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

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ABSTRACT

The main goal of this study was to develop, apply, and validate a new method to predict an indicator for CH₄ eructed by dairy cows using milk mid-infrared (MIR) spectra. A novel feature of this model was the consideration of lactation stage to reflect changes in the metabolic status of the cow. A total of 446 daily CH₄ measurements were obtained using the SF₆ method on 142 Jersey, Holstein, and Holstein-Jersey cows. The corresponding milk samples were collected during these CH₄ measurements and were analyzed using MIR spectroscopy. A first derivative was applied to the milk MIR spectra. To validate the novel calibration equation incorporating days in milk (DIM), 2 calibration processes were developed: the first was based only on CH₄ measurements and milk MIR spectra (independent of lactation stage; ILS); the second included milk MIR spectra and DIM information (dependent on lactation stage; DLS) by using linear and quadratic modified Legendre polynomials. The coefficients of determination of ILS and DLS equations were 0.77 and 0.75, respectively, with standard error of calibration of 63 g/d of CH₄ for both calibration equations. These equations were applied to 1,674,763 milk MIR spectra from Holstein cows in the first 3 parities and between 5 and 365 DIM. The average CH₄ indicators were 428, 444, and 448 g/d by ILS and 444, 467, and 471 g/d by DLS for cows in first, second, and third lactation, respectively. Behavior of the DLS indicator throughout the lactations was in agreement with the literature with values increasing between 0 and 100 DIM and decreasing thereafter. Conversely, the ILS indicator of CH₄ emission decreased at

the beginning of the lactation and increased until the end of the lactation, which differs from the literature. Therefore, the DLS indicator seems to better reflect biological processes that drive CH₄ emissions than the ILS indicator. The ILS and DLS equations were applied to an independent data set, which included 59 respiration chamber measurements of CH₄ obtained from animals of a different breed across a different production system. Results indicated that the DLS equation was much more robust than the ILS equation allowing development of indicators of CH₄ emissions by dairy cows. Integration of DIM information into the prediction equation was found to be a good strategy to obtain biologically meaningful CH₄ values from lactating cows by accounting for biological changes that occur throughout the lactation.

Key words: dairy cattle, mid-infrared, methane, lactation stage, milk

Hot Topic

Feed costs are the largest expense in dairy production and limit the profitability of dairy farms (Vallimont et al., 2011). Methane emissions, besides contributing to the carbon footprint of dairy products, represent a loss of use of energy intake (Johnson and Johnson, 1995). Therefore, interest is growing in mitigating CH₄ emissions, thereby reducing environmental footprint but also potentially improving feed efficiency and reducing related feed costs. Most of the CH₄ emitted by cattle is eructed. However, the measurement of eructed CH₄ emissions is difficult and expensive. To mitigate these emissions, it is important to have a cheap, fast, and robust method to measure the CH₄ emissions of individual cows on a large scale. Traits that can be easily recorded and that are correlated with CH₄ emissions are therefore of interest. De novo synthesis of milk FA in the mammary gland is dependent on the products of

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ruminal fermentation (Chilliard et al., 2000), as is the production of eructed CH₄ (Chilliard et al., 2009; Dijkstra et al., 2011). Hence, changes in the FA profile of milk fat may be relevant. Because mid-infrared (MIR) spectrometry is known to be useful in predicting milk FA contents (Soyeurt et al., 2011), Dehareng et al. (2012) developed prediction equations to obtain individual CH₄ emissions of dairy cows directly from milk MIR spectra. However, results obtained using those equations did not fit with what was expected from the literature, showing the lowest emissions in early lactation and an increase thereafter. A hypothesis could be that milk FA profile, and therefore the relationship between CH₄ and MIR spectra, is strongly influenced by the evolution of body-tissue mobilization during lactation, leading to suboptimal predictions. Consequently, MIR prediction equations that incorporate stage of lactation may better take into account the metabolic changes that occur throughout lactation. The objective of this study was to develop, apply, and test on MIR data, a novel and readily adoptable method that includes lactation stage as a proxy for metabolic status in the CH₄ prediction equation based on milk MIR spectra. The ability of the novel method to predict a CH₄ indicator was tested on a completely unrelated external data set.

Development of Mid-Infrared-Based CH₄ Indicators

Methane Reference Measurements. Measurements of CH₄ emissions from dairy cows were obtained from the Teagasc Animal and Grassland Research and Innovation Center (Moorepark, Co. Cork, Ireland), the Walloon Agricultural Research Centre (Gembloux, Belgium), and from one commercial herd in Belgium. A total of 446 individual daily CH₄ measurements were recorded: 270 Irish records from 117 individual Holstein, Jersey, and Holstein-Jersey crossbred cows, including 49 first-, 30 second-, and 38 third- or later-parity animals; and 176 Walloon records from 25 Holstein cows including 10 first-, 6 second-, and 9 third- or later-parity animals. Measurements were conducted at

different lactation stages (Table 1). This data set of CH₄ values was characterized by a minimum of 180 g/d, a maximum of 942 g/d, a mean of 416 g/d, and a standard deviation of 128 g/d. To achieve the largest possible variability required for robust calibration equations, cows were fed with very different diets: grass or high silage diets (maize vs. grass silages), with or without linseed supplementation, and synchronized or not in terms of fermentable energy and nitrogen supplies in the rumen. Daily CH₄ emissions of individual cows were determined using the sulfur hexafluoride (SF₆) tracer gas technique with a gas collection period of 24 h. Details about the methodology used to collect the breath samples of emitted gases and to measure the SF₆ and the CH₄ content were described by Dehareng et al. (2012). By using CH₄ emission data collected from different countries, environments, and feeding strategies, an increase in the variability of CH₄ emission was expected, ensuring improved robustness of the equation developed to predict CH₄ emissions.

Spectral Reference Data. On each day of the CH₄ measurements, a 40-mL aliquot of milk was collected from each cow at both the morning and evening milking. Sodium azide (0.32 g/L) was added to each sample before storage at 4°C and MIR analysis was subsequently undertaken using a MilkoScan FT6000 spectrometer (Foss, Hillerød, Denmark). Two spectrometers were used for this study, one located at the Teagasc Animal and Grassland Research and Innovation Center (Co. Cork, Ireland) and one at the milk laboratory Comité du Lait (Battice, Belgium). Two spectra were available per day and per CH₄ measurement. To obtain 1 spectrum per CH₄ record, the 2 spectra were averaged proportionally to the milk yield at each milking.

Development of CH₄ Indicator Equations. To determine the effect of inclusion of DIM information as a predictor variable, 2 prediction equations were developed using independent calibration processes; CH₄ was the dependent variable for both equations. The first prediction equation was independent of lactation stage (ILS) and included milk MIR spectra as the sole pre-

Table 1. Distribution of the number of records in the calibration data set, by parity and stage of lactation of cows, and country from where records were obtained, used to develop methane emissions indicators

Classes of DIM	Parity					
	First		Second		Third or greater	
	Ireland	Belgium	Ireland	Belgium	Ireland	Belgium
1 to 50 DIM	1	7	1	0	4	0
51 to 100 DIM	19	8	21	16	31	0
101 to 150 DIM	31	13	19	10	27	15
151 to 200 DIM	46	19	18	6	12	3
>200 DIM	19	33	13	6	8	40

dictor variable. The ILS equation was therefore equivalent to that developed by Dehareng et al. (2012). The second prediction equation was dependent on lactation stage (**DLS**) and included both DIM of each cow to describe the stage of lactation at which the data were collected and MIR spectra as predictor variables.

The calibration data set contained 446 average daily milk spectral records matched with the daily CH₄ emission data of each cow on the day the milk sample was collected. Following observation of the spectral variation, the calibrations were developed from 3 spectral regions: between wavenumbers 968 and 1,577 cm⁻¹, 1,720 and 1,809 cm⁻¹, and 2,561 and 2,966 cm⁻¹, respectively ($n = 289$ data points). Using Winisi software (Foss, Hillerød, Denmark), partial least squares regression was used to develop the MIR CH₄ prediction equation after the application of a first derivative (Soyeurt et al., 2011) on the recorded spectral data (hereafter called the ILS spectral data set).

To obtain the DLS calibration equation, each first derivative value of the spectrum was multiplied by (1) a constant (i.e., 1), (2) a linear ($\sqrt{3} \times x$), and (3) a quadratic $\left[\sqrt{5/4} \times (3x^2 - 1)\right]$ modified Legendre polynomial (Gengler et al., 1999), where

$$x = -1 + 2[(\text{DIM} - 5)/(365 - 5)].$$

Through this process, a modified spectral data set was generated containing 867 data points (289 data points for each constant, linear, and quadratic part) referred to hereafter as the DLS spectral data set. The linear and the quadratic parts of these modified spectra take into account the lactation stage of cows. Because the constant component was 1, the ILS spectral data set was a subset of the DLS spectral data set. The range of application of the DLS equation was between 5 and 365 DIM given the range of lactation covered in the definition of the Legendre polynomials.

To check the independence of residuals between the corresponding measured and predicted ruminal CH₄ emissions of cows (DLS and ILS MIR CH₄ indicators) across DIM, a linear regression of those residuals on DIM was fitted using the PROC REG procedure (SAS Institute Inc., Cary, NC).

Application of Prediction Equations to Milk Mid-Infrared Spectra

A general requirement for any CH₄ indicator and, therefore, the underlying prediction equation, is that its observed evolution and variation make biological sense when applied to a data set of measured spectra that cover a similar range of spectral variability

as the reference data. To test the observed evolution and variation of CH₄ indicators, the developed MIR CH₄ prediction equations (ILS and DLS equations) were applied to the spectral database of milk recording collected in the Walloon Region of Belgium since 2007. These milk samples are routinely collected by the Walloon Breeding Association (Ciney, Belgium) during farm milk recording and the spectral data records were generated by the milk laboratory Comité du Lait (Battice, Belgium) using a MIR MilkoScan FT6000 spectrometer (Foss, Hillerød, Denmark). The ILS equation was directly applied to the milk spectra after applying the first derivative. To use the DLS equation, the same pretreatment to each spectrum was used as in the creation of the DLS spectral data set (i.e., first derivative and multiplication by modified Legendre polynomials). Equations were applied only to records from Holstein cows in the first 3 lactations and collected between 5 and 365 DIM (the range of modified Legendre polynomials) leading to a total of 1,951,669 spectral records. To achieve a spectral data range that is well underpinned by spectra used for the calibration, the data set was cleared from spectrally too different data using the standardized Mahalanobis distance (**GH**). This value was calculated between each considered spectrum and those used for the calibration to estimate how different a MIR spectrum of the Walloon milk-recording data set was from spectra of the calibration set. Spectra with a GH greater than 3 were not used in this study, representing only 14% of the 1,951,669 spectra according to the huge variability of Walloon spectra and relatively limited variability of the prediction equations. Extreme values of predicted CH₄ were also detected and estimates of CH₄ below 150 and above 950 g/d of CH₄ (the extended minimum and maximum were obtained by subtracting or adding 50 g/d to minimum and maximum of actual CH₄ measurements from the data set of calibration as shown in Figure 1) were deleted. The final data set contained 1,674,763 MIR spectra and milk yield records collected between January 2007 and March 2014 in 1,654 herds from 176,692 cows including 1,649,750 and 1,672,441 ILS and DLS CH₄ records, respectively. The normality of the distributions for ILS and DLS CH₄ indicators from MIR were studied based on the calculation of Skewness and Kurtosis parameters using PROC UNIVARIATE in the SAS system (SAS Institute Inc., Cary, NC).

The observed variability of ILS and DLS MIR CH₄ indicators should reflect several effects known to influence CH₄ production such as effects of herd, year and month of test-date, lactation number, lactation stage, animal, and milk yield. Therefore, these effects were investigated for the MIR predicted CH₄ traits using the following model:

$$y_{pqrstu} = \mu + h_p \times y_q + mo_r + l_s \times d_t + c_u + m\alpha + (m \times m)\beta + e_{pqrstu}, \quad (1)$$

where y_{pqrstu} was the predicted CH₄ trait (1,649,750 and 1,672,441 for the ILS and DLS indicators described above); μ was the general mean; $h_p \times y_q$ was the crossed fixed effect of herd p and year of test-date q (8 yr from 2007 to 2014); mo_r was the fixed effect of month r ; $l_s \times d_t$ was the crossed fixed effect of lactation number s (3 classes: first, second, and third lactations) and lactation stage t (18 classes of 20 DIM from 5 to 365 DIM, the last class including 365 DIM); c_u was the random effect of cow u ; m was the daily milk yield (kg/d); α was the linear regression coefficient on m ; β was the quadratic regression coefficient on m ; and e_{pqrstu} was the associated random residual. The milk yield effect was included in the model to avoid a potential artifact due to milk yield. Variance components were estimated using the EM-REML and AI-REML algorithms implemented in the REMLF90 and AIREMLF90 software packages, and $\hat{y}$ and the $l_s \times d_t$ effect were estimated using the preconditioned conjugate gradient algorithm as implemented in the BLUP90IOD software (Misztal, 2012). The root mean square error (RMSE) and the mean absolute error (MAE) of ILS and DLS indicators were computed as follows (Willmott and Matsuura, 2005):

$$RMSE = \sqrt{\frac{\sum_{i=1}^n |\varepsilon_i|^2}{n}},$$

$$MAE = \frac{\sum_{i=1}^n |\varepsilon_i|}{n},$$

where n was the number of data, ε_i was the difference between $\hat{y}$ and DLS or ILS indicators for the i th observation. The RMSE and MAE were divided by the calibration coefficient of determination (R^2c) of prediction equations to approximate RMSE and MAE of $\hat{y}$ (from DLS and ILS indicators) and direct measurements of CH₄ emissions.

Independent Comparison of Methane Indicators with Actual Measurements

A small calibration data set with specific breeds, feed, and CH₄ measurement method was used to develop the prediction equations. However, the different sources of variation of milk composition interact during lactation. An independent comparison (correlations, bias, RMSE, and MAE) of ILS, and DLS CH₄ indicator values with actual CH₄ measurements from an independent data set was undertaken to assess if the DLS prediction equation was able to disentangle the captured sources of variation in a sufficient manner.

Moreover, to test whether or not the introduction of DLS calibration coefficients was capturing more than only a similarity in the lactation curve shape, a polynomial regression of measured CH₄ based only on DIM was fitted without milk MIR spectra. This was done using a multiple regression of the 446 CH₄ reference measurements (calibration data set) on the 3 regression variables constant, linear, quadratic modified Legendre polynomial of lactation stage, hereafter called regression on lactation stage (RLS). By doing this, the CH₄ measurements were linked to 3 regression variables including only DIM information instead of the 289 and 867 data points used for the ILS and DLS equations,

Figure 1. Relationship between measured reference CH₄ emissions of cows from the calibration data set and the corresponding indicators of these emissions from mid-infrared (MIR) spectra obtained from the equations independent of lactation stage (ILS) or dependent on lactation stage (DLS).

respectively. Through this strategy, RLS equation excluded milk MIR spectra information, whereas ILS and DLS equations included this information. The RLS equation was then applied on an independent data set described hereafter to assess if a simple polynomial regression of measured CH_4 on DIM would capture the major part of intra-lactation variability of CH_4 .

A total of 60 respiration chamber measurements of CH_4 were obtained from studies made at the Agrovet-Strickhof research station (Lindau-Eschikon, Switzerland). These records were collected during 2 d in open-circuit chambers described in detail by Buehler and Wanner (2012) using 30 lactating cows, and milk collected at that period was analyzed by MIR spectroscopy. These data were of a clearly different origin compared with the calibration data as they were obtained from different breed (Brown-Swiss), feeding system [half were exclusively fed on forage (grass hay, corn silage, and pellets made from artificially dried grass), and the other half received limited amounts of concentrate in addition to the same forage mix], and method of CH_4 measurement (chambers). Following application of the GH edit used on the commercial spectral data, one record was removed, leaving 59 spectra available for this study. The ILS, DLS, and RLS equations were applied on these data. Correlation, RMSE, and MAE between actual CH_4 measurements and corresponding CH_4 indicators were computed.

Results and Discussion

Development of CH_4 Indicator Prediction Equations. The average value for the 446 reference CH_4 measurements was 416 g of CH_4 /d with a standard deviation of 128 g/d. The coefficient of variation of 31% suggested a good variability of the studied trait. Figure 1 shows the relationship between the measured and predicted CH_4 data (i.e., ILS and DLS MIR CH_4 indicators) used in the calibration process. The ILS equation had a R^2 of 0.77 with a standard error of calibration (SEC) of 63 g/d. The R^2 and SEC of the DLS equation were 0.75 and 63 g/d, respectively. Based on the statistical calibration parameters, the ILS and DLS equations showed very similar abilities to predict the eructed CH_4 . Residuals between corresponding CH_4 measurements and predicted CH_4 emissions of dairy cows (ILS or DLS MIR CH_4 indicators) across DIM are shown in Figure 2. Using linear regression of residuals on DIM, the regression coefficients for the DLS residuals were closer to 0 than those obtained for the ILS residuals [ILS residual = $0.1032 (\pm 0.0459)$ DIM - 15.801 (± 7.551), and DLS residual = $-0.0011 (\pm 0.0490)$ DIM + 0.1662 (± 8.067)]. As regression coefficients were close to 0, residuals of the DLS indicator showed a bet-

ter fit over the lactation because the residuals were less influenced by DIM than the ILS indicator.

Descriptive Statistics of Indicators When Computed on Field Data. After application of the ILS prediction equation to the Walloon milk recording spectral MIR database, the average indicators of daily CH_4 emissions were 428 (± 141), 444 (± 144), and 448 (± 144) g/d for cows in first, second, and third lactation, respectively. The average indicators of CH_4 emissions obtained from the DLS equation were 444 (± 73), 467 (± 75), and 471 (± 77) g/d for first-, second-, and third-parity cows, respectively. These values were within the range of CH_4 measurements reported in the literature (e.g., Aguerre et al., 2011; van Zijderveld et al., 2011; Garnsworthy et al., 2012). The variability of the DLS MIR CH_4 indicator values was lower than that obtained using the ILS equation. Skewness and Kurtosis parameters of MIR CH_4 indicators were low (0.27 and -0.36 for ILS and -0.18 and 0.93 for DLS, respectively), suggesting an approximate normal distribution of the studied traits. More values for the ILS indicator were considered out of the range of the calibration in the final data set (less than 150 and greater than 950 g/d of CH_4) compared with those estimated using the DLS equation (1.5% vs. 0.1% of the 1,674,763 records, respectively).

Modeling Methane Indicators Traits. Both the ILS and DLS MIR CH_4 indicators were significantly affected by all studied fixed effects (i.e., herd $\times$ year of test-day, month of test-day, lactation number $\times$ lactation stage, and milk yield). The residual variance obtained from the estimation of variance components of the ILS indicator was higher than the residual variance of the DLS indicator (10,280 vs. 3,183 g^2 , respectively). Compared with those obtained using the ILS equation, DLS values conserved more of the intra-cow variability as the associated repeatability (relative cow variance) was 0.15 (± 0.001) for DLS indicators compared with 0.04 (± 0.000) for ILS indicators. As expected, given these results, adjusted RMSE and MAE of DLS indicators were lower than those of ILS indicators (RMSE of 81 and 135 g/d of CH_4 and MAE of 61 and 103 g/d of CH_4 for DLS and ILS indicators, respectively). These results indicate that ILS indicator values, even after fitting fixed effects, had a much higher overall variance. Also, the ILS indicator did not retain the expected intra-cow repeatability as did DLS. Therefore, the DLS indicator appeared to reflect better biological processes than the ILS indicator. Figure 3 shows $l_s \times d_t$ effect solutions on predicted CH_4 emissions of cows in first-, second-, and third-parity for 18 classes of 20 DIM. Through the use of the model (1) described previously, observed differences were corrected for all other effects, including milk yield. Both ILS and DLS indi-

Figure 2. Residuals between corresponding reference CH_4 measurements and CH_4 emissions indicators of dairy cows across DIM obtained from the calibration process. Indicators of CH_4 emissions were calculated by applying prediction equations that were independent of (ILS; Δ) or dependent on (DLS; +) lactation stage. Linear trends of residuals (ILS - - -, or DLS —) are also indicated.

cators of multiparous cows appear to be higher than those of primiparous cows as expected. The pattern of DLS values throughout lactation showed an increase during the first 15 wk of lactation followed by a decrease thereafter. In contrast, ILS values decreased at the beginning of lactation and then increased until the end of the lactation. The pattern of the DLS indicator of CH_4 emissions was similar to the pattern obtained by Garnsworthy et al. (2012). Actually, this curve is expected as it is known that an increase in feed intake takes place in cows postpartum (e.g., Berry et al., 2007) and feed intake is strongly positively associated with CH_4 emissions (Mills et al., 2003). By the joint fitting of milk yield the observed pattern in Figure 3 should reflect the corrected effect of lactation stage on CH_4 emission and not a correlated effect from the evolution of milk yield over the lactation. Results support the hypothesis that the difference between the pattern of ILS and DLS indicator could be explained by the fact that the DLS equation better accounts for biological changes by integrating modified Legendre polynomials related to DIM. Regression solutions for milk yield were also used as a way to assess the biological meaning of indicators. These solutions for the DLS indicator were as expected with 1.52 g/kg and -0.04 g/kg^2 for the linear and the quadratic regression coefficient on milk, respectively. Therefore, these results emphasized the expected link between daily CH_4 and milk yield. However, solutions for ILS were unexpected with -0.05 g/kg and -0.02 g/kg^2 for the linear and the quadratic re-

gression coefficient on milk, respectively. This result is another indication that ILS did not adequately predict the expected increase in CH_4 emission with milk yield.

Independent Comparison of Methane Indicators with Actual Measurements. Correlations (bias) between actual independent CH_4 measurements from respiration chambers and ILS, DLS, and RLS CH_4 indicators were 0.09 (-53 g/d of CH_4), 0.48 (-52 g/d of CH_4), and -0.29 (47 g/d of CH_4), respectively. The observed values for RMSE (MAE) were 113 (89), 90 (68), and 102 (80) g/d of CH_4 for the ILS, DLS, and RLS indicators, respectively. These results indicate that the DLS indicator prediction equation was much more robust than the ILS equation. When comparing with the results obtained using the RLS prediction, the DLS indicator also seemed to better describe the intra-lactation variability in the actual CH_4 measurements. Therefore, these results indicate that the DLS indicator was capturing more than only similarities in lactation curve shapes, as did RLS.

Additionally, it has to be stressed that this comparison was done in a completely different setting (e.g., breed, feed, CH_4 measurement method). Therefore, even if a correlation of the DLS indicator with actual CH_4 measurements of 0.48 might seem low, this result is close to expectations. These expectations could be approximated as the product between the square root of R^2 of the DLS equation (i.e., 0.75), the correlation between CH_4 measurements from chambers and SF_6 method [~ 0.80 as reported by Muñoz et al. (2012)], and

Figure 3. Crossed fixed effect solutions of lactation number and lactation stage on CH_4 emissions (g/d) of first- (L1), second- (L2), and third-lactation (L3) Holstein cows for each class of DIM (18 classes of 20 d in milk) predicted from lactation-stage-independent (a) and lactation-stage-dependent (b) prediction equations applied to milk mid-infrared (MIR) spectra from the spectral database related to the Walloon milk recording.

the hypothetical correlation of CH_4 prediction equations obtained for given breeds and feeding system but apply to others, which could be as low as 0.80.

Conclusions and Perspectives

In conclusion, when considering only the statistical calibration parameters of the 2 tested equations, the ILS equation provided numerically slightly better values regarding R^2c and SEC than the DLS equation. However, it is very important to also take into account the behavior of the obtained indicators on real-life field data sets. When prediction equations were applied to a Walloon spectral data set, the observed results indicated that the DLS indicator better reflected biological processes than the ILS indicator. Moreover, on a completely independent data set (different breed, feeding, and CH_4 measurement method), results indicated the clear superiority of the DLS compared with the ILS indicator and a lactation-stage based prediction (RLS indicator). These results also showed that the better fit

of DLS throughout the lactation compared with ILS was due to it incorporating more information than only the lactation curve shape as does RLS.

The main objective of this study was to demonstrate the ability to incorporate lactation stage information in the CH_4 prediction from MIR spectra. Based on these results, we can conclude that the novel DLS equation provided an adequate method to take into consideration the changes in expected metabolic status during lactation. In addition, the results of the present study suggest that the DLS equation enables development of CH_4 indicators. Nevertheless, the accuracy and robustness of this equation still need to be enhanced and verified in other environments.

The DLS equation can be considered appropriate to predict a useful indicator of CH_4 emission of dairy cows. However, the inclusion of additional reference data (e.g., other breeds, feeding systems) into the calibration set would increase the variability (at both CH_4 and spectral levels) and consequently improve this equation as already shown for other MIR equations (e.g., Soyeurt et al., 2011). Based on the strategy used for FA, a typical cycle of updating the DLS equation would incorporate 3 steps. First, the existing equation will be tested on newly available CH_4 reference data, simultaneously validating and harmonizing the corresponding MIR data. In the second step, predictions will be scrutinized and the new variability assessed to determine the relevant reference values that have to be added. After, most CH_4 reference values would be added to the calibration data set. Finally, a new calibration will be developed and tested for its robustness. With increasing coverage of the variability, the changes in the equation in the updating process will be less and less important leading to fewer and less frequent updates of the DLS equation. A specific complication compared with other traits is that different methods exist to acquire CH_4 reference values. Additional research will be needed to make the indicator robust in these circumstances. Moreover, further improvement of the DLS equation may also be achieved by adding relevant measurements of CH_4 emission to the calibration data set which better cover the entire lactation. For example, as shown in Table 1, few data are available from very early in lactation (first 50 d). Therefore, it would be interesting to add early lactation data to the calibration set.

The primary objective of this study was to test the usefulness of DLS coefficients. Therefore, the input variables were intentionally limited to using only MIR milk spectra and DIM information. The developed tool could be applicable routinely, for example in the milk-recording process where the milk analysis is often separated from the actual recording of yields. The next research steps should include other potentially useful

traits (e.g., milk yield, DMI). This would also allow the link between CH₄ and milk composition, as described by MIR spectral data, to be better separated, and to extend the reach of this research to feed energy loss and feed efficiency traits.

Finally, before including a CH₄ indicator into any index to select for a (climate-smart) selection objective, additional research is required to establish the relationships between the CH₄ indicator and other traits of interest.

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