Predicting enteric methane emission of dairy cows with milk Fourier-transform infrared spectra and gas chromatography–based milk fatty acid profiles

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ABSTRACT

The objective of the present study was to compare the prediction potential of milk Fourier-transform infrared spectroscopy (FTIR) for CH₄ emissions of dairy cows with that of gas chromatography (GC)–based milk fatty acids (MFA). Data from 9 experiments with lactating Holstein-Friesian cows, with a total of 30 dietary treatments and 218 observations, were used. Methane emissions were measured for 3 consecutive days in climate respiration chambers and expressed as production (g/d), yield (g/kg of dry matter intake; DMI), and intensity (g/kg of fat- and protein-corrected milk; FPCM). Dry matter intake was 16.3 ± 2.18 kg/d (mean $\pm$ standard deviation), FPCM yield was 25.9 ± 5.06 kg/d, CH₄ production was 366 ± 53.9 g/d, CH₄ yield was 22.5 ± 2.10 g/kg of DMI, and CH₄ intensity was 14.4 ± 2.58 g/kg of FPCM. Milk was sampled during the same days and analyzed by GC and by FTIR. Multivariate GC-determined MFA-based and FTIR-based CH₄ prediction models were developed, and subsequently, the final CH₄ prediction models were evaluated with root mean squared error of prediction and concordance correlation coefficient analysis. Further, we performed a random 10-fold cross validation to calculate the performance parameters of the models (e.g., the coefficient of determination of cross

validation). The final GC-determined MFA-based CH₄ prediction models estimate CH₄ production, yield, and intensity with a root mean squared error of prediction of 35.7 g/d, 1.6 g/kg of DMI, and 1.6 g/kg of FPCM and with a concordance correlation coefficient of 0.72, 0.59, and 0.77, respectively. The final FTIR-based CH₄ prediction models estimate CH₄ production, yield, and intensity with a root mean squared error of prediction of 43.2 g/d, 1.9 g/kg of DMI, and 1.7 g/kg of FPCM and with a concordance correlation coefficient of 0.52, 0.40, and 0.72, respectively. The GC-determined MFA-based prediction models described a greater part of the observed variation in CH₄ emission than did the FTIR-based models. The cross validation results indicate that all CH₄ prediction models (both GC-determined MFA-based and FTIR-based models) are robust; the difference between the coefficient of determination and the coefficient of determination of cross validation ranged from 0.01 to 0.07. The results indicate that GC-determined MFA have a greater potential than FTIR spectra to estimate CH₄ production, yield, and intensity. Both techniques hold potential but may not yet be ready to predict CH₄ emission of dairy cows in practice. Additional CH₄ measurements are needed to improve the accuracy and robustness of GC-determined MFA and FTIR spectra for CH₄ prediction.

Key words: dairy cow, enteric methane production, milk fatty acid concentration, milk Fourier-transform infrared spectroscopy

INTRODUCTION

Enteric CH₄ is produced in the gastrointestinal tract of livestock, mainly ruminants, and composes ~40% of global CH₄ emissions (Gerber et al., 2013). Enteric CH₄ is one of the main targets of mitigation strategies in the dairy cattle sector (Knapp et al., 2014). Quantification of CH₄ emission is thus important. Several in vivo CH₄ measurement techniques have been developed

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but are not suitable for precise and accurate large-scale measurements (Hammond et al., 2016). Cost-effective, efficient, robust, and fast CH₄ measurement techniques applicable on a large scale to estimate CH₄ emission of individual dairy cows are required. Therefore, identifying proxies (i.e., indicators or indirect traits related to CH₄ emission) might serve as a good alternative (Negussie et al., 2017).

Milk fatty acid (MFA) profiles have been suggested as a proxy to estimate CH₄ emission in dairy cattle, and many studies have evaluated this proposed relationship between MFA concentrations and CH₄ emission (e.g., Chilliard et al., 2009; Mohammed et al., 2011; Rico et al., 2016). However, the GC procedure required to obtain the MFA profiles is time consuming, labor intensive, and requires expensive instruments and trained personnel (Capuano et al., 2014) and is, therefore, unsuitable for large-scale measurements. Fourier-transform infrared spectroscopy (FTIR), on the other hand, is a rapid, cost-effective, and high-throughput technique. Currently, major milk components such as fat, protein, lactose, and urea are routinely measured with FTIR by milk recording organizations. Diverse milk phenotypes can be estimated by FTIR, as illustrated by De Marchi et al. (2014), including MFA composition (e.g., Rutten et al., 2009; Soyeurt et al., 2011), milk protein composition (Bonfatti et al., 2011), technological properties of milk (DeMarchi et al., 2009), and cow health and energy status (van Knegsel et al., 2010; McParland et al., 2011).

Dehareng et al. (2012) and Vanlierde et al. (2015) used FTIR to predict CH₄ emission of dairy cattle. The reported prediction accuracy of the models developed for CH₄ emission was high in both studies, with a cross-validated coefficient of determination ranging from 0.68 to 0.79. However, the CH₄ predictions of Dehareng et al. (2012) at different stages of lactation were not biologically meaningful, whereas Vanlierde et al. (2015) demonstrated that a lactation stage-dependent CH₄ prediction model was more robust and biologically more meaningful. The CH₄ prediction potential of FTIR spectra seems moderate (reviewed by van Gastelen and Dijkstra, 2016), based on experiments only using the SF₆-tracer technique to measure CH₄ emission. More recently, Shetty et al. (2017) demonstrated low prediction accuracy (coefficient of determination for validation being 0.13) for CH₄ emission (in L/d) when models were obtained using FTIR spectra and CH₄ emission measured by the sniffer method in automated milking stations. To date, no research has assessed the CH₄ prediction potential of milk FTIR spectra for CH₄ data obtained in climate respiration chambers and for all 3 units of CH₄ emission; namely, CH₄ production (in

g/d), CH₄ yield (in g/kg of DMI), and CH₄ intensity (in g/kg of fat- and protein-corrected milk; **FPCM**). The objective of the present study was to compare the prediction potential for CH₄ production, yield, and intensity of milk FTIR spectra with that of the GC-determined MFA profile, using CH₄ data obtained in climate respiration chambers.

MATERIALS AND METHODS

Data Collection

Data from 9 studies, designed as randomized block experiments, from Wageningen University & Research (Wageningen, the Netherlands) were used (Table 1). The experiments were conducted in accordance with Dutch law and approved by the Animal Care and Use Committee of Wageningen University & Research. The 9 studies represented 30 dietary treatments and 218 individual observations from lactating Holstein-Friesian cows. The data set included multiple observations from a small number of dairy cows (218 individual observations from 189 unique dairy cows). We consider these particular observations as unique and not as repeated measurements, because of the large differences in conditions between the observations of the same dairy cows (i.e., different experiment, different dietary treatment, different parity, and different lactation stage). The experimental setup was similar for all experiments. After an adaptation period of 12 d, cows were housed individually in open-circuit, indirect climate respiration chambers (described by van Gastelen et al., 2015) for a 5-d period to determine CH₄ emission (expressed as production, yield, and intensity). Diets were fed twice daily, and intake was restricted to 95% of the voluntarily DMI of the cow consuming the least within a block.

Cows were milked twice daily, and water was freely available during the entire experiment. While cows were housed in the climate respiration chambers, milk yield was recorded and representative milk samples (i.e., 5 g/kg of milk production from each cow) were collected at each milking according to van Gastelen et al. (2015). These milk samples were pooled per period and cow and subsequently analyzed for MFA composition (g/100 g of fatty acids) using GC as described by van Gastelen et al. (2015). The pooled milk samples were also analyzed in the laboratory of Qlip B.V. (Zutphen, the Netherlands) to determine the content of fat, protein, and lactose according to regular test-day procedures using MilkoScan FT 6000 equipment with diamond cuvettes (Foss Analytical A/S, Hillerød, Denmark) using the manufacturer supplied basic calibra-

tion models in conformity with ISO 9622 (International Organization for Standardization, 2013). The applied reference methods were ISO 1211 (International Organization for Standardization, 2010) for fat, ISO 8968–1 (International Organization for Standardization, 2014) for total protein, and an HPLC method based on ISO 22662 (International Organization for Standardization, 2007) for lactose. The FTIR absorption spectra consisted of 1,060 infrared frequencies (wavenumbers) representing infrared light absorption through the milk samples ranging from 925 to 5,008 cm^{-1} .

Statistical Analyses

Model Development: GC-Determined MFA. Multivariate models were developed using a stepwise procedure (PROC GLMSELECT of SAS; SAS Institute Inc., Cary, NC, version 9.2) with CH_4 emission (i.e., production, yield, and intensity) as the independent variable and stepwise selection of only GC-determined MFA (g/100 g total fatty acids). The significance level for a GC-determined MFA to enter or stay in the model was 0.01 and 0.05, respectively. The final models were selected based on the minimum Akaike's information criterion statistic. The selected models were evaluated

in PROC REG in terms of multicollinearity (variation inflation factor >10), but no multicollinearity was observed.

Model Development: FTIR. Prediction models for CH_4 production, yield, and intensity were developed only on preprocessed data of selected wavenumbers as linear regression models using partial least squares calculated with the SIMPLS algorithm of the PLS toolbox (Eigenvector Research Inc., Manson, WA). In the partial least squares method, spectroscopic data were reduced to a set of orthogonal, uncorrelated components (i.e., latent variables; **LV**). Selected wavenumbers ($n = 218$) were in the ranges 964 to 1,581 cm^{-1} , 1,715 to 1,773 cm^{-1} , and 2,814 to 2,968 cm^{-1} . These wavenumbers were selected because these contain valuable information on milk composition and are thus most relevant for milk analysis (Capuano et al., 2014). Additionally, parts of the infrared spectrum that are disturbed by high water absorption were omitted, because these can interfere with the quantification of other major milk components (Capuano et al., 2014). The selected wavenumbers were preprocessed by applying the Savitzky–Golay (Savitzky and Golay, 1964), first derivative with polynomial order 2 and window width 7, and subsequently mean centered.

Table 1. Data sources and characteristics of included studies

Study	Reference	n ¹	No. of treatments	Diet composition and treatments ²
1	Warner et al. (2015)	25	4	15% concentrate, 85% grass herbage. Grass herbage was cut after 3 vs. 5 wk of regrowth, after receiving a low (20 kg of N/ha) vs. high (90 kg of N/ha) fertilization rate after initial cut.
2	van Gastelen et al. (2015)	30	4	20% concentrate, 80% roughage. Roughage consisted of 100:0 vs. 67:33 vs. 33:67 vs. 0:100 grass silage:corn silage.
3	Warner et al. (2016)	42	6	20% concentrate, 80% grass silage. Grass silage received a low (65 kg of N/ha) vs. high (150 kg of N/ha) fertilization rate preceding a growth period of 28 vs. 41 vs. 62 d of regrowth.
4	Klop et al. (2016)	6	1	30% concentrate, 21% grass silage, 49% corn silage. Control diet, with concentrate containing urea as nonprotein N source.
5	Warner et al. (2017)	55	8	20% concentrate, 10% corn silage, 70% grass silage. Grass silage was cut at 4 growth stages (leafy vs. boot vs. early heading vs. late heading) and fed at 2 intake levels (15.5 vs. 16.6 kg of DMI/d).
6	Hatew et al. (2016)	25	4	20% concentrate, 5% wheat straw, 75% corn silage. Whole-plant corn was harvested at a very early (25% DM) vs. early (28% DM) vs. medium (32% DM) vs. late (40% DM) stage of maturity.
7	Klop et al. (2017)	7	1	30% concentrate, 30% grass silage, 40% corn silage (control diet).
8	Van Lingen et al. (2017)	4	1	30% concentrate, 30% grass silage, 40% corn silage (control diet).
9	van Gastelen et al. (2017b)	24	1	30% concentrate, 30% grass silage, 40% corn silage (control diet). Cows with <i>DGAT1</i> KK vs. <i>DGAT1</i> AA genotype.

¹The total number of observations, which equals the number of dairy cows, used for the present study.

²Proportion (%) on DM basis.

Model Evaluation. All CH₄ prediction models, GC-determined MFA-based and FTIR-based models, were evaluated using 2 methods. First, the mean squared error of prediction (MSEP) was calculated as

$$\text{MSEP} = \sum_{i=1}^n (O_i - P_i)^2 / n,$$

where n is the total number of observations, O_i is the observed value, and P_i is the predicted value. The square root of the mean squared error of prediction (**RMSEP**) gives an estimate of the overall error of prediction and is expressed as percentage of the observed mean or expressed in grams per day, grams per kilogram of DMI, and grams per kilogram of FPCM for CH₄ production, yield, and intensity, respectively. Second, concordance correlation coefficient analysis (**CCC**; Lin, 1989) was performed, where CCC is calculated as

$$\text{CCC} = r \times C_b,$$

where r is the correlation coefficient providing a measure of precision and C_b is a bias correction factor providing a measure of accuracy. The C_b variable is calculated as

$$C_b = 2 / [(v + 1/v + \mu^2)'],$$

where

$$v = S_o / S_p,$$

$$\mu = \frac{\bar{O} - \bar{P}}{(S_o \times S_p)^{0.5}},$$

where v provides a measure of scale shift, μ provides a measure of location shift, S_o and S_p are the observed and predicted standard deviations, and $\bar{O}$ and $\bar{P}$ are the observed and predicted means. A CCC of 0.20 or less indicates poor predictive ability, between 0.21 and 0.40 indicates fair predictive ability, between 0.41 and 0.60 indicates moderate predictive ability, between 0.61 and 0.80 indicates substantial predictive ability, and between 0.81 and 1.00 indicates accurate predictive ability (Altman, 1997). Furthermore, the predictive power of the calibration was evaluated through the ratio of performance to deviation (**RPD**) statistic, which is the ratio of the standard deviation of the original data to the standard error of cross validation (Dehareng et al., 2012). The RPD values are preferably as high as possible; RPD values between 5 and 10 are adequate for quality control and process control and are potentially suitable for application (Williams, 2014). Additionally,

PROC CORR in SAS was used to determine the Pearson correlation between the MFA-predicted CH₄ emissions and the FTIR-predicted CH₄ emissions.

Cross Validation MFA and FTIR. To calculate the models performance parameters [i.e., root mean squared error of cross validation (**RMSECV**) and the coefficient of determination of cross validation (**R²CV**)], we performed a random cross validation with 10 splits and 10 iterations as recommended by Rodriguez et al. (2010) for all MFA- and FTIR-based CH₄ prediction models. For each iteration, a model was developed as described above using 9 splits of the data set, and the selected model was subsequently evaluated as described above on the remaining part of the data set (i.e., 1 split). With this approach, all observations were used for both calibration and validation, and each observation was used for validation exactly once. The cross validation performance values represent the average of the 10-fold cross validation.

This random 10-fold cross validation was also used for selection of the number of LV for the FTIR-based CH₄ prediction models. The selected number of LV for the final models was based on the suggestion by PLS toolbox (Eigenvector Research Inc.) and visual assessment of the graphs of the root mean squared error of calculation and RMSECV against the number of LV. The number of LV before the RMSECV starts increasing or the RMSECV starts deviating considerably from the root mean squared error of calculation was the number selected.

RESULTS

The descriptive statistics of animal performance, dietary characteristics, CH₄ emission, and GC-determined MFA concentrations are presented in Table 2. The GC-determined MFA-based CH₄ production, yield, and intensity prediction models are shown in Table 3. In the final models, considering the odd- and branched-chain fatty acids (**OBCFA**), CH₄ production was positively associated with C15:0 ($P = 0.002$), CH₄ yield was positively associated with *iso* C15:0 and C17:0 ($P < 0.003$) but negatively associated with *anteiso* C15:0 ($P < 0.001$), and CH₄ intensity was positively associated with *iso* C15:0 and *iso* C17:0 ($P < 0.001$). The relation between CH₄ emissions and the C18:1, C18:2, C18:3 isomers was generally negative ($P < 0.010$), with the exception of the positive association between CH₄ production and C18:2n-6 ($P = 0.005$). Additionally, CH₄ production was negatively associated with C24:0 ($P = 0.007$) and positively associated with C20:4n-3 ($P = 0.002$), and CH₄ intensity was positively associated with C22:5n-3 ($P < 0.001$). The FTIR-based CH₄ prediction models were based on the regression between

the wavenumbers and CH₄ production, yield, or intensity, as illustrated in Figure 1. Certain wavenumbers were not related to CH₄ emissions (i.e., regression vector close to 0), whereas other wavenumbers were clearly positively or negatively related to CH₄ emissions. The strength and the direction (positive or negative) of the correlations as well as the correlated wavenumbers differed between the different units of CH₄ emission (i.e., production, yield, and intensity; Figure 1).

The evaluation results (i.e., R², RMSEP, and CCC analysis) of the GC-determined MFA-based and FTIR-based CH₄ prediction models are shown in Table 4. The observed versus predicted CH₄ production, yield, and intensity plots of the GC-determined MFA-based and FTIR-based CH₄ prediction models are shown in Figures 2A and 3A, respectively. The residual (i.e., observed minus predicted) versus predicted CH₄ production, yield, and intensity plots of the GC-determined MFA-based and FTIR-based CH₄ prediction models are shown in Figures 2B and 3B, respectively. The R², RMSEP (%), and CCC of the GC-determined MFA-based CH₄ prediction models ranged from 0.40 to 0.62, from 7.1 to 10.9%, and from 0.59 to 0.77, respectively (Table 4). The R², RMSEP (%), and CCC of the FTIR-based CH₄ prediction models ranged from 0.25 to 0.56, from 8.2 to 11.8%, and from 0.40 to 0.72, respectively. Based on the CCC, for both GC-determined MFA and FTIR, the prediction model for CH₄ yield had the lowest prediction potential (moderate predicting ability for both MFA- and FTIR-based models), and the prediction model for CH₄ intensity had the highest prediction potential (substantial predicting ability for both MFA- and FTIR-based models). The MFA- and FTIR-based prediction models for CH₄ production had substantial and moderate predicting ability, respectively. The variation in predicted CH₄ emission was smaller than that in the observed CH₄ emission, in particular for CH₄ yield, as indicated by the variable *v* (scale shift; the relative difference in standard deviation between predicted and observed values). The scale shift was greater for FTIR-based prediction models (*v* ranged from 1.33 to 2.00) than for GC-determined MFA-based prediction models (*v* ranged from 1.26 to 1.55).

The RPD statistic, that relates the standard error of prediction to the standard deviation of the original reference data, was smaller than 1.58 for the GC-determined MFA-based CH₄ prediction models and smaller than 1.39 for the FTIR-based CH₄ prediction models (Table 4), suggesting unsatisfactory prediction ability. The Pearson correlations between GC-determined MFA-predicted and FTIR-predicted CH₄ production, CH₄ yield, and CH₄ intensity were 0.62 ($P < 0.001$), 0.51 ($P < 0.001$), and 0.69 ($P < 0.001$), respectively (Figure 4).

The results of the internal cross validation of all GC-determined MFA-based and FTIR-based CH₄ prediction models are also shown in Table 4. The average number of GC-determined MFA included in the GC-determined MFA internal cross validation models varied between 4 and 5, and the average number of LV in the FTIR internal cross validation models varied between 4 and 6. The R²CV and the RMSECV of the GC-determined MFA-based CH₄ prediction models ranged from 0.38 to 0.63 and from 8.1 to 11.6%, respectively. The R²CV and the RMSECV of the FTIR-based CH₄ prediction models ranged from 0.19 to 0.49 and from 8.6 to 12.8%, respectively.

DISCUSSION

This is the first study evaluating and comparing the CH₄ prediction potential of GC-determined MFA and milk FTIR spectra for CH₄ data obtained in climate respiration chambers. Data were obtained from dairy cattle experiments where type of forage, forage quality, and forage-to-concentrate ratio were varied, without use of CH₄ mitigating additives. Our results indicated that GC-determined MFA-based prediction models had a higher prediction potential than did the FTIR-based models and described a larger amount of the observed variation in CH₄ emission.

GC-Determined MFA-Based CH₄ Prediction Models

All CH₄ prediction models were based on OBCFA and long-chain fatty acids (>16 carbons). No short- and medium-straight, even-chain fatty acids (≤16 carbons) were included in any of the GC-determined MFA-based CH₄ prediction models, despite the fact that these are synthesized *de novo* in the mammary gland from acetate and BHB produced in the rumen, which are both reported to be positively associated with CH₄ emission (Ellis et al., 2008). As reviewed by van Gastelen and Dijkstra (2016), these short- and medium-straight, even-chain fatty acids were usually not included in the GC-determined MFA-based CH₄ prediction equations (*n* = 6) previously developed, except for C4:0 and C16:0, which were included in one equation each. The association found between CH₄ emissions and both *iso* and *anteiso* OBCFA in the present study is in agreement with *iso* OBCFA being more abundant in fibrolytic bacteria and *anteiso* OBCFA being more abundant in amylolytic bacteria (Vlaeminck et al., 2006). Both C15:0 and C17:0 were found to be positively associated with CH₄ emissions, which is in disagreement with the findings of Vlaeminck et al. (2006) and Rico et al. (2016) but in agreement with the findings of Chilliard et al. (2009), Dijkstra et

Table 2. Descriptive statistics of animal performance, dietary characteristics, methane emission, and the milk fatty acid profile determined with GC (n = 218)

Variable	Mean	Median	SD	Minimum	Maximum
Animal performance					
BW (kg)	617	617	59.7	462	817
Parity	2.7	3.0	1.38	1.0	7.0
DIM	179	185	85.2	59	567
Milk yield (kg/d)	24.3	23.9	5.42	11.3	36.8
FPCM ¹ (kg/d)	25.9	25.3	5.06	12.3	39.9
Milk fat content (g/100 g milk)	4.67	4.67	0.659	2.94	6.70
Milk CP content (g/100 g milk)	3.37	3.30	0.406	2.62	5.00
Milk anhydrous lactose content (g/100 g milk)	4.57	4.59	0.221	3.80	5.03
DMI (kg/d)	16.3	16.1	2.18	10.8	24.5
Dietary characteristics (g/kg of DM, unless stated otherwise)					
DM (g/kg)	502	502	101.5	306	797
Ash	77	79	13.5	53	103
CP	176	172	40.1	82	251
NDF	380	372	49.9	242	501
ADF	221	218	25.7	183	291
ADL	14	14	4.2	6	26
Crude fat	31	33	6.7	21	46
Starch	118	79	85.5	5	326
Sugar	89	70	59.0	21	265
Gross energy (MJ/kg of DM)	18.6	18.6	0.41	17.6	19.3
NDF-to-starch ratio (g/g)	8.2	4.8	15.76	1.0	86.2
Methane emission					
Production (g/d)	366	365	53.9	234	535
Yield (g/kg of DMI)	22.5	22.6	2.10	17.2	28.0
Intensity (g/kg of FPCM)	14.4	14.4	2.58	8.5	24.8
Milk fatty acids (g/100 g of fatty acids) determined with GC					
C4:0	3.5	3.5	0.35	1.8	4.4
C6:0	2.1	2.2	0.21	1.5	2.6
C8:0	1.1	1.1	0.17	0.6	1.6
C10:0	2.5	2.4	0.53	1.1	4.1
C12:0	2.8	2.8	0.69	1.3	4.9
C14:0	10.4	10.5	1.39	6.7	14.1
<i>iso</i> C14:0	0.08	0.08	0.017	0.04	0.13
C14:1 <i>cis</i> -9	0.99	0.97	0.238	0.47	1.95
C15:0	0.97	0.97	0.168	0.53	1.56
<i>iso</i> C15:0	0.23	0.23	0.041	0.13	0.37
<i>anteiso</i> C15:0	0.40	0.40	0.068	0.24	0.62
C16:0	31.7	31.7	3.35	24.6	42.3
<i>iso</i> C16:0	0.18	0.18	0.035	0.12	0.34
C16:1 <i>trans</i> -9	0.21	0.21	0.037	0.13	0.35
C16:1 <i>cis</i> -9	1.9	1.8	0.38	1.0	3.0
C17:0	0.65	0.64	0.099	0.44	0.96
<i>iso</i> C17:0	0.40	0.39	0.060	0.25	0.63
<i>anteiso</i> C17:0	0.42	0.41	0.056	0.32	0.61
C17:1 <i>cis</i> -9	0.31	0.30	0.087	0.15	0.69
C18:0	9.6	9.7	1.61	5.0	15.2
C18:1 <i>cis</i> -9 ²	21.0	20.7	3.83	12.3	30.5
C18:1 <i>cis</i> -12	0.18	0.15	0.075	0.07	0.47
C18:1 <i>cis</i> -13	0.13	0.13	0.037	0.05	0.27
C18:1 <i>trans</i> -6	0.20	0.19	0.051	0.06	0.42
C18:1 <i>trans</i> -9	0.15	0.14	0.026	0.08	0.25
C18:1 <i>trans</i> -10	0.19	0.16	0.091	0.00	0.65
C18:1 <i>trans</i> -11	0.89	0.88	0.221	0.17	2.18
C18:1 <i>trans</i> -15 + C18:1 <i>cis</i> -11	0.77	0.75	0.171	0.33	1.23
C18:2 <i>cis</i> -9, <i>trans</i> -11	0.42	0.40	0.116	0.20	1.29
C18:2n-6	1.5	1.5	0.24	0.9	2.4
C18:3n-3	0.47	0.48	0.154	0.14	0.98
C18:3n-6	0.07	0.07	0.014	0.04	0.13
C20:0	0.13	0.13	0.019	0.08	0.19
C20:1 <i>cis</i> -11	0.06	0.06	0.022	0.00	0.12
C20:2n-6	0.04	0.04	0.007	0.02	0.07
C20:3n-6	0.07	0.07	0.019	0.03	0.13
C20:4n-3	0.03	0.03	0.026	0.00	0.13
C20:4n-6	0.11	0.11	0.024	0.05	0.18
C20:5n-3	0.06	0.06	0.013	0.03	0.09
C22:0	0.06	0.06	0.014	0.00	0.11
C22:5n-3	0.08	0.08	0.019	0.04	0.14
C24:0	0.04	0.04	0.013	0.00	0.08

¹Fat- and protein-corrected milk (FPCM; kg/d) = $[0.337 + 0.116 \times \text{fat (g/100 g of milk)} + 0.06 \times \text{protein (g/100 g of milk)}] \times \text{milk yield (kg/d)}$ (CVB, 2012).²C18:1 *cis*-9 represents the sum of C18:1 *cis*-9 and C18:1 *trans*-12; these 2 fatty acids could not be separated in the analysis. The portion of C18:1 *trans*-12 is considered to be negligible because this fatty acid is always present in small amounts.

al. (2011) and van Lingen et al. (2014). The negative relations between C18:1, C18:2, and C18:3 isomers in milk and CH₄ emission are in agreement with several other studies (e.g., van Lingen et al., 2014 and Rico et al., 2016). The associations between CH₄ emissions and long-chain fatty acids have been reported before (i.e., Chilliard et al., 2009; Rico et al., 2016; van Gastelen et al., 2017a), suggesting that these GC-determined MFA are important in terms of CH₄ prediction.

In general, the prediction potential of the GC-determined MFA-based CH₄ prediction models appeared to be moderate to substantial, with the CCC ranging from 0.40 to 0.77. The observed R² values ranged from 0.40 to 0.62 and were lower than the ones reported by Dijkstra et al. (2011) for CH₄ yield and by Chilliard et al. (2009), Mohammed et al. (2011), and Rico et al. (2016) for CH₄ production, but the observed R² values were of similar magnitude to those of van Lingen et al. (2014) and van Gastelen et al. (2017a). Recent research, including the present study, on the relationship between GC-determined MFA and CH₄ emission gives inconsistent results. Although some studies found a clear and strong relation between GC-determined MFA and CH₄ emission (e.g., Chilliard et al., 2009; Dijkstra et al., 2011), other studies concluded that GC-determined MFA alone might not be suitable to develop universal CH₄ prediction models (e.g., Mohammed et al., 2011), and more recently, Castro-Montoya et al. (2017) concluded that GC-determined MFA are not reliable predictors for specific amounts of CH₄ emitted by a cow based on the coefficient of determination of validation

ranging from 0.18 to 0.41. Even the studies that do find a clear relation between GC-determined MFA and CH₄ emissions do not describe similar prediction models using the same GC-determined MFA. The discrepancies between these studies have been reviewed by van Gastelen and Dijkstra (2016). Many factors can influence GC-determined MFA concentrations and, therefore, the relation between GC-determined MFA and CH₄ emissions (Gengler et al., 2016), such as dietary composition (e.g., Mohammed et al., 2011; Dijkstra et al., 2016) and lactation stage (Vanrobays et al., 2016). Moreover, it should be noted that previous analyses were often based on data of cattle fed lipid supplements or feed additives, whereas in the present study dietary contrasts included variation in forage-to-concentrate ratio, type of forage, and forage quality only.

The difference between R² and R²CV for the GC-determined MFA-based CH₄ prediction models was small (0.07 for CH₄ production, 0.02 for CH₄ yield, and 0.01 for CH₄ intensity; Table 4). These small differences indicate that all GC-determined MFA-based CH₄ prediction models are robust in terms of CH₄ prediction. The GC-determined MFA-based CH₄ prediction models were also assessed for robustness in terms of composition of the prediction models. All 4 GC-determined MFA that were part of the overall prediction model for CH₄ intensity (Table 3) were also selected in the prediction models developed in the 10-fold cross validation (results not shown). Three of the four GC-determined MFA were included in all 10 models (i.e., *iso* C15:0, *iso* C17:0, and C18:1 *trans*-15 + C18:1 *cis*-

Table 3. The prediction models developed for methane production (g/d), yield (g/kg of DMI), and intensity (g/kg of FPCM¹) based on milk fatty acids determined with GC

Methane emission	Milk fatty acid	Estimate	SE	P-value
Methane production (g/d)	Intercept	507.9	28.66	<0.001
	C15:0	62.9	17.22	0.002
	C17:1 <i>cis</i> -9	-240.6	32.29	0.007
	C18:1 <i>trans</i> -10	-202.8	47.75	0.010
	C18:1 <i>trans</i> -11	-59.3	12.70	<0.001
	C18:2n-6	48.1	14.08	0.005
	C18:3n-3	-187.1	24.40	<0.001
	C20:4n-3	326.4	104.30	0.002
	C24:0	-816.8	230.89	0.007
	Intercept	22.9	1.27	<0.001
Methane yield (g/kg of DMI)	<i>iso</i> C15:0	20.9	4.17	0.003
	<i>anteiso</i> C15:0	-9.6	2.34	<0.001
	C17:0	7.6	1.26	<0.001
	C18:1 <i>trans</i> -11	-2.4	0.52	<0.001
	C18:1 <i>trans</i> -15 + C18:1 <i>cis</i> -11	-2.7	0.84	<0.001
	C18:3n-3	-4.4	0.81	<0.001
	Intercept	8.0	1.13	<0.001
Methane intensity (g/kg of FPCM)	<i>iso</i> C15:0	24.8	3.66	<0.001
	<i>iso</i> C17:0	10.3	2.30	<0.001
	C18:1 <i>trans</i> -15 + C18:1 <i>cis</i> -11	-6.6	0.95	<0.001
	C22:5n-3	22.7	6.61	<0.001
	Intercept	8.0	1.13	<0.001

¹Fat- and protein-corrected milk (FPCM; kg/d) = [0.337 + 0.116 × fat (g/100 g of milk) + 0.06 × protein (g/100 g of milk)] × milk yield (kg/d) (CVB, 2012).

Figure 1. The regression vectors of the partial least squares models for methane (A) production (g/d), (B) yield (g/kg of DMI), and (C) intensity [g/kg of fat- and protein-corrected milk (FPCM)] plotted against wavenumbers (cm^{-1}).

11), which shows the robustness of the GC-determined MFA-based prediction model for CH_4 intensity in terms of composition. In comparison, all 6 GC-determined MFA of the MFA-based prediction model for CH_4 yield were selected in the 10-fold cross validation. Although only 1 GC-determined MFA of the GC-determined MFA-based model (i.e., C18:3n-3) was included in all 10 models of the cross validation, the other 5 GC-determined MFA were included in 6 to 8 of the 10 models. However, of the 8 GC-determined MFA in the MFA-based prediction model for CH_4 production, only 5 were also selected in the 10-fold cross validation, of which 1 GC-determined MFA (i.e., C18:3n-3) was included in all 10 models. Moreover, 3 of the GC-determined MFA in the GC-determined MFA-based CH_4 production prediction model were not selected in any of the 10 models of the cross validation (i.e., C18:1 *trans*-10, C18:2n-6, and C20:4n-3). This illustrates that the GC-determined MFA-based prediction model for CH_4 production in particular is less robust in comparison with the GC-determined MFA-based prediction model for CH_4 intensity and CH_4 yield.

FTIR-Based CH_4 Prediction Models

In general, the prediction potential of the FTIR-based CH_4 prediction models appeared to be moderate to substantial, with the CCC ranging from 0.40 to 0.72 and the R^2 ranging from 0.25 to 0.56. From the regression vector (Figure 1), it appears that bands around 975 cm^{-1} , $1,075 \text{ cm}^{-1}$, $1,150 \text{ cm}^{-1}$, $1,450 \text{ cm}^{-1}$, $1,500$ to $1,575 \text{ cm}^{-1}$, $1,750 \text{ cm}^{-1}$, and $2,850$ to $3,000 \text{ cm}^{-1}$ are important for the prediction of CH_4 emissions. The latter region and the bands around $1,175 \text{ cm}^{-1}$ and $1,750 \text{ cm}^{-1}$ are commonly used to quantify milk fat content (Safar et al., 1994; Dupuy et al., 1996; Yang and Irudayaraj, 2000). Protein is expected to have absorption peaks around wavenumbers $1,500$ to $1,700 \text{ cm}^{-1}$ (Osborne and Fearn, 1986; McQueen et al., 1995; Dufour et al., 1998), with the bands around $1,500$ to $1,575 \text{ cm}^{-1}$ coinciding with the amide II band (Etzion et al., 2004). Additionally, the infrared region between $1,000$ and $1,100 \text{ cm}^{-1}$ provides information on sugar molecules (Hashimoto and Kameoka, 2008). This suggests that the bands of the FTIR spectra that are important to determine the milk composition, such as fat and protein content, are also important for the prediction of CH_4 emission. However, as illustrated by Negussie et al. (2017), milk fat and milk protein content have low CH_4 prediction potential. This is also observed in the present study, in which milk protein and milk fat contents were relatively weakly associated with CH_4 emissions measured in the climate respiration chambers, except for CH_4 intensity, for which the calculation includes milk fat and

Table 4. The R² and concordance correlation coefficient (CCC) analysis of the prediction equations and the 10-fold cross validation results

Methane emission	Overall						10-Fold cross validation					
	Adjusted R ²	RMSEP ²	RMSEP % ³	CCC ⁴	r ⁵	C _b ⁶	v ⁷	μ ⁸	RPD ¹	No. of LV or MFA ⁹	R ² CV ¹⁰	RMSECV ¹¹ %
Methane production (g/d)												
GC-determined MFA ¹²	0.54	35.7	9.8	0.72	0.75	0.96	1.34	0	1.27	4	0.47	11.6
FTIR	0.36	43.2	11.8	0.52	0.60	0.88	1.68	0	1.19	4	0.30	12.4
Methane yield (g/kg of DMI)												
GC-determined MFA	0.40	1.6	7.1	0.59	0.64	0.91	1.55	0	1.15	5	0.38	8.1
FTIR	0.25	1.9	8.2	0.40	0.50	0.80	2.00	0	1.09	5	0.19	8.6
Methane intensity (g/kg of FPCM ¹³)												
GC-determined MFA	0.62	1.6	10.9	0.77	0.79	0.97	1.26	0	1.58	5	0.63	11.4
FTIR	0.56	1.7	11.8	0.72	0.75	0.96	1.33	0	1.39	6	0.49	12.8

¹Ratio of performance to deviation.
²Root mean squared error of prediction expressed in grams per day, grams per kilogram of DMI, and grams per kilogram of fat- and protein-corrected milk (FPCM) for methane production, yield, and intensity, respectively.
³RMSEP expressed as a percentage of the observed mean.
⁴CCC = $r \times C_b$.
⁵r = Pearson correlation coefficient, a measure of precision.
⁶C_b = bias correction factor, a measure of accuracy.
⁷v = scale shift; change in SD between predicted and observed methane emission.
⁸μ = location shift; if positive, underprediction; if negative, overprediction.
⁹Number of latent variables (LV) included in the Fourier-transform infrared spectra (FTIR)-based models or the number of milk fatty acids (MFA) included in the MFA-based models.
¹⁰R² of cross validation
¹¹Root mean squared error of cross validation.
¹²Milk fatty acids in g/100 g of fatty acids determined with GC.
¹³Fat- and protein-corrected milk (FPCM; kg/d) = [0.337 + 0.116 × fat (g/100 g of milk) + 0.06 × protein (g/100 g of milk)] × milk yield (kg/d) (CVB, 2012).

Figure 2. (A) Observed and predicted and (B) residual (i.e., observed – predicted) (1) methane production (g/d), (2) methane yield (g/kg of DMI), and (3) methane intensity [g/kg of fat- and protein-corrected milk (FPCM)] from the regression analyses based on milk fatty acid profiles (g/100 g fatty acids) determined with GC. The slope of residuals regressed on predicted values did not differ significantly from zero. The different symbols identify the 9 individual experiments (Exp.) described in Table 1.

Figure 3. (A) Observed and predicted and (B) residual (i.e., observed – predicted) (1) methane production (g/d), (2) methane yield (g/kg of DMI), and (3) methane intensity [g/kg of fat- and protein-corrected milk (FPCM)] from the partial least squares regression analyses based on Fourier-transform infrared wavenumbers (cm⁻¹). The slope of residuals regressed on predicted values did not differ significantly from zero. The different symbols identify the 9 individual experiments (Exp.) described in Table 1.

Figure 4. The relationship between methane production (g/d), yield (g/kg of DMI), and intensity [g/kg of fat- and protein-corrected milk (FPCM)] predicted with milk fatty acid (MFA) profiles determined with GC and predicted with milk Fourier-transform infrared spectra (FTIR). The different symbols identify the 9 individual experiments (Exp.) described in Table 1.

protein contents. Methane yield was correlated with fat content ($r = 0.17$, $P = 0.010$) and tended to be related to protein content ($r = 0.12$, $P = 0.066$), whereas no significant correlations were observed for CH_4 production. However, as expected from the similarity in FTIR spectra bands, FTIR-predicted CH_4 emissions were more strongly related to milk protein content ($r = 0.11$, $P = 0.096$ for CH_4 production; $r = 0.32$, $P < 0.001$ for CH_4 yield; $r = 0.64$, $P < 0.001$ for CH_4 intensity) and to milk fat content ($r = -0.11$, $P = 0.094$ for CH_4 production; $r = 0.37$, $P < 0.001$ for CH_4 yield; $r = 0.13$, $P = 0.053$ for CH_4 intensity).

The differences between R^2 and $R^2\text{CV}$ for the milk FTIR-based CH_4 prediction models were 0.06 for CH_4 production, 0.06 for CH_4 yield, and 0.07 for CH_4 intensity (Table 4). For CH_4 yield and intensity, these differences between R^2 and $R^2\text{CV}$ of FTIR-based models are somewhat larger than for GC-determined MFA-based models, indicating that GC-determined MFA-based models are slightly more robust. The number of studies on FTIR-based CH_4 prediction models is limited. Dehareng et al. (2012) reported FTIR-based prediction models for CH_4 production and CH_4 intensity (g/kg of milk) using the SF_6 -tracer technique, involving 11 lactating dairy cows and 3 dietary treatments. The prediction potentials of the FTIR-based prediction models reported by Dehareng et al. (2012) were higher than the ones reported in the present study, with the R^2 ranging from 0.77 to 0.93 and the $R^2\text{CV}$ ranging from 0.68 to 0.79. Additionally, Vanlierde et al. (2015) developed lactation stage-independent (i.e., including only FTIR spectra) and lactation stage-dependent (i.e., including FTIR spectra and DIM) CH_4 prediction models using the SF_6 -tracer technique, involving 142 lactating dairy cows fed a wide range of diets. Vanlierde et al. (2015) reported, for the lactation stage-independent CH_4 prediction model (i.e., comparable to present study), a strong correlation ($R^2 = 0.77$) between observed and predicted CH_4 production, which is also higher than that in the present study. However, the previous studies developed FTIR-based CH_4 prediction models using multiple measurements of the same cows in a shorter time frame than in our study. Consequently, cows were in a rather similar lactation stage and parity and received the same dietary treatment. The study by Dehareng et al. (2012) involved 11 dairy cows, whereas the prediction models were developed using 77 observations (i.e., 7.00 observations per individual), and the study by Vanlierde et al. (2015) involved 142 dairy cows, whereas the prediction models were developed using 446 observations (i.e., 3.14 observations per individual). This could have positively influenced the performance parameters of their CH_4 prediction models; repeated measures are generally more closely related than independent obser-

variations. Contrary, the present study involved multiple distinct measurements of a limited number of cows (i.e., out of 189 individual dairy cows, 29 cows had 2 observations; 1.15 observations per individual). These observations on the same individual are considered as separate measurements because they were obtained at a different parity and lactation stage as well as a different dietary treatment. Furthermore, the large range of CH₄ emissions measured using the SF₆-tracer technique might have contributed to the high prediction potentials found in both studies. In the study by Dehareng et al. (2012), CH₄ production ranged from 218 to 653 g/d and CH₄ intensity ranged from 10.2 to 47.1 g/kg of milk, and in the study by Vanlierde et al. (2015), CH₄ production ranged from approximately 180 to 950 g/d, which is not within the range of CH₄ measurements reported in literature (Appuhamy et al., 2016).

More recently, Shetty et al. (2017) concluded that it is not feasible to predict CH₄ emission based on FTIR spectra alone, because of the low prediction accuracies found when models were obtained using FTIR spectra and because of the marginal added value of FTIR spectra in combination with traits such as milk yield and lactation stage. Hence, considerable discrepancy exists between the results obtained in the present study and the 3 aforementioned studies. This discrepancy might be the results of different CH₄ emission measurement techniques (i.e., climate respiration chambers, SF₆-tracer technique, and the sniffer technique), the size as well as the structure of the population [i.e., ranging from 11 dairy cows in the study by Dehareng et al. (2012) to 490 dairy cows in the study by Shetty et al. (2017)], the prediction and validation methods (i.e., internal cross validation and external validation), and the duration of measurement and the time between CH₄ measurements and milk FTIR sampling (Shetty et al., 2017).

Comparison of GC-Determined MFA-Based and FTIR-Based CH₄ Prediction Models

For all CH₄ emission units, but particularly for CH₄ production and CH₄ yield, GC-determined MFA-based prediction models had a higher prediction potential than did the FTIR-based models. This is evident by the lower RMSEP values and higher R² and CCC values. The higher CCC values are caused by the higher accuracy (C_b) and, in particular, higher precision (r) of the GC-determined MFA-based CH₄ prediction models (Table 4). The relatively larger differences between the GC-determined MFA-based and FTIR-based prediction models for CH₄ production and CH₄ yield might be explained by GC-determined MFA being more closely linked to the ruminal CH₄ production pathways than FTIR spectra. It is known that GC-determined MFA

are related to CH₄ production because of the common biochemical pathway between CH₄ and fatty acids in the rumen (Ellis et al., 2008; Chilliard et al., 2009). As discussed above, the FTIR spectra represent the absorbed light by vibrations at several wavelengths of many milk components, including some GC-determined MFA, urea, citrate, free fatty acids, and fat, protein, and lactose content. The latter 3 solid major milk components have a low CH₄ prediction potential (Negussie et al., 2017) and do not seem to be directly linked with ruminal CH₄ pathways. The relatively small difference between the GC-determined MFA-based and FTIR-based prediction models for CH₄ intensity might be explained by the fact that CH₄ intensity takes milk yield into account, which is directly associated with enteric CH₄ production by cows and reflected by both the FTIR spectral data and the GC-determined MFA profile, due to dilution effects (Dehareng et al., 2012). This is also illustrated by the somewhat stronger correlation between GC-determined MFA-predicted CH₄ intensity and FTIR-predicted CH₄ intensity ($r = 0.69$) compared with the correlation between both methods for CH₄ production ($r = 0.62$) and CH₄ yield ($r = 0.51$).

All CH₄ prediction models, GC-determined MFA-based and FTIR-based models, had a scale shift that was different from one ($v > 1.26$). This indicates that a change in standard deviation exists between predicted and observed CH₄ values for all CH₄ prediction models, which is also visualized in Figures 2 and 3 for GC-determined MFA-based and FTIR-based models, respectively. The variation in predicted CH₄ values was clearly smaller than that in observed CH₄ values for all CH₄ prediction models. However, the scale shift was greater for all the FTIR-based CH₄ prediction models (v ranged from 1.33 to 2.00) than for the GC-determined MFA-based CH₄ prediction models (v ranged from 1.26 to 1.55), which indicates that GC-determined MFA-based CH₄ prediction models have the ability to describe more of the observed variation in CH₄ emissions compared with FTIR-based prediction models.

The RPD values from the present study are lower than the RPD values reported by Dehareng et al. (2012). The low RPD values from the present study (i.e., <1.58 for the GC-determined MFA-based CH₄ prediction models and <1.39 for the FTIR-based CH₄ prediction models) suggest that the prediction ability of these models can be regarded as poor (Williams, 2014). According to Williams and Sobering (1993), a RPD value of 2.5 and above would suggest that the model is satisfactory for screening. A narrow range in the variability of the observations is known to negatively affect predictability of methods of interest (Manley, 2014). Indeed, the coefficient of variation (SD relative to mean) is highest for CH₄ intensity (17.9%), and the models for

CH₄ intensity had relatively the best RPD. The lowest coefficient of variation is for CH₄ yield (9.3%), and the models for CH₄ yield had the smallest RPD values. Moreover, although the respiration chamber method is generally considered to be the golden standard for CH₄ measurements (Hammond et al., 2016), its reproducibility as compared with many chemical analyses, for which the RPD statistic was originally developed, is much lower, hence reducing prediction accuracy of the prediction methods. The RPD values would suggest that the CH₄ prediction models presented in the current study, GC-determined MFA-based and FTIR-based models, would not be able to classify dairy cows from populations with low variation in CH₄ emission into low and high CH₄ producers. More variation in the dairy population under evaluation, such as greater variation in animal genetics, in dietary composition, and in production management, could potentially improve the ability of the models to predict CH₄ emission (Dehareng et al., 2012).

The present study did not take lactation stage into account. Although lactation stage is a poor CH₄ proxy when considered alone (Negussie et al., 2017), Vanlierde et al. (2015) demonstrated that lactation stage in combination with FTIR improved the CH₄ prediction model. Vanlierde et al. (2015) developed both lactation stage-independent and lactation stage-dependent CH₄ prediction models. The average CH₄ production (g/d) predicted by both models was similar (416 ± 63 g/d, mean $\pm$ SD). However, in contrast to the lactation stage-independent prediction model, the lactation stage-dependent prediction model showed biologically meaningful behavior throughout lactation: an increase in CH₄ production (g/d) after calving up to approximately 100 DIM, followed by a gradual decline toward the end of lactation (Vanlierde et al., 2015). This effect of lactation stage could also be important for the MFA-based CH₄ prediction models, because Vanrobays et al. (2016) clearly demonstrated that the correlations between GC-determined MFA and CH₄ production in dairy cows vary according to lactation stage. We therefore acknowledge that the CH₄ prediction models of the present study may be improved in terms of predictive power and robustness, when combining GC-determined MFA or FTIR with lactation stage. We were, however, not able to confirm this, because differences in lactation stage were confounded by differences in dietary composition in the data set used in the present study. Additionally, it should be noted that this study was based on 9 experiments with forage-based diets only (forage varied between 700 and 850 g/kg of DM). Furthermore, the milk production of the cows did not exceed 36.8 kg/d, and all cows were restricted in their feed intake to avoid confounding effects of DMI on CH₄

production. Hence, the area of validity of the CH₄ prediction models that have been established in this study is limited to these conditions.

Application of CH₄ Prediction Models in Practice

In the present study, we show that GC-determined MFA have a higher prediction potential for CH₄ emissions than FTIR spectra. However, the GC procedure required to obtain the GC-determined MFA profile is unsuitable for routine milk recording, whereas the prediction of CH₄ emission using FTIR has the potential for practical high throughput application.

Although the RPD results suggest that the GC-determined MFA-based and FTIR-based CH₄ prediction models currently have limited applicability, the CCC results demonstrated that the models had at least moderate predictive ability. Potential practical applications for these models include (1) as a farm management tool, (2) to evaluate CH₄ mitigation strategies, and (3) as a tool to breed for dairy cows with lower CH₄ emissions (Cottle et al., 2011). When a dietary strategy is applied in practice, the proxy for CH₄ emission should be able to evaluate whether CH₄ emission is affected by the new dietary strategy. Therefore, within each study that had at least 2 dietary treatments, we evaluated whether the GC-determined MFA-based and FTIR-based CH₄ prediction models were able to estimate the same difference in CH₄ emission as measured in the climate respiration chambers, by comparing CH₄ emission at 2 extreme diets (i.e., furthest apart from one another in terms of dietary composition). The results of this evaluation are shown in Table 5. In general, most CH₄ prediction models predicted a difference in CH₄ emission similar to the climate respiration chambers in terms of trend (i.e., increase or decrease), with a few exceptions (viz., 2 for the GC-determined MFA-based and 5 for the FTIR-based CH₄ prediction models). Furthermore, estimated differences in CH₄ emission between the 2 diets were generally more in line with observed differences as measured in the climate respiration chambers when the GC-determined MFA-based CH₄ prediction models compared with the FTIR-based CH₄ prediction models were used to estimate differences. This suggests that the FTIR-based CH₄ prediction models might have less accuracy relative to the GC-determined MFA-based CH₄ prediction models to evaluate the effect of forage level and quality on CH₄ emission of dairy cattle.

Breeding for reduced CH₄ emission can be achieved with, for example, improved productivity, increased longevity, or shorter calving interval (Bell et al., 2011) but also by breeding for actual lower enteric CH₄ production (Wall et al., 2010). Several studies have

Table 5. Differences in methane emissions between 2 extreme dietary treatments within each study, measured in climate respiration chambers and estimated with the MFA-based and FTIR-based prediction models

Study	Reference	Difference between treatments	Methane emission	Difference		
				measured in CRC ¹	MFA ²	FTIR ³
1	Warner et al. (2015)	Grass herbage with 5 wk of regrowth receiving high fertilization compared with grass herbage with 3 wk of regrowth receiving low fertilization	Production (g/d) Yield (g/kg of DMI) Intensity (g/kg of FPCM ⁴)	31 2.0 1.3	36 1.9 1.3	8 0.4 1.0
2	van Gastelen et al. (2015)	Roughage consisting of 100% corn silage compared with roughage consisting of 100% grass silage	Production (g/d) Yield (g/kg of DMI) Intensity (g/kg of FPCM)	-12 -2.6 -1.3	-13 -2.9 -3.3	-15 -0.9 -0.8
3	Warner et al. (2016)	Grass silage with 62 d of regrowth and a high fertilization rate compared with grass silage with 28 d of regrowth and a low fertilization rate	Production (g/d) Yield (g/kg of DMI) Intensity (g/kg of FPCM)	-39 1.6 4.6	-4 1.5 2.6	8 0.0 1.6
5	Warner et al. (2017)	Late heading stage grass silage at low DMI compared with leafy stage grass silage at high DMI	Production (g/d) Yield (g/kg of DMI) Intensity (g/kg of FPCM)	24 5.0 4.0	32 2.8 3.1	-1 1.5 2.6
6	Hatew et al. (2016)	Late-harvested whole-plant corn silage compared with early-harvested whole-plant corn silage	Production (g/d) Yield (g/kg of DMI) Intensity (g/kg of FPCM)	-29 -1.6 -0.9	13 0.0 -1.1	-0.4 0.3 0.4

¹Climate respiration chambers.

²Milk fatty acids in g/100 g of fatty acids determined with GC.

³Fourier-transform infrared spectra.

⁴Fat- and protein-corrected milk (kg/d) = $[0.337 + 0.116 \times \text{fat (g/100 g of milk)} + 0.06 \times \text{protein (g/100 g of milk)}] \times \text{milk yield (kg/d)}$ (CVB, 2012).

shown that CH₄ emissions of dairy cows have a genetic component, with heritability ranging from 0.20 to 0.30 (e.g., De Haas et al., 2011, for predicted CH₄ emission based on feed intake; Lassen and Løvendahl, 2016, for CH₄ emission measured with a portable air sampler), indicating that breeding for dairy cows with lower CH₄ emission may be possible. Recently, Vanlierde et al. (2016) reported that FTIR can distinguish cows with low or high daily CH₄ emissions. Direct breeding for lower enteric CH₄ production requires CH₄ production measurements of a large number of individual dairy cows to determine the genetic component of the CH₄ phenotype as well as to determine the genetic correlations of CH₄ emissions with other traits. Although the feasibility needs to be assessed in actual commercial environments before implementation, the FTIR technique has the potential to assist in breeding for reduced CH₄ emission because it can be used routinely to estimate CH₄ on commercial dairy farms.

CONCLUSIONS

This study is the first to assess and compare the CH₄ emission prediction potential of GC-determined MFA profiles and FTIR spectra based on CH₄ emission data obtained in climate respiration chambers and for 3 different units of CH₄ emission (viz., CH₄ production, yield, and intensity). For both GC-determined MFA and FTIR, the prediction model for CH₄ yield had the lowest prediction potential and the prediction model for CH₄ intensity had the highest prediction potential. For all CH₄ emission units, but particularly for CH₄ production and yield, GC-determined MFA-based prediction models had a higher prediction potential than the FTIR-based models, and GC-determined MFA-based prediction models described a greater part of the observed variation in CH₄ emission than did FTIR-based models. Results indicate that the current GC-determined MFA-based and FTIR-based CH₄ prediction models have potential but limited current applicability. Additional CH₄ measurements are needed to improve prediction models in terms of accuracy and robustness of both GC-determined MFA and FTIR spectra for CH₄ prediction.

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