Direct and indirect predictions of enteric methane daily production, yield, and intensity per unit of milk and cheese, from fatty acids and milk Fourier-transform infrared spectra

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ABSTRACT

Mitigating the dairy chain's contribution to climate change requires cheap, rapid methods of predicting enteric CH₄ emissions (EME) of dairy cows in the field. Such methods may also be useful for genetically improving cows to reduce EME. Our objective was to evaluate different procedures for predicting EME traits from infrared spectra of milk samples taken at routine milk recording of cows. As a reference method, we used EME traits estimated from published equations developed from a meta-analysis of data from respiration chambers through analysis of various fatty acids in milk fat by gas chromatography (FA_{GC}). We analyzed individual milk samples of 1,150 Brown Swiss cows from 85 farms operating different dairy systems (from very traditional to modern), and obtained the cheese yields of individual model cheeses from these samples. We also obtained Fourier-transform infrared absorbance spectra on 1,060 wavelengths (5,000 to 930 waves/cm) from the same samples. Five reference enteric CH₄ traits were calculated: CH₄ yield (CH₄/DMI, g/kg) per unit of dry matter intake (DMI), and CH₄ intensity (CH₄/CM, g/kg) per unit of corrected milk (CM) from the FA_{GC} profiles; CH₄ intensity per unit of fresh cheese (CH₄/CY_{CURD}, g/kg) and cheese solids (CH₄/CY_{SOLIDS}, g/kg) from individual cheese yields (CY); and daily CH₄ production (dCH₄, g/d). Direct infrared (IR) calibrations were obtained by BayesB modeling; the determination coefficients of cross-validation varied from 0.36 for dCH₄ to 0.57 for CH₄/CM, and were similar to the coefficient of determination values of the equations based on FA_{GC} used as the reference method (0.47 for CH₄/DMI and 0.54 for CH₄/CM). The models allowed us to select the most informative wavelengths for each EME trait and to infer the milk chemical features underlying the predictions. Aside from the 5 direct infrared

prediction calibrations, we tested another 8 indirect prediction models. Using IR-predicted informative fatty acids (FA_{IR}) instead of FA_{GC}, we were able to obtain indirect predictions with about the same precision (correlation with reference values) as direct IR predictions of CH₄/DMI (0.78 vs. 0.76, respectively) and CH₄/CM (0.82 vs. 0.83). The indirect EME predictions based on IR-predicted CY were less precise than the direct IR predictions of both CH₄/CY_{CURD} (0.67 vs. 0.81) and CH₄/CY_{SOLIDS} (0.62 vs. 0.78). Four indirect dCH₄ predictions were obtained by multiplying the measured or IR-predicted daily CM production by the direct or indirect CH₄/CM. Combining recorded daily CM and predicted CH₄/CM greatly increased precision over direct dCH₄ predictions (0.96–0.96 vs. 0.68). The estimates obtained from the majority of direct and indirect IR-based prediction models exhibited herd and individual cow variability and effects of the main sources of variation (dairy system, parity, days in milk) similar to the reference data. Some rapid, cheap, direct and indirect IR prediction models appear to be useful for monitoring EME in the field and possibly for genetic/genomic selection, but future studies directly measuring CH₄ with different breeds and dairy systems are needed to validate our findings.

Key words: mid-infrared, near-infrared, ecological footprint, greenhouse gas, global warming

INTRODUCTION

Simple, noninvasive, cheap methods are needed that may be used with individual dairy cows at the population level to monitor herds for enteric CH₄ emissions (EME) and possibly to reduce them through genetic improvement of dairy cattle. Some studies have shown that the phenotypic variability of EME has a genetic basis (Pryce and Bell, 2017), but a major problem in genetically evaluating animals lies in the fact that the gold standard for accurate, precise EME quantification (i.e., the respiration chamber) is an expensive tool that can be used only in an artificial environment and on few animals, which is a problem in a genetic approach,

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and is available only in some research centers (de Haas et al., 2017).

Simpler, direct methods involve taking samples of the air near the cows' nostrils and mouths, and from these estimating the CH_4 eructated from the rumen and, in part, that emitted from the intestine through the lungs, which represents about 6 to 14% of total EME (Immig, 1996). Theoretically more precise and accurate methods involve the continuous release of a gas tracer in the rumen at a known rate, which is used as a reference for calculating CH_4 emission (Beauchemin et al., 2012), often using instruments mounted on the back of the cows. These methods are, in any case, expensive and involve ethical/animal welfare issues. The most practical methods, easy to use at the farm level, involve placing sampling instruments in the automatic milking systems or the automatic concentrate feeders (de Haas et al., 2017). These methods can sample the air at every visit of the cow (i.e., several times per day), but it is not clear to what extent the CH_4 emitted during milking or feeding is representative of the complete daily balance. Lastly, detectors that can function at a distance (laser, infrared) can be used during different phases of the cows' daily routine, but technicians are needed to operate them (Chagunda and Yan, 2011). The methods that do not use rumen tracers rely on measurement of the ratio between CH_4 and CO_2 in the air sample to predict daily CH_4 release on the basis of theoretical CO_2 emission predicted from one or more cow's traits (BW, milk yield, DMI, and so on; Madsen et al., 2010). These methods are less accurate and precise than respiration chambers, as the various EME patterns according to diet differ from the gold standard (Hammond et al., 2016) and from each other (Hristov et al., 2016), so their use is controversial (Wu et al., 2018).

Among the indirect methods based on genetic correlations with other traits, the favorable relation between EME and feed efficiency is very interesting (Basarab et al., 2013). However, the problem here is how to measure the feed DMI of individual animals at the population level. Interest is also growing in other indirect predictions of EME traits, as reviewed by Negussie et al. (2017). Among the more promising methods, studies have shown that the fatty acid (FA) profile of milk reflects microbial activity in the rumen, and therefore, EME, as reviewed by van Gastelen and Dijkstra (2016). Here, too, the gold standard for FA analysis [i.e., gas chromatography (FA_{GC})], despite being much simpler and cheaper than EME analyzed by respiration chambers, cannot be easily used in the context of routine milk recording schemes.

Infrared (IR) spectrometry has been widely used for predicting FA (FA_{IR}) in milk (Rutten et al., 2009;

Soyeurt et al., 2011; Ferrand-Calmels et al., 2014) and meat (Cecchinato et al., 2012), with interesting results. However, the major limitation is that, despite improvements in prediction methods (Eskildsen et al., 2014; Fleming et al., 2017), IR spectrometry yields good results for those milk FA with the highest concentrations, whereas some of the most interesting FA for predicting EME are those derived from microbial activity in the rumen, which are found in low concentrations (van Gastelen and Dijkstra, 2016). An alternative is to use IR spectrometry for direct prediction of EME traits from milk spectra, which is the most attractive option as IR spectra are routinely obtained from almost all individual milk samples processed within milk recording schemes and from bulk farm samples taken for milk quality pricing systems. In a preliminary study carried out on a very small number of cows, Dehareng et al. (2012) obtained promising results using Fourier-transform infrared (FTIR) spectra to predict the daily CH_4 production (dCH₄, g/d) of individual cows, and CH_4 intensity (CH_4/CM , g/kg) expressed per unit of fat- and protein-corrected milk (CM) produced. Later, the same research group published promising results from a study of a larger number of cows (Vanlierde et al., 2015, 2016; Vanrobays et al., 2016), although Shetty et al. (2017), using similar approaches, obtained unsatisfactory results.

As various EME traits have been analyzed in different studies (van Gastelen and Dijkstra, 2016; de Haas et al., 2017), but have not been compared in large surveys, in a recent study we used the method of van Lingen et al. (2014) based on selected FA_{GC} to estimate CH_4/CM and CH_4 yield per unit of DMI (CH_4/DMI), and to study the effect of dairy system, herd within dairy system, and parity and lactation stage of dairy cows (Bittante et al., 2018). In addition, we calculated dCH₄ by multiplying daily CM yield (dCMY, kg/d) by CH_4/CM , and as no study has previously reported this, we also estimated CH_4 intensity per kilogram of fresh cheese ($\text{CH}_4/\text{CY}_{\text{CURD}}$, g/kg) and cheese solids ($\text{CH}_4/\text{CY}_{\text{SOLIDS}}$, g/kg) using the cheese yield (CY) obtained from model cheeses made from milk samples from every individual cow.

Following previous research, the objective of the present study was to compare 5 reference EME traits, estimated on the basis of FA_{GC} and individual CY, with the same EME traits predicted directly by IR spectrometry from milk spectra, or indirectly calculated on the basis of other IR-predicted traits (FA_{IR}, CY, dCMY). The EME traits studied were CH_4 production (g/d), CH_4 intensity (g/kg) per unit of milk, fresh cheese and cheese solids, and CH_4 yield (g/kg) expressed per unit of DMI.

MATERIALS AND METHODS

Farms, Animals, and Samples

We sampled 85 herds in Trento Province (north-eastern Italian Alps) registered in the milk-recording program of the Provincial Breeders' Federation (FPA, Trento, Italy). The herds were selected from 610 farms to represent different environments and 4 dairy farming systems (Sturaro et al., 2013), ranging from very traditional (small farms, tied cows, hay-based diets) to intermediate (loose cattle, milking parlors, hay, and more concentrates), and modern (large farms using total mixed diets), with or without the use of silage. Details of the dairy systems and their effects on the reference EME traits are given in the previous study within the same project (Bittante et al., 2018).

Over a period of 1 yr, a total of 1,150 Brown Swiss cows from the 85 herds (15 cows per herd, with a few exceptions), at different parities and lactation stages, were sampled once (each herd on a different date) during the morning milking. Two milk subsamples per cow, representative of the whole milking, were taken and immediately refrigerated (4°C) without preservative. One subsample (50 mL) was used to analyze milk quality traits, whereas the other (2,000 mL) was taken to the Cheese-Making Laboratory of the Department of Agronomy, Food, Natural Resources, Animals and Environment of the University of Padova (Legnaro, Padova, Italy) for individual model cheese making.

The milk was analyzed for fat and protein content according to the IR procedure approved by ICAR (2017) (see the section "Development of Direct and Indirect FTIR Calibration Models"). To correct the milk yield for milk fat and protein contents, in accordance with van Lingen et al. (2014), a correcting multiplicative factor (**CF**) was calculated as follows (CVB, 2008):

$$\text{CF} = 0.337 + 0.116 \times \text{milk fat (\%)} \\ + 0.06 \times \text{milk protein (\%)}.$$

The value (kg/d) of each cow's dCMY was calculated on the basis of its daily milk yield (**dMY**) on the day of sampling as follows:

$$\text{dCMY (kg/d)} = \text{dMY (kg/d)} \times \text{CF}.$$

Individual Model Cheese Making

The percentage fresh cheese yield (**%CY_{CURD}**) of individual cows was obtained through the model cheese-making procedure described in detail by Cipolat-Gotet et al. (2013), and was expressed as

$$\% \text{CY}_{\text{CURD}} = \frac{\text{weight of wheel, g}}{\text{weight of milk, g}} \times 100.$$

Similarly, the percentage cheese solids yield (**%CY_{SOLIDS}**) was calculated as the ratio between the percentage DM multiplied by the weight of the fresh wheel (in the numerator) and the weight of the milk (in the denominator).

Each cow's daily production (kg/d) of fresh cheese (**dCY_{CURD}**) and cheese solids (**dCY_{SOLIDS}**) was calculated by multiplying the traits concerned (**%CY_{CURD}** or **%CY_{SOLIDS}**) by dMY:

$$\text{dCY}_{\text{CURD}} = \text{dMY} \times \% \text{CY}_{\text{CURD}} / 100,$$

$$\text{dCY}_{\text{SOLIDS}} = \text{dMY} \times \% \text{CY}_{\text{SOLIDS}} / 100.$$

Milk FA Profiling

Detailed FA profiles of the milk samples were obtained according to the method described in detail by Mele et al. (2016). Briefly, FAME were prepared by the direct extraction and alkali-catalyzed trans-methylation procedure, described by Feng et al. (2004). The FA composition was determined using a ThermoQuest gas chromatograph (Thermo Electron Corp., Waltham, MA) in split mode, fitted with a flame-ionization detector and a high polar fused-silica capillary column (Chrompack CP-Sil88, Varian, Middelburg, the Netherlands; 100 m, 0.25 mm i.d., film thickness 0.20 µm). Inter- and intraassay coefficients of variation and correction factors for the short-chain FA (Mele et al., 2008) were also calculated using a reference standard butter (BCR 164, Commission of the European Communities, Community Bureau of Reference, Brussels, Belgium) with the analytical limit of detection set at 0.001% above that of the total amount of FA. Milk FA composition was expressed as grams per 100 g of total FA. In conformity with the equations of van Lingen et al. (2014), we examined the following FA: 4:0 (butyric acid); 16:0 *iso* (iso-palmitic acid); 18:1 *trans*-10 (iso-oleic acid); 18:1 *trans*-11 (vaccenic acid); 18:1 *cis*-9 (oleic acid); and 18:2 *cis*-9,*cis*-12 (linoleic acid).

Definition of Reference Enteric Methane Emission Traits

In the previous study (Bittante et al., 2018), we used 2 prediction equations for estimating CH₄ yield (g/kg of DMI) and CH₄ intensity (g/kg of CM) from milk FA_{GC}. These equations were developed by van Lingen et al. (2014) from a meta-analysis of 148 observations from 8 trials carried out with dairy cows on 30 differ-

ent diets in respiration chambers in the Netherlands and the United Kingdom [coefficient of determination (R^2) = 0.54 and 0.47, for CH_4 yield and CH_4 intensity, respectively]. From these 2 equations, and from measurements of daily milk yield and composition and of individual %CY (fresh cheese and cheese solids), we derived the other EME traits as described in the aforementioned study (Bittante et al., 2018).

In accordance with the definitions of de Haas et al. (2017), we examined the following 5 traits as reference data for subsequent development of the IR predictions of EME traits:

- Methane yield: EME (g) per kg of DMI ($\text{CH}_4/\text{DMI}_{\text{FAGC}}$) was predicted according to the equation of van Lingen et al. (2014):

$$\begin{aligned} \text{CH}_4/\text{DMI}_{\text{FAGC}} \text{ (g/kg)} &= 23.39 + 9.74 \\ &\times \text{C16:0 } iso - 1.06 \times \text{C18:1 } trans-10 \\ &+ trans-11 - 1.75 \times \text{C18:2 } cis-9, cis-12, \end{aligned}$$

where all FA are obtained from CG and expressed as % of the sum of all milk FA.

- Methane intensity-milk: EME (g) per kg of CM ($\text{CH}_4/\text{CM}_{\text{FAGC}}$) was predicted according to the equation of van Lingen et al. (2014):

$$\begin{aligned} \text{CH}_4/\text{CM}_{\text{FAGC}} \text{ (g/kg)} &= 21.13 - 1.38 \times \text{C4:0} \\ &+ 8.53 \times \text{C16:0 } iso - 0.22 \times \text{C18:1 } cis-9 - 0.59 \\ &\times \text{C18:1 } trans-10 + trans-11. \end{aligned}$$

- Methane production: daily EME (g) per cow (dCH_4) was calculated as

$$\text{dCH}_4 \text{ (g/d)} = \text{CH}_4/\text{CM}_{\text{FAGC}} \times \text{dCMY}.$$

- Methane intensity-cheese: EME (g) per kg of fresh cheese ($\text{CH}_4/\text{CY}_{\text{CURD}}$) was calculated as

$$\text{CH}_4/\text{CY}_{\text{CURD}} \text{ (g/kg)} = \text{dCH}_4/\text{dCY}_{\text{CURD}}.$$

- Methane intensity-cheese solids: EME (g) per kg of cheese solids ($\text{CH}_4/\text{CY}_{\text{SOLIDS}}$) was calculated as

$$\text{CH}_4/\text{CY}_{\text{SOLIDS}} \text{ (g/kg)} = \text{dCH}_4/\text{dCY}_{\text{SOLIDS}}.$$

Infrared Spectra Acquisition and Editing

Within 20 h of milking, the full FTIR spectrum of absorbance of each sample was obtained from an FTIR spectrometer (MilkoScan FT 6000, Foss Electric,

Hillerød, Denmark). In total, 1,060 absorbance values were recorded for each milk sample covering the IR wavenumbers ranging from 5,000/cm (corresponding to a wavelength of 2.0 μm) in the near-IR subdivision of the IR area, through the mid-infrared (MIR) to wavenumber 930/cm (corresponding to a wavelength of 10.8 μm) in the far infrared subdivision.

Prior to data analysis, each single wavenumber of the spectra was centered and standardized to a null mean and a unit sample variance (Ferragina et al., 2015). Mahalanobis distances were calculated by means of the Mahalanobis function in the R software (R Core Team, 2015, R Foundation for Statistical Computing, Vienna, Austria), the inverse of the spectral covariance matrix, and the “center” statement as a vector of 0. Spectra with a distance greater than the mean ± 3 standard deviations were classified as outliers (16 spectra). The spectra were not subjected to any other mathematical pretreatment. Preliminary analyses using the first and second derivatives of the absorbance values showed no relevant advantage in using these data rather than raw data without mathematical pretreatments (-0.03 to $+0.06$ of R^2), which could be attributed to the Bayesian approach used for calibration.

Calibration of EME Traits Using FTIR Spectra

Separate models were fitted for all the EME traits and for the traits used for estimating them.

We used a Bayesian model, specifically the BayesB model, implemented in the BGLR package of the R software (de los Campos and Perez Rodriguez, 2015). Details of the procedure are described in Ferragina et al. (2015). Basically, phenotypes were regressed on standardized spectra covariates using the linear model:

$$y_i = \beta_0 + \sum_{j=1}^{1,060} x_{ij} \beta_j + \varepsilon_i,$$

where y_i is the measured phenotype of the i th sample, β_0 is an intercept, $\{x_{ij}\}$ are standardized FTIR wavelength data ($j = 1, \dots, 1,060$), β_j is the effect of each wavelength, and ε_i are the model residuals assumed to be *iid* (independent and identically distributed) with normal distribution centered at zero with variance σ_ε^2 . Given the above assumption, the conditional distribution of the data, given the effects and variance parameters, is

$$P(\mathbf{y}|\boldsymbol{\theta}) = \prod_{i=1}^n N(\mu_i, \sigma_\varepsilon^2),$$

where $\mathbf{y} = \{y_{ij}\}$, $\boldsymbol{\theta}$ represents the collection of model parameters $\boldsymbol{\theta} = \{\beta_0, \beta, \sigma_\varepsilon^2\}$, $N(\mu_i, \sigma_\varepsilon^2)$ is a normal distribution centered at μ_i (mean) $= \beta_0 + \sum_{j=1}^{1,060} x_{ij}\beta_j$ and with variance σ_ε^2 , and $\beta = \{\beta_j\}$ is a vector containing the effects of the individual spectra-derived wavelengths. Specification of the Bayesian model is completed by assigning prior distribution to the unknowns, $\boldsymbol{\theta}$. The default values of the built-in BGLR rules were used for all the model's hyperparameters, and the inferences were based on 30,000 iterations and a burn-in of 10,000.

Assessment of Prediction Accuracy Through Cross-Validation

The accuracy of the prediction equations was assessed by a training-testing procedure. A training data set (80% of the total records) was used to build the equation, and a testing data set (20% of the total) was used as validation. Samples were randomly assigned to the training or testing sets, and the training-testing procedure was repeated 10 times for each trait with different training and testing set samples each time. This procedure was used for all the traits studied. In each of the 10 training-testing trials of the prediction procedure for each trait, the observed and predicted values of the testing data set were used to calculate the coefficient of determination of cross-validation (R^2_{CV}) and the root mean square error of cross-validation ($RMSE_{CV}$). The coefficient of determination of calibration (R^2_{CAL}) was also calculated using the measured and predicted values of each training data set. The final R^2_{CAL} , R^2_{CV} , and $RMSE_{CV}$ for each trait are averages of the 10 training-testing trials.

To identify the wavelengths relevant for predicting a given trait, the 1,060 equation coefficients corresponding to each wavelength for the considered trait were plotted together. The corresponding Pearson correlation values relative to the absorbance of each sample for every wavelength and the corresponding measured value of the trait of interest were also added to the figure. To facilitate description of the results throughout the spectral range, we divided the spectrum into 5 regions (Bittante and Cecchinato, 2013): the short-wave infrared (**SWIR**, often called near-infrared); the transition from near- to mid-infrared (**SWIR-MWIR**), often referred to as the first “water” region; the first mid-infrared region (**MWIR1**); the second mid-infrared region (**MWIR2**), often referred to as the second “water” region, and the transition from mid- to long-infrared (**MWIR-LWIR**).

Development of Direct and Indirect FTIR Calibration Models

The above-described Bayesian approach was used for direct (**direct-IR**) prediction of the 5 EME traits. “Direct” refers to use of the 5 reference EME data (descriptive statistics are listed in Table 1) as dependent variables regressed against the 1,060 absorbance values of each FTIR spectrum. The direct-IR predicted EME traits were as follows:

- predicted CH_4 yield (**CH₄/DMI_{IR}**, g/kg);
- predicted CH_4 intensity-milk (**CH₄/CM_{IR}**, g/kg);
- predicted CH_4 intensity-fresh cheese (**CH₄/CY_{CURD-IR}**, g/kg);
- predicted CH_4 intensity-cheese solids (**CH₄/CY_{SOLID-IR}**, g/kg); and
- predicted daily CH_4 production (**dCH_{4-IR}**, g/d).

The same direct-IR model was also used to predict a further 15 informative traits (listed in Table 1) from the milk FTIR spectrum, which were previously used to calculate the reference EME traits (5 production traits, 4 cheese yield traits, and 6 milk FA proportions). The reference milk fat and protein contents shown in Table 1 were obtained using the same FTIR spectrometer and calibration equations pre-installed by the supplier company according to ICAR official methods (ICAR, 2017). Given that we used the same FTIR spectra, our fat and protein calibrations are then “calibrations of calibrations,” and have the sole objective of testing the ability of our Bayesian approach to capture all the variability in the same spectra generated by the unknown calibration procedure used by the instrument supplier. The results are very satisfactory, with R^2_{CAL} and R^2_{CV} both greater than 0.99 (Table 1).

These direct-IR predictions of the informative traits were used to develop the following 8 indirect (**indirect-IR**) procedures for predicting the same 5 EME traits:

- **CH₄/DMI_{FAIR}**, using the same equations of van Lingen et al. (2014) for calculating the reference data, but with FA_{IR} instead of FA_{GC};
- **CH₄/CM_{FAIR}**, again using equations of van Lingen et al. (2014), but with FA_{IR} instead of FA_{GC};
- **CH₄/CY_{CURD-IR-IR}**, using the same procedure as for the reference values, but substituting the reference CH_4/CM_{FAGC} with its IR prediction (CH_4/CM_{IR}) and the measured %CY_{CURD} with its IR prediction;
- **CH₄/CY_{SOLIDS-IR-IR}**, using the same procedure as for the reference values, but substituting the reference CH_4/CM_{FAGC} with its IR prediction

(CH₄/CM_{IR}) and the measured %CY_{SOLIDS} with its IR prediction;

- **dCH₄-CM-IR**, by multiplying the measured corrected milk yield (dCMY) by the direct-IR CH₄ intensity-milk (CH₄/CM_{IR});
- **dCH₄-CM-FAIR**, by multiplying the dCMY by the indirect-IR CH₄ intensity-milk based on FA_{IR} (CH₄/CM_{FAIR});
- **dCH₄-IR-IR**, by multiplying the IR predicted daily corrected milk yield (dCM_{IR}) by the CH₄/CM_{IR};
- **dCH₄-IR-FAIR**, by multiplying the dCM_{IR} by the CH₄/CM_{FAIR}.

Analysis of the Sources of Variation in the Reference and Predicted EME Traits

The parameters often used for evaluating IR predictions (R^2_{CV} , RMSE_{CV}, and so on) are available only for direct-IR predictions of EME and other informative traits. To evaluate the indirect-IR EME predictions (but also the direct-IR EME predictions), we carried

out another type of validation that involved analyzing their sources of variation and comparing them with those of the reference EME values. The objective was to assess whether the IR predictions are capable of capturing the relevant technological and biological information in the reference values. In particular, we analyzed the variability among the different herds and different animals, and in the effects of the dairy systems on the former, and of parity and lactation stage on the latter in both the reference and predicted traits. The fixed effects were assessed in terms of least squares means and the significance of the contrasts, and the random effects in terms of variances and their incidence on total variance. To do this, we adopted the same mixed linear model used in the previous study for analyzing the reference EME values (Bittante et al., 2018) to analyze the direct-IR and indirect-IR predictions:

$$y_{ijklm} = \mu + \text{dairy system}_i + \text{herd} / \text{date}_j (\text{dairy system})_i + \text{parity}_k + \text{DIM}_l + e_{ijklm},$$

Table 1. Descriptive statistics and Fourier transform infrared milk spectra calibrations of enteric CH₄ emissions traits and of the traits used for their estimation (N = 1,150 cows from 85 herds)¹

Trait	Mean	SD	R ² _{CAL}	R ² _{CV}	RMSE _{CV}
Estimated CH ₄ emissions					
CH ₄ /DMI (g/kg)	21.3	1.6	0.64	0.49	1.18
CH ₄ /CM (g/kg)	14.2	1.8	0.70	0.57	1.17
dCH ₄ (g/d)	357	108	0.48	0.36	86.0
CH ₄ /CY _{CURD} (g/kg)	100	16	0.71	0.55	11.2
CH ₄ /CY _{SOLIDS} (g/kg)	208	31	0.64	0.47	22.6
Milk production trait ²					
Milk yield (kg/d)	24.5	7.9	0.57	0.44	5.9
Milk fat (%)	4.22	0.69	0.999	0.994	0.07
Milk protein (%)	3.71	0.43	0.999	0.995	0.03
Correcting factor	1.05	0.09	1.000	0.997	0.01
dCMY ² (kg/d)	25.5	8.1	0.55	0.41	6.2
Cheese yield trait ³					
%CY _{CURD} (%)	15.05	1.90	0.81	0.72	1.02
%CY _{SOLIDS} (%)	7.22	0.94	0.83	0.79	0.43
dCY _{CURD} (kg/d)	3.66	1.17	0.50	0.36	0.93
dCY _{SOLIDS} (kg/d)	1.76	0.57	0.55	0.41	0.43
Informative milk FA _{GC} (%ΣFA _{GC}) ⁴					
4:0, butyric acid	3.45	0.90	0.59	0.47	0.66
16:0 <i>iso</i> , iso-palmitic acid	0.32	0.09	0.40	0.30	0.08
18:1 <i>trans</i> -10, iso-oleic acid	0.29	0.09	0.56	0.42	0.07
18:1 <i>trans</i> -11, vaccenic acid	1.20	0.37	0.60	0.43	0.28
18:1 <i>cis</i> -9, oleic acid	18.30	3.15	0.79	0.67	1.85
18:2 <i>cis</i> -9, <i>cis</i> -12, linoleic acid	2.05	0.60	0.64	0.49	0.44

¹R²_{CAL} = coefficient of determination of calibration; R²_{CV} = coefficient of determination of cross-validation; RMSE_{CV} = root mean square error of cross-validation; CH₄/DMI = CH₄ yield per kilogram of DMI; CH₄/CM = CH₄ intensity of milk; dCH₄ = daily CH₄ production per cow; CH₄/CY_{CURD} (CH₄/CY_{SOLIDS}) = CH₄ intensity of fresh cheese (or cheese solids).

²dCMY = daily fat- and protein-corrected milk yield.

³%CY_{CURD} = weight of fresh cheese as % of processed milk; %CY_{SOLIDS} = weight of cheese solids as % of processed milk; dCY_{CURD} = daily production of fresh cheese per cow; dCY_{SOLIDS} = daily production of cheese solids.

⁴Fatty acids (%ΣFA_{GC}, expressed as a percentage of gas total fatty acids in milk obtained by GC) included as independent variables in the equations used to estimate the enteric CH₄ emissions (van Lingen et al., 2014).

where y_{ijklm} is the observed trait; μ is the overall mean; *dairy system*_{*i*} is the fixed effect of the *i*th dairy system (*i* = 1 to 4); *herd/date*_{*j*}(*dairy system*)_{*i*} is the random effect of the *j*th herd/date (*j* = 1 to 85) within the *i*th dairy system; *parity*_{*k*} is the fixed effect of the *k*th parity (*k* = 1 to 5 for 1 to 5 or more lactations); *DIM*_{*l*} is the *l*th 60-d class of DIM (*l* = 1 to 6 DIM classes); and e_{ijklm} is the residual random error term $\sim N(0, \sigma_e^2)$. The significance of dairy system was tested on the error line of herd within dairy system, that of parity and DIM class on the error line of the residual variance.

Orthogonal post hoc contrasts were built to test the significance ($P < 0.05$) of dairy system and parity factors: (1) the “traditional” dairy system was compared with the “modern” systems; (2) within the modern systems, the “no TMR” herds were compared with the “TMR” herds; (3) within the TMR herds, those using silage were compared with those using water. First, second, third, and fourth parities were each compared with greater parities (e.g., 1st parity vs. 2nd–5th parities, 2nd parity vs. 3rd–5th parities, and so on). In addition, linear, quadratic, and cubic contrasts were tested for the effect of DIM (Bittante et al., 2018).

RESULTS

Descriptive Statistics and FTIR Calibrations

Descriptive statistics and direct-IR calibration parameters of the EME traits and of the traits used to calculate them (milk production, cheese yield, and informative milk FA) are given in Table 1.

The reference (mean $\pm$ SD) CH₄ yield (CH₄/DMI) was 21.3 ± 1.6 g/kg, and CH₄ intensity-corrected milk (CH₄/CM) 14.2 ± 1.8 g/kg. We also predicted EME per cow per day (dCH₄, 357 ± 108 g/d), CH₄ intensity-fresh cheese (CH₄/CY_{CURD}: 99.8 ± 16.3 g/kg), and CH₄ intensity-cheese solids (CH₄/CY_{SOLIDS}: 207.6 ± 30.8 g/kg).

The R^2_{CAL} and R^2_{CV} of the direct-IR predictions of CH₄/DMI were, respectively, 0.64 and 0.49. Those of CH₄/CM were slightly more accurate (0.70 and 0.57), similar to those of CH₄/CY_{CURD} (0.71 and 0.55) and slightly better than those of CH₄/CY_{SOLIDS} (0.64 and 0.47). These calibration and cross-validation parameters are in the range of those obtained for the direct-IR predictions of the informative FA used for calculating the reference values (R^2_{CAL} : 0.40 to 0.79; R^2_{CV} : 0.30 to 0.67; Table 1).

Direct-IR prediction of dCH₄ was less accurate (R^2_{CAL} 0.48, R^2_{VAL} 0.36) than direct-IR prediction of the other EME traits. These calibration and cross-validation parameters are only slightly lower than those obtained

for the other “daily quantity” traits (dMY, dCMY, dCY_{CURD}, and dCY_{SOLIDS}).

The coefficients of each wavenumber for every direct-IR predicted EME trait are plotted in Figures 1 and 2, together with the Pearson correlations between each absorbance and EME trait. To facilitate interpretation of the results, the 5 most informative wavenumbers of each prediction equation of the EME traits (those with the highest coefficients in absolute value) are given in Table 2, which also shows the major wavenumbers of the other informative traits in the same area of the spectrum obtained in this or in previous studies. We can see that the near-IR (SWIR) region of the FTIR spectrum (5,000 to 3,673 waves/cm) is not important for predicting EME traits, as only one of the 25 important signals included in Table 2 was found there (3,978 /cm) and was related to predicting CH₄/CM. No important signals were observed in either of the 2 “water absorption” regions of the FTIR spectrum (SWIR-MWIR 3,669 to 3,052, and MWIR2 1,698 to 1,586 waves/cm). The large majority of important signals are concentrated in the central part of the MIR (MWIR1) section (3,048 to 1,701/cm), where we found 3 signals of dCH₄, 3 of CH₄/DMI, and 8 of CH₄ intensities, and in the transition area from MIR to far infrared wavenumbers (MWIR/LWIR, 1,582 to 930/cm), where we found 2 signals of dCH₄, 2 of CH₄/DMI, and 6 of CH₄ intensities.

Sources of Variation in the Reference and in the Directly or Indirectly IR-Predicted EME Traits

Table 3 summarizes the results of the regressions of the predicted (direct-IR and indirect-IR) CH₄ yield and CH₄ intensity-milk traits on the corresponding reference traits, and also shows the results of the statistical analyses (effects of dairy system, herd within dairy system, parity, and DIM) of the reference and predicted (direct-IR and indirect-IR) EME traits.

The linear regressions of the direct-IR predicted EME traits on the corresponding reference traits (derived from FA_{GC}) yielded intercepts that did not differ significantly from the expected 0.00, slopes that did not differ from the expected 1.00, and correlation coefficients of 0.76 for CH₄/DMI_{IR} and 0.83 for CH₄/CM_{IR}. The ANOVA of the 2 direct-IR predictions was substantially equivalent to that of the corresponding reference data, both having very similar levels of significance and least squares means for all the factors included in the model, as well as similar incidences of herd variance on total variance. The only appreciable difference is that both direct-IR-predicted EME traits have a lower

RMSE than the corresponding reference data, probably because milk spectra have greater repeatability. The 2 indirect-IR predictions ($\text{CH}_4/\text{DMI}_{\text{FAIR}}$ and $\text{CH}_4/\text{CM}_{\text{FAIR}}$) based on IR predictions of informative FA and the equations of van Lingen et al. (2014) were also substantially equivalent to the direct-IR predictions in terms of the regression on the reference data and sources of variation.

Table 4 displays the same information as Table 3 (reference data, direct-IR, and indirect-IR predictions),

but for $\text{CH}_4/\text{CY}_{\text{CURD}}$ and $\text{CH}_4/\text{CY}_{\text{SOLIDS}}$. Here, too, the intercepts and slopes of the linear regressions of the direct-IR predictions did not differ from theoretical expectations, and the correlation coefficients were equivalent to or lower than those for $\text{CH}_4/\text{CM}_{\text{IR}}$. The least squares means and the levels of significance of the sources of variation obtained by an ANOVA of both direct-IR predictions differed slightly from those obtained from the reference data. The lower residual variability of the direct-IR predictions was not, in this

Figure 1. Direct-infrared prediction equation coefficients obtained using the variable selection Bayesian (BayesB) method (solid line) and simple correlation (CORR) coefficients (dashed line) for the absorbance of each wavenumber of the milk Fourier transform infrared spectrum for the prediction of CH_4 yield (CH_4/DMI , g/kg) and CH_4 intensity-corrected milk (CH_4/CM , g/kg). SWIR = short-wave infrared (i.e., near-infrared); MWIR = mid-wave infrared (i.e., mid-infrared), where MWIR1 is the first mid-infrared region and MWIR2 is the second mid-infrared region; and LWIR = long-wave infrared (i.e., far-infrared). Color version available online.

Figure 2. Direct-infrared prediction equation coefficients obtained using the variable selection Bayesian (BayesB) method (solid line) and simple correlation (CORR) coefficients (dashed line) for the absorbance of each wavenumber of the milk Fourier transform infrared spectrum for the prediction of daily CH_4 production per cow (dCH_4 , g/d) and of CH_4 intensity per unit of fresh cheese ($\text{CH}_4/\text{CY}_{\text{CURD}}$, g/kg) and cheese solids ($\text{CH}_4/\text{CY}_{\text{SOLIDS}}$, g/kg). SWIR = short-wave infrared (i.e., near-infrared), where MWIR1 is the first mid-infrared region and MWIR2 is the second mid-infrared region; MWIR = mid-wave infrared (i.e., mid-infrared); and LWIR = long-wave infrared (i.e., far-infrared). Color version available online.

case, accompanied by a parallel decrease in herd variability, so that the incidence of this variance on total variance was greater.

Unlike CH_4 yield and CH_4 intensity-milk, shown in Table 3, the indirect-IR predictions of CH_4 intensity-fresh cheese ($\text{CH}_4/\text{CY}_{\text{CURD-IR-IR}}$) and CH_4 intensity-cheese solids ($\text{CH}_4/\text{CY}_{\text{SOLIDS-IR-IR}}$) were not based on FA_{IR} , but on the IR predictions of CH_4 production ($\text{dCH}_{4\text{-IR}}$) divided by the IR predictions of daily cheese production ($\text{dCY}_{\text{CURD-IR}}$ and $\text{dCY}_{\text{SOLIDS-IR}}$). The results in both cases are much less satisfactory than the results of the direct-IR predictions of the 2 EME traits. In fact, the intercepts and slopes of the linear regressions on the reference data differed significantly from the expected 0.00 and 1.00, respectively, and the correlation coefficients were lower (0.67 and 0.62 vs. 0.81 and 0.78, respectively). Moreover, the least squares means estimations and levels of significance obtained by an ANOVA of these 2 indirect-IR predictions differed

from the reference and direct-IR predicted EME traits. Lastly, the residual variability was in both these cases higher than the reference and the direct-IR predicted traits, but herd variability was not, so its incidence on total variance was lower.

In the case of dCH_4 (g/d) per cow, aside from the reference trait, there are 5 alternative IR predictions (Table 5): the direct-IR and the 4 indirect-IR predicted traits. Two of the latter were obtained by multiplying the measured dCMY (kg/d) by the $\text{CH}_4/\text{CM}_{\text{IR}}$ or by the $\text{CH}_4/\text{CM}_{\text{FAIR}}$ predicted CH_4 intensity, whereas the other 2 were obtained in the same way, but using the IR predicted dCM (dCM_{IR} , kg/d) instead of the measured value.

The intercept and slope of the regression of the direct-IR predicted $\text{dCH}_{4\text{-IR}}$ with the reference values did not differ from expectations, although the correlation coefficient was not very high (0.68). The ANOVA of direct-IR CH_4 production resulted in lower residual

Table 2. Most important electromagnetic waves for predicting enteric CH_4 emissions (EME) and other milk traits¹

Major BayesB calibration coefficient				Other trait	
Wavenumber (N/cm)	Wavelength (nm)	Coefficient/SD	EME trait	Present study	Other studies
3,978	2.51	−1.55	CH_4/CM	—	—
2,982	3.35	−0.51	dCH_4	$\text{dCY}_{\text{SOLIDS}}$, 16:0 <i>iso</i> , 18:1 <i>cis</i> -9	RCT , ² k_{20} , ² CF_P , ² $\text{CH}_4\cdot\text{CO}_2$, ³ dCH_4 ³
2,893	3.46	−1.10	dCH_4	—	—
2,832	3.53	−1.74	CH_4/CM	Fat, $\text{dCY}_{\text{SOLIDS}}$, 18:1 <i>cis</i> -9, 18:2 <i>cis</i> -9, <i>cis</i> -12	Fat B ⁴
2,828	3.54	−1.85	CH_4/DMI	—	—
2,824	3.54	−1.20	CH_4/DMI	—	—
2,812	3.56	+0.96	CH_4/SO	—	—
2,639	3.79	−0.85	CH_4/SO	dCY_{CURD}	REC_{FAT} ⁵
2,539	3.94	−1.48	CH_4/CU	—	$\%\text{CY}_{\text{CURD}}$ ⁵
2,442	4.09	−0.55	dCH_4	16:0 <i>iso</i>	—
1,798	5.56	+1.69	CH_4/CU	—	—
1,771	5.65	−2.10	CH_4/CU	$\%\text{CY}_{\text{SOLIDS}}$, 16:0 <i>iso</i>	Fat A, ⁴ REC_{PROT} ⁵
1,771	5.65	−1.48	CH_4/DMI	—	—
1,767	5.66	−1.74	CH_4/SO	—	—
1,701	5.88	−1.39	CH_4/CM	—	—
1,493	6.70	−0.82	dCH_4	Protein dCY_{CURD}	Protein, ⁴ dCH_4 ³
1,489	6.72	−0.56	dCH_4	—	—
1,447	6.91	−3.03	CH_4/CM	$\%\text{CY}_{\text{SOLIDS}}$	$\%\text{CY}_{\text{CURD}}$ ⁵
1,443	6.93	−1.23	CH_4/SO	dCY_{CURD}	REC_{PROT} ⁵
1,443	6.93	−4.29	CH_4/CU	—	—
1,439	6.95	−2–10	CH_4/SO	—	—
1,335	7.49	+1.36	CH_4/CU	—	RCT ⁵
1,223	8.18	−1.34	CH_4/DMI	18:2 <i>cis</i> -9, <i>cis</i> -12	Lactose ⁴
1,019	9.82	−1.74	CH_4/CM	4:0	$\text{CH}_4\cdot\text{CO}_2$, ³ dCH_4 ³
968	10.33	−4.79	CH_4/DMI	18:1 <i>trans</i> -10, 18:1 <i>trans</i> -11, 18:2 <i>cis</i> -9, <i>cis</i> -12	REC_{PROT} ⁵

¹BayesB = Bayesian selection method; $\text{dCY}_{\text{SOLIDS}}$ = daily production of cheese solids; dCY_{CURD} = daily production of fresh cheese per cow; $\%\text{CY}_{\text{CURD}}$ = percentage fresh cheese yield; $\%\text{CY}_{\text{SOLIDS}}$ = percentage cheese solids yield; dCH_4 = daily CH_4 production per cow; CM = corrected milk; SO = $\%\text{CY}_{\text{SOLIDS}}$; CU = $\%\text{CY}_{\text{CURD}}$; RCT = rennet coagulation time, k_{20} = curd firming time, CF_P = potential curd firmness, REC_{FAT} = recovery of milk fat in cheese, REC_{PROT} = recovery of milk protein in cheese.

²BayesB coefficients according to Ferragina et al. (2017).

³Recursive partial least squares variable importance in projection scores according to Shetty et al. (2017).

⁴Wavenumber of filters used in monochromatic infrared according to Lynch et al. (2006) and Kaylegian et al. (2009).

⁵BayesB coefficients according to Ferragina et al. (2015).

Table 3. Linear regression and ANOVA for CH₄ yield (CH₄/DMI, g/kg) and CH₄ intensity (CH₄/CM, g/kg) estimated on the basis of milk fatty acids analyzed by GC (reference, _{FAGC}), directly predicted by milk infrared spectra (direct-IR), or indirectly estimated on the basis of fatty acids predicted by infrared (indirect IR, _{FAIR})

Item	Methane yield (g/kg)			Methane intensity-milk (g/kg)		
	Reference CH ₄ /DMI _{FAGC}	Direct-IR CH ₄ /DMI _{IR}	Indirect-IR CH ₄ /DMI _{FAIR}	Reference CH ₄ /CM _{FAGC}	Direct-IR CH ₄ /CM _{IR}	Indirect-IR CH ₄ /CM _{FAIR}
Regression on reference						
Correlation (r)	—	0.76	0.78	—	0.83	0.82
Intercept ¹ (a)	—	0.03 ^{NS}	−0.9 ^{NS}	—	−0.1 ^{NS}	0.3 ^{NS}
Slope ² (b)	—	1.00 ^{NS}	1.04 ^{NS}	—	1.01 ^{NS}	0.98 ^{NS}
ANOVA						
Dairy system ³ (LSM)						
Traditional	22.2***	22.0***	22.0***	14.4	14.4	14.4
Modern no TMR	21.2	21.2	21.2	14.2	14.2	14.2
Modern TMR _W	20.0***	20.4**	20.5**	13.2***	13.5***	13.5***
Modern TMR _S	21.9	21.5	21.7	15.0	14.9	15.0
Herd/date effect ⁴	0.61	0.61	0.63	0.51	0.52	0.56
Parity ⁵ (LSM)						
1st	21.3	21.3	21.2**	14.0**	14.1	14.0***
2nd	21.3	21.3	21.3	14.2	14.2	14.2*
3rd	21.3	21.3	21.4	14.3	14.3	14.4
4th	21.2	21.2	21.4	14.2	14.2	14.3*
≥5th	21.3	21.3	21.4	14.3	14.3	14.5
DIM (LSM)						
<60	20.5	20.8	20.6	12.6	12.6	12.7
61–120	21.1	21.4	21.3	14.2	14.2	14.2
121–180	21.5	21.5	21.6	14.6	14.5	14.6
181–240	21.5	21.4	21.5	14.6	14.6	14.6
241–300	21.4	21.3	21.5	14.5	14.6	14.7
>300	21.9	21.4	21.6	14.8	14.8	14.8
DIM contrast (<i>P</i> -value)						
Linear	***	***	***	***	***	***
Quadratic	***	***	***	***	***	***
Cubic	***	***	***	***	***	***
RMSE ⁶	0.85	0.68	0.63	1.12	0.87	0.84

¹Significance (NS and asterisks) refers to the difference between intercept estimate and the expected 0.00 value.²Significance (NS and asterisks) refers to the difference between slope estimate and the expected 1.00 value.³Asterisks refers to the significance of the orthogonal contrasts between the traditional dairy system versus all the modern ones (in the line “Traditional”); among modern dairy systems, the farms not using total mixed diets versus those using it (in the line “Modern no TMR”); among farms using TMR between those adding water (TMR_W) versus those adding silages (TMR_S) to moisturize the diet (in the line “Modern TMR_W”).⁴The variance of herd/date within dairy system, expressed as ratio with total variance.⁵Asterisks refer to the orthogonal contrast between primiparous and multiparous (in the line “1st”); between second calvers and third and more calvers (in the line “2nd”); between third calvers and fourth and more calvers (in the line “3rd”); between fourth calvers and cows with 5 or more calvings (in the line “4th”).⁶RMSE = root mean square error.**P* < 0.05; ***P* < 0.01; ****P* < 0.001.

and herd variances, some differences in the significance levels of the effects of parity and DIM, and in general, the least squares means of the various fixed factors (dairy system, parity, and DIM class) there being fewer differences among them than among the corresponding reference values.

The indirect-IR predictions of dCH₄ obtained by multiplying measured dCM by CH₄/CM_{IR} or by CH₄/CM_{FAIR} were much closer to the reference values than the direct-IR predictions, and had a very high correlation (0.96) and yielded very similar information to the ANOVA. When we used IR-predicted milk yield (dCMY_{IR}) instead of the measured dCMY to indirectly predict CH₄ production, the linear regressions with the

reference data and the ANOVA of the predictions were very similar to or even less satisfactory than the direct-IR predictions.

DISCUSSION

The estimated EME traits obtained with the reference method based on the FA profiles of milk analyzed by GC, together with the other traits (milk production, cheese-yields) used for the calculations, were reported and discussed in a previous study within the same project (Bittante et al., 2018) and will not be discussed here. Nor will we discuss the detailed FA profiles of all the cows in this study, as they were previously analyzed

Table 4. Linear regression and ANOVA of CH₄ intensity-fresh cheese (CH₄/CY_{CURD}, g/kg) and -cheese solids (CH₄/CY_{SOLIDS}, g/kg) estimated on the basis of reference CH₄/CM divided by measured cheese yield (reference, CH₄/CM/CY), directly predicted by milk infrared spectra (direct-IR), or indirectly estimated dividing CH₄/CM by CY, both predicted by infrared (indirect-IR)

Item	Methane intensity fresh cheese (g/kg)			Methane intensity cheese solids (g/kg)		
	Reference CH ₄ /CY _{CURD}	Direct-IR CH ₄ /CY _{CURD-IR}	Indirect-IR CH ₄ /CY _{CURD-IR-IR}	Reference CH ₄ /CY _{SOLIDS}	Direct-IR CH ₄ /CY _{SOLIDS-IR}	Indirect-IR CH ₄ /CY _{SOLIDS-IR-IR}
Regression on reference						
Correlation (r)	—	0.81	0.67	—	0.78	0.62
Intercept ¹ (a)	—	−4 ^{NS}	32***	—	−10 ^{NS}	83***
Slope ² (b)	—	1.04 ^{NS}	0.71***	—	1.05 ^{NS}	0.63***
ANOVA						
Dairy system ³ (LSM)						
Traditional	104	101	100*	218**	213	207**
Modern no TMR	101	100	97	207	207	201*
Modern TMR _W	91**	93**	89*	189**	193**	185
Modern TMR _S	106	107	98	215	216	197
Herd/date effect ⁴	0.54	0.60	0.35	0.48	0.60	0.25
Parity ⁵ (LSM)						
1st	97***	99*	92***	204**	208	195
2nd	99**	100	94***	207	207	195*
3rd	102	101	97	208	207	198
4th	102	101	97*	208	206	197
≥5th	102	101	100	209	208	202
DIM (LSM)						
<60	90	91	91	187	188	188
61–120	102	101	102	211	209	209
121–180	103	103	99	214	213	204
181–240	104	103	100	213	212	200
241–300	101	102	94	208	211	193
>300	101	101	92	210	210	190
DIM contrast (<i>P</i> -value)						
Linear	***	***	**	***	***	**
Quadratic	***	***	***	***	***	***
Cubic	***	***	***	***	***	***
RMSE ⁶	10	8	11	21	13	24

¹Significance (NS and asterisks) refers to the difference between intercept estimate and the expected 0.00 value.²Significance (NS and asterisks) refers to the difference between slope estimate and the expected 1.00 value.³Asterisks refer to the significance of the orthogonal contrasts between the traditional dairy system versus all the modern ones (in the line “Traditional”); among modern dairy systems, the farms not using total mixed diets versus those using it (in the line “Modern no TMR”); among farms using TMR between those adding water (TMR_W) versus those adding silages (TMR_S) to moisturize the diet (in the line “Modern TMR_W”).⁴The variance of herd/date within dairy system, expressed as a ratio with total variance.⁵Asterisks refer to the orthogonal contrast between primiparous and multiparous (in the line “1st”); between second calvers and third and more calvers (in the line “2nd”); between third calvers and fourth and more calvers (in the line “3rd”); between fourth calvers and cows with 5 or more calvings (in the line “4th”).⁶RMSE = root mean square error.**P* < 0.05; ***P* < 0.01; ****P* < 0.001.

with the aim of identifying their latent explanatory factors (Mele et al., 2016), and their heritability and genetic, herd and residual correlations and clustering (Pegolo et al., 2016), and were subjected to genome-wide association and network analysis (Pegolo et al., 2017).

The equations for estimating the EME traits based on FA_{GC} were developed by van Lingen et al. (2014) by regressing the FA_{GC} profiles of milk against the emissions obtained with the gold standard method in respiration chambers, using data obtained from a meta-analysis of 8 trials comprising 148 observations of cows on 30 different diets in 2 countries (the United Kingdom and the Netherlands). The coefficient of determination of the 2

equations used (0.54 for CH₄ yield, 0.47 for CH₄ intensity-milk) were, as often happens with meta-analyses, lower than those drawn from single-experiment studies (van Gastelen and Dijkstra, 2016), but produced results that were appreciably accurate and precise. We preferred to use these equations because they were based on a large data set representing a wide variety of feeding and management conditions, and the cows' average milk production was very similar to that in our study. The results using these equations differed little to those obtained with other direct methods that did not use respiration chambers but were instead based on sampling the air near the cows' nostrils in an open environment (Negussie et al., 2017). It should, however,

be noted that the value of these estimates depends on the characteristics of the method used (with or without a tracer gas in the rumen, frequency and distribution during the 24 h of air sampling, type of instrument, and so on) and they have recently been questioned (Hristov et al., 2016; Wu et al., 2018).

The major difference between our survey at field level and van Lingen et al. (2014) is the breed of the cows (Brown Swiss vs. Holstein Friesian). However, we should point out that breed is a minor source of variation in the FA profiles (FA expressed as proportions of their sum) of milk produced by cows in the same type

of environment, with the exception of Jersey cows, because their milk has a greater fat content and fat globule size (Kelsey et al., 2003; Soyeurt et al., 2006; Maurice-Van Eijndhoven et al., 2013). Almost no information is available on the effect of breed on EME traits, although there is reason to assume that the cow's breed has an almost null effect on its rumen microbiota activity when feed regimen and milk production are comparable. Xue et al. (2011) compared Jersey crossbreds with Holsteins purebreds and found many productive and metabolic differences between the 2 genotypes, but the energy loss from EME as a ratio of total energy intake was very

Table 5. Linear regression and ANOVA of daily CH₄ production (dCH₄, g/d) calculated multiplying the measured daily corrected milk yield (dCM) by the CH₄ intensity-milk (reference), directly predicted by milk infrared spectra (direct-IR), or indirectly calculated multiplying the measured dCM or the infrared predicted dCM by the CH₄ intensity directly predicted by infrared or indirectly estimated by IR-predicted fatty acids (FAIR)

Item	Methane production (g/d)					
	Reference dCH ₄	Direct-IR dCH _{4-IR}	Measured milk yield		IR predicted milk yield	
			dCH _{4-CM-IR}	dCH _{4-CM-FAIR}	dCH _{4-IR-IR}	dCH _{4-IR-FAIR}
Regression on reference						
Correlation (r)	—	0.68	0.96	0.96	0.65	0.63
Intercept ¹ (a)	—	16 ^{NS}	4 ^{NS}	8**	68***	84***
Slope ² (b)	—	0.96 ^{NS}	0.99 ^{NS}	0.98 ^{NS}	0.81***	0.77***
ANOVA						
Dairy system ³ (LSM)						
Traditional	307***	335***	305***	308***	332***	335***
Modern no TMR	368**	357**	367**	368***	351***	351***
Modern TMR _W	390*	377*	399	398*	376*	375**
Modern TMR _S	444	416	441	444	421	425
Herd/date effect ⁴	0.34	0.27	0.36	0.34	0.30	0.28
Parity ⁵ (LSM)						
1st	331***	346***	334***	331***	352***	349***
2nd	379	365***	378**	378**	368	368*
3rd	397	376	397	400	377	380
4th	393	383	393	395	377	380
≥5th	387	386	390	394	376	381
DIM (LSM)						
<60	399	402	402	405	407	411
61–120	422	400	423	423	396	398
121–180	402	381	403	403	379	379
181–240	377	366	377	377	364	365
241–300	338	349	342	342	347	348
>300	325	330	327	327	326	328
DIM contrast (<i>P</i> -value)						
Linear	***	***	***	***	***	***
Quadratic	***	NS	***	***	NS	NS
Cubic	***	NS	***	***	NS	NS
RMSE ⁶	73	56	70	71	63	67

¹Significance (NS and asterisks) refers to the difference between intercept estimate and the expected 0.00 value.

²Significance (NS and asterisks) refers to the difference between slope estimate and the expected 1.00 value.

³Asterisks refer to the significance of the orthogonal contrasts between the traditional dairy system versus all the modern ones (in the line “Traditional”); among modern dairy systems, the farms not using total mixed diets versus those using it (in the line “Modern no TMR”); among farms using TMR between those adding water (TMR_W) versus those adding silages (TMR_S) to moisturize the diet (in the line “Modern TMR_W”).

⁴The variance of herd/date within dairy system, expressed as a ratio with total variance.

⁵Asterisks refer to the orthogonal contrast between primiparous and multiparous (in the line “1st”); between second calvers and third and more calvers (in the line “2nd”); between third calvers and fourth and more calvers (in the line “3rd”); between fourth calvers and cows with 5 or more calvings (in the line “4th”).

⁶RMSE = root mean square error.

P* < 0.05; *P* < 0.01; ****P* < 0.001.

similar in the 2 breeds, even when their diets had very different roughage:concentrate ratios.

Direct Prediction of EME Traits from Milk FTIR Spectra

Some studies have been carried out on the prediction of EME traits from IR spectra of milk, and none is based on reference data obtained with the gold standard method. Although the most accurate and precise measurements are obtained with respiration chambers, the number of cows that can be tested is necessarily limited and the artificial environment is very different from that on commercial farms.

Dehareng et al. (2012) is the first attempt that we are aware of to predict EME traits from IR milk spectra. To predict CH_4 emissions, they sampled air through a capillary tube situated between the cows' nostrils using SF_6 released in the rumen as a tracer (Martin et al., 2008). Analyses were made of the SF_6 and CH_4 contents of 77 air samples, and of the FTIR spectra and FA_{GC} profiles of 154 milk samples taken 1.5 d after air analysis from 11 Holstein cows (dMY : 19.7 ± 5.1 kg/d) on 3 different diets. The R^2 values of the direct-IR prediction equations, obtained from this preliminary study, were in the range of 0.68 to 0.72 with a corresponding root mean square error of 84 to 105 g/d for dCH_4 , and in the range of 0.69 to 0.79 with a root mean square error of 5.00 to 6.22 g/kg for CH_4/CM , although given the small number of diets, cows, and samples, these results should be treated with caution. It is worth noting that Dehareng et al. (2012) also analyzed the FA profiles of milk samples and obtained an R^2 coefficient of the multiple correlation with CH_4/CM (0.76) that was very similar to that obtained from the direct-IR validations.

In a later study by the same research group (Vanlierde et al., 2015) on a larger data set (446 dCH_4 measurements from 142 Jersey, Holstein, and Holstein-Jersey cows from Belgium and Ireland), again using the SF_6 method, the direct-IR prediction equation was modified by incorporating DIM on the day of milk sampling using linear and quadratic Legendre polynomials. The reason for this was that if it had not been included in the prediction equation the results obtained using only FTIR spectra would not have reflected the expected pattern throughout lactation due to productive and metabolic modifications with advancing DIM (de Haas et al., 2011). The R^2 of the $\text{dCH}_{4\text{-IR}}$ prediction equation was 0.75, whereas the root mean square error was 63 g/d. The study reported only daily CH_4 production, but a follow-up study on the genetic parameters of predicted EME traits also included CH_4/CM (Kandel et al., 2017).

Shetty et al. (2017) recently completed a large experiment on 500 Danish Holstein cows on 1 experimental and 2 commercial farms. The reference values were obtained using "sniffers" placed within the automated milking stations, which produced 3,623 measures of $\text{CH}_4:\text{CO}_2$ ratios. Rather than estimating dCH_4 using the flow of SF_6 gas from the rumen, they estimated the cows' daily CO_2 production from BW, milk yield, and gestation age (Madsen et al., 2010), which provided 2,202 estimates of dCH_4 production. Validation of the prediction equations based only on milk FTIR spectra obtained using partial least squares regression yielded R^2 values of 0.21 for the $\text{CH}_4:\text{CO}_2$ ratio, and 0.13 for dCH_4 (i.e., much lower than those obtained in this study), and the authors judged that prediction of EME traits using only FTIR spectra was not feasible. When they added information on dMY , herd, parity, DIM, and season to the spectra, the R^2 values increased to 0.31 and 0.39, respectively, but the contribution of the FTIR spectra was considered to be modest.

It is worth noting that in our study, where we did not include DIM or other information in the calibration equations, the reference data and 10 of the 13 direct-IR and indirect-IR predictions of the 5 EME traits exhibited a 3rd order pattern during lactation, with lower values in early lactation (Tables 3, 4, and 5), in accordance with theoretical expectations for these traits (de Haas et al., 2011). The only exceptions were $\text{dCH}_{4\text{-IR}}$, $\text{dCH}_{4\text{-IR-IR}}$, and $\text{dCH}_{4\text{-IR-FAIR}}$, where a linear decrease occurred over the course of lactation (Table 5). It is not surprising that the R^2 values of our direct-IR prediction equations for CH_4/DMI and CH_4/CM (0.49 and 0.57, respectively) differ little from the equations of van Lingen et al. (2014), if we take into account the large number of herds and cows in our study, the variability in the systems sampled, and the year-round distribution of samplings.

Informative Signals of Milk FTIR Spectra for Predicting EME Traits

Regarding the calibration procedure, Vanlierde et al. (2015) used partial least squares (PLS) and pre-selected only 289 out of the 1,060 wavelengths of the entire spectral range (2,966 to 2,561, 1,809 to 1,720, and 1,577 to 968/cm) to reduce the size of the model and avoid noisy areas of the FTIR spectrum. For our study, we adopted a variable selection Bayesian model (BayesB), commonly used in genomic studies, and used the absorbances of all 1,060 waves without the need for wave preselection as the Bayesian model itself selects the most informative wavelengths for prediction (Ferragina et al., 2015). This also gave us an insight

into the calibration equations for identifying the chemical bonds and compounds most involved in the predictions of EME traits or, at least, of the FA related to them. Moreover, this model has proven to be more effective than conventional PLS models for milk traits with modest determination coefficients of calibration (Ferragina et al., 2015).

Like Vanlierde et al. (2015), Shetty et al. (2017) also used 3 ranges of FTIR milk spectra (2,955 to 2,700, 1,820 to 1,705, and 1,550 to 1,000 waves/cm), but having obtained VIP scores from recursive PLS analysis, they identified the most important areas of the spectrum for EME trait prediction.

Figures 1 and 2 allow us to clearly identify the more informative wavelengths of each predicted EME trait according to the Bayesian model we adopted, and to compare the equation coefficients with the Pearson correlations of each wavelength with the same trait. The first result that stands out is that the most informative wavelengths identified by the Bayesian model are seldom those with the highest simple correlations with the EME trait to be predicted, confirming our previous research (Ferragina et al., 2015).

Table 2 shows that of the 25 most informative FTIR signals for predicting EME traits (5 for each EME trait predicted), in our study we found 5 in areas of the spectrum that had not been considered in previous studies (Vanlierde et al., 2015). One signal was found in the near-IR section (SWIR, 3,978 waves/cm), 2 in the MIR section (MWIR, 2,539 and 2,442 waves/cm), and 2 very close to the 2 “water absorption” sections of the milk FTIR spectrum (2,982 and 1,701 waves/cm). These correspond to 2 of the 5 signals related to dCH_{4-IR} , 2 of the 5 signals of CH_4/CM_{IR} , and 1 of the 5 signals of $CH_4/CY_{CURD-IR}$.

The data in Table 2 show that the areas of the milk FTIR spectrum that are informative for predicting EME traits are often also important for predicting other chemical or technological characteristics of milk.

The 5 signals related to dCH_4 are also important for dCY_{CURD} and dCY_{SOLIDS} , the protein content of milk (Lynch et al., 2006; Kaylegian et al., 2009), milk coagulation and curd firming patterns (Ferragina et al., 2017), and iso-palmitic and oleic acids. Four of the 5 signals listed in Table 2 for predicting dCH_4 (excluding 2,442 waves/cm) are very close to the waves for the same trait and for predicting the $CH_4:CO_2$ ratio identified by Shetty et al. (2017) with a different methodology, whereas the 5th signal (2,442 waves/cm) is in one of the areas of the spectrum these authors excluded from their analysis.

Methane yield, which reflects feedstuff fermentation in the rumen, is associated with the signals of the spectrum related to direct-IR prediction of almost all the

informative FA included in the equation of van Lingen et al. (2014), which we used to estimate the reference data, and also of oleic acids, which were not included. Moreover, these signals are directly or closely related to prediction of milk fat and lactose (Lynch et al., 2006; Kaylegian et al., 2009) and to milk protein recovery in cheese (Ferragina et al., 2015).

The 3 CH_4 intensity traits expressed per unit of corrected milk, fresh cheese, and cheese solids are associated with the spectral signals related to informative FA, milk fat content (Kaylegian et al., 2009), and in particular and as expected, to cheese yields expressed as % of milk processed and daily production per cow, and to milk protein recovery in cheese and rennet coagulation time (Ferragina et al., 2015).

Indirect Predictions of EME Traits from Milk FTIR Spectra

An indirect use of the IR calibrations could be to predict informative FA to be used in estimating EME traits (FA_{IR}). The ability of the direct-IR and indirect-IR predictions to analyze and quantify the effects of the major sources of variation in the CH_4/DMI and CH_4/CM traits were almost equivalent to each other and to the reference data (Table 3).

In the case of CH_4/CY_{CURD} and CH_4/CY_{SOLIDS} , an indirect use of the IR calibrations has been to predict dCH_{4-IR} and dCY_{-IR} and then to calculate their ratios. This indirect-IR method proved to be much less efficient than direct-IR predictions, and the reason for this may lie in the difficulty of predicting daily traits.

This is particularly evident in predicting dCH_4 , where neither direct-IR prediction nor IR prediction of $dCMY$ multiplied by the direct or indirect predictions of CH_4/CM were satisfactory compared with the reference method (Table 5). Daily CH_4 production is a combination of a dimensional scaling factor, which depends on the cow's size, feed intake, and milk yield, and a more qualitative factor related to the relative production of CH_4 per unit of feed ingested or milk produced. The difficulty in predicting the quantity of CH_4 produced using only characteristics of the milk (FTIR spectra), and not the quantity, is evident, as Shetty et al. (2017) have also clearly shown. It is not surprising that much more satisfactory results were obtained when the dimensional aspect of dCH_4 was represented by the measured $dCMY$ value, and the qualitative aspect was represented by the direct and indirect predictions of CH_4/CM (Table 5).

The reference data based on FA_{GC} have been found to be heritable in this population (G. Bittante and A. Cecchinato, University of Padova, Legnaro, Italy, unpublished data). Direct-IR prediction of dCH_4 has

been put forward as the major trait for selecting cows with the aim of genetically improving the ecological footprint of the dairy sector, but the feasibility of genetic selection based on direct-IR predictions is highly controversial (Vanrobays et al., 2016; Shetty et al., 2017). Our results suggest that dCH₄ calculated from measured dCMY or dCY and direct-IR or indirect-IR predictions of CH₄ intensity per unit of milk or cheese is more accurate than direct-IR prediction. In any case, further research is needed to explore the feasibility of using EME traits predicted with different direct-IR and indirect-IR methods to genetically improve dairy cattle populations.

CONCLUSIONS

In this study, we examined the accuracy of EME traits predicted directly from milk FTIR spectra compared with reference data based on equations developed from a meta-analysis of the relationships between the EME traits and milk FA profiles analyzed by GC (FA_{GC}). Direct prediction of EME traits from milk FTIR spectra seems a viable option for estimating EME in the field. Possible alternatives, potentially more accurate, could be to combine daily milk yield with FTIR predictions to estimate daily CH₄ production, or to use FTIR predictions to estimate informative milk FA proportions for use in estimating EME traits. Their application in genetic or genomic selection is appealing, but more detailed analysis of the genetic correlations between the reference and predicted data, and of the molecular basis of both, is required.

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