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Vanlierde, A. and Soyeurt, H. and Gengler, N. and Colinet, F.G. and Froidmont, E. and Kreuzer, M. and Grandl, F. and Bell, M. and Lund, P. and Olijhoek, D.W. and Eugène, M. and Martin, C. and Kuhla, B. and Dehareng, F. (2018) Short communication: Development of an equation for estimating methane emissions of dairy cows from milk Fourier transform mid-infrared spectra by using reference data obtained exclusively from respiration chambers. *Journal of Dairy Science* . ISSN 0022-0302

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J. Dairy Sci. 101:1–7
<https://doi.org/10.3168/jds.2018-14472>

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Short communication: Development of an equation for estimating methane emissions of dairy cows from milk Fourier transform mid-infrared spectra by using reference data obtained exclusively from respiration chambers

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ABSTRACT

Evaluation and mitigation of enteric methane (CH₄) emissions from ruminant livestock, in particular from dairy cows, have acquired global importance for sustainable, climate-smart cattle production. Based on CH₄ reference measurements obtained with the SF₆ tracer technique to determine ruminal CH₄ production, a current equation permits evaluation of individual daily CH₄ emissions of dairy cows based on milk Fourier transform mid-infrared (FT-MIR) spectra. However, the respiration chamber (RC) technique is considered to be more accurate than SF₆ to measure CH₄ production from cattle. This study aimed to develop an equation that allows estimating CH₄ emissions of lactating cows recorded in an RC from corresponding milk FT-MIR spectra and to challenge its robustness and relevance through validation processes and its application on a milk spectral database. This would permit confirming the conclusions drawn with the existing equation based on SF₆ reference measurements regarding the potential to estimate daily CH₄ emissions of dairy cows from milk FT-MIR spectra. A total of 584 RC reference CH₄ measurements (mean ± standard deviation of 400 ± 72 g of CH₄/d) and corresponding standardized milk mid-infrared spectra were obtained from 148 individual lactating cows between 7 and 321 d in milk in 5 European countries (Germany, Switzerland, Denmark, France, and Northern Ireland). The developed equation based on RC measurements showed calibration and cross-validation coefficients of determination

of 0.65 and 0.57, respectively, which is lower than those obtained earlier by the equation based on 532 SF₆ measurements (0.74 and 0.70, respectively). This means that the RC-based model is unable to explain the variability observed in the corresponding reference data as well as the SF₆-based model. The standard errors of calibration and cross-validation were lower for the RC model (43 and 47 g/d vs. 66 and 70 g/d for the SF₆ version, respectively), indicating that the model based on RC data was closer to actual values. The root mean squared error (RMSE) of calibration of 42 g/d represents only 10% of the overall daily CH₄ production, which is 23 g/d lower than the RMSE for the SF₆-based equation. During the external validation step an RMSE of 62 g/d was observed. When the RC equation was applied to a standardized spectral database of milk recordings collected in the Walloon region of Belgium between January 2012 and December 2017 (1,515,137 spectra from 132,658 lactating cows in 1,176 different herds), an average ± standard deviation of 446 ± 51 g of CH₄/d was estimated, which is consistent with the range of the values measured using both RC and SF₆ techniques. This study confirmed that milk FT-MIR spectra could be used as a potential proxy to estimate daily CH₄ emissions from dairy cows provided that the variability to predict is covered by the model.

Key words: cattle, greenhouse gas, spectroscopy, proxy

Short Communication

Increasing sustainability and profitability while reducing the environmental footprint of dairy production is, among others, a major challenge for the breeding sector. The reduction of greenhouse gas emissions is

Received January 19, 2018.

Accepted April 1, 2018.

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one of the key factors in meeting these intentions. A reliable method for obtaining CH₄ measurements on large numbers of individual cows in commercial farms is needed. Reliable measurement of CH₄ emissions from these dairy cows is time consuming and expensive; thus, the development of proxies to estimate individual emissions is warranted (Negussie et al., 2017).

Predicting daily CH₄ emissions of dairy cows from milk Fourier transform mid-infrared (**FT-MIR**) spectra is a promising approach (Dehareng et al., 2012; Vanlierde et al., 2015; Vanlierde et al., 2016). In industrial countries, milk FT-MIR spectra are already collected routinely at a reasonable cost in the context of milk recording programs. However, the existing prediction equation for daily CH₄ emissions from milk FT-MIR spectra is based on reference CH₄ measurements obtained using the SF₆ tracer technique (Vanlierde et al., 2016), which only quantifies enteric CH₄. On the other hand, recent investigations have developed similar models using the “sniffer” method to measure CH₄ emissions on a large number of dairy cows. However, results obtained did not confirm the potential to estimate CH₄ from milk FT-MIR spectra only (Shetty et al., 2017). Considering that the respiration chamber (**RC**) technique is praised as the gold standard method capable of measuring total CH₄ emitted by cattle (Hammond et al., 2016), the objective of the present study was to develop a new prediction equation based exclusively on reference data obtained from RC measurements. The robustness and relevance of this equation were tested through validation processes and the application on a milk spectral database. This work will also permit developing an equation based on the RC measurement technique, which gives predictions in an RC range as RC take into account emissions from the lower digestive tract (unlike the SF₆ technique). The ability to predict CH₄ emission from milk FT-MIR spectra will be discussed.

A total of 584 reference CH₄ measurements (400 ± 72 g/d) from 148 individual lactating cows were obtained in open-circuit RC from studies across Europe: Germany, Switzerland, Denmark, France, and Northern Ireland (the facilities used are described in Yan et al., 2000; Derno et al., 2009; Hellwing et al., 2012; Guyader et al., 2015; Grandl et al., 2016). Consequently, this data set represents different feeding regimens, climates, breeds, and feed types. Cows were housed in individual RC for a minimum of 3 d, and CH₄ production was measured for at least 2 consecutive 24-h periods while cows were fed ad libitum. Individual milk samples were taken during a.m. and p.m. milkings from the same days. The corresponding milk samples were collected and analyzed after sampling to obtain FT-MIR spectra. The a.m. and p.m. spectra were linked to a daily spectrum by a weighted average based on the a.m. and p.m.

Table 1. Distribution of parity and stage of lactation of cows used to develop methane emission prediction equations based on respiration chamber reference measurements

DIM	Parity			Total
	1	2	>2	
1–50	1	28	13	42
51–100	3	5	9	17
101–150	3	8	13	24
151–200	1	9	18	28
>200	8	15	14	37
Total	16	65	67	148

milk yields. The averaged milk FT-MIR spectra were then related to the corresponding 24-h CH₄ measurements. The reference data sets consisted of 211 data from Germany [50 Holstein (**HO**) cows, 406 ± 60 g/d of CH₄], 138 data from Switzerland [40 Brown Swiss (**BS**), 9 HO, 8 Red HO, and 1 HO × Simmental cows, 450 ± 76 g/d of CH₄], 130 data from Denmark (9 HO and 10 Jersey cows, 366 ± 64 g/d of CH₄), 81 data from France (9 HO cows, 366 ± 61 g/d of CH₄), and 24 data from Northern Ireland (6 HO and 6 Swedish Red Cross cows, 365 ± 44 g/d of CH₄). These cows were of varying parities and DIM (Table 1).

To avoid any instrument interference and ensure that the milk FT-MIR spectra are comparable regardless of the spectrometer used and the date of analysis, the milk FT-MIR spectra were standardized according to the procedure described in Grelet et al. (2017). A first derivative was applied to the milk FT-MIR spectra as recommended by Soyeurt et al. (2011). The calibration process was 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 (n = 289 data points). Constant (P0), linear (P1), and quadratic (P2) modified Legendre polynomials were computed from DIM the day of CH₄ measurement of the cows (Gengler et al., 1999) and applied to each wavenumber of spectra to take into account the metabolic status of cows during lactation (Vanlierde et al., 2015). The final modified spectra were based on 3 × 289 data points (867 data points). A modified partial least square (**PLS**) regression as implemented in the WINISI software (version 4.6; Foss, Hillerød, Denmark) was used.

The robustness of the calibration model was tested with a 5-group internal cross-validation procedure. Reference data were divided randomly into 5 groups, and 5 calibration models were developed by removing 1 individual group for each calibration development. Then, the removed group was predicted by the calibration model based on the 4 other groups. Moreover, as several measurements per cow are included in the database, a cow- and country-dependent external valida-

tion (**CCDEV**) was conducted using R (R Core Team, Vienna, Austria). To carry out this external validation, 20% of the cows were removed randomly and simultaneously per country (i.e., 10 cows from Germany, 12 from Switzerland, 4 from Denmark, 1 from France, and 2 from Northern Ireland). The calibration model was developed on the remaining 80% of cows, and subsequently the model was tested by predicting the removed data (Shetty et al., 2017). The country-dependent step ensured that the country's variability information (e.g., diet, management) would be considered in the model. Given that the database was not built for the purpose of developing this equation, the removal of some specific cows can have a deep effect on the statistics and, thus, on the conclusions. Therefore, the CCDEV process was repeated 500 times to test a maximum number of combinations, and the variations of the coefficient of determination (R^2) of CCDEV were observed.

The percentage of cows of each breed in the RC data set (percentage of data values) was 55% (68%) HO, 27% (14%) BS, 7% (11%) Jersey, 5% (4%) Red HO, 5% (2%) Swedish Red Cross, and less than 1% (less than 1%) HO \times Simmental, meaning that there were 2 main breeds (HO and BS). A breed-specific model has been tested on these 2 most represented breeds to observe whether this permits more accuracy within each specific breed. The HO model was based on 398 reference values from 82 cows (399 ± 68 g/d of CH_4), and the BS model was based on 80 reference values from 40 cows (458 ± 68 g/d of CH_4). A PLS regression was performed as described before, with a 5-group cross-validation process and a cow- and breed-dependent external validation (**CBDEV**). Regarding this CBDEV step, 20% of cows were removed randomly and simul-

taneously per breed, and the calibration models were developed for each breed on the remaining cows of this breed and subsequently tested using the removed data. Regarding the lower number of possible combinations in comparison with the entire RC data set, the CBDEV process was repeated 20 times per breed.

To evaluate the accuracy of the model for each data set, the root mean squared errors of calibration (**RMSEC**) for the total data set and for each country's data set independently and the root mean squared errors of prediction (**RMSEP**) for the CCDEV and CBDEV steps were computed as follows:

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

where n was the number of observations and ε_i was the difference between the measured CH_4 and corresponding estimated CH_4 values for the i th observation.

To assess the consistency of the predictions obtained with this new equation based on RC measurements, this equation was applied to the Walloon milk recording standardized spectral database, which contains spectral data collected between January 2012 and December 2017. In the present analysis, only records from HO cows collected between 5 and 365 DIM (the range of modified Legendre polynomials) in the first 3 lactations were considered, leading to a total of 1,679,728 standardized spectral records. To ensure that the spectral variability of the milk recording samples was covered by the equation, only the spectra with a standardized Mahalanobis distance (global H distance) lower than 3 were used in this study (e.g., Shenk and

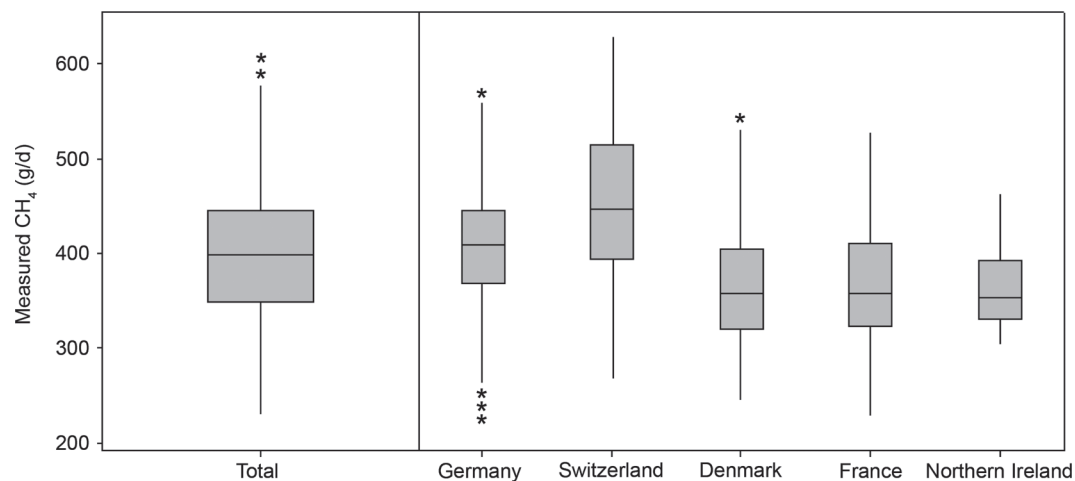


Figure 1. Boxplot of methane measurements obtained in respiration chambers for the entire data set and per country. Boxplot of methane measurements obtained in respiration chambers for the entire data set (Total) and per country. The boxes identify interquartile ranges (Q1–Q3), the solid black mid line indicates the median, whiskers end at the lowest and highest values that are not extreme values, and asterisks represent extreme values.

Table 2. Distribution and general statistics regarding reference methane measurements in a respiration chamber (g/d) and methane predictions (g/d) from milk Fourier transform mid-infrared spectra in individual European countries

Country	No. of data	Reference data				Predicted data				RMSEC ¹
		Mean	SD	Minimum	Maximum	Mean	SD	Minimum	Maximum	
Germany	211	406	60	233	566	408	50	247	494	40
Switzerland	138	450	76	266	630	444	58	283	557	47
Denmark	130	366	64	244	556	368	51	226	472	38
France	81	366	61	229	527	366	38	293	439	47
Northern Ireland	24	365	44	304	464	366	16	342	398	36
Total	584	400	72	229	630	400	58	226	557	42

¹Root mean squared error of calibration.

Westerhaus, 1991; Soyeurt et al., 2012). During this process a moderate amount (9.8%) of the spectra was removed. Finally, CH₄ predictions lower than 150 g/d and higher than 950 g/d were considered outliers (Vanlierde et al., 2015) and excluded from this study (206 predicted data). In the end, 1,515,137 CH₄ predicted values were used to test the practical application of the equation. These represented 1,176 different herds and 132,658 individual cows.

The results obtained with the milk FT-MIR model designed to predict CH₄ emissions (g/d) based on RC measurements were encouraging. The correlations between measured and predicted values during calibration and cross-validation were 0.8 and 0.75, respectively, and confirmed the relevance of evaluating the CH₄ emissions from milk FT-MIR spectra. Regarding the statistical performance of the RC-based model built on 14 terms (vs. 10 terms for the SF₆-based equation), the calibration R² was 0.65 for the RC-based equation versus 0.74 for the SF₆-based equation (Vanlierde et al., 2016). The cross-validation R² was 0.57 for the RC-based equation versus 0.70 for the SF₆-based equation. The R² are highly dependent on the range and variability of the data. In comparison with SF₆ measurements, the lower observed R² for RC is probably partly due to the lower range of reference values [from 229 to 630 g/d of CH₄ (vs. 180 to 802 g/d for the SF₆ data set) with a mean ± standard deviation of 400 ± 72 g/d (vs. 430 ± 129 g/d for the SF₆ data set)]. Moreover, the development of a calibration equation requires a sufficiently varied set of data (Davies and Fearn, 2006). However, this European data set was collated from independent studies in each country, usually for the purpose of comparing CH₄ emissions in response to different dietary treatments. Therefore, animals that were closely comparable in terms of milk yield and lactation stages were used in each country. This explains why the data sets regarding the number of animals, data, diets, RC type, and so on are different among countries; ideally, they should be more comparable. Figure 1 illustrates the distribution of CH₄ measurements for the entire

data set and for each individual country. As expected regarding the previous remarks, the distribution of the reference data appeared to vary between countries and can induce a “country effect.” Ideally, these issues could be dealt with by enlarging the number and therefore the variability of the reference data in each country. Nevertheless, in the present study, collaboration between research institutes allowed merging the available data sets and gave the opportunity to design a European data set with RC measurements from individual cows of different genetic background and fed differing diets. This translated into greater variability than that obtained within individual countries as well as in terms of range of reference CH₄ measurements (required for the calibration process) regarding the zootechnical aspects (e.g., covered breeds, diets, DIM). In the present case, the variability in lactation stages, parity, milk yield, feed intake, and CH₄ emission of this Europe-wide RC data set was not as well-balanced as that in the data set obtained from SF₆ measurements. Indeed, the reference data set for the SF₆-based equation was deliberately collected for the purpose of creating an equation able to predict CH₄ values from milk FT-MIR spectra, meaning that a maximum number of scenarios (e.g., measurements on cows of different parities, with lactation stage not represented in the model, fed with specific diets) were intentionally researched. Nonetheless, even with nonoptimal variability, the observed linear relationship between measured and predicted CH₄ emissions (Figure 2) implies that FT-MIR spectra predictions are correlated with CH₄ production measured in RC. The standard error of calibration was 43 g/d for RC (vs. 66 g/d for SF₆), and the standard error of cross-validation was 47 g/d (vs. 70 g/d for SF₆). With a lower standard error of cross-validation, the RC-based predictions are closer to the actual values than the SF₆ equation. The predictions of calibration data ranged between 226 and 557 g/d of CH₄, with an average ± standard deviation of 400 ± 58 g/d. As shown in Table 2, the means of the predictions are similar to the means of the measured data for each country as well as for the overall data

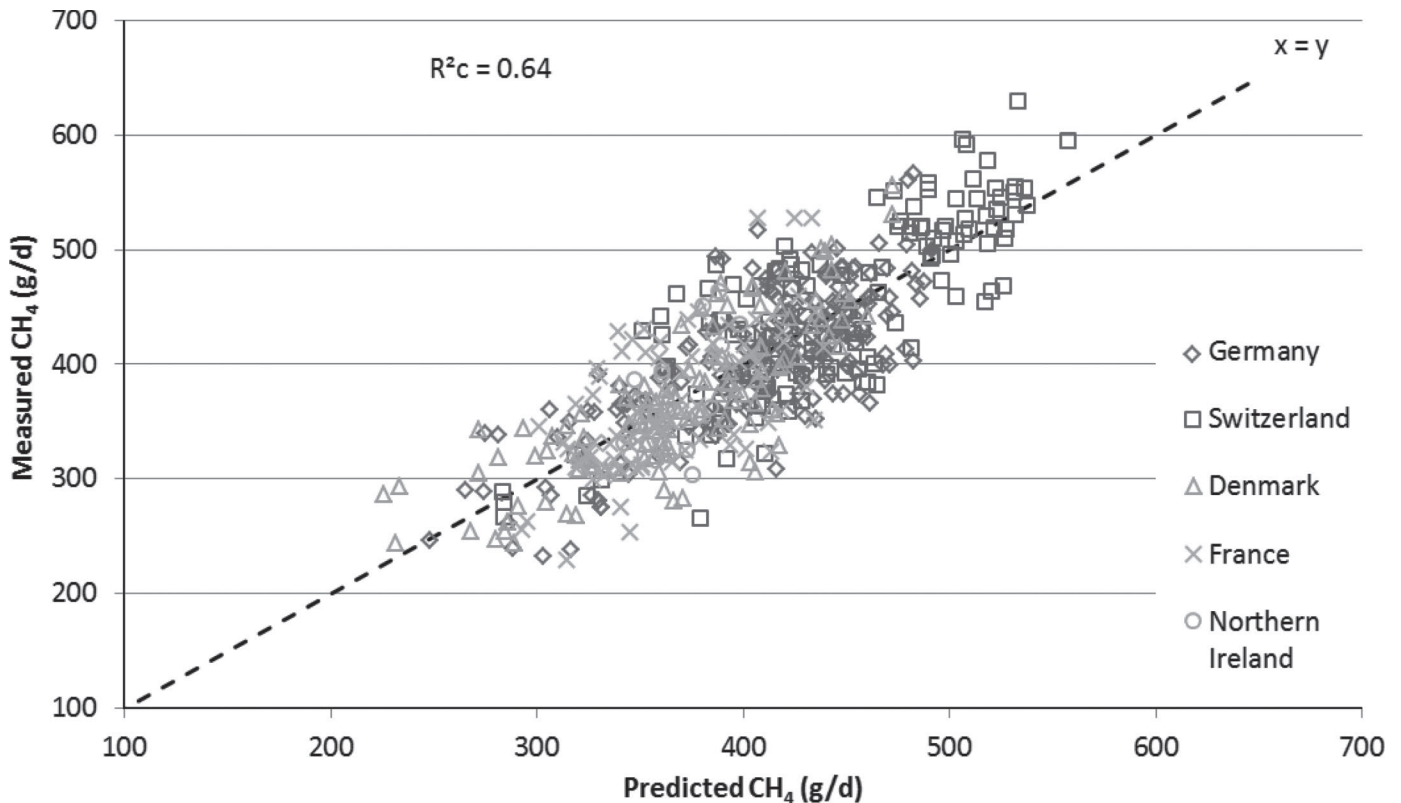


Figure 2. Relationship between predicted CH_4 emissions (g/d) of dairy cows determined from milk mid-infrared spectra and CH_4 emissions (g/d) of dairy cows measured in a respiration chamber. Data obtained from 584 daily CH_4 measurements and corresponding milk Fourier transform mid-infrared spectra recorded in 5 European countries. Color version available online.

set with RC. Noticeably, the standard deviations are systematically lower for the predictions than for the measurements. This could be a result of the modified PLS regression process, which tends to reduce the amplitude of prediction values compared with the amplitude of reference values. Indeed, this is intrinsic to the PLS regression. The lower the correlation, the more this phenomenon is accentuated.

The RMSEC of different countries ranged from 36 to 47 g/d of CH_4 , and the overall RMSEC of 42 g/d represented 10% of the overall mean CH_4 value. This is lower than the RMSEC observed for the SF_6 -based model (65 g/d, which represents 15% of the SF_6 -based data set mean of 430 g/d of CH_4).

Interestingly, from Figures 1 and 2, only few data have measured or predicted values lower than 300 g/d (low, $n = 40$) and almost only the Swiss data are >500 g/d (high, $n = 54$). Because these low and high CH_4 values are not often present and occur with less variability (animals, diets) than values between 350 and 450 g/d (mid, $n = 295$), where most of the reference data are observed (Figure 1), it can be assumed that predictions for values lower than 300 g/d of CH_4 or greater than 500 g/d of CH_4 are less accurate. Indeed,

the RMSEC were 51, 36, and 55 g/d of CH_4 for low, mid, and high values, respectively.

Concerning the CCDEV step, the averaged statistics after randomly removing 20% of the cows per country in a loop of 500 repetitions showed an R^2 of CCDEV varying between 0.08 and 0.69 (with a mean of 0.40) and an RMSEP of CCDEV varying between 44 and 106 g/d of CH_4 (with a mean of 64 g/d) depending on the cows removed. These results demonstrate that the ability of the model to predict the validation data varies greatly depending on whether the cows included in the calibration data set include the necessary information for a valid prediction equation. This indicates that the model is not very robust in its current state, which is to be expected considering that the available data set was not collected for this purpose, but when the required variability is included in the model it is able to estimate the CH_4 emission independently of the representation of a specific cow. The distribution of the RMSEP of CCDEV is detailed in Figure 3. It can be observed that in most cases RMSEP of CCDEV range between 55 and 75 g/d, meaning that they are higher than during the cross-validation process, which is expected because this validation is more stringent than the cross-validation.

Regarding the breed-specific test, the HO and BS models permitted us to obtain calibration R^2 of 0.61 and 0.78, RMSEC of 42 and 32 g/d of CH_4 , cross-validation R^2 of 0.48 and 0.46, R^2 of CBDEV of 0.33 (minimum–maximum: 0.09–0.59) and 0.31 (minimum–maximum: 0.01–0.74), and RMSEP of CBDEV of 70 (minimum–maximum: 46–125) and 72 (minimum–maximum: 44–118) g/d of CH_4 , respectively. This shows that the calibration statistics are similar or even better for the breed-specific models than for the model including all available breeds, but during the cross-validation and the CBDEV steps statistics are worse. This means that these breed-specific models are less robust and that, at least at this stage, the other breeds add value to the global model.

When the equation developed with all the RC data was applied to the Walloon milk recording spectral database, the average predicted CH_4 emissions were 444 ± 51 g/d for first-lactation ($n = 659,457$ milk samples from 105,349 dairy cows in 1,166 herds), 449 ± 51 g/d for second-lactation ($n = 506,606$ milk samples from 81,863 dairy cows in 1,159 herds), and 448 ± 50 g/d for third-lactation cows ($n = 349,074$ milk samples from 57,112 dairy cows in 1,145 herds). The standard deviation in CH_4 emissions was similar between parities. Even with the absence of Belgian data in the reference RC-based model, these predictions on the Belgian spectrum are consistent with regard to the expected range for lactating dairy cows and are included in the range of measured values of this study and the SF_6 version of the equation (Vanlierde et al., 2016). The RC-based model confirms that the equation gives logical results in practice provided that the conditions of application are considered.

Regarding the R^2 and the errors observed, the current potential of the RC-based equation permits at least distinguishing trends between animals (high or low daily CH_4 emissions) provided that the variability (e.g., breed, diet) of the predicted sample is covered by the reference data available. The best assumptions for the lower statistics observed by Shetty et al. (2017) could be attributed to the sniffer reference technique to measure CH_4 , the different structures of the tested population, the duration of measurements (not a 24-h measurement, in contrast to SF_6 or RC methods), and the time between CH_4 measurement and milk sampling, which differs significantly between both studies.

As identified in the literature (Davies and Fearn, 2006) and previously observed in the development process of the equation based on SF_6 measurements, the addition of new RC values with variability not yet included in the model (e.g., additional CH_4 measurements linked to corresponding milk FT-MIR spectra from more cows with different diets) is expected to

increase the accuracy and robustness of this equation. For the purpose of enlarging the variability covered by the equation, further research could combine RC and SF_6 data sets toward the development of a prediction model.

To conclude, an equation based on CH_4 measurements obtained in RC and the corresponding milk FT-MIR spectra has been developed. Even without an ideal composition of the reference data set collated from multiple research institutes, using different facilities, and during diet trials in which similar animals are often selected (factors that are not ideal), the final conclusions are comparable with previous findings with the SF_6 reference technique to measure CH_4 (Vanlierde et al., 2016). The lower R^2 values obtained with the RC rather than the SF_6 version of the equation can be attributed to the lower variability of the RC data set. This last point highlights the importance of collaborating in the development of more efficient calibration models by combining existing data sets and efforts to obtain a final data set as varied as possible. The study confirmed that milk FT-MIR spectra data are a relevant proxy to estimate individual daily CH_4 emissions on commercial farms from individual milk samples. Existing equations still need to be improved through the inclusion of additional reference data covering more of the naturally occurring variability in the present reference data set. However, this approach provides a method for carrying out large-scale studies on individual lactating cows to estimate daily CH_4 emissions and identifying mitigation options. Additionally, it would allow enteric CH_4 to be included in genetic selection strategies of dairy cattle along with other current phenotypes of interest (e.g., milk yield, health, fertility).

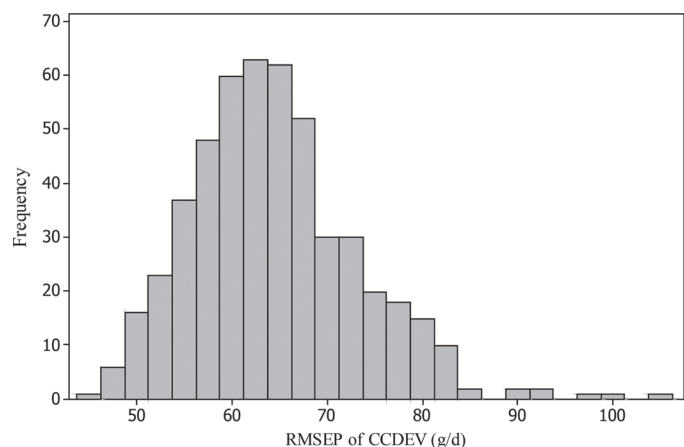


Figure 3. Distribution of the root mean squared error of prediction (RMSEP) during 500 repetitions of the cow- and country-dependent external validation (CCDEV).

ACKNOWLEDGMENTS

The authors are grateful to their fellow researchers with regard to collecting methane and milk samples. The OptiMIR (INTERREG IVB North-West Europe funded; www.optimir.eu) and the COST Methagene (COST-Horizon 2020 funded; www.methagene.eu) European projects are gratefully acknowledged for facilitating networking between research teams and data exchange. The authors thank the Walloon Breeding Association (Ciney, Belgium) and Comité du lait (Battice, Belgium) for providing access to data related to the Walloon milk recording. The Association of Swiss Cattle Breeders (Zollkofen, Switzerland) is acknowledged for providing the milk Fourier transform mid-infrared spectral data of the Swiss samples. Part of the work has been funded by the EU project GplusE (<http://www.gpluse.eu>), especially research conducted at the Agri-Food and Biosciences Institute (AFBI) in Northern Ireland, and facilitation of data exchanges. The GplusE project has received funding from the European Union's Seventh Framework Programme for research, technological development, and demonstration under grant agreement n° 613689. The views expressed in this publication are the sole responsibility of the authors and do not necessarily reflect the views of the European Commission. Research conducted at Leibniz Institute for Farm Animal Biology (FBN) in Germany was partially funded by the optiKuh project supported by the German Federal Ministry of Food and Agriculture (BMBL) through the Federal Office for Agriculture and Food (BLE). Research conducted in France at the French National Institute for Agricultural Research (INRA) was funded by the French National Research Agency (ANR) through the Joint Programming Initiative on Agriculture, Food Security and Climate Change (FACCE-JPI). Global Network program (ANR-13-JFAC-0003-01). Research conducted in Denmark at Aarhus University was funded by the Danish Milk Levy Fund and Aarhus University.

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