield or compositions. However, relationships between MU and other important traits such as longevity, metabolic diseases, and fertility are needed. The current study is also a first step to do comparative GWAS analysis, potentially highlighting genomic specificity of this special local breed.

Predictive ability of MIR Spectra for detecting non-coagulating milk of Sarda ewes

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Milk coagulation ability is crucial in the dairy industry. Non-coagulating (NC) samples are defined as milk not forming a curd within the testing time (RCT) of 30 min. In Sheep milk, up to 10% NC samples has been reported both in individual and bulk milk. Although the clotting properties of individual milk have been widely studied, little attention has been given to NC milk and these samples are often removed from the analysis. MIR spectra can be exploited both to predict cheese-making aptitude and to discriminate between coagulating and NC samples. Milk sample from 1,018 Sarda ewes from 47 flocks located in Sardinia (Italy) were analysed and served as training dataset (TD). Validation dataset (VD) were of 662 ewes sampled from the same flocks but one year later (1-5 controls) for 2,656 records in total. Three classical MCP were measured: rennet coagulation time (RCT), curd firmness (a_{30}) and curd firming time (k_{20}). MIR spectra were recorded in the region between 925.92 and 5,011.54 cm^{-1}. In order to predict the coagulation status (binary trait: 0=NC, 1=Coagulating): (1) principal component of MIR spectra and logistic regression (PC-LR) on coagulation status; and (2) linear discriminant analysis (DA) were applied. The effect of different MIR regions combinations/exclusion on predictive ability of NC sample was also assessed. About 9.5 and 5.5% did not coagulate at 30 min for TD and VD, respectively. The use of logistic regression on PC extracted from MIR spectra gave the poorer results (correct assignment of NC samples <57% for TD). PC-LR selecting and combining different regions of MIR spectra did not improve the % of correct assignment. DA combined to step-wise variables selection raised up to 87% the correct assignment in TD. As far as external validation concern DA was carried out on VD for different number of test day available (23.1-100% of correct assignment, >90% if at least 3 test day were available per animal).

Session 70 Theatre 1

Environmental impacts of pork production: what can we learn from life cycle assessment studies?

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The key drivers for the environmental impacts of pork production are the production efficiency (mainly the feed conversion ratio), the composition of the feed ration, the production system for the feedstuffs, the animal husbandry system, and the direct emissions from the animals. Certain trade-offs are observed between animal-friendly husbandry systems and conventional systems, but these trade-offs are less marked than for cattle or poultry and mainly limited to higher ammonia emissions from animal-friendly label housing systems. Agricultural production dominates the impacts of the pork value chain; transports, slaughtering, processing, packaging and retail have comparatively lower impacts. In comparison with meat from other animal species, pork has lower climate change, eutrophication and acidification impacts than ruminant meat, but higher than poultry meat. Water scar-city in turn tends to be higher for pigs than for ruminants, when irrigation is used in feedstuff production. The land use is also lower than for ruminants, but we have to bear in mind that feedstuffs for pigs are almost exclusively produced on arable land, while ruminants can forage on grassland. Due to the digestion systems of pigs, which is very similar to humans, the nutrition of pigs is competing with human nutrition. This feed-food competition can be mitigated by feeding mainly by-products of the food value chain, which have lower than food grade quality. The most promising options to reduce the environmental impacts of pork production are: improve feed conversion ratios by breeding and management, include environmental criteria in the optimisation models for feed rations, increase the use of animal by-products for human consumption ('nose-to-tail' strategies), and develop animal-friendly and low-emission husbandry systems.

Session 70 Theatre 2

Sustainable pig production in Switzerland thanks to knowledge building and transfer

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The production of healthy, productive pigs is the core objective of the Pig Health Service of Switzerland (SGD). Due to close cooperation with various research institutes, the SGD is technically up to date. The regular transfer of knowledge between the SGD and the farmers takes place in the form of advisory meetings and courses. During farm visits, feeding, hygiene and management, animal health, use of veterinary medicines and animal traffic are discussed and recorded in a database. This is an important tool for targeted advice and for the early detection of trends in the spread of diseases. The Swiss pig health program covers about 90% of all sows and about 60% of all fatteners in Switzerland. Health data digitally recorded and evaluated The SuisSano health program aims at a transparent and targeted use of medicines. As of March 2021, just under 3,000 farms had joined the program. Animal treatments and departures are recorded in the Electronic Treatment Journal. For each farm, an index for antibiotic treatments is calculated and compared with the average of all participating farms. In 2020, the antibiotic use of over 1,800 participating farms totalled almost 1 million defined course doses (DCD). Evaluations show that 8% of fattening farms are responsible for over 57% of total antibiotic usage. In lactating sows, 54% of treatments are for PPDS (postpartum dysgalactia syndrome) and 27% for lameness. Only a small proportion of the agents used (2-6%) are HPCIA's (highest priority critically important antimicrobials). This percentage has been significantly reduced in recent years. Identified farms with high antibiotic usage are advised on management measures and use of appropriate active substances. Biosecurity – discovering deficiencies In connection with the spreading of African swine fever (ASF), SUISAG launched 2020 the Swiss ASF risk assessment tool. SGD farms can assess their farm and receive a to do list with suggestions for improvement, which are in turn discussed at advisory meetings. The data from the individual farms are also used for evaluations of the biosecurity level throughout Switzerland. This allows general recommendations to be made.

Concepts, production and marketing of pork animal welfare programs from a slaughterhouse perspective

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Animal welfare concepts and the societal demand for these concepts were discussed in the scientific community for several years in Germany. Results of the research showed that the consumer should be ready for new products that entail higher degree of animal welfare standards. The general public – following news, media reports und political discussions – seemed to be quite ready indeed. While discussions on the topic became more numerous by the week, the actual demand for these products in supermarkets and butcheries did not grow in proportion. Even nowadays, only a very small part of the German pork industry actually is animal welfare. At Brand Qualitätsfleisch, the first animal welfare programs started as early as 2014. Over the years, the experience of the company in dealing with these programs increased dramatically and has made Brand Qualitätsfleisch a specialist in conceptualizing, marketing and growing animal welfare programs in pig production. Despite the before mentioned, lagging demand for animal welfare products, the company and its partners were able to successfully increase these concepts in terms of size and depth over the last years. By extending the scope of the company from slaughter-only to a wider range of responsibilities such as marketing, communication, conceptualizing, coordination of stakeholder, farm optimization and welfare criteria definition, the company was able to grow and develop new animal welfare programs of all sizes. This presentation gives an overview on the activities of the company in the day-today operations of animal welfare production and marketing. In addition, the presentation shares the company's outlook on the future of this market in Germany.

Session 70 Theatre 4

Transformation towards sustainable pig production systems: how to make a game changer from approach

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The continued growth of the livestock sector to meet rising demand for animal products stresses the urgent need of a just transition towards sustainable livestock systems, including environmentally friendly production processes, economic fair and balanced remuneration for all actors and social demands, such as social equity and healthy food. More specific, modern pig production faces challenges such as pollution of air and water, loss of biodiversity, use of energy, animal welfare and use of antibiotics. It is now widely accepted that we need to redirect the future path and built additional capacities to address these challenges. First, we need to understand the current pig production system. Second, we need to have more knowledge on what a resilient and sustainable pig production system could entail. Then, we can discuss possible transformative pathways, which are strategies and solutions that can bring the production system from its current state to a desired resilient and sustainable state. The variety of actors involved have many different, often contradictory, perspectives and changing visions. Because of this incomplete knowledge, the complex interdependencies and different visions, we can call this a wicked problem. So, although technical solutions are and will stay very important, they are not sufficient anymore to deal with the challenges ahead. We need new tools and methodologies to find and integrate innovations and we need more capacity to understand and support interaction between people. Therefore, in this presentation, we discuss what is necessary to reach systemic innovation, what it can deliver but also explicit some bottlenecks.

Session 71 Theatre 1

Grass silage for dairy cows: impact of harvest stage on yield, value, production and income

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In France increasing protein self-sufficiency is the major challenge for dairy farms. Producing more home grown proteins can decrease the feeding cost, secure the production system, improve products traceability and reduce the potential negative impacts on environment. Ryegrass and red clover mixtures can meet these expectations. During four years, an experiment was led in Western France on two bovine dairy systems, one conventional and one organic. The aim was to produce grass silage with a higher content in proteins by reducing cutting intervals and harvesting mixtures of hybrid ryegrass and red clover at an earlier stage (Early: 5 weeks of regrowth versus 7 for the Control). In the conventional system, the early silage led to 6 to 7 cuts per year with a reduced yield per hectare two years out of four, compared to the control system (5 cuts per year). However, the early harvested forage offered higher energy and protein yields by hectare (respectively +5% and +18%) and its nutritive value in terms of energy and protein contents was always better than in the control silage. However, an increase in working time by 2 ½ hours per hectare was necessary to realise these early cuts. The cost of the two first early cuts reached 55 €/t DM compared to 35 €/t DM for the first control late cut. During winter, these silages were delivered to separate groups of cows. In the conventional system, grass silage represented 40% of the forage diet. In the Early group the total intake increased by 1.7 kg DM/d/cow and cows produced significantly more milk (+1.9 kg/d) with no impact on milk quality. In the organic system, grass silage represented 70% of the forage diet. The intake increased by 4.1 kg DM/d/cow and cows produced significantly more milk (+2.4 kg/d for primiparous and 4.8 for multiparous cows). The margin over feeding cost for a herd of 50 cows was increased by 675 € per month (resp. 2. 061 €) in the conventional (resp. organic) system. The high increase in intake remains a challenge for the latter. In both systems producing early cut grass silage of ryegrass and clover mixtures is an efficient way to improve feeding self-sufficiency and milk production.

Session 71 Theatre 2

Environmental cost analysis in organic Dehesa farms (SW Spain)

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Agriculture, food production and processing systems are currently being questioned since they have been considered to be unsustainable on account of their excessive resource use, their fossil fuel use and their heavy environmental impacts on water, soil, air and biodiversity. The fight against climate change has certainly become a major challenge in our society, as environmental impacts are associated with the emission of greenhouse gases (GHG) However, not all the animal production systems generate GHG emissions with the same intensity on the environment and society. In this sense, livestock production based on grazing animals are as a more sustainable model that involves improved environmental practices and provides society with various ecosystem services, including carbon sequestration. In this scenario, the establishment a price to CO_2 emitted could be a valid tool for reducing these emissions. The main purpose of this paper is to estimate the maximum price per tonne of CO_2 equivalent (eq) that could be borne by the various models of extensive organic livestock farms. The study shows the economic-environmental balance of a case analysis for three hypothetical scenarios considering farm emissions and CO_2 sequestration levels. The economic indicators were developed on the basis of the system of integrated environmental and economic accounting, applied to the economic accounting for agriculture and forestry and the EC Regulation no. 549/2013. On the other hand, the life cycle analysis has been carried out following the UNE-EN-ISO 2006 standards and for the calculation of the carbon footprint the IPCC guidelines. The results show that the maximum price that farms can bear is within a range of € 0.20 to € 792/tn of CO_2 eq depending on the scenario analysed and the production model. In some cases, there is an economic income (net) due to the weight of carbon sequestration in their emissions balance. On the other hand, the time horizon to be considered for the calculation of sequestration is a key aspect of debate, together with the need to establish a clear framework for the allocation of the price of CO_2 eq and to create the necessary tools to favour the development of a standardized compensation market.

Farmer and consumer attitudes towards utilizing composting bedding materials from cattle barns

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The lying and walking area in FW cattle housing systems consists of organic bedding material, which is cultivated and aerated to stimulate the composting process. The compost area can also be utilized for food production during the grazing period of the herd. However, it is important to understand how farmers and consumers perceive this multi-functional use of the barn in order to gauge its market potential. Therefore, the multi-functional use of the FW system was demonstrated by utilizing the composting bedding for fattening pigs during grazing time. In a survey across six EU countries, 80 farmers who are familiar with FW and cubicle housing were questioned about some technical and marketing aspects of the FW housing with composting material vs cubicle housing with slurry, using a 7-point scale. Results from consumer focus groups conducted in Austria, Germany, and Slovenia and a corresponding quantitative survey in eight EU countries (total of 3,693 respondents) were used to assess consumer perception of re-using the compost material from the FW system for food production. Farmers found the composted bedding material to be a significantly better soil improver than slurry; however, the cost of bedding material was seen as the biggest disadvantage of this system. The FW system was also seen as having a higher market potential, delivering high quality manure and dairy products. Most consumer focus group participants had a positive view of re-using the compost for other products, but some concerns were raised regarding using the compost. The quantitative survey results indicate consumers mostly feel safe consuming food products grown with the compost despite participants stating to be somewhat worried about the safety of the food they eat in general. However, more than half of consumers think the compost can be used for non-consumable products (e.g. soil, energy) compared to around 25% thinking the compost is suitable for growing produce that is normally un-cooked and for raising of animals. Overall, farmers and consumers view the multi-functional use of compost from the FW system to be promising.

Styrian agriculture – the past as a window to the future

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Within the framework of a thesis, the development of agriculture in Styria over the past 20 years is examined and development trends for the coming 20 years are highlighted on the basis of current and future framework conditions and indicators. Important parameters are the diverse landscape types and different climatic conditions aa well as the different agricultural production branches, which can vary between favourable and disadvantaged areas. Findings from this work for land managers can be informative and interesting for other alpine regions with similar economic and cultural backgrounds. The thesis covers Styrian agriculture in general and a focus on two reference farms for which a sustainable farm concept is developed. The first farm is located in Upper Styria, which is a conventional dairy farm with about 25 dairy cows in tied-in housing system. In contrast to this is the farm of the Reisinger family in eastern Styria, which has been focusing on diversification for some time. First run as a dairy farm, it was converted to sheep milk production and processing in the nineties, and in the last 10 years has changed its strategy to farm vacations and the sale of hay for pets. For this project, the first step is to make an inventory in 2000, which is done through archival material and on-farm records, as well as interviews. Then the actual state is considered, using the same sources as in the previous point. In order to be able to make a forecast for the further development of the farm, an online survey of prospective and active farmers and interviews with agricultural officials, decision-makes and experts are carried out. At the same time, the development trends on biodiversity and future challenges of agriculture, especially in countries with a high diversity in landscape structure as Austria, are included in the results of the work.

Session 71 Theatre 5

The whole and the parts – effects of impaired animal health on farms' economic viability

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In a systemic understanding, a farm is the superordinate system for individual cows that defines their living conditions from which certain traits emerge. A prominent production trait across dairy farms is milk yield; another is animal health, partly reflected by milk contents and the somatic cell count, available from test day records. An important cost item related to diseases is the cows' death (on farm or slaughter). Cows which finally failed to cope with their living conditions indicate a poor state of animal health and welfare. From an economic perspective the cows' death defines the end of refunding costs for rearing and keeping by sales of milk and meat. Individual cows support the viability of a farm when revenues exceed the costs for raising the heifer, the daily full costs for feed and keep and the proportional share of the fixed costs of the farm. These cows are 'profit cows'. Milk recordings and economic data of 32 dairy farms in Germany were used to aggregate farms according to their emerging output (yield and production diseases) by means of a principal component and a cluster analysis. The proportion of profit cows and other characteristics of the herd within clusters were assessed. The analysis based on the factors milk yield, metabolism and udder health resulted in five clusters of farms: (1) high performer with good health status (n=6); (2) low performer with impaired udder health (n=4); (3) high performer with impaired health status (n=8); (4) average performer with good udder health (n=9); (5) poor performer with metabolic problems (n=5). The proportion of profit cows was highest in clusters 1 and 2 (57.5 and 55.6%) and lowest in clusters 4 and 5 (29.4 and 19.5%). It seems incompatible to have a big proportion of profit cows in farms with a high proportion of cows with metabolic problems, a low milk price and high culling and mortality rates. Changing the perception of production diseases from collateral damage to a main cause of losses might foster farm specific iterative processes which aim for less production diseases.

Session 71 Theatre 6

Assessing nitrogen use efficiency through blood and milk 15N enrichment in grazing dairy cows

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Genetic selection for feed efficiency is constrained by the cost and difficulty of measuring individual feed intake. Animal proteins are naturally ^{15}N enriched ($\delta^{15}N$ isotopic fractionation) relative to the diet and the extent of this difference ($\Delta^{15}N_{animal-diet}$) has been correlated to nitrogen use efficiency (NUE) in stall-fed dairy cows. The study investigated whether $\delta^{15}N$ and $\Delta^{15}N_{animal-diet}$ of blood and milk can be used as a predictor of NUE (milk N yield/N intake) and residual nitrogen intake (RNI: actual - expected nitrogen intake). Furthermore, it was intended to identify between and within breeds variation in grazing dairy cows. Two groups of 14 Holstein (HO) and 14 Swiss Fleckvieh (FV) dairy cows were investigated, twice in mid, and once in late-lactation over a 2 year period. During each 7-day measuring periods, a blood and a pooled (3 days) milk sample of each cow were obtained and analysed for ^{15}N, using a gas chromatograph-combustion-isotope ratio mass spectrometer. The individual herbage intake of each cow was estimated using n-alkane marker technique. The data was statistically analysed with mixed linear regression with breed and lactation period as fixed factors and cow as random effect. The ^{15}N obtained from different N pools in dairy cows were correlated (R^2=0.16-0.61) with feed efficiency. The NUE and RNI were best explained by $\delta^{15}N$ of milk (R^2=0.59) and $\Delta^{15}N$ of milk (R^2=0.61), respectively. NUE was significantly different (P=0.047) between breeds with HO 0.27 (SD=0.06) and FV 0.29 (SD=0.07). As well as for RNI significant difference (P=0.007) between HO 152 g N/d (SD=62.5) and FV 120 g N/d (SD=26.0) was observed. The blood's $\delta^{15}N$ 4.7 ‰ (SD=0.2, P=0.73) and $\Delta^{15}N$ 3.7 ‰ (SD=1.1, P=0.86), as well as milk's $\delta^{15}N$ 4.7 ‰ (SD=0.3, vs=0.46) and $\Delta^{15}N$ 3.7 ‰ (SD=0.9, vs=0.66) were not significantly different between breeds. Overall, there is potential to use ^{15}N as a low-cost biomarker in milk and blood for nitrogen use efficiency in grazing dairy cows.

Relationship between proxies of nitrogen use efficiency for dairy cows in early lactation

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Abstract: Nitrogen use efficiency (NUE) of dairy cows is an important trait that farmers as an indicator of sustainability in dairy production. However, many traits definition may represent NUE of dairy cows involving multiple N-related traits. The purposes of our research were: (1) to explore the curves of N-related traits in the first 50 days in milk (DIM); (2) to analyse the relationship between predicted NUE (PNUE), predicted N losses (PNL), predicted N intake (PNintake), and milk urea (MU). PNUE, PNL, and PNintake were predicted based on milk mid-infrared spectra, parity, and milk yield. Each N-related trait was divided between primiparous and multiparous. After editing, the data included 143,595 records within 5 to 50 DIM from 53,660 cows on 766 farms. The genetic parameters were estimated using a multiple-trait repeatability model. In the first 50 DIM, the multiparous PNUE, PNL, PNintake, and PNout were all greater than the first class, but MU was reversed. In addition, the PNL and PNintake increased as DIM increased, but PNUE decreased gradually. MU first decreased and then increased (around DIM 17 was the minimum). The range of estimated heritabilities for PNUE, PNL, MU, and PNintake in the first and second class were from 0.09 to 0.19. Among the different traits, the novel N traits (PNUE, PNL, and PNintake) had a low genetic correlation with MU (from -0.14 to 0.03), but the genetic correlation between PNL and PNintake was high (from 0.76 to 0.94). Therefore, we believe that the novel N traits are different from MU, and PNL is affected by PNintake.

Influence of inbreeding on milk production and fertility traits in Swiss Holstein cows

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In the present study, the relationships between the inbreeding coefficient and milk yield, milk fat milk protein, first service conception rate (FSC) and interval from first insemination to conception (FIC) were studied in Swiss Holstein cows. For the study, the dairy cattle breeding association Holstein Switzerland provided milk production and pedigree data for a total of 36,548 Swiss Holstein dairy cows with a total of 40,686 standard lactations (years 2000-2018). The mean pedigree completeness of the first 5 generations was between 90 and 100%. The mean inbreeding coefficient was 4.9%. Mixed linear models (milk production traits and FIC) and a logical model with binomial distribution (FSC) were used for the investigations. The models included the fixed effects month of calving (only for milk, protein and fat), age of the cow, milk yield (only for fat, protein, FSC and FIC), inbreeding coefficient, number of lactations (only for FIC), year of calving, gestation day, as well as farm and animal as random effects and residual effect. The regression coefficients and the standard errors of the milk yield, milk fat and milk protein were -12.22±3.6 kg, -0.46±0.1 kg and -0.31±0.06 kg per percent inbreeding increase, respectively. Inbreeding showed no significant impact on delay time. The number of lactations and the milk yield influenced the FIC. The FIC in the first lactation was around 80 days and decreased continuously with increasing lactation down to 30 days by the sixth lactation. The FIC also increased by higher milk yield. In cows with a milk yield of up to 6,000 kg, the FIC was on average 54 days while in animals with a milk yield of more than 11,000 kg it was on average 83 days. No inbreeding depression was identified in FSC. However, there was a string influence of milk yield on FSC. In cows with milk yields of up to 6,000 kg, the likelihood of FSC was 62%. In animals with a milk yield of more than 11,000 kg, only 37% had a successful conception from their first insemination. The effects of inbreeding reported in this study were small and can be compensated by additive genetic breeding progress.

Elevated vaginal and reticular temperature, and heart rate indicate early heat stress in dairy cows

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Even in moderate climate zones, as Switzerland, increasing frequencies of heat periods or extreme weather events are recorded nowadays. Particularly dairy cows on pasture are directly exposed to climate conditions, what emphasizes the importance of a reliable to detection of signs of heat stress signs reliably. The objective of the present study was therefore to investigate the suitability of physiological parameters as early indicators of heat stress in grazing dairy cows in Switzerland. Therefore, the vaginal (VT) and the reticular (RT) temperatures of 24 lactating dairy cows were recorded during two summer periods (2018 and 2019). Additionally, the heart rate (HR) for each cow was measured between 9 am and 2.30 pm. On pasture the ambient climatic conditions were recorded continuously to calculate the temperature-humidity-index (THI) as well as the comprehensive-climate-index (CCI). For the analysis, 12 periods of up to 6 consecutive days with increasing THI and CCI were selected to display conditions of increasing heat stress. The mean THI amounted 63±5.7 and ranged from 44.8 to a maximum of 77.8. In total, VT was on average 38.5±0.3 °C and RT 39.0±0.9 °C. The measured HR averaged 74.6±13.7 bpm and ranged from 50 to 180 bpm. Both, the body temperatures as well as the HR were affected by climatic conditions and increased with rising ambient THI ($P<0.001$). The RT showed higher variations than VT, which might be caused by increasing water intake during heat load. In conclusion, this study showed that dairy cows even in moderate climate zones experience heat load and the measurement of physiological parameters could indicate early signs of heat stress.

Positive health-related effects of offering veal calves warm water from 1 to 3 months of age

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Newly shipped 2-6 weeks old calves born in dairy herds encounter numerus environmental changes upon arrival in the fattening unit. Often the supply of milk is lower than in the dairy herd, which forces calves to increase intake of dry feed, which requires easy access to fresh water. The water temperature in drinking cups is often cold (6-8 °C) but uptake of water can be increased if tempered (16-18 °C) or warm (36-38 °C). So, the water supply installed in the pens might not be enough at this stage and age. The objective was to test if an extra source of warm water had positive effects on health and/or performance of rosé veal calves. The study included 107 Holstein male calves, divided into 2 batches. For each batch, calves were divided into 12 pens with 4-6 calves per pen, and 6 pens were assigned to control (CON) and 6 pens to extra water (EXW). All pens were equipped with drinking cups (intake not recorded), but EXW was also offered 2×3 l water at 38 °C in an open surface trough per calf per day from arrival until 93 d of age. In the statistical analysis, fixed effects of water, batch and the interaction were included, and pen was considered random and was the experimental unit ($n=24$). The water offered was usually drunk in all pens. Calves were (MEAN±SE) 29±1.5 d with a BW of 58.3±0.9 kg at arrival. ADG from arrival until 40±1.6 d was not different between CON and EXW calves (816±34 g/d) but tended to decrease in batch 1 and increase in batch 2 ($P<0.07$). ADG from 40 to 93 d (1,058±19 g/d) was not affected. However, number of treatments for diseases per calf was lower ($P<0.01$) for EXW (0.2) than for CON (0.8) and clinical scores were numerically better for EXW (0.4 vs 1.0, $P=0.30$). Three calves died in the CON group compared with 0 calves in the EXW group ($P=0.29$). All calves ($n=104$) were treated, fed and housed similarly from 3 to 10 months where calves were slaughtered. Age (297±1 d), carcass weight (213±2 kg), carcass gain (648±7 g/d) were similar, but EUROP carcass conformation (3.6 vs 3.4), proportion of carcasses receiving premium (93 vs 80%) and lung remarks (2 vs 8%) were numerically improved in EXW calves (all $P=0.20$). The findings suggest improved health of calves when supplied extra warm water from 1 to 3 months of age.

Session 71 Theatre 11

Efficiency of an essential oil-based mixture to cure clinical mastitis in dairy cows

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As a promising non-antibiotic treatment for mild and moderate clinical mastitis in lactating dairy cows, an essential oil mixture (EO) was evaluated. Treatments performed on 132 cows experiencing clinical mastitis were recorded in 41 dairy herds in Bretagne (B) and Auvergne Rhône Alpes (AURA). Cows were randomly assigned to essential oil (EO, n=71) or antibiotic (AT, n=61) group. AT cows were treated according to the farm health protocol. The EO mixture was composed of *Litsea citrata* EO, *Leptospermum scoparium* EO, *Corydothymus capitatus* EO, *Cymbopogon martinii* var *motia* diluted with grape seed oil. It was applied topically on affected quarter of EO cows during 14 consecutive milkings. Milk samples were collected from affected quarters for microbiological analysis at the onset of clinical mastitis and 28 days after. Clinical signs were registered at 2, 5 and 28 days after occurrence; individual somatic cell count (SCC) was registered each month. Statistical analyses were performed using Rstudio and generalized linear model accounted for fixed effect. 25.3% of EO cows received an additional intramammary antibiotic treatment because of perceived lack of response to the initial treatment 2 or 5 days after occurrence (vs 8.2% in AT group). Clinical cure rate was lower in the EO group than in AT group (87.7 vs 68.8%, $P<0.05$). Cell Cure Rate (CCR) was defined by 2 consecutive individual SCC inferior to 300,000 cell/ml after occurrence. CCR tended to be different in the two groups regarding the last individual SCC before occurrence ($P<10\%$). CCR tended to be higher in EO group than in AT group in B ($P<10\%$). No effect of the treatment was observed on CCR in AURA. No effect of the treatment was observed on bacteriological cure rate (75% in EO group vs 83% in AT group; ns). We showed that using hybrid therapy with EO as first-line treatment and antibiotic as second-line treatment seemed to be a promising way to deal with clinical mastitis in order to reduce the use of antibiotics in dairy herds.

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Economic value of milkability in Czech Holstein

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Breeding for improved milkability is getting still more emphases especially due to enlarged data availability e.g. from automatic milking system. Though the positive correlation with cow longevity, udder morphology and overall cow mobility, this trait represents a candidate for desirable selection response in such traits. Recently, milkability and some udder traits were employed into the separate robotic index of the Czech Holstein. Selection index of this population is based on the complex of 17 production, functional and exterior traits. To construct a comprehensive selection index and to reach the appropriate selection gain in cow milking ability beside the current traits defined as breeding objectives, economic value (EV) of the trait should also be known. Milkability is usually expressed as milking speed, i.e. as the average amount of milk in kg harvested during cow milking per minute. In economic terms, higher milking ability causes a shorter milking time that saves the costs for milking labour, electrical power, and wear on the milking equipment. Therefore, the bio-economic model EWDC of the software ECOWEIGHT was enriched to provide calculation of EV for this trait. Current production and economic parameters of the local Holstein population were considered in the program as well. For milking speed, an EV of 96.0 €/(kg of milk/min) and per cow and year was calculated. It corresponds to the costs saved by milking a cow with milking speed higher by 1 kg/min than milking a cow with a herd average milking speed. Improving this trait, the yearly milking time of a cow can be reduced by 18 hours on average. About 75% of the EV was coming from saving labour costs and the rest mostly from reduced electricity expenses. Taking into account the overall economic importance of all the 12 breeding goal traits, the economic importance of milkability represented about 6%. Therefore this new trait would noticeably participate in the enhanced selection index of the breed. The study was supported by project MZE-RO0718-V003 and QK1810253 of the Czech Republic.

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Ecobreed – the economic value of a cow as selection criterion

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At the level of a single farm, the decision to keep or cull a cow is key to the economic success. Every culling decision is complex because the costs of rearing a heifer, the profit from selling the cow and the different performance of a producing cow compared to a replacement heifer must be taken into account. If the replacement of a cow by a heifer results in a net loss, the culling decision incurs costs. An increased longevity enables to distribute the fixed rearing costs of a replacement heifer over a longer productive lifetime. In addition, the milk yield is generally higher in older than first-lactating cows. However, if the replacement rate is too low, non-profitable cows might stay in the herd for too long while more profitable and genetically superior heifers cannot enter the herd. Different approaches have been proposed to determine the optimal point in time to replace an old cow by a young heifer. One of these approaches is based on the replacement theory. Here, the expected profit of a cow is calculated using its net present value and compared to the net present value of a replacement heifer. However, the net present value is has limitations regarding the modelling of potential genetic improvements as well as in depicting the random effects in a herd structure. Markov chain-based models are able to accommodate a higher level of complexity including different developments of traits such as age, milk yield, fertility and potential changes in interactions between the traits. In the first step of a Markov chain-based analysis, all possible states of a cow must be defined. The transition probabilities between the different states of a cow are defined in a second step. In the last step, an optimum solution is derived from the stochastic simulation of a herd where cows are expected to traverse as many states of the model as possible. The goal of this project is to develop a Markov chain-based model for the Swiss dairy farming industry with the additional aim to design and implement a decision support tool for dairy farmers.

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NIRS potential for the prediction of feed efficiency in organic grazing dairy cows

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Optimization of feed efficiency in dairy cows may help limiting nitrogen and greenhouse emissions associated with milk production while improving the use of resources and reducing production costs. Monitoring of individual feed intake for efficiency estimation being highly time-consuming and costly, proxy tools for feed efficiency would be an asset. This paper, as part of a bigger project on the identification of biomarkers involving organic grazing cows, explores the potential of NIRS for the prediction of feed efficiency. 54 lactating cows (38 Holstein, 16 Swiss Fleckvieh), primiparous and multiparous, in early and late lactation were included in this paper. The overall dataset included feed milk and faeces amount and composition, blood metabolites, feed intake (n-alkane method), ruminating, grazing and physical activity, thermal imaging and methane emissions. The cows were milked twice a day (morning and evening). Daily milk yield was recorded and the milk composition was determined in a pooled morning-evening sample on days 1, 4 and 7 of each measurement period. Milk samples were stored at 4 °C in vials with Broad Spectrum Microtabs II. In addition to cell count, enzymatic urea and $\delta^{15}N$ were determined. Fatty acids profile, nitrogen content and NIRS were determined in week-pooled milk samples after freeze drying. Spectra (diffuse reflectance) were taken from a total of 102 freeze-dried milk samples with a NIRFlex N-500 equipped with a rotary cup. Per sample, 3 replicates were taken with 21 scans per replicate, in the range of 4,000 to 10,000 cm^{-1}. NIRS models were developed with PLS algorithms including data pretreatment: SNV (normalization standard normal variate), mf (normalization MSC Full), db1g2 (1st derivative BCAP Gap 2) and dg1g2 (1st derivative Savitzky-Golay 9 points Gap 2). The NIRS models (independent calibration and validation sets) presented moderate to good predictive potential (R^2: 0.56 to 0.99) for N content, urea, fatty acids and feed efficiency parameters in milk samples.

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Better lactation start in dairy cattle with a protected standardised grape extract supplementation

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Parturition is well known for being one of the most crucial periods in dairy cattle due to the important metabolic shift happening and the high energy demand, increasing sanitary risks. The present study aimed at evaluating the effect of a supplementation of dairy cattle in the days prior to calving and monitor its effects on the beginning of milk production. 42 cows were paired according to their production history and parity and divided in two groups (n=21/group, average parity = 2.4): a control group (CTL) and a supplemented group (BP-O) receiving 3 g of a commercial protected standardised grape extract per day for 15 days prior to calving (Nor-Grape® BP-O, Nor-Feed) on top of the CTL diet. Individual daily dry matter intake and daily milk production were recorded for 8 weeks from calving. Daily production average per week (DPA) and average daily dry matter intake per week (ADDI) were then computed to compare groups. Statistical analysis was performed using ANOVA or Wilcoxon when data did not follow normality. ADDI was significantly influenced by the dietary treatment (P<0,001) and tended to be higher in the supplemented group on W1 (+2.0 kg/cow/day), W6 (+2.1 kg/A/D) and W8 (+2.6 kg/A/D) post-calving (P<0,10) and was significantly higher on W2 post-calving (+3.5 kg/A/D, P<0,05). PDA was also influenced by the supplementation (P=0.07) and tended to be higher in the BP-O group (+3.0 L/A/D on W2, +2.8 L/A/D on W8). Whilst the supplementation level represented only a minimal part of the diet (3 g/A/D), it significantly influenced the dry matter intake and milk production although the encapsulation of the product means it is highly unlikely to affect palatability. Further research is required on the use of this small dose of rumen-protected standardised grape extract on liver protection, since it could represent a potential explanation so as to why the dry matter intake was positively influenced in this transition period of high metabolic demand for the cows.

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Consequences of hyperketolactia in early lactation dairy cows

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FTIR allows for the determination of milk acetone (mACE) and β-hydroxybutyrate (mBHB), providing a monitoring tool for hyperketolactia, defined as elevated ketone bodies in milk. In this study we characterized the consequences of hyperketolactia during early lactation in Polish Holstein-Friesian cows. A total of ≈7.15 M milk samples were collected within 6-60 DIM, over a 6-year period (from 2013 to 2019), in the milk recording system. Published mACE (≥0.15 mmol/l) and mBHB (≥0.10 mmol/l) threshold concentrations considered to reflect a hyperketolactic state were used to classify milk samples into ketolactia subpopulations: Normal (mACE<0.15 and mBHB<0.10) and hyperketolactic-either (HYKL, mACE≥0.15 or mBHB≥0.10). Additionally, HYKL samples were further categorized as hyperketolactic-both (HYKLACE&BHB, mACE≥0.15 and mBHB≥0.10), hyperketolactic-BHB (HYKLBHB, mACE<0.15 and mBHB≥0.10), and hyperketolactic-ACE (HYKLACE, mACE≥0.15 and mBHB<0.10). Results for the same cow but for different test-days were considered as independent values. The first and second test-day milk yield (1TDMY and 2TDMY, respectively), as well as 305 d lactation milk yield (305MY) were studied in relation to hyperketolactia found on either the first (1TD) or second (2TD) test-day. HYKL decreased 1TDMY, 2TDMY, especially on the TD it was found. It also decreased 305MY, but this effect was more apparent when HYKL was found on 2TD or both TD (Normal 7,928 kg, HYKL on 1TD 7,697 kg, HYKL on 2TD 7,363 kg, HYKL on both 7,215 kg). Among ketolactia subpopulations, the lowest 1TDMY and 2TDMY were found in HYKLACE&BHB, as well as in HYKLACE found on 1TD. The 305MY of HYKLACE cows, found on both TD, was about 250 kg less than in Normal. In conclusion, HYKL impairs milk yield, depending on the day of early lactation as well as on ketolactia subpopulation.

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KetoMIR as a new indicator trait for subclinical ketosis and its use in herd management

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Milk analysis using mid-infrared spectroscopy (MIR) is a fast and inexpensive way of examining milk samples on a large scale for fat, protein, lactose, urea, ketone bodies and fatty acids. As the milk composition is related to the health status and metabolism of a cow, it is possible to use MIR spectra to detect diseases. A new indicator trait for subclinical ketosis, KetoMIR, was developed by LKV Baden-Württemberg. The new concept of KetoMIR is to offer a ketosis risk index via milk samples that is more accurate than the fat-protein ratio. Based on veterinary ketosis diagnoses and the milk components, KetoMIR, a three-class ketosis index, was developed: 1 = low ketosis risk, 2 = medium ketosis risk and 3 = high ketosis risk. The aim of the current study was to analyse the phenotypic relationships between the KetoMIR index and milk yield, fertility and health at the farm level. The average frequency of cows with a medium to high ketosis risk at the farm level was 13.9%. Increased ketosis risk based on the KetoMIR index had a significant negative impact on average herd milk yield (-1,975 kg milk). Calving to first service interval was prolonged (+ 36 days) as well as calving interval (+ 58 days). Mean herd somatic cell count in first and higher lactations was increased by 60,000 and 134,000 cells/ml, respectively. So far, KetoMIR results have only been used for herd management. KetoMIR is used, for example, by feeding advisors to assess and, if necessary, adjust the feeding situation on the farm in the dry cow period and early lactation.

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How to manage dairy cattle indoor-feeding period with insufficient grass silage stocks?

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Poor weather conditions during growing season may result in an inadequate grass silage stock. A dairy farm should prepare for this by storing a good quality late harvested grass silage and straw and supplement it with a high amount of concentrate to avoid loss in milk production. However, high concentrate level (CL) in the diet increases a risk for acidosis and hoof disorders. The aim of this study was to evaluate the effects of high concentrate feeding model on dairy cows' health and milk yield. Thirty mid- and late lactating dairy cows were used in a periodical study. The experiment started with a 19-d covariate (P0) period. The diet consisted of high quality first cut grass silage supplemented with barley/oats/rapeseed meal mixture. After P0 the silage was replaced with an advanced maturity grass silage and CL of total mixed ration was raised by 50 g/kg dry matter (DM) every 10-14 days. The realized CL were 395, 503, 558, 606 and 655 g/kg DM for P0, P1, P2, P3 and P4, respectively. The P4 included straw 70 g/kg DM. Indwelling ruminal pH sensors were used to record rumen pH of cows and claw health was also followed. Data were analysed using the PROC MIXED of SAS with repeated measures. The lowest daily average ruminal pH (6.52) was measured within P3 ($P<0.001$) which was probably due to the lowest fibre content (370 g/kg DM) in the diet. pH minimum levels decreased (<0.05) and standard deviation increased (<0.001) during the trial. Nevertheless, ruminal pH did not decrease below the threshold level 5.8 except occasional short periods. There were no changes in claw health of cows. Milk fat content (52 g/kg) and the ratio of fat to protein (1:40) were the highest with P1. The differences between P2 and P4 were not significant and fat content remained over 48 g/kg and fat:protein ratio over 1:26. Energy corrected milk yields were 34.0, 31.4, 33.3, 33.7 and 33.0 kg/d for P0-P4, respectively. Increasing CL increased the energy corrected milk yield when advancing lactation was taken into account. The results suggest that increasing CL up to 650 g/kg DM together with a combination of suitable roughage did not have major negative effects on mid-lactating dairy cows' health or milk production.

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Breeder's preferences in the choice of AI bulls at herd level

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A herd management app for smartphones, called 'SmartCow' has been developed by breeding organisations and Swissgenetics, an AI organisation, in cooperation with Identitas, the national animal tracing organisation. Around 13,400 cattle farmers used this app in 2020. We conducted an extensive online survey among 'SmartCow'-users, to assess breeder's preferences in AI-bull selection at herd level. The plan is to add a sire selection tool to the app based on these results. After data validation and exclusion of responses from non-dairy cattle farms, the answers from 3,261 cattle milk producers remained for analysis. Their answers regarding the location of the farms, the average milk production per cow and average herd-size agree with the results from annual economic evaluation of the Swiss agricultural sector. Therefore, we conclude that the collected answers are quite representative for the Swiss dairy cattle sector. 91% (2,971) of the responding users are member of a breeding organisation. 969 (30%) of the users never use sexed semen, while 504 (15%) indicate that they use progeny-tested bulls only. These proportions differ between breeds and depend on average milk yield of the herd and the region where the farm is located. Among all users, milk production traits (milk yield and milk components) followed by somatic cell score/resistance to mastitis and fertility traits are the most important traits to select AI-bulls. Less than 40% of the users regularly consider kappa-caseine-, beta-caseine- or polled-genotypes. In a next step, the data will be submitted to a cluster-analysis. The results will serve to develop a tool that assists users in finding AI-bulls that fit their breeding strategy at herd level best.

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Inbreeding rate in the conservative population of Red Polish cattle

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The Polish Red (RP) cattle is a rare and native breed and one of the oldest cattle breeds in Europe. In order to protect the breed against extinction, a genetic resources conservation programme was established. A programme was started in 2000 and is coordinated by the National Research Institute of Animal Production in Balice. Annual inbreeding rate and inbreeding rate per generation in the conservative population of RP were calculated. Data for the study were drawn from the SYMLEK database (basic population) belonging to Polish Federation of Cattle Breeders and Dairy Farmers and included pedigrees of 6,191 cows and 515 bulls heads, born between 1960 to and 2014. The average completeness of the pedigree for the first 4 generations was 78.68% (parents), 69.70% (grandparents), 66.16% (great-grandparents), 62.43% (great-great-grandparents), respectively. The inbreeding coefficients were computed using a model with genetic groups, according to the algorithm of VanRaden. It was found that in the analysed population 99,71% animals are inbreed with the mean coefficient of inbreeding ranging from 0.00 to 28.29%. Pedigree analysis also showed a relationship between the dynamics of population inbreeding of RP cattle over the years of birth and generation, as the generation interval. In results, annual inbreeding rate was 0.02 for animals born between 1960 and 1998, and 0.05 for animals born from 1999 to 2014. Inbreeding rate per generation and generation interval in the conservative population of Polish Red cattle irregularly increased from 0.15 and 3.88% in tenth generation to 1.48 and 5.94% in first generation of ancestors, respectively. In conclusion, the results of our study showed an tendency to reduce genetic variation in the conservative population of Polish Red cattle, although the dynamics of this phenomenon is relatively low. It also proves the need for constant monitoring of the genetic variability of this breed. The results obtained also indicate the need to calculate and analyse other parameters of the genetic structure of the conservative population of RP cattle, for a more complete knowledge of changes in the genetic diversity in the population. Elaborated as a part of task 03-18-21-09.

Relationship between cows' activity and the level of inbreeding

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The objective of this study was to study the relationship between the mean number of holstein-friesian cow steps per hour measured by pedometers over first lactation, and the average inbreeding coefficients. In total, we used 1,217 cows genotypes collected on four experimental farms belonging to the National Research Institute of Animal Production in Krakow (NRIoAP). The genotypes for low and high-density chips with pedigree information were deposited in Polish cattle SNPdb. The average number of steps per hour over cow first lactation was calculated based on behavioural database NRIoAP. The imputation and LD-based SNP pruning preceded the primary analysis. The final set of 43,772 SNPs was used to identification coefficient of genomic inbreeding (FROH) by the using of KING software. Cows were assigned to 3 groups similar in number depending on mean cow activity (A): A<130 steps/h = 1st group; A≥130 steps/h & <150 steps/h = 2nd group; A≥150 steps/h = 3rd group. The results shown that in the analysed population, all cows are inbred, with the mean FROH ranging from 0.02 to 17.03%. The average coefficient of inbreeding decreased from the level of 9.79% in 1st group, 9.55% in 2nd group and 9.17% in 3rd group of animals. In conclusion, the results of our research suggest a decrease in cows' step activity along with an increase in inbreeding. This phenomenon may indicate the impact of inbreeding depression on the activity of cows, associated in decreasing their vitality.

A retrospective evaluation of production performance of Butana cattle kept in researech stations

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The Butana cattle of Central Sudan is nowadays gaining attention due to recent advocacy pertinent to the establishment of breeding program for the breed. The aim of the current study was to evaluate milk production and reproduction performance achieved in Butana cattle over five decades starting from 1961, and to estimate breeding values of the sires used during the period. Data consisting of 380 cows from Atbara Livestock Research Station, with 958 total records of lactation, 674 records of calving interval and 616 records of dry period over 50 calving years were evaluated. Traits studied were lactation milk yield (LMY), lactation length (LL), calving interval (CI) and Age at first calving (AFC). A mixed linear model with function 'Lme4' was applied for the data analysis and 'Best Linear Unbiased Prediction' (BLUP) estimates of breeding value of sires for the production and reproduction traits were derived. Variables used as fixed effects were calving years and cow's parturition number. Our result showed an overall mean estimate of 1,367.2±694.2 kg for LMY, 272.2±66.4 days for LL, 370.5±46.9 days for CI, and 46.9±10.1 months for AFC. LMY increased significantly during the calving years between 2000 and 2010, however, LL and CI were not significantly influenced by the calving years (P>0.05). AFC declined from 55 months to 45.9 months between 2001 and 2010. Negative estimates of breeding value were found for some of the sires used actively for breeding. This points to an ineffective method of sire selection in the station. To enhance the performance of Butana cattle, better selection criteria and breeding managements are needed.

Feeding concentrated colostrum ensures sufficient uptake of IgG in newborn Holstein calves

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Failure of passive transfer of IgG is one of the major reasons for increased morbidity and mortality among neonatal calves. This is mainly due to lack of a sufficient amount of high-quality colostrum with a minimum of 50 g IgG/l to the newborn calves. By applying a modified processing method that concentrates IgG in colostrum, the total volume of high-quality colostrum can be increased. Hence, the objective of this study was to investigate whether calves fed with concentrated colostrum within 0-2 h after birth attain a sufficient passive immunization compared to calves fed non-processed (i.e. 50 g IgG/l) colostrum. This pilot experiment included 6 newborn Holstein calves divided into 2 groups, with 3 calves assigned to control colostrum (CC) and processed colostrum (PC), respectively. Colostrum from both treatments was heat treated at 60 °C for 60 min and had an IgG concentration of 44 and 61 g/l in CC and PC, respectively. Immediately after birth calves were weighed and fed with either CC or PC (i.e. 10% of birth BW) within 0-2 h after birth using an oesophageal tube. Calves were 1.20±0.32 h old at first colostrum feeding, had a birth BW of 38.7±2.2 kg, and were fed 3.88±0.20 l of colostrum. Feeding PC compared to CC resulted in a numerically higher volume of ingested IgG pr kg birth BW (6.10±0.09 vs 4.50±0.13 g/kg birth BW), a numerically higher concentration of IgG in plasma at 69±12 h of age (34.6±5.3 vs 22.0±1.8 g/l) and a numerically higher apparent efficiency of absorption (56±9 vs 50±3%). The findings indicate that colostrum concentrated to a well-defined high-quality colostrum (≥50 g IgG/l) can be used as a substitute to high-quality colostrum. Thus, the quantity of high-quality colostrum can be increased and thereby the total rate of passive immunized newborn calves can be ensured. As the results of this pilot study look promising, further studies are currently evaluating short- and long-term effects of feeding processed colostrum to newborn calves on their passive immunity, health, and productivity.

Comparison of performances of 7 models to predict enteric methane emissions usable for dairies

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Dairies are key players in lowering milk carbon footprint, especially by encouraging the reduction of enteric methane (CH_4) emissions of dairy cows. Indeed, CH_4 emission represents 54% of milk CO_2 footprint. To evaluate the reduction of CH_4 emitted in farms, it is necessary to quantify CH_4 emissions, e.g. by using prediction models. There are many of them, using all kinds of input data, mainly diet composition and feed intake data. However, dairies have only easy access to data from milk production, milk fat and protein productions, and milk fatty acids measured thanks to infra-red methods, which reduces considerably the number of usable models. After an inventory of more than 250 equations (found in scientific literature) to predict CH_4, only 7 equations were found to be suitable for a use by dairies, i.e. using easily the accessible data listed above. The performance of these 7 models were compared on a database containing data from 25 scientific publications: measured CH_4 emissions (used as reference), description of cow diets, and milk data (production, composition and fatty acids measurable with infra-red methods). Comparisons have been made on 2 perimeters: an 'on farm' perimeter, in which diets were representative of what is commonly done in farms (n=73); and a 'linseed' perimeter, in which diets contained linseed as a solution to decrease CH_4 emission (n=18). On the 'on farm' perimeter, the model with both the best RMSEP and R^2 criteria was a model from Niu *et al.* (RMSEP=3.3 and R^2=0.59). On the 'linseed' perimeter, this model was less performant with RMSEP=3.4 and R^2=0.19, which does not allow the recognition of addition of linseed as a solution to decrease CH_4 emission. The bests models on the 'linseed' perimeter were the model of Weill *et al.* (RMSEP=2.1) and a model from Engelke *et al.* (R^2=0.65). However, the model of Engelke *et al.* was not very performant on the 'on farm' perimeter with RMSEP=4.6 and R^2=0.37. The best compromise of performance between 'on farm' (RMSEP=3.4 and R^2=0.54) and 'linseed' (RMSEP=2.1 and R^2=0.48) perimeters was the model of Weill *et al.*, which allow a correct prediction whatever the dairy cow diets contain.

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Experiences with GPS sensors for dairy / suckler cows on Slovenian Alpine pastures

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During the last decades, Slovenian and also European mountain areas have been subjected to a decrease of agro-pastoral activities. Some young farmers in Slovenia look for new form of mountain grazing of cattle in Alps. Together with HipSquare team, they tested the use of GPS sensors for dairy and beef cattle on Slovenian Alpine pastures last year. With the use of GPS sensors, farmers can check animal positions in real time by using a smartphone or personal computer. The aim of this study was to verify providers' product data based on real-life testing data (GPS quality, battery run-time, server communication, etc.), gain further information on GPRS signal quality in different regions, get insights about farmers daily problems and discover new use cases. Activity data from 15 GPS-collared cattle were collected throughout the grazing season of 2020. The GPS collars recorded movements over 24 hours. On the base of experiences till now, we can say that in general it is possible to provide a GPS service to farmers based on the Tracker V34 but only for outdoor tracking use cases. Use of GPS sensors covered next: find cow, alert when a cow is escaping, and collecting km per cow for activity tracking. Battery lifetime is enough for 4-5 month depending on the position of the tracker. Animal-carried sensors offer the opportunity to monitor activities of grazing animals continuously over long time periods without disturbing their natural behaviour. Animal-carried GPS sensors offer new insights into mountain livestock behaviour. However, most devices were developed for in-house deployment and need careful adaptation and testing for their application in the harsh mountain environment.

Session 71 Poster 26

Land use conflict between farmers and herdsmen – implication for rural development

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Farmers and Herdsmen are having a lot of conflict in areas of land matter. This is due to the inadequacy of grazing resources, climate change and the effect of this is noted on the household welfare, loss of material resources, Agricultural produce and reduce income. This presentation study the different conflict suggest ways of ending such, as creating awareness of land use regulations among farmers and herdsmen's, provision of grazing land, extension services to teach farmers and herdsmen on conflict coping mechanisms, educating the farmers and herdsmen's for peaceful co-existence an mutual benefit, viable NGOs on farmers-herdsmen conflict management, especially in areas of awareness, education prevention, support livestock-centred live hoods including cattle herding, and conflict mitigation. Finally conflict management framework is required to curb the danger posed by farmer – herdsmen conflict and Traditional and local leaders should be well involved in finding solutions to farmer herdsmen.

The incidence and management of bovine cystic ovarian disease in dairy herds across Atlantic Canada

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Cystic ovarian disease (COD) is one of the most common reproductive disorders in dairy cows, resulting in substantial profit losses due to reduced milk yield and increased culling. Despite the negative impact of COD, little data is available on COD incidence in Atlantic Canadian dairy herds. The objective of this study was to investigate the recording and management practices of Atlantic Canadian dairy producers to manage COD in their herd and to estimate the incidence of COD in Atlantic Canada. Dairy producers from the four Atlantic Provinces were surveyed online from December 2020 to February 2021. Participants were asked questions about disease incidence, herd health, milk yield, and disease management practices. A total of 31 questionnaires were analysed (11.39% response rate). The mean lactational incidence rate of COD across all provinces was 9.56, 1.56% above the national incidence rate. For analysis farms were divided into high incidence (>9%) and low incidence (<9%) groups. Regression analysis, Chi-square tests, and t-tests were conducted. Low incidence farms had lower milk yields ($P<0.05$) and were less likely to record COD cases ($P<0.01$). However, low incidence farms were more likely ($P<0.05$) to only treat COD cases on veterinary advice or in late presenting cows, and to have mixed herds including Jersey, Ayrshire and other breeds. High incidence farms were more likely ($P<0.01$) to record COD cases, treat all or only early presenting COD cases, and to have Holstein only herds. No significant difference in the frequency of herd health checks or fresh cow checks was noted between high and low incidence farms. No producers stated that they used genetic programs to reduce herd COD incidence and only two producers stated that they used feeding strategies, vitamins and body condition scoring to prevent COD in their herds. The higher than national average COD incidence and a lack of using preventative COD measures in Atlantic Canada suggests room for improvement in COD management. Finally, lower COD incidences may be associated with mixed herds, milk yields, and treating on veterinary advice but also may be due to improper recording of disease on low incidence farms skewing incidence rates to be lower than normal.

SmartCow: integrating research infrastructures to support innovation in European cattle breeding

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The SmartCow project (H2020 N°730924; https://www.smartcow.eu/) integrates key European cattle research infrastructures (RIs) to promote their coordinated use and development, and thereby help the European cattle sector face the challenge of sustainable production. SmartCow RIs cover the diversity of cattle types and production systems (dairy vs beef, extensive vs intensive). They benefit from strong scientific and technical skills in animal physiology and behaviour, and in ethics in animal experimentation. Thanks to networking activities, SmartCow developed a book of methods to harmonize experimental procedures in cattle physiology and behaviour, improved animal trait ontology to standardize the vocabulary and built a cloud-based data-platform to facilitate data sharing and interoperability. Joint research activities were conducted to refine reference methods in cattle nutrition, produce proxies to evaluate feed efficiency, nitrogen and enteric methane emissions, and new methods to exploit data from sensors monitoring animal behaviour. SmartCow transnational access activity provided academic and private research communities with easy access to 11 major RIs from 7 countries for around 10,000 experimental cow-weeks distributed in 24 research projects. A Stakeholder Platform composed of pre- and post-farm gate industry, farmer organizations and policy makers at national and EU levels, and a training program targeted to young scientists supported the dissemination of SmartCow outcomes. This session will highlight how SmartCow improved RIs phenotyping capacities and services to foster innovation in the European cattle sector towards ethical solutions for efficient use of animal and feed resources that promote animal welfare and healthy livestock.

Session 72 Theatre 2

Using N more efficiently in cattle production systems – challenges and opportunities

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Diet N use efficiency (NUE) is affected by multiple dietary and animal factors that determine the supply of essential amino acids (EAA) relative to requirements and associated faecal and urinary N losses. NUE in ruminants is low relative to nonruminants, largely due to effects of rumen fermentation, with 75% or more of diet N excreted in manure. Increasing concerns over the impacts of ammonia, nitrates, and N_20 highlight the need to develop sustainable approaches for milk and meat production by cattle that minimize losses of diet N to the environment. Variation in NUE between animals is substantial and related to feed efficiency. Recent studies demonstrate the improvements in NUE that can be achieved by feeding diets with lower total protein concentration, as long as variation in diet composition does not lead to long-term undersupply of EAA or an imbalance of energy and protein. In addition, dietary starch can also increase NUE through both rumen and metabolic effects. Historically, NUE and body N balance of individual animals has been measured using digestion trials with total faecal and urine (and milk) collection to determine total intake and excretion of N, with body tissue N retention or loss calculated by difference. Meta-analysis of individual measurements of N balance obtained at multiple locations undertaken in the SmartCow project (H2020 N°730924) highlights the variation attributable to methodologies, including volatile N losses, and the need for standardized approaches to reduce experimental error and increase precision and comparability of results. Moreover, measurement of N balance of individual animals is time consuming, laborious, and restricts animal movements. The SmartCow project confirms faecal NIRS and natural ^{15}N abundance of animal proteins as biomarkers of digestion and NUE, respectively, highlighting their potential for replacing digestion trials and time consuming phenotyping.

Session 72 Theatre 3

Prediction of between-animal variation in N use efficiency in dairy cows from isotopic N signatures

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Animal proteins are naturally enriched in 15N relative to the consumed diet and the 15N enrichment of animal proteins over the diet (Δ15N) has been recently shown to capture the between-animal variation in N use efficiency (NUE). The aim of this study was to evaluate the performance of a previous model to predict between-animal variability in NUE from measured Δ15N in lactating cows. The data included 990 records from 17 experiments comprising 164 contemporary groups (CG; group of cows fed the same diet at the same place and measuring period) with an average of 6 cows per CG. Samples of milk or plasma and feed were analysed for 15N and Δ15N was calculated as the 15N difference between animal proteins and diet. Measured NUE and Δ15N of each cow were expressed as their deviations from the average value of the CG they belonged to and noted as observed deviations. Predicted differences in NUE between each cow and its CG average value was calculated as 0.035 × observed deviations of Δ15N (according to the previous published model) and noted as predicted deviations. The coefficient of variation was 10 and 13% for observed NUE and Δ15N within CG, respectively. The correlation between observed and predicted deviations of NUE was positive in 84% of CG. Overall correlation coefficient between predicted and observed deviation of NUE was moderate but significant ($r=0.50$, $P<0.001$). The model error showed that Δ15N was able to identify between-animal variability in NUE when the phenotypic deviations among cows surpassed 2.90 percent units. This study confirmed that regardless of the modest prediction accuracy of the validated model, the Δ15N biomarker was able to identify phenotypes of contrasting NUE.

Digestibility and intake become repeatable traits in young bulls with at least 7 days of measurement

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Gold standard for measuring digestibility in cattle consists in placing animals in digestibility stalls for several consecutive days (d). This is constraining for the animal whose movements become very limited. According to the 3 R principles (Reduce, Replace and Refine) applied to cattle experimentation, our objective was to determine the minimum length of measurement period to obtain repeatable measurements of total tract digestibility (TTD) and voluntary dry matter intake (VDMI). Eight Charolais bulls, previously adapted to their diet in free stalls, were submitted to two 15 d periods of VDMI and TTD measurements in digestibility tie stalls (5 d of adaptation to the individual stalls and 10 d of measurement). Four bulls received a high crude protein content diet (173 g/kg DM) and the other four a low one (116 g/kg DM). At the beginning of the first (P1) and second (P2) digestibility periods, the animals were aged respectively (mean ± sd) 294±11.9 and 371±11.8 d and weighted 417±15.6 and 516±25.0 kg. The VDMI and total amount of excreted faeces were measured daily for 10 d. Differences between P1 and P2 for TTD and intake level were tested by paired t test over 10 d. Repeatability of TTD and VDMI measured during 2, 3, 4, 5, 6, 7, 8, 9 and 10 consecutive days was assessed through their respective within-diet correlation coefficients between P1 and P2. Animals digested more efficiently during P2 than P1 (+2 g/100 g; $P<0.05$) in relation to their lower intake level (-0.12 kg DM/100 kg bodyweight, $P<0.05$). Correlation coefficients increased with length of measurements: from -0.42 to 0.88 for TTD and from 0.33 to 0.76 for VDMI, between 2 and 10 d, respectively. A high correlation was observed when TTD was measured over 7 days ($r=0.78$), with only minor improvements (+12.8%) thereafter ($r=0.88$ at d10). When VDMI was also measured over 7 d, we obtained a good correlation ($r=0.61$) which however improved slightly (+24.6%) thereafter ($r=0.76$ at d10). We conclude that a minimal length of measurement period of 7 d may be necessary to obtain repeatable results on TTD and VDMI in young bulls fed *ad libitum*.

Milk mid-infrared spectra to estimate rumen fermentation parameters

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Measurement of rumen fermentation parameters is difficult, particularly under practical conditions, requiring expensive material and restrictive handling of animals, e.g. oral stomach tubing, cannulation, bolus administration, housing in respiration chambers, etc. However, rumen disorders or suboptimal fermentation processes of dairy cows could be a consequence of unsuitable diet composition, insufficient feed intake, or health problems, and imply a loss of performance in dairy farming. If such malfunction would be detected long-term health, physiological, and economic consequences could be avoided and animals may achieve higher efficiency and well-being. Thus, developing a technique to monitor rumen fermentation parameters at reasonable cost while ensuring animal welfare is an interesting prospect for herd management. Early detection of rumen dysfunction may give farmers and nutritionists the opportunity to act well before the manifestation of marked symptoms and avoid meaningful losses in animal efficiency. Ammonia (NH_3) and volatile fatty acids (VFA) concentrations, and pH from rumen fluids, as well as enteric CH_4 emissions were considered relevant parameters to be measured in this context. Regarding the known metabolic links between these rumen parameters and milk composition, milk mid-infrared (MIR) spectra were studied as a potential proxy. In the framework of the Smartcow project, reference datasets including animal phenotypes and corresponding milk MIR spectra from different research institutes have been gathered with the purpose of validating the estimation of CH_4 and to investigate the development of new proxies for the aforementioned rumen fermentation parameters. This data-collection effort highlighted the importance of common protocols to collect data with a view to allow the merging of datasets and to mutualize time and economic efforts in the development of robust proxies. The first results will be presented and discussed.

Methane emission, microbiome and immune function in dairy cattle

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The aim was to delineate interactions between enteric methane (CH_4) emissions, rumen microbiome, and immune function in early and late lactating cows. Low CH_4 emissions were associated with higher feed conversion efficiency (FCE), but also with impaired immune response of cows probably lacking the energy to sustain an adequate immune response. In addition, how does microbial community structure reflect low or high CH_4 emissions? We hypothesized that (1) low and high CH_4 emitting cows differ in the rumen microbiome; and that low compared to high CH_4 emitters are (2) characterized by a higher FCE and milk production efficiency (MPE) and (3) a lower immune response. Dry matter intake (DMI), body weight (BW), milk yield and constituents and CH_4 production in respiration chambers were studied in early (n=20, 31 days in milk, DIM) and late (n=14, 390 DIM) lactating, multiparous Holstein cows. Immune response to concanavalin A (ConA) and phytohaemagglutinin (PHA) was studied *in vitro* using whole blood and peripheral blood mononuclear cells (PBMC). Analyses of 16S rRNA in rumen fluid (oesophageal tubing) and TNAα in supernatants are underway. Statistical analyses were performed within lactation stage with SAS 9.4. Cows weighted 545-917 kg, had 9-24 kg DMI/d, 16-51 kg energy corrected milk (ECM)/d, 363-751 g CH_4 production/d and PBMC proliferation indices (PI) of 1.9-5.5 (ConA) and 1.4-3.8 (PHA). As hypothesized, cows producing less CH_4 (g/d) were characterized by a higher FCE (ECM/DMI; early lactation: P=0.002, late lactation: P=0.055), and by a higher (P=0.045) MPE (ECM/BW) at the late, but not early lactation stage (P=0.9). CH_4/d, CH_4/DMI, CH_4/ECM and CH_4/BW were not correlated (P>0.1) to immune response (PI). Accordingly, cows retrospectively grouped into low and high emitters (CH_4/d, CH_4/DMI, CH_4/ECM, CH_4/BW) did not differ (P>0.1) in immune response (PI), except for low compared to high CH_4/DMI emitters in early lactation who showed lower PI (P<0.052). Preliminary results confirm that greater CH_4 emissions are related to decreased FCE yet improved immune function in early lactation.

Detection of complex animal traits from data provided by activity sensors

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Behaviour impacts on an animal's functioning (e.g. eating determines nutrition). In turn the internal state of an animal impacts on its behaviour (e.g. over stress reactions). We thus assume that behaviour is related to complex traits such as production, efficiency, health, welfare. We carried out several studies to investigate such links. First, we analysed the impact of animal behaviour on its functioning. Feed intake for cows fed indoor depends on the time spent eating and the rate of eating (DMI/min). Production (e.g. milk yield) depends on feeding behaviour and other activities. Indeed, more active cows (characterised by high number of steps per day) yield less milk (r=-0.37), presumably because walking increases energy expenditure. Also higher feeding rates seem detrimental to milk yield (r=-0.36). We are investigating ways to characterise precisely the feeding behaviour of cows (number and size of meals, ratio of eating/chewing per meal, etc.) and its links with feed efficiency. Second, we analysed the impacts of internal states on behaviour. Infectious diseases and stress alter the activity. This was observed through drinking behaviour (increased in case of fever) or alteration of the daily rhythm of activity (less marked in more than 90% health disorders). Such alterations can be detected before appearance of clinical signs of a disease. Finally, the behavioural phenotype of an animal during dry period (level of activity, regularity and periodicity of activity measured) seems to determine its robustness after calving, especially in relation to health disorders. Characterising the behaviour of animals can now be done on farms on a routine basis thanks to sensors automatically recording activities. This opens opportunities to better predict or monitor animal health, welfare and production, which in turn can help farm management at strategic (e.g. use of behavioural criteria for selection) and operational (refinement of the daily interventions) levels.

Session 72 Theatre 8

Can a virtual cow model help precision feeding in dairy cattle?

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Precision farming allows automatic and massive data collection aiming at improving animal productivity and welfare among many goals. Animal models are needed to interpret these data and individual variability in order to better adjust management strategies (i.e. feeding strategies). The GARUNS model of dairy cow lifetime performance initially proposed by Martin and Sauvant has evolved through the last ten years, including a reproductive module, able to take into account individual reproductive management, and more recently a 'feed plan' module, taking into account the composition of the ration distributed to the cow during its life. The first objective of this study was to quantify the effect of this 'feed plan' module on the fitting accuracy of production variables. The second objective was to evaluate the fitting quality of the second lactation when the fitting was done on first lactation data only. The new model, with the 'feed plan' module, was fitted with a step-by-step procedure on the first lactation data of 16 Holstein cows. These data were collected during an experimental trial on extended lactation conducted from 2012 to 2015 at the Danish Cattle Centre in Aarhus University (Denmark). Data used concerned production (dry matter intake, milk yield, body weight, body condition score, milk components) and reproduction (insemination and parturition time) data as well as rations' composition. Preliminary results indicated that the adjustment with the new model was more accurate for the three production variables studied (dry matter intake, milk yield and body weight) during the first lactation than the one with the model without 'feed plan' module. Based on the first lactation fitting, the new model was also able to fit the second lactation data to a certain extend. To conclude, this first approach indicated the relevance of the feed module to improve the model fitting. It could therefore serve as management tool and help predicting the individual productivity of the next lactation. Further work should include perturbations (i.e. health incidents) in the model to obtain more accurate predictions.

Session 72 Theatre 9

Prediction of total feed intake of dairy cows using a portable near-infrared microspectrometer

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Near Infrared Spectroscopy (NIRS) is widely used for the assessment of animal feed composition, including compound feeds, raw materials, forages and total mixed rations (TMR). Usually, these composition data are incorporated into feeding equations or models to predict the animal response. However, a NIR spectrum is a powerful digital print of the product, containing not only information about the chemical composition of the product, but also information about other parameters related to its texture, origin, structure or organoleptic characteristics. Thus, the objective of this work is to evaluate the potential of NIRS to directly predict the voluntary intake of dairy cows from TMR spectra. Sixty cows belonging to Aarhus University farm were divided in four groups and fed with four different TMR diets (control diet, and three other diets with an increasing level of 5, 10 and 15% of straw instead of silage). Cows were monitored during eight weeks and feed intake was measured individually. A total of 142 samples of TMR were analysed using a portable micro-NIRS instrument, based on LFV (Linear Filter Variable) technology, working on reflectance in the range 910-1676 nm. A PLS (Partial Least Squares) model was developed for predicting the total TMR intake with a population characterized by a total feed intake mean value of 49.52 kg, resulting a coefficient of determination of 0.81 and a standard error of cross validation (SECV) value of 2.52 kg. These results show the feasibility of NIRS for the *in situ* direct prediction of the animal response associated to a specific diet, which could be of great importance to match animal needs and diets, and to increase the farm efficiency.

Session 72 Theatre 10

Evaluation of strategies for feed efficiency phenotyping in the Norwegian red dairy cattle

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The feed is one of the largest costs in dairy production. However, measuring feed intake to breed more efficient cattle is very expensive. Due to high costs, it is important to optimise the recording of feed intake to maximise genetic gain. Here we evaluated different strategies for feed intake recording in the Norwegian red dairy cattle using a stochastic simulation. Using AlphaSimR we have developed in silico breeding programme of the Norwegian red dairy cattle breed. We start with the creation of a historical population, continue with 45 years of progeny testing scheme, and finish with the recent genomic selection scheme and the future strategies. The in-silico breeding program simulates the whole population of 200,000 cows across 5 lactation groups and their progeny, all distributed across 7,000 herds of variable size. Animals have associated genome, genetic and breeding value, phenotypes, SNP array genotypes and externally estimated breeding values. We generated phenotypes as a function of genetic value, permanent environment, herd, herd-year effects. We tested phenotyping feed intake in the different number of cows (500, 1000, 1,500, 2,000 and 3,000) across the different number of contract herds (10, 20, 50, and 100). We compared these scenarios on the accuracy of estimated breeding values and genetic gain. Since there are different alternatives of feed efficiency traits (such as dry matter intake, energy intake, feed conversion ratio, residual feed intake, feed saved, etc.), we tested selection on a feed efficiency trait with heritabilities of 0.1, 0.2, and 0.3. Accuracy of estimated breeding values and genetic gain increased with an increased number of phenotyped cows as expected. Detailed modelling of the population structure showed a substantial impact on environmental effects. The scenarios applied in this study can guide strategies for collecting phenotypic data for other expensive recordings.

Session 72 Theatre 11

Which methodology to study the effect of farming practices on cheese sensory properties?

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To understand and quantify the effects of changes in farming practice on sensory properties of cheese, experimental approaches controlling environment and cheesemaking process are needed. In many previous experiments, variability was obtained by repeated samplings of the blended milk of all animals submitted to an experimental treatment. As replicates result from the same animals sampled repeatedly, this approach raises statistical issues. We tested if sampling subgroups of animals submitted to the experimental treatments to get independent cheese replicates leads to the same conclusions. Twenty-four grazing cows were randomly allocated to a permanent grassland with over 60 plant species or to a temporary grassland with 31 plant species for 1 month. On 3 days per group, morning bulk milk of 3 subgroups of 4 cows and their yield-weighted blend were collected and simultaneously processed to Cantal-type cheese. Ten trained panellists assessed appearance, texture, odour, and flavour of the 9-wk ripened cheeses. Sensory data on cheeses derived from the group blends approach (n=3) or from the subgroup blends approach (n=9 or n=3, when considering only 1 cheese per subgroup) were analysed with mixed linear models that included group as fixed effect, panellist and replicate or subgroup (according to the approach) as random effects, and subgroup as repeated effect. Comparing either 1 cheese per subgroup or cheeses from group blends did not result in significant sensory differences depending on pasture type. Considering all cheeses derived from subgroup blends (n=9) revealed that the more biodiverse pasture leads to less firm (-11%) cheeses with stronger dry fruit odour (+23%) and flavour (+24%). Beyond the chosen experimental approach, statistical significance of the sensory differences is mainly driven by the number of replicates.

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Mapping of cattle infrastructures, equipment and sample banks within the SmartCow consortium

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Mapping of cattle infrastructures, equipment and sample banks is needed to foster synergies between RIs. Awareness of the research infrastructures (RIs) available in Europe within the SmartCow consortium was achieved by creating an interactive map outlining facilities both within and outside the consortium where research is carried out based within the beef and dairy sector in Europe. This is the first interactive map developed within Europe outlining RIs across the beef and dairy sectors. The map is available at https://www.smartcow.eu/map/ which can be filtered by sector and by membership (consortium/non-consortium). There are 10 consortium members (INRAE, SRUC, Teagasc, Aarhus, IRTA,FBN,CRAW,CEDAR, WUR, WU) amounting to 18 RIs in which 14 are dairy focused, 3 beef focused, and 1 beef and dairy focused. Data was gathered on each consortium members RIs which outlines the general infrastructure to include - wet chemistry lab analysis facilities. The structure of the institute in terms of area (ha), soil type and number of dairy/beef cattle was documented. Other elements outlined include climate and animal facilities. Information with regards to equipment and techniques used within each RI was compiled with particular attention paid to methane methodology and analysis. This allows RIs to become a support network for one another as it opens the lines of communication, sharing of expertise and aids in troubleshooting for both equipment and techniques used though the RIs. Information was gathered from each RI in terms of Database Management while also outlining personnel responsible for sample banks. All the data collected from each RI has been compiled in an excel sheet which is available to all SmartCow members though either the SharePoint or Agrimetrics (both within consortium databases). This Work Package clearly shows that considerable expertise within Europe, across RI can be shared, which can have synergistic effects on across country collaborative research programs.

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Encouraging research complementarities through mapping of cattle research infrastructures in Europe

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The European Strategy Forum on Research Infrastructures (ESFRI) roadmap clearly identified the need for improved coordination, harmonisation, and access to European research infrastructures (RIs) of farm animals. The SmartCow project (www.smartcow.eu H2020 Grant N°730924) had the objective to link the research infrastructure, resource capability and research needs across Europe. One aspect was to identify, characterize and map the research infrastructures across Europe. An online survey was created and send by the EAAP to their database inviting to fill general information regarding the research infrastructure: type of production (beef or dairy, number of animals, land type, etc.), research topics, type of equipment and facilities available on the infrastructure. From the 43 answers received, 2 were not relevant, 21 came from experimental only farms, 2 from demonstrative only farms and 17 from demonstrative and experimental farms. The 41 RIs came from 19 countries (Austria, Belgium, Bulgaria, France, Germany, Hungary, Ireland, Israel, Italy, Poland, Portugal, Romania, Slovakia, Spain, Sweden, Switzerland and the United Kingdom). 34 RIs had dairy herds, with mostly Holstein and Simmentals breeds and herds varying from 25 to 400 cows. 17 RIs had beef production, 8 with a suckler herd varying from 25 to 220 cows and mostly cross breeds, 15 with a fattening unit for heifer, steers, or cows mostly for dairy or crossbreeds. The type of research equipment found on those RIs are behavioural monitoring (35 farms), Individual feed intake facilities (28), Greenhouse gas measurement (19), digestion facilities (15), grazing studies equipment (15) and metabolism facilities (13). Those RIs were added to the interactive map available on the project website (https://www.smartcow.eu/map/) along with the 18 RIs from the SmartCow consortium. They were also invited to complete the full SmartCow database. The identification of possible missing RIs is underway to complete the map and updates of the RIs' data displayed on the map is planned.

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Towards common standards, guidelines for measurement and data management in cattle research

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SmartCow project developed a common framework regarding standards, measurements, and data management to ensure high quality data production with regard to routine and experimental measurements and recordings in cattle physiology and behaviour. Experimental guidelines and protocols were published as an Open Access living handbook on PUBLISSO entitled 'Methods in cattle physiology and behaviour research – Recommendations from the SmartCow consortium'. The book currently includes 19 chapters covering ethics in experiments on live cattle, intake and behaviour, body condition and anatomy, reproductive, stress and health assessment, rumen function, nutrient digestibility and balance studies, respiratory chamber facility and techniques to measure gas emissions (https://books.publisso.de/en/publisso_gold/publishing/books/overview/53/199). Each chapter lists specific animal traits referring to their identifiers in the Animal Trait Ontology of Livestock (ATOL) and the Environmental Ontology of Livestock (EOL). Vice versa, 92 new traits identified in the published guidelines were included in the existing ontologies. The notable new traits are: milk ketone body content, milking volume, lactation number, stage of lactation, rumen volatile fatty acid content, days of pregnancy, etc. All data providers have access for to the ontologies (http://www.atol-ontology.com/en/erter-2/). Agrimetrics authored a data management plan to set out the approach to managing data within the project, and delivered a Cloud-Based Data Platform (CBDP). The CBDP is designed to ensure the interoperability, sharing and exploitation of the data according FAIR principles. A SmartCow ontology including ATOL and ETOL represents the data and allows researchers to upload and semantically link data with that of other groups of researchers. Data that was not collected or observed at the same time can now be queried together and exported, into an environment of the user's choice, for further analysis.

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Transnational access to leading cattle research facilities within the EU SmartCow project

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The Transnational access (TNA) programme makes available cattle research facilities of SmartCow partners for research by academic or industry colleagues from other (mainly EU/associated) countries. There are 11 major research infrastructures (18 installations) in 7 countries. The facilities cover a range of cattle types (dairy, beef), different breeds and genotypes and diverse husbandry and feeding systems. The installations include the most advanced animal science technologies applied to cattle in the fields of nutrition, physiology, ethology and animal husbandry. In addition to subject priorities, we sought to encourage new users, early-career scientists and users without access to facilities in their region. Total budget was €1.5 million and 10,000 experimental 'cow-weeks' were available across the cattle research facilities of Aarhus University, FBN, INRAE, IRTA, SRUC, Teagasc, University of Reading, Wageningen University and WUR-DLO. Project funding supports the operating costs of facilities running experiments designed by the users, from intensive physiological studies using few animals to large production ones with cows on pasture. Four calls for proposals were made from 2018 to 2020 and we received 47 full proposals, 19 from academic users and 28 from industry users. Proposals were evaluated by at least two independent experts, as well as our ethics committee, and we expect to complete work for 24 of these projects by end of SmartCow in January 2022. Successful proposals were led by teams from Austria, Bulgaria, Denmark, France, Germany, Ireland, Macedonia, Norway, Spain, Switzerland and the UK. Six projects have already completed their work, including studies of diet effects on rumen functioning and methane emissions, diet effects on cheese quality, multi-species sward effects on performance, amino acid nutrition of dairy cows, and sensor technologies for both feed evaluation and assessment of cow stressors.

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Titanium dioxide as digestibility marker – between and within cow variation in faecal concentrations

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Quantification of between and within cow variation in faecal nutrient concentrations is important when identifying the optimal sampling procedure for using digestibility markers in feeding trials. The aim of this experiment was to quantify the between cow variation in faecal concentration of titanium dioxide (TiO_2), N and N: TiO_2 ratio, and to assess if within cow variation was affected by diet composition. Twelve Holstein cows were used in a 4×4 balanced Latin square with 4 diets, with 60:40 forage:concentrate ratio as dry matter (DM), and 4 periods of 21 days each. The 4 diets were 2×2 factorial arranged, including low vs high grass silage digestibility (LDGS vs HDGS) and 18 vs 42% of diet DM from maize silage (18MS vs 42MS). Faecal spot samples (300 g) were collected twice daily from d 18 to 21 at h 08:00 and 14:00. Samples were analysed individually (8 samples/cow/period) for TiO_2 and N. Dependent variables (TiO_2, N and N:TiO_2 ratio in faeces) were analysed in a linear mixed model including interaction between dietary factors and interactions between period, day and time as fixed effects, and cow and cow within period as random effects. Between cow variation was the standard deviation (SD) of the random cow effect from the model. Effect of diet on within cow variation was assessed using the raw data SD of cow within period (SD for the 8 samples/cow/period) for each given nutrient as dependent variable in a linear mixed model with interaction of dietary factors and period as fixed effects, and cow as random effect. Average and between cow variation (model mean ± SD for random cow) were 3.77±0.08 and 2.89±0.06 g/kg faecal DM for TiO_2 and N, and 0.77±0.03 g/g for N:TiO_2 ratio. Within cow variation was not affected by treatments, with the exception of faecal N SD, which tended to be higher for cows fed 42MS diets than cow fed 18MS diets (SD 0.28 vs 0.21 g/kg DM; P=0.05). Within cow variation resulted in average and between cow variation of 0.36±0.06 and 0.25±0.03 g/kg faecal DM for TiO_2 and N, and 0.041±0.005 g/g for N:TiO_2 ratio. Eight spot samples were sufficient to have a stable within cow variation for TiO_2 and N:TiO_2 ratio, but high maize inclusion increased the within cow variation for faecal N concentration.

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Influence of measurement method of methane on the performances of faecal NIRS models in cattle

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Enteric methane (CH_4) emissions in cattle is one of the largest greenhouse gas contributor from agriculture. Measuring CH_4 emissions is important in order to assess mitigation strategies but existing methods cannot be used on large number of animals on farm. Spectroscopic methods using milk have been suggested as an alternative for predicting emissions on farm but are limited to lactating dairy cows. Faeces could be a more universal matrix but few studies are available regarding the use of spectroscopic methods on the prediction of CH_4 emissions. The objective of this study was to asses faecal near-infrared spectroscopic (NIRS) prediction models, based on CH_4 emissions measured with different methods (respiration chambers (RC), sulphur hexafluoride tracer gas (SF_6) and GreenFeed® (GF)). In this work based on two *in vivo* studies, we used SF_6 measurements as the reference. In study 1, RC and SF_6 measurements were performed simultaneously leading to two NIRS models based on 32 RC and SF_6 methane values in non-lactating cows. In study 2, SF_6 and GF values were collected simultaneously and two NIRS models were developed using 24 values from dairy cows. In both studies, bias between measured and predicted values was non-significant compared to the standard error of cross-validation corrected by the bias (SECV(C)), when values issued from models based on SF_6 method were compared to SF_6 values (bias=0.54 g/d and SECV(C)=45.1 g/d for study 1 and bias=3.00 g/d and SECV(C)=95.4 g/d for study 2). However, if the cross-validation is done on RC or GF and compared to SF6 values, bias greatly increased (-38.0 g/d and 101.2 g/d in study 1 and 2, respectively whereas SECV(C) in each study were 50.3 and 100.4 g/d). These results suggest that SECV(C) values were similar between methods within each study but there was a bias. This bias was more important for GF than for RC when compared to SF_6 method. These results could help to minimize errors due to the combination of CH_4 emission databases obtained from several measurement methods.

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Can we identify low CH_4 emitter dairy cattle by their milk carbon isotopic signatures?

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Enteric methane produced by ruminants is extremely depleted in 13C compared to the substrates from which it is formed. This is the consequence of the strong isotopic fractionation occurring in the rumen when CO_2 is reduced into methane. As a result, it can be expected that the more methane is emitted by a ruminant, highly depleted in 13C, the greater is the enrichment in 13C of body-pools such as the secreted milk. Based on this hypothesis, we aimed to explore whether the natural 13C abundances in milk (milk δ13C) could be used as a proxy of enteric methane emissions in dairy cows fed the same diet. We measured daily methane emissions from 56 dairy cows during 11 weeks using 2 GreenFeed systems after 6 weeks of adaptation. Dairy cows (156±37 days in milk) were reared in identical conditions and fed the same basal diet made (on a dry matter basis) of hay (25%), haylage (50%) and concentrate (25%) and supplemented or not with phytogenic additives (25 g/cow/day). Milk was sampled in the morning milking at the end of weeks 7 and 11 and subjected to isotopic analysis by elemental analyser-isotopic ratio mass spectrometer. Methane emissions (g/d) were calculated for each animal as the average of the 11-wk measurements (91±31 visits) and their values were regressed on milk δ13C obtained either in week 7 or week 11 or their average. Milk δ13C values were moderately repeatable across the two milk samples ($r=0.66$; $P<0.001$). The average milk δ13C values for each animal was positively correlated with their average measured 11-wk methane emissions ($r=0.56$; $P<0.001$). However, we obtained different relationships depending on the milk sampling period with higher correlations obtained in week 7 ($r=0.59$; $P<0.001$) compared to week 11 ($r=0.39$; $P=0.01$). Our preliminary results highlight the potential of milk δ13C to reflect the animal-to-animal variation in methane emissions for dairy cows fed a similar diet. Further studies in contrasted farming conditions should be conducted to evaluate whether this proxy could be proposed as an alternative phenotyping tool of methane emissions in dairy cattle.

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Investigating components of cow breath with potential for rumen diagnostics

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This work explored the potential of breath analysis as a non-intrusive way to study rumen function. Grazing dairy cows at the Lincoln University Dairy Farm (New Zealand) were held in a crush adjacent to an open collecting yard and breath samples from immediately adjacent to the nostril pumped into tedlar bags for approximately 30 seconds. Samples were transported to the laboratory (within 3 hours) for analysis of breath components by Selected-Ion Flow Tube Mass Spectrometry (SIFT-MS; Syft Instruments, Christchurch, New Zealand). Samples were collected on six separate sampling dates (20, 20, 12, 12, 12 and 12 cows respectively). There were significant negative relationships between concentrations of methane and ammonia on most occasions ($P<0.05$; $P<0.001$; $P<0.01$; NS; $P<0.01$; $P<0.05$)). This is interpreted to represent mixtures of breath (averaging 1,333; 1,691; 821; 530; 1,117 and 1,751 ppm methane) and ambient air (10.1 and 8.4 (12 and 13 September); 169.1 and 144.5 (23 and 24 March); 90.0 and 51.8 (17 and 18 April) ppb ammonia). Breath sampled at the nostril of cows is a mixture of eructed rumen gases, exhaled (alveolar) breath and ambient air. Since rumen gas contains roughly 25% methane, this suggests an average 200-fold dilution of rumen gas in exhaled breath. After correction for dilution with ambient air, the average concentrations of gases most related to rumen function were: acetic acid = 128 (s.d. =165.3) ppb; propionic acid = 29 (s.d.=48.6) ppb; butyric acid = 62 (s.d.=48.3) ppb; hydrogen sulphide = 10.9 (s.d.=19.65) ppb; methyl sulphide = 3.8 (s.d.=3.00) ppb; and dimethyl sulphide (DMS) = 471 (s.d.=934.9) ppb. One-third of samples contained no hydrogen sulphide (which is exclusive to rumen gas), but all contained much higher levels of DMS from alveolar breath. Aside from methane, DMS is the most promising rumen diagnostic (for excess rumen degradable protein), though low concentrations of all metabolites in breath (sub ppm) preclude easy application. Future work should collect or directly analyse breath over longer periods to ensure representative sampling of rumen gas.

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Effects of diet protein concentration on digestion and N balance of dairy cows over 3 lactations

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Our objective was to measure effects of diet crude protein (CP) level over 3 full lactations on digestion and N utilization in dairy cows. In total 215 Holstein heifers were assigned to one of 3 rations fed *ad libitum* from first calving (71 or 72 heifers each) and formulated to contain 14, 16, and 18% CP and provide metabolizable protein below, at, and above predicted requirements. Treatments were maintained over 3 full lactations. During each lactation, diet digestion and N balance were measured using 4 cows per treatment at 6, 20 and 34 weeks postpartum using 5-d total collection of faeces and urine. Digestion data for each lactation (n=12) were analysed for effects of diet, week, and their interaction using Mixed Models. Overall, DMI and milk yield were not affected by diet, but milk yield tended to be lower for the 14% CP diet in parity 1 and 2. Intake of N and milk urea increased linearly, and N use efficiency (NUE; milk N/N intake) decreased linearly, with increasing diet CP concentration. Digestibility of DM increased with increasing diet CP concentration for 2nd and 3rd parity cows, but not primiparous cows with lower DMI. In contrast, faecal N as a percent of intake N decreased linearly, whilst N digestibility increased linearly, with increasing diet CP concentration. As N intake increased with increasing diet CP concentration, amounts of N digested, excreted in urine, and excreted in urine as urea increased linearly, regardless of parity. Body N retention also increased with increased N intake in 2nd and 3rd parity cows. Urine N as a portion of manure N, and urea N as a portion of urine N, both increased linearly with increasing diet CP concentration, but faecal N output was not affected by diet CP concentration. Decreasing N intake through reduced diet CP concentration was associated with improved NUE and reduced urine urea and total N excretion.

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The effect of a molasses based liquid feed on fibre digestion, nitrogen utilisation and rumen pH

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Previous *in vitro* rumen fermentation studies found increased nitrogen efficiency, fibre digestion and improved pH when a molasses-based liquid feed was added to a dairy cow diet. Improving fibre digestion can allow greater energy extraction from home grown feeds and reduce the reliance on bought in alternatives. Additionally, improving N retention can lower the environmental impact of animal production therefore supporting greater sustainability on farm. This study investigated the effect of incremental diet inclusion of a molasses-based liquid feed (Regumix) in a lactating dairy cow ration on fibre digestibility, nitrogen utilisation and rumen pH. Four mid-lactation Holstein dairy cows were randomly assigned to one of four dietary treatments in a 4×4 Latin Square experiment with 4-week periods. Measurements of milk yield and composition, diet intake and digestion, and urinary N excretion were obtained using total faecal and urine collection over the last five days of each period. Reticular pH was measured using a wire-less rumen pH bolus. Cows were fed a control diet composed of grass silage, maize silage and concentrate blend. Treatments were three levels of Regumix included to achieve intakes of 0.7, 1.4 and 2.1 kg as fed, proportionally diluting the other diet components. This resulted in isonitrogenous and iso energetic diets with differing concentrations of carbohydrate fractions. Increasing inclusion of Regumix increased neutral-detergent fibre ($P<0.024$) and acid-detergent fibre ($P<0.088$) digestibility in a quadratic manner, with an increase in NDF and ADF digestibility over control of 33 ($P<0.052$) and 29 ($P<0.043$) g/kg respectively for the 1.4 kg/d Regumix inclusion. N retention increased numerically with increasing Regumix and milk N output numerically decreased, but the effect was not significant. Milk yield and composition, digestion of other diet components and rumen pH were unaffected by Regumix inclusion. In conclusion, adding 1.4 kg of Regumix to a lactating dairy cow ration significantly increased fibre digestion without negatively effecting the rumen environment.

Impact of physically effective fibre on chewing, nitrogen balance, and performance of lactating cow

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The study hypothesized that increasing physically effective fibre (peNDF) concentration of a total mixed ration (TMR) quadratically affects feed intake, chewing time, rumen microbial protein synthesis (MPS), and performance of dairy cows, which in turn will alter partitioning in their nitrogen (N) excretion. Four rumen-fistulated Holstein cows with a mean (±standard deviation) milk yield of 31.9±2.69 kg/d and days in milk (DIM) of 75±8.4 d were assigned to a 4×4 Latin Square with four periods (13 d adaptation + 8 d sampling). The peNDF concentration of a TMR low in rumen-degradable crude protein supply was adjusted by varying its mixing time: 15, 30, 45, and 60 min, corresponding to peNDF concentrations (particles >8.0 mm) of 202, 208, 221, and 238 g/kg dry matter. Data were analysed by PROC MIXED (SAS V9.4) with peNDF and period as main effects, DIM as covariable, and animal as random factor and tested for linear and quadratic contrasts. Nutrient intakes ($P \leq 0.03$), apparent total tract digestibility of organic matter ($P=0.06$), and MPS ($P=0.02$) responded quadratically to increasing peNDF concentration with lower values for the high and low peNDF than both medium peNDF diets. Similarly, eating and total chewing times were longer for both medium peNDF diets ($P \leq 0.02$), while rumination time was similar ($P \geq 0.28$) across diets (all in min/d). Milk yield and composition did not differ between diets ($P \geq 0.10$). Partitioning of N excretion responded quadratically to increasing peNDF concentration, with a lower proportion of the ingested N excreted via urine ($P<0.01$) and a greater proportion secreted via milk ($P<0.01$) for the high and low peNDF than both medium peNDF diets. Results confirm the quadratic effects of increasing peNDF concentration on tested variables. Partitioning in N excretion of dairy cows can be adjusted by changing the peNDF concentration of their TMR. Financial support by the European Union's Horizon 2020 research and innovation program (Project 'SmartCow'; grant agreement no. 730924) and the H. Wilhelm Schaumann Stiftung is acknowledged.

Validation of eating duration assessment from automatic feeder systems

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The aim of the study was to test if automatic recordings of when a cow enters and exits a feed bin were comparable with visual observations of the same, and how well total visit duration corresponds to eating duration. Three feed bin systems were tested: Insentec Roughage Intake Control (RIC); BioControl Control and Record Feed Intake (CRFI); MooSystem Intake Control Feeding System (MF). All cows were used to the systems before the study started. The cows' behaviour was recorded using direct observations by a person standing in front of the bins (RIC, MF) or from video recordings (BC). Eating duration/visit was the summary of head angled down, taking a bite and chewing. Total visit duration included timestamps of the cow entering the bin until exiting. Observations with visit durations <10 s (biologically irrelevant visits) or >2,333 s (1% longest durations) were considered outliers and removed. We divided visits into three classes according to their observed duration: short <150 s; medium 150-599 s; long ≥600 s. To analyse how accurate the system can predict total visit duration, and whether the total visit duration can be used to predict eating duration, the observed duration of visits and duration of eating were tested in mixed models, with class of visit duration, the type of feed bin system, and the interaction between the two as fixed effects. Cow was included as random effect. The analysis was repeated for short, medium and long visits. Duration of a visit to the feeder can be estimated with very high accuracy in all three systems. The mean difference between observed and system duration was -0.21±0.14 s for RIC, -2.63±0.29 s for BC and 0.40±0.19 s for MF and differed between systems ($P<0.01$). Duration of eating can also be estimated with high accuracy, slope was 1.00±0.035 for short, 0.98±0.016 for medium and 1.00±0.011 for long visits. Cows with RIC spent more time not eating when visiting the feeders than cows in BC or MF, which could be due to the feeder design.

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Definition of indicators of activity from data provided by wearable sensors in dairy cattle

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The use of animal sensors offers the opportunity to continuously monitor and record specific behavioural or other biological/physiological measures in livestock species, including dairy or beef cattle. The sensors provide continuous dynamic signals that can be analysed and modelled to obtain quantitative characteristics as possible novel biomarkers for phenotyping cows. These biomarkers vary in time and between animals, and might have predictive value with respect to the ability of the animal to respond to disturbances. We investigated ways to acquire quantitative metrics describing dairy cow phenotypes from their behaviour recorded with the use of sensors. We examined ways to visualize data (e.g. so-called actogram), as well as basic mathematical operators (sum, average, weighted sum) and statistical methods (variance, RMSSD, autocorrelation, multivariate analyses). More sophisticated modelling approaches were used such as Fast Fourier Transform, and a number of ad-hoc operators were developed. These calculations were applied to data from accelerometers (Nedap Smarttags) and a position system (GEA CowView). Descriptors are proposed for the time budget of cows, the activity level, the distribution of activities or their level within days and across days, the rhythmicity or regularity of behaviour (i.e. diurnal rhythm, the extent to which an animal performs the same behaviour at the same times from one day to the next), and the synchronization of activities between cows from the same groups. These descriptors can then be used to predict complex aspects such as health disorders or welfare state, contributing to the monitoring and the phenotyping of cows for both operational and strategic management purposes.

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Evaluation of strategies for summarizing acceleration data to monitor calving in rangeland systems

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PLF technologies offer a great potential to improve efficiency. In the case of cattle, calving is a key event both in terms of production and animal welfare. Calving monitoring based on accelerometer data has been widely addressed in scientific literature and some commercial devices are available, most of them designed to be used indoors. However, those PLF tools are not valid for rangeland systems due to connectivity constraints. In rangelands, acceleration data need to be transmitted wirelessly over long distances to allow real-time monitoring of calving. Low Power Wide Area (LPWA) networks have been deployed for the Internet of Things, allowing long-range data transmission, but the number and size of data packages that can be sent through them are limited. Thus, high-resolution data gathered by accelerometers cannot be directly transmitted using LPWA networks. The objective of this work is to evaluate different data summarization strategies to build meaningful indicators for calving detection that can be transmitted through LPWA networks. Fifteen beef and dairy cows were equipped with 3D accelerometers mounted on collars, which gathered high-resolution data. Cow behaviour around calving was videorecorded and labelled. In most cases, acceleration data showed an increased motion index before calving and changes in the axes registering gravity during and after calving. Based on these results and considering the restrictions on the size of data to be transmitted imposed by LPWA networks, various data summarization strategies were designed and evaluated in relation to their ability to detect calving. Large differences in calving detection capacity among strategies were found, as well as the need for further work to implement project results on commercial devices. Additional acceleration data are currently being gathered at INRAE Le Pin as part of SmartCow EU project.

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Daily activity budget of beef cows under occasional feed restriction periods throughout lactation

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The behaviour of cows can be affected by environmental conditions, farm management and social structure of the herd. The aim of this study was to evaluate how the daily activity budget of beef cows was affected by feed restriction, energy balance and stage of lactation, by using an activity sensor. Thirty-one lactating Parda de Montaña beef cows were individually fed at a flat rate with a diet that met 100% of the energy requirements of the average cow. On months 2, 3 and 4 post-calving they were under feed restriction (55% of the requirements) during 4 days (d). All cows were equipped with a Medria® Axel collar accelerometer, which logged physical activity continuously at 5 min intervals throughout lactation. On each month data were collected before, during and after restriction (Basal, Restriction and Refeeding periods, 4 d each), selecting only the single most dominant activity among the 5 recorded (ingestion, rumination, rest, other and over-activity). The cows were clustered according to their performance and energy balance (EB) into Balanced (BAL) and Imbalanced (IMBAL) cows. Differences in ingestion, rumination and rest were found among periods, ingestion (142, 115, 138 min/d in Basal, Restriction, Refeeding) and rumination (385, 332, 371 min/d) decreased in Restriction period while rest increased (544, 626, 479 min/d). The magnitude of these changes differed among months ($P<0.001$), the largest differences in ingestion occurred in month 2 and in rumination and rest in months 3 and 4. Ingestion and rest were affected by the interaction between EB cluster and period ($P<0.001$). The IMBAL cows showed differences in ingestion (159, 121, 159 min/d in Basal, Restriction and Refeeding periods) and rest (529, 639, 447 min/d) whereas ingestion did not change among feeding periods in BAL cows (125, 110, 117 min/d) and differences in rest were lower (560, 614, 511 min/d). We can not ascertain whether this was partially the cause or the effect of their different energy balance. In conclusion, under feed restriction the time saved from eating and rumination was reflected in longer resting time, and patterns were affected by cow energy balance and lactation stage.

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Evaluation of the ingestive behaviour of the dairy cow under two systems of rotation with slope

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The ingestive behaviour of grazing animals is modulated by the vegetation characteristics, topography and the type of stocking method. This research was carried out in 2019, at the Rumipamba CADER-UCE. It aimed to evaluate the impact of two contrasting stocking methods of dairy cows grazing a pasture with an average of slope >8.5%. Four dairy cows were set to graze a 0.4 ha paddock for 5 days for continuous stocking methods, while for the electric fence methods the dairy cows were restricted to 0.2 ha and the fence was moved uphill every 3 hours, repeating this process four times a day. Cow were equipped with activity sensors for 12 h per day. The whole procedure was repeated 2 times after realizing an equalization cuts and both paddocks, a rest time of 30 days and a random reassignment of paddocks to one of the treatments. The cows showed a difference in terms of the percentage of grazing $P=0.0072$, being higher with the electric fence (55% of the measurement time). From rising-plate-meter estimates of available biomass along the grazing periods, we calculated despite similar forage allowances (electric fence = 48.06 kg DM/cow/d and continuous = 48.21 DM/cow/d) a higher forage intake was obtained in the electric fence treatment (17.5 kg DM/cow/d) compared the continuous stocking (15.7 kg DM/cow/d) ($P=0.006$). In terms of milk production animals grazing under the differences electrical fence stocking method tended ($P=0.0985$) to produce more milk (17.39 kg/d) than those grazing in the continuous system (15.16 kg/d) due to the influence of the slope ($P=0.05$), while for milk quality the protein content was higher for the electric fence (33.7 g/l) than the continuous method (30.5 g/l) ($P=0.039$). None of the other milk properties differed between methods ($P>0.05$).

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Effects of phytogenic feed additives on performance and enteric methane emissions in dairy cattle

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Methane (CH_4) is a by-product of rumen fermentation and potent greenhouse gas. Due to the rising challenge of climate change strategies to mitigate ruminal CH_4 emissions are increasingly required. Phytogenic feed additives (PFA) are in discussion to fit this need as they are known to have the potential to influence ruminal fermentation. The objective of the current study was to use 3 different PFA with the same level of Cinnamaldehyde (CIN) and Condensed tannins (CT) and increase the level of Garlic oil (GO) to investigate their long-term efficacy on total dry matter intake (DMI, kg/d), CH_4 production (g/d), milk yield (kg), and milk composition in dairy cows. For that, 56 mid-lactating multiparous Holstein cows (120±46 days in milk) were allocated to 4 balanced groups fed with 4 dietary treatments (n=14 animals/group/treatment): control (CON), without PFA in the diet; prototype 1 (PP1), prototype 2 (PP2), and prototype 3 (PP3) with the addition of PFA (25 g/cow/day). After 4 weeks of the basal diet (75% forages based on hay:haylage 25:50, and 25% concentre, DM basis) cows were gradually switched to an experimental diet for 2 weeks and continued the experimental diets for 3 months. Feed intake, body weight (BW), and milk yield were automatically recorded daily during the whole trial as well as CH_4 emissions (g/day) using 2 GreenFeed systems. Intake and milk yield were similar for diets supplemented with PFA compared to CON [CON (15.6±2.92), PP1 (17.9±2.44), PP2 (17.3±4.14) and PP3 (18.9±4.52)] (kg/d DMI) and (kg/d) [CON(23.2±4.07), PP1 (23.2±3.73), PP2 (22.5±4.57) and PP3 (24.2±4.28), Average ±SD] kg/d milk, respectively. Methane emissions when expressed in g/kg NDF intake appear lower at least for PP3 (49.9±19.17) and PP1 (45.5±9.24) seems to be lower [CON(53.67±12.62), PP2 (51.15±13.38), Average ±SD]. Further statistical analysis is required to evaluate the effect of tested PFA on methanogenesis in dairy cows in the present study.

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Chewing activity and rumen pH of cows adapting to a high grain diet with phytogenic supplementation

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Milk production systems require dense-energy diets, which may have negative impacts on rumen health. The reduction in physical effective fibre impairs rumination and increases rumen fermentation, depressing ruminal pH of cows. The objective of this study was to evaluate chewing behaviour and rumen pH during adaptation to a high grain diet supplemented with a phytogenic mixture of menthol and thymol (PHY) or without (CON). The study included 8 rumen cannulated non-lactating Holstein cows. The experiment consisted of 8 days; whereby, in day 0 cows were fed a forage TMR (45% grass silage, 45% corn silage and 10% grass hay), and thereafter dietary grain was gradually increased by approximately 10% daily. At the last day of adaptation, the diet contained 65% concentrate, 26.85% grass silage and 8.75% corn silage. Chewing activity was recorded using noseband sensors (RumiWacthSystem). Ruminal pH was continuously recorded at 15-minute intervals using indwelling pH systems (Dascor Inc.) placed in rumen ventral sac. Data were analysed with Proc Mixed of SAS with cow as random and day as fixed effect. Mean ruminal pH decreased from day 0 to 7 for both CON and PHY ($P<0.01$). Interestingly, the duration of rumen pH below 5.8 was reduced for PHY compared to CON (210 vs 635 min, and 395 vs 830 min on days 6 and 7, respectively). There was a consistent reduction on rumination time independent of PHY supplementation. However, on day 2, rumination time was greater for PHY compared to CON (592, 453 min, $P<0.05$). In addition, chewing index decreased for CON and PHY during adaptation from 74.2 to 38.7 min/kg DMI for day 0 and 7, respectively. Our 8-day adaptation experiment demonstrated that rumen pH and chewing behaviour are highly influenced by diet composition, particularly by level of fibre inclusion. Our findings indicate that PHY effectively mitigated the detrimental effects of the reduction of effective fibre during adaptation to high grain diet, reducing the duration that pH stayed below 5.8 on days 6 and 7, and increasing rumination time on days 1 and 2 of the adaptation.

Effect of origin and quantity of starch on *in vitro* digestibility and acidogenicity of cow diets

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The aim of the study was to determine the effect of increasing the proportion of cereal grain (10-90% DM of diet) and the origin of starch (fast or slowly degradable) in TMR diets on *in vitro* true digestibility (IVTD, 48 h of incubation) and on the production of organic acids (acidogenicity; AV). The experiment was carried out on 18 model TMR made from corn silage, alfalfa silage, grass hay, soybean meal, and cereal grain prepared in two series; with maize (M) or wheat (W) grains. Initial concentrate to forage ratio was established based on a diet for the lactating cows (35 kg of milk per day). In representative samples of TMR, the chemical composition, IVTD (Ankom, USA) and AV according to Wadhwa *et al*. method were determined. Regardless of starch origin, the growing share of cereal grains in TMR diet resulted in their increased IVTD. In turn, diets containing W grains, compared to M, were characterized by higher IVTD only for 10 and 20% share of grain in TMR (P<0.05). A positive correlation (P<0.01) between the share of grain in TMR and its AV was found. Regardless of cereal grain share, TMR made with W were characterized by higher AV. The equations the estimation of AV value of TMR based on its nutrients concentration were proposed. In conclusion, the determination of AV of feeds and TMR may be a useful method for proper selection of feed ingredients for TMR formulation. In this context, AV is a helpful parameter to define an optimal quantity of cereal grains in TMR which differ in the rate and extend of starch degradation in the rumen.

Long-term effect of feeding a potentiated ZnO source on milk profile and serum Zn in dairy cows

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The effects of feeding a potentiated ZnO (HiZox®; Animine, France) on milk composition, milk fatty acids (FA) profile and serum Zn in lactating dairy cows were evaluated in 12 primiparous and 16 multiparous Holstein cows (milk yield (MY) 32±5 kg/d, DIM 162±90, BW 673±70 kg). Cows were blocked by LN and DIM and randomly assigned to 1 of 2 treatments (HZ, n=14; or CON, n=14) for 12 weeks. Cows were fed a partial mixed ration in feed bin scales (1.68 Mcal/kg NEL, 131 g/kg CP, 375 g/kg NDF, 41.1 mg/kg DM of Zn; DM basis). At each milking, a mix of 500 g of soybean pellets (SB) and SB pellets with 2.4 mg Zn/g of SB were offered to achieve a daily dose of 80 mg Zn/kg DM for HZ group. Cows were fed *ad libitum* and milked twice a day, then DM intake and MY data daily recorded. On week 11, milk samples were analysed for FA profile and milk composition biweekly using MilkoScan FT 6000. On the first and last day, blood samples from 5 cows were collected for Zn concentration analysis. All collected data on performance and milk characteristics was analysed using the MIXED procedure of SAS. The statistical model included the effects of block (random), treatment (fixed) and the random residual error. For the serum Zn, values measured at the start of the trial were added to the model as a covariate. HZ and CON did not significantly differ for MY (28.3 vs 28.4 kg/d; P=0.97) but HZ had a higher DMI than the CON (27.8 vs 24.9 kg/d; P=0.06). Serum Zn concentration tended to be greater for HZ compared to CON (0.94 vs 0.82 mg/l; P=0.08). Milk fat concentration (4.0 vs 4.1%; P=0.60) and yield (1.0 vs 1.0 kg/d; P=0.6) was the same for the HZ and CON. Milk protein and lactose concentrations (%; P=0.43, P=0.99) and yield (kg/d; P=0.42, P=0.99) did not differ among experimental groups. HZ and CON were not significantly different for saturated (71.0 vs 71.4; P=0.85), monounsaturated (24.8 vs 24.7; P=0.93) and polyunsaturated (4.1 vs 3.9; P=0.39) FA. 7 CON cows had a mastitis event vs 2 in HZ. Long-term feeding of a potentiated ZnO improved plasma Zn but didn't change milk production, composition or FA profile.

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Effect of type of silage and concentrate on eating behaviour and relation to enteric methane

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While the relationship between diet composition and enteric methane emission from ruminants is well understood there is a lack of data that compares methane emission with eating behaviour. The aim of this study was to evaluate the effect of 2 silages and 2 concentrates on eating behaviour recorded using the Rumiwatch system, and to quantify the between-cow variation in eating behaviour and methane emission and possible correlations. Twenty-four lactating Holstein cows were used in a crossover study with two 21 days periods. The cows were divided in 6 blocks of 4 cows each, with either 1st or 2nd lactation cows. Each cow received 2 of 4 diets, in a design balanced for carryover effects. Diets were 2×2 factorial arranged, with maize silage (MS) or grass-clover silage (GS), as 100% of forage inclusion and 55% of diet dry matter (DM), and barley (BA) or dried beet pulp (DB), as 21.5% of diet DM. Eight Rumiwatch units were used on 2 blocks simultaneously for 4 consecutive days (d 12-15 for even blocks and d 15-18 for odd blocks). In a staggered progression, each block spent d 19-21 in respiration chambers to record gas exchange. Eating, ruminating and total chewing time, as well as methane emission and DM intake (DMI) were averaged within cow and period. Dependent variables were analysed in a linear mixed model including interaction between dietary factors, and effect of period and block as fixed effects, and cow as random effect. Cows fed MS had lower eating time (358 vs 384 min/d; P=0.04), eating rate (17.2 vs 19.2 min/kg DM; P<0.01) and total chewing time (796 vs 862 min/d; P=0.02) compared to cows fed GS diets. Cows fed BA had lower eating rate than cows fed DB (17.3 vs 19.2 min/kg DM; P<0.01). Average and between-cow variation (model mean ± sd for random cow) were 440±30, 508±10 and 947±40 min/d for eating, rumination and total chewing. Average and between-cow variation for eating rate was 21.5±1.5 min/kg DMI. A negative correlation was found between random cow solutions for eating rate and daily methane production (r=−0.61; P<0.01) but no correlation was found when methane was related to DMI. In this study dietary factors affected eating behaviour, but no relationship between eating behaviour and methane per kg DMI was found.

Session 73 Theatre 1

Dietary tannins affect differently cow milk fatty acid profile according to grazing season

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Ruminant extensive farming systems are characterized by periods with different pasture availability along the year, resulting in nutritional imbalance with well-known implications. Tannins are polyphenolic compounds commonly found in forages that can affect ruminal N metabolism and fatty acid (FA) profile of milk. However, little is known about the effectiveness of dietary tannins according to pasture availability in extensive farming. The aim of the present study was to compare the effect of dietary tannins on cow milk FA profile in two different grazing seasons in the Mediterranean. Two experiments were performed on 14 dairy cows reared in extensive system. The first experiment took place in the wet season (WS) with green pasture availability and the second one in the dry season (DS), when cows grazed on dry stubble. In both experiments, the cows were divided into two balanced groups: a control group and a group receiving 150 g/head/d of tannin extract (TAN). During 23 days of trial, individual milk was collected at day 1, 2, 3, 4, 5, 8, 11, 15, 18 and 23 and analysed. Data from WS and DS experiments was statistically analysed separately with an ANCOVA mixed model. Measurements on milk sampled before the beginning of the trial were included as covariate. Dietary tannins depressed *de novo* FA and increased monounsaturated FA concentration in the first days of WS experiment, but the effect vanished after the first week. Conversely, in DS experiment TAN milk consistently showed lower branched-chain FA concentration and lower C18:1 *t*10 to C18:1 *t*11 ratio and rumenic to linoleic acid ratio. Dietary tannins effectiveness on cow milk quality varies according to the grazing season. Tannins bioactivity seems to be enhanced when green pasture is not available, with potentially positive effects on FA profile and N metabolism. The findings of this study contribute to the European project ProYoungStock.

Effect of nanocurcumin and fish oil as natural anti-inflammatory compounds on calves' performance

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The interest in using natural anti-inflammatory compounds as feed supplements in dairy calves' diets has been increasing over the past decades. Curcumin (CUR) and fish oil (FO) have been known as anti-inflammatory compounds among natural products, which attracted the researcher's attention to use it as a feed additive in animal diets. The present study is the first that compared the effect of CUR vs FO on calves' performance. The aim of this study was to evaluate the effect of supplementation with CUR and FO on Holstein calves' performance. A total of 44 Holstein calves were randomized into four groups: (1) Whole milk with no supplement (CON); (2) Whole milk + 350 mg/kg BW per day fish oil (FO); (3) Whole milk + 350 mg/kg BW per day tallow (TAL, isocaloric control); and (4) Whole milk + 4 mg/kg BW per day nano curcumin (CUR). Treatments were mixed with whole milk and used from day 7 to 60 of age. All calves received the same amount of whole milk in the pre-weaning period (d 7-60) and were monitored until day 75 post-weaning period. All groups received the same diet, based on NRC (2001) recommendations, which contained a mixture of starter and chopped alfalfa hay. Individual daily feed intake was recorded throughout the experiment, while body weight data were collected every 15 days. Blood samples were collected on days 30 and 60 of age. The data were analysed as repeated measures in JMP 13.2 software. The treatment's significance was determined by the LSMEANS Tukey HSD test. Treatments did not have any effect on starter DMI intake, body weight, average daily gain, feed efficiency, and rectal temperature in the pre or post-weaning period and from d 7-75 ($P>0.05$). However, CUR improved calves' health status through a significant effect on blood metabolites, especially in the first 30 days old. The concentrations of glucose, BUN, creatinine, triacylglycerol, and ALT were affected by CUR ($P<0.05$). In conclusion, although treatments did not exert any significant effects on calves' performance, it seems that using nano curcumin could improve calves' health status.

Effect of the administration of a high sugar diet on beef cattle growth performance and rumen health

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The aim of the study was to evaluate the effects of a diet with higher sugar content in partial substitution of starch, on production performances, health status and rumen environment and functionality in fattening beef cattle. At the arrival, 106 Charolaise beef cattle males were blocked by weight and conformation and then divided into two experimental groups differing in the characteristics of the diets: (1) Control (CON) diet (dietary sugar: 3.5% of DM, dietary starch: 40% DM); (2) Treatment (TREAT) diet (dietary sugar: 8.5% DM; dietary starch: 35% DM) obtained through the inclusion of 1 kg/head/day of sugar liquid feed. Growth performances were analysed in terms of body weights and average daily gain (ADG) at the arrival (d0), at day 126 (d126) and at the slaughter (d186). The feed intake and the feed conversion rate (FCR) were evaluated weekly. All the animals were evaluated, by physical examination, daily and data about health status were recorded. The rumen functionality and environment were evaluated in 10 animals per group through the use of ruminal boluses (smaXtec Premium Bolus SX-1042A). Growth performance resulted to be significantly influenced by the treatment ($P<0.001$). Treated animals were heavier at d186 (695.01 vs 682.56 kg; $P<0.001$), due to a better ADG (1.486 vs 1.419; $P<0.001$), resulting also in a statistically higher carcass weight (413.26 vs 404.08 kg, $P<0.001$). No statistically significant differences were found in dressing percentage, and carcass SEUROP score. In terms of health status, animals in the TREAT group had a lower incidence of both bovine respiratory disease (BRD) and lameness, with, respectively 20 and 48.5% reductions. Indeed, TREAT animals showed a lower average daily pH than CON (6.68 vs 6.74, $P<0.05$) and significantly less time with pH lower than 5.8 (0.67 vs 0.14 h, $P<0.05$). The use of a high sugar diet, in partial substitution of starch from corn meal, positively affects rumen health and efficiency, resulting also in better growth performances.

Session 73 Theatre 4

Feed restriction affects protein and microRNA levels in dairy cows' milk

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The aim of this study was to observe the effect of feed restriction (FR) on milk concentrations of major proteins and microRNA in order to identify potential biomarkers of negative energy balance in cows. We hypothesized that the effects of FR on mammary gland metabolism are reflected in milk and its different fractions. Two FR trials were conducted at mid lactation to induce negative energy balance: a short duration and high intensity one (SH) and a long duration and moderate intensity one (LM). The SH experiment involved 8 Holstein cows. The LM experiment involved 19 Holstein cows either in control (n=9) or restriction conditions (n=10). FR lasted 6 and 29 days with a dry matter intake reduced by 64 and 20% of *ad libitum* intake, and induced a negative energy balance of -42 and -21 MJ/d, respectively for SH and LM experiments. Milk was sampled before, during (3 times for LM) and after the restriction periods. MicroRNA, extracted from whole milk, fat globules, milk isolated mammary epithelial cells and extracellular vesicles were sequenced and protein concentrations analysed using LC-MS. Concentration of several major proteins decreased during FR: αS2-casein (LM and SH), αS1-casein (SH), κ-casein (LM), α-lactalbumin (LM), β-lactoglobulin (LM). Among 2,896 microRNA detected in milk, the abundance of 159 microRNA was affected by FR, mostly in extracellular vesicles (n=83) and whole milk (n=85), few in fat globules (n=6), but none in mammary epithelial cells. Among these 159 microRNA, 15 varied in two compartments. These experiments showed that FR affects the abundance of proteins and microRNA in milk. It may reflect modifications of mammary gland metabolism. These results will be further explored by proteomic analysis and by RT-qPCR on more samples. These preliminary results are promising in the search for non-invasive biomarkers of negative energy balance in milk.

Session 73 Theatre 5

Co-expression of miRNAs in rumen fluid and tissue in cattle transitioned to high-grain diet

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MicroRNAs are expressed in several bovine tissues, but their presence and function in the rumen remain unexplored. The aim of this study was to investigate the presence of miRNAs in rumen fluid of dairy cattle and the possible co-expression of miRNA in the rumen wall tissue upon transition from forage to high-grain (HG). Four ruminally-cannulated Holstein cows were transitioned from forage feeding to 65% HG diet over a period of two weeks. Rumen fluid and papillae biopsies were collected at week 1 (forage) and week 2 (HG). Small RNAs were sequenced with Illumina NovaSeq 6000 (1x 50 bp) and demultiplexed reads were analysed using sRNAbench. Sequences were mapped to the bovine genome using Bowtie. Bovine miRNAs and human miRNA homologues were identified using miRBase 22. Using R statistical software, the results were filtered and the number of shared and unique miRNAs between matrices, diets and individuals were compared. Differential expression was calculated using DESeq2. Overall, 377 miRNAs were identified in the rumen fluid, while 638 miRNAs were detected in the papilla samples. Of these, 373 miRNAs were shared between the two matrices. Three miRNAs were found to be exclusively present in rumen fluid (bta-miR-137, bta-miR-2285bh, bta-miR-2285ak-5p). Three members of the miR-2285 family in rumen fluid were uniquely expressed either in forage diet or in HG diet. However, DESeq2 did not identify significant variations in rumen fluid due to the diet (FDR<0.05). To the best of our knowledge, this is the first report of miRNA expression in rumen fluid of cattle under different feeding regimes, and the first co-expression study between rumen fluid and tissue. The high number of shared miRNAs between the rumen fluid and rumen wall suggests a continuous release of miRNAs from the epithelium into the ruminal environment, which may imply a role of these small molecules in the regulation of host-microbiota interactions.

Session 73 Theatre 6

Evaluation of miRNAs in the plasma and leucocytes of cattle fed forage or high-grain diets

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MicroRNAs (miRNAs) are an auspicious prospect of biomarkers, that occur abundantly in circulation. Sub-acute ruminal acidosis (SARA) is a digestive disorder associated with a high-grain diet and identified by a low ruminal pH. Biomarkers are needed to identify cows that are better able to cope with SARA. Four rumen-cannulated Holstein cows were fed a control diet of 100% forage and were also sequenced after one week of adaption to a 65% grain diet. After diet transition, SARA was verified using a threshold of ruminal pH<5.8 for 320 min/d. Using the Illumina NovaSeq 6000 platform, small RNA libraries were sequenced from blood plasma and blood leucocytes of these four Holstein cows for the two time points with different diets. Comprehensive identification of miRNA profiles was carried out with the aim to evaluate miRNAs as biomarkers in cattle exposed to SARA through a high grain feeding period. Read processing and pre-filtering was conducted in sRNAbench and R. Read counts were filtered (>10 reads) and unique and shared miRNAs between diets were identified, comprising a total of 520 miRNAs in plasma and 730 miRNAs in leucocytes. From these, 498 miRNAs were found to be shared between plasma and leucocytes, with 22 being exclusively expressed in plasma and 232 unique in leucocytes. In the plasma, 63 circulating miRNAs were found when cows were fed the high-grain diet that could not be found during forage feeding. A DE analyses revealed ten upregulated (bta-miR-6524, bta-miR-769, bta-miR-1388-5p, hsa-miR-23a-5p, bta-miR-12034, bta-miR-2454-3p, bta-miR-2285aa, bta-miR-331-5p, bta-miR-338, bta-miR-664b9) and two downregulated miRNAs (bta-miR-1306, bta-miR-7857-5p) (FDR<0.05) differentiating between forage and high-grain diet. These results show that miRNAs in systemic circulation are impacted by dietary changes and, as such, need to be assessed for their potential as a biomarker of SARA in cattle.

Session 73 Theatre 7

Effect of *Ceratonia siliqua* on coccidian faecal egg count and dry matter of faeces in light lambs

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Coccidiosis is one of the most common parasitic diseases in young lambs. To control that, alternative methods are required to reduce the risk of drug resistance and food safety issues. This study evaluated the effect of carob pulp (*Ceratonia siliqua*) as a source of condensed tannins in lambs' feed on the coccidian egg count and dry matter (DM) of faeces. A total of 144 weaned male and female crossbreed lambs (41 days old and 15.0 kg of BW) were randomly submitted to one of three isoenergetic and isoproteic concentrates: C0 (no carob), C15 (15% of carob) and C30 (30% of carob) plus straw. The lambs were raised in two batches (winter and summer) and were allocated in 12 pens in each batch (mixed sex per pen). For the coccidian egg count (CFEC, egg/g), pooled faeces samples were collected by rectal stimulation in all lambs of each pen (8 replicates/treatment) at weaning, 50, 65 and 80 days of age. Faeces samples to assess the DM of faeces were collected using the same procedure as CFEC. For each parameter, the CFEC and faeces, were analysed through mixed models with repeated measurements. The results showed similar CFEC at weaning (3,450, 20,475 and 9,981 egg/g; for C0, C15 and C30, respectively, P>0.05). At the age of 65, there were differences in CFEC between C0 and C30 (79,312 vs 28,018±8,755 egg/g; P<0.05), but no effects were observed at day 80 (12,775, 10,344 and 5,169 egg/g; for C0, C15 and C30, respectively, P>0.05). The lambs from the winter batch had lower overall coccidial egg excretion than those from the summer batch at day 65 (33,108 vs 65,287±7,148 egg/g; P<0.05). Nevertheless, results were similar in the rest of days, regardless of batch (P>0.05). The DM of faeces were affected by the treatment, being lower in C0 than C30 (31.8 vs 34.8±0.8%; P<0.05), but similar respect to C15 (33.4%). In addition, the interaction between batch and age showed an increase of DM in faeces for winter compared to summer batch at days 50 and 65 (36.2 vs 30.6 and 35.6 vs 30.2±1.01%; respectively, P<0.05). In conclusion, the inclusion of carob has a positive effect on reducing coccidial excretion at peak of infection (mid finishing period) and consequently improving faecal consistency.

Plasmatic and ruminal metabolomes of lambs divergently selected on residual feed intake

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In ruminants, feed efficiency is a complex trait involving the host and its ruminal microbiota. In meat sheep, little is known about their joint action on animal efficiency. The objective of this work was to explore the ruminal and plasmatic metabolome differences between genetically divergent lines on residual feed intake (RFI). Rumen and blood samples were taken from male lambs belonging to the two divergent lines selected on RFI estimated under a 100% concentrate diet. Samples were taken twice: firstly when lambs were around 3 month-old and fed the same diet as their ancestors did during selection and secondly when lambs were 7-9 month-old and fed a diet with 1/3 concentrate and 2/3 dactylis hay. Rumen samples were taken using an oesophageal probe and blood samples were taken at the jugular vein before being centrifuged to separate plasma from blood cells. All the samples were frozen. Preparation of the samples consisted in several centrifugations and addition of a phosphate buffer. Samples were then analysed using NMR spectrometry and integrated with the *ASICS* R package. Data was then analysed both using a mixed linear model using the sasLM R package and multivariate analysis using *mixOmics* R package (PLS and PLS-DA). Because of the great effect of diet, concentrate and forage based data must be separately analysed. Rumen samples did not present many differences between lines under the concentrate diet and under the forage diet, no metabolite was differently expressed. Multivariate analysis on rumen samples did not separate the two lines well. On the contrary, plasma samples showed interesting differences between the 2 lines with amino acids being (under both diets) more present in plasma samples from less efficient animals than in samples from efficient ones. In both diets, plasma samples from efficient lambs had significantly ($P<0.05$) more citrate than samples from less efficient lambs. It seems that plasmatic metabolome gives a better insight in the understanding of the differences between lines, ruminal metabolome will be analysed with microbiota data in ulterior works.

Effect of inclusion of concentrates and exogenous butyrate on rumen wall development in sheep

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Increased intake of concentrates is known to stimulate ruminal epithelium growth, which is related mostly to increased butyrate (BU) production in the rumen (RU). Thus, BU supplementation can be used to enhance RU epithelium development and efficiency of milk and meat production. However, the effect of BU supplementation on RU may depend on concentrate inclusion in the diet. The aim of this study was to determine the effect of concentrates inclusion in the diet and exogenous BU supplementation on RU wall development in sheep. Forty two rams (27.8±7.3 kg; 9 to 14 months of age) were allocated into 6 groups and fed a diet with low (22.5% of dry matter (DM) of the diet; L) or high (60% DM of the diet; H) inclusion rate of concentrates in combination with high (3.2% of DM of the diet; SBH), low (1.6% of DM of the diet; SBL) or none (SBN) sodium BU supplementation. Hay was used in the diet as a main source of forage whereas barley grain as a main source of concentrates. Dry matter intake was limited to 3% of body weight of animals. After 21 days, animals were slaughtered. The RU tissue was weighted and pH of the reticulorumen (RR) fluid was measured. The samples of RR fluid and RU tissues (from ventral and dorsal sac) were taken for analysis of short-chain fatty acids (SCFA) concentration and epithelium and muscle DM weight, respectively. The pH of RR fluid was lower for H than L groups ($P<0.01$). Butyrate concentration (mmol/l) in the RR fluid was greater for H than L groups and linearly increased with increasing dose of supplemental BU ($P<0.01$). The RU tissue weight was the highest when H was combined with SBH (concentrate inclusion × BU supplementation interaction, $P<0.01$). There was no significant impact of investigated factors on ruminal epithelium and muscle DM weight (mg/cm^2). Results show no impact of inclusion rate of concentrates and exogenous BU in a diet on ruminal epithelium and muscle DM weight but the impact of BU on RU tissue weight depended on inclusion of concentrates in the diet.

Session 73 Theatre 10

Tetraselmis **sp. incorporation in the post-weaning piglet diets: effects on productive performance**

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The microalgae *Tetraselmis* sp. (TSM) is a novel feedstuff with an interesting nutritional profile, particularly regarding protein, pigment and n-3 PUFA contents. It is a putative alternative to conventional feedstuffs such as soybean meal. Such feedstuffs are widely used in pig feeding and production, raising important sustainability concerns. The objective of this study was to evaluate the effect of dietary TSM on growth performance, digestibility and development of the digestive tract of weaned piglets. Piglets were randomly distributed into 4 experimental groups (n=6): Control (no TSM) and three groups fed with 5, 10 and 15% dietary incorporation of TSM, as a replacement of the basal diet. Each piglet was individually housed in a metabolic cage (within a digestibility-driven study) with *ad libitum* access to water. After an adaptation period of 4 days, the trial lasted two weeks. Piglets were weighed each week to determine animal performance. At the end of the experiment piglets were slaughtered, viscera were collected, weighed (stomach, small and large intestine, spleen, liver, vesicle and pancreas) and measured (small and large intestine length). Data was analysed using the polynomial contrasts method to test the effect of dietary TSM. Average daily gain was consecutively lower with increasing TSM ($P<0.05$, linear), similarly to the average daily intake ($P<0.01$, linear). The feed conversion ratio was not significantly affected. The weight of the full stomach decreased with TSM inclusion ($P<0.05$, linear), as the weight of its content ($P<0.01$, linear). Regarding the empty stomach, there was a weight increase in TSM groups compared to the control group ($P<0.05$, linear). The weight of the empty and full small intestine and the weight of the pancreas increased with TSM incorporation ($P<0.01$, linear). The incorporation of TSM negatively influenced the growth performance of piglets. Future information provided by digestibility coefficients will complement these results, allowing a better knowledge of the nutritional values of TSM for weaned piglets.

Session 73 Theatre 11

Influence of fish oil in gestation and lactation diets on faecal microbiota of lactating piglets

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Very little is known about the impact of maternal dietary n-3 LCFA on piglet's microbiota composition. The aim of this study was to evaluate the effect of replacing 1.5% (w/w) of animal fat in the gestation and lactation diets of sows by a fish oil source on faecal microbiota of lactating piglets. Twenty-two sows [Landrace × Large white] were divided in blocks of two as similar as possible regarding body weight and parity, and were randomly assigned to a control or a fish oil (n-3 LCFA) diet. At birth, two low and two high birth weight piglets per sow were selected and faecal samples were collected at weaning. Bacterial V3-V4 region of 16S gene was amplified by PCR and sequenced with an Illumina MiSeq platform. An average of 50,000 sequences were obtained for each sample and, after a quality filtering, they were compared to the NCBI database with the BLAST tool to determine their taxonomies. Statistical analysis were made with R software, using the libraries vegan, for the diversity analysis, and DESeq2 for the differential presence study. No effect of birth body weight was observed and it was removed from the statistical analysis. At *phylum* level, n-3 LCFA faecal samples were more diverse according to the Simpson ($P=0.033$) and Shannon ($P=0.020$) indices than control samples. Independently of dietary treatment, the faecal microbiota was dominated by *Firmicutes*, *Bacteroidetes*, *Actinobacteria* and *Proteobacteria* but no differences were observed between treatments. Only an increase of *Verrucomicrobia* was observed by dietary n-3 LCFA ($P=0.006$). Fifteen differences were observed at family level between treatments, a decrease of *Ruminococcaceae* ($P<0.001$) and an increase of *Clostridiaceae* ($P<0.001$) in n-3 LCFA samples stand out. Regarding known genera that could directly affect animal health, it should be note decreases of *Ruminoccocus* ($P=0.009$) and *Gemmiger* ($P=0.008$), and increases of *Clostridium* ($P<0.001$) and *Akkermansia* ($P=0.011$) in n-3 LCFA samples. To conclude, the inclusion of fish oil in sow diets increases microbiota diversity and modifies bacterial populations in lactating piglets.

An alternative *in vitro* model to investigate immunomodulatory functions of feed additives in chicken

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The functional *ex vivo* analysis, as an alternative to animal testing *in vivo*, of adaptive immune cell responses to stimuli as feed additives requires efficient and pure isolation of peripheral blood mononuclear cells (PBMCs). We established an *in vitro* model with chicken PBMCs to explore direct interactions with feed additives, especially probiotics. The cell culture system was established by testing different anticoagulants for blood sampling, different PBMC isolation methods, different sera, and additional L-glutamine as cell culture supplementation. The cells were cultured in RPMI 1640 medium at 41 °C and 5% CO_2. For immunophenotyping using flow cytometry, antibody sets for the differentiation of immune cell types were established. For validation of a response capacity, a control medium containing 10 µg/ml concanavalin A (conA) was used. The citrate tubes revealed the highest live cell count compared to EDTA ($P<0.05$) and heparin ($P<0.01$) tubes and a higher lymphocytes count compared to 200 µl 0.5 M EDTA ($P<0.05$). The relative leukocyte count was highest when blood was sampled in heparin compared to citrate ($P<0.01$) and EDTA ($P<0.01$) tubes. The relative thrombocyte count was highest in citrate tubes compared to heparin ($P<0.01$) and 200 µl 0.5 M EDTA ($P<0.1$) tubes. However, due to the highest live cell count, we used citrate tubes as the anticoagulant. The dextran-ficoll isolation decreased the relative thrombocyte count significantly ($P<0.01$). For PBMC culture, the addition of chicken serum revealed the highest relative leukocyte count ($P<0.05$) and the lowest relative thrombocyte count compared to foetal calf serum (FCS) ($P<0.05$) after 1 day of cultivation. The cell viability did not differ. Additional L-glutamine had no effect. ConA treatment revealed an activation of CD4 T-helper cells ($P<0.05$) and CD8 cytotoxic T-cells ($P<0.1$) and can serve as a positive control. The *in vitro* model with chicken PBMCs is established that can be used to investigate the immunomodulatory functions of feed additives.

The effect of linseed oil on *in vitro* fermentation kinetics using horse caecal fluid as inoculum

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Oil is commonly used to increase the energy density of the diet for horses with elevated energy requirements. It has been reported that oil can be fed in relatively large amounts without affecting nutrient digestion, but it might affect fibre fermentation negatively. The objective of this study was to investigate how increasing the proportion of linseed oil in relation to sugar beet pulp (SBP) affects *in vitro* fermentation kinetics when using horse caecal fluid as inoculum. *In vitro* fermentation was measured using the ANKOM RF wireless gas production system. Bottles were incubated for 48 h with an SBP:linseed oil ratio of 100:0, 75:25, 50:50 and 25:75. Caecal fluid (inoculum) was collected from 4 cecum cannulated horses 3 h after the morning meal. In triplicate, feeds (1 g) were weighted into 250 ml incubation bottles and mixed with 34 ml of inoculum and 66 ml of buffer and gas production was measured every ten minutes. In SAS, total gas production (TGP; ml gas/g DM) was fitted to the Groot model $TGP=A/(1+(B^C/t^C))$ where A is the asymptotic gas production, B is time at which half of the maximum gas production is reached and C a constant related to the shape of the curve. The effects of feed combination on A, B and C were compared in SAS. Parameter A was highest when using only SBP and decreased with increasing amount of oil ($P<0.05$). Parameter B was lower when using the lowest proportion of SBP ($P<0.05$) compared to the other treatments. Shape of the curve changed as reflected in the constant C, when the SBP:oil ratio decreased ($P<0.05$). Interestingly, the ratio of SBP:oil changed the parameter A more than expected when decreasing the proportion of SBP (100% SBP:238.6 ml gas/g DM). When the proportion of SBP decreased to 75, 50 and 25% the A was 75.1, 46.0 and 21.6% of expected. As the parameters B and C also change with increasing proportions oil, these results indicate that oil has a negative effect on the fermentation in horses when reaching a certain level. Further research on oil supplementation to horses should focus on how oil affects digestion and fermentation in horses.

Relationship between CH_4 measured with sniffer method and dry matter intake in dairy cows

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The environmental impact of dairy cows largely comprises of the methane emissions from their digestion. Methane production is known to have a close relationship with feed/energy intake. Hence, it is important to investigate and understand how much variations in dry matter intake (DMI) could contribute to methane production. Several different methods are used to measure methane emissions from dairy cows. The objective of this study is to investigate the relationship between methane gas production measured with sniffer (infrared (IR)) method and dry matter intake in Swedish Red and Holsteins cows. Methane data from a total of 37 lactating cows from April 2017 – May 2018 was collected. The cows were fed with low starch concentrates restrictedly and grass-clover silage *ad libitum*. We performed a linear regression model with data aggregated in 3 different periods (weekly, fortnight, monthly) to compare the relationships. Pearson Correlation coefficient analysis was also calculated to determine the relationships between the methane production and the total corrected DMI for each period. The average methane production and total DMI were 409.26±57.25 g/day and 24.54±3.50 kg/day, respectively. While the milk yields each day averaged 33.41±6.96 kg. There were significant relationships ($P<0.05$) between the CH_4 production and the total DMI in all periods. However, the adjusted R-squared (R2) values from all analyses have fairly low prediction power, approximately 5-7% of the variation in the measure of methane (g/day) could be predicted by total DMI. The weekly, fortnight, and monthly correlation (R) between total corrected DMI and CH_4 production/day were 0.19, 0.25, and 0.23, respectively. While the relationships between total corrected DMI and CH_4/kg milk yield were -0.28, -0.28, and -0.31, respectively. The weak relationship between DMI and methane gas emission in this study could be due to the variation between cows, the accuracy of the IR measurements, and airflow systems. In conclusion, in the present study, there was a weak but significant correlation between methane production and DMI.

A new integrative mathematic approach to study metabolic trajectories of grazing dairy cows

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Mathematic approaches are needed to study integrated metabolic trajectories of dairy cows during lactation. This study aimed to assess the suitability of the perturbation vector (PERTv) for evaluating individual metabolic trajectories of multiparous cows belonging to two Holstein genetic strains (North American, NAH, n=20; New Zealand, NZH, n=20). At calving, cows were assigned to two feeding strategies differing on the inclusion of directly grazed pasture: 30% (P30, n=20) vs 60% (PMAX, n=20) of dry matter intake. The P30 cows were also fed with a total mixed ration (80:20, concentrate:forage ratio), while the PMAX cows were supplemented with concentrate at the milking parlour and conserved forage if needed. A plasma metabolic dataset corresponding to -45, 21, 100 and 180 days in milk (DIM) was used. It was assumed that the metabolic state at any given time could be described by plasma concentrations of several energy (glucose, non-esterified fatty acids, β-hydroxybutyrate, insulin), protein (3-methylhistidine, urea, total protein, albumin) and redox (thiobarbituric acid reactive substances, protein carbonyls) metabolites. Therefore, the PERTv was calculated as follow: $|PERTv| = \sqrt{(\sum xi^2)}$, in which xi denote the foldchange of each metabolite concentration after log-normalization over the median. It was assumed that $|PERTv| = 0$ reflected the non-perturbated states, while increasing values reflected states of greater perturbation. The results were analysed through ANOVA as repeated measures considering genetic strain, feeding strategy, DIM and its interactions as fixed effects, and the cow as random effect. The PERTv changed ($P=0.05$) through time in a genetic strain by feeding strategy dependent-manner, as it increased ($P<0.01$) between -45 DIM and 21 DIM and then decreased ($P<0.05$) at 100 DIM for all cows, except for NZH-PMAX in which the PERTv remained unchanged until 180 DIM. This determined that NZH-PMAX had the lowest ($P<0.01$) values at 21 DIM compared with the rest. Our results showed the PERTv was able to reflect the increased metabolic load at the onset of lactation and it seems to be useful to address the effect of genotype and feeding strategy on metabolic trajectory.

Skills and knowledge expected by MSc students and professionals in animal sciences

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With the collaboration of the students of the training education Science and Engineering in Animal Production. The young generation wants to play an active role in climate change mitigation and adaptation. They expect adjustments in their education and training. However, the question is what to change? How? Do the students and professionals have similar expectations about training and skills to develop? A survey on students' and professionals' opinions was performed in Jan. 2021 by 17 MSc students involved in animal sciences at the Institut Agro, a high education institution in agricultural sciences in France. A total of 141 professionals in animal sciences (from 6 mo. to 40 yr. after graduation), 142 students from the Institut Agro (BSc, MSc), and 170 undergraduate students intending to candidate to such curriculum (A level, higher national diploma, undergraduate intensive preparatory course) completed an online survey. The survey focussed on: expectations and feedback about the format and content of the training programme; skills required for an MSc student and optimal professional integration. One third of the students from the Institut Agro and the young professionals emphasized the importance of interdisciplinarity, good theoretical knowledge, methodology and soft skills developed during their training. Animal sciences is be attractive to undergraduate students (33%) particularly in the fields related to current agricultural issues such as animal health and welfare, environment and communication. Work-linked training (55% of the Institut Agro's students), limited filed work or knowledge about the business world were the main shortcomings identified. Professional recruiters look mainly for: soft skills (57%) such as learning desire, interpersonal skills; methodology (43%) like organisation, information research; basic farm knowledge (40%); and teamwork (13%). Those skills mainly matched the ones wanted by the students and currently developed at the Institut Agro. This survey showed the high determination of students to be involved in their training, their willingness to take initiatives, and to link their education to the current and future agricultural issues and the demands of the professional sector.

Perspectives for the animal nutrition higher education, employment, and research

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Global forces are demanding more from the agri-food systems. A growing world population with changing patterns of diet requires more sustainable food, including those of animal origin. Thus, livestock production must take place integrating and combining the needs of people and the environment. This challenge implies a revised approach in animal nutrition research, for which is of critical importance the integration of information and decision making for defining feeding plans based not only on productive objectives, but also on health, product quality, and sustainability aspects. In this scenario the future of animal nutrition will be shaped by a human capital trained in livestock applied science, basic disciplines as well as soft skills, process engineering, economics, computer science and communication Young researchers in animal nutrition will be able to deal with production data, environmental factors, health biomarkers, sustainability, nutrigenomics, quality of final products, and safety of the entire production chain, as an integrated whole. The integration of all of these disciplines will be an essential element for the development of a holistic approach to animal nutrition.

Designing a metabolomics approach for identification of novel biomarkers in animal science

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Metabolites are small molecules, present in cells and tissues of all organisms. These metabolites, play important roles in the body, and are involved in various activities. The metabolome, which is defined as the thousands of metabolites in each individual, can be measured by metabolomics analyses. Metabolomics analyses allow to analyse simultaneously a high number of metabolites in various biological sample types. Liquid chromatography mass spectrometry (LC-MS) is a cutting-edge analytical tool utilized in metabolomics studies. While in humans this field is more developed, it has not been extensively applied in non-human animals so far. Our objective was to develop optimized methods for saliva sample extraction and LC-MS data acquisition and analyses in order to identify biomarkers for a specific condition. In this research, a pig saliva model was used for the identification of novel stress biomarkers. Study design included saliva samples collected from 200 pigs, at a group level of 5-10 pigs in their familiar environment and again at the slaughterhouse after 24 hours in the unfamiliar environment, transport, and regrouping. Metabolites were extracted from pig saliva samples using several parallel extraction procedures in order to obtain both polar and apolar molecules for broader metabolite coverage. Then, LC-MS analyses were performed. Initial data analysis of obtained LC-MS data revealed 936 putative annotated compounds. 216 metabolites were significantly upregulated with high confidence, 2.1 to 54.2 times higher on the second sampling when animal welfare was impaired. 630 metabolites were found to be downregulated 2.0 to 333.3 times lower on the second sampling when animal welfare was impaired, as compared to the first sampling, when welfare was higher. In conclusion, the current study suggests a multi-extraction protocol following LC-MS data acquisition protocols for broad metabolite coverage. Moreover, performing untargeted metabolomics methods for the identification of novel biomarkers suggests a promising direction in the future research of stress and animal welfare.

The C3 gene as a potential mediator of metabolic status and reproductive development in bull calves

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Enhanced nutrition and metabolic status during early life can advance sexual development in the bull calf, mediated through the hypothalamic-pituitary-testicular biochemical axis. The arcuate nucleus region (ARC) of the hypothalamus is particularly important in integrating peripheral metabolic cues with GnRH signalling, however, the precise molecular mechanisms regulating this effect remain to be fully elucidated. The objective of this study was to conduct an integrative analysis of global transcriptomic and proteomic datasets derived from the ARC of bull calves offered either a high or moderate plane of nutrition from 2 to 12 weeks of life. Holstein-Friesian bull calves (mean age: 17.5 days; mean bodyweight 48.8 kg), were assigned to either a high (H; n=15) or moderate (M; n=15) energy dietary treatment group. Target growth rates for H and M, groups were 1.0 and 0.5 kg/day, respectively. At 12 weeks of age, all calves were euthanised, the ARC region harvested and global miRNAseq, mRNAseq and proteomics analyses undertaken. Bioinformatic analyses were undertaken to determine differentially expressed (DE) miRNA and DE mRNA and resulted in the identification of 1 DE-miRNA (miR-2419-3p) and 83 DE-mRNA between the two contrasting dietary groups. The C3 mRNA gene was identified as a target mRNA gene of miR-2419-3p, indicating a direct relationship between the two transcripts. This gene was further evaluated through co-regulation network analysis of the proteomics data to determine the interaction of the C3 protein with other proteins identified within the ARC. Network analysis of proteomics data revealed C3 as a key hub protein, as well as indicated a role for proteins involved in insulin signalling towards the regulation of C3, with C3 in turn regulating proteins involved in GnRH signalling. Results from this study indicate a role for miR-2419-3p and C3 in mediating the interaction between metabolic status and reproductive development in bull calves. Funded by Science Foundation Ireland (16/IA/4474).

Session 74 Theatre 3

NMR spectroscopy reveals seminal plasma metabolites as sperm quality/functionality biomarkers in pig

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Metabolomic approaches allow the study of the downstream events of gene expression. Many studies have been conducted in humans in order to determine the influence of seminal plasma (SP)-metabolites in sperm physiology. However, in livestock species, these relationships have not been uncovered yet. The aim of the present work was to evaluate the potential relationship between SP-metabolites with sperm quality and functionality parameters. Twenty-eight ejaculates from healthy and fertile Artificial Insemination-boars were collected and split into two aliquots, one for the metabolomic analysis of SP using nuclear magnetic resonance spectroscopy and the other one for sperm quality and functionality assessments using computer-assisted sperm analysis and flow cytometry. Twenty-three metabolites were identified and quantified in all SP samples and weak to moderate correlations between SP-metabolites and sperm quality and functionality parameters were found. The most relevant relationships with the highest R scores were: (1) the percentage of viable sperm was positively correlated with glutamate ($R=0.46$, $P<0.05$); (2) the percentage of viable sperm with high intracellular H_2O_2 levels showed a positive correlation with methanol ($R=0.42$, $P<0.05$); (3) the percentage of viable sperm with high membrane destabilization was negatively correlated with trimethylamine N-oxide ($R=-0.38$, $P<0.05$); and (4) the percentage of progressive motile sperm was found to be negatively correlated with carnitine ($R=-0.39$, $P<0.05$) and isoleucine ($R=-0.39$, $P<0.05$). The results of this study demonstrated that some SP-metabolites are related to sperm quality and functionality parameters in pigs. These findings suggest that these metabolites could be used as biomarkers to predict quality and functionality of boar sperm.

Session 74 Theatre 4

Prediction of bull fertility based on the sperm methylome

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In humans and model species, alterations of sperm DNA methylation have been reported in cases of spermatogenesis defects, male infertility and poor developmental outcomes. This study was therefore designed to assess the potential of sperm DNA methylation in refining bull fertility predictions. A unique collection of 100 sperm samples was constituted by pooling 2-5 ejaculates per bull, for 100 Montbéliarde bulls of consistent ages, assessed as fertile or subfertile based on non-return rates after 56 days post insemination. DNA methylation profiles of these sperm samples were obtained by Reduced Representation Bisulfite Sequencing. Using methylKit, fertility-related differentially methylated cytosines (DMCs) were identified in 188 genes. Interestingly, 73 genes targeted by DMCs are involved in sperm differentiation and function, fertility and embryo development; out of which 5 were further analysed by pyrosequencing. In order to evaluate the predictive ability of the fertility-related DMCs, the sperm samples were split into a training (n=66) and a testing (n=34) set. Using a Random Forest approach, a predictive model was built from the methylation values obtained on the training set at DMCs. The predictive accuracy of this model was 72% on the testing set, and 69% on individual ejaculates collected on an independent cohort of 20 bulls. This study, conducted on the largest set of bull sperm samples so far examined in epigenetic analyses, shows that the sperm methylome is a valuable source of fertility biomarkers. The next challenge is to combine these results with other data measured on the same sperm samples, in order to improve the quality of the model and better understand the interplay between DNA methylation and other molecular features in the regulation of fertility. Funding: Grant ANR-13-LAB3-0008-01, Apis-Gene and ANRT.

Session 74 Theatre 5

Transcriptomic profiling and functional assessment of omega-3 fatty acids in porcine enterocytes

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Marine and plant-based omega-3 polyunsaturated fatty acids (ω-3 PUFAs) are widely incorporated in animal diet to improve growth and immunity. Especially, ω-3 PUFAs such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) are known to exhibit strong immunomodulatory effects as anti-inflammation and anti-oxidation. However, its molecular activity on intestinal epithelium under inflammatory and oxidative stress is not fully understood. Presently, we evaluated the dose-response, anti-inflammatory and anti-oxidative effects of EPA and DHA against lipopolysaccharides (LPS) challenge in a non-transformed porcine enterocyte model, IPEC-J2. The results showed 24 h treatment with EPA or DHA exhibited proliferative effects in IPEC-J2 cells at concentrations of 6.25-50 μM ($P<0.05$). Further, 24 h pre-treatment of DHA (3.3 μM), EPA (6.7 μM) or DHA:EPA (1:2; 10 μM) increased the mitochondrial activity, decreased apoptotic caspase-3/7 release by two-fold post-LPS (24 h) challenge ($P<0.05$). For the first time, we demonstrated the proliferative and cytoprotective properties of EPA/DHA at low concentrations in IPEC-J2 cells. Increased intracellular mitochondrial activity by ω-3 PUFAs can play a crucial role in preventing enterocyte apoptosis during inflammatory and oxidative stress. Further, to identify the novel molecular pathway of ω-3 PUFAs activity, the gene expression was evaluated by high-throughput transcriptomics technique. cDNA library was constructed and sequenced by Illumina NextSeq. The sequences were further processed and aligned to reference pig genome. Around 293 common and 149 unique differentially expressed genes corresponding to LPS challenge with and without ω-3 PUFAs pre-treatment was identified. Thus, the present outcomes highlight ω-3 PUFA mediated cellular mechanisms underpinning their function in pig nutrition.

Session 74 Theatre 6

Preliminary application of dynamic proteomics to study broiler chicken growth and health status

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Protein fractional synthesis rate (FSR) has been proposed as an early marker for assessing the effect of novel feed compounds in broiler chickens. Measurement of protein FSR has been historically limited due to the necessity to isolate individual proteins for measuring the incorporation of isotopic tracers. Dynamic proteomics has emerged as a novel approach for measuring individual FSR of multiple proteins in a complex mixture. Two Ross 308 broiler chickens were administered an oral bolus of 10 g D2O/kg body weight as an isotopic tracer. One hour later, chickens were injected intravenously with either 2 mg/kg *Escherichia coli* lipopolysaccharide (LPS) or a saline solution. Both chickens were blood sampled and culled 24 h after D2O administration. A third chicken which was not administered D2O was also sampled. Deuterium enrichment in body water was quantified by FTIR analysis of plasma. GC-MS analysis of albumin-bound amino acids was performed to quantify the number of deuterium atoms each amino acid can incorporate, which serves as a proxy for the intrahepatic pool. Plasma peptide deuterium enrichment was measured by high resolution HPLC-Orbitrap MS analysis of FASP-digested plasma proteins with MS1 data mining using Skyline proteomics software. Peptide FSR calculation was performed using a linear model that integrates deuterium enrichment data over time. Protein FSR values are expressed as the mean of FSR estimates in 4 peptides from each protein. LPS-challenged bird and unchallenged bird showed respectively an albumin FSR of 3.5%/day and 12.3%/day, apo A1 FSR of 17.7%/day and 20.7%/day, apo A4 FSR of 22.9%/day and 25.6%/day, fibrinogen FSR of 27.3%/day and 23.9%/day and ovotransferrin FSR of 18.4%/day and 13.3%/day. Simultaneous measurement of individual peptide FSR from multiple plasma proteins has been achieved. This novel approach shows potential for assessing chicken growth and health status and to evaluate the effect of novel feed compounds on broiler chicken protein FSR.

Session 74 Theatre 7

Machine learning tracking immune-related proteins in serum of periparturient dairy cows

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Almost all dairy cows undergo a state of temporary immunosuppression accompanied by some degree of systemic inflammation during transition from late gestation to early lactation. Comparing the serum proteome profile of dairy cows during periparturient period allows insight into the molecular fingerprint of the immune system and may help developing strategies to enhance its functions. Serum samples collected from 16 Holstein dairy cows at d -21, +1, and +28 relative to parturition were depleted of highly abundant proteins and subjected to label-free proteomics analyses based on liquid chromatography coupled with tandem mass spectrometry. Supervised machine learning algorithm 'Random-Forest' (in R software) ranked top differentially abundant proteins during the time. From the proteins associated with immune response, a cluster of six acute-phase proteins, 10 Complement regulatory proteins, and other immune-related proteins were fluctuated during the time. Gene ontology and KEGG analysis revealed enrichment of following pathways: from d -21 to +1, acute-phase response (two up and three downregulated proteins), Complement and coagulation cascades (7 downregulated proteins), response to stress (five down and three upregulated proteins), and cellular oxidant detoxification (one up and one downregulated proteins), and from d +1 to +28 acute-phase response (three down and one upregulated proteins), Complement and coagulation cascades (seven upregulated proteins), regulation of inflammatory response (three up and three downregulated proteins), NF-kappa B signalling pathway (two up and one downregulated proteins), and Toll-like receptor signalling pathway (one up and one downregulated proteins). Overall, this study improved our understanding of the molecular signature of periparturient immunosuppression and inflammation.

Session 74 Theatre 8

Intramammary infection with non-aureus staphylococci modifies water buffalo milk lipidome

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Water buffalo (*Bubalus bubalis*) provides an essential source of milk for human consumptions world-wide. Although regarded as more resistant, dairy water buffaloes can be affected by mastitis with a frequency overlapping those of dairy cows, when hygiene conditions are limited, or when genetic and performance pressures are high. Among the pathogens associated with intramammary infection (IMI) in water buffaloes, non-aureus staphylococci (NAS) have become the most frequent. Given the high percentage of lipid in water buffalo milk (8%) and their importance as critical molecules involved in inflammation, we determined the untargeted lipidome of water buffalo milk during NAS-IMI using a liquid chromatography-quadrupole time-of-flight mass spectrometry (LC-QTOF-MS) approach. The study was carried out on milk from 34 quarters, of which 17 were affected by NAS-IMI, and 17 were healthy (H) ($SCC<200\times10^3$ cells/ml and negative for udder pathogen growth). Sixteen subclasses of lipids were identified in both groups of animals. A total of 2,021 lipid species were identified, of which 82 were significantly changed ($FDR<0.05$) in milk with NAS-IMI. In particular, the abundance of 65 and 17 lipids increased, or decreased, respectively in IMI quarter milk as compared to H. Increased molecules mostly belonged to free fatty acids. We demonstrated that water buffalo milk lipidome changes during NAS-IMI, and identified some fatty acids as potential novel biomarkers for improving mastitis diagnosis. Part of this work was carried out in 'OMICs', an advanced mass spectrometry platform established by the Università degli Studi di Milano.

Session 74 Theatre 9

Altered serum proteomes in newly weaned piglets born from the sows receiving a low ratio of ω6:ω3

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The serum proteome in weaned piglets can respond to nutrition but has not been assessed in relation to the sow's diet. We aimed to determine changes in serum proteomes of weaned piglets that were born from sows receiving diets differing in the ratio of ω6:ω3 fatty acid during gestation and lactation. Multiparous sows were fed one of the following diets: a ω6:ω3 ratio of 13:1 during gestation and ratio of 10:1 during lactation (control ratio or CR, n=8) or ω6:ω3 ratio of 4:1 (low ratio or LR, n=8) from day 28 of gestation onwards. Blood was collected at weaning from their piglets (n=10 per group) and serum aspirated after coagulation. Serum samples were analysed using Tandem Mass Tag-based quantitative proteomics. Differentially abundant proteins were determined by the Mann-Whitney U test. Pathway enrichment analysis was performed using the Reactome database. A total of 552 proteins were identified with >1 unique peptides. Of these, 18 were increased and 12 were decreased in abundance ($P<0.05$) in LR vs CR serum. Serum proteins linked to the innate immune system, including immunoglobulins-like (A1BG), and acute-phase proteins (C5, SERPINA1, SERPING1, TF, VTN, TTR), were over-abundant while immunoglobulin (IGJ), lipoprotein (APOA2), and glycoprotein (F12) were decreased in LR vs CR piglets. Pathway analyses revealed that innate immune system, haemostasis, and platelet degranulation, activation, signalling, and aggregation were the most enriched terms in the LR vs CR groups, while plasma lipoprotein assembly, remodelling, and clearance and diseases of haemostasis were down-regulated. These data suggest how enrichment in ω3 fatty acids in sows' colostrum and milk could positively affect piglet's weaning survival rate and weight gain. Overall, these preliminary results may open up a new perspective of how maternal diet during pregnancy and lactation could alter the serum proteome in their newly weaned piglets.

Session 74 Theatre 10

Muscle proteomics of pre-weaned piglets from sows fed with extreme ω-6/ω-3 fatty acid ratio diets

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An optimal ratio of omega-6 and omega-3 polyunsaturated fatty acids (PUFAs) plays an essential role to maintain metabolic modulations and homeostasis, mainly due to their contrasting inflammatory functions. At present, there are a few studies on how sow nutrition directly affects piglet fat and muscle deposition, especially before weaning. The present study was conducted to determine on how the sow's milk, fed with extreme ω-6/ω-3 fatty acid ratio diets, directly affects the meat quality of the piglets and the expression profiles of proteins. A total of 8 multiparous sows, with an average body weight of 256.56±10.76 kg (mean ± SEM), were used and divided between two dietary treatments with ω-6/ω-3 fatty acid ratios of 13 and 4. The ω6 and ω3 fatty acids for this study were derived from soybean oil and linseed oil, respectively. Piglets were nourished only with sow's milk for 28 d. At the end of lactation, a total of 48 piglets (24 males and 24 females) with an average body weight of 8.16±0.17 kg (mean ± SEM) were selected. Longissimus dorsi muscle samples were collected and stored at -80 °C, and will be subjected to fatty acid and proteomics analyses. The fatty acid analysis will be measured using gas chromatography to obtain the fatty acid methyl esters (FAME) and peak retention times will be verified using standard mixtures and pure standard methyl esters. Moreover, untargeted shotgun proteomics approach based on liquid chromatography-mass spectrometry (LC-MS) will be performed on muscle samples from piglets. Resulting protein profiles will be analysed for differential abundance and will be annotated for enrichment analysis such as gene ontologies and pathways.

Impact of the ratio of ω6:ω3 fatty acids in the maternal diet on milk pig exosome proteome profiles

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Lowering the ratio of ω6:ω3 polyunsaturated fatty acids (PUFA) in maternal diets may promote the health and performance of sows and their piglets. Exosomes are of increasing interest for their prospective use in delivering bioactive cargo, yet the transmission of dietary effects from mother to offspring via exosomes is largely unknown. The effects of two different ω6:ω3 ratios in the sows' diet on the proteome profile in milk exosomes were tested. Sows were randomly allocated to either the control group (CR, n=5) receiving a standard diet (ω6:ω3 = 10:1), or the treatment group (LR, n=5) fed at a lower dietary ω6:ω3 ratio (4:1) during gestation and lactation. Milk samples were collected at day 0, 7 and 14 post-partum. Exosomes were isolated by ultracentrifugation coupled with size exclusion chromatography and characterized by nanoparticle tracking analysis, transmission electron microscopy, and Western blotting for exosome markers. Isolated exosomes were in-gel digested and after TMT-labelling of the peptides, they were subjected to LC-MS/MS. The statistical analyses were performed in R using an in-house developed workflow. The P-values were adjusted for multiple testing by Benjamini-Hochberg method. After exclusion criteria were applied, 319 proteins in total were statistically analysed in each timepoint. Differentially expressed proteins (DAP) were detected at day 7 (2 DAP upregulated in CR) and day 14 (1 DAP upregulated in LR). Albeit few, the presence of DAP provides new insights on how dietary changes could potentially affect the composition of milk exosomes, which could possibly affect health via milk consumption, and newborns' health and metabolism.

Milk-derived exosomes modulate porcine monocyte immune function

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Exosomes are nanovesicles (30 to 150 nm) with an endosome-derived membrane that mediate intercellular communication. They are secreted by almost all cell types to modulate short and long-range signalling events by transferring their cargo (e.g. proteins, lipids, DNA, RNA) to recipient cells and altering their function. Milk exosomes (ME) can enter and modulate immune cells by transferring their regulatory molecules. Since no information on their immunomodulatory ability on porcine monocytes is available, this study aimed to evaluate the *in vitro* impact of ME on porcine monocyte (CD14+) immune response. Monocytes were isolated from the blood of twenty 100 kg healthy pigs (TOPIGS), using magnetic-activated cell sorting technique (MACS), while ME were isolated by ultracentrifugation coupled with size exclusion chromatography. After the removal of LPS, ME were characterized with: (1) Nanoparticle Tracking Analysis (NTA) to determine their size and concentration, (2) Transmission Electron Microscopy (TEM) to visualize their morphology and structure, and (3) Western Blotting to identify the exosome marker TSG-101. First, monocytes were treated with increasing numbers of ME (10^3, 10^5, 10^7, and 10^8), and apoptosis and viability were measured to assess potential cytotoxic effects. Second, ME [10^7] were added to the cells and their phagocytosis, killing capability, reactive oxygen species (ROS) production and chemotaxis were evaluated. ME did not cause cytotoxic effects as apoptosis and viability remained unchanged. ME decreased monocyte phagocytosis and increased their ROS production under normal and pro-inflammatory conditions. No effects on chemotaxis and killing capability were observed. In conclusion, this study provided insights into the role of ME in pig immune response, demonstrating that ME can exert immunomodulatory roles in two inflammatory functions of monocytes.

Session 74 Theatre 13

In vitro immunomodulatory effects of PUFA on porcine monocytes

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Docosahexaenoic acid (DHA) and Eicosapentaenoic acid (EPA) are omega-3 long-chain polyunsaturated fatty acids (PUFA) found in fish oil. They have been commonly used as dietary integrators in human and animal nutrition, modulating the immune system *in vivo* and *in vitro*, mostly by exerting anti-inflammatory activities. EPA and DHA can alter immune cell functions, mainly by causing structural and functional changes in the cell membrane after incorporating or interacting directly with the anti-inflammatory transcription factor PPARγ. Both PUFA can modulate goat neutrophil and monocyte immune function *in vitro*. However, their mechanisms of action are still not fully elucidated, and no information on porcine monocyte immune response is available yet. Therefore, this study aimed to evaluate DHA and EPA's *in vitro* effect on porcine monocytes (CD14+) immune response. Briefly, monocytes were isolated from the blood of twenty 100 kg healthy pigs (TOPIGS), using a magnetic-activated cell sorting technique (MACS). Monocytes were first treated with increasing concentrations of DHA and EPA (25 µM, 50 µM, 100 µM and 200 µM) and apoptosis and viability were measured to assess potential cytotoxic effects. Once determined the molecules' working concentrations (100 µM, 50 µM and 25 µM), their effects on phagocytosis, chemotaxis, and reactive oxygen species (ROS) production were evaluated. DHA and EPA (200 µM) decreased porcine monocyte viability but not their apoptosis. DHA increased the cells' ROS production at 25 µM and 50 µM and decreased it at 100 µM at 60 min in normal conditions. In contrast, EPA increased it at 100 µM and decreased it at 25 µM and 50 µM at 60 min. No effects were observed in pro-inflammatory conditions (PMA challenge). In conclusion, this study suggests that DHA and EPA can exert differential *in vitro* immunomodulatory effects in pigs, by potentiating or dampening their oxidative burst in a dose-specific manner.

Session 74 Theatre 14

The role of citrus and cucumber diets in chicken gut microbiota profile using 16s rRNA sequencing

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The need to find good alternatives to antibiotic growth promoters is a major challenge for the broiler industry. The presence of active molecules such as pectin, limonene, carotenoids and polyphenols in novel plant extracts makes them potential candidates. A dietary trial (10 birds × 4 replicates × 3 dietary treatments) was performed in the experimental poultry facility at University of Glasgow to test the hypothesis that a baseline diet (diet 1) supplemented with citrus (diet 2) or cucumber extract (diet 3) can improve gut health of broiler chickens through the modulation of the gut microbiota. To study the microbiota profile 12 birds per treatment were culled at day 14 and 28 and the DNA of the jejunum and caeca was extracted and amplified by a 16S specific-PCR, sequenced using Illumina Sequencer and analysed with QIIME 2™ at Polyomics, Glasgow. Alpha and beta diversity analysis showed that diet 2 and 3 did not significantly affect the entire bacterial taxonomy. However, a significant difference in the microbial composition was found between the two different tissues (jejunum vs caeca). Bird age also had a small effect (day 14 vs day 28). LEfSe (Linear discriminant analysis Effect Size) was used to identify taxonomic biomarkers modulated by the dietary regimen. Diet 2 influenced the abundance of *Lactobacillus, Rhodococcus* genus and *Streptomycetaceae, Nocardiaceae* families in the jejunum at day 14 and 28 while diet 3 had no effect on this region. On the other hand, diet 3 caused a strong modulation of some bacteria strains (*Bacillales, Rickettsiales* order and *Planococcaceae* family) in the caeca at days 14 and 28. Pycrust and STAMP were used to explore the functions of these bacteria: the majority of bacteria that varied with site, age or diet were mainly involved in chicken's metabolism. In conclusion, results from this study will be used to search novel biomarkers of health status and develop new dietary plans.

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Impact of NGS data trimming on differential gene expression analysis

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First step of every NGS data analysis is performing quality control and filtering. The purpose of this process is to remove low-quality and unreliable data. The aim of this study was to analyse how NGS data trimming impacts the results of differential gene expression analysis. The dataset was taken from European Nucleotide Archive (study accession: PRJNA175445). Data consisted of six RNA-seq samples collected from two groups of bees (*Apis mellifera*): the control group, which was fed honey and pollen, as well as the experimental group fed only honey. First, data filtering was performed using Trimmomatic software. All samples were trimmed five times, each run differing in only one parameter, which was the quality threshold in SLIDINGWINDOW function (respectively: 10, 15, 20, 25 and 30). Then, quantification of gene expression was performed using Kallisto software. Next, using DESeq2 program, differential gene expression analysis was performed, comparing case and control groups within each run. The Kobas software was applied to find significant pathways related to differentially expressed transcripts. The bioinformatic pipeline was also performed for raw (untrimmed) data. The quality threshold for trimmed data had a significant impact on performed analysis. More severe trimming resulted in less transcripts with significant difference between control and experimental groups. What is more important, the results for each run contained transcripts unique for itself which means that information about some transcripts was lost (e.g. differentially expressed transcripts present in severely trimmed runs were not significant for less severely trimmed). Consequently, different biological pathways and gene ontologies were found for each run. In conclusion, NGS data filtering can significantly impact performed analysis. The threshold quality value impacts not only the amount of available data, but also particular transcripts that are detected in differential gene expression analysis, which might lead to wrong biological conclusions. This project was supported by Poznan Supercomputing and Networking Centre.

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Effect of enhanced early life nutrition on the anterior pituitary transcriptome of heifer calves

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Early onset of puberty to facilitate calving at two years of age is a key economically important trait in beef cattle. Enhanced nutrition during the early calfhood period has been shown to hasten the onset of puberty in heifer calves. The hypothalamic-pituitary-ovarian biochemical signalling axis regulates reproductive development in heifers, with the dynamics of gonadotropin pulsatility within the anterior pituitary gland, in particular, central to final sexual maturity. However, the precise molecular mechanisms regulating the influence of metabolic status on this signalling axis in heifer calves is yet to be fully elucidated. The objective of this study was to determine the effect of an enhanced plane of nutrition during early life on the transcriptional profile of the anterior pituitary gland in heifer calves. Angus × Holstein-Friesian heifer calves were offered either a high (HP, n=14) or moderate plane of nutrition (MP, n=15) from 3 to 21 weeks of age. Target growth rates were 1.2 kg/d and 0.5 kg/d, for HP and MP groups, respectively. At 21 weeks of age, all calves were euthanized and anterior pituitary tissue harvested from all calves. RNA was isolated from all anterior pituitary samples and subsequently subjected to RNAseq analysis. Average daily gain was affected by diet ($P<0.001$) and was 1.18 and 0.50 kg/day, for HP and MP calves, respectively. RNAseq analysis resulted in the identification of 178 differentially expressed genes (Padj<0.1; fold change>1.5). Biochemical pathway analysis of DE genes, identified the GnRH signalling pathway as enriched (P.adj.<0.01). Additionally, pathway analysis predicted reproductive system development and function to be affected based on prevailing early life dietary management (P.adj.<0.001). Results from this study show that an enhanced dietary intake during early calfhood affected transcripts involved in subsequent reproductive development in heifer calves, suggesting advanced sexual development as a consequence of enhanced early life nutrition. Funded by Irish Department of Agriculture, Food and the Marine (RSF 13/S/515).

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Major urinary protein knockout leads to lipid accumulation in young male but not in female mice

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The 21 isoforms of the major urinary protein (Mup) are produced mainly in the liver and excreted via urine. In addition to their function in scent communication, they may be also involved in lipid metabolism via a yet unknown mechanism. To elucidate the metabolic phenotype of the entire Mup gene cluster deletion, homozygous male and female CRISPR/Cas9-mediated Mup knockout (KO) mice and their wild type (WT) littermates (n=12 for each sex×genotype combination) with a C57/BL6N background were fed a standard chow *ad libitum*. At three months of age, body weight (BW) was recorded, visceral adipose tissue (VAT) volume was assessed by microCT and feed intake was recorded during 48 h. From a subset of animals (n=6), liver and plasma samples were collected for transcriptomic profiling and analyses of triglycerides (TG). A linear model including sex, phenotype and their interaction as fixed factors was used for data analysis of BW, VAT, and plasma TG. The cut-off for differential expressed genes (DEG) was set to $P<0.05$. The KO mice were heavier (25.8 vs 24.7±SD 3.3 g, P=0.02) and showed a higher VAT volume (381 vs 264±SD 210 mm^3, P=0.03) than the WT mice but had a lower feed intake (8.1 vs 9.7±SD 2.5 g, P=0.02). Plasma TG were higher in male (65.3 vs 45.5±SD 15.7 ng/ml, P=0.01) but not in female (58.0 vs 53.1±SD 7.7 ng/ml, P=0.84) KO mice compared to their WT counterparts. We identified 747 upregulated and 725 downregulated DEG in male KO compared to male WT mice. Subsequent pathway enrichment analysis indicated an activation of monocarboxylic acid metabolism and endogenous accumulation of triglycerides, in male KO compared to male WT mice. Interestingly, this was not observed for female KO mice. In conclusion, we observed a sexual dimorphic role of Mup in lipid metabolism. The Mup accounts for 4.5% of the total hepatic protein synthesis in males and to a much lower extent in females. Consequently, the absence of the Mup gene cluster may reduce the basal metabolic rate to a greater extent in male than in female Mup KO mice, leading to a positive energy balance despite the lower feed intake and, thus, towards increased lipid accumulation.

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Differentiation of intramuscular preadipocytes in low marbling beef cattle

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Intramuscular fat (IMF) is one of the key factors affecting beef quality. It is though a late maturing tissue and the last to be deposited, varying to great extent between genotypes. Some systems and rearing practices produce beef with lower level of IMF of what is considered optimum, such as those in the Pyrenees and adjacent areas, based in local breeds with low tendency to accumulate fat and end-points settled at early ages. In order to investigate the underlying mechanisms leading to a low development of IMF, a model of primary bovine adipocyte culture was used. Samples of *Longissimus thoracis* muscle were aseptically taken from Pirenaica young bulls (374±5.2 d; 585±1.37 kg LW; n=4) after slaughter, immediately placed in saline serum at 37 °C and processed in the laboratory. Intramuscular preadipocytes were obtained by collagenase digestion, cultured and challenged to differentiate. Total RNA was extracted at days 0, 2, 5, 7 and 10 after induction of differentiation. Gene expression of adipogenic and antiadipogenic transcription factors and adipogenic enzymes were determined by relative quantitative RT-PCR using SYBR Green probes; relative gene expression was calculated by the DDCt method, normalizing against β-actin. Expression of the key adipogenic factors PPARG and CEBPA and SREBP increased up to day 5, then decreased and stabilized. WNT10B and NR2F2 are considered to negatively regulate PPARG but while WNT10B seemed to follow a pattern of expression contrary to PPARG (diminishing from day 0 to day 2 and increasing thereafter), NR2F2 had a pattern of expression similar to PPARG and CEBPA. On the other hand, expression of sFRP5, which might play a role in the regulation of WNT10B, increased by day 5, maintaining that level thereafter. Expression of adipogenic genes FABP4, ACACA and LPL were upregulated after the expression of PPARG and CEBPA increased while FASN showed little variation. Then, the results suggest that all the candidate genes studied were upregulated by factors that trigger preadipocyte differentiation in a coordinate manner, allowing the low expression of WINT10B during the first 48 h, the overexpression of the key adipogenic transcription factors PPARG and CEBPA, which in turn, upregulate the expression of adipogenic enzymes.

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Impacts of body condition score on reproductive performance and overall productivity of beef cows

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This study compared reproductive and productive responses of beef cows according to their body condition score (BCS) at initiation of their annual breeding season. Non-pregnant, suckling multiparous *Bos taurus* (Angus-influenced; n=358) and *B. indicus* (Nelore; n=370) cows were assigned to an oestrus synchronization + fixed-time artificial insemination (AI) protocol. *Bos taurus* cows were assessed for BCS at the time of AI, and *B. indicus* cows during the first processing for oestrus synchronization. Cows were classified as BCS<5.0 (*B. taurus*, n=158; *B. indicus*, n=248) or BCS≥5.0 (*B. taurus*, n=173; *B. indicus*, n=122). All *B. taurus* cows were exposed to natural service for 50 d (beginning 18 d after AI), and pregnancy status verified 40 d after AI. *Bos indicus* cows were evaluated for pregnancy status 30 d after AI, and those diagnosed as non-pregnant immediately exposed to natural service for 60 d. Calf birth date and sex were recorded during the subsequent calving season. Calves were weaned at ~7 mo of age, and calf weaning BW also recorded. Pregnancy rates to AI did not differ (vs≥0.16) between cows with BCS≥5.0 or <5.0 (60.9 vs 53.5% for *B. taurus;* 37.0 vs 31.4% for *B. indicus*; respectively). Pregnancy rates to natural service were greater (vs=0.03) in *B. taurus* cows with BCS≥5.0 compared with BCS<5.0 (82.7 vs 67.9%; respectively), but did not differ in *B. indicus* cows (78.9 vs 71.5%; respectively). Calving rate was greater (vs<0.03) in cows with BCS≥5.0, which also calved earlier (vs<0.01) compared with BCS<5.0 (93.7 vs 85.1% in *B. taurus,* 83.6 vs 73.3% in *B. indicus*; respectively). Cows with BCS≥5.0 weaned more (vs≤0.05) calves compared with BCS<5.0, which were older (vs≤0.04) (215 vs 211 d in *B. taurus*, 219 vs 209 d in *B. indicus*; respectively) and heavier (vs≤0.09) at weaning (258 vs 252 kg in *B. taurus*, 213 vs 203 d in *B. indicus*; respectively). Kilo of calf weaned/cow exposed to breeding was greater (vs≤0.03) in cows with BCS≥5.0 compared with BCS<5.0 (221 vs 200 d in *B. taurus,* 159 vs 129 d in *B. indicus*; respectively). Therefore, *B. taurus* and *B. indicus* cows with BCS≥5.0 at initiation of the breeding season had greater reproductive efficiency and overall productivity during a cow-calf cycle.

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Idiopathic male subfertility in a Holstein Friesian AI bull: a case study

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Poor bull fertility can seriously impact herd reproductive performance, particularly in seasonal systems of production. Despite passing routine laboratory tests of semen quality, a significant range in field fertility exists amongst bulls used in artificial insemination (AI). While the contribution of an individual bull's fertility to overall herd pregnancy rate is difficult to quantify precisely, subfertility can have a major impact on productivity and economic returns on farms that heavily use a low fertility bull in a season. One such example is a Holstein Friesian bull released in the Irish market in Spring 2020 which, despite passing all of the routine quality control checks in the semen laboratory, had a phenotypic pregnancy rate of approximately 19% from 2,856 inseminations. To investigate the potential causes of this subfertility, frozen-thawed semen from this bull (3 ejaculates) was independently assessed using computer-assisted sperm analysis (CASA), flow cytometry and *in vitro* fertilisation (IVF). CASA did not reveal any difference in sperm motility or kinematics relative to a control high fertile bull. Furthermore, flow cytometry analysis for a range of parameters including sperm viability, acrosome integrity, membrane fluidity, mitochondria membrane potential, superoxide production, DNA fragmentation and protamine content did not detect any differences between semen from this bull and a control bull (P>0.05). Yet cleavage rate (41.6 vs 91.2%, P<0.05), proportion of 5- to 8-cell embryos at 48 h (a good indicator of developmental competence; 6.4 v 51.8%, P<0.05) and blastocyst yield (7.8 vs 36.8%, P<0.05) following IVF were markedly reduced compared to a control bull. Increasing the sperm concentration used in IVF from 1 to 4 million/ml did not alter the outcome. The genome of this bull is currently being sequenced to investigate the presence of potential causative mutations. Supported by Science Foundation Ireland (16/IA/4474).

The effect of pasteurization in the expression of bovine milk microRNA

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The edaphoclimatic conditions in Asturias (northwest Spain) enable the presence of milk production systems oscillating between farms based on grazing and low consumption of conserved feed and forages as well as a low density of animals per surface, and intensive farms with confined animals, with a higher density, and higher consumption of conserved feed and forages. Identifying milk according to its origin is a useful way to highlight the positive effects of grazing farms on the environment, milk quality, animal welfare, and social aspects. MicroRNAs (miRNAs) are 21-25-nucleotide small RNAs that perform various functions within cells, including gene expression regulation. They can be profiled through microarray, quantitative real-time Polymerase Chain Reaction (RT-qPCR), or sequencing. Their expressions vary according to the genetic context and external factors to the animal (feeding, handling, etc.). Besides, these molecules are resistant to adverse physicochemical conditions, which make them potential biomarkers. In previous studies, a set of miRNAs from raw milk with differential expression according to some ingredients of the diet were determined. However, it is important to evaluate the effect of technological treatments, in milk factories, (pasteurization, fermentation, sterilization, etc.) on these miRNAs. Furthermore, taking into account the potential bioactivity of milk according to its miRNAs content, it is important to assess miRNAs milk content intended for human consumption. In the present study, we analysed the effect of pasteurization on the miRNAs determined as biomarkers. For that purpose, the expression of nine miRNAs was analysed in the same volume of twenty tank milk samples before and after pasteurization. The expression analysis of the different biomarkers in pasteurized and non-pasteurized milk showed that the expression of most miRNAs was lower after pasteurization, although without significant differences ($P<0.05$). Moreover, pasteurization of raw milk affects differently fat and cell fraction. These results suggest that miRNAs are quite resistant to pasteurization.

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