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Genetic Relationships between Methane-Related Traits and Milk Composition in Lactating Dairy Cows

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GENETIC RELATIONSHIPS BETWEEN METHANE-RELATED TRAITS AND MILK COMPOSITION IN LACTATING DAIRY COWS

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Dissertation originale présentée en vue de l'obtention du grade de docteur en sciences agronomiques et ingénierie biologique

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2018

Résumé

Kandel Purna. (2018). Relations génétiques entre les caractères liés aux émissions de méthane et la composition du lait chez les vaches laitières en production (Thèse de doctorat en anglais). Gembloux, Belgique, Gembloux Agro-Bio Tech, Université de Liège, 113p., 16 table, 16 fig.

Les émissions de méthane (**CH_4**) représentent un caractère d'évaluation de l'impact environnemental des vaches laitières parmi les plus importants. La mise en place de programmes de sélection génétique visant à réduire l'impact des émissions de CH_4 requiert l'estimation des paramètres génétiques du CH_4, l'estimation des corrélations avec les autres caractères économiquement importants et une appréciation de la réponse à la sélection sur les caractères visés. Dans la première partie de cette thèse, les émissions de CH_4 (g/j ; **PME**) ont été prédites à partir des spectres moyen infrarouge (**MIR**)d'échantillons de lait de vaches Holstein en utilisant des équations utilisant les acides gras comme variables de référence. Les héritabilités de PME ainsi obtenues étaient modérées, allant de 0.21 à 0.40. La variabilité génétique estimée des pères était suffisamment large que pour espérer une réponse à la sélection. Dans la deuxième partie de cette thèse et afin de minimiser les erreurs de prédictions, les paramètres génétiques ont été estimés à partir de prédictions directes du CH_4à partir des spectres MIR en utilisant une équation de prédiction basée sur de vraies valeurs CH_4 mesurées par la méthode SF_6. L'intensité prédite du CH_4 (**PMI**, g/kg de lait) a été obtenue en faisant le rapport de la valeur du CH_4 (g/j) sur le rendement total en lait enregistré au jour de contrôle, ce caractère présentant l'intérêtd'être comparable entre différentes intensités de systèmes de production. La relation entre PMI et le rendement en lait (**MY**) était curvilinéaire. La distribution du PMI, n'étant pas normale, ce caractère a subi une transformation logarithmique (**LMI**) avant d'être modélisé. Les analyses génétiques ont été réalisées en utilisant deux modèles génétiques. En plus des effets fixes, le premier modèle incluait un effet aléatoire lié à l'environnement permanent et un effet génétique additif. Le deuxième contenait en plus un effet aléatoire intra-troupeau. Il a été ainsi montré que l'inclusion d'une courbe de lactation intra-troupeau permettait une meilleure modélisation. L'héritabilité de PME était de 0.26 et celle de PMI était de 0.27. La contribution à la variance totale del'effet aléatoirelié à la courbe de lactation intra-troupeau suggère un impact de la conduite spécifique du troupeau sur les émissions de CH_4. Après l'obtention de la confirmation de la composante génétique des caractères liés au CH_4, les corrélations génétiques entre ces caractères et ceux liés à la production laitière ont été estimées à partir de données issues de vaches en première et deuxième lactation. Les corrélations phénotypiques entre PME et MY, les rendements en matières grasses et en protéines étaient nulles, par contre les corrélations phénotypiques avec LMI étaient fortement négatives. La corrélation génétique était faiblement négative entre PME et les caractères de production laitière mais fortement négative avec LMI. L'héritabilité intra-lactation et les corrélations changeaient toutes deux d'une lactation à l'autre, suggérant que la relation entre les caractères liés au CH_4 et ceux de production laitière était dynamique. Après avoir montré les relations avec les caractères de production laitière, les corrélations génétiques des émissions de CH_4 avec les caractères fonctionnels [fertilité, note d'embonpoint (**BCS**), longévité], les caractères de santé (santé mammaire etles caractères de conformationont été estimées. Les corrélations entre les caractères fonctionnels et ceux liés aux émissions de CH_4 étaient positives, suggérant un certain équilibre entre ces caractères lors de la sélection. Les caractères de conformationliés à la capacité d'ingestion montraient des corrélations génétiques positives avec PME mais négatives avec LMI. Finalement, en adaptantl'actuel index de sélection wallon pour inclure PME ou LMI, il a été montré qu'il était possible de réduire les émissions, sans toutefois compromettre les caractères de production laitière mais avec des conséquences négatives sur la fertilité, la note d'embonpoint et la longévité. En conclusion, cette recherche suggère la faisabilité d'une sélection génétique permettant d'optimiser les émissions de CH_4 chez les vaches laitières.

Mots-clés. Méthane, moyen infrarouge, héritabilité, corrélations génétiques vaches laitières

Abstract

Kandel Purna. (2018). Genetic relationships between methane-related traits and milk composition in lactating dairy cows. (PhD Dissertation in English).Gembloux, Belgium, Gembloux Agro-Bio Tech, University of Liège, 113 p., 16 table, 16 fig.

Methane **(CH_4)** emission is one of the most important environmental traits from dairy cows. Genetic selection programs aiming to mitigate CH_4 emissions require the estimation of genetic parameters, correlations with other economically important traits and predicted selection response of these traits. In first part of this thesis, CH_4 emissions (g/d; **PME**) were predicted from several milk fatty acid based prediction equations using mid-infrared (**MIR**) spectra of milk samples from Holstein cows. The heritability of PME was moderate and ranged from 0.21 to 0.40. The sires genetic variability were large enough to respond selection pressure. In second part and to minimize prediction errors, genetic parameters were estimated from direct prediction of CH_4 (i.e. based on SF_6 measurements) from milk MIR spectra. Predicted CH_4 intensity (**PMI**, g/kg of milk) was derived from the ratio of CH_4 (g/d) value divided by the total milk yield recorded for the considered test-day which is a trait that is comparable across different production systems. The relationship between PMI and milk yield (**MY**) was curvilinear and the distribution of PMI being non-normal, it was log-transformed (**LMI**) in further analyses. The genetic analyses were performed using two genetic models with or without random within-herd lactation curve effects along with random permanent and additive genetic effects. The results showed that the model with random within-herd lactation curve effects had a better fitting. The heritability of PME was 0.26 and PMI was 0.27. The contribution of random herd-specific lactation curve effects to the total variance also suggested an impact of herd specific management on the CH_4 emission traits. After confirming genetic component of CH_4 traits, genetic correlations of these traits with milk production traits were explored and expanded to second lactation. The phenotypic correlations between PME and MY, fat yield and protein yield were not different than zero but with LMI, the phenotypic correlations were highly negative. The genetic correlation was low negative between PME and milk production traits but high negative with LMI. The intra-lactation heritability and correlation were changing across lactation suggested there was dynamic relationship between CH_4 traits and milk production traits. After demonstrating correlation between milk production traits, the genetic correlation between CH_4 traits and functional traits [fertility, body condition score (**BCS**), longevity], health traits (udder health) and type traits were estimated. There were positive correlations between CH_4 emission traits and functional trait suggested there were tradeoffs between these traits in selection. The ingestion ability related type traits had positive genetic correlations with PME but negative genetic correlation with LMI. Finally, using the current Walloon selection index and by selecting PME and LMI, the emission traits responded by a reduction in CH_4emission, without jeopardizing in milk production traits but having negative consequences in fertility, BCS and longevity. In conclusion, this study shows the feasibility to adapt the selection index to mitigate the CH_4 emitted by dairy cows.

Keywords: Methane, Mid infrared, heritability, genetic correlation, dairy cows

Acknowledgements

After years of work, it is my honor to thank all individuals who helped me directly and indirectly to accomplish my PhD research. I would like to express my gratitude to my co-supervisor Dr. Hélène Soyeurt for her guidance, purposefulness, encouragement and academic support during these years. Starting from the first day, her support was incredible. Her guidance and insights have been reflected in many parts of this thesis and will be useful for my future career too. I also sincere appreciate to my supervisor, Prof. Nicolas Gengler for his continuous support and friendly advice in work place as well as in publication and for financial support. Prof. Gengler is an inspiration for students to get succeeded in their lives, both academically and scientifically. I have learned many things from him to be successful and working as perseverance.

I have had a great experience of collaborative efforts with my colleagues Marie-Laure Vanrobays and Sylvie Vanderick from Gembloux Agro-Bio Tech as well as Amélie Vanlierde from Walloon Agriculture research center. They have contributed significant part of my research and were reflected as co-authors in various publications, presentation and posters. Similarly, collaborative work with other scientists from Walloon Agriculture Research Center, namely, Frédéric Dehareng, Eric Froidmont and Pierre Dardenne, was highly productive. I sincerely thank them for their scientific input and their roles as co-authors.

As a secondment at GreenhouseMilk project, my stay was successful due to the guidance of Dr. Donagh Berry and Dr. Sinead McParland at Teagasc Moorpark. I want to thank sincerely to both of them for their scientific as well as logistic supports. Similarly, I would like to offer my sincere thanks to the supervisors of GreenhouseMilk project, namely, E. Wall, Y. de Haas, N. Friggens and L. Shalloo, who provided a very good platform for me to learn about science and improve my presentation and communication skills. I also thank D.H. Deighton and Eva Lewis from Teagasc, Moorepark for their scientific help. In addition, thanks go to my colleges of GreenhouseMilk, namely, Marcin, Phuong, Elodie and Frank.

It was a pleasure to experience a great working environment at Gembloux Agro-bio Tech and having friendship and scientific discussion with my colleges, namely, Pierre, Catherine, Hedi, Frédéric, Marie, Valerie, Hana, Aurélie and Jérémie as well as front office assistance by Cecile and Geneviève.

Continuous support and understanding from my beloved wife Radhika throughout my PhD study and travels were highly commendable. Similarly, my lovely daughter, Preetika, and our new born baby, Liam, always inspire me to do something innovative in my life. I was fortunate to stay blessed and encouraged from both of my parents as well as from Hari Mammy.

Finally, I would like express my sincere thanks to my thesis committee members Prof. Y. Beckers, Y. Brostaux and E. Froidmont. I would also thanks to the

GreenhouseMilk project from European Union and supplementary funding from Methamilk Project; Ministry of Agriculture-Wallonia and Administration R&D of University of Liège.

Finally, I also want to remember to all teachers of my academic journey from Kiran Nimavi, Narayani Vidya Mandir, IAAS Lamung and Rampur; and Wageningen. It has been great journey and I may have missed a lot of people who encouraged me during my thesis research; I would like to thank them all.

Purna Kandel
2018

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List of Abbreviations

AIC	Akaike information criteria
BCS	Body condition score
BIC	Bayesian information criteria
BLUP	best linear unbiased method
CH_4	Methane
d	day
DCE	Direct calving ease
DFF	Direct female fertility
DIM	Days in milk
DMI	Dry matter intake
EBV	Estimated breeding value
FA	Fatty acid
FY	Fat yield
GCFA	Gas chromatography fatty acids
g/d	gram per day
LMI	Log-transformed methane intensity
LRT	Likelihood ratio test
MACE	Multiple Across Country Evaluation
MCE	Maternal calving ease
MFA	Milk fatty acid
MI	Methane intensity
MIR	mid infrared
MY	Milk yield
PME	Predicted methane emission
PMI	Predicted methane intensity
PY	Protein yield
R^2cv	Coefficient of determination of the cross-validation
REML	Restricted maximum likelihood
SCS	Somatic cell score
SECV	Standard errors of cross validation
SF_6	Sulfur hexafluoride
UDH	Udder health

1

General Introduction

*Methane (**CH_4**) emission is of major concern in term of environmental impact of dairy cow breeding.The selection of CH_4 emission traits predicted from milk composition was the main aim of this thesis. In this chapter, the context, aim, outline and framework of this research thesis are presented.*

1. Context

Major objective of dairy production is to maximize farm profitability; however, consumer preferences and environmental concerns associated with milk production have also been considered as integral components of sustainable dairy farming these days. Sustainable livestock production requires economic, societal and environmental impacts to be considered (Boichard and Brochard, 2012). Lower carbon footprint can be achieved by reducing enteric CH_4 emissions. Indeed, livestock sector produces about 80 million tons of enteric CH_4 annually (Beauchemin et al., 2008). Enteric CH_4 fermentation by ruminants accounts for about 32% of total non-CO_2 emissions from agriculture. In terms of greenhouse effects, CH_4 is 25 times more potent than CO_2; therefore, the reduction of CH_4 would make an important contribution to decrease the impact of greenhouse gas (**GHG**) emissions. Among others methods like the feeding changes or the use of feeding supplements that reduce CH_4 emissions, selective breeding of animals with low CH_4 emissions without (relative) compromising production characteristics could be promising area to reduce carbon footprint from milk production. This breeding goal requires a large number of accurate and repeatable phenotypes for genetic analysis, preferably from commercial farms. Several direct and indirect methods have been used to quantify CH_4 emissions *in vivo* and they all have certain advantages and limitations. Conventional methods of enteric CH_4 measurements (e.g. respiration chambers, SF_6 tracer techniques and others) are difficult and not cost effective yet to get a large number of phenotypes for genetic analyses. Therefore, the recording of phenotypes that is highly correlated with CH_4 emission and easier to record for a large number of cows would be an ideal alternative. These indirect biomarkers can then be used to predict the emissions of a larger number of animals and are therefore feasible to estimate the dairy cattle variability of CH_4.

Organizations involved in the performance recording of dairy cattle collect individual milk related traits through the analysis of individual milk samples by milk laboratories. A particularly promising group of milk components, in regard to this research context, are milk fatty acids (**FA**s). The use of milk FAs records is biologically relevant for predicting CH_4emission. Indeed, some milk FAs are indirectly linked with volatile FAs formed during rumination. Based on this indirect link, equations using gas chromatographic FA measurements to predict CH_4 emissions have been derived (*e.g.*, Chilliard et al., 2009; Dijkstra et al., 2011). Gas chromatography is also expensive technique and not in routine. Previous studies have shown that mid infrared (**MIR**) spectroscopy can be used to predict milk FAs (Soyeurt et al., 2011). These milk MIR predicted phenotypes are considered as cheap, rapidly obtained, usable on a large scale, robust and reliable. Initially, CH_4 emissions used in this PhD work were predicted from literature equations based on milk FAs predicted by MIR. In the second approach, CH_4 emissions were directly predicted from MIR spectra to minimize prediction errors (Dehareng et al., 2012; Vanlierde et al., 2015 and 2016). Additionally, an interesting feature of MIR based prediction of CH_4 emissions is its repeatable characteristic. Indeed, approximately every four weeks during lactation, milk samples are collected for a considered cow and analyzed by MIR allowing a prediction of CH_4. Therefore, this can generate a large amount of phenotypic data for genetic analysis.

The consideration of new traits within the breeding selection goals starts by an acquisition of phenotypic data, and then the estimation of their heritability and genetic variability. After quantification of heritability of the considered trait using appropriate models, a number of correlated effects with traits of interests (milk production traits or functional traits like fertility and body condition scores, health traits (udder health), longevity and type traits) can be calculated. Finally, based on these correlated effects, selection response can be quantified based on the present and future selection scenarios. This PhD work followed this framework. Particularly, this thesis focused on simulated scenarios of genetic progress which helps to decide the ultimate goal of reducing CH_4 emission from dairy production system through genetic selection using proxies of CH_4 emissions. To achieve this objective, different hypothesis were validated:

- predicted CH_4 traits would be heritable;
- those traits would have sufficient genetic variability to rank sires into low and high CH_4 emitters;
- those environmental traits and other traits included in the selection index would be selected simultaneously without jeopardizing animal production and efficiency.

2. Aim of the Thesis

This thesis aims to explore the potential of using genetic selection of CH_4 emission traits predicted from milk composition using MIR spectroscopy. Towards the objective, genetic parameters of CH_4 production (g/day) and intensity (g/kg of milk) were estimated from equations developed from MIR milk FAs and direct MIR milk spectra using models including or not random within-herd lactation effects. The phenotypic and genetic correlations of these CH_4 traits with other economic traits were estimated from the dairy cattle in the Walloon region of Belgium. Finally, consequences of genetic selection of these traits were explored to provide estimates of selection response.

3. Outline

This manuscript is a compilation of published scientific papers. After general introduction in Chapter 1,the variability of literature based CH_4 indicator traits predicted from milk fatty acids using MIR spectroscopy is exposed for the Holstein dairy cow population of Walloon region of Belgium(Chapter 2).Chapter 3 discusses the CH_4 traits directly predicted from MIR-spectra to estimate the genetic parameters of mid-infrared predicted CH_4 emissions in first parity Holstein cows with or without random within-herd lactation effects. These genetic parameters of CH_4 emissions are expanded up to second lactation and exposed in Chapter 4as well as the genetic correlations with milk production traits. In Chapter 5, the consequences of genetic selection of environmental impact traits on economically important traits are analyzed. Finally, in Chapter 6, all results from previous chapters are discussed. The document draws the conclusion and the future perspectives of the present work.

4. Thesis Framework

This thesis research was initiated in September 2011 within the GreenhouseMilk- a Marie Curie Initial Training Network which was funded by the European Commission - Framework 7, to develop genetic tools to mitigate the environmental impact of dairy systems. Within this project, the objective was to harness genetic tools to elucidate the genetics of emissions in dairy cattle to select “environmentally friendly” bulls to suit dairy production systems. After November 2013, the research was supported by the Methamilk project (SPW-DGO3) funded by Ministry of Agriculture of Walloon Region of Belgium. Additional financial support was provided from University of Liège, Administration R&D. This thesis is an outcome of active collaborations with the Walloon Agriculture Research Center, Gembloux for CH_4 equation development; the Walloon Breeding Association (Ciney, Belgium) for the pedigree and milk recording database; the Comité du Lait (Battice, Belgium) for the MIR analysis.

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2

Assessing variability of literature based methane indicator traits in a large dairy cow population

In this chapter, CH_4 emissions were predicted from literature equations using fatty acids predicted by mid-infrared spectrometry. Moderate heritability (0.20-0.40) and large genetic variability was estimated. The genetic correlation with milk yield was low negative but high positive with fat content and protein contents. This study showed the feasibility to predict fatty acid derived CH_4 indicator traits developed in the literature from MIR spectrometry. Moreover, the estimated genetic parameters of these traits suggested a potential genetic variability of the daily quantity of CH_4 eructed by Holstein dairy cows.

From Kandel, P. B., N. Gengler, and H. Soyeurt. 2015. Assessing variability of literature based methane indicators traits in a large dairy cow population. Biotech. Agron. Soc. Environ. 19:11-19.

Abstract

Dairy production is identified as a major source of CH_4 emissions. Selective breeding can be one method to mitigate CH_4 emissions but practical and cheap measurements of this trait are not currently available. Four CH_4 indicator traits based on milk fatty acid (**FA**) contents were referenced from literature. The aim of this study was to use these literature CH_4 indicators to assess the variability of CH_4 emission emitted by dairy cows. Literature indicator traits were originally based on gas chromatography derived FA. As those were not available for all available cows in our population, a sample of 602 gas chromatographic analyses was used to develop a calibration equation to predict the CH_4 indicators based on milk MIR spectra which were available for all studied cows. Then, in a second step, MIR prediction equations were applied to the 604,028 recorded spectral data collected from 2007 to 2011 for 70,872 cows in their first three lactations in order to predict the literature CH_4 indicator traits. Genetic parameters for these traits were estimated using single trait test-day random regression animal models. The predicted MIR CH_4 estimates were in the expected range from 350±40 to 449±65 g/d. The averaged predicted MIR CH_4 emission (g/d) increased from the beginning of lactation, reached the highest level at the peak of lactation and then decreased towards the end of the lactation. The average daily heritabilities were 0.29-0.35, 0.26-0.40, and 0.22-0.37 for the different studied CH_4 indicators for the first three lactations, respectively. The largest differences between estimated breeding values of sires having daughters in production eructing the highest and the lowest CH_4 content was 24.18, 29.33 and 27.77 kg per lactation for the first three parities. Low negative correlations were observed between CH_4 indicator traits and milk yield. Positive genetic correlations were estimated between CH_4 indicator traits and milk fat and protein content. This study showed the feasibility to predict fatty acid derived CH_4 indicator traits developed in the literature from MIR spectrometry. Moreover, the estimated genetic parameters of these traits suggested a potential phenotypic and genetic variability of the daily quantity of CH_4 eructed by Holstein dairy cows.

1. Introduction

Livestock production is considered as one of the key sources of greenhouse gas emission, the main gas produced being CH_4. Globally, livestock produces about 80 million tons of enteric CH_4 annually (Beauchemin et al., 2008). The enteric CH_4 fermentation by ruminants accounted for about 32% of total non-CO_2 emissions from agriculture. CH_4 is 25 times more potent than CO_2 in its greenhouse effect; therefore, the reduction of CH_4 would make an important contribution to decrease the impact of greenhouse emissions (Smith et al., 2007). Furthermore, CH_4 emission from ruminants accounts for 2% to 12% of gross energy loss of feeds depending on the type of diets (Johnson et al., 1995). Therefore the mitigation of CH_4 emission from livestock has dual advantages: the decrease of its environmental impact and the increase of energy efficiency.

Currently a certain number of methods are available to obtain CH_4 emission data on a low scale. It is largely admitted that the most accurate measurement of CH_4 emission can be obtained from the calorimetry method using respiration chambers. However, the cost, handling of lactating dairy cows and the CH_4 measurement time, limit the number of animals that can be measured which reduces its usefulness in large populations. The sulfur hexafluoride gas (**SF_6**) measurement (Johnson et al., 1994) is an alternative method that can be used because of its relatively low cost and its ability to keep the animal in a more natural environment (i.e. grazing). However, obtaining an accurate direct measurement of CH_4, using this method is complex and the recording of such data is also unfeasible at a medium to large scale. Other methods are under scrutiny but all depend on the acquisition of extra data outside current data acquisition strategies in regular recording system of animal production.

The most used data acquisition in dairy cattle is through the performance recording. Consequently, some studies have focused on the creation of indicator traits indirectly related to the CH_4 emission based on milk composition which is or could be routinely appreciated through milk recording. A particular promising group of components are FAs. The use of FA traits seems to be biologically relevant to predict CH_4. Indeed, CH_4 output from ruminants is directly linked to the microbial digestion in the rumen. The fermentation process of feed carbohydrates leads to the production of hydrogen (H_2) and the methanogenesis is the essential pathway to expel this H_2 (Moss et al., 2000). The synthesis of acetate (C2) and beta-hydroxybutyrate (C4) produces H_2 that is then converted to CH_4, while propionate (C3) synthesis consumes H_2 (Demeyer et al., 1975). The de novo synthesis of milk FA in the mammary gland uses mostly C2 (85% of de novo synthesized FA) but also C4 (10 to 15%), and a limited amount of C3 to make short chain FA (C4, C6, and C8), nearly all medium chain FA (C10, C12, and C14), and approximately 60% of C16 (Chilliard et al., 2000; Couvreur et al., 2007). Based on this indirect link, equations using gas chromatographic FA measurements (**GCFA**) to predict CH_4 emission have been derived (e.g., Chilliard et al., 2009; Dijkstra et al., 2011). Unfortunately, gas chromatography has not been adapted for large scale use in dairy cow population especially due to its cost.

Recent research showed the feasibility of the prediction of FA using MIR spectrometry (e.g., Soyeurt et al., 2011).Therefore, the aims of this study were 1) to develop MIR equations to predict fatty acid derived CH_4 indicator traits developed in the literature directly from MIR spectrometry and to apply those for the Walloon Holstein cow population and 2) to estimate the phenotypic and genetic parameters for these traits and their relationship with milk production traits in order to evaluate indirectly the CH_4 emission of Holstein dairy cows.

2. Material and Methods

2.1. Computation of Methane Indicator Traits

Several different CH_4 indicator traits derived from FA are given in the literature. Well known and already used in several studies are those defined by Chilliard et al., (2009) and Dijkstra et al., (2011). In this study CH_4 indicator traits presented by Chilliard et al. (2009) were investigated, as the CH_4 indicator trait defined by Dijkstra et al. (2011) was developed from less abundant milk FA and uses dry matter intake (**DMI**) in its formula. The CH_4 emissions in Chilliard et al., (2009) were measured using SF_6 technique. As the latter information was not available in the current study, the CH_4 indicator trait developed by Dijkstra et al., (2011) was not used in this research. CH_4 indicators defined by Chilliard et al., (2009) are based on major milk FA or class of major milk FA, and the accuracy of MIR prediction of these FA was *a priori* known to be very high (0.87 to 0.94) (Soyeurt et al., 2011). As the MIR spectrometry is used by nearly all milk recording organizations, adapted milk MIR spectra prediction equations for FA could be used to derive potentially informative CH_4 indicator traits. In this context, two options are possible: 1) use of the equations for FA developed by Soyeurt et al., (2011), then computation of the CH_4 indicators using these MIR based FA or 2) computing reference values for CH_4 indicator traits using the GCFA database used by Soyeurt et al., (2011) and then use of these reference values for CH_4 indicators with associated MIR data to obtain direct MIR equations. The second proposal was used in this study for two reasons. First, one of the indicator traits was based on a sum of different FA, and using individual estimates would have led to an accumulation of prediction errors for this CH_4 indicator. Also, by re-computing the calibrations for all traits, the obtained equations were comparable and adapted to the range of variation in the reference data.

The calibration dataset used to develop the MIR calibration equations for the CH_4 indicator traits contained 602 Walloon milk samples and their corresponding GCFA profile and MIR spectra (i.e., those samples included in the calibration dataset used by Soyeurt et al., (2011)). The sampling procedure was explained in detail by Soyeurt et al., (2011).

The reference values for the CH_4 indicator traits (called hereafter Methane_1 to

Methane_4) were computed from the GCFA contents by using the equations published by Chilliard et al., (2009) (Table 2-1). The equations developed by Chilliard et al., (2009) predicted the CH_4 emission in g/d. The coefficient of determination reported in literature by Chilliard et al., (2009) for the 4 indicators (R^2ref) varied between 0.81 (i.e., Methane_3) and 0.88 (Methane_1 and Methane_2). From the reference CH_4 data and their corresponding milk MIR spectra, partial least squares regressions were used to develop four CH_4 indicator trait equations after applying a first derivative pre-treatment on the recorded spectral data. A t-outlier test was used to delete potential outliers during the calibration process. This process explained why the number of samples used for each developed equation was slightly different. The robustness of the developed MIR equations was assessed by cross-validation using 50 groups. The cross-validation coefficient of determination (**R^2cv**) and the cross-validation standard error (**SECV**) were calculated.

2.2. Milk Samples and Predictions of Methane Indicator Traits

Milk samples were collected from Holstein cows in 1207 herds between January 2007 and October 2011 through the Walloon milk recording managed by the Walloon Breeding Association (Ciney, Belgium). All samples were analyzed using a Milkoscan FT6000 spectrometer (Foss, Hillerød, Denmark) by the milk laboratory ‘Comité du Lait’ (Battice, Belgium) to quantify the contents of protein and fat and to record the spectral raw data.

The CH_4 prediction equations developed by Chilliard et al., (2009) and adapted for MIR spectra in this study were applied to the recorded MIR spectral data to predict MIR literature indicators of the quantity of the eructed CH_4. The dataset contained 262,759 records from 53,481 first parity, 203,092 records from 41,419 second parity, and 138,177 records from 28,978 third parity Holstein cows. All records used were observed between 5 and 365 days in milk (**DIM**). The pedigree data was extracted from the pedigree used for the regular Walloon genetic evaluation for milk production traits.

2.3. Estimation of Genetic Parameters

Single trait test-day random regression animal models were used to model the variability of the studied traits:

$$\mathbf{y=X\beta+Q(Zp+Zu)+e}$$

where $\mathbf{y}$ was the vector of observations for each trait (Methane_1 to Methane_4, milk yield, fat and protein contents), $\boldsymbol{\beta}$ was the vector of fixed effects (herd x test-day, DIM (24 classes; 15 days each starting from day 6 to day 365), and age at calving (9 classes: 21 to 28 months, 29 to 32 months, and 33 months and more for first lactation; 31 to 44 months, 44 to 48 months, and 49 months and more for second lactation and 41 to 57 months, 57 to 60 months, and 60 months and more for third lactation); **p** was the vector of permanent environmental random effects, **u** was the vector of additive genetic effects; **Q** was the matrix containing the coefficients of 2^{nd} order Legendre polynomials; **e** was the vector of residuals; **X** and **Z** were incidence matrices assigning observations to effects.

Variance components were estimated using the average information REML method (AI-REML, Misztal, 2011). Average daily heritability **(h^2)** was the averaged h^2 estimated separately for each DIM between 5 and 305 DIM as the ratio of the genetic variance at the considered DIM to the total variance. The estimated breeding values (**EBV**) were calculated using a BLUP approach. Approximate daily genetic correlations were computed between traits using the following method. First, daily breeding values **(EBVd)** for each DIM between 5 and 305 and for cows with records were calculated as following:

$$\mathbf{EBVd}_{\mathbf{htk}} = \sum_{m=0}^{2} a_{hkm} z_{tm},$$

where $EBVd_{htk}$ was the daily breeding value of cow k, for trait h, for each DIM t between 5 and 305; a_{hkm} was the random regression coefficient for the additive genetic effects; z_{tm} was the covariate for Legendre polynomials associated with DIM t; and z_{t0}= 1.0, zt_1= $3.0^{0.5}$x, z_{t2}=$5.0^{0.5}(1.5x^2– 0.5)$, where x= 2[(*t*– 5)/300] – 1.

Second, daily genetic correlations between 2 traits were estimated as correlations between EBVd values of the 2 traits of interest for each DIM from 5 to 305. Finally, average daily correlations were defined as the average correlations across the entire lactation.

3. Results and Discussion

3.1. Development of MIR Predictions for Methane Indicator Traits

After applying the equations of Chilliard et al., (2009) on the GCFA data included in the calibration set (i.e., 602 samples), the mean and the SD obtained for the reference values for Methane_1 to Methane_4 were within the range of estimated CH_4 emission values from the results published by Chilliard et al., (2009; Table 2-1). The values for Methane_3 had a clear tendency to be lower than the values for the other three indicators.

The robustness of the MIR equation developed to predict the literature CH_4 indicator traits can be assessed by R^2cv (Table 2-1). For all traits except Methane_3, R^2cv was of 0.91 and higher. Methane_1 and Methane_2 seem to be the most relevant MIR traits because they had the highest R^2ref (as reported by Chilliard et al., 2009), and high R^2cv were obtained suggesting that they were theoretically the best CH_4 indicators. Methane_2 and Methane_1 had also the lowest SECV (18.42 and 19.30 g of CH_4/d, respectively). In addition to this, the FA used in Methane_3 and Methane_4 were also a subset of those used for Methane_1.

Table 2-1: Methane equations developed by Chilliard et al., (2009) and used in the calibration procedure and statistical parameters calculated for the developed MIR prediction equations for methane emission based on Walloon data (g/d)

Indicator trait	Equation (fatty acids in g/100g of fat)	R^2ref	N	Mean	SD	SECV	R^2cv
Methane_1 (g/d)	9.97 x (C8:0 to C16:0) – 80	0.88	597	447	68	19	0.92
Methane_2 (g/d)	-8.72 x C18:0 + 729	0.88	602	422	61	18	0.91
Methane_3 (g/d)	282 x C8:0 +11	0.81	595	369	43	23	0.72
Methane_4 (g/d)	16.8 x C16:0 – 77	0.82	588	460	88	26	0.92

R^2ref = literature coefficient of determination between the CH_4 predicted from gas chromatographic data and the SF_6 CH_4 data (for Methane_1 to Methane_4); N = number of samples used in the calibration set; Mean = mean of the reference values; SD = standard deviation of the reference values; SECV = standard error of cross validation; R^2cv = cross validation coefficient of determination

3.2. MIR Indicators of Methane Eructed by Walloon Holstein Cows

Descriptive statistics for the common production traits and the developed MIR CH_4 indicator traits for the first three lactations of studied Walloon Holstein cows are shown in Table 2-2. By taking into account all CH_4 indicators, the MIR CH_4 predictions ranged from 350 g to 443 g/d in the first lactation which corresponds to 128 to 162 kg per year.

For the second lactation, the CH_4 MIR prediction was 360 g to 449 g/d and almost similar for the third lactation (Table 2-2). MIR predicted values stayed in the range observed on reference values (Table 2-1) in terms of means and standard deviations. For all studied MIR traits, a larger amount of CH_4 was estimated for the second lactation compared to the first lactation. However, this trend was almost flat from the second to the third lactation. Even if Mohammed et al., (2011) suggested an overestimation of CH_4 production obtained from the equations of Chilliard et al., (2009), the obtained MIR CH_4 predictions were within the range of the published CH_4 emissions. Except for Methane_3, the MIR based predictions of CH_4 in our study were in agreement with the daily CH_4 emissions (429± 128 g/d) measured in Holstein cows in Belgium (Dehareng et al., 2012), as well as the CH_4 quantity measured from the group where these equations were derived (418.1 g/d, Martin et al., 2008). Similarly, predictions were on par with CH_4 emission measured through the SF_6 method from other authors like Heimeier et al., (2011), and also measured from the respiration chambers (van Zijderveld et al., 2011).

Table 2-2: Descriptive statistics for the common production traits and the CH4 indicator traits for the first three lactations (with their corresponding SD)

Traits	Lactation 1 (N=262,759)	Lactation 2 (N=203,092)	Lactation 3 (N=138,177)
Milk (kg/d)	23.61±5.88	26.57±7.59	27.51±8.36
Fat (g/dl of milk)	4.07±0.62	4.17±0.67	4.19±0.67
Protein (g/dl of milk)	3.44±0.35	3.53±0.38	3.50±0.38
Methane_1 (g/d)	436±65	449±64	448±65
Methane_2 (g/d)	401±58	414±57	415±57
Methane_3 (g/d)	350±40	360±38	363±38
Methane_4 (g/d)	443±73	448±76	444±75

The estimated MIR CH_4 indicators increased until the peak of lactation, which occurred during the second or third month of lactation, and then decreased (Figure 2-1a). This pattern could be explained by the evolution of milk production within the lactation; an increase in milk production requires more energy and therefore led to increased CH_4 emission per day, albeit a low amount (Capper et al., 2009). Similarly the DMI increases as the lactation progresses. Also, DMI is one of the primary drivers for CH_4 emission (Hegarty et al., 2007). The peak of lactation don’t corresponds to the peak of the ingestion andat the beginning of the lactation energy required to produce milk is also obtained by mobilizing body reserves (Banos et al., 2010), which could explain the delay in the peak of the CH_4 indicators compared to the peak in milk production.

The difference in MIR CH_4 predictions between the beginning and their peak during lactation was around 50-60 g/d. A similar observation was obtained by IPCC (2006) and Garnsworthy et al., (2012). The evolution pattern of the CH_4 emission for the second and third lactations (data not shown) was similar to the one observed for the first lactation but the overall level of estimated MIR CH_4 predictions was slightly higher (1-2%)(Figure 2-1b). It can be attributed mostly to the increase in milk production, DMI and body weight gains (Grainger et al., 2008; Garnsworthy et al., 2012). Despite their very simple nature, the evolution of the MIR based CH_4 indicator traits were in line with expectations.

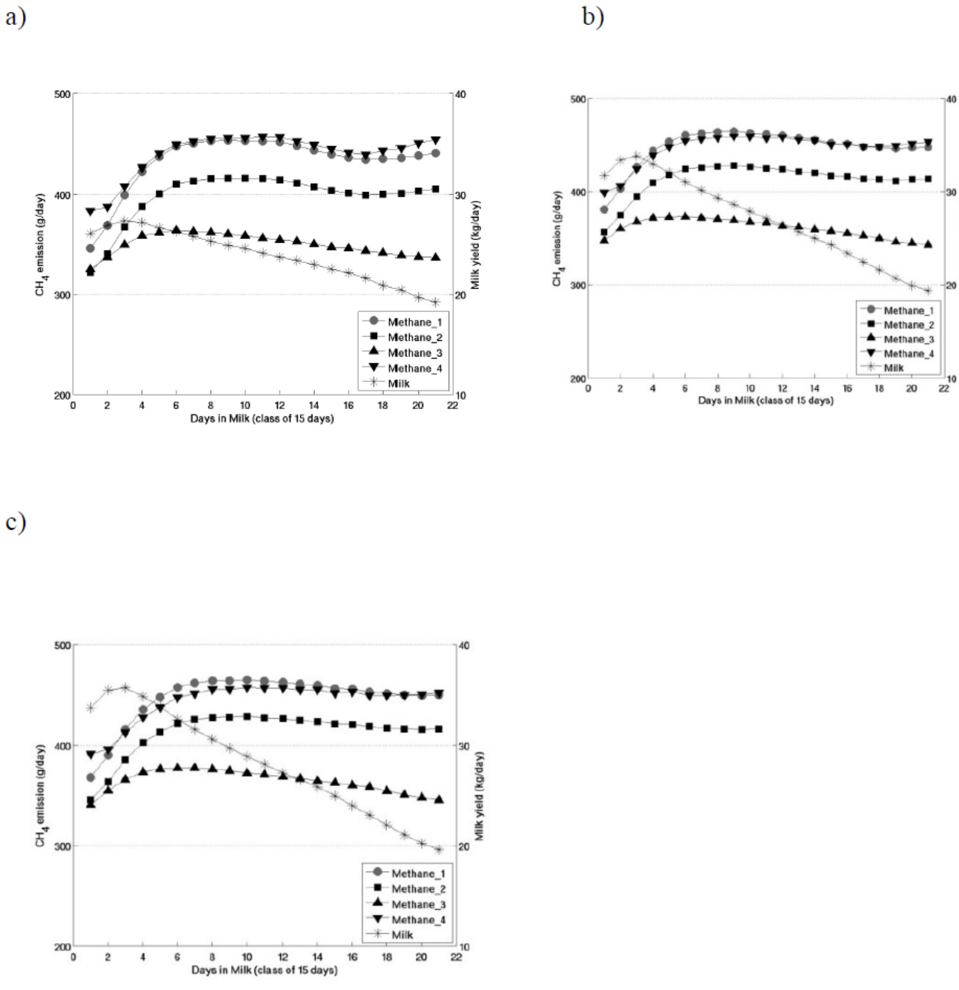

Figure 2-1: Evolution of the four CH_4 indicator traits and milk yield for the first (Figure 2-1a), second (Figure 2-1b) and third (Figure 2-1c) parity Holstein cows throughout lactation

3.3. Heritability

Average daily heritability for the four MIR CH_4 indicator traits ranged between 0.29 and 0.35 for the first lactation, 0.26 to 0.40 for the second lactation, and 0.22 to 0.37 for the third lactation. For Methane_1, which seems to be the most relevant indicator, the average daily heritabilities were 0.35 (±0.01), 0.38 (±0.01), and 0.34 (±0.01) for the first three lactations, respectively. The estimated heritability suggested a potential transmission of the capacity of dairy cows for emitting high or low quantity of CH_4 from generation to generation. However, this CH_4 indicator trait was basically derived from the saturated FA which has reported heritabilities around 0.40 (Bastin et al., 2011); therefore, the values observed for daily heritabilities in this study were expected. Previously reported heritability for the predicted CH_4 production in Holstein cow was 0.12 (Cassandro et al., 2010) and 0.35 (de Haas et al., 2011). In the study by Cassandro et al., (2010), the CH_4 production was calculated using predicted DMI for dairy cattle; de Haas et al., (2011) predicted CH_4 emission as 6% of gross energy intake corrected for the energy content of milk. Another study which estimated heritability of enteric CH_4 emission measured by Fourier transform infrared gas analyzer obtained a heritability of 0.21 (Lassen et al., 2012). The difference in heritability between different methods was presumably due to a prediction of CH_4 obtained from very different methodology. However, the tendency found by these previous authors and the current results are in line to suggest a potential genetic variability of the quantity of CH_4 eructed by dairy cows per day.

The DIM heritabilities ranged from 0.29 to 0.41 for Methane_1, from 0.26 to 0.37 for Methane_2, from 0.24 to 0.35 for Methane_3, from 0.29 to 0.41 for Methane_4 in first lactation. The DIM heritabilities from all CH_4 indicator traits increased from mid-lactation until late lactation and then decreased slightly until the end of the lactation for all studied lactations (Figure 2-2; data only shown for first lactation). This could be explained by the negative energy balance of a cow at the beginning of the lactation which involves a mobilization of lipids from adipose tissue. The energy is therefore partly obtained from metabolic processes which are controlled by different genetic processes. At later lactation stages, the cow is in positive energy balance; therefore, the energy comes mainly from the ingested food (Friggens et al., 2007) which, given the results obtained here, seems to be captured well by the studied CH_4 indicator traits. Another explanation could be the increase of DMI during the mid and later lactation. Indeed, the CH_4 emission is positively correlated with DMI (Grainger et al., 2008), which is partially controlled by genetic process (Berry et al., 2007). Finally, the shape of the heritability curves is also a function of the model used. The objective of this research was not to study the optimal model for the studied traits. Previous studies for yield traits (e.g., Gengler et al., 2005) showed the potential importance of herd-specific lactation curves and the differences in partitioning of phenotypic variances across the lactation according to types of herds. Differences among herds in their feeding management are expected; therefore, future research will need to address this issue.

3.4. Phenotypic and Approximate Genetic Correlations

The phenotypic correlations and the approximate genetic correlations calculated between the MIR CH_4 indicator traits and the common production traits are presented in Table 2-3 for the first lactation. Both phenotypic and approximate genetic correlations were positive among all studied MIR CH_4 indicators. Correlations between Methane_1 and Methane_2 were close to the unity indicating that they basically describe the same trait and lower correlations were observed with other CH_4 MIR traits but the correlations always stayed positive.

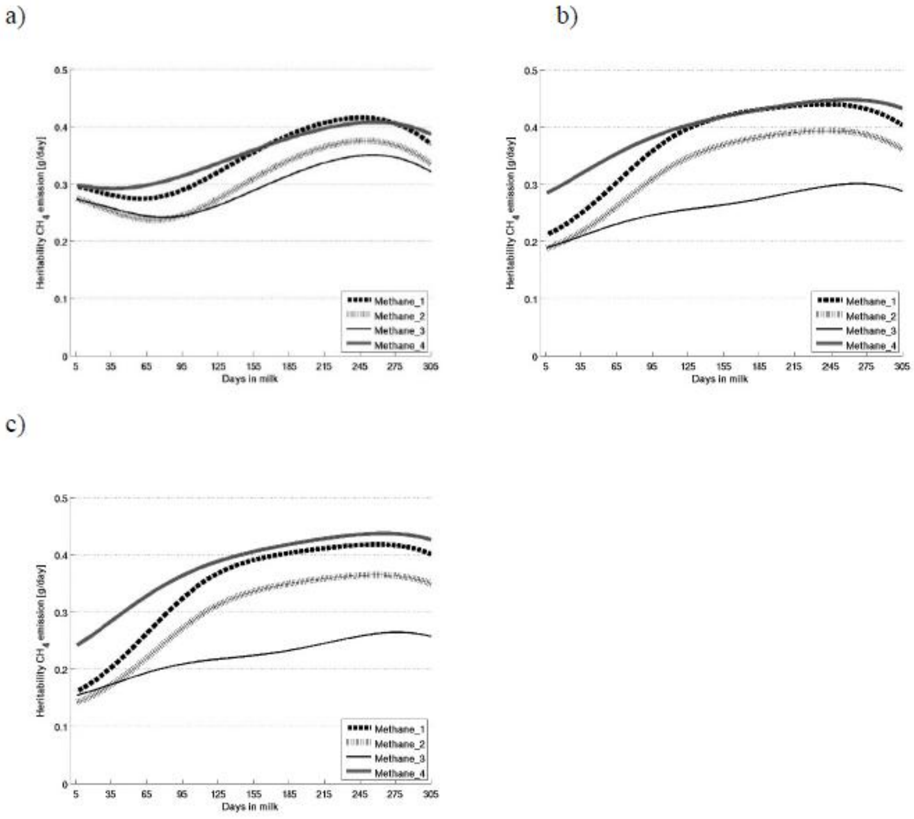

Figure 2-2: Evolution of the daily heritability of the four CH4 indicator traits throughout the first (Figure 2-2a), second (Figure 2-2b) and third (Figure 2-2c) lactations

The phenotypic correlations for the MIR CH_4 traits with milk yield were nearly zero (-0.18 to -.06) except for Methane_3 which was positive (0.24), indicating again that this trait behaved differently. IPCC model indicates a linear relationship between milk yield and CH_4 emission due to fact that milk yield and feed intake as predictor of CH_4 emissions in this model. However, other complex model like life cycle assessment model indicates lack of linear relationship (Sonesson et al., 2009). Wall et al., (2010) mentioned cows with higher genetic merit for milk production produced less CH_4/kg of milk on different diets possibly due to their low maintenance requirement and their higher feed efficiency suggesting a curvilinear relationship between these traits. Madsen et al., (2010) found a slight positive correlation between milk production and the ratio CH_4:CO_2 (*i.e.*, proxy to the CH_4 emission).

The observed phenotypic correlations were positive between MIR CH_4 indicators and fat content (0.31-0.54) and protein content (0.14-0.38). This suggests a higher CH_4 emission if the milk is rich in protein and/or fat. This could be partly explained by the fact that more energy is required to produce higher content of fat and protein (NRC, 2001). More specifically, fat content is influenced by the fiber content of feed. However, the relationship with protein content should be evaluated because protein will be enhanced with high energy diet containing starch and producing propionate in the rumen. Grainger et al., (2010) found a reduction of CH_4 emission for dairy cows fed with a supplemented whole cottonseed meal with no change in fat content but a small decrease in protein content. However, except that, there is no direct evidence in the literature that an increase in fat and protein content proportionately increases the CH_4 in g/d.

The approximate genetic correlations between the studied MIR CH_4 traits and milk yield were low and negative (-0.11 to -0.18), but positive with the fat content (0.31 to 0.55) and protein content (0.14 to 0.36; Table 2-3). Therefore, Selecting for increased milk productioncan slightly decrease the CH_4 emissions as suggested previously by Wall et al., (2010).

Table 2-3: Phenotypic (below the diagonal) and approximate genetic (above the diagonal) correlations between the studied CH4 indicator traits and production traits in first parity Holstein cows

	Methane_1	Methane_2	Methane_3	Methane_4	Milk	Fat	Protein
Methane_1		0.98	0.55	0.81	-0.18	0.55	0.32
Methane_2	0.99		0.59	0.75	-0.17	0.54	0.36
Methane_3	0.56	0.57		0.18	-0.17	0.31	0.17
Methane_4	0.88	0.87	0.32		-0.11	0.43	0.14
Milk	-0.07	-0.06	0.24	-0.18		-0.50	-0.38
Fat	0.23	0.21	0.01	0.29	-0.39		0.59
Protein	0.23	0.25	0.05	0.17	-0.38	0.51	

Milk=Milk kg/d; Fat = Fat content g/dl of milk; Protein=Protein content g/dl of milk

EBVs for all studied CH_4 indicator traits were estimated for cows with MIR CH_4 predictions and for sires that had daughters with MIR CH_4 predictions. Given the heritabilities, as expected, substantial differences in EBVs between animals were observed. For instance, the lowest EBV of sire for Methane_1 cumulated at 305 days was -11.12 kg and the highest was 13.06 kg. The range between EBVs of Methane_1 was 24.18 kg for the first lactation, 29.33 kg for the second lactation and 27.77 kg for the third lactation. Similarly, the lowest EBV for cow for Methane_1 was -14.46 kg and the highest was 14.87 kg. The range was equal to 30.36 kg for the first parity cows, 35.93 kg for the second parity cows, and 32.92 kg for the third parity cows. The EBV for extreme animals of all MIR CH_4 traits was higher in the second lactation compared to the first one and slightly lower in the third lactation (data not shown). The Pearson correlations of EBV ranged from 0.77 to 0.80 between first and second lactation, from 0.68 to 0.72 between first and third lactation and from 0.75 to 0.81 between second and third lactation. The relatively strong rank correlation suggested that the rankings of animal were consistent between lactations.

Lower EBV for sires were observed for all MIR CH_4 indicator traits compared to the ones calculated for the cows with MIR records as expected. Commonly used dairy sires are intensively selected for production traits. Given the estimated approximate genetic correlations, selecting for milk yield only should reduce slightly MIR CH_4 indicator traits which are in line with expectations that animals with high genetic merit for yield are more efficient. However, selection is more on milk solids than on milk yield alone, and therefore the relationship between CH_4 emissions and production traits is complicated and additional studies are required.

4. Conclusions

In conclusion, this study showed the potential to predict CH_4 indicator traits from MIR spectral data based on CH_4 indicator traits published in the literature and therefore, its potential use to screen a large dairy cow population. The obtained results showed also the existence of large phenotypic and genetic variability of these MIR CH_4 indicator traits suggesting a potential phenotypic and genetic variability of CH_4 content eructed by dairy cows.

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3

With or without random within-herd lactation curve effects to estimate the genetic parameters of mid-infrared predicted methane emissions in first parity Holstein cows

*In this chapter, CH_4 emissions from first parity Holstein cows were predicted directly from milk mid-infrared spectra to minimize prediction errors. Two genetic models with or without random within-herd lactation curves effects models were tested and model with random within-herd lactation curves effects fitted better than without it. The estimates of heritability of predicted CH_4 emission (**PME**) was 0.26 and log-transformed predicted methane intensity (**LMI**) was 0.27 from model with herd-specific lactation curves random effects and within herd-lactation curve effect has significant effect.*

From: Kandel, P. B., M.-L. Vanrobays, A. Vanlierde, F. Dehareng, E. Froidmont, N. Gengler, and H. Soyeurt. 2017. unpublished. With or without random within-herd lactation curve effects to estimate the genetic parameters of mid-infrared predicted methane emissions in first parity Holstein cows.

Abstract

The carbon footprint of milk production can be improved by reducing the enteric CH_4 emissions from cows through genetic selection. In this study, CH_4 emission **(**PME**;** g/d**)** was predicted from milk mid-infrared spectra recorded longitudinally across the first lactation Holstein cows. Predicted CH_4 intensity (**PMI**; g/kg of milk) was derived as the ratio of PME to milk yield for a given test-day and log-transformed (LMI) in subsequent analysis. The objectives were to compare two statistical models; to infer variance components and heritability for predicted CH_4 traits (PME and LMI).Data included 366,126 predicted CH_4 emissions test-day records on 56,991 cows in 935 herds. The first model included herd x test-day, days in milk, and age at calving as fixed effects; and permanent environment, additive genetic and residual effects as random effects. In order to allow herd-specific lactation curves effects, the second model also included random effects modeling herd-specific lactation curves. Firstly, two models were compared from the log-likelihood ratio test (**LRT**), Akaike information criterion (**AIC**), Bayesian information criterion (**BIC**), the coefficient of calibration (**R^2C**) and the root mean square error of prediction (RMSEP). Based on LRT, AIC, BIC, second model fit better than first model. Average daily heritability (±se) of PME was 0.26(±0.01) and LMI was 0.27(±0.01) and daily heritability ranged from 0.22 to 0.32 for PME and from 0.20 to 0.36 for LMI from second model. The relative variance for random herd-specific lactation curve effects for PME was 0.10 and LMI was 0.05. The contribution of random herd-specific lactation curve effects to the total variance also suggested an impact of herd specific management on the CH_4 emission traits. In conclusion, model with random herd-specific lactation curve effects fit better for both traits and MIR predicted CH_4 emission traits had moderate heritable genetic components. This information can be used breeding programs that aimed to reduce the carbon footprint of dairy products.

1. Introduction

Livestock production is considered to be responsible for 14.5% of global anthropogenic CH_4 emissions (Gerber et al., 2013). The enteric fermentation of feed in rumen accounts for the major part of the total CH_4 emitted by ruminant livestock. CH_4 is a potent greenhouse gas with a global warming potential 25 times that of CO_2. In addition to the environmental impact, enteric CH_4 production also represents a loss of dietary energy for ruminants considered to range from 2 to 12% of gross energy intake depending on factors such as feed intake, diet composition and the animal itself (Johnson and Johnson, 1995). Therefore, reducing the enteric CH_4 emissions of dairy cows is expected to improve both economic and environmental performance of livestock industries.

Although the use of animal selection is interesting to mitigate the CH_4 emission of dairy cows due to its additive specificity, current selection breeding objectives do not include enteric CH_4 emissions trait because this inclusion is difficult due to a paucity of records. Direct CH_4 measurements are done using respiration chambers; SF_6 technique or other recent techniques have a too low throughput and are too expensive to generate sufficient data needed for the estimation of genetic parameters. Therefore, the use of indirect biomarkers that allow predictions of the CH_4 emissions for a large number of animals is desirable. Previous studies have shown that MIR spectroscopy can be used to predict milk fatty acid concentrations (Soyeurt et al.,2011), which were found by several authors to be indirectly related to CH_4 emission (e.g., Chilliard et al., 2009; Dijkstra et al., 2011). Recently, Dehareng et al., (2012) and Vanlierde et al., (2015 and 2016) demonstrated the prediction feasibility of CH_4 emission by milk MIR spectra. This MIR-based CH_4 emission indicator trait that can be used easily to generate a large number of CH_4 emission phenotypes as this technology is routinely used for the milk analysis used for the milk payment and the milk recording.

However, despite this difficulty to obtain enough phenotypes, a few studies had already investigated the genetic aspects of CH_4 quantities emitted by dairy cows. Between-cow coefficient of variation (**CV**) of daily CH_4 production (g/d) of 17.8% (measured in respiration chamber) and CV of 19.6% (measured by SF_6 technique) was reported in sixteen lactating dairy cows (Grainger et al., 2007). However, as dry matter intake **(DMI)** is the primary determinant of CH_4 emission (Hegarty et al., 2007), a substantial proportion of variability in daily CH_4 emission between cows can be attributed to between-cow variation in feed intake and indirectly milk yield. A more accurate estimation of the true genetic variation in CH_4 emission between cows is provided by measurement of CH_4 yield expressed in g/kg of dry matter intake (DMI). The CV of CH_4 yield (g/kg of DMI) of eight lactating Holstein cows fed a forage diet was recently estimated to be approximately 7% using both respiration chambers and SF_6 techniques (Deighton et al., 2014). A heritability of 0.35 for CH_4 emission (g/d) predicted from gross energy intake was estimated by de Haas et al., (2011). Similarly, the heritability of CH_4 emission (g/d) predicted from the MIR milk fatty acids using prediction equations published by Chilliard et al., (2009) was 0.22 to 0.40 (Kandel et al., 2015). The heritability of CH_4 emission (g/d) and emission intensity (g/kg of milk) indirectly obtained by Fourier transform infrared gas analyzers were both estimated at 0.21±0.06 (Lassen and Løvendahl 2016). Therefore, even though these studies were based on different methodologies, all supported the existence of a genetic contribution to the enteric CH_4 emission of dairy cows. Still, the number of samples and cows used in these studies limited an accurate prediction of genetic parameters of CH_4 emission and abundance of MIR spectral predicted CH_4 emissions would permit estimation of genetic parameters more accurately.

Different sources of variation of CH_4 emission in dairy cows are already highlighted by several studies (e.g., Garnsworthy et al., 2012). One of the most influent sources is the feeding practices) which changes often throughout the year (i.e., pasture during the spring and summer for having sufficient grass areas) (Ulyatt et al., 2002). Therefore, the inclusion of effect(s) in the descriptive model which take this particularity into account is also required. Previous studies showed the potential importance of herd-specific lactation curves for production traits (e.g. Gengler et al., 2005) and therefore, models with and without herd-specific lactation curves effects were also had to be examined in MIR predicted CH_4 emissions traits. Therefore, the main objective of this study was to estimate accurately the genetic parameters of CH_4 emission for first parity dairy cows through the use of a large dataset containing MIR-predicted CH_4 traits (g/d and g/kg of milk). To achieve this objective, this study also compared two statistical models with the difference of the inclusion of random within-herd lactation curve effects.

2. Materials and Methods

2.1. Data

Milk samples were collected from first parity Holstein cows (i.e., at least 75% of Holstein gene) between January 2010 and April 2014 as part of the routine milk recording undertaken in the Walloon region of Belgium by the Walloon Breeding Association (Ciney, Belgium). All samples were analyzed using a Foss Milkoscan FT6000 spectrometer (Hillerød, Denmark) by the milk laboratory ‘Comité du Lait’ (Battice, Belgium) to quantify the contents of fat and protein and to provide the spectral data. Only test-day records observed between 5 and 365 days in milk **(DIM)** were kept. As applied by the International Committee for Animal Recording, records were retained in this study if they were comprised between of 3 and 99 kg of milk yield, 1 and 7 % of milk protein content, and 1.5 and 9 % of milk fat content.

The daily CH_4 emission (PME; g/d) was predicted from the recorded milk MIR spectra using the lactation-stage-dependent equation developed by Vanlierde et al., (2015) which is an extended work of Dehareng et al., (2012). The CH_4 MIR prediction equation was developed from 446 SF_6 CH_4 measurements taken from 142 cows. The standard error of calibration for the equation was of 63 g/d with a calibration coefficient of determination equal to 75%. More detail about the development of this equation was provided in Vanlierde et al., (2015). Predicted methane intensity (**PMI**; g/kg of milk) was derived from PME (g/d) divided by the total milk yield (kg/d) recorded on the same test-day. Based on the observed skewness and kurtosis for PMI (Table 3-1), this trait was log-transformed to be normally distributed (**LMI**).

In order to eliminate potential abnormal records, PME values below the 0.1 percentile and above the 99.9 percentile were deleted. Moreover, herds were only kept in the study if there were at least 100 records from January 2010 to April 2014. The final dataset contained 366,126 daily CH_4 emission test-day records on 56,991 first parity cows from 935 herds. In total, 1.93% of all available records were deleted after the applied edits.

Pedigree data were extracted from the database used for the official Walloon genetic evaluation and were limited to animals born after 1990 which permitted to pedigree up to three generations back. The pedigree file included 120,503 animals.

2.2. Estimation of Genetic Parameters

In order to test the interest of taking into account the herd variability, two different single trait random regression test-day models were compared. The first model (called hereafter model_1) was similar to the model used routinely for the Walloon genetic evaluation of dairy production traits where a fixed herd x test-day effect is included to take into account the fixed differences in herd management between studied herds (Croquet et al., 2006). The second model (model_2) differed from the model_1 by the addition of a random within-herd lactation curve effect regressed using the second order Legendre polynomials.

Model_1 and model_2 can be summarized as follows:

$$\mathbf{y=X\beta+Q(Zp+Zu)+e} \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\textbf{(model\_1)}$$

and

$$\mathbf{y=X\beta+Q(Hh+Zp+Zu)+e} \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\textbf{(model\_2)}$$

where **y** was the vector of observations for PME or LMI, **β** was the vector of fixed effects (herd x test-day, DIM (24 classes of 15 days interval), and age at calving (3 classes: 21 to 28 mo, 29 to 32 mo, and 33 mo to 49 mo), **h** was the vector of random within-herd lactation curve effects, **p** was the vector of random permanent environmental (**PE**) effects, u was the vector of random additive genetic effects; **Q** was the matrix containing the coefficients of second order Legendre polynomials; e was the vector of residuals; **X**, **H** and **Z** were incidence matrices assigning observations to effects. (Co) variance components were estimated by using the Restricted Maximum Likelihood **(REML)** method (Misztal, 2012). The convergence of REML for variance components estimation was set at 10^{-11}. Average daily heritability was the average of heritability values estimated for each DIM between 5 and 305 DIM as the ratio of the genetic variance to the total variance at the considered DIM. Variance components mentioned in this article were also averaged between DIM 5 and 305.

The two studied models were compared based on the log-likelihood ratio test (**LRT**), Akaike information criterion (**AIC**), Bayesian information criterion (**BIC**), the coefficient of determination (R^2) and the root mean square error of prediction (**RMSEP**). The LRT compared the fit of two models, one of which (model_1, number of parameter = 13) is a reduced version of the full model (model_2, number of parameter = 19). LRT value was calculated for all studied traits as follows:

$$\text{LRT} = 2\log \text{likelihood of model\_2} - 2\log \text{likelihood of model\_1}$$

This calculated LRT value was compared with a critical value to decide whether to reject the model_1 in the favor of model_2. The critical value was determined based on the difference of degrees of freedom between the two models and a significance level equal to 0.05 from a chi-squared distribution. The AIC is also related to the maximum likelihood estimation and is defined as:

$$-2L_m+2_m$$

Where L_m is the maximized log-likelihood and m is the number of parameters in the model. The model with the minimum AIC is considered as the best model. The BIC is a criterion for model selection among a finite set of models; it is based on likelihood function and considers a penalty term for the number of parameters in the model by which a model with smaller value is better. The calibration coefficient of determination (R^2 calibration) was calculated as the square of the correlation coefficient between the solutions of on model_1 to their corresponding predicted values calculated from the model_2. Similarly, RMSEP between model_1 and model_2 were also computed.

2.3. Estimated Emission Pattern for each Random Effects

The estimated breeding values **(EBV)** were calculated for appreciate the variability of genetic component potentially transmission towards offspring. The EBVs as well as the solutions of the fixed effects were obtained by solving the associated mixed model equations using the best linear unbiased method (BLUP) implemented in the program created by Mistzal, (2012).

3. Results

3.1. Descriptive Statistics

The descriptive statistics for studied traits are presented on Table 3-1. The average and standard deviation of PME was 440 ± 80 g CH_4/d with a minimum of 100 g/d and a maximum of 913 g/d. The average PMI was 19.7 ± 6.5 g CH_4/kg of milk with a minimum of 5 g/kg and a maximum of 72 g/kg of milk. The average LMI was 2.94±0.32 with a minimum of 1.60 and a maximum of 4.27. The coefficient of variation of PME and LMI was 18.6 % and 11.1%. Within lactation, PME increased from early lactation to 120-150 DIM and then decreased towards the end of lactation whereas PMI (or LMI) was decreased from early lactation to 40-50 DIM and then increased gradually to the end of lactation reaching maximum at 250-280 DIM and almost flat after that (Figure 3-1).

Table 3-1: Descriptive statistics of studied traits on first parity Holstein cows (n = 366,126 records from 56,991 cows in 935 herds)

Traits	Mean	SD	Min	Max	Skewness	Kurtosis
Predicted methane emission (PME; g/d)	440	80	100	913	-0.28	1.16
Predicted methane intensity (PMI; g/kg of milk)	19.6	6.5	5.0	71.9	1.54	5.08
Log-transformed methane intensity (LMI)	2.9	0.3	1.6	4.2	0.04	0.82
Milk yield (kg/d)	23.9	5.9	3.40	81.6	0.16	0.15
Fat content (g/dl of milk)	3.9	0.6	1.5	9.0	0.39	0.92
Protein content (g/dl of milk)	3.3	0.4	1.0	7.0	0.51	1.76

3.2. Choice of Model

The variance components were calculated using models with and without random within-herd lactation curve effects. The comparison of the two models was performed using the statistical parameters LRT, AIC, BIC and R^2 of calibration coefficient and RMSEP and results are presented in Table 3-2. The calculated value of LRT for PME was 3441 and for LMI was 3468. These values were a significant difference (P<0.001) at six degrees of freedom between the two models and was in favor of model_2 for both CH_4 traits. Similarly, the AIC values were smaller for both CH_4 traits and smaller values of AIC are considered better fit model. However, the R^2 of calibration and RMSEP were so similar therefore, this test was not conclusive on model selection. However, random within-herd lactation curve effect was significantly than zero (0.11 and 0.05), therefore, it was still important to incorporate in model. Therefore, model_2 was regarded better fit than model_1.

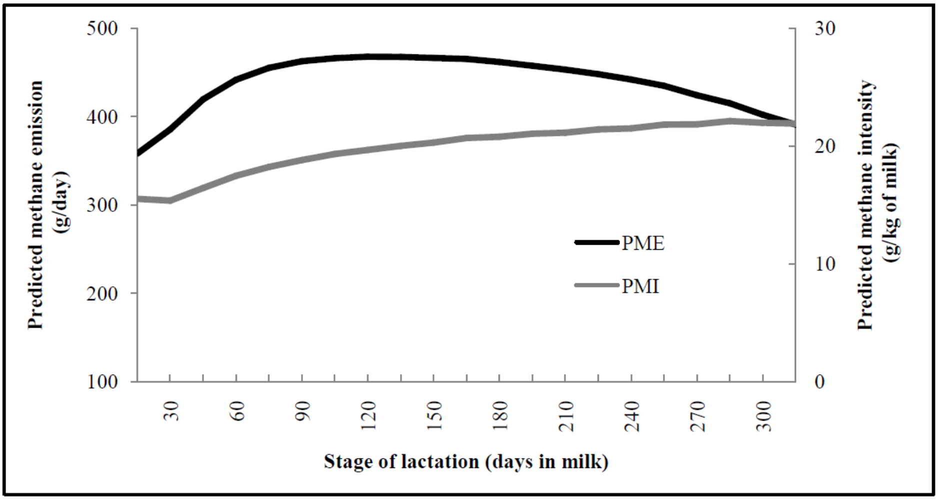

Figure 3-1: Change in daily predicted methane emissions (PME, g/d) and daily predicted methane intensity (PMI, g/kg of milk) with stage of lactation (days in milk) for first lactation Holstein cows

3.3. Partitioning of Variances and Heritability

The average daily variance components with their corresponding relative variances from both models and traits are presented in Table 3-3. Residual variances were same across lactation because model used as constant residual effects. The average relative 305-d variance of the random within-herd lactation effects obtained using model_2 was 0.11 for PME and 0.05 for LMI. By comparing the absolute variance results from the two studied models, 305-d random within herd-specific lactation variance came partly from the 305-d genetic effect along with an increase of the 305-d total variance. The partitioning of 305-d variances within lactation from both models for PME and LMI are presented in Figure 3-2 and Figure 3-3, respectively. The smallest permanent environment variance was observed during mid-lactation. There were slight changes in random within-herd lactation effect variances throughout lactation. Both PME and LMI genetic variances were larger at the beginning and at the end of lactation.

Table 3-2: Comparison of the two models*

Statistical parameters	Predicted methane emission (PME)		Log-transformed methane intensity (LMI)	
	Model_1	Model_2	Model_1	Model_2
2 log Likelihood value	-3,662,149	-3,658,708	-3,024,079	-3,020,611
Log-likelihood ratio (LRT)	3,411[a]		3,464[b]	
Akaike information criterion (AIC)[c]	3,662,175[d]	3,658,746[e]	3,024,105[f]	3,020,649[g]
Bayesian information criteria (BIC)[h]	3,662,315[i]	3,658,952[j]	3,024,245[k]	3,020,855[l]
R^2 coefficient of Calibration	84.89	84.84	86.96	86.90
Root mean square error of prediction (RMSEP)	31.60	31.64	1.19	1.19

*Model_1 had fixed effects as herd x test-day, days in milk and age at calving and random effects as permanent environment, genetic and residual effects. Model_2 had an extra random within-herd lactation effect than model_1. [a, b]Highly significant (<0.001 at 6 degree of freedom); [c,d,e]AIC (Smaller is better; model_2 has 3429 less than model_1);[c,f,g]AIC (Smaller is better; model_2 has 3456 less than model_1); [h,i,j]BIC (Smaller is better; model_2 has 3363 less than model_1);[h,k,l]BIC (Smaller is better; model_2 has 3390 less than model_1).

Average and standard error of daily heritability of PME was 0.32 ± 0.01 and 0.26 ± 0.01, from model_1 and model_2 respectively. Similarly heritability and standard errors of LMI was 0.32±0.01 and 0.27±0.01 from model_1 and model_2 respectively. The differences in heritability values between model_1 and model_2 are attributed to the inclusion of the random within-herd lactation effects in model_2. As mentioned previously, the genetic variability decreased when random within-herd lactation effects were considered. Therefore, it was expected that a lesser estimated heritability would result from model_2 compared to model_1. Daily heritability of PME ranged from 0.27 to 0.42 and from 0.22 to 0.32 from model_1 and model_2, respectively. Similarly, daily heritability of LMI ranged from 0.27 to 0.44 and from 0.20 to 0.36 from model_1 and model_2 respectively (Figure 3-4). Overall, different heritability patterns were observed between PME and LMI. Daily heritability of PME decreased from the beginning of lactation until around 210 DIM and then increased; whereas daily heritability of LMI increased from the beginning to the end of lactation.

Table 3-3: Average daily variances of studied random effects estimated for the Mid-infrared predicted methane emission (PME) and log-transformed predicted methane intensity (LMI) in first parity Holstein cows

		Variance	Herd specific lactation curve	Permanent environment	Genetic	Residual	Total
PME(kg^2/d^2)	Model_1	Absolute	N/A	743	1153	1488	3384
		Relative	N/A	0.22	0.34	0.44	1.00
	Model_2	Absolute	369.58	803	938	1485	3597
		Relative	0.10	0.22	0.26	0.41	1.00
LMI	Model_1	Absolute	N/A	2.20	1.96	2.01	6.16
		Relative	N/A	0.36	0.32	0.33	1.00
	Model_2	Absolute	0.28	2.30	1.67	2.00	6.25
		Relative	0.05	0.37	0.27	0.32	1.00

Model_1 had fixed effects as herd x test-day, days in milk and age at calving and random effects as permanent environment, genetic and residual effects. Model_2 had an extra random within-herd lactation effect than model_1.

3.4. Estimated Emission Pattern for each Random Effects

The EBVs for both predicted CH_4 traits were estimated for sires that had daughters with observed MIR CH_4 predictions. Given the obtained heritability and observed phenotypic variations of PME and LMI, it was expected that substantial differences in EBVs between sires would be observed. The PME EBVs of sires that had daughters with PME using model_2 was ranged from -21.8 to 17.2 kg CH_4 when cumulated over 305-d of lactation. Similarly, the LMI EBVs of sires that had daughters with LMI ranged from -9.8 to 10.7. Therefore, there is significant ranges existed in both that which supports genetic variability of both traits.

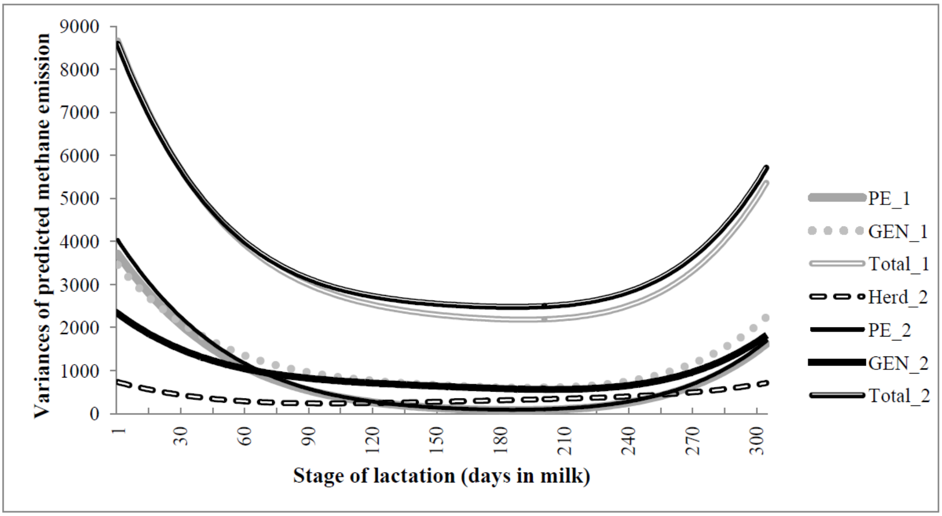

Figure 3-2: Partitioning of mid-infrared predicted methane emission (PME) variances (g^2/d^2) in first lactation for random within-herd lactation curve effect (Herd_2), permanent environmental (PE), genetic and total variances using two models. The residual variance was assumed constant throughout lactation (not shown). Subscript 1 corresponds to model_1 and subscript 2 corresponds to model_2. Model_2 had an extra random within-herd lactation effect than model_1

4. Discussion

Current selective breeding objectives for dairy cattle do not include enteric CH_4 emissions. Selection objective traits should be measurable, have genetic variation and economic value. These MIR predicted CH_4 traits fulfill at least first two criteria. However environment concerns are not yet addressed in breeding goal due to the knowledge gap on how to improve them most efficiently without jeopardy of profitability and how to measure these traits in large scale and accurately. In the future due to socio-economic importance, the third criterion might also be met. The abundance of MIR spectra through routine milk collection is a key to generate these large numbers of CH_4 phenotypes to proceed for genetic analysis.

4.1. Mid-Infrared Predicted Methane Emission Phenotypes

The average PME (440 g/d) and PMI (19.7 g/kg of milk) (Table 3-1) were within the range of several CH_4 emission studies reported in Holstein cows. The normal distribution of PME permitted strait forward to fit in genetic analysis model however the distribution of PMI was non-normal and values were skewed (Table 3-1); therefore the PMI was log-transformed (LMI). Even though, the PMI was log-transformed for genetic analyses, for reasonable comparison at phenotypic level with published literature, PMI was also discussed below. CH_4 emissions and CH_4

intensity measured in respiration chamber from UK Holstein cows were 430 g/d and 21.4 g/kg of milk (Veneman et al., 2015); 360 g/d and 26.5 g/kg of milk were mentioned by Enriquez-Hidalgo et al., (2014) from Irish Holstein; and 418 g/d and 17.4 g/kg of milk were measuring SF_6 techniques in French Holstein by Martin et al., (2008). All of these published CH_4 emission values were based on a relatively small number of cows per experiment (n = 6 to 30). Recently, Moate et al., (2014) reported a CH_4 intensity of 20.2 g/l of milk from 220 Australian dairy cows measured in respiration chambers which are similar to the average (19.7 g/kg milk) of PMI observed in this study. Additionally, a low coefficient of variation could cause difficulties in quantifying genetic variance; however, these predicted CH_4 phenotypes had relatively high coefficient of variations. Therefore, we concluded from the above mentioned references and also those reported by Vanlierde et al., (2015), that PME and PMI values obtained were within the expected range for CH_4 traits.

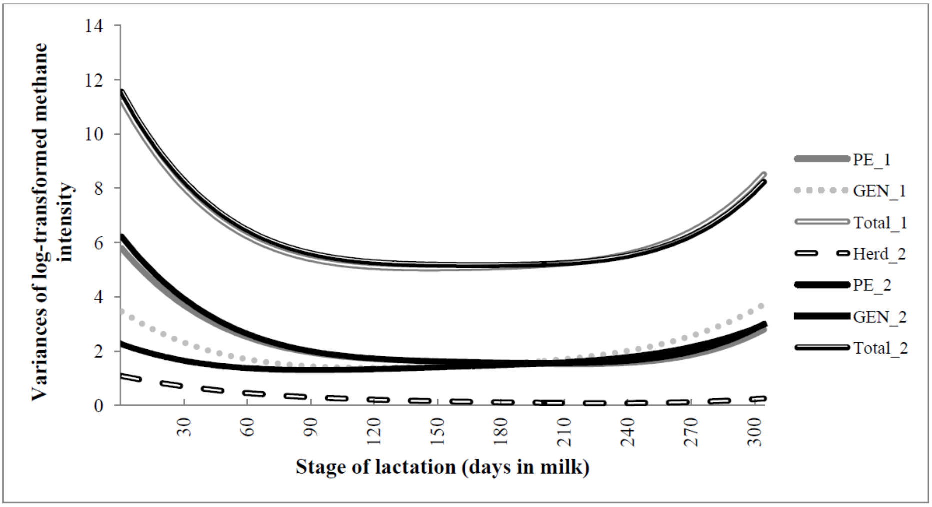

Figure 3-3: Partitioning of mid-infrared predicted log-transformed methane intensity (LMI) variances in first lactation for random within-herd lactation curve effect (Herd_2), permanent environmental (PE), genetic and total variances using two models. The residual variance was assumed constant throughout lactation (not shown). Subscript 1 corresponds to model_1 and subscript 2 corresponds to model_2. Model_2 had an extra random within-herd lactation effect than model_1

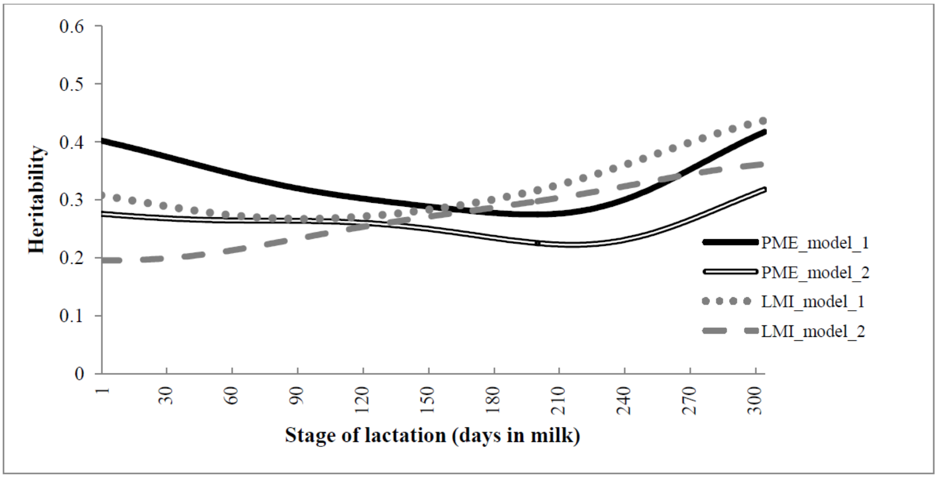

Figure 3-4: Daily heritability of mid-infrared predicted methane emission (PME) and Log-transformed predicted methane intensity (LMI) in first lactation Holstein cows obtained from model_1 and model_2. Model_2 had an extra random within-herd lactation effect than model_1

The prediction of PME pattern within lactation (Figure 3-1) was similar to that obtained by Garnsworthy et al., (2012). The largest amount of CH_4 was predicted in 120-150 DIM which is later than peak milk yield and standard DMI curve during lactation. The PMI pattern (Figure 3-1) differed from the PME pattern as expected due to the definition of PMI which had milk as denominator. Both PME and PMI patterns can be explained by differences in the energy partitioning, feed intake and milk yield at the different stages of lactation. The pattern of PME was explained by the increase of feed intake by cows postpartum as feed intake is positively associated with CH_4 emissions (Hegarty et al., 2007). The PMI records were obtained by dividing PME by the daily milk yield observed at the considered test-day. Therefore, the pattern of PMI within the lactation can be explained by changes in milk production throughout lactation. Lower PMI was therefore observed when the milk production was higher. In nutshell, both predicted CH_4 emission phenotypes values were as expected CH_4 emission from dairy cows and enough to proceed forward for genetic analyses.

4.2. Model Selection and Variance Components Analysis

Based on the obtained LRT, AIC tests, model_2 which had an extra random herd-specific lactation effects better fit of model. Even though R^2 co-efficient calibration and RMPSE results didn’t show improvement in model_2, the relative variance of 11 percent in PME and 5 percent in LMI in total variance explained by herd-specific lactation curves random effects was a good argument for model_2 was better than model_1. Even though, residual variances were same using both models, the total variances were larger in model_2 which reduce relative residual variance by model_2 by 3 percent in LMI and 4 percent in PME also signify that model_2 was better fit than model_1. PME values on some herds had higher PME and 2 to 3 fold greater LMI than the lowest emitting herd (data not shown) which is in agreement with the results reported by O'Brien et al., (2014). Based on their case study to assess the carbon footprint of milk between top-performing and average herds in Irish, UK, and US dairy systems, the top performing herds had carbon footprints 32% less than average herds. Differences between herds observed in both PME and LMI in the present study was explained by analyzing the non-genetic component of the total variance of MIR CH_4 traits (i.e. random herd-specific lactation curve effects in model_2). This enabled the observed differences to be related to herd characteristics which can be the result of variation in CH_4 emission and milk yield; and low CH_4 intensity due to low CH_4 emission and high milk yield across herds. The relative variance of 11 percent of random herd specific lactation effect also support the hypothesis that existence of herd difference of CH_4 emissions of dairy cows. Previous studies for production traits (e.g. Gengler et al., 2005) showed the potential importance of herd-specific lactation curves, here modeled using herd-specific lactation curve effects, and the differences in partitioning of phenotypic variances across the lactation according to the herd type. Understanding the cause of the observed random herd-specific lactation effects could help to adapt the management of cows in a given herd to reduce their CH_4 emission of milk production.

4.3. Genetic Parameters

Average daily heritability values obtained for both CH_4 traits were similar to those estimated for production traits in dairy cattle breeding. These heritability estimates were lower than most short-chain fatty acids predicted from MIR spectra and higher than polyunsaturated fatty acids (Bastin et al., 2013). Heritability differences were noted within lactation. After a slow decrease, an increase was observed from early to late lactation for PME, while LMI heritability increased from towards late lactation. These features were common in both models (Figure 3-4). Differing daily heritability throughout lactation suggests that there might be dynamic genetic regulations within intra-lactation. Differences in heritability estimates at the beginning of lactation within these primiparous cows may reflect differences in metabolism, such as a need for young cows to partition energy toward growth (Wathes et al., 2007) and mobilizing body lipid. This phenomenon might be due to in early lactation, milk production increased rapidly towards peak production but DMI increased at a slower rate (Berry et al., 2007) requiring the energy for milk production to be partly derived from body reserves (VandeHaar and St-Pierre, 2006) resulting in reduced LMI. Conversely, during late lactation, cows increased the deposition of body tissue whilst milk yield declines, thereby increasing LMI during the later stage of lactation. Therefore, milk production, DMI regulation, body fat mobilization and CH_4 emissions should have a dynamic relationship throughout lactation. The difference in physiological stages in growing heifers (primiparous) and multiparous cows with different energy demands and the age of the animal at measurement can substantially affect phenotypes for methane traits, which in turn, affects genetic parameter estimation (Manzanilla-Pech et al., 2016).This could be one of the most important areas for further research to explore genetic regulation of CH_4 emissions which disentangle effects of other factors verses CH_4 emissions. Nevertheless, the genetic parameters obtained from milk MIR spectra predictions are also potential selection traits because of high predictive ability of CH_4 emissions. Previous study estimated the heritability of CH_4 emission (g/d) predicted from milk MIR fatty acids based on equations developed by Chilliard et al., (2009) were estimated to be between 0.22 and 0.40 (Kandel et al., 2015). Similarly, heritability of CH_4 yield (g/kg of DMI) predicted from several groups of fatty acids were reported from 0.12 to 0.44 (van Engelen et al., 2015). The heritability of CH_4 emission g/d and emission intensity (g/kg of milk) indirectly obtained by Fourier transform infra-red gas analyzers was estimated at 0.21±0.06 (Lassen and Løvendahl 2016). Various other proxies have been published that predict CH_4 emissions from dairy cows from milk composition, feed intake and other indirect measurements. Feed intake based prediction of CH_4 emission had a heritability of 0.12 if the indicator was predicted from DMI (Cassandro et al., 2010) and of 0.35 if the indicator was predicted from 6% of gross energy intake (de Haas et al., 2011). The observed differences in heritability values between the literatures and those obtained in the current study may be due to the diversity of CH_4 predictions and their correlations with actual measured traits. However, all studies confirmed the heritable nature of CH_4 related traits.

4.4. Estimated Emission Pattern during Lactation

Substantial differences in EBVs among animals were observed as expected which permit the ranking of sires based on their EBV values. However, additional research is required to obtain data from further lactations and multiple generations to improve the accuracy of a sire EBV for both PME and LMI. This study was only intended to compare two genetic model to estimate genetic parameters of predicted CH_4 traits, however in real life selection of new traits is based on not only genetic parameter of that trait but also correlated response of other economic important traits like milk, fat, protein yield and functional traits. We didn’t cover those traits in this manuscript however research is underway to estimate all correlated responses from all economic traits. Nevertheless, these results provided first insights of partitioning of variances and heritability of predicted CH_4 traits to proceed further.

5. Conclusions

The present study found that the MIR CH_4 traits had moderate heritable components and genetic variability between cows could be exploited in breeding programs. A model including random herd-specific lactation effects provided a better fit than a model containing random permanent environmental and genetic effects. There was substantial range of EBV further reinforce the genetic variability of studied traits. These genetic parameters of MIR CH_4 traits provide a starting point for the selective breeding of dairy cattle with a reduced carbon footprint of milk production.

6. Acknowledgements

This research received financial support from GreenHouseMilk, a Marie Curie initial training network, funded by the European Commission FP-7 and the Methamilk project (SPW-DGO3). The authors acknowledge the Walloon Breeding Association (Ciney, Belgium) for providing the pedigree database, the Comite du Lait (Battice, Belgium) for supply of MIR spectra and University of Liège (SEGI facility) for the use of the NIC3 supercomputer.

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4

Genetic parameters of mid-infrared methane predictions and their relationships with milk production traits in Holstein cattle

In this chapter, using better fit model from chapter 3, genetic parameters for predicted methane traits (PME; g/d and LMI) and their correlation with milk production traits in the first and second lactation dairy cows were estimated. Heritability values of CH_4 *traits were moderate (from 0.17 to 0.24) which confirmed a genetic contribution for the predicted* CH_4 *emissions. The genetic correlation between PME and milk and protein yield were low negatives but fat yield was positive. Genetic correlations between LMI and milk, fat and protein yields were negative implying that selection for higher producing cows would favour a lower* CH_4 *emission intensity.*

From Kandel, P. B., M.-L. Vanrobays, A. Vanlierde, F. Dehareng, E. Froidmont, N. Gengler, and H. Soyeurt. 2017. Genetic parameters of mid-infrared methane predictions and their relationships with milk production traits in Holstein cattle. J. Dairy Sci. 100:5578–5591.

Abstract

Many countries have pledged to reduce greenhouse gases. In this context, the dairy sector is one of the identified sectors to adapt production circumstances to address socio-environmental constraints due to its large carbon footprint related to CH_4 emission. This study aimed mainly to estimate 1) the genetic parameters of two milk mid-infrared based CH_4 proxies (PME; g/d and LMI) and 2) their genetic correlations with milk production traits [milk **(MY)**, fat **(FY)** and protein **(PY)** yields] from first and second parity Holstein cows. A total of 336,126 and 231,400 MIR CH_4 phenotypes were collected from 56,957 and 34,992 first and second parity cows, respectively. PME increased from the first to the second lactation (433 vs. 453 g/d). LMI decreased (2.93 vs. 2.86). We used 20 bivariate random regression test-day models to estimate the variance components. Moderate heritability values were observed for both CH_4 traits and those values decreased slightly from the first to the second lactation (0.25 ± 0.01 and 0.22 ± 0.01 for PME; 0.18 ± 0.01 and 0.17 ± 0.02 for LMI). Lactation phenotypic and genetic correlations were negative between PME and MY in both first and second lactations (-0.07 vs. -0.07 and -0.19 vs. -0.24, respectively). More close scrutiny revealed that relative increase of PME was lower with high MY levels even reverting to decrease and therefore explaining the negative correlations indicating that higher producing cows could be a mitigation option for CH_4 emission. PME phenotypic correlations were nearly equal to zero with FY and PY for both lactations. However, the genetic correlations between PME and FY were slightly positive (0.11 and 0.12) whereas with PY the correlations were slightly negative (-0.05 and -0.04). Both phenotypic and genetic correlations between LMI and MY or PY or FY were always relatively highly negative (from -0.21 to -0.88). As the genetic correlations between PME and LMI were strong (0.71 and 0.72 in first and second lactation); the selection of one trait would also strongly influence the other trait. However, in animal breeding context PME, as direct quantity CH_4 proxy, would be preferred to LMI which is a ratio trait of PME with a trait already in the index. The range of PME sire estimated breeding values were 22.1 and 29.41 kg per lactation in first and second parity. Further studies must be conducted to evaluate the impact of the introduction of PME in a selection index on the other traits already included in this index such as, for instance, fertility or longevity.

1. Introduction

Due to its large carbon footprint, the dairy sector must to adapt its production circumstances to address the current socio-environmental constraints (Wollenberg et al., 2016). This environmental impact is mainly related to the emission of enteric CH_4 by dairy cows occurring during the microbial fermentation of feed in rumen. Changes in feeding or adapted animal genetics are two relevant solutions to mitigate the CH_4 emission. Compared to feeding, genetic selection has a slower response on the field but its effect is permanent and cumulative. To conduct successful genetic mitigation of CH_4, three pre-requisites are needed. First, CH_4 trait must be sufficiently heritable from generation to generation to allow a relatively fast significant improvement. Second, a sufficient genetic variability of this studied trait must exist in the considered dairy cow population. Third, genetic correlations of CH_4 with other traits of interest need to be known. To verify these 3 pre-requisites, genetic analysis must be performed.

Currently, more and more researches focus on the genetic variability of CH_4 emitted by dairy cows. Heritability (**h^2**) of CH_4 emission quantified using respiration chambers, considered as the gold standard for CH_4 measurements, is not available due to technical and financial difficulties to obtain sufficient phenotypes. However, few studies report h^2 values using gas analyzer technique. Pickering et al. (2015) found a h^2 of 0.05 for the daily CH_4 emission. For the same trait, Lassen and Løvendahl (2016) reported higher values (0.25). All of these results came from relatively low scale studies (i.e., low number of cows and herds). To increase the number of phenotypes, the use of CH_4 correlated traits easier to be collected on the field and in many herds is relevant. Due to the high relationship between the quantity of CH_4 eructed by dairy cows and the feed intake, some authors estimated the h^2 of CH_4 from feed intake based CH_4 predictions. So, Pickering et al. (2015) found a higher h^2 compared to the one obtained from gas analyzer (0.13). Cassandro et al. (2010) reported a similar h^2 value (0.10). However, de Haas et al. (2011) estimated a higher h^2 value (0.35). Even if this methodology allows the collection of a higher number of phenotypes, the acquisition of feed intake and composition records is not largely generalized on a routine basis.

Another alternative is the use of milk composition as a proxy of CH_4 emission. The FA profile seems to be valuable information (Chilliard et al., 2009; Dijkstra et al., 2011). So, van Engelen et al. (2015) estimated the h^2 of CH_4 yield (g/kg of DMI) predicted from several groups of FAs. Obtained h^2 ranged from 0.12 to 0.44. As the FA profile can be predicted using MIR spectrometry (Soyeurt et al., 2011), this method could be used to predict directly the quantity of CH_4 eructed daily by dairy cows. So, Dehareng et al. (2012) developed the first MIR CH_4 equation which was later improved by Vanlierde et al. (2015 and 2016). As this methodology is used currently for the milk recording which implies an individual milk sample collection from all productive cows in all participated herds every 4 or 6 weeks, the MIR CH_4 phenotypes have the advantage to be fast, cheap and allow a large scale data recording. Moreover, as it is known that the quantity of CH_4 eructed by dairy cows varies within and between lactations (Garnsworthy et al., 2012), the first objective of this study is the estimation of the genetic parameters of MIR CH_4 emission (PME, g/d) and intensity (PMI, g/kg of milk) traits from first and second parity Holstein cows. Moreover as the relationships of CH_4 emission and intensity with other economically important traits are relatively unknown at large scale, the second objective of this paper is to estimate the phenotypic and genetic correlations between those two MIR CH_4 traits with MY, FY, PY.

2. Materials and Methods

2.1. Data

Milk samples were collected from first and second parity Holstein cows between January 2010 and April 2014 as part of the routine milk recording undertaken in the Walloon region of Belgium by the Walloon Breeding Association (Ciney, Belgium). All milk samples were analyzed using Foss Milkoscan FT6000 spectrometers (Hillerød, Denmark) by the milk laboratory 'Comité du Lait' (Battice, Belgium) to quantify the contents of fat and protein and to generate the spectral data.

PME (g/d) was predicted from the recorded milk MIR spectra using the lactation-stage-dependent equation developed by Vanlierde et al. (2015). In few words, this CH_4 MIR prediction equation was developed from 446 CH_4 measurements from 142 Belgian and Irish cows. Reference daily CH_4 emissions of individual cows were determined using the SF_6 tracer gas technique with a gas collection period of 24 h. The calibration dataset was characterized by a mean of 416 ± 128 g of SF_6 CH_4/d with minimum and maximum of 180 and 942 g/d. The lactation-stage-dependent equation was developed from those reference values and their corresponding daily milk MIR spectra. The standard error of calibration of this equation was of 63 g/d. The calibration coefficient of determination was equal to 75%. More details about the sample collection and data treatment are reported in Vanlierde et al., (2015). PMI (g/kg of milk) was defined as the ratio of PME divided by the daily MY (kg/d) recorded on the same test-day. This trait was then log-transformed to be normally distributed and called LMI. In order to eliminate potential abnormal records, the predicted MIR CH_4 traits values below the 0.1 percentile and above the 99.9 percentile were deleted.

Only cows between 5 and 365 DIM and with at least 75% of Holstein genes were studied. If a cow had CH_4 records for second parity, this cow must have also records for first parity to be considered in the study. As proposed by the International Committee for Animal Recording, milk production records were retained if they were between 3 and 99 kg of milk yield, 1 and 7% of protein, and 1.5 and 9% of fat. Moreover, only herds having at least 100 MIR CH_4 phenotypes were kept in this study. Therefore, the final dataset contained 366,126 and 231,400 test-day records collected from 56,957 and 34,992 first and second parity Holstein cows belonging to

935 herds. Pedigree data were obtained from the pedigree database used for the Walloon genetic evaluation. The pedigree file contained 120,504 animals born after 1990. The average number of daughters per sire was 84 with a minimum of 15 daughters and a maximum of 9,762 daughters from a total of 581 sires.

2.2. Genetic Analyses

Twenty bivariate random regression test-day models were performed to estimate the variance components of studied traits. The pairs were PME and MY; PME and FY; PME and PY; LMI and MY; LMI and FY; LMI and PY; MY and FY; MY and PY; FY and PY; PME and LMI in first and second lactation. The model can be summarized as follows:

$$\mathbf{y=X\beta+Q(Hh+Zp+Zu)+e}$$

where **y** was the vector of observations of pair of two traits, **β** was the vector of fixed effects (herd x test-day, DIM (24 classes of 15 days interval), and age at calving (3 classes for each lactation: 21 to 28 mo, 29 to 32 mo, and 33 mo and more for first lactation and 31 to 44 mo, 44 to 48 mo, and 49 mo and more for second lactation),**h** was the vector of random within-herd lactation curve effects, **p** was the vector of permanent environmental random effects, **u** was the vector of additive genetic random effects; **Q** was the matrix containing the coefficients of 2nd order Legendre polynomials; **e** was the vector of residuals; **X** was the incidence matrix assigning observations to fixed effects; **H** was the incidence matrix assigning observations to random within-herd lactation curve effects and **Z** was the incidence matrix assigning observations to the additive genetic or permanent environmental effects.

Variance components were estimated by Bayesian method using Gibbs sampling (Misztal, 2012). Priors of variance components were estimated from univariate models using average information REML method (Misztal, 2012). Posterior means of (co)variance components were calculated using 100,000 iterations after a burn-in of 10,000 iterations. As three replicates were available for the variance components for each studied trait due to the number of used bivariate models, the variance estimates were averaged.

Average daily h^2 value was the average of h^2 estimated for each DIM between 5 and 305 DIM. These daily h^2 at a specific DIM was calculated as the ratio of the genetic variance to the total variance (i.e., the sum of variances estimated from the within-herd lactation curve, genetic variances, permanent environment, and residual) at the considered DIM. Standard errors of h^2 estimates were computed using the method reported by Fischer et al., (2004) based on variance estimates from the inverse of the average information matrix.

Daily phenotypic and genetic correlations between trait a and trait b at DIM i were calculated as followed:

$$r_{a_i,b_i} = \frac{t \sum_{a,b} t'}{\sqrt{\left(t_i \sum_a t_i'\right)\left(t_i \sum_b t_i'\right)}}$$

where t was the vector created by summing coefficients of Legendre polynomials for DIM 5 to 305; $\Sigma_{a,b}$ was the matrix including the genetic or phenotypic covariances between trait a and trait b; and Σ_a and Σ_b were the genetic or phenotypic variance matrices for traits a and b, respectively.

The breeding values were estimated (EBV) using a BLUP approach. Daily EBV for each DIM between 1 and 305 and for cows with records were calculated as following:

$$\text{daily EBV}_{htk} = \sum_{m=0}^{2} a_{hkm} z_{tm},$$

where $EBVd_{htk}$ was the daily breeding value of cow k, for trait h, for each DIM t between 5 and 305; $a_{hkm}$was the BLUP solution of the additive genetic effect of order m; $z_{tm}$was the covariate of Legendre polynomial of order m associated with DIM t; and $z_{t0} = 1.0$, $z_{t1} = 3.0^{0.5}x$, $z_{t2} = 5.0^{0.5}(1.5x^2 - 0.5)$, where $x = 2[(t - 5)/305] - 1$. Only EBV of sire having daughters with MIR CH_4 phenotypes were kept for this step. All daily EBVs were cumulated to get 305d-EBV of animal for each trait. The Spearman correlations were calculated between EBVs of sires estimated using first and second parity records in order to assess the differences between sire rankings for all MIR CH_4 traits.

3. Results

3.1. MIR Methane Traits and their Observed Relationships with Milk Yield

Table 4-1 provides the mean and standard deviation of all studied traits in first and second lactation. PME increased from the first to the second lactation (433 vs. 453 g/d). Inversely, PMI decreased (19.8 vs. 18.8 g/kg of milk) as LMI (2.93 vs. 2.86). The coefficients of variation between lactations stayed relatively stable for all studied CH_4 traits (18.6% and 17.1% for PME; 11.1% and 12.8% for LMI in first and second lactation, respectively).

Figure 4-1a illustrates the relationship between PME and MY. An increase of MY increased PME until on average 20 kg of milk/d then the relationship was nearly flat and was slightly negative for high daily productive cows. PME increased at the beginning of lactation, but in contrast to MY, the peak of PME reached later (after 120th DIM; Figure 4-2a). Similar patterns were observed for both studied lactations even if the values were slightly higher for the second lactation (Figure 4-2a).

Table 4-1: Mean ± standard deviation of all studied traits

Traits	Lactation 1 (N=366,435)	Lactation 2 (N=231,743)
MIR CH4 emission (g/d)	433 ± 80	453 ± 78
MIR CH4 intensity (g/kg of milk)	19.8 ± 6.8	18.8 ± 7.6
Log-transformed MIR CH4 intensity	2.93 ± 0.33	2.86 ± 0.37
Milk yield (kg/d)	23.41 ± 6.42	26.79 ± 8.24
Fat yield (kg/d)	0.92 ± 0.23	1.07 ± 0.32
Protein yield (kg/d)	0.78 ± 0.19	0.90 ± 0.25

The relationship between MY and PMI was curvilinear and after log-transformation, the relationship was linearized (Figure 4-1b). LMI pattern within lactation was the lowest around 50th DIM and higher values at beginning and late lactation. Due to the higher MY in second lactation, PMI was lower throughout second lactation than first lactation (Figure 4-2b).

3.2. Heritability Estimates of MIR Methane Traits

We have observed moderate h^2 values for PME and those values decreased slightly between first and second lactation (0.25 ± 0.01 and 0.22 ± 0.01). Lower values were observed for LMI compared to PME and stayed stable between lactations (0.18 ± 0.01 and 0.17 ± 0.02; Table 4-2). Values of h^2 estimated for MY, FY and PY were similar to those previously reported by Bastin et al. (2013) from Walloon Holstein cattle.

Within lactation, daily h^2 of PME ranged from 0.20 to 0.27 in first lactation and from 0.16 to 0.26 in second lactation. In first lactation, h^2 estimates of PME increased from beginning, were higher in mid lactation and decreased towards the end of lactation. In second lactation, PME h^2 decreased from early lactation towards the end of lactation (Figure 4-3). Within lactation, LMI ranged from 0.12 to 0.24 and from 0.13 to 0.19 in first and second lactation, respectively. The h^2 values of LMI in both lactations increased linearly from the beginning to the end of lactation. LMI h^2 pattern observed for the two lactations were globally similar even if larger differences of h^2 appeared at the end of the lactation (Figure 4-3).

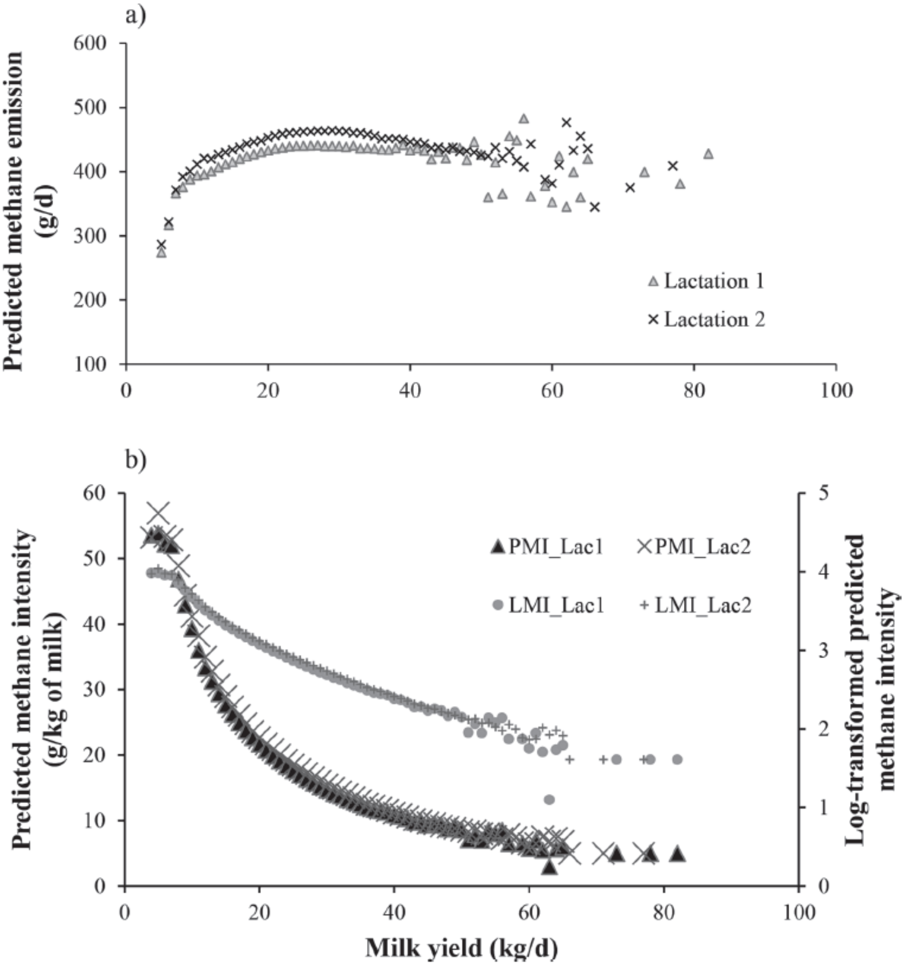


Figure 4-1: Relationship between mid-infrared predicted CH_4 emission (g/d; 4.1a) or intensity [PMI; g/kg of milk, and $\log_{10}$(PMI), called LMI; 4.1b] and milk yield (kg/d) from first- and second-parity Holstein cows. Lac1 = lactation 1; Lac2 = lactation 2

3.3. Phenotypic and Genetic Correlations between MIR Methane Traits and Milk Yield

Phenotypic correlations between PME and MY were slightly negative in both lactations (-0.07). The estimated within-lactation phenotypic correlations were equal to -0.11 at the beginning of lactations and then increased gradually towards zero until the end of lactation (Figure 4-4a). However, at genetic level, the estimated correlations were higher. Indeed, the lactation genetic correlations were equal to -0.19 and -0.24 for the first and second lactation, respectively (Table 4-2). Compared to phenotypic level, the changes of genetic correlations throughout the lactation was more marked notably for the first lactation. Indeed, the genetic correlations decreased until around 200th DIM and then increased rapidly until the end of lactation (Figure 4-4a).

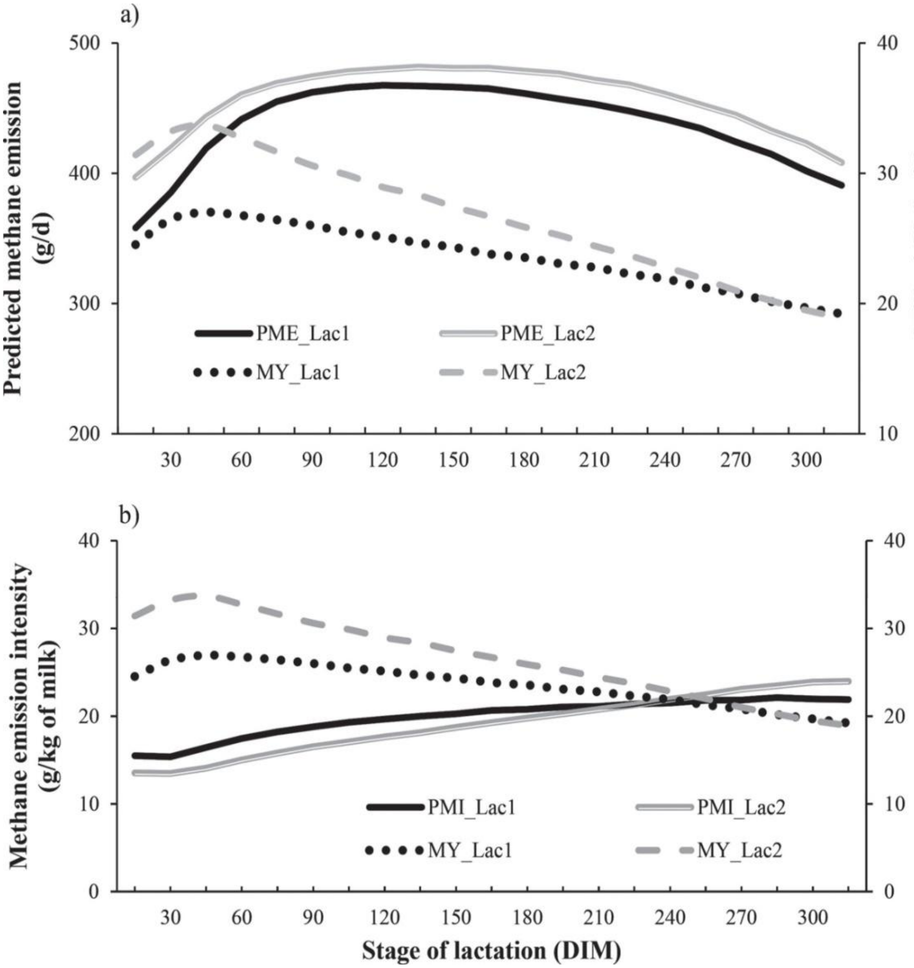


Figure 4-2: Change in daily mid-infrared (MIR) CH_4 emission (PME), milk yield (MY), MIR CH_4 intensity (PMI), and $\log_{10}$(PMI), called LMI, across lactation from first- and second-parity Holstein cows. Lac1 = lactation 1; Lac2 = lactation 2

Phenotypic correlations between LMI and MY were highly negative in both lactations (-0.68 and -0.72; Table 4-2) and stayed relatively constant throughout the lactation even if a slight decrease of values can be observed for the second parity at the end of the lactation (Figure 4-4b). Highly negative correlations were also observed at the genetic level. The values ranged between -0.60 and around -0.85 for both lactations. The correlations decreased until around 180th DIM and then increased untilthe end of lactation (Figure 4-4b).

Table 4-2: Heritability (diagonal and bold), phenotypic (below the diagonal) and genetic (above the diagonal) correlations between mid-infrared (MIR) CH4 traits and production traits in the first (first row) and second (second row) lactation Holstein cows

	PME	LMI	Milk yield	Fat yield	Protein yield
PME	**0.25**	0.71	-0.19	0.11	-0.05
	0.22	0.72	-0.24	0.12	-0.04
LMI	0.47	**0.18**	-0.68	-0.21	-0.66
	0.46	**0.17**	-0.88	-0.26	-0.62
Milk yield	-0.07	-0.68	**0.16**	0.79	0.92
	-0.07	-0.72	**0.14**	0.84	0.94
Fat yield	-0.01	-0.43	0.79	**0.13**	0.82
	0.01	-0.51	0.83	**0.12**	0.86
Protein yield	-0.02	-0.55	0.92	0.82	**0.14**
	0.01	-0.61	0.93	0.86	**0.11**

PME = MIR CH_4 emission (g/d); LMI = Log-transformed MIR CH_4 intensity

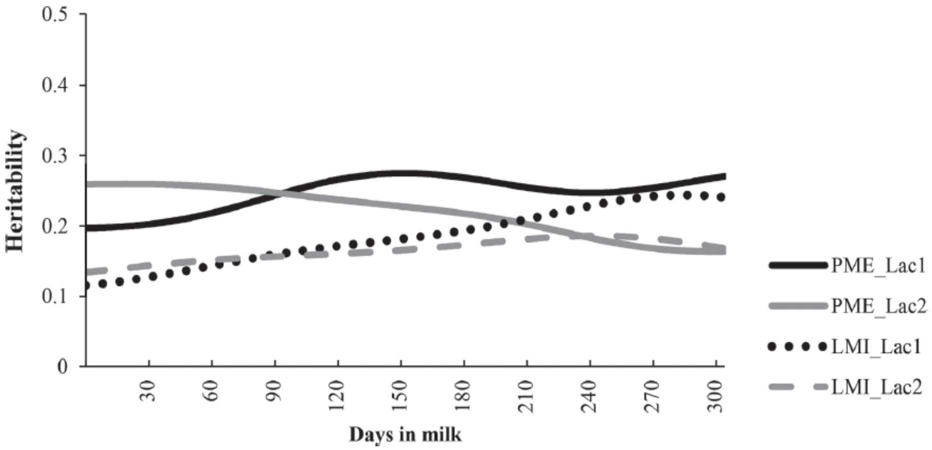


Figure 4-3: Daily heritability estimates for MIR CH_4 emission (PME) and log-transformed MIR CH_4 intensity (LMI) in first and second lactation

3.4. Phenotypic and Genetic Correlations between MIR Methane Traits and Fat Yield

Lactation phenotypic correlations between PME and FY were nearly equal to zero in both lactations (-0.01 and 0.01; Table 4-2). However, marked changes of phenotypic correlations were observed throughout the lactation. Indeed, the within lactation phenotypic correlations between PME and FY were negative at early lactation, zero at mid-lactation and positive at late lactation. We have observed this tendency in both studied lactations (Figure 4-5a). At the genetic level, lactation correlations were positively higher (0.11 and 0.12; Table 4-2); however negative correlations were observed at the beginning of second lactation until 90th DIM. In first lactation, the correlation values were globally positive throughout the lactation (Figure 4-5a).

Lactation phenotypic correlations between LMI and FY were moderately negative for both lactations (-0.43 and -0.51; Table 4-2). Compared to PME, the correlations values were always negative throughout the lactation (Figure 4-5b).

3.5. Phenotypic and Genetic Correlations between MIR Methane Traits and Protein Yield

As observed with FY, lactation phenotypic correlations estimated between PME and PY were nearly equal to zero in both lactations (-0.02 and 0.01; Table 4-2). Similarly to FY, the within lactation phenotypic correlations between PME and PY were negative at early lactation, zero at mid-lactation and positive at late lactation in both studied lactations (Figure 4-6a). Compared to FY, the genetic correlations estimated between PME and PY stayed relatively similar to the ones obtained at the phenotypic level (-0.05 and -0.04; Table 4-2). However the pattern of genetic correlation changes was more fluctuating (Figure 4-6a).

Lactation phenotypic correlations between LMI and PY were higher than the ones observed with FY but lower than the ones observed for MY (-0.55 and -0.61; Table 4-2). At the genetic level, the correlations were also negative and the values were similar to the ones observed at the phenotypic level (-0.66 and -0.62; Table 4-2). Within lactation correlations between LMI and PY were relatively stable at the phenotypic level but more fluctuating at the genetic level (Figure 4-6b).

3.6. Genetic Variability of Methane MIR Traits

EBVs of MIR CH_4 traits in both lactations were estimated for sires that had daughters with MIR CH_4 phenotypes. The lowest sire EBV for PME cumulated at 305 days was -11.15 kg and the highest was 11.01 kg in first lactation and was -15.68 kg and the highest was 13.73 kg in second lactation. The range was therefore equal to 22.15 kg in first parity and 29.41 kg in second parity. The Spearman correlations of EBV of PME between first and second lactation was 0.92 and 0.95 for LMI.

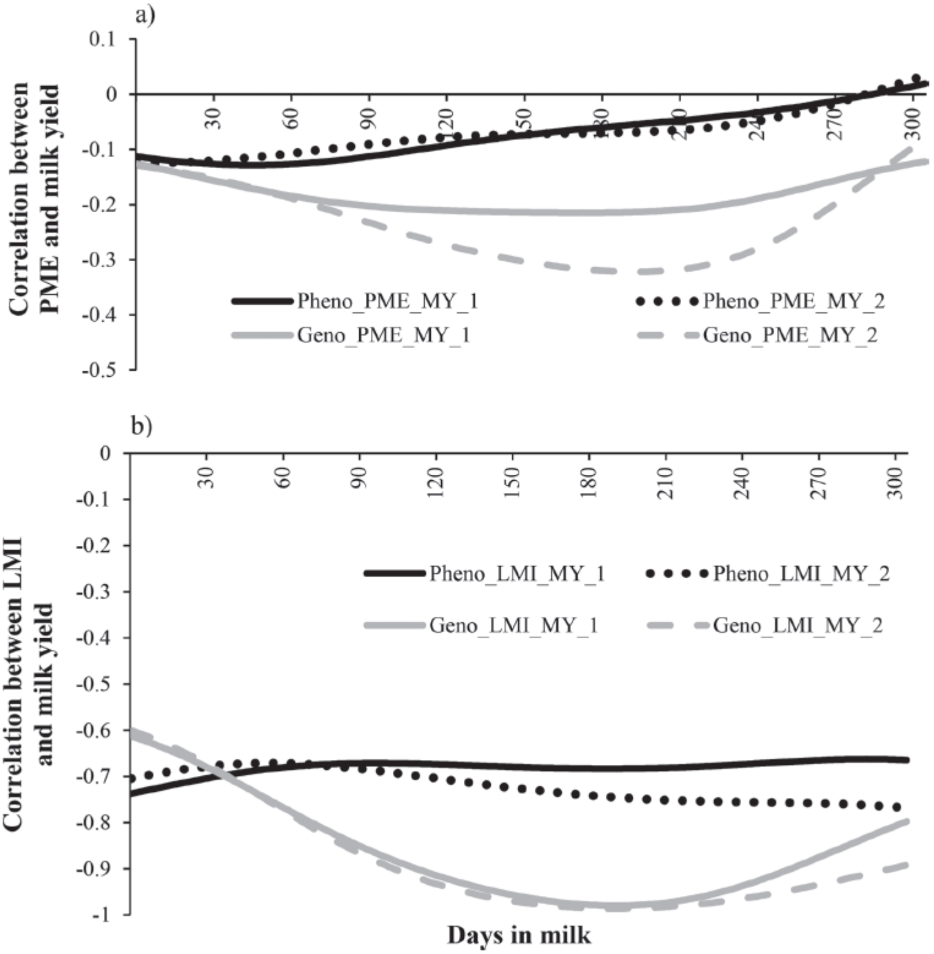


Figure 4-4: Genetic and phenotypic correlations between MIR CH_4 emission (PME; Figure 4-4a) or log-transformed MIR CH_4 intensity (PMI; Figure 4-4b) and milk yield within the first and second lactation

4. Discussion

Current selective breeding objectives for dairy cattle do not include enteric CH_4 traits. However, the improvement of livestock through genetics is particularly an effective technology, producing permanent and cumulative changes of trait in a desired direction (Wall et al., 2010). In this context, a study about the genetic variability of MIR CH_4 predictions can be a starting point for the inclusion of such environmental trait in future selection index. To achieve this, 3 pre-requisites must be verified: 1) MIR CH_4 traits must have a behavior similar to the one observed from gold standard CH_4 measurements, 2) MIR CH_4 traits must be heritable and 3)

MIR CH_4 traits must present a sufficient genetic variability in the studied dairy cow population. Finally if all of these pre-requisites are satisfied, the knowledge of the relationships between these MIR traits with high economic interest traits is required. We have considered only the relationships with milk production traits in this paper.

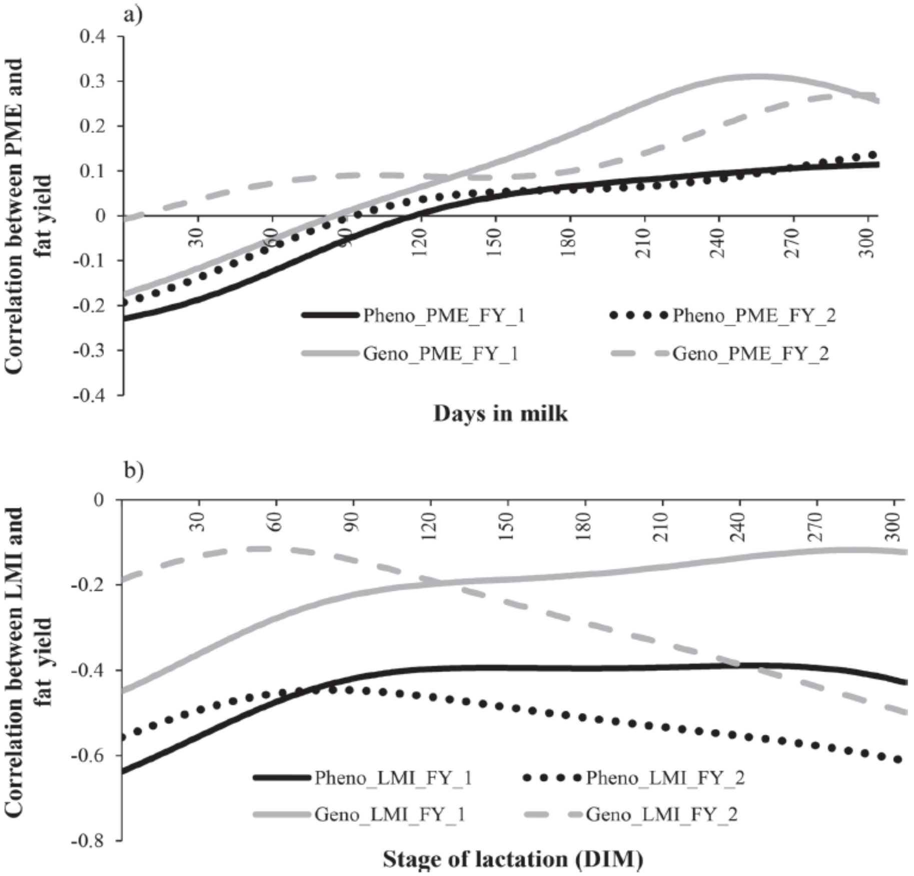


Figure 4-5: Genetic and phenotypic correlations between MIR CH4 emission (PME; Figure 4-5a) or log-transformed MIR CH4 intensity (PMI; Figure 4-5b) and fat yield within the first and second lactation

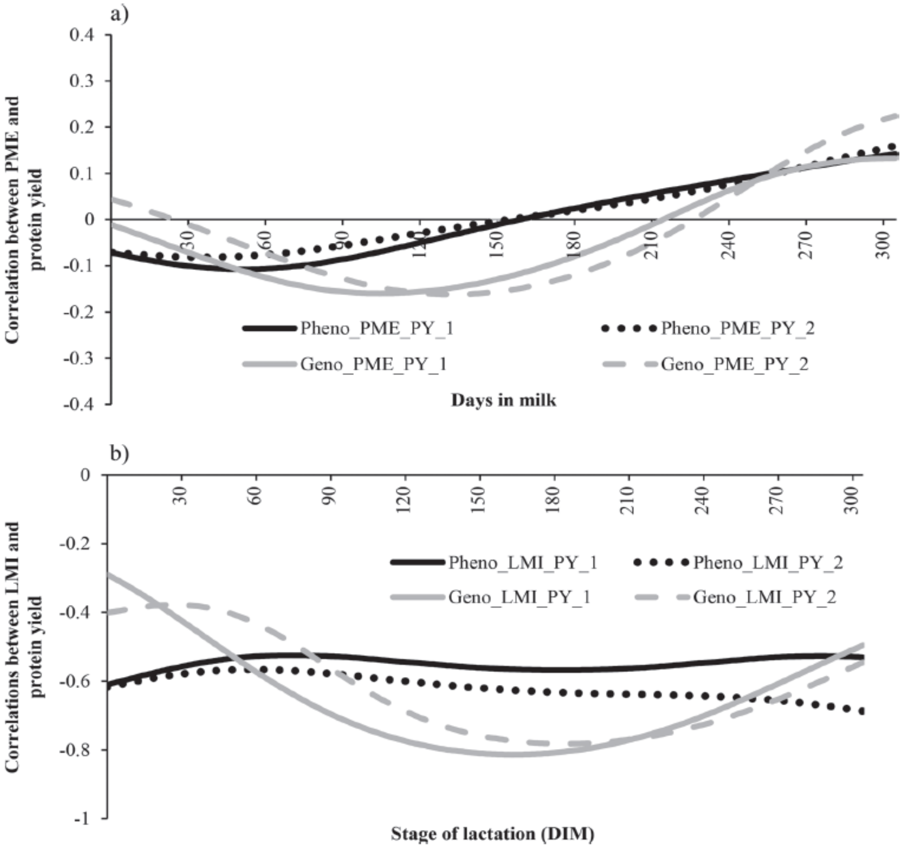


Figure 4-6: Genetic and phenotypic correlations between MIR CH4 emission (PME; Figure 4-6a) or log-transformed MIR CH4 intensity (PMI; Figure 4-6b) and protein yield within the first and second lactation

4.1. Behavior of MIR Methane Traits compared to Reference Methane measurements

This study used CH_4 phenotypes predicted using milk MIR spectrometry. The calibration coefficient of determination for the MIR CH_4 equation used was equal to 0.75 and their calibration standard error was of 63 g/d (Vanlierde et al., 2015). Therefore the phenotypes used in this study must be considered as a proxy of the enteric quantity of CH_4 eructed by dairy cows. So, it is necessary to verify if these MIR CH_4 phenotypes have the expected behavior compared to the findings published in the literature.

The means of PME (433 and 453 g/d) and PMI (19.8 and 18.8 g/kg of milk; Table 4-1) were within the range reported in several studies conducted on dairy cows. For instance, Veneman et al. (2015) reported 430 g of CH_4/d and 21.4 g of CH_4/kg of milk from Holstein cows installed in respiration chambers. Enriquez-Hidalgo et al. (2014) mentioned 360 g/d and 26.5 g/kg of milk. Martin et al. (2008) obtained 418 g/d and 17.4 g/kg of milk from SF_6 experiment. Moate et al. (2014) reported a CH_4 intensity of 20.2 g/l of milk.

More than only the similarities with CH_4 estimates, the between and within lactation tendencies were also in agreement with the literature. The within lactation changes of daily PME (g/d) depicted in Figure 4-2 were similar to the one reported by Garnsworthy et al. (2012) using gas analyzer technique. Based on the same authors, the higher values of PME and the lower of PMI observed in second lactation were also expected and can be mainly explained by the changes of feed intake, feed efficiency, energy partitioning and the evolution of milk production.

MIR CH_4 phenotypes should also have the expected behavior when they are compared to the evolution of MY. The curvilinear relationship between PMI and MY observed in this study (Figure 4-1b) suggested the same pattern between CH_4 intensity and MY. This type of curve was also reported by Moate et al. (2014). Concerning PME, the correlation observed between these values and MY was equal to 0.33 which was a very low value compared to the expected relationship and may be considered controversial. There are however several elements that appear under closer scrutiny in our data that can explain this value. First there is obviously no doubt about the fact that PME is related to the quantity of feed intake and there is no indication that this relationship differs significantly from a linear one. However the relationship between the quantity of milk produced by the cow and its feed intake is not as linear but also lactation stage dependent. Interestingly Figure 4-1a shows the pattern of the evolution of the relationship between PME and MY was not linear. Figure 4-7a represents the evolution between PME and MY also in regards to the evolution of DIM and the number of records in function of classes of 1 kg MY. By observing more closely the pattern of this evolution (Figure 4-7a) and limiting to MY classes with at least 1,500 test-day records (Figure 4-7b), 2 distinct areas appeared: one from 9 to 23 kg of milk/d and another one from 24 kg to 38 kg of milk/d. The first area showed a nearly linear increase of PME with MY and a strong positive correlation between them (0.83) and the second area showed first a stagnation of the increase of PME and then after 30 kg/d a slight, nearly linear, decrease of PME leading to a negative correlation (-0.43). Under more close scrutiny, we can observe that the evolution of PME as a function of MY class in the first area (i.e., positive correlation) can be largely explained by the average DIM of each MY class (R=-0.95). Therefore if DIM increased, PME decreased. This is expected due to the relationship between feed intake and DIM in late lactation, as all average DIM were > 170 days. The second area (until 38 kg/d in order to have a sufficient number of records to calculate the average PME; Figure 4-7b) was less explained by DIM and showed a positive relationship between PME and DIM (0.56). This is consistent with the fact that this area was more associated with cows in the first part (< 165 DIM) of their lactation, therefore increasing their feed intake and their PME with DIM. Moreover, there are three associated elements that we might put forward to explain the behavior for high classes of MY. First high yielding cows might be more efficient which leads to increasing MY without higher emissions of CH_4. But we have no elements to proof this. Second, cows in early lactation produce more milk, but potentially reach their feed intake limits too, therefore mobilizing energy from their adipose tissue to produce additional milk, this milk being not related to an increase of PME. Third, in Belgium currently the feeding systems used increase the ratio of concentrates to roughage strongly for MY over 30 kg, in many farms even for lower levels of production, because of the wide-spread use of concentrate feeding stations. These increasing levels of concentrates in the feeding of higher MY imply relatively lower CH_4 emissions. Unfortunately, no data about feed intake or composition as well as feed efficiency is available in the Walloon Region of Belgium to confirm all these hypotheses.

Even if the calibration coefficient of determination was nearly equal to 1, all of these results confirmed that the MIR CH_4 phenotypes (i.e., indirect CH_4 proxy) had a behavior in line with the expectations based on the findings obtained from direct CH_4 measurements or estimates.

4.2. Heritability of MIR Methane Traits

Several recent studies described the genetic regulation of either CH_4 emissions or proxies of CH_4 emissions predicted from feed intake. The estimation of h^2 from direct CH_4 measurements on dairy cows using respiration chambers, considered as the gold standard method, is not feasible due to an insufficient acquisition of CH_4 phenotypes explaining by technical and financial reasons. Currently, gas analyzer instrument allows a larger CH_4 data acquisition. Various results of h^2 were published in the literature: for instance, 0.05 by Pickering et al. (2015) and 0.21 ± 0.06 by Lassen and Løvendahl (2016). However, such studies were also conducted at relatively low scale even if the study of Lassen and Løvendahl covered 20 herds and 1,745 cows. To conduct larger scale studies, the use of CH_4 proxies are interesting. A proxy is a trait directly or indirectly correlated with the interest trait. By using MIR CH_4 phenotypes, the current study enters in this context. Indeed, MIR CH_4 phenotypes can be considered as indirect CH4 proxies. From more than 230,000 records and using random regression test-day models, this study found moderate h^2 for PME suggesting a moderate genetic component of the quantity of CH_4 eructed by dairy cows. These results are in agreement with other studies conducted from CH_4 direct measurements (e.g., Lassen and Løvendahl, 2016) and from CH_4 proxies. Indeed, Kandel et al. (2015) found h^2 ranged from 0.22 to 0.40 from CH_4 proxies estimated from milk MIR FAs based on equations developed by Chilliard et al. (2009). The h^2 for CH_4 proxies (g/d) derived from feed intake information was estimated to 0.13 by Pickering et al. (2015), 0.10 by Cassandro et al. (2010) and 0.35 by De Haas et al. (2011). Similarly, van Engelen et al. (2015) reported h^2 of CH_4 yield (g/kg of DMI) predicted from several groups of FAs. The h^2 values ranged from 0.12 to 0.44. Even if all of these studies were based on different methodologies, all support the existence of a genetic component of the enteric CH_4

quantity eructed by dairy cows.

Moreover, the daily h² tendencies within and between two lactations were also shown in this study. A slightly decrease of h² was observed between the first and second lactation (0.25 vs. 0.22). Kandel et al. (2013) reported also this decrease using MIR CH_4 phenotypes predicted using an independent-lactation-stage MIR CH_4 equation. However the heritability values obtained by these authors were lower (0.12, 0.10 and 0.09 from first, second and third parity cows). These h² differences could be only explained by the changes of the prediction equation because the studied cow population having the same origin. Within lactation evolution of MIR CH_4 phenotypes predicted using an independent-lactation-stage equation, as done by Kandel et al. (2013), had not the expected shape (Vanlierde et al., 2015). By opposition, the use of a dependent-lactation-stage equation, as done in this study, allows the obtaining of MIR CH_4 phenotypes which have the expected DIM trend (Vanlierde et al., 2015). Within lactation differences in PME h² curves from primiparous and second parity cows were observed and could reflect differences in the partitioning of energy between first and second parity cows (Wathes et al., 2007).

We found that LMI was less heritable than PME (0.18 and 0.17; Table 4-2) and increased linearly throughout the lactation. This h² values were similar to the ones observed for milk yield (0.16 and 0.14). Similar results were obtained by Lassen and Løvendahl (2016). These authors found 0.21 ± 0.06 for the CH_4 intensity measured using gas analyzer technique. De Haas et al. (2011) found a h² of 0.58 using CH_4proxies based on feed intake information. However, the CH_4 intensity trait was not log-transformed in those 2 studies.

4.3. Genetic Variability of MIR Methane Traits

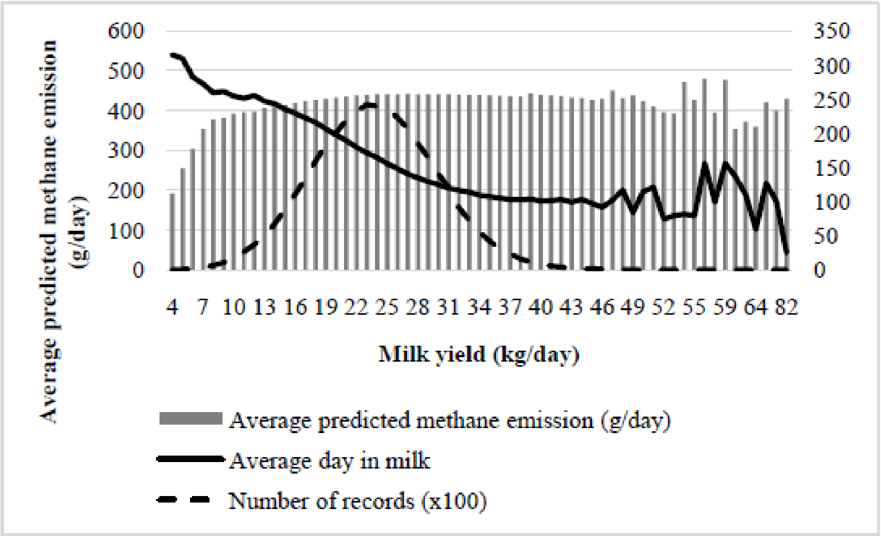
The calculated ranges of 305-d EBV for sires having daughters with MIR CH4 phenotypes were equal to 22.15 kg and 29.41 kg in first and second lactation. These substantial EBV differences between sires were expected because of the obtained moderate h² (Table 4-2) and the observed phenotypic variability of PME (Table 4-1). De Haas et al. (2011) reported a genetic standard deviation around 6 kg of CH_4 per lactation.

The high Spearman correlations values of sire EBVs between first and second lactation for MIR CH_4 traits (0.92 for PME and 0.95 for LMI) suggested that the rankings of animal were similar between the 2 studied lactations.

4.4. Phenotypic and Genetic Correlations between MIR Methane Traits and Milk Production Traits

Negative phenotypic and genetic correlations were observed between PME andMY; genetic correlations were higher (-0.07 and -0.07 vs. -0.19 and -0.24 for first and second lactation, respectively; Table 4-2). These findings are surprising becauseolder researches mentioned a positive genetic correlation (De Haas et al., 2011; Dong et al., 2015; Kandel et al., 2013 and Lassen and Løvendahl, 2016). As

a)



b)

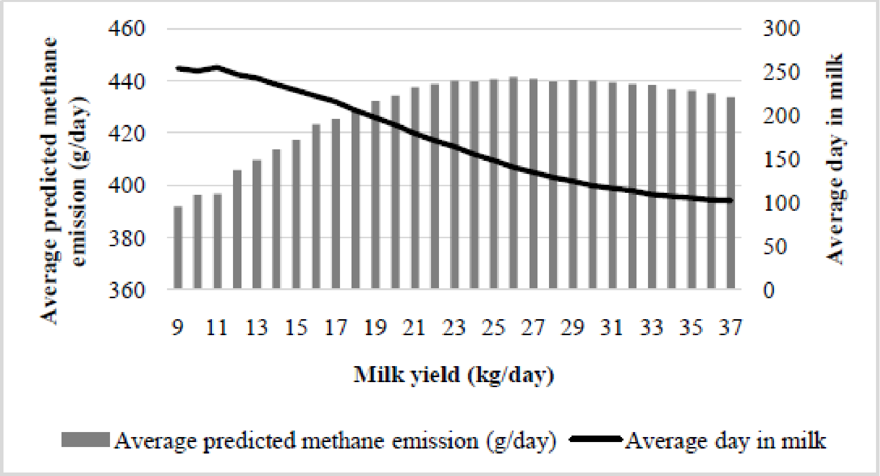


Figure 4-7: Evolution of predicted methane emission (g/d) and day in milk in function of milk yield (g/d) from the entire first lactation dataset (4-7a) and from CH_4 and day in milk averages calculated from at least 1,500 test-day records (4-7b)

mentioned previously, the results obtained by Kandel et al. (2013) must be considered with caution due to the use of a dependent-lactation-stage MIR CH_4 equation. However, from gas analyzer technique, Lassen and Løvendahl (2016) found also a positive genetic correlation between CH_4 emission (g/d) and fat and protein corrected milk (0.42 ± 0.10). The way of measurement for CH_4 in g/d used by these authors forced a positive correlation with MY. Indeed, the CH_4 proxy was estimated by using the CH_4:CO_2 ratio multiplied by the daily CO_2 emission and the heat-producing unit which was calculated using the following formula: (5.6 x live weight$^{0.75}$ + 22 x fat and protein corrected milk + 1.6 x 10^{-5} x days carried calf). De Haas et al. (2011) found also a positive correlation (0.26) from feed intake based CH_4 proxy; the very strong link between this proxy and dry matter intake (0.99) could explained this correlation. Dong et al. (2015) mentioned that there was no apparent influence of genetic merit of cows. Phenotypically, the correlation observed in this study was close to zero. This was expected based on the observed relationship between MY and PME (Figure 4-1a, 4-7a and 4-7b). Indeed, as you had a mix of positive, and negative relationships between MY and PME, it was expected to observe a nearly zero phenotypic correlations. At genetic level, the negative correlations between MY and PME were higher (-0.19 and -0.24; Table 4-2) but stayed relatively low. Two remarks from these results: First, as the value was low, the MY information cannot be considered as a good proxy of CH_4 at the genetic level. Second, if a breeding selection is conducted for high producing cows, the PME will decrease slightly. Unfortunately, it is known that high milk producing cows tend to have more problems of fertility and longevity. This is not desired. Therefore, further studies must be conducted about the links between PME and the other traits included in the selection index such as, for instance, fertility or longevity.

Based on the positive genetic correlations between MY and FY (0.79 and 0.84), it was expected to observe negative correlations between PME and FY but the correlations were positive (0.11 and 0.12). This can be explained. Soyeurt et al. (2008) showed that if MY increased, fat content decreased and the unsaturated FA increased (Soyeurt et al., 2008). Based on the findings of Van Lingen et al. (2014), this involved a decrease of CH_4 emission (g/d). Indeed, those authors reported negative correlations between the concentrations of trans-6+7+8+9 C18:1, trans-10+11 C18:1, cis-11 C18:1, cis-12 C18:1, cis-13 C18:1, trans-16+cis-14C18:1, and cis-9,12 C18:2 in milk fat and CH_4 emission. Therefore the slight positive correlation can be explained by the combination of fat dilution in higher milk production and the changes of milk fat composition in milk produced by high daily milk productive cows. The changes of genetic correlations within lactation were also expected because as mentioned previously CH_4 is influenced by the changes of milk FA composition and these FAs vary within lactation (Bastin et al., 2013). The phenotypic correlations between PME and FY were equal to zero but the changes of correlation value throughout the lactation were important. For instance, in first lactation, negative correlations were observed until 120th DIM and then became positive.

PY is more related to the nitrogen efficiency than CH_4 emissions but MY and PY were strongly positively genetically correlated (0.92 and 0.94; Table 4-2). Therefore the slight negative correlations between PME and PY (-0.05 and -0.04) were expected. The same conclusion can be formulated at the phenotypic level.

The observed phenotypic and genetic correlations between LMI and production traits (MY, FY and PY) were all highly negative for the 2 studied lactations (Table 4-2). Therefore these results support the hypothesis that an increase of cow productivity (i.e., higher MY, FY and PY) will lead to a decrease of CH_4 intensity per kg of milk (Gerber et al., 2013). This relationship confirmed the suggestion of Wall et al. (2010) that the use of higher milk producing cows in a herd can be regarded as one of the mitigation options to reduce environmental impact of the dairy production system. However, this approach could imply fertility and longevity which could also impact the final calculation of carbon footprint of a specific herd. Future investigations must be conducted based on life cycle assessment to verify this.

4.5. Predicted Methane Emission as Selection Trait

Selection for CH_4 mitigation has to be conceived inside adapted breeding programs using updated breeding objectives obtained with selection indexes containing also CH_4 indicator traits. In this study, 2 CH_4 predictions were studied (PME and LMI). Both traits were heritable and presented a genetic variability on the studied dairy cattle. Compared to LMI, PME is a direct CH_4 quantity trait. For all of these reasons, PME would be preferred for a future inclusion in a selection index. Moreover, the relationship between PME and LMI was genetically strong (0.71 and 0.72 in first and second lactation) suggesting to the inclusion of one of these traits will impact the other.PME selection would select sustainable cows while a selection on the LMI would select a sustainable milk production. Before inclusion of PME in a selection index, further studies must be conducted. Indeed, the relationship between PME and other economic interest traits must to be known in order to avoid any undesired impact on cow health, longevity and fertility as well as on milk production and composition.

5. Conclusions

In summary, due to their heritability and genetic variation, PME and LMI can be introduced in selection index to consider the environmental impact of milk production in the future breeding objectives but PME would be preferred as it is a direct CH_4 quantity proxy. However, the genetic correlations between PME and LMI were high, suggesting that a selection of one will trigger improvement of the other. The genetic regulation of PME and LMI differed between early and later lactation especially in first lactation. Different within lactation correlations suggested dynamic relationships between milk production traits and CH_4 emissions. Before the introduction of PME in a selection index, further studies must be conducted to evaluate the impact of this inclusion in all traits already present in the current selection index and not only the common production traits as done in this study; notably because the results suggested that the use of higher milk productive cows can be a mitigation option but it is known that such cows have more problem of robustness, fertility and longevity. Moreover, the improvement and the validation of MIR phenotypes must continue to ensure that the MIR CH_4 proxy used is reliable and accurate.

6. Acknowledgments

The authors acknowledge the support provided by the European Commission under the Seventh Framework Program for the GreenhouseMilk and GplusE projects, Grant Agreements FP7-PEOPLEITN-2008 238562 and FP7-KBBE-613689, the contribution of the COST Action FA1302 Methagene (http://www.methagene.eu) and of the Public Service of the Walloon Region of Belgium (SPW-DGO3) through the Methamilk project. The content of the paper reflects only the view of the authors; the European Community is not liable for any use that may be made of the information contained in this publication. The authors acknowledge the Walloon Breeding Association (Ciney, Belgium) for providing the pedigree database, the Comite du Lait (Battice, Belgium) for the supplying of the MIR spectra and University of Liège (SEGI facility) for the use of the NIC3 and NIC4 supercomputers.

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5

Consequences of genetic selection for environmental impact traits on economically important traits in dairy cow

After demonstrating correlation between milk production traits in chapter 5, the genetic correlation between CH_4 *traits and functional traits (fertility, BCS, longevity), health traits (udder health) and type traits were estimated. There were positive correlations between* CH_4 *emission traits and functional traits suggested there would be tradeoffs between these traits in selection. The capacity related type traits had positive genetic correlations with PME but negative genetic correlation with LMI. Finally, using present Walloon selection program and by selecting PME and LMI, the emission traits would responded by reduction in emission, without jeopardizing in milk production traits but negative consequence in fertility, BCS and health traits.*

Based on: Kandel, P. B., S. Vanderick, M.-L. Vanrobays, H. Soyeurt and N. Gengler. 2017. Consequences of genetic selection of environmental impact traits on economically important traits in Dairy Cows. Anim. Prod. Sci. 57:xxxxx

Abstract

Methane emission is an important environmental trait in dairy cows. Breeding aiming to mitigate CH_4 emissions require the estimation of genetic correlations with other economically important traits and the prediction of their selection response. In this study, test-day CH_4 emissions were predicted from milk mid-infrared spectra of Holstein cows. Predicted CH_4 emissions (PME) and log-transformed CH_4 intensity (LMI) computed as the natural logarithm of PME divided by MY. Genetic correlations of PME and LMI with traits used currently were approximated from correlations between estimated breeding values of sires. Values were for PME with MY 0.06, FY 0.09; PY 0.13; fertility 0.17; BCS -0.02; udder health (**UDH**) 0.22; and longevity 0.22. As expected by its definition, values were negative for LMI with production traits (MY -0.61; FY -0.15 and PY -0.40) and positive with fertility (0.36); BCS (0.20); UDH (0.08) and longevity (0.06). The genetic correlations of 33 type traits with PME ranged from -0.12 to 0.25 and for LMI ranged from -0.22 to 0.18. Without selecting PME and LMI (*status quo*) the relative genetic change through correlated responses of other traits were in PME by 2% and in LMI by -15%, but only due to the correlated response to MY. Results showed for PME that direct selection of this environmental trait would reduce milk carbon foot print but would also affect negatively fertility. Therefore more profound changes in current indexes will be required than simply adding environmental traits as these traits also affect the expected progress of other traits.

1. Introduction

The breeding goal in dairy cattle should support the profitability of milk production. Genetic correlations between MY and reproduction, health and fitness traits are negative, and a decline in many functional traits was reported by many studies (Egger-Danner et al., 2015). Accordingly reproduction, health and fitness traits have been included in breeding goal and also selection indices over the past decade. This has resulted in improvement in these traits (Egger-Danner et al., 2015). However a novel class of traits will need to be considered in the future, those linked to environment concerns. There are at least two major reasons why they are not yet addressed. First direct accurate measurements of these traits on a large scale are difficult to impossible, making their use as selection index traits difficult. Second introduction of environment concerns into breeding goal is also very difficult due to the knowledge gap on how to improve them most efficiently without putting profitability into jeopardy. A major source of the environmental footprint from dairy system is CH_4emissions which is responsible for 4% of the anthropogenic CH_4 emission (FAO, 2010). The enteric fermentation in the rumen accounts for a major part of total CH_4 emitted from dairy cows. In addition to the environmental impact, CH_4 is associated in the literature to a loss of 2 to 12% of gross energy intake (Johnson and Johnson, 1995). Therefore, reducing the CH_4 emitted by dairy cows is of both, economic and environmental, interests. Genetic gains are cumulative and small improvements per generation can build over time. To select any new trait, it must have genetic variation and show heritability. Even with currently only limited research available, CH_4 traits predicted from milk fatty acids (Kandel et al., 2015) and measured through non-invasive method (Lassen and Løvendahl 2016) have shown sufficient heritability. Previous studies have shown that MIR spectroscopy can be used to predict milk fatty acids (Soyeurt et al., 2011) and that milk FAs are indirectly related to CH_4 emission (Chilliard et al., 2009; Dijkstra et al., 2011). Also, the heritability of MIR milk FAs predicted CH_4 emission was estimated between 0.21 to 0.40 (Kandel et al., 2015). Moreover, direct prediction of CH_4 from MIR spectra without the use of milk fatty acids would be a step forward because by avoiding intermediate steps, prediction errors could be minimized (Gengler et al., 2016). Dehareng et al., (2012) and Vanlierde et al., (2015 and 2016) demonstrated that quantification of CH_4 emission directly by MIR spectroscopy from milk samples was feasible and can be useful to generate a large number of indirect CH_4 phenotypes. Vanlierde et al., (2015) supported by results from Vanrobays et al., (2016) showed that links between CH_4 and milk composition are lactation stage specific.

Genetic selection of CH_4 emission traits predicted from MIR spectra of milk samples can be imagined because recent research demonstrated genetic variance and sufficient heritability (Kandel et al., 2017). However, the addition of environmental impact traits into the selection goal needs the careful consideration of its impact on other traits in this goal. Before adding any novel traits, additional information about genetic correlations with other objective traits that are already in place and their predicted response are needed. Amongst the correlations needed are those with milk production traits, with functional traits like fertility and with health traits. Udder health was represented by somatic cell score (**SCS**) on a reversed scale. Even if they are not in the breeding objective, correlations to type and BCS, will allow assessing the impact on these traits too.

Therefore, the objectives of this study were twofold, first to estimate the genetic correlations between environmental impact traits and other traits of interest, and second to quantify their predicted selection response in simple scenarios.

2. Materials and Methods

2.1. Genetic Valuation of Environmental Impact Traits

Currently no routine genetic evaluation exists in the Walloon region of Belgium for environmental impact traits linked to CH_4 emissions. However in order to approximate genetic correlations amongst traits, preliminary evaluations were necessary.

2.2. Milk Samples and Prediction of Environmental Traits

Milk samples were collected from Holstein cows in their first three lactations from January 2010 and March 2014 as routine Walloon milk recording. All milk samples were analyzed using a Milkoscan FT6000 spectrometer (Foss, Hillerød, Denmark) by the milk laboratory ‘Comité du Lait’ (Battice, Belgium) to quantify the contents of fat and protein and to record the spectral data. Production records ranged between 5 and 365 DIM. Official International Committee of Animal Recording **(ICAR)** norms were applied. Therefore observations outside of ranges of 3 to 99 kg milk yield, 1 to 7% protein content and 1.5 to 9% fat content were not used for the calculations as suggested in these norms (ICAR, 2016).

The CH_4 emission (PME; g/d) was predicted from the recorded and standardized (Grelet et. al., 2015) milk MIR spectral database of Walloon milk recording using the equation developed by Vanlierde et al., (2015). The PMI (g/kg of milk) was defined as the ratio of PME divided by the total milk MY recorded for the considered test-day. The distribution of PMI was non-normal and skewed therefore (Figure 5-1) presenting a log-normal aspect. Therefore PMI was log-transformed and called LMI using the natural logarithm. The data sets of predicted environmental traits had 700,505 test-day records from 58,412 first three parity cows sired by 2455 bulls. The heritabilities of PME and LMI were estimated to be 0.25 and 0.18 respectively (Kandel et al., 2017). Within cow, if parity 3 was present, parities 1 and 2 had to be present, and if parity 2 was present, parity 1 had to be present. Animals which had, based on their pedigree, at least 75% of confirm Holstein genetics in their breed composition were kept for this study. Pedigree data were extracted from pedigree used for routine Walloon genetic evaluation and contained 119,068 animals born after 1990 which permitted pedigree up to three generations back.

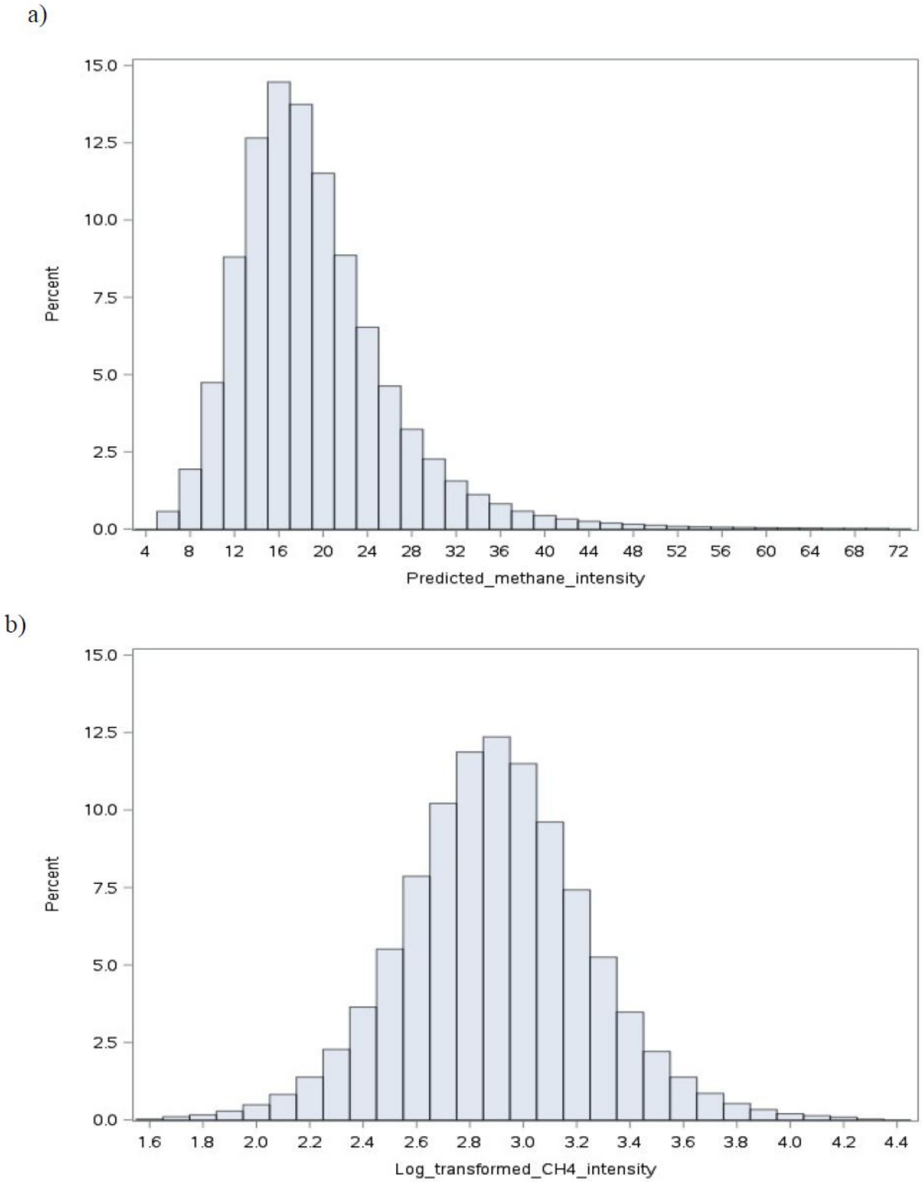

Figure 5-1: Frequency distribution of 700,505 records of (5-1a) predicted methane intensity (g/kg of milk) and (5-1b) log-transformed methane intensity for 58,412 Holstein cows.

2.3. Model

A single trait multiple lactation random regression test-day model was used to estimate the genetic parameters and breeding values of each of PME and LMI. The model can be presented as follows:

$$\mathbf{y} = \mathbf{X}\boldsymbol{\beta} + \mathbf{Q}(\mathbf{Hh} + \mathbf{Zp} + \mathbf{Zu}) + \mathbf{e}$$

where **y** was the vector of observations for each trait (PME or LMI), **β** was the vector of fixed effects (herd x test-day, days in milk (24 classes of 15 days interval), and age at calving (9 classes: 21 to 28 months, 29 to 32 months, and 33 months and more for first lactation; 31 to 44 months, 44 to 48 months, and 49 months and more for second lactation and 41 to 57 months, 57 to 60 months, and 60 months and more for third lactation), **h** was the vector of random within-herd lactation curve effects, **p** was the vector of permanent environmental (**PE**) random effects, **u** was the vector of additive genetic effects; **Q** was the matrix containing the coefficients of 2nd order Legendre polynomial regressors; **e** was the vector of residuals; **X** was an incidence matrix assigning observations to levels of fixed effects**,** **H** and **Z** were incidence matrices assigning regressors to random regression coefficients.

2.4. Variance Components and Solutions of Mixed Model Equations

The variance components were estimated by Bayesian method with Gibbs sampling. Priors of variance components were estimated using univariate models using the average information REML method (Misztal, 2014). Posterior means of (co)variance components were calculated using 90,000 samples after a burn-in of 10,000 samples. The EBVs were calculated using a BLUP approach using obtained variance components.

2.5. Economically Important Traits

The Walloon Breeding Association (Ciney, Belgium) uses for Holstein dairy cows a selection index called V€G (Vanderick et al., 2015). Table 5-1 gives the relative importance of the different traits used in the current index. This index was obtained to select for a breeding goal that was derived based on a lifetime economic function including production and functional traits (N Gengler, pers. comm.). The three categories of traits under routine genetic evaluation in Wallonia and included in the selection index are production, functional traits and type traits. The later were not considered having an economic value on their own, but contributing to the traits in the breeding goal (N Gengler, pers. comm.). Production traits included MY, FY and PY, functional traits were UDH and longevity and more recently fertility and calving traits. These traits were a. combined female fertility (**CFF**), b. direct calving ease (**DCE**) and c. maternal calving ease (**MCE**). The genetic correlations were calculated for all fertility related traits however response to selection was only calculated for combined female fertility. CFF representing pregnancy rate and higher values are better. Direct calving ease and maternal calving ease were just recently added in selection index; therefore, the responses were not calculated in this study however genetic correlations were calculated. The trait BCS is currently used in computations of EBV for combined fertility and not directly in the index or even breeding goal. However there are indications (e.g. Vanrobays et al., 2016) that CH_4 production through its links to fatty acids and intake interacts with body fat mobilization. Effects of selection on CH_4 were also computed for BCS, an indicator of body fat mobilization and, indirectly, an important element for a long term effect on fertility. Longevity was also calculated from direct longevity trait plus genetically correlated type traits. In addition to production and functional traits, a total of 33 type traits (recorded and derived) are also part of Walloon animal genetic evaluation system. Type traits were broadly classified as body capacity, udder and feet and leg traits. Details of all these traits definitions, their genetic model for parameter estimations are described in Vanderick et al., 2015 and Croquet et al., 2006. The EBV of sires for production, functional and type traits were extracted from the database containing the EBV computed for the official Walloon genetic evaluation 2016 March run. These EBV were of domestic, but more often of Multiple Across Country Evaluation (**MACE**) origin, provided by the INTERBULL Center (Uppsala, Sweden).

2.6. Approximated Genetic Correlations

Very few genetic evaluation systems are completely multivariate across all the index traits. An implication of this is also that genetic correlations amongst these traits are not known. In order to achieve the objective of this study approximated values were needed. Pearson's correlations among EBVs of sires were computed in order to get lower-bound estimates of genetic correlations. The productive life of higher yielding cows goes over 2.5 to 3.2 parities before being culled (e.g., Hare et al., 2006). As life (3 parities) genetic correlations and selection response is easy to understand and interpret, therefore in this study we studied all traits cumulated over life time (at least 3 parities). A total of 2455 bulls had daughters with environmental records and subsequently EBV. These EBV were centered and expressed as average daily values but based on cumulative 305 day emissions over the three lactations (Table 5-2). For this 2455 sires the corresponding sire EBV for current official genetic evaluations were selected when they showed sufficient reliability (limits depending upon the traits: 50-99% for production traits and 25-99% for functional and type traits). Table 5-2 gives the figures of selected bulls ranging from 1369 to 1427 for production and functional traits. The equivalent figure was 1422 sires for type traits.

2.7. Selection Scenarios and Predicted Responses

Five selection scenarios were proposed to calculate the selection response. Scenario I was the current Walloon selection index V€G (*status quo*), and from second to fifth selection scenarios were 5, 12.5 and 25 and 50 percent addition of CH_4 emission traits (PME respectively LMI) and proportional reduction on other traits present in current index (Table 5-1). The weight of CH_4 traits were put negative because we were interested to reduce the CH_4 emission from our dairy production. Relative genetic changes for each trait from selection based on these alternative total indexes were estimated as $\mathbf{r} = \mathbf{b}'\mathbf{G}$ where $\mathbf{r}$ = vector of relative genetic gain on all traits; and **b** = vector of proportional index weights; **G** = matrix of genetic correlations between index traits and goal traits. As only relative changes were relevant for this study, selection intensity was set to 1 and response was calculated for one generation.

3. Results

3.1. Environmental Traits and Economic Important Traits Descriptions

The average ± sd PME was 443.86 ± 77.04 (g/d) and LMI was 2.87 ± 0.36 for first three lactations. The sire EBVs of CH_4 emissions traits that had daughters in production were accumulated over three parities, expressed on a daily basis and presented in Table 5-2. Similarly the corresponding sire EBVs obtained from official Walloon genetic evaluation for production (MY, FY and PY) and functional traits (Fertility, BCS, UDH and longevity) were also presented in Table 5-2. Average reliabilities of selected groups ranged between 61 (for maternal calving ease) and 91 (for UDH). The selected sire EBV for type traits are presented in Table 5-3 with average reliabilities between 74 and 91.

3.2. Genetic Correlations between Environmental Traits and Economic Important Traits

The approximate genetic correlations based on correlation between sire EBV, hereafter called simply genetic correlation, between PME and LMI and production and functional traits are presented in Table 5-4. The genetic correlation between PME and LMI was estimated 0.33. PME had small positive genetic correlations with milk production traits i.e. 0.06 with MY, 0.09 with FY and 0.13 with PY. However, the genetic correlation between LMI and milk production traits were negative and in case with MY was highly negative (-0.61) and moderate negative with PY (-0.40) and low negative (-0.15) with FY. The genetic correlation with combined female fertility with both CH_4 traits was positive but higher in case of LMI (0.36 vs 0.17). Other reproductive traits (DCE and MCE) also had positive genetic correlation with PME however negative correlation were observed between LMI and MCE. The correlation between PME and BCS was very close to zero but 0.20 between LMI and BCS. UDH had positive genetic correlation with both CH_4 traits. Finally, longevity had positive genetic correlation with both CH_4 traits (Table 5-4).

The genetic correlations between CH_4 traits and type traits are reported in Table 5-3. The genetic correlation between PME and 33 type traits ranged from -0.12 to 0.25 and between LMI and 33 type traits ranged from -0.22 to 0.18. The body capacity traits also had in general positive genetic correlation with PME and negative genetic correlations with LMI. The body weight related traits like stature and angularity has positive genetic correlation with PME and negative genetic correlation with LMI. The udder capacity traits also had in general positive genetic correlation with

PMEand negative genetic correlations with LMI.

3.3. Expected Genetic Changes under Selection Scenarios

The selection response to each scenario of selecting PME is reported in Table 5-5. The PME would be increased by 2% without selecting this trait but through correlated responses of other traits. A relative weight of 12.5% on PME (selection scenario III) was necessary to decrease PME. A relative weight of 25 % of PME (selection scenario IV) generated a response of PME by -6%, MY by 15%, FY by 6%, PY by 11%, fertility by -4%, BCS by -11%, UDH by 13% and longevity by 22% . In all scenarios MY, FY and PY also increased except with the extreme selection scenario V (50% weight on PME) which decreased FY and PY.

In all PME reduction scenarios, fertility, BCS and UDH would decrease. Given that the longevity has currently a very high weight in Walloon index (~21 percent) longevity has a very positive response in selection scenario I, however the progress would be reduced with each scenario selecting for lower PME.

The favorable genetic gain would be achieved for LMI in all selection scenarios (Table 5-6). The expected response of LMI would range from -15% to -33% from selection scenario I to selection scenario V. MY, FY and PY would increase in each scenario. For example by the addition of 25% of LMI, the resulting response would be for LMI by -24%, MY by 29%, FY by 16%, PY by 28%, fertility by -10%, BCS by -13%, UDH by 13% and longevity by 23%.

Table 5-1: Current Walloon selection index V€G for dairy cows (Scenario I) and selection scenarios for environmental traits (II-V)

				Selection Scenarios				
Sub-index	2nd order sub-index	Traits	Weight	I (= V€G)	II	III	IV	V
Production (V€L)			48	48	45.60	42	36	24
		Milk (kg)		10	9.50	8.75	7.50	5
		Fat (kg)		9	8.55	7.87	6.75	4.50
		Protein (kg)		29	27.55	25.37	21.75	14.50
Functional (V€F)			28	28	26.60	24.5	21	14
		Udder health		3.36	3.19	2.94	2.52	1.68
		Longevity		20.72	19.68	18.13	15.54	10.36
		Total fertility		1.96	1.86	1.71	1.47	0.98
		Direct calving ease		0.84	0.79	0.73	0.63	0.42
		Maternal calving ease		1.12	1.06	0.98	0.84	0.56
Type (V€T)			24	24	22.8	21	18	12
	V€M	Rear leg set		1.40	1.33	1.22	1.05	0.70
		Rear leg rear view		0.70	0.66	0.61	0.52	0.35
		Bone quality		3.70	3.51	3.23	2.77	1.85
		Feet and legs		3.20	3.04	2.80	2.4	1.60
	V€C	Overall development		0.30	0.28	0.26	0.22	0.15
		Final conformation		0.50	0.47	0.43	0.37	0.25
		Overall udder		0.20	0.19	0.17	0.15	0.10
	V€P	Fore udder		2.00	1.90	1.75	1.50	1

Sub-index	2nd order sub-index	Traits	Weight	Selection Scenarios I (= V€G)	II	III	IV	V
		Rear udder height		3.20	3.04	2.80	2.40	1.60
		Udder support		1.30	1.23	1.13	0.97	0.65
		Udder depth		3.20	3.04	2.80	2.40	1.60
		Front teat placement		0.50	0.47	0.43	0.37	0.25
		Rear teat placement		2.50	2.37	2.18	1.87	1.25
		Teat length		1.30	1.23	1.13	0.97	0.65
Environment (V€E)		PME or LMI			5	12.50	25	50
Total (V€G)				100	100	100	100	100

V€M = feet and leg economic index; V€C = capacity economic index; V€P = udder economic index; PME = predicted methane emission (g/d); LMI = log-transformed methane intensity.

Table 5-2: Sire estimated breeding values (EBV) of environmental impact, production and functional traits in the Walloon Holstein dairy population used in this study, all traits except environmental traits are from official genetic evaluation of March 2016 (domestic and MACE EBV)

Traits	Sires	Mean	SD	Minimum	Maximum	Average Reliability
PME (g/d)	2455	0.00	9.57	-31.80	33.67	
LMI (/d)	2455	0.00	0.46	-2.17	2.04	
Milk Yield (kg/lactation)	1427	331.97	492.74	-1988	1995	86
Fat Yield (kg/lactation)	1427	11.99	19.43	-50	77	86
Protein Yield (kg/lactation)	1427	9.92	15.30	-58	56	86
Combined Female Fertility (mean = 100, SD = 10)	1425	97.94	10.20	65	141	81
Direct Calving Ease (mean = 100, SD = 10)	1406	93.42	15.29	34	144	67
Maternal Calving Ease (mean = 100, SD = 10)	1369	96.55	15.19	41	150	61
Body Condition score (mean = 0, SD = 1)	1392	-0.27	0.91	-2.94	3.49	71
Udder health (mean = 100, SD = 10)	1426	96.19	13.24	48	134	91
Longevity (mean = 100, SD = 10)	1425	93.41	17.45	29	147	78

Estimated breeding values for predicted methane emission (PME) and log-transformed methane intensity (LMI) were computed in this study. PME and LMI were centered for the 2455 sires, all other traits expressed in their original scales.

Table 5-3: Sire estimated relative breeding values (RBV) of type traits (mean = 0; SD = 1) in the Walloon Holstein dairy population for the genetic evaluations of March 2016 (domestic and MACE RBV) used and their genetic correlations with environmental impact traits (PME and LMI)

Traits	Mean	SD	Minimum	Maximum	Average Reliability	Genetic correlations	
						PME	LMI
Stature	0.18	0.96	-3.11	3.22	90	0.15	-0.06
Chest width	0.12	1.34	-4.42	4.52	82	0.01	-0.11
Body depth	0.16	1.15	-3.98	4.40	86	0.00	-0.10
Chest depth	0.14	1.14	-3.85	3.65	86	0.04	-0.12
Loin strength	-0.03	1.08	-3.93	3.72	87	0.00	-0.01
Rump length	0.21	1.13	-3.61	4.02	89	0.17	-0.07
Rump angle	-0.14	1.42	-5.75	5.28	91	0.01	-0.09
Hips width	0.17	1.08	-3.50	3.74	89	0.03	-0.16
Rump width	0.10	1.29	-4.81	4.85	89	-0.01	-0.10
Foot angle	0.14	1.45	-4.47	4.79	75	0.06	-0.04
Rear leg set	0.08	1.38	-3.92	5.04	87	0.00	0.05
Bone quality	0.13	1.03	-3.40	3.34	87	0.10	0.10
Rear leg rear view	-0.04	1.55	-5.12	5.64	81	0.04	-0.03
Udder balance	0.13	1.01	-3.99	3.05	89	0.06	-0.08
Udder depth	0.09	1.29	-4.16	4.47	91	0.25	0.18
Teat placement side	0.06	1.17	-4.18	3.67	85	-0.09	-0.02
Udder support	0.28	1.23	-4.61	3.91	79	0.02	-0.13
Udder texture	0.23	0.99	-3.29	3.37	84	0.07	-0.13

Traits	Mean	SD	Minimum	Maximum	Average Reliability	Genetic correlations	
						PME	LMI
Fore udder	0.14	1.25	-4.25	3.30	80	0.11	0.07
Front teat placement	0.17	1.23	-3.72	4.03	90	0.07	-0.11
Teat length	-0.01	1.30	-5.39	4.97	92	-0.12	-0.13
Rear udder height	0.21	1.12	-3.85	3.79	83	0.13	-0.13
Rear udder width	0.16	0.91	-3.38	2.91	86	0.02	-0.22
Rear teat placement	0.29	1.21	-3.99	4.03	90	0.02	-0.12
Angularity	0.19	0.90	-3.21	3.11	76	0.12	-0.11
Overall development	0.14	1.24	-4.19	4.05	87	0.09	-0.10
Overall rump	0.03	1.29	-4.23	4.85	86	0.01	-0.17
Overall feet and leg score	0.10	1.24	-4.73	4.03	75	0.07	-0.03
Overall udder score	0.18	1.00	-3.59	3.39	78	0.16	0.02
Overall fore udder	0.20	1.16	-4.33	3.56	85	0.17	0.06
Overall rear udder	0.10	1.09	-4.11	3.30	85	0.13	-0.05
Overall dairy trait	0.13	0.95	-3.51	3.07	83	0.12	-0.09
Overall conformation score	0.19	0.89	-3.29	2.89	74	0.15	-0.06

PME = predicted methane emissions (g/d); LMI = log-transformed methane intensity

Table 5-4: Genetic correlation between environmental traits with production and functional traits of selection of dairy cows

Traits	Predicted methane emission	Log-transformed methane intensity
LMI	0.33	/
Milk yield	0.06	-0.61
Fat yield	0.09	-0.15
Protein yield	0.13	-0.40
Fertility	0.17	0.36
Direct calving ease	0.37	0.00
Maternal calving ease	0.15	-0.11
Body condition score	-0.02	0.20
Udder health	0.22	0.08
Longevity	0.22	0.06

LMI = log-transformed methane intensity

Table 5-5: Selection responses (percentage of change) of environmental, production and functional traits to PME selection scenarios

Selection Scenario	PME	MY	FY	PY	Fertility	BCS	UDH	Longevity
I	1.94	16.68	17.25	22.25	-3.33	-7.97	15.94	27.83
II	0.36	16.35	14.95	20.07	-3.44	-8.57	15.36	26.73
III	-2.01	15.85	11.49	16.80	-3.61	-9.46	14.48	25.09
IV	-5.95	15.02	5.73	11.35	-3.88	-10.95	13.03	22.35
V	-13.84	13.35	-5.80	0.46	-4.44	-13.92	10.12	16.86

PME = predicted methane emissions; MY = milk yield; FY = fat yield; PY = protein yield; Fertility = Combined female fertility; BCS = body condition score; UDH=udder health (reversed somatic cell score); Selection scenario 1= current Walloon dairy cattle index (V€G), from second to fifth selection scenarios were addition of PME by 5, 12.5, 25 and 50 percent and proportional decrease on other traits respectively

Table 5-6: Selection responses (percentage of change) of environmental, production and functional traits to LMI selection scenarios

Selection Scenario	LMI	MY	FY	PY	Fertility	BCS	UDH	Longevity
I	-14.51	16.68	17.25	22.25	-3.33	-7.97	15.94	27.83
II	-16.38	19.24	17.06	23.48	-4.70	-8.94	15.26	26.79
III	-19.19	23.07	16.78	25.33	-6.77	-10.40	14.23	25.23
IV	-23.85	29.45	16.29	28.40	-10.20	-12.82	12.53	22.63
V	-33.19	42.21	15.33	34.56	-17.08	-17.66	9.12	17.43

LMI = log-transformed methane intensity; MY = milk yield; FY = fat yield; PY=protein yield; Fertility = Combined female fertility; BCS = body condition score; UDH = udder health (reversed somatic cell score); Selection scenario 1 = current Walloon dairy cattle selection program, from second to fifth selection scenarios were addition of PME by 5, 12.5, 25 and 50 percent and proportional decrease on other traits respectively

4. Discussion

The final objective of this study was to assess the response of selection for environmental traits by selecting them directly as well as the correlated responses of other economic important traits. Similarly, the motivation was also to improve the understanding of the genetic influence and their correlations on CH_4 emission by dairy cows. Currently, there is no direct economic incentive for a dairy producer to develop a program which reduces CH_4 emissions. Given that increasing significance of climate change, in national agendas but also for the dairy industry, environmental traits would need to be included in dairy cattle breeding. Similarly, societal demands are changing from both environmental and economic perspectives and CH_4 emission traits could be added in the breeding goals defined for dairy cows in the near future (Hayes et al., 2013).

All of genetic correlations between production, functional and type traits and CH_4 emissions traits were revolving around the efficiency and inefficiency of animal from intake, digestion, production, reproduction and survival. Dairy cows seem to partition energy for production, then for reproduction and finally for survival. More efficient dairy cows will produce more milk relative to the amount of feed ingested and less energy lost as CH_4. All production traits had small positive correlations with PME and high negative correlations with LMI, as was expected given its definition. The very negligible positive genetic correlation of PME with production traits suggested that these traits are not able to predict CH_4 emissions alone in dairy cows on a genetic level. Similarly, positive genetic correlations observed between PME and fat and protein corrected milk yield (0.07±0.09) (Lassen and Løvendahl, 2016) was similar to this study (0.06 with MY). There is ongoing debate on those figures because they appear low, but one should not forget that even if PME is also driven by intake it is also strongly related to energy lost or energy efficiency, a different mechanism *a priori* not (strongly) linked to intake. On a phenotypic level our recent research (unpublished data) showed that with increasing MY, the correlation with PME is decreasing and eventually inversing as always higher producing animals produce more and more from body reserve mobilization than intake. In beef cattle, using small preliminary analysis using genomic selection, response to selection of CH_4 yield (CH_4/kg dry matter intake) was estimated to be reduction by 4 percent in 10 years (Hayes et al., 2016). In dairy cows, using prediction from feed intake, PME would at least theoretically decrease in the order of 11 to 26% in 10 years (de Haas et al., 2011). However those predictions were totally different than prediction used in this study as they assumed that the major driving factor behind PME were only intake driven, therefore, direct comparison was difficult.

The reduction of LMI by 15% through the current Walloon selection index was similar to results obtained by Bell et al., (2011). These authors demonstrated that genetic selection for energy corrected milk (ECM) reduced CH_4/ECM (which is similar to LMI) by 15% for the first three lactations until mature size and maximum milk yield are achieved. Moreover, increasing selection pressure for reduced LMI gives a strong positive reaction of MY and associated traits. Therefore, as expected from these results, the functional traits would have negative to strongly negative correlated response. Fertility and BCS would be mostly affected but also longevity.

The genetic correlation between environmental traits and fertility could indicate that more resource inefficient cows show better female fertility and therefore simultaneous selection for both traits might be difficult. On the other hand, a breeding strategy emphasizing female fertility traits would improve cow fertility and reduce within-herd replacement rates and consequently reduced replacements contribute to decreasing CH_4 emissions in herd level (Knapp et al., 2014) but the relationship in individual level is not known yet.

The body condition score would decrease in all scenarios of selection on either PME or LMI. The substantial genetic correlation between BCS and LMI reduced BCS and positive genetic correlation between BCS and fertility (Bastin et al., 2013) had led to reduction in both traits. It is also well known that the early lactation period is characterized by body fat mobilization, negative energy balance (van Knegsel et al., 2007) which is also related to CH_4 emissions, so test-day genetic correlations are more important than average of whole lactations.

The fact that longevity had also positive genetic correlations with emissions could indicate that the higher CH_4 producing cows might be more efficient in survival. However like for improved fertility, by promoting longevity emissions from replacement would be diluted. However their effect in individual level of emission is unresolved (Grandl et al., 2016).

In sheep, it was demonstrated that smaller body confirmation animal had smaller rumen and shorter duration of ruminal passage which leads to less CH_4 (Goopy et al., 2014). In this study, almost all capacity and body size related traits like stature, chest width, rump length and angularity had positive genetic correlations with PME which suggested increased body capacity and body weight increased also PME. On the other hand, the body capacity type traits had negative genetic correlations with LMI, suggesting that selection for LMI would preserve these traits.

Even without selection on LMI the reduction in CH_4 intensity was already substantial due to the negative correlation with production traits. The speed of reduction would be faster if we add this new trait to the selection index, however the decrease in fertility would be substantial unless fertility traits were also added in the selection index.

This study has some limitations. First, the analyses were only based on correlations of sire EBV. A more direct method would be to estimate genetic correlations from the data using bivariate models. However, such approach would have required variance components estimation for a great number of bivariate models including random regression. Therefore, for this study, approximation were used and as presented by Calo et al. (1973), correlations between breeding values do not fully reflect the genetic relationships between two traits and they might underestimate them. Second, a better approach to create a selection index would be to put appropriate economic weights to environmental traits instead of adding a linear percentage in selection scenarios. However, even if there is an economic value of CH_4 emission in the industrial sector, this is not yet the case in agriculture. An alternative strategy would be to optimize expected gains, developing weights retrospectively. Third, the responses presented in Table 5-5 and 5-6 assume that all breeding values for all traits have equal reliability. That might not be the case at the moment of selection. It is therefore somewhat idealized scenarios but in practice accuracy will differ due to heritability and different recording (e.g. longevity and fertility). However, this study showed practical significance of current selection and its effect on PME and LMI where PME is increasing but CH_4 intensity decreasing.

5. Conclusions

This study presented novel results. First, under the hypothesis to continue using the current Walloon index, without directly selection for environmental traits, PME would be increased but LMI would be decreased through correlated responses to the selection for correlated traits. This is the expected result that gains are currently only achieved per unit produced. Second, by giving direct selection pressure on environmental traits, they would respond to selection, but would also change fundamentally the responses in other traits. These responses were quantified in various scenarios. One of the scenarios – reducing all traits weight by 25% of current index and addition of 25% of PME would reduce gains in FY and PY and almost all functional traits (fertility, BCS, and longevity) would need to be protected. The addition of 25% of LMI would shift the emphasis on production traits, especially MY, and affect even stronger functional traits. In conclusion, direct selection of environmental traits would reduce methane emission (a part of carbon footprint of milk) but more profound changes in current indexes will be required than simply adding environmental traits, as adding these traits to the selection index would affect the equilibrium between the other traits.

6. Acknowledgements

The authors acknowledge the support provided by the European Commission under the Seventh Framework Program for the GreenhouseMilk and GplusE projects, Grant Agreements FP7-PEOPLEITN-2008 238562 and FP7-KBBE-613689, the contribution of the COST Action FA1302 Methagene (http://www.methagene.eu) and of the Public Service of the Walloon Region of Belgium (SPW-DGO3) through the Methamilk project. The content of the paper reflects only the view of the authors; the European Community is not liable for any use that may be made of the information contained in this publication. The authors acknowledge the Walloon Breeding Association (Ciney, Belgium) for providing the pedigree database and the Comite du Lait (Battice, Belgium) for the supplying of the MIR spectra. Computational resources have been provided by the Plateau de Calcul et Modélisation informatique (CAMI) of the University of Liège, Gembloux Agro-Bio Tech and the Consortium des Equipements de Calcul Intensif (CECI) of the Federation Wallonia-Brussels (Brussels, Belgium), funded by the National Fund for Scientific Research (Brussels, Belgium) funded under grant 2.5020.11.

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6

Key Results, General Discussion, Conclusion and Future Prospects

Previous chapters demonstrated that mid-infrared prediction of CH_4 *emission traits can be considered as indicator of* CH_4 *measurements obtained by* SF_6 *reference method. These predicted* CH_4 *emission traits had a large genetic variability which suggested that these traits would likely respond to selection pressure. Selection of* CH_4 *traits would reduce milk carbon footprint without jeopardizing production traits, but energy balance and efficiency related (fertility, body condition score) traits have to be protected. This requires the use of innovative selection index useful for climate-smart breeding. In this chapter, key results, their discussion and implications have been summarized and future research directions are discussed.*

1. Selection of Environmental Impact Traits in Dairy Cows

Livestock production affects and is affected by global warming. First, it contributes to global warming through GHG emission and, second, it also suffers from global warming through the changes of quality and yield of animal feed and a potential climatic stress (Kipling et al., 2016). Therefore, many researchers have been striving to put environmental impact traits in their breeding goal for dairy cattle (Pickering et al., 2015). Similarly, from the consumer's perspective, it is believed that the negative impact of climate change can be minimized by consuming environmental friendly products. The consumer behavior, by choosing low carbon footprint milk products, may also provide a competitive edge for 'greener' milk production (Feucht and Zander, 2017). Beside the carbon footprint of milk and consumer's behavior, CH_4 emission from dairy cows is also a loss of energy from feed offered to animals (Johnson and Johnson, 1995). This loss of energy could be decreased by reducing CH_4 emissions, offering potential economic profit for farmers. In these multifaceted problems and opportunities, the genetic selection of dairy cows for environmental traits without jeopardizing farm profitability and animal welfare would be one of the objectives for future dairy production system. Genetic selection provides a reliable route towards permanent and cumulative reductions in enteric CH_4 emissions. In this PhD thesis, only environmental traits based on enteric CH_4 were studied and carbon footprint is colloquially used for CH_4 emissions. However, this thesis doesn't address nitrogenous emission and higher concentrate in animal feeds may lead to higher nitrogen rejection and nitrous oxide emission. If other production parameters are the same, a genetic selection for decreasing CH_4 emission will decrease carbon footprint.

2. Large scale methane related phenotypes

The pre-requisite for selective breeding is the obtaining of a large amount of phenotypic data. Several direct *in vivo* methods are used to quantity CH_4 emitted by dairy cows but they are expensive and labor intensive. This limits the availability of phenotypes. Therefore, there is a necessity to obtain or predict CH_4 proxies using large scale, cost and labor effective techniques. In this context, milk FAs were deemed to be biologically relevant indicators to predict CH_4. Indeed, CH_4 output from ruminants is directly linked to the microbial digestion in the rumen. So, several equations from milk gas chromatography FAs were published by different authors to predict CH_4 emissions (i.e., Chilliard et al., 2009; Dijkstra et al., 2011; van Lingen et al., 2015; van Gastelen et al., 2017). Major milk FA composition can be measured through the analysis of individual milk samples by MIR spectrometry (Soyeurt et al., 2011; de Roos et al., 2007). This approach has the advantage to be rapid, cheap, environmentally friendly and already used in routine by milk laboratories around the world. Similarly, in the context of performance recording, an interesting feature of MIR based prediction of CH_4 emissions is there are longitudinal records repeated approximately every four weeks during lactation for each cow. First, in this thesis, the literature CH_4 emission phenotypes (g/day) predicted from Chilliard et al. (2009) equations using gas chromatography FAs were used as reference values to developMIR equations. This allowed obtaining CH_4 phenotypes predicted from milk MIR spectra used in Chapter 2. Unfortunately, those predictions included multiple prediction errors related to the prediction of CH_4 using major FA in milk itself as well as the prediction of FA by MIR. The best methane prediction equation had R^2 of 0.80 with R^2cv of 0.92. This provided the final R^2 is about 0.72. Another factor was the prediction accuracy of milk FAs themselves, which ranged from 0.80 to 0.90. This was the good start to explore possibility of milk fatty acids as a predictor of CH_4 emissions; however, GC is unsuitable for routine analysis. Methane emissions prediction can be improved from integration with other factors, like feed intake, nutrient composition of the feed, parity, and lactation stage using MIR spectra (van Gastelen and Dijkstra, 2016.) Therefore, to minimize the prediction error and to add additional milk composition information, direct CH_4 predictions (g/day) from milk MIR spectra using Vanlierde et al. (2015) equations were considered as second approach where parity and lactation stage dependent factors were considered. The equation developed by Vanlierde et al., (2015) was used to predict methane emissions traits in this thesis (Chapter 3, 4 and 5). This CH_4 MIR prediction equation was developed from 446 CH_4 measurements from 142 Belgian and Irish cows. Reference daily CH_4 emissions of individual cows were determined using the sulfur hexafluoride (SF_6) tracer gas technique with a gas collection period of 24 h. The lactation-stage-dependent equation was developed from those reference values and their corresponding daily milk MIR spectra. The standard error of calibration of this equation was of 63 g/d. The calibration coefficient of determination was equal to 76% (Vanlierde et al., 2015).

Environmental traits used in this thesis were only based on predicted enteric CH_4. CH_4 emission **(**PME**;** g/d**)** was predicted from milk MIR spectra collected from Holstein cows using Chilliard et al. (2009) equations (Chapter 2) and Vanlierde et al. (2015) equations (Chapter 3, 4 and 5). Predicted CH_4 intensity (PMI; g/kg of milk) was derived as the ratio of PME to milk yield for a given test-day. Due to the statistical property of this trait, PMI was log-transformed (LMI). Genetic parameters were estimated for those traits in order to test the following hypotheses:

- Are predicted CH_4 traits heritable? ;
- Do those traits show sufficient genetic variability to rank sires into low and high CH_4 emitters? ;
- Is it possible to include those environmental traits in selection indexes without jeopardizing animal production, health, fertility and longevity?

3. Phenotypic variability of MIR Methane proxies

The MIR CH_4 emissions (g/d) varied throughout the lactation with an increase mid-lactation and then a linear decrease. The observed pattern of MIR CH_4 emissions (g/d) in function of the days in milk (Chapter 3 and 4;Figure 6-1) was in overall comparable with the one observed from other methods of CH_4 measurements (Garnsworthy et al., 2012; Lassen and Løvendahl, 2016). The coefficients of variation between lactations stayed relatively stable for all studied CH_4 traits (15-18% for PME and 11-14% for LMI; Chapter 2 and 4).

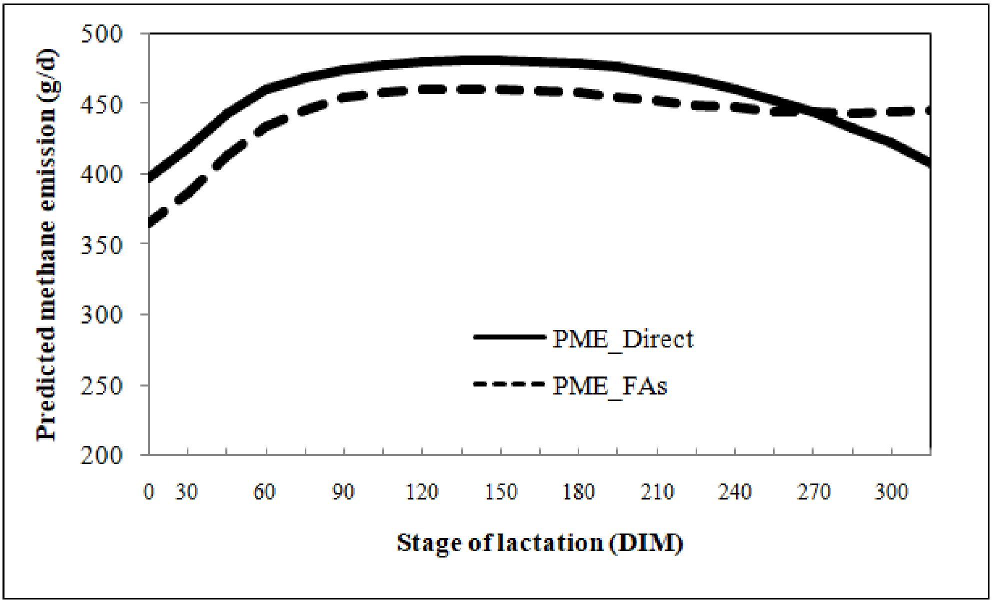

Figure 6-1:.Change in daily predicted methane emission (PME_direct) and milk fatty acids predicted (PME_FAs) across lactation. The PME was averaged for first three lactations from the data from Chapters 3 and 4 for illustration.

The phenotypic correlation of CH_4 and milk yield (MY) were very low along with FY and PY, however the correlations with fat and protein percentages had moderate correlations (Chapter 2 and Chapter 4). LMI had high negative phenotypic correlations with MY, FY and PY (Chapter 4) but had positive phenotypic correlations with fat and protein content (Chapter 2; Williams et al., 2014). The curvilinear relationship between MIR CH_4 emission intensity and MY suggested that high yielding cows have lower milk carbon footprint (Chapter 4). This curvilinear relationship was also observed by Moate et al., (2016) from Australian dairy population measured using respiration chamber of CH_4 measurement method (Figure 6-2). Similar curvilinear relationship was also observed by Watt et al., (2015). This relationship could be attributed to a dilution of fixed requirements for maintenance, and to a lower extent to an improved efficiency for milk production (Dijkstra et al., 2013; Knapp et al., 2014). Therefore, this relationship also suggested that the promotion of higher milk producing cow is regarded as one of the mitigation options to reduce the environmental impact for dairy production system (Wall et al., 2010).

For all studied MIR CH_4 traits (g/d), second lactation CH_4 emissions were higher than the first lactation. However, the MIR predicted CH_4 emission was almost flat from the second lactation to the third lactation (Table 2-2; Table 4-1 and Figure 6-3). This agrees with the findings of Grandl et al. (2016) who mentioned that older cows did not have increased CH_4 emissions than second lactation cows. Similar results were also reported by Haar and Pierre (2006) that maximum lifetime energy efficiency is typically reached after 2.5 lactations, when mature size and maximum milk production are achieved. In case of CH_4 emissions, from the emission patterns between second and third lactation, first two lactations records look sufficient for complete picture that allows shorter phenotype collection period. These authors suggested that when maximum energetic efficiency is reached, environmental waste such as energy loss as enteric or manure CH_4 will be minimized (Haar and Pierre, 2006).

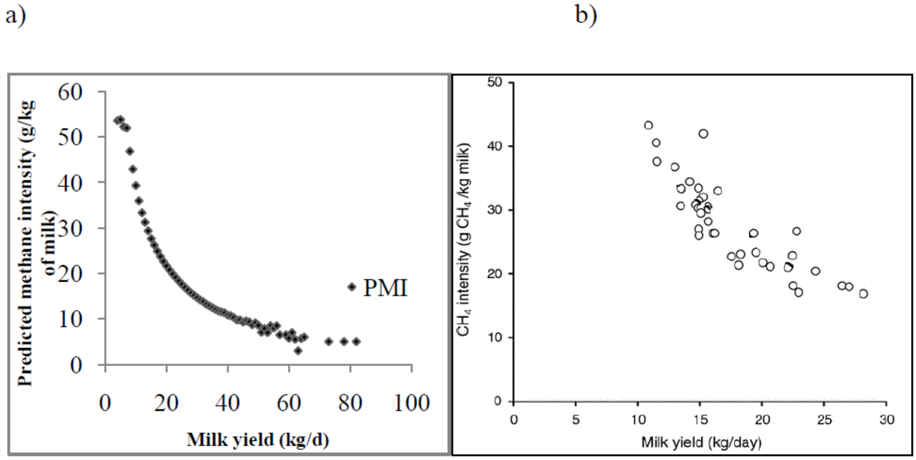

Figure 6-2: 6-2a. Relationship between predicted methane intensity (PMI; g/kg of milk) and milk yield (kg/d) in the averaged first three lactation Holstein cows. PMI=Predicted methane intensity (from Chapter 5) 6-2b. The relationship obtained from Australian dairy cows (adapted from Moate et al., 2016 figure ©CSIRO publishing)

4. Genetic Parameters of Predicted Methane Emission Traits

The heritability values of predicted MIR CH_4 emissions from Chilliard et al. (2009) equations were moderate (from 0.26 to 0.40). Heritability values obtained in this study were similar to the findings of van Engelen et al., (2015) which were also obtained from milk FAs. The average heritability increased from the beginning of the lactation towards the end of lactation with the changes in body lipid mobilization and energy balance which is seen as a change of body condition score (Chapter 2). The range of EBV of sires having their daughters in production with the highest and the lowest CH_4 content was around 27 kg per lactation. These ranges of estimated breeding values were large enough to appreciate genetic variability. The genetic correlation between MIR FAs predicted CH_4 emissions and milk yield was low negative while the correlations between CH_4 emissions and fat and protein contents were positive. This study supported the hypothesis of genetic component of predicted CH_4 emissions from dairy cows and can be used to rank sires based on their EBV. This should allow to select future generations for reduced CH_4 emissions (Chapter 2).

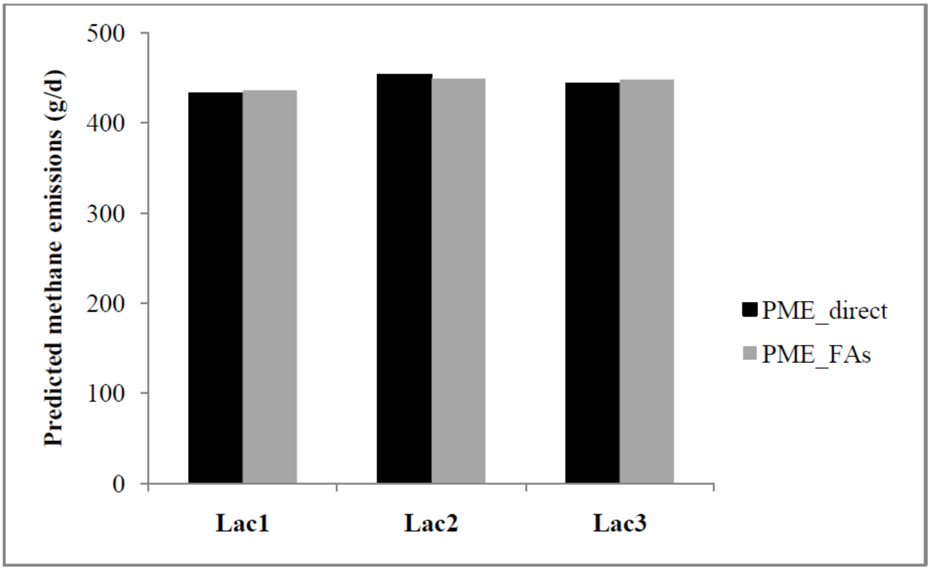

Figure 6-3: Predicted methane emissions from MIR milk fatty acids (PME_FAs) and direct prediction from MIR spectra (PME_direct) in the first, second and third lactation Holstein cows (results from Chapter 3, 4 and 5); Lac=Lactation.

Genetic analyses of the directly predicted MIR CH_4 phenotypes (PME and LMI) were carried out using two models to select better fit model. One model had extra random within-herd lactation curve effects, including permanent environment and additive genetic effects. The random within herd lactation curve effects model fitted better than without herd lactation curve effects model (Chapter 3). The contribution of random herd-specific lactation curve effects was around 10 percent of the total variance. This random herd-specific lactation curve effects suggested an impact of herd specific management on the CH_4 emission traits. Using this model, the estimates of heritability for PME (0.26) and log-transformed CH_4 intensity (0.27) were moderate. The heritability values were higher (0.34 vs. 0.26 for PME and 0.32

vs. 0.27 for LMI) from the model without random within herd lactation curve effects. The most part of these differences were attributed to the random within herd lactation curve effects and PE effects (Chapter 3). These heritability values were lower (0.35 vs. 0.26) compared to the ones found for the milk FAs predicted methane emissions (g/d; Chapter 2). From these results, further investigation about the genetic parameters in second lactation and the relationships with other milk production traits was carried out (Chapter 4). These heritability results were in general within the range of various methods of CH_4 measurements (Table 6-1).

5. Genetic Correlations between MIR Methane Proxies and Economic Important Traits of Selection

Model including a random within herd lactation curve effects had a better fit (Chapter 3) and was used to estimate the genetic correlations of PME and LMI with the common milk production traits (MY, FY and PY). The low negative genetic correlation between PME and MY from this study was similar to the findings obtained in other studies (Dong et al., 2015; Lassen and Løvendahl (2016). Across lactations, genetic correlation between PME and FY increased from the beginning toward late lactation and this evolution persisted in both first and second lactations. The observed changing genetic correlations between PME and FY within lactation suggest the complex relationship between CH_4 traits and FY. The fat composition is indirectly related to CH_4 emissions, they share mechanism during rumen fermentation (Bielak et al., 2016). Genetic correlations of LMI and MY with FY and PY were negative and became more pronounced with increasing days in milk. These relationships were consistent with the pattern of body lipid mobilization during lactation. Indeed, an early lactation cow is able to mobilize body energy reserves towards milk production and body fat mobilization does not contribute in CH_4 production which leads to lower CH_4 intensity. Mid and later lactation cow has to replenishing fat stores, towards fetus in addition to milk production and also higher feed intake that results higher amount of CH_4 intensity. Therefore, there was dynamic intra-lactation genetic correlation between both PME and LMI and FY(Chapter 4). PY is more related to the nitrogen efficiency than CH_4 emissions butMY and PY were strongly positively genetically correlated. Therefore the slight negative correlation between PME and PY was expected (Table 4-2).

After estimating genetic correlations between MIR CH_4 traits and milk production traits, the genetic correlations with functional and type traits were estimated (Chapter 5). Genetic correlations between CH_4 traits and all traits in the selection index were approximated in Chapter 5 from the correlations between their corresponding EBVs rather than bi-variate models. This way of doing does not fully reflect the genetic relationships between two traits and can underestimate the intensity of the relationship. This could explain the small differences in genetic correlation values between CH_4 traits and milk production traits estimated in Chapter 4 (i.e., from bivariate models) and Chapter 5 (i.e., from approximated

Table 6-1: Heritability Estimates of predicted and measured methane emissions traits in dairy cows from prediction methods

Traits	Prediction/ Measurements	Average/ unspecified Lac	Lac 1	Lac 2	Lac3	Reference
PME (g/d)	Milk MIR spectra	0.22	0.25	0.22	0.19	Chapter 4
LMI	Milk MIR spectra	0.17	0.18	0.17	0.16	Chapter 4
PME (g/d)	MFAs	0.36	0.35	0.38	0.34	Chapter 2
PME (g/d)	MFAs	0.31	0.30	0.33	0.30	Chapter 2
PME (g/d)	MFAs	0.29	0.29	0.33	0.26	Chapter 2
PME (g/d)	MFAs	0.38	0.35	0.40	0.38	Chapter 2
PMY (g/kg of DMI)	MFAs	0.16	0.16	0.17	0.14	unpublished[a]
ME (g/d)	Infrared-breathalyzer	0.21				Lassen and Løvendahl, (2016)
MI(g/kg of milk)	Infrared-breathalyzer	0.21				Lassen and Løvendahl, (2016)
PME (g/d)	6% gross energy	0.35				De Haas et al., (2011)
PME(g/d)	Gross energy	0.10				Cassandro et al., (2011)
PME (g/d)	Gross Energy	0.13				Pickering et al., (2015b)
ME (g/d)	Laser detected	0.05				Pickering et al., (2015)
PMY (g/kg of DMI)	MFAs	0.12				van Engelen et. al., (2015)
PMY (g/kg of DMI)	MFAs	0.20				van Engelen et. al., (2015)
PMY (g/kg of DMI)	MFAs	0.44				van Engelen et. al., (2015)

PME= predicted methane emission (g/d); LMI= Log-transformed methane intensity; ME= methane emission (g/d), PMY= predicted methane yield (g/kg of DMI); MI=methane intensity (g/kg of milk); DMI=Dry matter intake; MFAs=Milk fatty acids; Lac=Lactation; [a]prediction equation used from Dijkstra et al., 2011 correlation from EBV estimation). Genetic correlations between PME and functional traits (fertility, UDH and longevity) were low positive (from 0.17 to 0.22) except BCS (-0.02). Genetic correlations between LMI and all studied functional traits were positive (from 0.06 to 0.36). The CH_4 emission is inefficiency of animal but production, reproduction and survival are efficiency from animal. The relation between inefficiency and efficiency should be antagonistic and the genetic correlation should be reflected on that way. Dairy cows seem to partition first energy for production, then for reproduction and finally for survival (Puillet et al., 2016). More efficient dairy cows will produce more milk relative to the amount of feed ingested and less energy lost as CH_4 and this mechanism is likely controlled by genetics. This hypothesis also supported by Grandl et al., (2016) was that CH_4 emissions feeding diets. Even if measurement methods and techniques are different, the conclusion about the genetic regulation of CH_4 emissions from dairy cows was similar between studies. Older cows do not have increased CH_4 emissions than second lactation cows (Grandl et al., 2016) and also observed by our data set. Similar results were found by Haar and Pierre (2006) that maximum lifetime energy efficiency is typically reached after three lactations, when mature size and maximum milk production are achieved. These authors suggested that when maximum energetic efficiency is reached, environmental waste such as energy loss as enteric or manure CH_4 would be minimized. The correlation with BCS found in this this should be taken with extra care because of its dynamic nature intra-lactation (i.e., BCS changes) and further study in this area is required.

Udder health was represented by SCS on a reversed scale. The genetic correlations were low positive between MIR CH_4 traits and UDH (Table 5-4; Chapter 5). Direct biological reasoning of this relationship has to be established, however, we could argue that increased production has a toll on health traits.

The genetic correlation between 33 type traits and PME were ranged from -0.12 to 0.25 while the correlation between same 33 traits and LMI were ranged from -0.13 to 0.18. Particularly, the capacity related traits (e.g. overall conformation, udder and development score) had positive genetic correlations with PME and negative genetic correlations with LMI. It suggested that through CH_4 intensity selection, these type traits could be preserved. Body weight (**BW**) is not a part of selection index however a higher BW animal has a large rumen capacity. A large rumen capacity results in a lower passage rate of feed in rumen causing in a higher CH_4 production (Moraes et al., 2014). The fluctuation of BW should be considered as previously mentioned genetic correlations of BCS.

6. Consequences of Selection of Environmental Impact Traits

The breeding goal is the direction in which we want to improve the population in future. In this study, the breeding goal is to include the reduction of carbon footprint of milk production. Generally, the breeding goals are based on economic model of the production system. The economic value for each trait is determined by modeling the effect of that trait on the profit of a production herd. A profit equation is derived that is a function of the breeding goal traits. Subsequently, the economic value of each breeding goal trait is found as the partial derivative of profit with respect to that trait. The goal of selection index is to estimate the genetic component of the breeding objective by assembling traits to be selected into a weighted linear combination. The current selection index of the Walloon region of Belgium considers the following weights (Vanderick et al., 2015):

Table 6-2: Current Walloon selection index

Sub-index	Traits	Weight
Production Traits		48
	Milk (kg)	10
	Fat (kg)	9
	Protein (kg)	29
Functional Traits		28
	Udder health	3.3
	Longevity	20.7
	Total fertility	2.0
	Direct calving ease	0.8
	Maternal calving ease	1.1
Type Traits		24
	Feet and leg	9
	Capacity related traits	1
	Udder traits	14

(Detailed description on Table 5-1)

In some cases, indirect selection of a particular trait is considered if highly correlated traits are more regularly recorded, less expensive to record, measured at earlier life and/or more heritable. Methane emissions provide an example of a phenotype that is difficult to measure individually on a large scale and for which proxies can be assembled into an indirect selection index for CH_4 (i.e., MIR prediction in this thesis) (Negussie et al, 2017). Beside large-scale reliable records on the proxies, important building blocks of an indirect selection index are their heritability values and the genetic correlations between them and the other traits included in the selection index. For CH_4 proxies, heritability and correlations are known from this thesis, however their direct contribution to the profit functions (i.e., contribution of the given trait to the breeding goal) are unknown.

Without including PME and LMI in the Walloon selection index (*status quo*), the relative genetic change through correlated response of other traits, PME would be increased by 2% and LMI would be decreased by 14% per generation. Without direct inclusion of PME and LMI in the index, the relative change in MY, FY, PY and longevity would be increased by ~16% and fertility, BCS and UDH would be decreased by ~10%. Reducing the relative weights of all traits by 25% in the current index and adding 25% of PME (or LMI) would result PME to be decreased by 6% and LMI to be decreased by 23%. When selecting for environmental traits, the response of functional traits would be unfavorable; therefore, energy balance and efficiency related traits (fertility, BCS) and health traits (UDH) must be protected (Chapter 5).

Some other literatures have also predicted how much changes would come in CH_4 reduction from genetic selection. De Haas et al. (2011) estimated the possibility of reduction of CH_4 production of a cow by selecting more-efficient cows in the order of 11 to 26% in 10 year time from base year. That estimate was based on the selection of residual feed intake, not by directly selecting CH_4 traits. Eight percent reduction CH_4 yield was estimated in sheep after one generation of selection against CH_4 emissions (Pinares-Patiño et al., 2013). Similarly, through genomic selection, CH_4 emissions could be reduced by 5% in 10 years in beef cattle (Hayes et al., 2016). In Australian dairy cows, the selection response without any selection pressure in CH_4 emission traits was predicted ~1% in total emissions from the dairy industry per year (i.e., corresponds with *status quo* scenario of PME). Methane intensity is expected to be reduced by 0.55% per year (Pryce and Bell, 2017). Using life cycle analysis and estimated from correlated traits, 15% reduction in CH_4 intensity was projected over next 15 years from Irish dairy industry (Amer et al., 2017). Of course, these predictions are based on different assumptions and many parameters like accuracy of selection, reliability of traits are not precise yet. This leads to difficulty for direct comparison between methods but give an overall consensus that genetic selection is possible to reduce CH_4 emissions.

In Chapter 5, the reliabilities of the CH_4 EBV were assumed high (through high number of progeny as of MY and moderate heritability of trait; production traits have reliability of 0.8 or higher). Some unanswered questions remain in this research. How many animals/progeny with CH_4 records do we need to achieve reliable EBV? Also, do multi-traits analysis combing CH_4 traits with other traits improve the reliability of CH_4 EBV?

When including traits in the overall selection index or breeding program for a breed, it is important to be aware of potential unexpected consequences. For example: are there traits for which we do not want to select (against or in favor) but that are correlated with the breeding goals and would therefore be passively dragged by selection? A known illustration in dairy breeding is the somewhat inverse relation between milk yield, on one hand, and milk quality (e.g., fat and protein content) and animal functionality (e.g., fertility) on the other hand (Negussie et al, 2017). A careful construction of the overall selection index and its implementation in the breeding program is therefore needed. An important conclusion of this thesis and other studies is that innovative selection index theory will be needed to develop adapted indexes useful for climate-smart breeding strategies, especially because of first indications that CH_4 reduction influences fertility and animal robustness negatively were confirmed. Strategies to develop restricted selection index (Kempthorne and Nordskog, 1959) or direct economic weights for emission traits should be identified to mitigate CH4 emission from dairy system to reduce carbon foot-print of milk production. Other potential approaches are through desired gain index, where trait values are calculated indirectly from index-weighing factors based on a predetermined desired or restricted amount of genetic gain for one or more traits (Gibson & Kennedy, 1990) or with trait weightings in the formulation of breeding objectives (Amer et al., 2017).

Even though, direct pricing of CH_4 emissions from dairy industry is not in practice, future pricing mechanism cannot be ignored. A note should also be taken in consideration of global collaboration of greenhouse gas reduction from dairy industry. Paris climate accord (2015) might have large impact to formulate the exact policy in particular countries, regions depending upon price of carbon (if any). Even though, recently, second largest polluter of GHG, USA had withdrawn from its climate accord commitment. Nevertheless, Europe (including Belgium), Canada and other countries are still in Paris accord; therefore, the dialogue of economic selection index is still on the table.

7. Conclusions

In conclusion, the inclusion of environmental impact traits into dairy cattle selection seemed to be possible through selection index theory. The obtained results support the following hypotheses: a) predicted MIR CH_4 traits are heritable; b) those traits have sufficient genetic variability to rank animals into low and high CH_4 emitters; and c) environmental traits can be included in the current selection index, however, a new equilibrium between traits is needed in order to avoid jeopardizing animal production, udder health, fertility and longevity.

8. Implications

Some previous studies already supported the genetic contribution to the CH_4 emissions from dairy cows. This research confirmed those previous studies through the obtained heritability estimates of predicted MIR CH_4 emission. Moreover, this research provided a better understanding of genetic correlation between MIR based CH_4 emission traits and milk production, udder health, functional and type traits. The selection of CH_4 trait could have the following implications:

- At animal level: with the current Walloon index, the CH_4 intensity from dairy cows is decreasing; direct selection for reduced CH_4 would increase the reduction, however, other (functional) traits must be protected.

- At national herd level: Appropriate selection index can be formulated to minimize unwanted correlated response on functional traits.
- At policy level: Regional and federal governments are generally inclined to levy carbon tax on any sectors that produces GHG. This research showed that the CH_4 intensity from milk production is decreasing regardless of the selection of CH_4 emission traits. However, it also showed that incorporating these novel traits into the selection index offer more reduction of CH_4 emissions from dairy cows. However, integration of environmental traits may be problematic with fertility and BCS, and ultimately farm profit; therefore, the proper mechanism to compensate/incentive should be in place to ensure farm profit. Otherwise the end-users may not be interested to incorporate this trait in their breeding objective. Farmers are already in the tight-rope of the profit margin; therefore, it should be included in rural development policy through incentive to farmers who rear dairy herd more environmental friendly. In addition, these results may assist global collaboration to define a suitable measurement, and many thousands of records to ensure valid and accurate evaluations of environmental traits.

9. Future Research

This thesis is based on the prediction of CH_4 traits from MIR milk composition. Several authors have demonstrated that MIR spectra can predict this trait whereas some studies have skeptic (for e.g. Shetty et al., 2017). The selection index formulation for environmental traits has issues and use of these traits in era of genomic selection, following future research are recommended to get whole picture of mitigation of methane emission from genetics.

- The MIR predicted phenotypes used must be validated. An innovative method could be to estimate the genetic correlations between MIR phenotypes and direct CH_4 measurements.
- Direct genetic correlations between MIR CH_4 predictions and functional and type traits must be estimated.
- The fixed effects used in thesis to explain the CH_4 variability were based on those used in the Walloon genetic evaluation for dairy production traits. Addition research is therefore required to optimize the modeling of these effects as was already done for the random effects during this thesis.
- The economic weight of the CH_4 emissions traits are still blur. Some countries/regions had tried to quantify the price of carbon offset but their values

are still unclear. Therefore, further research is required to create economic weight or alternatively, with trait weightings in the formulation of breeding objectives or restrictive selection index or desired gain index.

- Most of the animals used in this study were also genotyped through various projects, therefore, genome wide association study to pinpoint the chromosomal regions of CH_4 emission. The future genetic evaluation of any novel trait in dairy cows would have both genetic and genomic analysis. These difficult to measure traits are one of the prime examples to be benefitted from genomic selection. The integration of both sources of information should be used into a genetic or a single-step genomic evaluation.

- Lastly, but not least; the microbes that convert methane in host rumen may have a significant role in methane emissions. Even though, this thesis didn’t touch the microbes and host-microbes interaction, which is one of the areas of potential research.

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Doctoral Trainings and Scientific Communications

1.	**Thematic Training** (minimum 15 credits)	Credits
1.1	Training for users of computing devices and mass storage, Université catholique de Louvain, Belgium	5
1.2	Dairy cows lactations, profiles, nutrient allocation and energy balance, Aarhus University, Denmark	4
1.3	Training in infrared spectroscopy and chemometrics, Walloon Agricultural Research Center, Gembloux	4
1.4	Modeling for Biologist, Agro Paris Tech, France	4
1.5	High Performance computing, Université catholique de Louvain, Belgium	5
1.6	Nutrition and fat metabolism in dairy cattle, Wageningen University, The Netherlands	1
1.7	Healthy foods from Healthy animals, BSAS, University of Nottingham, UK plus student sessions and GHM meeting	3
1.8	International conference on Quantitative Genetics: Understanding variation in quantitative traits, Edinburg, UK	5
	Subtotal	31
2.	**Transversal Training** (minimum 10 credits)	
2.1	Animal Use in Research Training: Ethics of Animal Use, Core and Livestock stream, University of Alberta, Canada	3
2.2	American Dairy Science Association Conference, Phoenix, AZ, USA	4
2.3	American Dairy Science Association Conference, Indianapolis, USA	4
2.4	Genetic Parameters for methane indicator traits based on milk fatty acids in dual purpose Belgian Blue cattle, 17th Symposium on Applied Biological Sciences, University of Leuven, Belgium	1
2.5	Scientific Stay aboard 'TEAGASC, Moorpark', Ireland	5
2.6	Peer -reviewing (1 article in journal 'Animal' and 2 articles in 'Journal of Dairy Science')	3
	Subtotal	20
3.	**Scientific Communications (minimum 25 credits)**	
3.1	**Kandel, P. B.**, M.-L. Vanrobays, A. Vanlierde, F. Dehareng, E. Froidmont, N. Gengler, and H. Soyeurt. 2017. Genetic parameters of mid-infrared methane predictions and their relationships with milk production traits in Holstein cattle. J. Dairy Sci. 100: 5578–5591.	8

3.2	**Kandel, P. B.**, S. Vanderick, M.-L. Vanrobays, H. Soyeurt, and N. Gengler. 2017. Consequences of genetic selection for environmental impact traits on economically important traits in Dairy Cows. Animal Prod. Sci. 57:x (online early)	8
3.3	**Kandel, P. B.**, N. Gengler, and H. Soyeurt. 2015. Assessing variability of literature based methane indicators traits in a large dairy cow population. Biotech. Agron. Soc. Environ. 19:11-19.	5
3.4	**Kandel, P. B.**, S. Vanderick, M.-L. Vanrobays, A. Vanlierde, F. Dehareng, E. Froidmont, H. Soyeurt, and N. Gengler. 2014. Consequences of selection for environmental impact traits in dairy cows. Proceeding of 10th World Congress on Genetics Applied to Livestock Production (WCGALP), Vancouver, Canada. Am. Soc. Anim. Sci., Champaign, IL.	5
3.5	**Kandel, P. B.**, M.-L. Vanrobays, A. Vanlierde, F. Dehareng, E. Froidmont, P. Dardenne, E. Lewis, F. Buckley, M.H. Deighton, S. McParland, N. Gengler, and H. Soyeurt. 2013. Genetic parameters for methane emission predicted from milk mid-infrared spectra in dairy cows. Adv. Anim. Biosci. 4:279.	5
3.6	**Kandel, P. B.**, H. Soyeurt and N. Gengler. 2012. Estimation of genetic parameters for methane indicator traits based on milk fatty acids in dual purpose Belgian blue cattle. Comm. Agri. Appl. BioSci. 771:21-25.	5
3.7	Genetic parameters for methane emission indicators in dairy cows, Département des Sciences Agronomiques, presentation des travaux de doctorat, University of Liege, Gembloux Agro-Bio Tech, Gembloux	3
	Subtotal	39
	Total	**90**