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

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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 erupting the highest and the lowest CH$_4$ content were 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
étant 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 CH₄ 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 fillettes 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 CH₄ 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 le lait 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 émises 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 (CH₄). Globally, livestock produces about 80 million tons of enteric CH₄ annually (Beauchemin et al., 2008). Enteric CH₄ fermentation by ruminants accounts for about 32% of total non-CO₂ emissions from agriculture. Methane is 21 times more potent than CO₂ in its greenhouse effect; therefore, the reduction of CH₄ would make an important contribution for decreasing the impact of greenhouse emissions (Smith et al., 2007). Furthermore, CH₄ 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 CH₄ 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 CH₄ emission data on a small scale. It is widely acknowledged that the most accurate measurement of CH₄ emissions can be obtained from the calorimetry method using respiration chambers. However, the cost, the handling of lactating dairy cows and the CH₄ 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 (SF₆) (Johnson et al., 1994) is an alternative method for obtaining CH₄ 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 CH₄ 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 CH₄ 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 CH₄. Indeed, CH₄ output from ruminants is directly linked to microbial digestion in the rumen. The fermentation process of feed carbohydrates leads to the production of hydrogen (H₂) and the methanogenesis is an essential pathway to expel this H₂ (Moss et al., 2000). The synthesis of acetate (C2) and beta-hydroxybutyrate (C4) produces H₂ that is then converted to CH₄, while propionate (C3) synthesis consumes H₂ (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 (GCF/A) to predict CH₄ 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 CH₄ 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 CH₄ emission of Holstein dairy cows.

2. MATERIALS AND METHODS

2.1. Computation of CH₄ indicator traits

Several different CH₄ 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 CH₄ indicator traits described by Chilliard et al. (2009) were investigated in the current study. The CH₄ 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 CH₄ indicator traits. In this context, two options are possible:
- using the MIR equations for FA developed by Soyeurt et al. (2011), then computing the CH₄ indicators by using these MIR FA predictions;
- computing reference values for CH₄ indicator traits using the gas chromatographic FA database used by Soyeurt et al. (2011) and then using these reference values for CH₄ indicators with associated MIR data to obtain direct MIR equations for these CH₄ 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 CH₄ 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 CH₄ 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 CH₄ 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 CH₄ emission in g per day. The coefficient of determination reported in literature by Chilliard et al. (2009) for

### Table 1. CH₄ equations developed by Chilliard et al. (2009) and used in the calibration procedure and statistical parameters calculated for the developed MIR prediction equations for CH₄ emission based on Walloon data (g per day)

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

R²ref: literature coefficient of determination between the CH₄ predicted from gas chromatographic data and the SF₆ CH₄ data (for Methane_1 to Methane_4) — coefficient de détermination publié dans la littérature entre les prédictions de CH₄ obtenues à partir des données chromatographiques en phase gazeuse et les données SF₆ ; 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 ($R^2_{\text{ref}}$) 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 ($R^2_{\text{cv}}$) 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:

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

where $y$ was the vector of observations for each trait (Methane_1 to Methane_4, milk yield, fat and protein contents), $\beta$ 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 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:

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

where $\text{EBVd}_{htk}$ 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 $\text{EBVd}$ 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 $R^2_{\text{cv}}$ (Table 1). For all traits except...
Methane_3, R\textsuperscript{cv} 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 R\textsuperscript{ref} (as reported by Chilliard et al., 2009) and high R\textsuperscript{cv} were obtained. Moreover, Methane_2 and Methane_1 had also the lowest SECV (18.42 and 19.30 g of CH\textsubscript{4} per day, respectively). This suggests that Methane_1 and Methane_2 were theoretically the best CH\textsubscript{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\textsubscript{4} eructed by Walloon Holstein cows

Descriptive statistics for the common production traits and the developed MIR CH\textsubscript{4} indicator traits for the first three lactations of studied Walloon Holstein cows are shown in Table 2. By taking into account all CH\textsubscript{4} indicators, the MIR CH\textsubscript{4} predictions ranged from 350 g to 443 g per day in the first lactation which corresponds to 128 to 162 kg of CH\textsubscript{4} per year.

For the second lactation, the CH\textsubscript{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\textsubscript{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\textsubscript{4} production obtained from the equations of Chilliard et al. (2009), the obtained MIR CH\textsubscript{4} predictions were within the range of the published CH\textsubscript{4} emissions. Except for Methane_3, the MIR based predictions of CH\textsubscript{4} in our study were in agreement with the daily CH\textsubscript{4} emissions (429 ± 128 g per day) measured in Holstein cows in Belgium (Dehareng et al., 2012), as well as the CH\textsubscript{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\textsubscript{4} emissions measured through the SF\textsubscript{6} method from other authors like Heimeier et al. (2010), and also measured from the respiration chambers (van Zijlderveld et al., 2011).

The estimated MIR CH\textsubscript{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\textsubscript{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\textsubscript{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\textsubscript{4} indicators compared to the peak in milk production.

The difference in MIR CH\textsubscript{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\textsubscript{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\textsubscript{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\textsubscript{4} indicator traits were in line with expectations.

### Table 2. Descriptive statistics for the common production traits and the CH\textsubscript{4} indicator traits for the first three lactations (with their corresponding SD) – Statistiques descriptives des caractéres laitiers communément utilisés et des émissions de CH\textsubscript{4} prédites par MIR pour les trois premières lactations (avec leurs écarts-types correspondants).

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

### 3.3. Heritability

Average daily heritability for the four MIR CH\textsubscript{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 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 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 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 1](image-url) 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.
3.4. Phenotypic and approximate genetic correlations

The phenotypic correlations and the approximate genetic correlations calculated between the MIR \( \text{CH}_4 \) 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 \( \text{CH}_4 \) 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 \( \text{CH}_4 \) MIR traits but the correlations always stayed positive.

The phenotypic correlations for the MIR \( \text{CH}_4 \) traits with milk yield were nearly zero (-0.18 to -0.06) 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 \( \text{CH}_4 \) emission due to fact that milk yield and feed intake as predictor of \( \text{CH}_4 \) 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 \( \text{CH}_4 \cdot \text{kg}^{-1} \) 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 \( \text{CH}_4: \text{CO}_2 \) (i.e., proxy to the \( \text{CH}_4 \) emission).

The approximate genetic correlations were positive between MIR \( \text{CH}_4 \) indicators and fat content (0.31-0.54) and protein content (0.14-0.38). This suggests a higher \( \text{CH}_4 \) 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 \( \text{CH}_4 \) 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 \( \text{CH}_4 \) daily content (gday\(^{-1}\)).

The approximate genetic correlations between the studied MIR \( \text{CH}_4 \) 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 \( \text{CH}_4 \) emissions as suggested previously by Wall et al. (2010).

EBVs for all studied \( \text{CH}_4 \) indicator traits were estimated for cows with MIR \( \text{CH}_4 \) predictions and for sires that had daughters with MIR \( \text{CH}_4 \) 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 CH₄ 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 CH₄ étudiés prédits par MIR et les caractères de production pour les vaches Holstein en première lactation.

<table>
<thead>
<tr>
<th>Methane_1</th>
<th>Methane_2</th>
<th>Methane_3</th>
<th>Methane_4</th>
<th>Milk</th>
<th>Fat</th>
<th>Protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methane_1</td>
<td>0.98</td>
<td>0.55</td>
<td>0.81</td>
<td>-0.18</td>
<td>0.55</td>
<td>0.32</td>
</tr>
<tr>
<td>Methane_2</td>
<td>0.99</td>
<td></td>
<td></td>
<td>0.59</td>
<td>-0.17</td>
<td>0.54</td>
</tr>
<tr>
<td>Methane_3</td>
<td>0.56</td>
<td>0.57</td>
<td></td>
<td>0.75</td>
<td>-0.17</td>
<td>0.31</td>
</tr>
<tr>
<td>Methane_4</td>
<td>0.88</td>
<td>0.87</td>
<td>0.32</td>
<td>0.18</td>
<td>-0.11</td>
<td>0.43</td>
</tr>
<tr>
<td>Milk</td>
<td>-0.07</td>
<td>-0.06</td>
<td>0.24</td>
<td>-0.18</td>
<td>-0.50</td>
<td>-0.38</td>
</tr>
<tr>
<td>Fat</td>
<td>0.23</td>
<td>0.21</td>
<td>0.01</td>
<td>0.29</td>
<td>-0.39</td>
<td>0.59</td>
</tr>
<tr>
<td>Protein</td>
<td>0.23</td>
<td>0.25</td>
<td>0.05</td>
<td>0.17</td>
<td>-0.38</td>
<td>0.51</td>
</tr>
</tbody>
</table>

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 CH₄ 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 CH₄ 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 CH₄ 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 CH₄ 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 CH₄ indicator traits suggesting a potential phenotypic and genetic variability of CH₄ content eructed by dairy cows.

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Bibliography


Variability of methane indicators in dairy cows


