



## Combining short-term breath measurements to develop methane prediction equations from cow milk mid-infrared spectra



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### ABSTRACT

Predicting methane (**CH<sub>4</sub>**) emission from milk mid-infrared (**MIR**) spectra provides large amounts of data which is necessary for genomic selection. Recent prediction equations were developed using the GreenFeed system, which required averaging multiple CH<sub>4</sub> measurements to obtain an accurate estimate, resulting in large data loss when animals unfrequently visit the GreenFeed. This study aimed to determine if calibrating equations on CH<sub>4</sub> emissions corrected for diurnal variations or modeled throughout lactation would improve the accuracy of the predictions by reducing data loss compared with standard averaging methods used with GreenFeed data. The calibration dataset included 1 822 spectra from 235 cows (Holstein, Montbéliarde, and Abondance), and the validation dataset included 104 spectra from 46 (Holstein and Montbéliarde). The predictive ability of the equations calibrated on MIR spectra only was low to moderate ( $R_v^2 = 0.22\text{--}0.36$ , RMSE = 57–70 g/d). Equations using CH<sub>4</sub> averages that had been pre-corrected for diurnal variations tended to perform better, especially with respect to the error of prediction. Furthermore, pre-correcting CH<sub>4</sub> values allowed to use all the data available without requiring a minimum number of spot measures at the GreenFeed device for calculating averages. This study provides advice for developing new prediction equations, in addition to a new set of equations based on a large and diverse population.

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### Implications

Methane emissions from dairy cows can be predicted using milk mid-infrared spectra. Recently developed prediction equations are calibrated on short-term breath measurements, requiring the averaging of 20 measurements for accurate daily estimates per cow. This results in data loss or imprecision. We developed equations indicating that precorrecting the individual methane measurements for diurnal variation before averaging them over 2 weeks can limit data loss and improve predictive performance. Some of them will soon be applied to milk spectra routinely collected by the French milk recording companies to perform a genomic evaluation aimed at reducing methane emissions from French dairy cows.

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### Introduction

In the context of reducing greenhouse gas emissions, one major mitigation strategy in the agricultural sector is decreasing methane (**CH<sub>4</sub>**) emissions from cattle (Gerber et al., 2013). Several studies have demonstrated the possibility of direct genetic selection against CH<sub>4</sub> emissions (Pickering et al., 2015; Pryce and Haile-Mariam, 2020), which would complement improvements in cattle nutrition and management (Beauchemin et al., 2022). However, genetic selection against CH<sub>4</sub> emissions requires large-scale phenotyping for this trait, which is challenging in several ways.

The methods that are largely considered the gold standards for measuring individual CH<sub>4</sub> emissions from cattle – respiration chambers and sulfur hexafluoride tracer gas – are low throughput, costly, and labor-intensive, and require technical skills to ensure accurate measurements (Hammond et al., 2016; Tedeschi et al., 2022). New direct phenotyping tools have been developed to

estimate CH<sub>4</sub> emissions from breath samples taken during milking or feeding, such as the GreenFeed system (GF, C-Lock Inc., Rapid City, SD, USA) and the “sniffer methods” (Garnsworthy et al., 2019). They are medium throughput, but their large-scale deployment on commercial farms – as would be required for genetic purposes – is hindered by cost, technical management, and medium reliability (Hammond et al., 2016; Tedeschi et al., 2022). Consequently, proxies have been proposed to more easily generate the amount of data needed for genetic analysis of CH<sub>4</sub> emissions (Negussie et al., 2017; van Gastelen and Dijkstra, 2016). Based on initial research by Dehareng et al. (2012), there is growing interest in predicting CH<sub>4</sub> emissions from milk mid-infrared (MIR) spectra (Coppa et al., 2022; Denninger et al., 2020; McParland et al., 2023; Vanlierde et al., 2018) which are routinely collected and already stored in many practical settings.

Several equations have been developed to predict CH<sub>4</sub> emissions using reference measurements collected with gold-standard methods (Dehareng et al., 2012; Shetty et al., 2017; Vanlierde et al., 2015), but it is only recently that these equations have been calibrated using measurements collected with GF and Sniffer (Coppa et al., 2022; Liu et al., 2022) more suited to rapidly develop larger and more diverse calibration populations. However, those latter equations were calibrated using homogeneous calibration populations – either in terms of breeds, feeding systems, living conditions, or parity and lactation stage – and therefore may not be applicable to spectra collected from cows not matching the calibration criteria.

Because GF measurements are short (a few minutes), one limitation of using them to calibrate equations is their sensitivity to feeding-related diurnal variations in CH<sub>4</sub> emissions. As a result, a single record is a poor proxy for daily CH<sub>4</sub> emissions (Hristov et al., 2015). This means that multiple measurements must be associated with individual milk MIR spectra, and they are usually averaged (Coppa et al., 2022; Denninger et al., 2020; Liu et al., 2022), with the recommendation of a minimum of 20 measurements per individual (Manafiazar et al., 2016). However, if a cow rarely visits the GF, it can take a long time to reach 20 measurements, which can lead to unequal recording periods for cows with very different visit frequencies (Coppa et al., 2022) or to data loss when averaging over fixed time periods.

In this study, we developed equations to predict CH<sub>4</sub> emissions from dairy cows based on GF measurements and milk MIR spectra from a heterogeneous population. We included individuals from three different breeds – Holstein, Montbéliarde, and Abondance – living under different conditions, representing a total of five farms and nine diets. The idea is to determine the best equation to apply to the spectra routinely collected by the French milk recording in order to develop a genomic evaluation of CH<sub>4</sub> emissions. Therefore, a specific aim of this study was to determine if considering the diurnal variations of CH<sub>4</sub> emissions or modeling GF data throughout lactation with random regression models would improve the accuracy of the predictions by reducing data loss compared with the standard averaging method used with GF data.

## Material and methods

### Animals and diet

To establish the most robust prediction equations possible, the experiment was designed to maximize the variability of CH<sub>4</sub> emissions in the dataset. To this end, data were obtained from nine experiments carried out on five French experimental or commercial farms, which will be divided into two groups to facilitate comprehension. In the first group, data were collected over a period of 3–5 weeks between September 2019 and October 2021. The cows

were of three different breeds: 29 Holstein, 112 Montbéliarde, and 18 Abondance. The cows were milked twice a day in milking parlors (at 0700 and 1600 h), and their parity ranged from 1 to 7. In the second group, data were collected from 76 first- and second-parity Holstein cows throughout lactation from March 2021 to January 2022. Cows were milked using an automated milking system (Lely Astronaut A4, Lely Holding, Maassluis, the Netherlands). Overall, the diets in the experiments varied from high-forage to high-energy diets (Supplementary Table S1). No dietary oil or CH<sub>4</sub>-reducing additive was present in any of the diets.

### Spectra collection

For cows in the first group, spectra were recorded once per cow per week from samples composed of 50% evening milk and 50% morning milk. Cows of the second group had free access to an automatic milking system. Once a week, milk samples were collected at each visit over a 24-hour period. One spectrum was obtained from each milk sample using MIR spectroscopy. If multiple spectra were recorded for the same cow during this 24-hour period, they were averaged proportionally to the milk yield at each visit, to obtain one final spectrum per cow per week.

### Methane emission recording

Methane measurements were obtained using GF devices that were freely available to cows. In the experiments of the first group, cows were trained to visit the GF and staff ensured that they used it, while this was not the case in the second group. The GF devices were calibrated daily following the manufacturer's instructions. Low-energy pellets or concentrate were distributed to bait the cows and ensure the proper head position in the bin. Following Vanlierde et al. (2015), CH<sub>4</sub> records of less than 150 g/d and greater than 950 g/d were discarded (0.7% of the data), resulting in 41 194 individual CH<sub>4</sub> measurements.

### Methane variables to calibrate the prediction equations

Methane emissions recorded using the GF method are usually averaged over several days to obtain an accurate estimate of the true CH<sub>4</sub> emission of the animal. These averages are then associated with milk MIR spectra to develop prediction equations. In this study, we defined this approach as the reference. As these averages are biased by the combination of the diurnal variations of CH<sub>4</sub> emission (Hammond et al., 2016) and the frequency of visit to the GF, we proposed new approaches trying to overcome these weaknesses. First, we wanted to determine if correcting CH<sub>4</sub> emissions for diurnal variations before averaging would improve the predictive ability of the equations. Second, we wanted to determine if modeling CH<sub>4</sub> emission throughout lactation using all the CH<sub>4</sub> records would improve the quality of the reference CH<sub>4</sub> associated with the milk MIR spectra. Third, we wanted to determine if using at least 20 CH<sub>4</sub> measurements to calculate the average (Manafiazar et al., 2016) was still recommended with the two new approaches or if they allowed to estimate a CH<sub>4</sub> emission robust enough to use all the data available.

### Reference approach: methane emission averaged over 1 or 2 weeks

In the first approach, simple averages were calculated over either 7 days (1-week average) or over 13 ± 1 day(s) (2-week average). These averages were calculated from raw CH<sub>4</sub> measurements taken before the day that MIR spectra were recorded (another scenario was tested with averages centered on the day of the spectrum recording, but no difference in prediction performances was observed and this scenario is not presented).

### Methane emission precorrected for diurnal variations

Individual CH<sub>4</sub> measurements were affected by the time of visit to the GF. These variations were completely independent of the MIR spectra. To reduce this noise, the CH<sub>4</sub> measurements were adjusted for these diurnal variations estimated within each farm ( $y_{ijhf} - H_{hf}$ , referred to as precorrected throughout the remainder of this article) according to the following model:

$$y_{ijhf} = \mu + DIM_t + P_j + H_{hf} + \alpha_i + e_i \quad (1)$$

where  $y_{ijhf}$  is the measured raw CH<sub>4</sub>,  $\mu$  is the overall mean,  $DIM_t$  is the fixed effect of the week in milk,  $P_j$  is the fixed effect of parity (1, 2, or 3+),  $H_{hf}$  is the fixed effect of hour h on farm f,  $\alpha_i$  is the random animal effect, and  $e_i$  is the random residual effect term of  $y_{ijhf}$ , normally distributed.

### Methane emission modeled throughout lactation

Another approach consisted of modeling CH<sub>4</sub> emissions with random regression models using each visit to the GF. The base model was the following:

$$y_{il} = \mu + FIXED + \sum_{n=1}^2 \beta_{ln} \gamma_n(t) + \sum_{n=1}^2 \alpha_{in} \gamma_n(t) + e_i \quad (2)$$

where  $y_{il}$  is the CH<sub>4</sub> performance of animal i; *FIXED* includes the fixed effects of year × month of recording, year × season of calving, breed, and experiment;  $\beta_{ln}$  is the n<sup>th</sup> fixed regression coefficient on Days in Milk (**DIM**) within parity class l;  $\gamma_n$  is the n<sup>th</sup> coefficient of a Legendre polynomial evaluated at DIM t;  $\alpha_{in}$  is the n<sup>th</sup> random regression coefficient of animal i; and  $e_{ijkl}$  is the random residual term, normally distributed.

Using the Blupf90 suite of programs (Miszta et al., 2002), three models were developed based on Eq. (2): M1 used raw CH<sub>4</sub> as  $y_{il}$ ; M2 used pre-corrected CH<sub>4</sub> as  $y_{il}$ ; and M3 used raw CH<sub>4</sub> as  $y_{il}$  and included an additional fixed effect of the time of measurement within the farm. Predictions were obtained by subtracting the residuals (M1 and M2) or the residuals and the fixed effect of the time of measurement within the farm (M3) to the phenotype.

### Restriction to a minimum number of visits per week for considering the data

To maximize the reliability of GF data, it is generally recommended to combine at least 20 measurements per individual (Manafiazar et al., 2016). However, this threshold would have excluded most of the data for the 1-week calculation. We therefore adapted these restrictions as follows: a minimum of 14 measurements for 1-week CH<sub>4</sub> averages and 20 measurements for 2-week CH<sub>4</sub> averages. We then calibrated equations using averages com-

plying with this threshold (restricted models) or using all the averages available (unrestricted models).

In conclusion, prediction equations were developed from 14 reference CH<sub>4</sub> variables. These variables included raw averages over 1 or 2 weeks, precorrected averages over 1 or 2 weeks, and three methane variables modeled throughout the lactation, each of them either restricted or unrestricted. Table 1 presents the distribution of the 14 CH<sub>4</sub> variables.

### Spectral data treatment

To calibrate the equations, we used CH<sub>4</sub> measurements recorded between 5 and 305 DIM. To ensure that the cows would have at least 7 days of CH<sub>4</sub> measurement before each of their spectra, only spectra collected from the 11th DIM were considered to calibrate the equations on 1-week CH<sub>4</sub> averages. Similarly, only spectra collected from the 16th DIM were used to calibrate the equations on 2-week CH<sub>4</sub> averages.

Spectra were standardized according to the procedure described in Grelet et al. (2017) to remove instrument interference. Three spectral regions were considered for the calibration process (968–1 577 cm<sup>-1</sup>, 1 720–1 809 cm<sup>-1</sup>, and 2 561–2 966 cm<sup>-1</sup>), resulting in the selection of 289 data points, to which a first derivative was applied (Soyeurt et al., 2011). To make the prediction equation coefficients lactation stage dependent, each wavenumber of the spectra was multiplied by a constant, a linear, and a quadratic modified Legendre polynomial factor defined for 5–365 DIM (Gengler et al., 1999). This resulted in modified spectra counting 867 data points (289 data points for each constant, linear, and quadratic part), which were used to develop the prediction equations using partial least square regression (Winisi software, Foss, Hillerød, Denmark). A five-fold internal cross-validation procedure was performed to test the performance of the models. The calibration and cross-validation accuracy of the models was evaluated using values of R<sup>2</sup> and SE.

The standardized Mahalanobis distance was calculated to estimate spectral homogeneity. This value was calculated between each spectrum and the mean of all spectra used for calibration and was used to exclude outlier spectra.

### Validation dataset

The validation dataset contained 104 spectra and the corresponding 1-week and 2-week CH<sub>4</sub> averages obtained from 46 cows, originating from three experiments. From this dataset, 64 spectra were taken under the same conditions as the second group, from 22 primiparous Holstein cows between February and September 2022. The other 40 spectra were from 24 cows (4 Hol-

**Table 1**  
Distribution of the reference methane (CH<sub>4</sub>) variables (g/day) measured from dairy cows used to calibrate the equations.

| Reference CH <sub>4</sub> variables <sup>1</sup> | Unrestricted <sup>2</sup> |      |    |     |     | Restricted <sup>2</sup> |      |    |     |     |
|--|---------------------------|------|----|-----|-----|-------------------------|------|----|-----|-----|
|  | n                         | Mean | SD | Min | Max | n                       | Mean | SD | Min | Max |
| Raw CH <sub>4</sub>                              |                           |      |    |     |     |                         |      |    |     |     |
| 1-week   | 1 822                     | 427  | 78 | 208 | 671 | 1 035                   | 438  | 79 | 216 | 640 |
| M1   | 1 822                     | 412  | 67 | 211 | 637 | 1 035                   | 421  | 71 | 217 | 637 |
| M3   | 1 822                     | 429  | 69 | 223 | 645 | 1 035                   | 439  | 74 | 233 | 645 |
| 2-week   | 897                       | 426  | 73 | 219 | 626 | 694                     | 432  | 76 | 219 | 626 |
| Precorrected CH <sub>4</sub>                     |                           |      |    |     |     |                         |      |    |     |     |
| 1-week   | 1 822                     | 410  | 74 | 200 | 649 | 1 035                   | 420  | 76 | 205 | 629 |
| M2   | 1 822                     | 361  | 64 | 164 | 553 | 1 035                   | 367  | 67 | 171 | 553 |
| 2-week   | 897                       | 409  | 71 | 208 | 616 | 694                     | 414  | 73 | 208 | 616 |

<sup>1</sup> Methane measurements were used raw or pre-corrected for a farm-specific hour effect, then were either combined by averaging them over one or 2 weeks or modeled across lactation using random regression models (M1 = raw CH<sub>4</sub>; M2 = pre-corrected CH<sub>4</sub>; and M3 = raw CH<sub>4</sub> + fixed effect of the time of measurement within the farm).

<sup>2</sup> Unrestricted models were calibrated using all data, whereas at least 14 or 20 measurements were required to calibrate 1-week or 2-week restricted models, respectively.

**Table 2**

Descriptive statistics of methane emissions in g/day of dairy cows for calibration and validation sets. These data were used to compute and analyze raw methane averaged over one week for individuals with a minimum of 14 measurements.

| Type of model          | n      | Mean | SD  | Min | Max |
|------------------------|--------|------|-----|-----|-----|
| Calibration population | 26 069 | 440  | 123 | 150 | 943 |
| Validation population  | 1 257  | 399  | 110 | 150 | 775 |

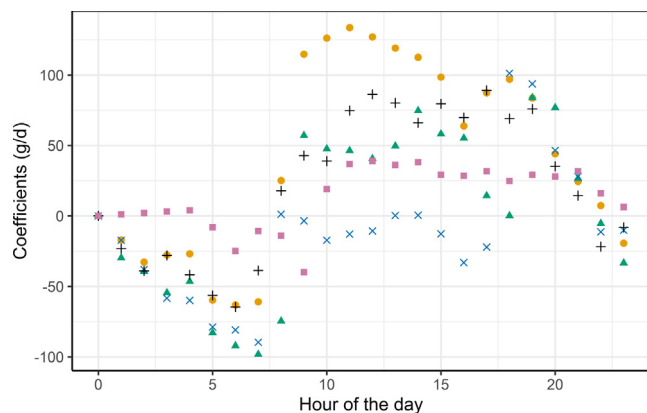
stein and 20 Montbéliarde) in two different experiments from two farms of the first group. There were no animals in common between the calibration set and the validation set; furthermore, the two sets were completely separated in time, with no overlap. We retained spectra between 16 and 305 DIM, CH<sub>4</sub> measurements between 150 and 950 g/d, and CH<sub>4</sub> averages calculated from at least 20 individual visits. The same treatment was applied to the validation spectra as for calibration spectra (selection of spectral regions, application of a first derivative, and integration of DIM information). We kept one spectrum every 2nd week to avoid overlapping CH<sub>4</sub> averages. Calculations of the standardized Mahalanobis distance between each validation spectrum and the spectra used for calibration did not reveal any outlier. The validation accuracy of the models was evaluated using values of R<sup>2</sup> and RMSE. Validation performances were nearly identical for both 1-week or 2-week CH<sub>4</sub> averages, so only the results from 2-week CH<sub>4</sub> averages are presented.

**Results**

*Variability of spectra and methane emissions*

Across all calibration experiments, the average value of raw CH<sub>4</sub> emissions per visit was 440 g/d (from the 26 069 visits used to compute restricted 1-week CH<sub>4</sub> averages), with a SD of 123 g/d (Table 2). The number of visits varied depending on how CH<sub>4</sub> was calculated (e.g., 1-week or 2-week averages, restricted or not), but the mean and SD were similar for all types of CH<sub>4</sub> variables. When CH<sub>4</sub> measurements were precorrected for farm-specific diurnal variations (Fig. 1), the average amount of CH<sub>4</sub> emitted per visit was 428 g/d (from the 26 069 visits used to compute restricted 1-week CH<sub>4</sub> averages), with a SD of 114 g/d. Again, these values were similar for all CH<sub>4</sub> variables.

The frequency of visits to the GF units varied from 2.1 to 4.5 per cow per day depending on the experiment; there were large differences between the first group and the second group, which had average (SD) visit frequencies of 4.0 (1.4) and 2.1 (1.0), respectively. Because of the low visit frequency in the second group,

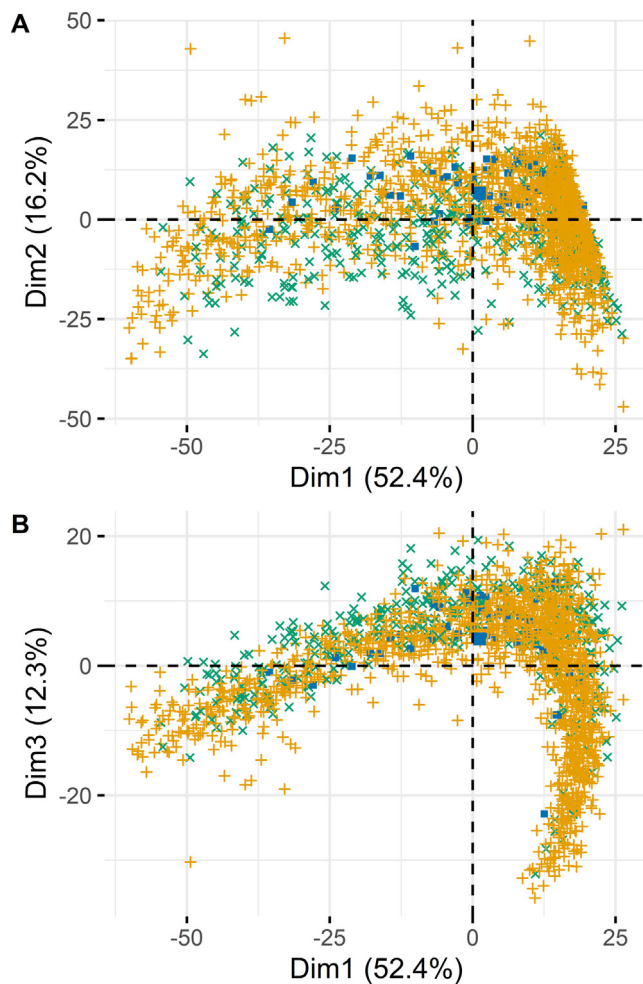


**Fig. 1.** Farm-specific effect of time of day on methane emissions of dairy cows. Each type of point and color represents a farm.

imposing restrictions based on the number of visits in a given period (i.e., excluding individuals who did not meet the minimum number of visits) removed a considerable amount of data (58.8 and 34.6% for 1-week and 2-week CH<sub>4</sub> averages, respectively). The impact of the restriction was less notable for the first group, in which the percentage of data removed varied from 0 to 33.3% and from 0 to 25% for the 1-week and 2-week CH<sub>4</sub> averages, respectively.

Analysis of Mahalanobis distances revealed that the spectra were homogeneously distributed around the centroid, with Montbéliarde spectra at the periphery (Fig. 2). To minimize data loss, we computed 2-week CH<sub>4</sub> averages over 13 ± 1 days instead of a strict 14-day period; otherwise, 75% of the data would have been removed due to the experimental designs.

For the validation set, the average CH<sub>4</sub> emissions per visit were 399 g/d, with a SD of 110 g/d. Descriptive statistics of CH<sub>4</sub> emissions are shown in Table 2. The analysis of the standardized Maha-



**Fig. 2.** Principal component analysis of the 1 822 milk mid-infrared spectra. (A) First and second dimensions, and (B) first and third dimensions, representing data from Holstein (▲), Montbéliarde (■), and Abondance (●) cows.

lanobis distance revealed that validation spectra were homogeneous with the calibration spectra.

#### Prediction of methane emissions from mid-infrared spectra

The calibration and validation performances of the 14 predictive models developed for the different CH<sub>4</sub> variables are shown in Table 3. The equations had R<sub>cv</sub><sup>2</sup> values ranging from 0.30 to 0.45 and SE<sub>cv</sub> varying from 53 to 63 g/d. In calibration, restricted models performed better than unrestricted models for all CH<sub>4</sub> variables, and pre-correction of CH<sub>4</sub> measurements did not improve the performance of the prediction equations. Generally, equations based on modeled values of CH<sub>4</sub> performed better than those based on averaged CH<sub>4</sub> with respect to both R<sub>cv</sub><sup>2</sup> and SE<sub>cv</sub>, with M1 performing better than M2 and M3.

For all validation models, R<sub>v</sub><sup>2</sup> ranged from 0.22 to 0.37 and RMSE ranged from 57 to 70 g/d. Regardless of how CH<sub>4</sub> was calculated, restricted models performed worse in terms of R<sup>2</sup> and RMSE than unrestricted models, except for raw 2-week CH<sub>4</sub> averages and pre-corrected 1-week averages. Pre-correction of CH<sub>4</sub> measures generally improved the performance of the prediction equations, except for the equation that used modeled values of CH<sub>4</sub> (M1, M2, and M3), for which pre-correction decreased R<sub>v</sub><sup>2</sup> while greatly improving RMSE. Unrestricted pre-corrected equations performed better than restricted raw equations when CH<sub>4</sub> measurements were averaged over 1 or 2 weeks.

#### Discussion

The objective of this study was to determine whether pre-correction of individual CH<sub>4</sub> measurements for diurnal variations or modeling CH<sub>4</sub> emissions throughout the lactation would allow to improve the performance of the prediction equations. This approach would avoid the high data loss resulting from the use of averages calculated from a minimum number of individual measurements (Manafiazar et al., 2016).

Methane emissions measured punctually by the GF system are sensitive to the diurnal variation due to an animal's feeding pattern (Hammond et al., 2016). Indeed, they are highest after a meal and decrease with time. In addition, a large measurement error is expected due to the limited recording time – a few minutes – of each measurement. To obtain a reliable estimation of daily CH<sub>4</sub> emission, a common recommendation is to combine 20 punctual measurements (Manafiazar et al., 2016), usually by averaging them. This follows the hypothesis that errors are independent between measurements and that the error variance is divided by 20. In addition, these 20 visits are better distributed throughout

the day and thus capture diurnal variations. We did observe that restricting a minimum number of visits to average CH<sub>4</sub> emissions leads to improve the predictive ability of the equation calibrated on 2-week CH<sub>4</sub> values averages. However, applying this restriction (minimum 20 visits to average CH<sub>4</sub> over 2 weeks) resulted in a large amount of data (22.6%) being excluded from the calibration dataset. To preserve as much data and variability as possible, we investigated other methods of combining GF data. First, we confirmed that using twice as many spectra by averaging CH<sub>4</sub> measurements averaged over 1 week – and restricting to at least 14 visits to compute the average – was not efficient, as we observed an increased prediction error of this equation compared to the equation calibrated on restricted 2-week averages. This could be related to both overfitting by using spectra and CH<sub>4</sub> averages recorded at short intervals, or due to the loss of accuracy of the reference CH<sub>4</sub> value for 1-week averages as the repeatability of GF measurements increases over 2 weeks (Arbre et al., 2016; Denninger et al., 2019).

A second proposal to increase the amount of data used to calibrate the equations was to model CH<sub>4</sub> emissions throughout the lactation using random regression models. Despite better R<sub>cv</sub><sup>2</sup> and SE<sub>cv</sub>, the equation calibrated on modeled raw CH<sub>4</sub> emissions had a higher prediction error in validation than the restricted 2-week averages equation. Modeling may be smoothing out too much of the variation in CH<sub>4</sub> relative to the spectra, which were not adjusted for the animal effect.

A third proposal was to account for the diurnal variations of CH<sub>4</sub> emissions, either by using pre-corrected individual measurements (averaged or modeled) or by including a farm-specific hour effect in the random regression model (M3). All three models showed improved performance in validation compared to the raw 2-week average models. In this study, using all available data was the best solution when using pre-corrected data, as the unrestricted pre-corrected 2-week averages equation was the best model. We hypothesized that by pre-correcting the individual CH<sub>4</sub> measurements for the farm-specific diurnal variations, that are directly related to the feeding pattern and not to the emissions of the animal itself (Garnsworthy et al., 2012; Hammond et al., 2016), we removed a significant proportion of the uninformative variability that required the restriction to at least 20 measurements for averaging over 2 weeks. Moreover, by using a spectrum every 2nd week, we limit the bias associated with overfitting the model – by using one spectrum per week that does not bring entirely new information – which can be observed by the best performance of the M1, M2, and M3 models in calibration but not in validation.

The 14 equations built from the different CH<sub>4</sub> variables had values of R<sub>v</sub><sup>2</sup> ranging from 0.22 to 0.36 and RMSE ranging from 57 to

**Table 3**  
Calibration (cv) and validation (v) statistics of the prediction equations for the different methane (CH<sub>4</sub>) variables (SE<sub>cv</sub> and RMSE in g/day) in dairy cows.

| Methane variables <sup>1</sup> | Unrestricted <sup>2</sup> |                              |                  |            |                             |      | Restricted <sup>2</sup> |                              |                  |            |                             |      |
|--------------------------------|---------------------------|------------------------------|------------------|------------|-----------------------------|------|-------------------------|------------------------------|------------------|------------|-----------------------------|------|
|                                | Calibration               |                              |                  | Validation |                             |      | Calibration             |                              |                  | Validation |                             |      |
|                                | n                         | R <sub>cv</sub> <sup>2</sup> | SE <sub>cv</sub> | n          | R <sub>v</sub> <sup>2</sup> | RMSE | n                       | R <sub>cv</sub> <sup>2</sup> | SE <sub>cv</sub> | n          | R <sub>v</sub> <sup>2</sup> | RMSE |
| Raw CH <sub>4</sub>            |                           |                              |                  |            |                             |      |                         |                              |                  |            |                             |      |
| 1-week                         | 1 822                     | 0.35                         | 63               | 104        | 0.36                        | 68   | 1 035                   | 0.41                         | 61               | 104        | 0.25                        | 69   |
| M1                             | 1 822                     | 0.40                         | 53               | 104        | 0.37                        | 68   | 1 035                   | 0.45                         | 55               | 104        | 0.28                        | 70   |
| M3                             | 1 822                     | 0.31                         | 53               | 104        | 0.27                        | 63   | 1 035                   | 0.33                         | 55               | 104        | 0.22                        | 66   |
| 2-week                         | 897                       | 0.34                         | 60               | 104        | 0.24                        | 69   | 694                     | 0.38                         | 60               | 104        | 0.28                        | 63   |
| Pre-corrected CH <sub>4</sub>  |                           |                              |                  |            |                             |      |                         |                              |                  |            |                             |      |
| 1-week                         | 1 822                     | 0.32                         | 61               | 104        | 0.36                        | 69   | 1 035                   | 0.37                         | 60               | 104        | 0.26                        | 61   |
| M2                             | 1 822                     | 0.38                         | 53               | 104        | 0.31                        | 59   | 1 035                   | 0.40                         | 55               | 104        | 0.26                        | 61   |
| 2-week                         | 897                       | 0.30                         | 59               | 104        | 0.36                        | 57   | 694                     | 0.38                         | 57               | 104        | 0.28                        | 61   |

<sup>1</sup> Methane measurements were used raw or pre-corrected for a farm-specific hour effect, then were either combined by averaging them over 1 or 2 weeks or modeled across lactation using random regression models (M1 = raw CH<sub>4</sub>; M2 = pre-corrected CH<sub>4</sub>; and M3 = raw CH<sub>4</sub> + fixed effect of the time of measurement within the farm).

<sup>2</sup> Unrestricted models were calibrated using all data, whereas at least 14 or 20 measurements were required to calibrate 1-week or 2-week restricted models, respectively.

70 g/d. The  $R^2$  of our equations was lower (maximum of 0.36) than the value of 0.46 reported by Coppa et al. (2022), working with averages of 20 GF measurements, and the value of 0.47 reported by Shadpour et al. (2022) working with weekly averages. It was also lower than the value of 0.66 reported by McParland et al. (2023) for weekly  $\text{CH}_4$  averages associated with an average of AM and PM milk spectra. On the contrary, our equations performed better than the one of Liu et al. (2022) ( $R^2 = 0.17$ ) who did not include DIM in their equation, which has been shown to improve the predictive ability of equations (Coppa et al., 2022; Vanlierde et al., 2015). However, our average prediction error (64.6 g/d) is among the lowest in the literature for comparable equations (Coppa et al., 2022; Liu et al., 2022; and Shadpour et al., 2022). In particular, the restricted raw 2-week model had a RMSE of 63 g/d, lower than the prediction errors of 66.2 and 70 g/d for averages of at least 20 measurements reported by Coppa et al. (2022) and Shadpour et al. (2022). Although McParland et al. (2023) reported the lowest prediction error (41 g/d), they calibrated their equation on less variable  $\text{CH}_4$  measurements, with an SD of daily  $\text{CH}_4$  averages of 51 g/d while the SD ranged from 74 to 91 g/d for daily or weekly averages in the other studies, including ours (Coppa et al., 2022; Liu et al., 2022; Shadpour et al., 2022). The unrestricted precorrected 2-week model performed even better, with a prediction error of 57 g/d. The restricted raw 1-week model had an RMSE of 69 g/d, lower than the value of 73.7 g/d reported by Shadpour et al. (2022) for weekly averages. These results seem to argue for more diversity in the calibration population. This would allow the identification of a more general relationship between  $\text{CH}_4$  emissions and MIR spectra and lead to a more robust prediction equation when applied to new data. Vanlierde et al. (2021) also found that the prediction equations were more robust when calibrated on a diverse population using MIR spectra and  $\text{CH}_4$  measurements from respiration chambers and sulfur hexafluoride tracer gas.

To obtain accurate predictions when prediction equations are applied to new spectra, the calibration population should be as diverse as possible. Although we would have liked our calibration population to be larger and more diverse for our ambitious goal, it was among the largest and most heterogeneous ones yet assembled. Our calibration population covered a large range of variability of the diets used on the French dairy farms but would benefit from including pasture and fresh herbage diets, as  $\text{CH}_4$  emissions are known to differ between grazing and non-grazing animals (Brito et al., 2022; O'Neill et al., 2010). Our calibration population also included several breeds, first because breed-specific equations poorly predict methane emissions from other breeds – as we found in this study when we tried to predict the  $\text{CH}_4$  emissions of Montbéliarde and Abondance cows using an equation calibrated only on Holstein data (results not shown) – and second because a multibreed equation is more efficient and cost-effective in the objective of implementing a  $\text{CH}_4$  genomic selection for multiple breeds. Even if our equations have a lower  $R^2$  than the other equations reported in the literature (Coppa et al., 2022; Liu et al., 2022; McParland et al., 2023; Shadpour et al., 2022), they have some of the lowest prediction error. Nevertheless, a phenotype predicted with a low accuracy is acceptable for genetic evaluation, as the quantity of phenotypes (millions, in routine application in French commercial farms) will dilute the imprecision of each individual prediction and result in a highly accurate prediction of breeding values.

## Conclusion

The work conducted in this study was intended to shed light on the best way to combine  $\text{CH}_4$  emissions measured using GF for use in calibrating prediction equations. We found that precorrecting

individual GF measurements before averaging them over 2 weeks compensated for the need for restriction to at least 20 measurements. The equations could be improved by further increasing the diversity of the calibration population, but the large-scale phenotyping required to establish an optimal calibration population is time-consuming and expensive. In the short term, global projects involving multiple research centers and their partners may be the most efficient solution for collecting the data needed to develop more accurate and robust prediction equations. Meanwhile, considering the growing interest to mitigate  $\text{CH}_4$  emissions, the equations developed in this study will soon be applied to the milk MIR spectra collected in France to provide a large population required to implement a genomic selection of  $\text{CH}_4$  emissions.

## Supplementary material

Supplementary material to this article can be found online at <https://doi.org/10.1016/j.animal.2024.101200>.

## Ethics approval

All experiments were carried out in accordance with the guidelines on animal research issued by the French Ministry of Agriculture (<https://www.legifrance.gouv.fr/eli/decret/2013/2/1/2013-118/jo/texte>).

## Data and model availability statement

The data were generated within a public-private project and cannot be made publicly available immediately. Data can be obtained from the authors upon reasonable request.

## Declaration of Generative AI and AI-assisted technologies in the writing process

During the preparation of this work the author(s) did not use any AI and AI-assisted technologies.

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## Declaration of interest

None.

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