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

Purna Bhadra Kandel [(1)], Nicolas Gengler [(1)], Hélène Soyeurt [(2)]

[(1)] University of Liege - Gembloux Agro-Bio Tech. Department of Agricultural Science. Animal Science Unit. Passage des Déportés, 2. B-5030 Gembloux (Belgium).
[(2)] University of Liege - Gembloux Agro-Bio Tech. Department of Agricultural Science. Passage des Déportés, 2. B-5030 Gembloux (Belgium). E-mail: hsoyeurt@ulg.ac.be

Description of the subject. Dairy production is identified as a major source of methane (CH_4) emissions. Selective breeding represents one method for mitigating CH_4 emissions but practical and cheap ways to measure this trait are not currently available. In the present study, four CH_4 indicator traits based on milk fatty acid (FA) contents were referenced from the literature.

Objectives. The aim of the study was to use these literature CH_4 indicators for assessing the variability of methane emissions emitted by dairy cows.

Method. Literature CH_4 indicator traits were originally defined based on the measurements of FA content by gas chromatography. However, these measurements were not available for all the available cows in our studied population. A sample of 602 gas chromatographic analyses was therefore used to develop a calibration equation for predicting the literature CH_4 indicators based on milk mid-infrared (MIR) spectra. This spectral information was available for all the studied cows. Then, in a second step, in order to predict the literature CH_4 indicator traits, the developed MIR prediction equations were applied to the 604,028 recorded spectral data collected between 2007 and 2011 for 70,872 cows in their first three lactations. Genetic parameters for these traits were then estimated using single trait test-day random regression animal models.

Results. The predicted MIR literature CH_4 estimates were in the expected range from 350 ± 40 to 449 ± 65 g per day. The averaged predicted MIR CH_4 emission (g per day) increased from the beginning of lactation, reached the highest level at the peak of lactation and then decreased towards the end of lactation. The average daily heritability values 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 the estimated breeding values of sires that had 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.

Conclusions. This study showed the feasibility of using MIR spectrometry results to predict fatty acid derived CH_4 indicator traits developed in the literature. 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.

Keywords. Milk, methane, fatty acids, infrared spectrophotometry, heritability, cows.

Appréciation de la variabilité d'indicateurs méthane issus de la littérature et appliqués sur une large population de vaches laitières

Description du sujet. La production laitière est reconnue comme une des sources majeures d'émissions de méthane (CH_4). Le recours à un programme de sélection spécifique pourrait être une bonne méthode pour optimiser les émissions de méthane par les vaches laitières. Le développement d'un tel programme nécessiterait un nombre important d'enregistrements relatifs aux émissions de méthane. Malheureusement, aucune méthode pratique et bon marché n'existe actuellement pour créer une telle base de données. Cependant, quatre indicateurs CH_4 basés sur les quantités en acides gras dans la matière grasse laitière ont été recensés dans la littérature.

Objectifs. L'objectif de cette étude est d'utiliser ces indicateurs de la littérature afin d'apprécier la variabilité des émissions de méthane éructées par les vaches laitières.

Méthode. Ces indicateurs utilisent les quantités en acides gras obtenues par chromatographie en phase gazeuse. Comme ce type de données n'est pas disponible pour toute la population laitière, un échantillon de 602 analyses chromatographiques a

été créé dans cette étude afin de développer une équation de calibrage permettant de prédire les quantités de méthane émises à partir du spectre moyen infrarouge (MIR) du lait qui est disponible pour toutes les vaches étudiées. Ensuite, l'équation de calibrage ainsi obtenue a été appliquée sur 604 028 données spectrales enregistrées entre 2007 et 2011 auprès de 70 872 vaches au cours de leurs trois premières lactations afin de prédire les quantités de méthane émises. Les paramètres génétiques de ces nouveaux indicateurs méthane prédits par MIR ont également été estimés en utilisant un modèle animal de type jour de test avec régressions aléatoires.

Résultats. Ces quantités prédites par MIR variaient selon une gamme attendue s'étalant entre 350 ± 40 et 449 ± 65 g par jour. L'émission prédite moyenne de CH_4 en g par jour augmentait au début de la lactation, atteignait sa plus haute concentration au pic de lactation et ensuite diminuait jusqu'à la fin de la lactation. Les héritabilités journalières moyennes variaient entre 0,29-0,35 ; 0,26-0,40 et 0,22-0,37 pour les différents indicateurs méthane étudiés au cours des trois premières lactations. Les plus grandes différences entre les valeurs d'élevage estimées pour des taureaux ayant des filles en production émettant le plus et le moins de méthane étaient de 24,18 ; 29,33 et 27,77 kg par lactation pour les trois premières lactations. Des corrélations faiblement négatives ont été observées entre les indicateurs CH_4 et la quantité de lait. À l'inverse, des corrélations positives ont été estimées entre ces mêmes indicateurs et les taux en matières grasses et en protéines.

Conclusions. Cette étude montre la possibilité de prédire des indicateurs méthane issus de la littérature et utilisant les concentrations en acides gras dans la matière grasse laitière à partir de la spectrométrie MIR. De plus, cette étude suggère également à partir des paramètres génétiques obtenus l'existence d'une variabilité phénotypique et génétique des quantités de méthane éructées par les vaches laitières Holstein.

Mots-clés. Lait, méthane, acide gras, spectroscopie infrarouge, héritabilité, vache.

1. INTRODUCTION

Livestock production is considered as one of the key sources of greenhouse gas emissions, the main gas produced being methane (CH_4). Globally, livestock 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. Methane is 21 times more potent than CO_2 in its greenhouse effect; therefore, the reduction of CH_4 would make an important contribution for decreasing the impact of greenhouse emissions (Smith et al., 2007). Furthermore, CH_4 emissions from ruminants account for 2% to 12% of gross energy loss from feed, depending on the type of diet (Johnson et al., 1995). Therefore the mitigation of CH_4 emissions from livestock has two advantages: a decrease in its environmental impact and an increase in energy efficiency.

There are currently several methods available for obtaining CH_4 emission data on a small scale. It is widely acknowledged that the most accurate measurement of CH_4 emissions can be obtained from the calorimetry method using respiration chambers. However, the cost, the handling of lactating dairy cows and the CH_4 measurement time, limit the number of animals that can be measured, which reduces the usefulness of this method in large populations. The measurement of sulfur hexafluoride gas (SF_6) (Johnson et al., 1994) is an alternative method for obtaining CH_4 emission data, which 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 on a medium to large scale. Other methods are under scrutiny but all depend on the acquisition of extra data from outside the current data acquisition strategies used in the usual recording systems of animal production.

The most used method of data acquisition in dairy cattle is through performance recording. Consequently, some studies have focused on the creation of indicator traits indirectly related to the CH_4 emissions, based on milk composition which is or could be routinely measured through milk recording. A particularly promising group of components, in this regard, are fatty acids (FA). The use of FA traits seems to be biologically relevant for predicting CH_4. Indeed, CH_4 output from ruminants is directly linked to microbial digestion in the rumen. The fermentation process of feed carbohydrates leads to the production of hydrogen (H_2) and the methanogenesis is an 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 emissions have been derived (*e.g.*, Chilliard et al., 2009; Dijkstra et al., 2011). Unfortunately, gas chromatographic method is not adapted for large scale use in dairy cow population especially due to its cost.

Recent research showed the feasibility of predicting FA using MIR spectrometry (*e.g.*, Soyeurt et al., 2011). Therefore, the aims of the current study were :

– 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 equations on the spectral data recorded for the Walloon Holstein population;

– to estimate the phenotypic and genetic parameters of these indicator traits and their relationship with milk production traits in order to evaluate indirectly the CH_4 emission of Holstein dairy cows.

2. MATERIALS AND METHODS

2.1. Computation of CH_4 indicator traits

Several different CH_4 indicator traits derived from FA are given in the literature. The indicators defined by Chilliard et al. (2009) and Dijkstra et al. (2011) are well known and already used in several studies. Only CH_4 indicator traits described by Chilliard et al. (2009) were investigated in the current study. The CH_4 indicator trait defined by Dijkstra et al. (2011) was not considered in this study because its formula was developed from less abundant milk FA and the dry matter intake (DMI), which were not available. Methane 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 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:

– using the MIR equations for FA developed by Soyeurt et al. (2011), then computing the CH_4 indicators by using these MIR FA predictions;

– computing reference values for CH_4 indicator traits using the gas chromatographic FA database used by Soyeurt et al. (2011) and then using these reference values for CH_4 indicators with associated MIR data to obtain direct MIR equations for these CH_4 indicators.

The second proposal was preferred 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 gas chromatographic FA 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 FA contents measured by gas chromatography by using the equations published by Chilliard et al. (2009) (**Table 1**). The equations developed by Chilliard et al. (2009) predicted the CH_4 emission in g per day. The coefficient of determination reported in literature by Chilliard et al. (2009) for

Table 1. CH_4 equations developed by Chilliard et al. (2009) and used in the calibration procedure and statistical parameters calculated for the developed MIR prediction equations for CH_4 emission based on Walloon data (g per day) — *Équations développées par Chilliard et al. (2009) et utilisées dans la procédure de calibrage développée dans cette étude ainsi que les paramètres statistiques calculés pour les équations MIR développées à partir des données wallonnes (g par jour).*

Indicator trait	Equation (fatty acids in g·100 g^{-1} of fat)	R²ref	N	Mean	SD	SECV	R²cv
Methane_1 (g per day)	9.97 x (C8:0 to C16:0) – 80	0.88	597	447	68	19	0.92
Methane_2 (g per day)	-8.72 x C18:0 + 729	0.88	602	422	61	18	0.91
Methane_3 (g per day)	282 x C8:0 +11	0.81	595	369	43	23	0.72
Methane_4 (g per day)	16.8 x C16:0 – 77	0.82	588	460	88	26	0.92

R²ref: 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) — *coefficient de détermination publié dans la littérature entre les prédictions de CH_4 obtenues à partir des données chromatographiques en phase gazeuse et les données SF_6*; N: number of samples used in the calibration set — *nombre d'échantillons utilisés dans le set de calibrage*; Mean: mean of the reference values — *moyenne des valeurs de référence*; SD: standard deviation of the reference values — *écart-type calculé pour les données de référence*; SECV: standard error of cross validation — *erreur standard de validation croisée*; R²cv: cross validation coefficient of determination — *coefficient de détermination obtenu pour la validation croisée*.

these four 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 performed 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 CH_4 indicator traits

Milk samples were collected from Holstein cows belonging to 1,207 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; 203,092; and 138,117 records collected from 53,481; 41,419; and 28,978 first, second, and third parity Holstein cows, respectively. All of these records 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:

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

where **y** was the vector of observations for each trait (Methane_1 to Methane_4, milk yield, fat and protein contents), **β** 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 2nd 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 breeding values were estimated (**EBV**) 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:

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

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, **z**$_{t1}$ = $3.0^{0.5}$x, **z**$_{t2}$ = $5.0^{0.5}(1.5x^2 - 0.5)$, where x = $2[(t - 5)/300] - 1$.

Second, daily genetic correlations between two traits were estimated as correlations between **EBVd** values of the two traits of interest for each DIM between 5 and 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 CH_4 indicator traits

After applying the equations of Chilliard et al. (2009) on the gas chromatographic FA data included in the calibration set (*i.e.*, 602 samples), the mean and SD obtained for the reference values for Methane_1 to Methane_4 were within the range of estimated CH_4 emission values published by Chilliard et al. (2009) (**Table 1**). The values for Methane_3 tended 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 1**). For all traits except

Methane_3, R²cv was at least 0.91. Based on the obtained results, Methane_1 and Methane_2 seem to be the most relevant MIR traits because they had the highest R²ref (as reported by Chilliard et al., 2009) and high R²cv were obtained. Moreover, Methane_2 and Methane_1 had also the lowest SECV (18.42 and 19.30 g of CH_4 per day, respectively). This suggests that Methane_1 and Methane_2 were theoretically the best CH_4 indicators. In addition to this, the FA used in Methane_3 and Methane_4 were also a subset of those used for Methane_1.

3.2. MIR indicators of CH_4 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**. By taking into account all CH_4 indicators, the MIR CH_4 predictions ranged from 350 g to 443 g per day in the first lactation which corresponds to 128 to 162 kg of CH_4 per year.

For the second lactation, the CH_4 MIR prediction was 360 g to 449 g per day. The value was almost similar for the third lactation (**Table 2**). MIR predicted values stayed within the range observed from the reference values (**Table 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 per day) 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 per day, 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. (2010), and also measured from the respiration chambers (van Zijderveld et al., 2011).

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 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). However, at 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 per day. 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 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%) (**Figures 1b** and **1c**). 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.

Table 2. Descriptive statistics for the common production traits and the CH_4 indicator traits for the first three lactations (with their corresponding SD) — *Statistiques descriptives des caractères laitiers communément utilisés et des émissions de CH_4 prédites par MIR pour les trois premières lactations (avec leurs écarts-types correspondants).*

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

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
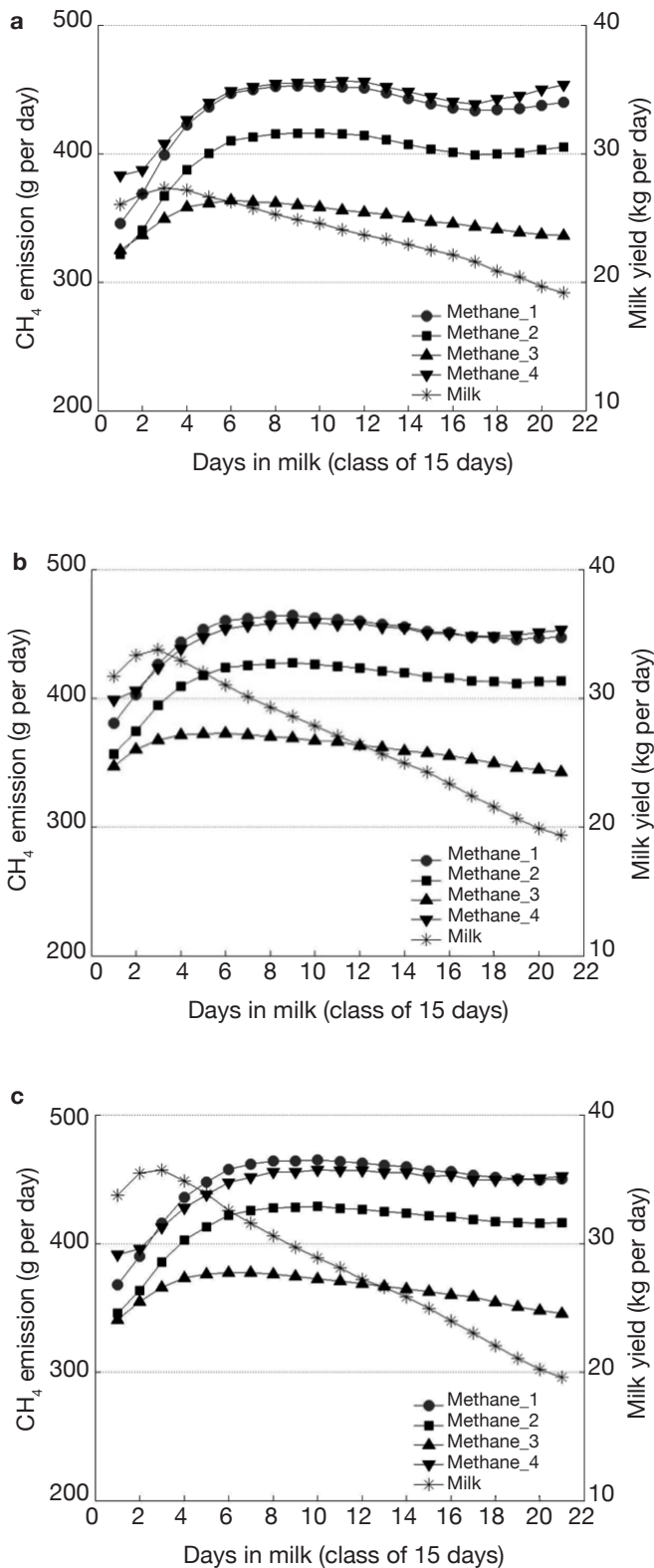
Figure 1. Evolution of the four CH_4 indicator traits and milk yield for the first (**a**), second (**b**) and third (**c**) parity Holstein cows throughout lactation — *Évolution des quatre indicateurs CH_4 prédits par MIR et de la quantité de lait pour les vaches Holstein au cours de leur première (**a**), deuxième (**b**) et troisième (**c**) lactation.*

CH_4 from generation to generation. However, this CH_4 indicator trait was basically derived from the saturated FA which has reported heritability values 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 Hass 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 of 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 heritability values 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**). 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.
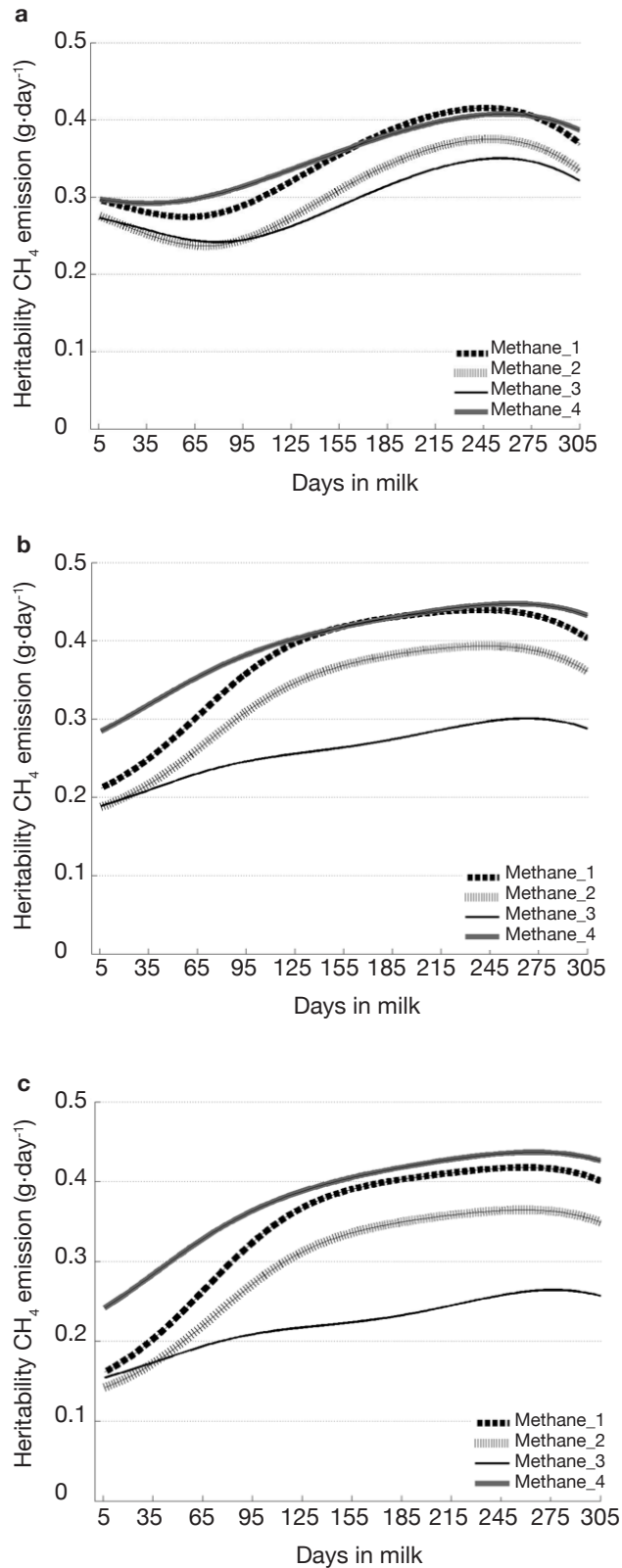
Figure 2. Evolution of the daily heritability of the four CH$_4$ indicator traits throughout the first (**a**), second (**b**) and third (**c**) lactations — *Évolution des héritabilités journalières pour les quatre indicateurs CH$_4$ au cours de leur première (**a**), deuxième (**b**) et troisième (**c**) lactation.*

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 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.

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^{-1} 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). 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$_4$ daily content (g·day^{-1}).

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 3**). Therefore, selecting for increased milk production can slightly decrease the CH$_4$ emissions as suggested previously by Wall et al. (2010).

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 heritability values and as expected, substantial differences of EBVs between animals were observed.

Table 3. Phenotypic (below the diagonal) and approximate genetic (above the diagonal) correlations between the studied CH_4 indicator traits and production traits in first parity Holstein cows — *Corrélations phénotypiques (en dessous de la diagonale) et génétiques approchées (au-dessus de la diagonale) entre les indicateurs CH_4 étudiés prédits par MIR et les caractères de production pour les vaches Holstein en première lactation.*

	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 per day) — *lait (kg par jour)*; Fat: fat content (g·dl^{-1} of milk) — *taux en matières grasses (g·dl^{-1} de lait)*; Protein: protein content (g·dl^{-1} of milk) — *taux en protéines (g·dl^{-1} de lait).*

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 is 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 methane 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 methane 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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