Using (mid-)infrared spectroscopy methods to measure milk composition, energy balance and beyond…. in dairy cows

N. Gengler

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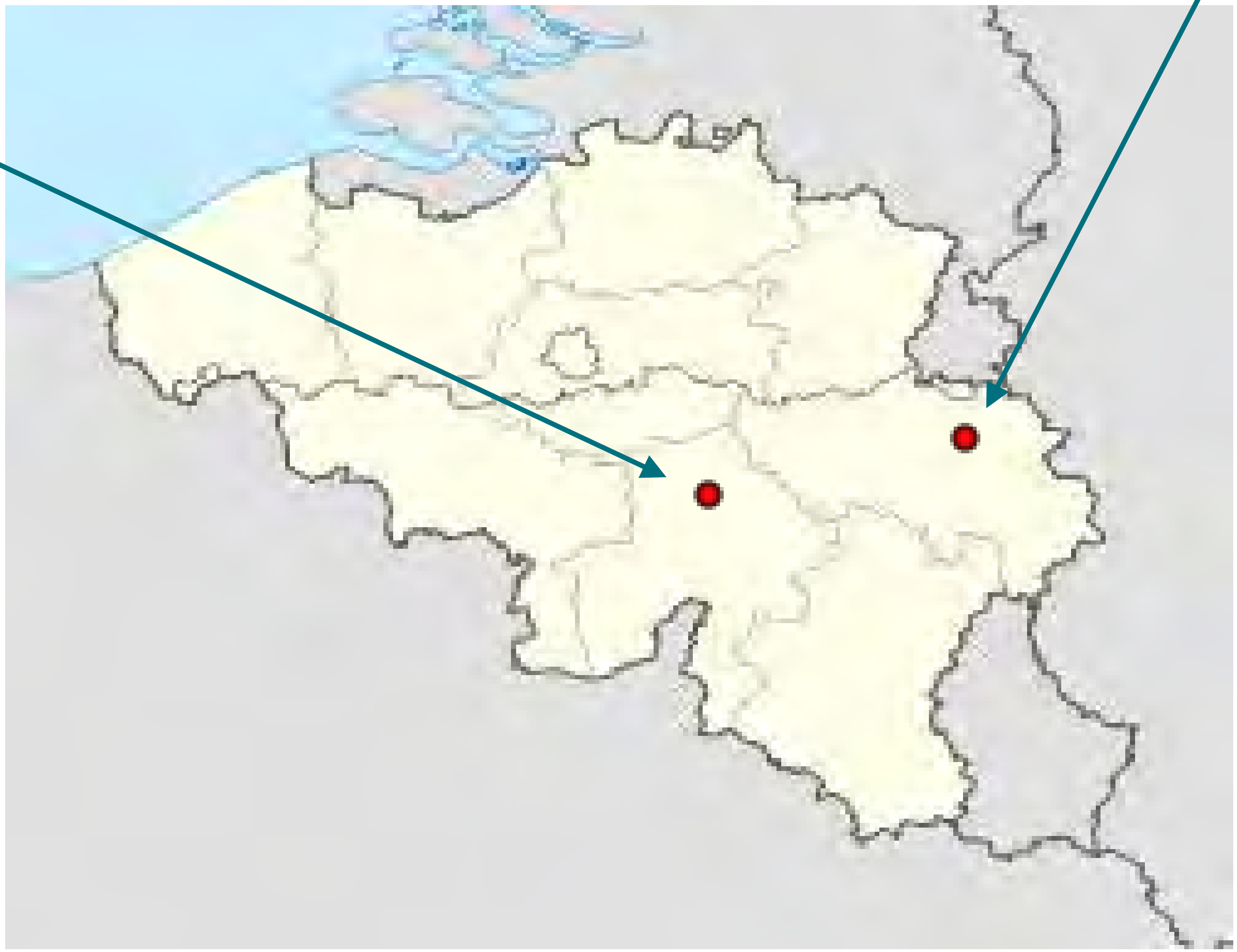
First: where I come from….

Gembloux Agro-Bio Tech and University of Liège (ULg)

LIÈGE université

LIÈGE université Gembloux Agro-Bio Tech



Gembloux and
Gembloux Agro-Bio Tech (GxABT)

LIÈGE université
Gembloux
Agro-Bio Tech

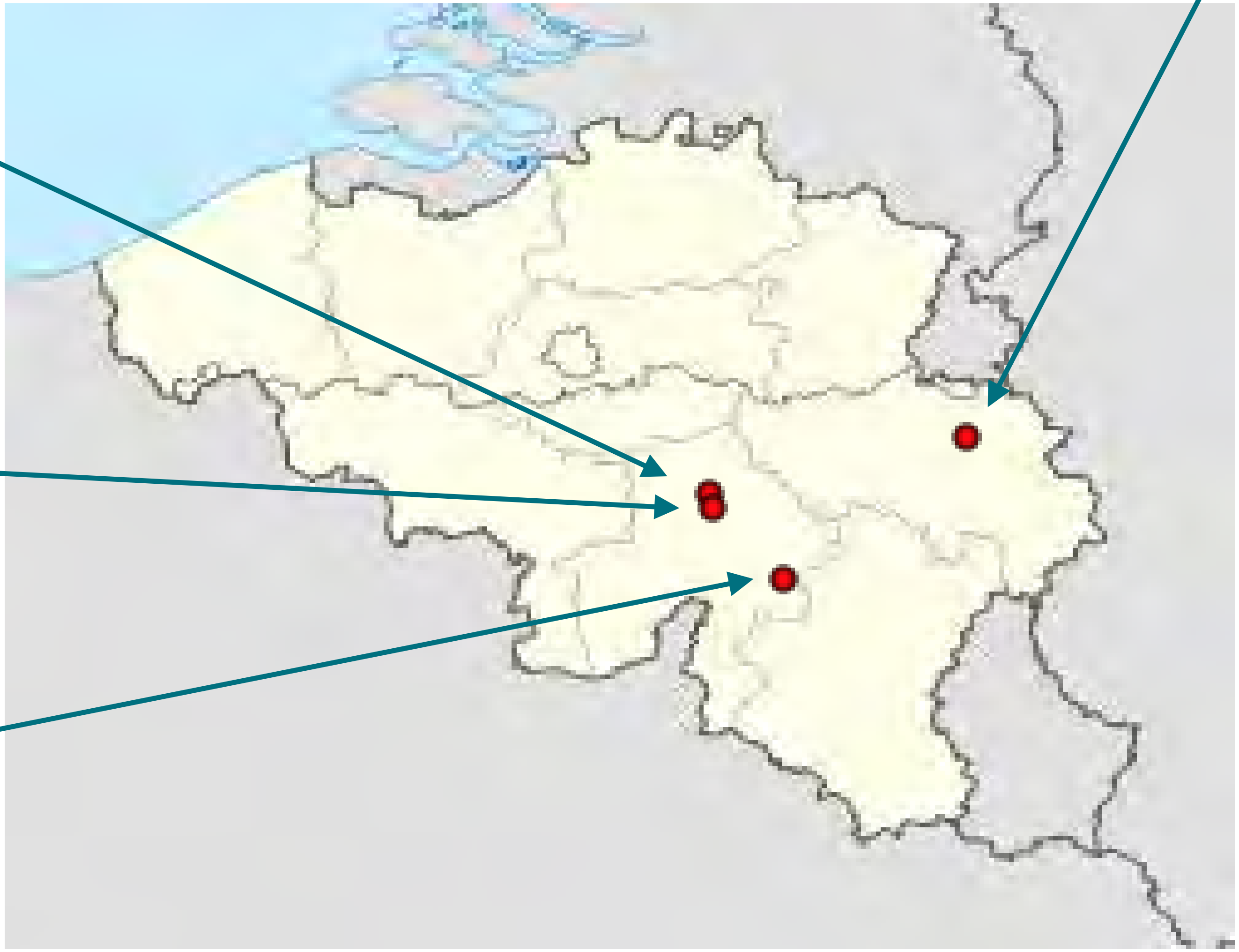
Collaborations inside Belgium

LIÈGE université

LIÈGE université
Gembloux
Agro-Bio Tech

cra-w

awe



Outline of Presentation

I. What is infrared (IR) spectroscopy?

II. Assessing fine milk composition from IR

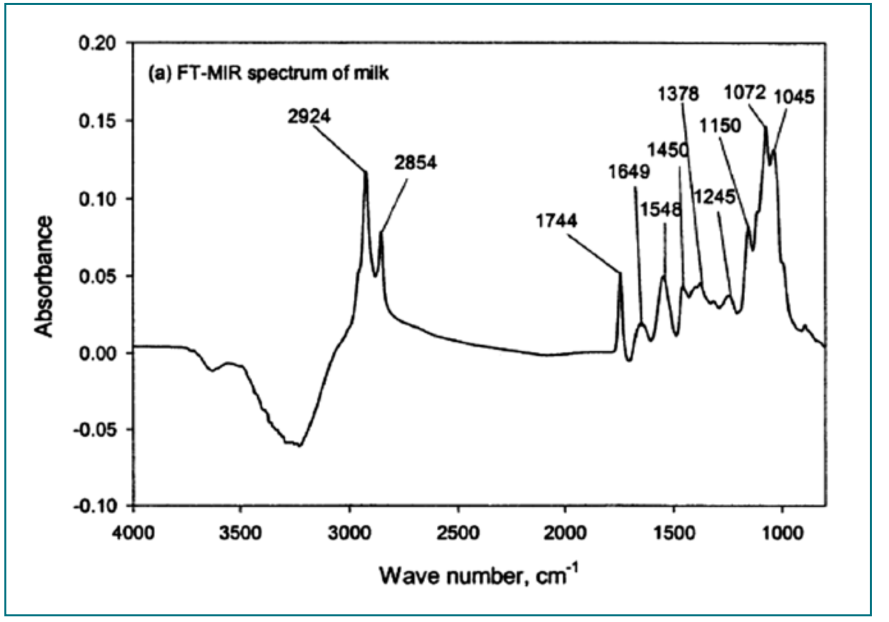
III. Beyond milk composition from IR

IV. Future of IR – ongoing research

What is Infrared (IR) Spectroscopy?

- IR spectroscopy or Vibrational spectroscopy
  - Interaction of infrared radiation with matter
- Large range of techniques, e.g.
  - Absorption spectroscopy (more liquids, gases)
  - Reflectance spectroscopy (more solids)
- Instruments called IR spectro(photo)meters
- Methods often called "Spectrometry"
  - As it is about quantification

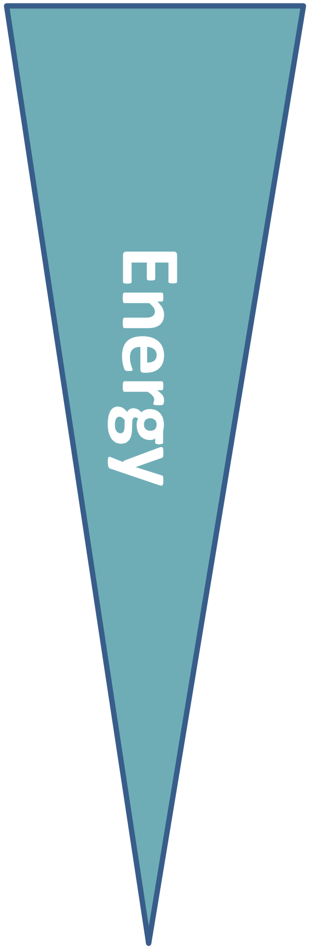
IR Spectrum



- ❑ IR light absorbances (or transmittances) for range of frequencies or wavelengths
  - ➢ Units of IR frequency ➔ reciprocal cm (cm^{-1})
    - ❖ also called "wave numbers"
  - ➢ Units of IR wavelength ➔ micrometers (μm)
    - ❖ also called microns
    - ❖ related to wave numbers in a reciprocal way
- ❑ Different IR ranges

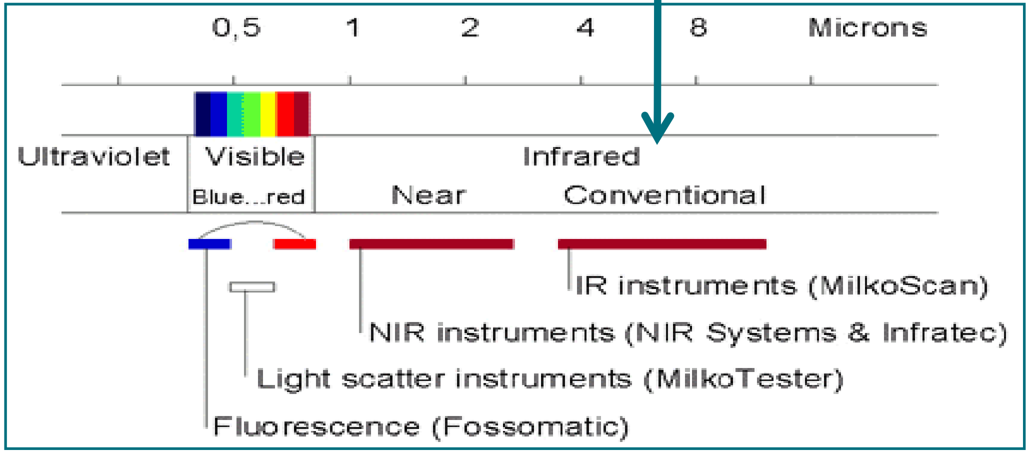
IR Spectral Ranges

- Near-IR (NIR)
  - Approximately 14000–4000 cm^{-1} (0.8–2.5 μm)
  - Can excite overtone or harmonic vibrations
- Mid-infrared (MIR)
  - Approximately 4000–400 cm^{-1} (2.5–25 μm)
  - May be used to study fundamental vibrations and associated rotational-vibrational structure
- Far-infrared (FIR)
  - Approximately 400–10 cm^{-1} (25–1000 μm)
  - Adjacent to the microwave region, low energy and may be used for rotational spectroscopy



IR Spectral Ranges

- Types of IR spectra ranges (here in milk applications)
  - Mid-Infrared (MIR)



FOSS

Typical MIR Spectrometers (milk testing)

- **FOSS** MilkoScan™ 7

FOSS

- **Bentley Instruments**
  DairySpec FT automatic

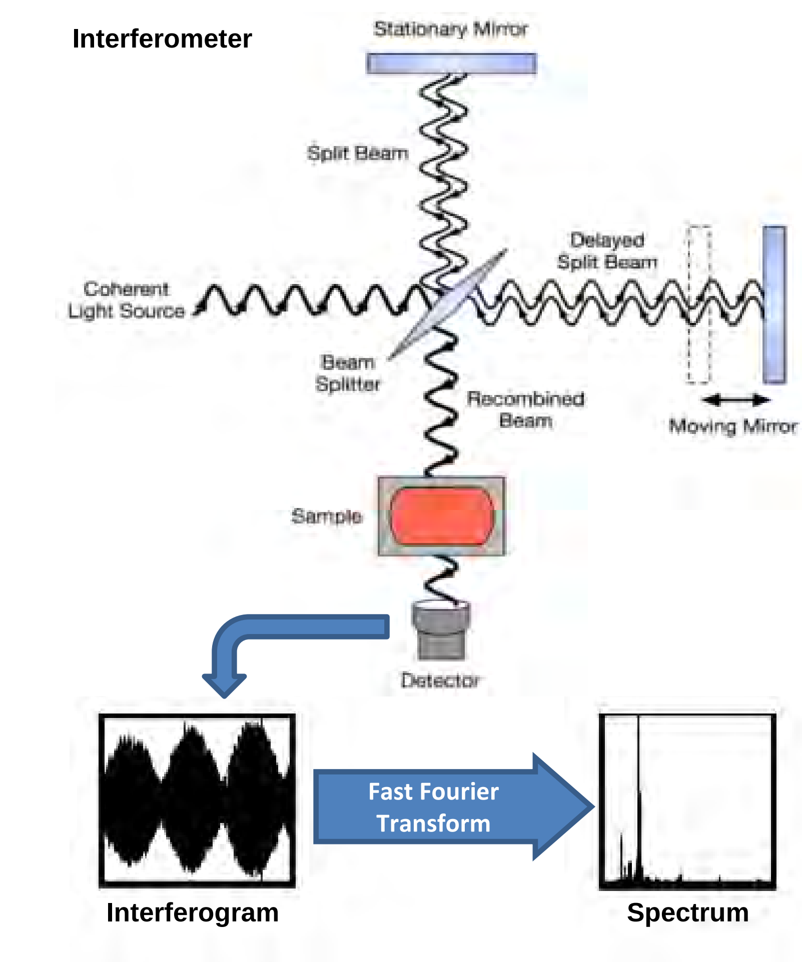
Bentley Instruments

- **Delta Instruments**
  LactoScope FTIR Advanced

Delta Instruments

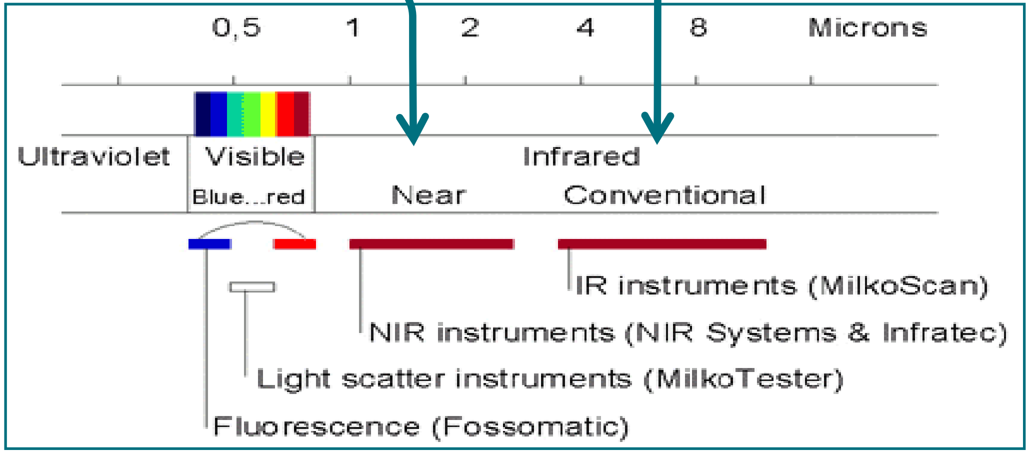
FTIR Spectrometry

- ❑ Use of Fourier-Transform (FT) based technology
  - ➢ (Fast) FT algorithm transforming an interferogram to a spectrum
- ❑ Generally associated to MIR ⇨ FT-MIR
- ❑ In commercial applications often called FTIR (= FT-MIR)
- ❑ But there is also FT-NIR etc.



IR Spectral Ranges

- Types of IR spectra ranges (here in milk applications)
  - Mid-Infrared (MIR)
  - Near-Infrared (NIR)



FOSS

NIR Spectrometry

- ❑ Often called NIRS
  - ➢ Can be absorbance or reflectance (often)
  - ➢ Often also FT based technologies
- ❑ NIR more energy then MIR
  - ➢ Often used on bulk material
  - ➢ Little preparation
    ➔ as feed stuff, cheese (as **FOSS** DairyScan™)
- ❑ NIR less "precise" then MIR
- ❑ NIR less sensitive ➔ ok for less controlled environments
  - ➢ Recently NIR started to be used in in-line on-farm applications
    (as AFILAB by **Afimilk**)

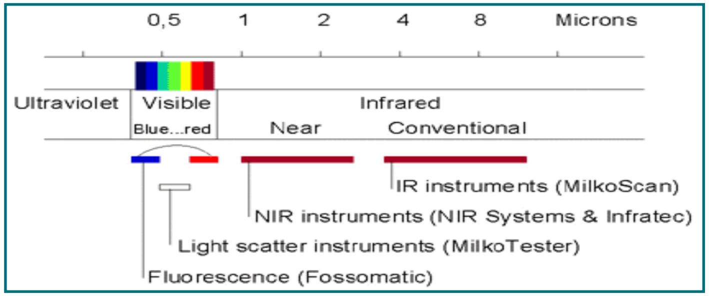
FOSS

Afimilk

II - Assessing fine milk composition from IR spectral data

Milk Composition from IR

- On-farm ➔ NIR (starting)
  - Useful for major components



FOSS

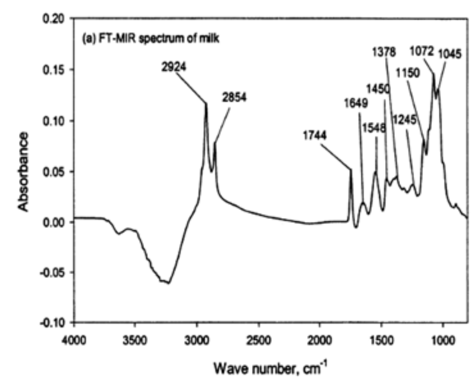
- More common: MIR in central milk test labs
  - Standard method for fat, protein, urea and lactose
  - Existing technology in (nearly) all milk testing labs
  - Used in milk payment and milk recording

MIR Spectrometry

Milk samples

(milk payment, milk recording)

MIR analysis



Raw data = MIR spectra

Calibration equations

Quantification:

fat

protein

urea

lactose

MIR Spectrometry

Milk samples

(milk payment, milk recording)

MIR analysis

Traditional data flow
(no MIR spectra stored)

Quantification:

fat

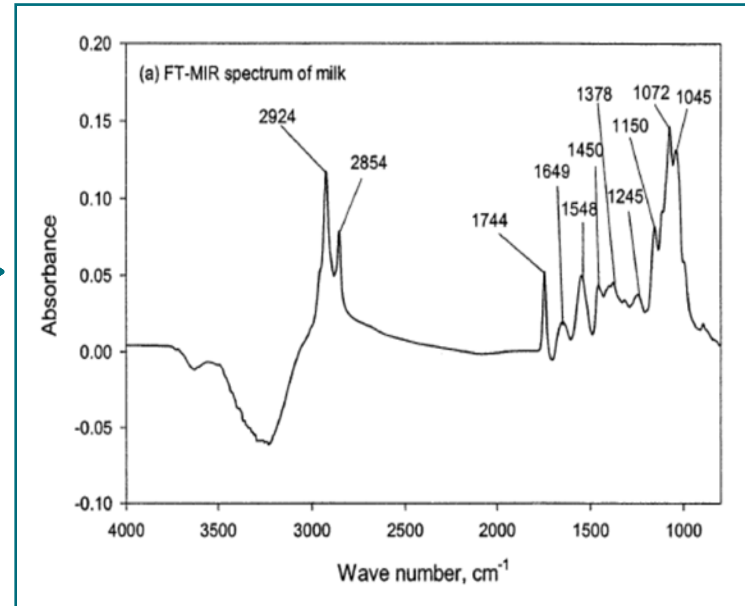
protein

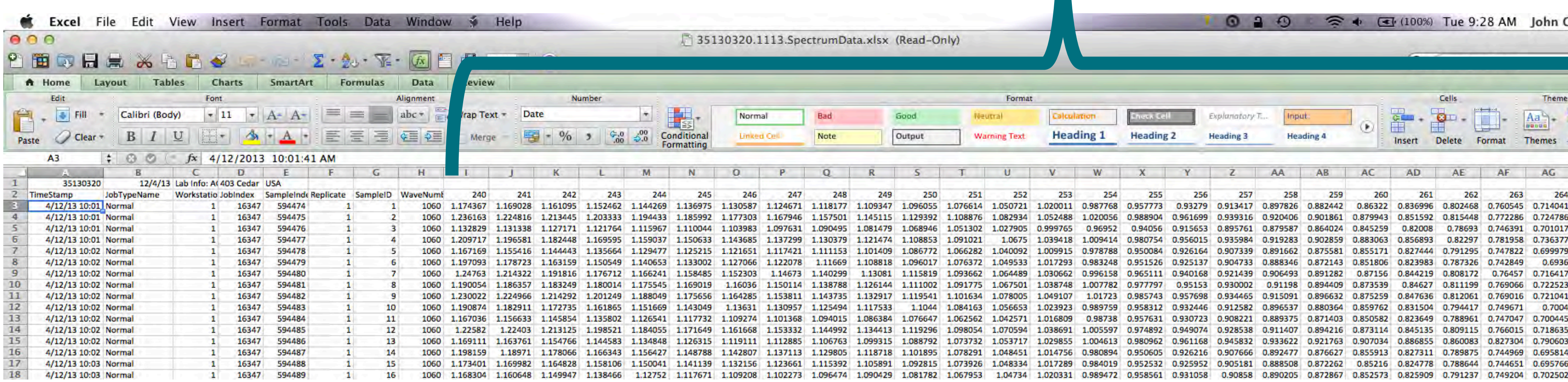
urea

lactose

MIR Spectrometry - Calibration

- Different between brands and models
  - Between 850 – 1060 absorptions values (abs)
- "Calibration"
  - Obtaining b coefficients e.g., Fat% = $b_0 + \sum b_i \, (abs)_i$





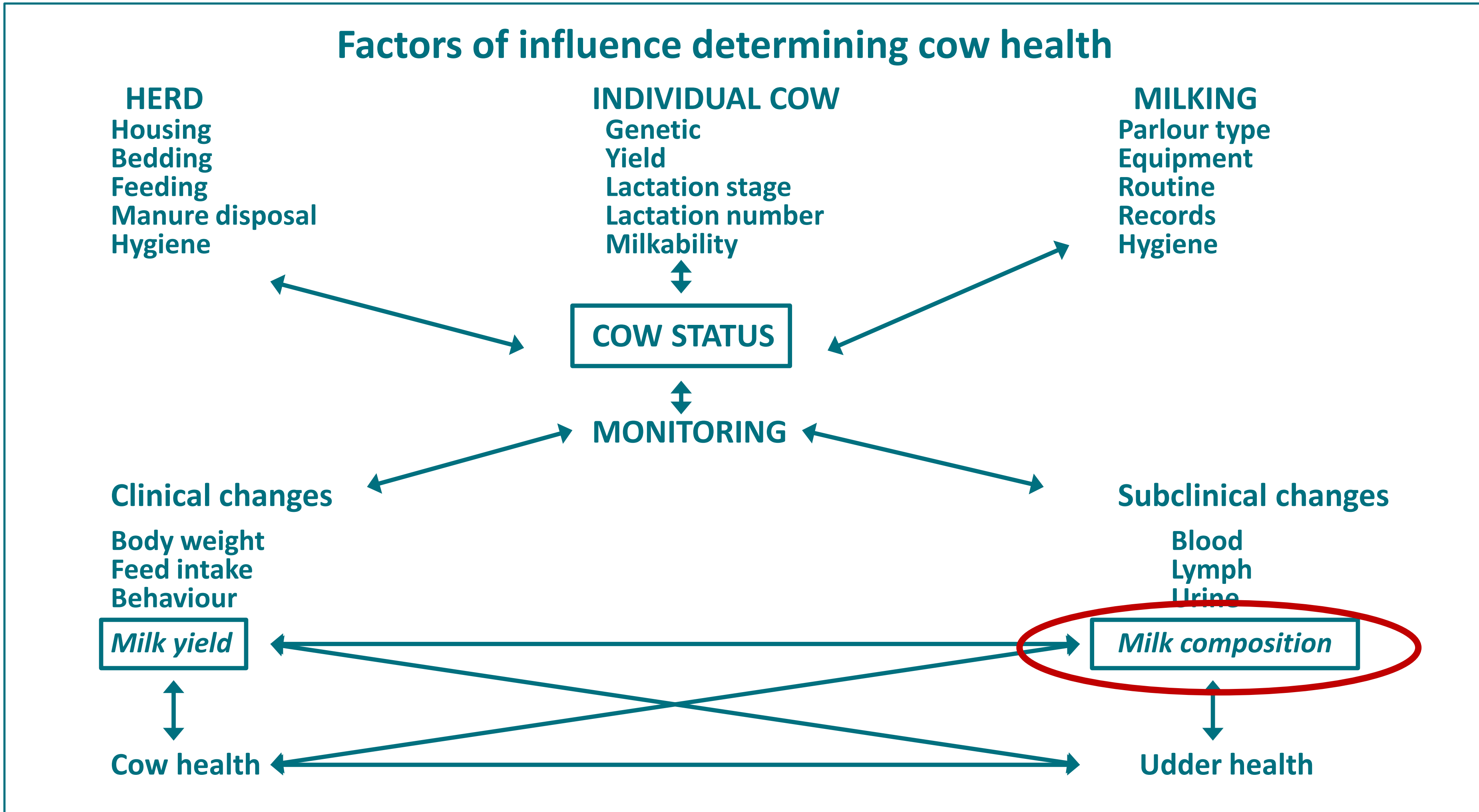
Major Challenge: Data

- Without data
  - No breeding or management possible!
- But data has also to be relevant
  - As close as possible to the processes we follow
  - But always also a cost-benefit issue
    (e.g., health and environmental traits)

Major Challenge: Relevant Data

- Without data
  - No breeding or management possible!
- But data has also to be relevant
  - As close as possible to the processes we follow
- Here enters relatively new concept of biomarkers defined as:
  - "… objectively measured and evaluated … indicator of normal biological processes, pathogenic processes, or … responses to an … intervention" (National Institutes of Health)

Usefulness of Milk Composition!



Hamann & Krömker 1997. Livest. Prod. Sci. 48: 201-208.

Milk Composition

- ❑ Until recently 5 major constituents
  - ➢ Milk fat, protein, urea nitrogen, lactose and somatic cell count (not IR !)
- ❑ However
  - ➢ Milk is a very complex substance with large number of constituents
  - ➢ Some major constituents themselves complex groupings of minor constituents

⇨ many potential Biomarkers

Fine Milk Composition

- Milk fat
  - Fatty acids mostly as triglycerides
  - Non-esterified fatty acids (NEFAs)
- Milk protein
  - Caseins
  - α-lactalbumins
  - β-lactoglobulins
  - Other minor proteins (e.g., lactoferrin)
- Other minor constituents
  - β-hydroxybutyrate (BHB or 3-hydroxybutyrate)
  - Acetone and acetoacetate
  - Minerals
  - Vitamins
  - ….

However Fundamental Problem

- How to get (fine) milk composition:
  - Fast and reliable
  - At reasonable costs

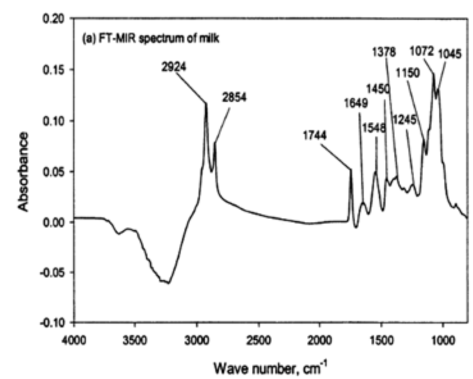
- Idea: following the example of major milk components
  - Using IR, in particular MIR as technology already widespread

Major Milk Components (except SCC)

Milk samples

(milk payment, milk recording)

MIR analysis



Raw data = MIR spectra

Calibration equations

Quantification:

fat

protein

urea

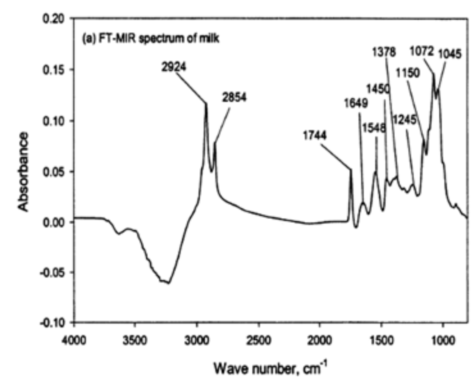
lactose

Novel Traits

Milk samples

(milk payment, milk recording)

MIR analysis



Raw data = MIR spectra

Novel
Calibration equations

Quantification:

Novel traits

Key Issue: Calibration

- Creating linear prediction equations from observed absorbances
  - $P(\text{trait of interest}) = b_0 + \sum b_i(\text{abs})_I$

- Calibration: Highly specialized field in itself

Calibration

- Important to assemble both
  - Reference phenotypic data (“Gold-standards“) and
  - Reference spectral data

- And to cover spectral and phenotypic variabilities
  - Expected range of phenotypes must be covered by range of reference data used in calibration
    - E.g., predicted values expected from 1 to 10, reference data used in calibration process needs to cover this range too
  - Multidimensional space defined by reference spectral data must cover the space expected in the field data
    - Often checked using the GH parameter (Global Standardized Mahalanobis Distance)

Calibration

- Computing spectral prediction equation coefficients
  - Field of “Chemometrics“
  - Numerous multivariate methods:
    - Partial Least Squares (often used),
      but also Ridge Regression, Bayesian methods, SVM, …
  - Also different pre-treatment of MIR data
  - Variable selection, etc….

- Very similar to genomic prediction
  - Spectral data ⇔ SNP Data
  - Methods
  - Variable selection
  - “Sample” selection….

Developing Calibrations - Collaborative Model

- Developing calibration equations through a concerted action
  - New partners join with data (reference ⇔ spectra) and help improve equations
  - Get in exchange access to equation + updates
- Until recently unknown in MIR
  - More usual in NIR ← feed composition
  - In collaboration with Walloon Agricultural Research Center (CRA-W)
  - Consortia were initiated for many novel traits

Indeed…

- Developed calibration equations
  - Have to be validated before use in new populations
  - Different breeds, feeding and production systems may influence prediction accuracies!

- Reasons why new reference data needed:
  1. Validation of existing equations
  2. Introduction of novel variability in calibration datasets

- Shows interest of gradual process with new “populations” joining calibration consortium leading over time to:
  - Variability represented in the calibration data ↗
  - Capacity of equations to adapt to novel circumstances ↗
  - Therefore: general ”Robustness” of equations ↗

Examples of Successful Consortia

- Milk fatty acid (FA) equations:
  - First equations developed in 2005
  - Improved through international collaborations:
    - Belgium, France, Germany, Ireland, UK, Luxembourg, Finland, ….
    - Developed and validated in multiple breeds, countries and production systems

J. Dairy Sci. 94:1657–1667
doi:10.3168/jds.2010-3408

Mid-infrared prediction of bovine milk fatty acids across multiple breeds, production systems, and countries

H. Soyeurt,*†[1,2] F. Dehareng,‡[1] N. Gengler,*† S. McParland,§ E. Wall,‡ D. P. Berry,§ M. Coffey,# and P. Dardenne‡

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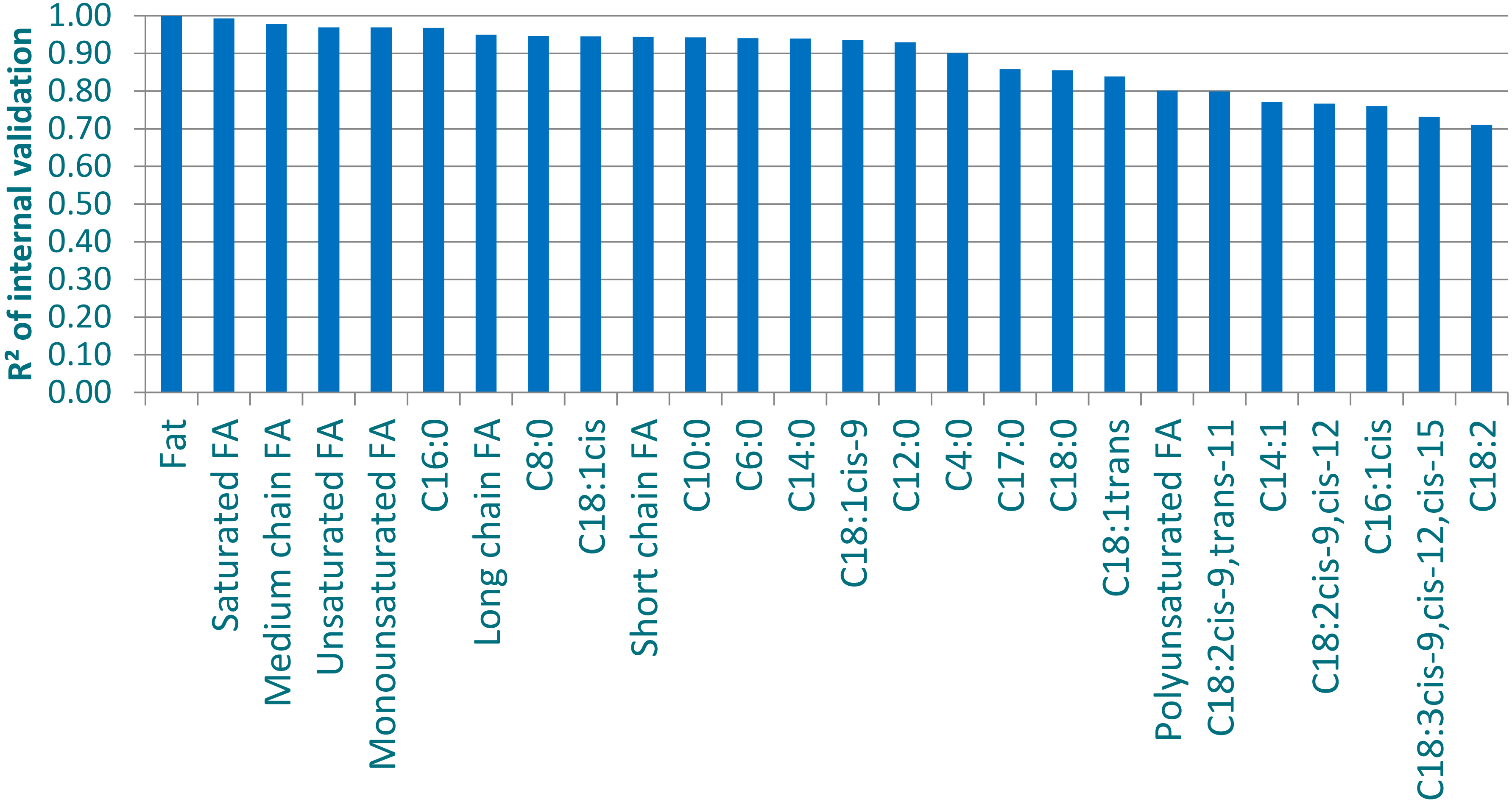
⇨ increased robustness

Accuracy of Fatty Acids Calibration Equations



Calibration equations were developed from at least 1,600 milk samples

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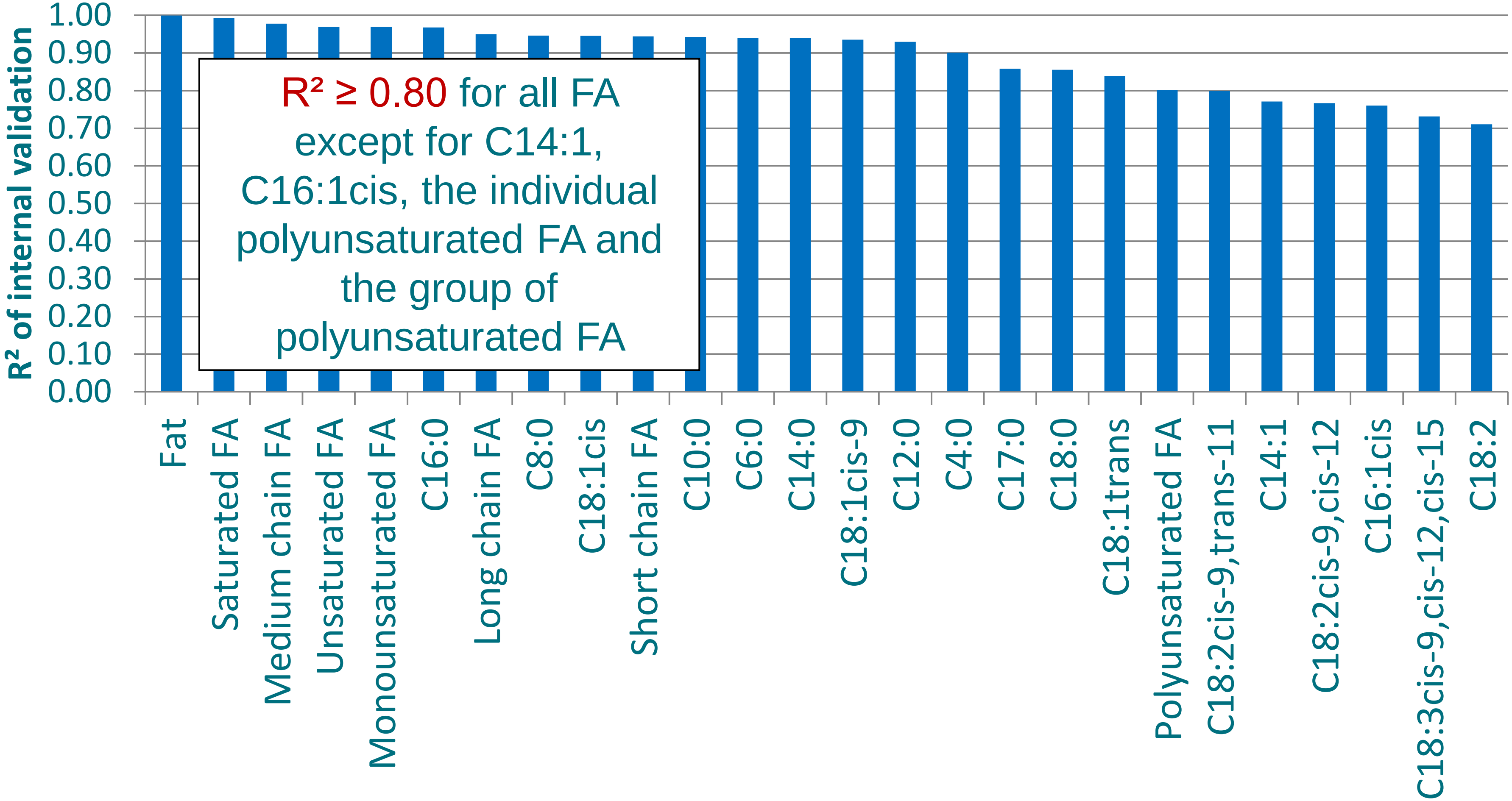

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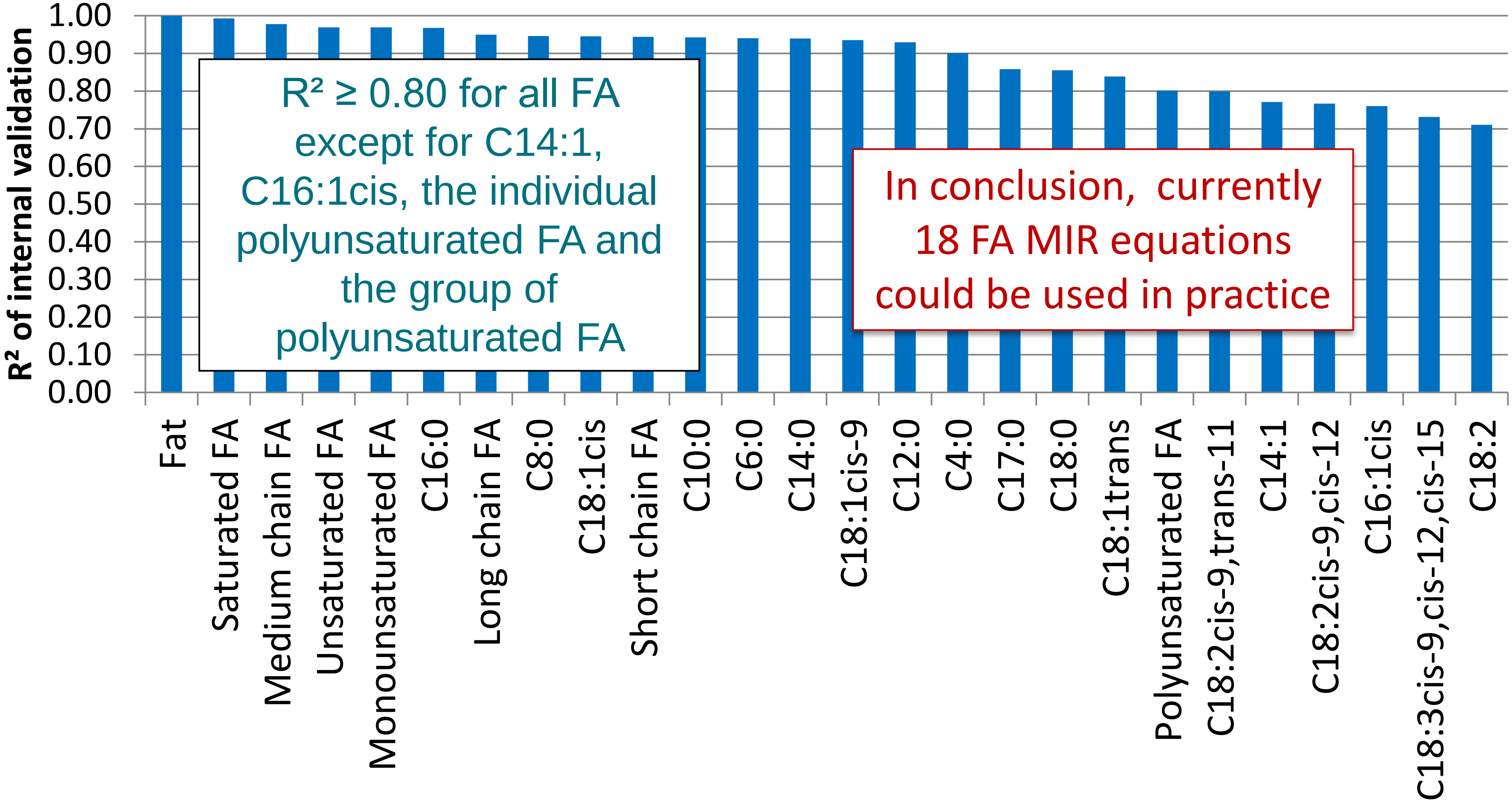

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- Milk mineral equations:
  - First equations developed in 2006
  - Improved through international collaborations:
    - Belgium, France, Germany, and Luxembourg

Examples of Successful Consortia

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  - Multiple breeds, countries and production systems
- Milk mineral equations:
  - First equations developed in 2006
  - Improved through international collaborations:
    - Belgium, France, Germany, and Luxembourg
- Lactoferrin equations:
  - Cooperative effort of Belgium, Ireland and UK ← France

Lactoferrin

- Glycoprotein present naturally in milk
- Involved in the immune system
- Interests:
  - Potential indicator of mastitis
  - Help to maintain a good immune system in Humans
- However R^2 of internal validation = 0.71

⇨ MIR predictor of lactoferrin

- Estimation of Biomarker not without errors

Fine Milk Composition → Biomarkers → "Status"

- Therefore complexity of fine milk composition very useful to assess (some examples):
  - Animal (health) status
    (e.g., ketosis using BHBA, acetone, acetoacetate and citrate)
  - Milk and milk product quality, technological properties
    (e.g., FA, caseins)
  - Udder health
    (e.g., lactoferrin, minerals)
  - And even, as shown by recent research, feeding behavior under heat stress
    (e.g., FA linked to body fat reserve mobilization)

J. Dairy Sci. 98:4956–4968
http://dx.doi.org/10.3168/jds.2014-9148

Genetic analysis of heat stress effects on yield traits, udder health, and fatty acids of Walloon Holstein cows

H. Hammami,*†[1] J. Vandenplas,*† M.-L. Vanrobays,* B. Rekik,‡ C. Bastin,* and N. Gengler*

III - Beyond milk composition from IR

Biomarker and Indicator Traits

- “Classical” objective of milk MIR spectrometry
  ➔ predicting “perfectly” the component

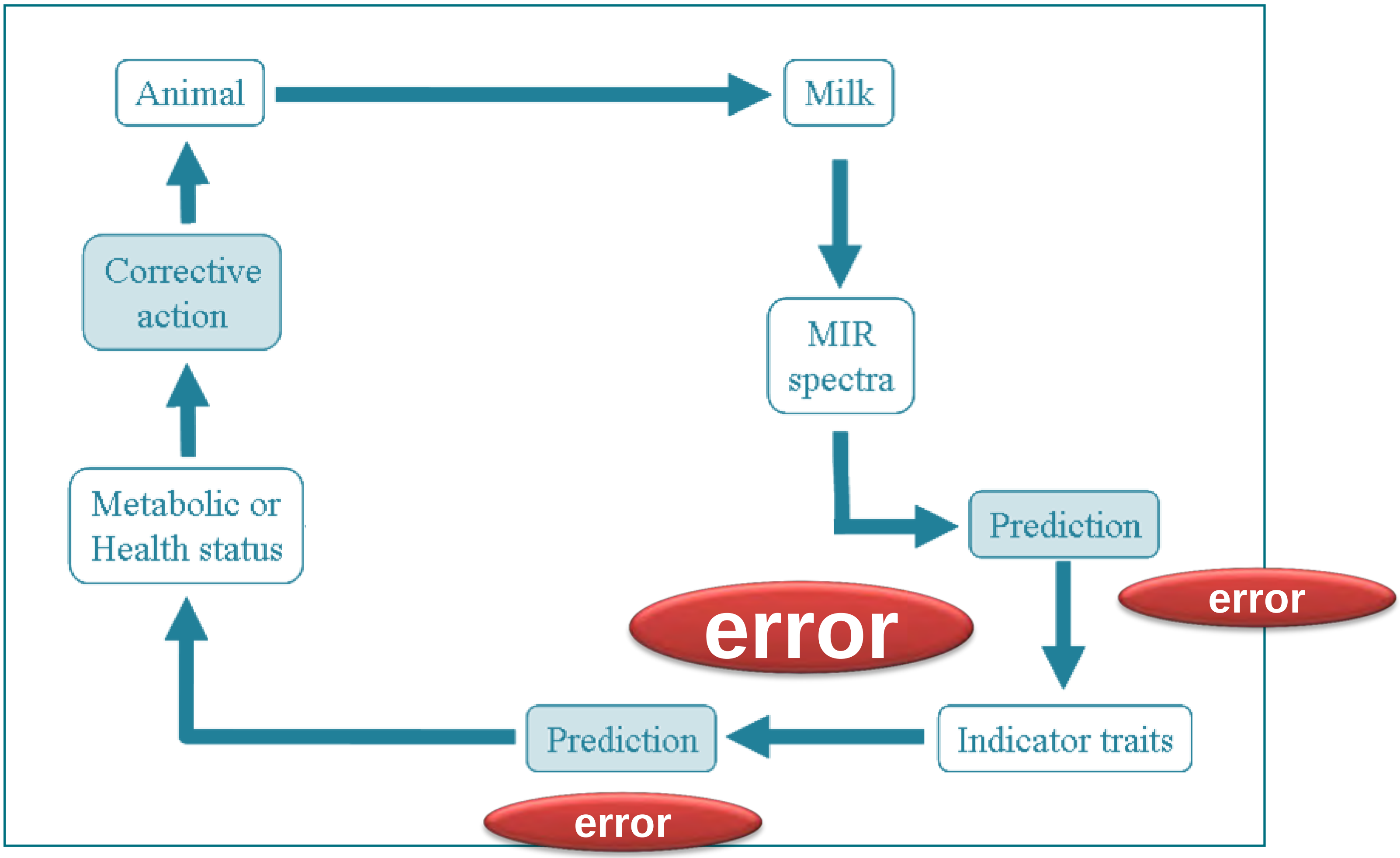
- However, many biomarkers or indicator traits can only be predicted rather imperfectly

Therefore Proposed Alternative

- ❑ Defining traits closer to “real” trait of interest
- ❑ Example from dairy cattle
  - ➢ Currently: MIR ➔ BHB, acetone ➔ Ketosis
  - ➢ Proposal: MIR ➔ Ketosis
- ❑ Concept of
  “Management (Information) Trait”
  ➔ OptiMIR project (www.optimir.eu)

WITH THE SUPPORT OF

MIR ⇨ Indicator ⇨ Management Trait



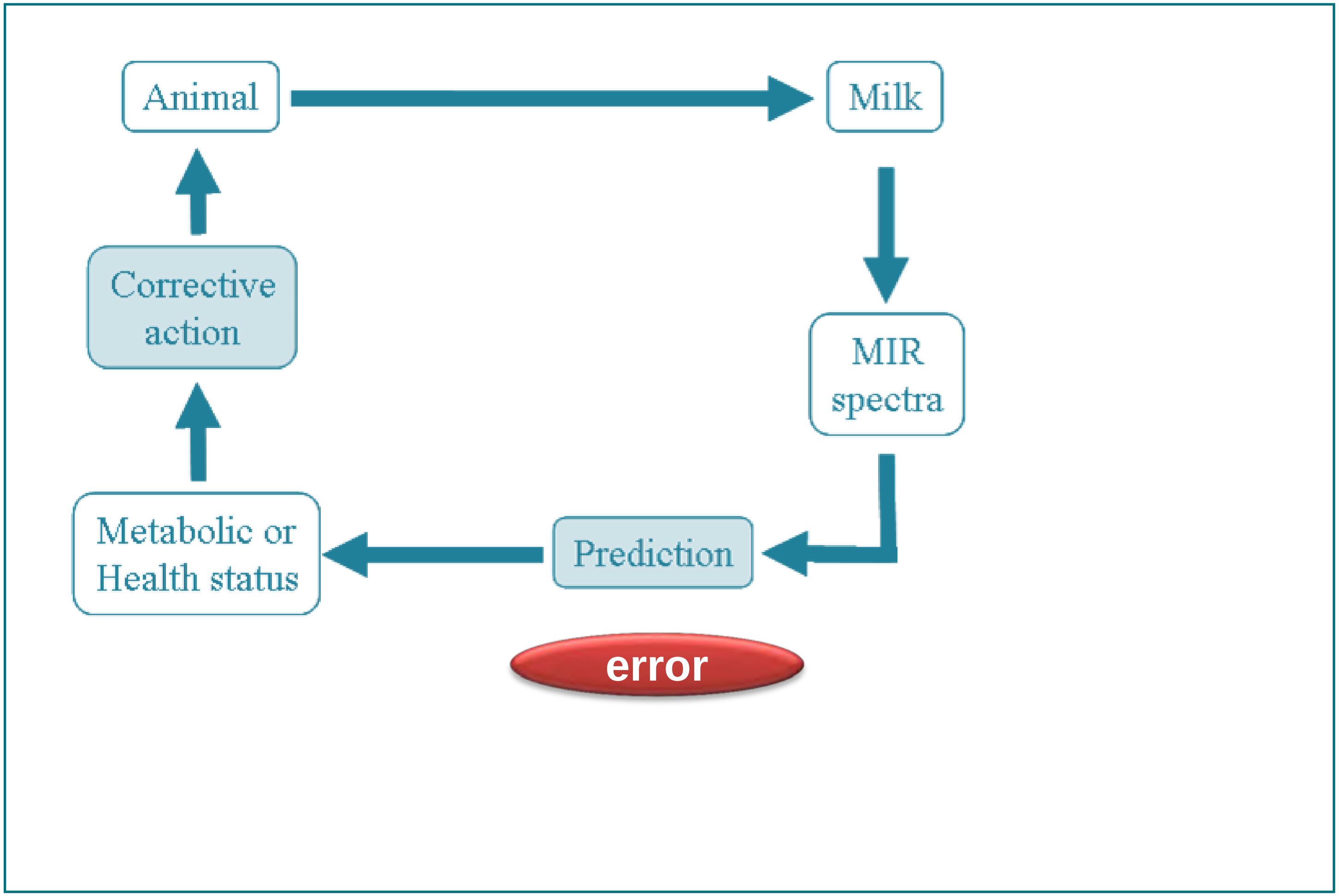
Direct Prediction of Traits of Interest

- "Classical" objective of milk MIR spectrometry
  ➔ predicting "perfectly" the component

- However many biomarkers or indicator traits can only be predicted rather imperfectly
  - Double "error"

- 1st Innovation
  - Direct prediction of "Management" Traits from MIR spectra
  - Not the direct component, but directly related to process/status

MIR ⇨ Management Trait

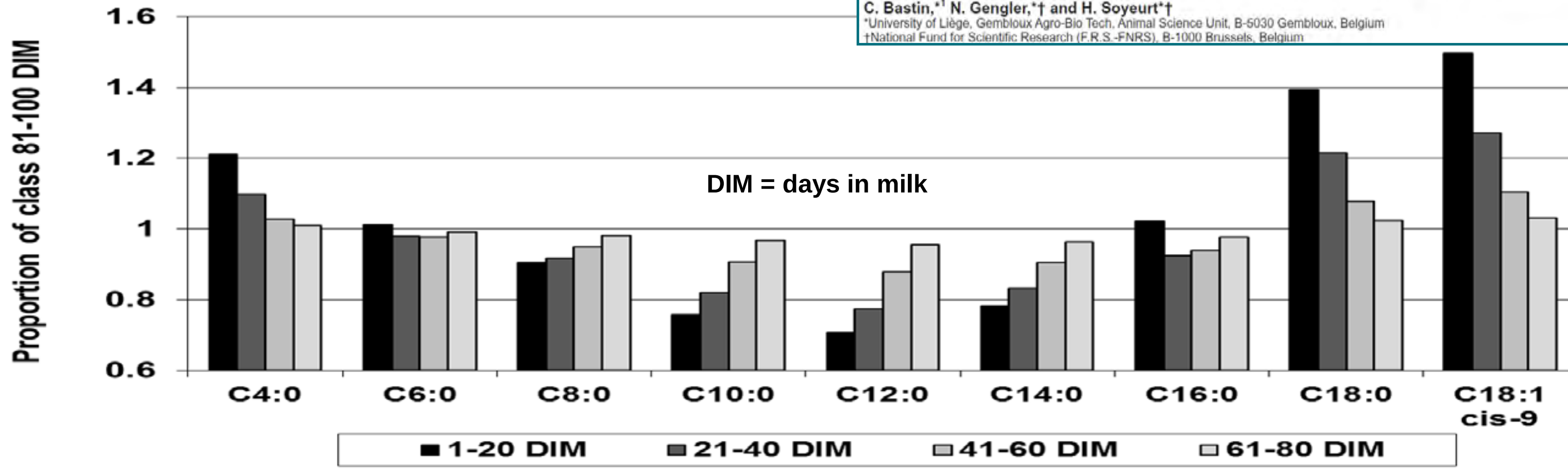


FA Profile Variable Throughout the Lactation

J. Dairy Sci. 94:4152–4163
doi:10.3168/jds.2010-4108

Phenotypic and genetic variability of production traits and milk fatty acid contents across days in milk for Walloon Holstein first-parity cows

C. Bastin,*[1] N. Gengler,*† and H. Soyeurt*†
*University of Liège, Gembloux Agro-Bio Tech, Animal Science Unit, B-5030 Gembloux, Belgium
†National Fund for Scientific Research (F.R.S.-FNRS), B-1000 Brussels, Belgium



- Indirect: reflecting equilibrium between: Body fat mobilization ⇔ Feed intake
  - Body fat mobilization → also heat stress
  - Feed intake → driving force for CH_4
- Direct calibration of energy balance and related traits

J. Dairy Sci. 98:4956–4968
http://dx.doi.org/10.3168/jds.2014-9148

Genetic analysis of heat stress effects on yield traits, udder health, and fatty acids of Walloon Holstein cows

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*Animal Science Unit, Gembloux Agro-Bio Tech, University of Liege, 5030 Gembloux, Belgium
†National Fund for Scientific Research, 1000 Brussels, Belgium
‡School of Higher Education in Agricultural of Mateur, TN-7030 Mateur, Tunisia

J. Dairy Sci. 99:4056–4070
http://dx.doi.org/10.3168/jds.2015-10051

The potential of Fourier transform infrared spectroscopy of milk samples to predict energy intake and efficiency in dairy cows[1]

S. McParland[2] and D. P. Berry
Animal and Grassland Research and Innovation Center, Teagasc, Moorepark, Fermoy, Co. Cork, Ireland

Other Sources of Variation Added to Calibration

- 2nd Innovation
  - Adding other sources of variation into calibration process
- Example for MIR predicted methane
  - Methane (⇔ FA) ⇔ MIR Spectra

Other Sources of Variation Added to Calibration

- 2nd Innovation
  - Adding other sources of variation into calibration process
- Example for MIR predicted methane
  - Methane (⇔ FA) ⇔ MIR Spectra
  - More details in article

J. Dairy Sci. 98:5740–5747
http://dx.doi.org/10.3168/jds.2014-8436

***Hot topic*: Innovative lactation-stage-dependent prediction of methane emissions from milk mid-infrared spectra**

A. Vanlierde,*[1] M.-L. Vanrobays,†[1] F. Dehareng,* E. Froidmont,‡ H. Soyeurt,† S. McParland,§ E. Lewis,§ M. H. Deighton,# F. Grandl,|| M. Kreuzer,|| B. Gredler,¶ P. Dardenne,* and N. Gengler†[2]

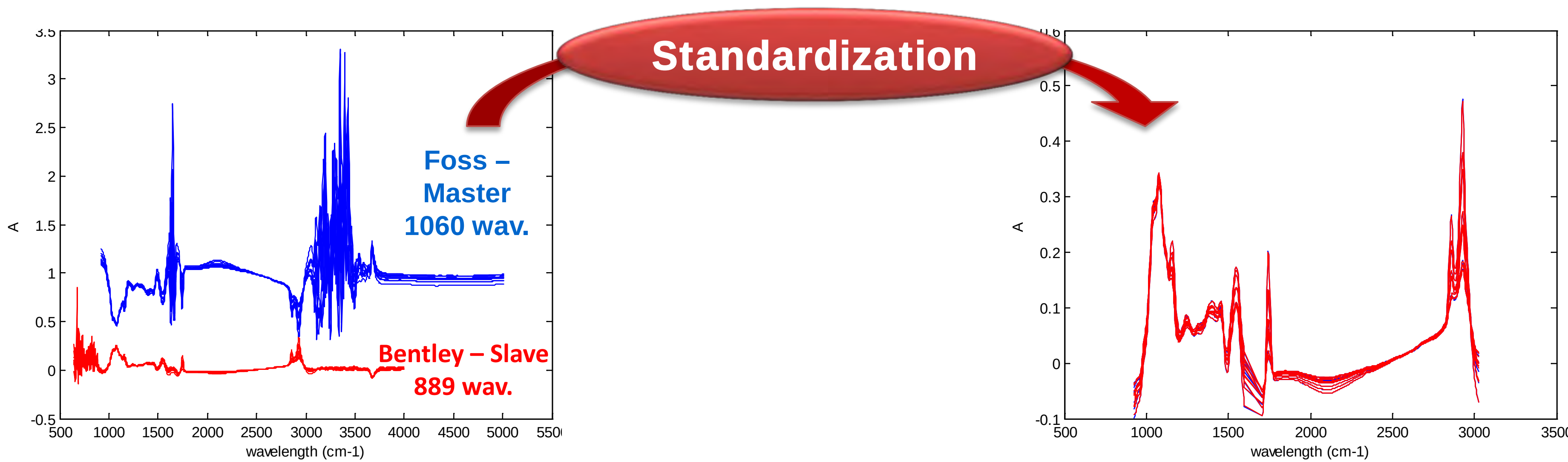
- Variable calibration equation coefficients
  - Here Days in Milk (DIM) dependent
  - $P(CH_4) = f_{b0}(DIM) + \sum f_{bi}(DIM) \times (abs)_i$
- But can be used in many other situations
  - When links between phenotype of interest and MIR data not constant

Other Issues…

- Each calibration equation
  - Normally only for the instruments used for the calibration
- At least two issues
  - Different brands ⇨ different spectral wavelength ranges
  - Individual spectrometers ⇨ over time generated MIR data not 100% stable
- In context of traditional calibrations
  - Brand specific equations (“Black box”)
  - Manufacturers using different “tricks” like “Standardization Solutions”
  - Post-prediction adjustments for “Bias” and “Slope” using reference samples with known values

⇨ but for novel trait, traits with no obvious reference samples?

3rd Innovation: Spectra Standardization



- ❑ Two steps to generate “standardized” (harmonized) spectral data
  1. Transforming from different ranges of wavelength to a common one
  2. Applying “bias“ and “slope” corrections for each wavelength
- ❑ Recent publication:

J. Dairy Sci. 98:2150–2160
http://dx.doi.org/10.3168/jds.2014-8764

Standardization of milk mid-infrared spectra from a European dairy network

C. Grelet,[1] J. A. Fernández Pierna,[1] P. Dardenne, V. Baeten, and F. Dehareng[2]
Walloon Agricultural Research Center, Valorisation of Agricultural Products Department, 24 Chaussée de Namur, 5030 Gembloux, Belgium

IV - Future of IR – ongoing research

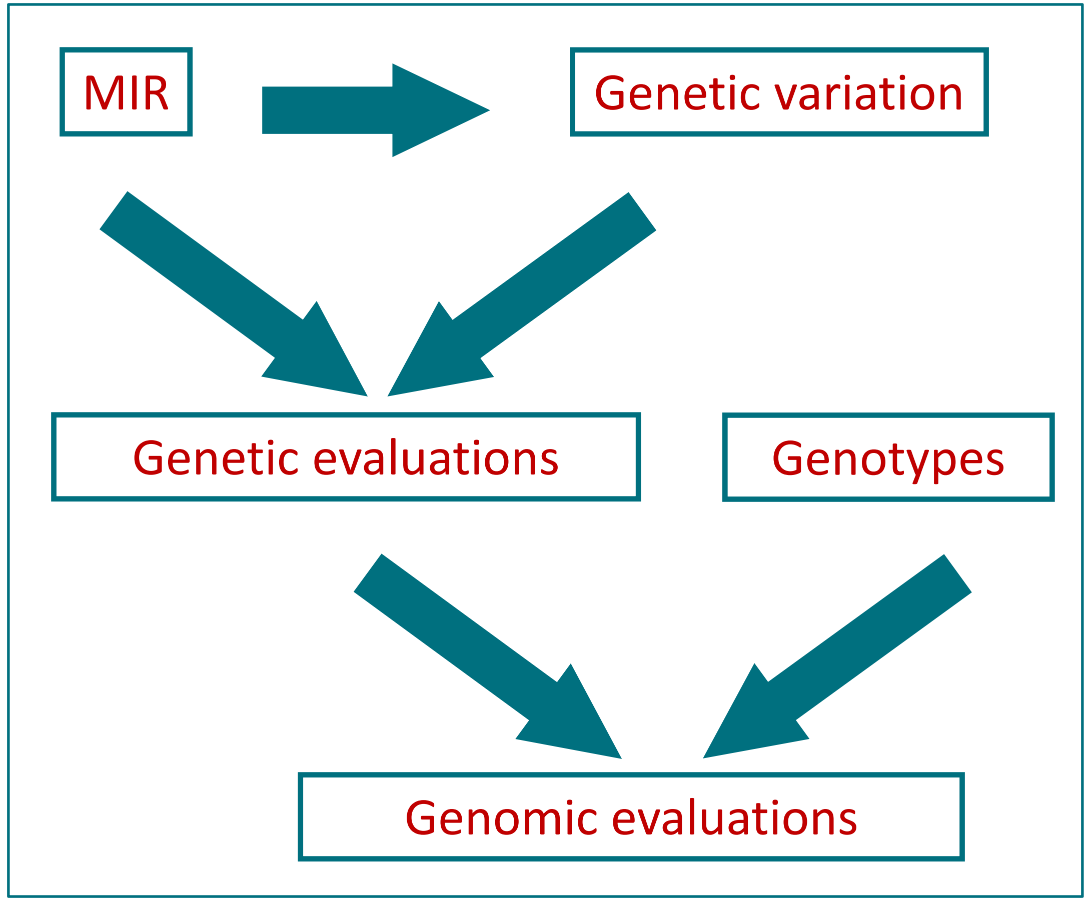
Trent in Animal Breeding: Direct Use of MIR

J. Dairy Sci. 93:1722–1728
doi:10.3168/jds.2009-2614

Genetic variability of milk components based on mid-infrared spectral data

H. Soyeurt,*†[1] I. Misztal,‡ and N. Gengler*†

- Traits: absorbance values at given wave numbers
- Avoiding "phenotypic" calibration and risk of low R^2_{CV}
- Problem of high nb of dimensions (many MIR traits) ➔ targeted combination of traits (My presentation at ICAR 2017 on the 15th of June)



Development ⇨ International MIR Projects

⇨ important to develop international collaborations

- ❑ Leading to several European projects
  - ➢ RobustMilk (FP7 – KBBE) – finished
    - ❖ FA and lactoferrin predictions
  - ➢ GreenHouseMilk (FP7 – Marie Curie – ITN) – finished
    - ❖ Methane predictions
  - ➢ OptiMIR (INTERREG-IVB North-West Europe) – finished
    - ❖ MIR tools implementation technology and management use
  - ➢ GplusE (FP7 – KBBE) – ongoing
    - ❖ Mostly health traits
- ❑ Collaboration in local projects in other countries (Germany, Australia)
- ❑ Continuing interested in other collaborations

MIR Spectral Databases and Standardization

- Creation of spectral databases related to milk recording needed
  - Already in Walloon Region of Belgium and in Luxembourg since several years

- At member milk recording organizations
  - European Milk Recording
    www.milkrecording.eu
  - Organizing “Standardization”

⇨ development of breeding and management tools

Conclusions

- Many opportunities in (M)IR based methods:
  - Illustrated by examples
  - Context of breeding and management of dairy cattle
    - But IR not only milk → not elaborated in this talk

Many Opportunities in (M)IR Based Methods

Animal Health

Fatty acids, minerals, lactoferrin, lactose, β-hydroxybutyrate, acetone…

Environment

Fatty acids, methane emission through fatty acid contents, urea

Herd Management

Urea, fat, protein, lactose…

(M)IR spectrum

Biodiversity

Changes in milk composition

Nutritional Quality of Milk

Fatty acids, lactoferrin, minerals…

Hygienic Quality of Milk

Antibiotics, somatic cells…

Technological Quality of Milk

Traits related to cheese making (casein, dornic acidity, coagulation time...)…

Conclusions

- Many opportunities in (M)IR based methods:
  - Illustrated by examples
  - Context of breeding and management of dairy cattle
    - But IR not only milk → not elaborated in this talk
- Help to avoid:
  - Bottleneck of getting relevant data → collaborations
- Simplifying concepts:
  - Researching direct link: MIR ⇔ "Management Information Traits"
  - In animal breeding: skipping phenotypic calibration
- Several other innovations
- Challenges (and opportunities ahead)
  - Integration into "Precision Livestock Farming"

Acknowledgments

- Support through the Futurospectre partnership:
  - awé – Milkcomite – CRA-W – ULg-GxABT

- Two core teams
  - Team ULg-GxABT: H. Soyeurt, C. Bastin, F.G. Colinet, H. Hammami, M.-L. Vanrobays, A. Lainé, S. Vanderick, ….
  - Team CRA-W: P. Dardenne, F. Dehareng, C. Grelet, A. Vanlierde, E. Froidmont, ….

Thank you!