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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 methane (**CH_4**) emissions of dairy cows with that of gas chromatography (**GC**)-based milk fatty acid (**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 dry matter intake; **DMI**), and intensity (g/kg fat- and protein-corrected milk; **FPCM**). Dry matter intake was 16.3 ± 2.18 kg/d, FPCM yield was 25.9 ± 5.06 kg/d, CH_4 production was 366 ± 53.9 g/d, CH_4 yield was 22.5 ± 2.10 g/kg DMI, and CH_4 intensity was 14.4 ± 2.58 g/kg FPCM (mean ± SD). Milk was sampled during the same days and analyzed by GC and by FTIR. Multivariate GC-determined MFA-based and FTIR-based CH_4 prediction models were developed and, subsequently, the final CH_4 prediction models were evaluated with root mean square error of prediction (**RMSEP**) and concordance correlation coefficient (**CCC**) analysis. Further, we performed a random 10-fold cross validation to calculate the models performance parameters (e.g., the coefficient of determination of cross validation; **R^2CV**). The final GC-determined MFA-based CH_4 prediction models estimate CH_4 production, yield, and intensity with a RMSEP of 35.7 g/d, 1.6 g/kg DMI, and 1.6 g/kg FPCM, and with a CCC of 0.72, 0.59, and 0.77, respectively. The final FTIR-based CH_4 prediction models estimate CH_4 production, yield, and intensity with a RMSEP of 43.2 g/d, 1.9 g/kg DMI, and 1.7 g/kg FPCM, and with a CCC 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 CH_4 emission than FTIR-based models. The cross validation results indicate that all CH_4 prediction models (both GC-determined MFA-based and FTIR-based) are robust, as the difference between R^2 and R^2CV ranged from 0.01 to 0.07. These results indicate that GC-determined MFA have a greater potential than FTIR spectra to estimate CH_4

production, yield, and intensity. Both techniques hold potential, but may not yet be ready to predict CH_4 emission of dairy cows in practice. Additional CH_4 measurements are therefore needed to improve the accuracy and robustness of both GC-determined MFA and FTIR spectra for CH_4 prediction.

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

INTRODUCTION

Enteric methane (**CH_4**) is produced in the gastrointestinal tract of livestock, mainly ruminants, and comprises ~40% of global CH_4 emissions (Gerber et al., 2013). Enteric CH_4 is one of the main targets of mitigation strategies in the dairy cattle sector (Knapp et al., 2014). Quantification of CH_4 emission is thus important. Several *in vivo* CH_4 measurement techniques have been developed, but are not suitable for precise and accurate large scale measurements (Hammond et al., 2016). Cost-effective, efficient, robust, and fast CH_4 measurement techniques applicable on a large scale to estimate CH_4 emission of individual dairy cows are required. Therefore, identifying proxies (i.e., indicators or indirect traits related to CH_4 emission), might serve as a good alternative (Negussie et al., 2017).

Milk fatty acid (**MFA**) profiles have been suggested as proxy to estimate CH_4 emission in dairy cattle, and many studies have evaluated this proposed relationship between MFA concentrations and CH_4 emission (e.g., Chilliard et al., 2009; Mohammed et al., 2011; Rico et al., 2016). However, the gas chromatography (**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 CH_4 emission of dairy cattle. The reported prediction accuracy of the models developed for CH_4 emission was high in both studies, with an cross validated coefficient of determination ranging from 0.68 to 0.79. However, the CH_4 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 CH_4 prediction model was more robust and biologically more meaningful. The CH_4 prediction potential of FTIR spectra seems moderate (reviewed by Van Gastelen and Dijkstra, 2016), which is based on experiments only using the SF_6-tracer technique to measure CH_4 emission. More recently, Shetty et al. (2017) demonstrated low prediction accuracy (coefficient of determination for validation being 0.13) for CH_4 emission (in L/d) when models were obtained using FTIR spectra and CH_4 emission measured by the sniffer method in automated milking stations. To date, no research has assessed the CH_4 prediction potential of milk FTIR spectra for CH_4 data obtained in climate respiration chambers and for all 3 units of CH_4 emission, viz. CH_4 production (in g/d), CH_4 yield (in g/kg dry matter intake; **DMI**), and CH_4 intensity (in g/kg fat- and protein-corrected milk; **FPCM**). The objective of the present study was to compare the prediction potential for CH_4 production, yield, and intensity of milk FTIR spectra with that of the GC-determined MFA profile, using CH_4 data obtained in climate respiration chambers.

MATERIAL 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 dataset 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 CH_4 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 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 FA) 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 calibration 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 1060 infrared frequencies (wavenumbers) representing infrared light absorption through the milk samples ranging from 925 to 5008 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, USA, 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 pre-processed data of selected wavenumbers as linear regression models using Partial Least Squares (PLS) calculated with the SIMPLS algorithm of the PLS toolbox (Eigenvector Research Inc., Manson, WA, USA). In the PLS method, spectroscopic data were reduced to a set of orthogonal, uncorrelated components (viz. latent variables; **LV**). Selected wavenumbers (n = 218) were in the ranges 964 - 1581 cm^{-1}, 1715 – 1773 cm^{-1}, and 2814 - 2968 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 pre-processed by applying the Savitzky-Golay (Savitzky and Golay,1964), first derivative with polynomial order 2 and window width 7, and subsequently mean centered.

Model evaluation. All CH_4 prediction models, GC-determined MFA-based and FTIR-based, were evaluated using 2 methods. Firstly, the mean square error of prediction (**MSEP**), calculated as

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

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 MSEP (**RMSEP**) gives an estimate of the overall error of prediction and is expressed as percentage of the observed mean or expressed in g/d, g/kg DMI, and g/kg FPCM for CH_4 production, yield, and intensity, respectively. Secondly, concordance correlation coefficient analysis (**CCC**; Lin, 1989) was performed, where CCC is calculated as

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

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

$$C_\mathrm{b} = \frac{2}{[v + 1 / v + \mu^2]},$$

where

$$v = \frac{S_o}{S_p},$$

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

where v provides a measure of scale shift, while 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 lower 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, process control, and potentially suitable for application (Williams et al., 2014). Additionally, PROC CORR in SAS was used to determine the Pearson correlation between the MFA predicted CH_4 emissions and the FTIR predicted CH_4 emissions.

Cross validation MFA and FTIR. In order to calculate the models performance parameters (i.e., root mean square error of cross validation (**RMSECV**) and the coefficient of determination of cross validation (**R^2CV**)), 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 CH_4 prediction models. For each iteration, a model was developed as described above using 9 splits of the dataset, and the selected model was subsequently evaluated as described above on the remaining part of the dataset (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 CH_4 prediction models. The selected number of LV for the final models was based on the suggestion by PLS toolbox and visual assessment of the graphs of the root means square error of calculation (**RMSEC**) and RMSECV against the number of LV. The number of

LV before the RMSECV starts increasing or the RMSECV starts deviating considerably from the RMSEC was the number selected.

RESULTS

The descriptive statistics of animal performance, dietary characteristics, CH_4 emission, and GC-determined MFA concentrations are presented in Table 2. The GC-determined MFA-based CH_4 production, yield, and intensity prediction models are shown in Table 3. In the final models, considering the odd- and branched-chain fatty acids (**OBCFA**), CH_4 production was positively associated with C15:0 (P = 0.002), CH_4 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 CH_4 intensity was positively associated with both *iso* C15:0 and *iso* C17:0 (P < 0.001). The relation between CH_4 emissions and the C18:1, C18:2, C18:3 isomers was generally negative (P < 0.010), with the exception of the positive association between CH_4 production and C18:2n-6 (P = 0.005). Additionally, CH_4 production was negatively associated with C24:0 (P = 0.007) and positively associated with C20:4n-3 (P = 0.002), and CH_4 intensity was positively associated with C22:5n-3 (P < 0.001). The FTIR-based CH_4 prediction models are based on the regression between the wavenumbers and CH_4 production, yield, or intensity, as illustrated in Figure 1. Certain wavenumbers were not related with CH_4 emissions (i.e., regression vector close to 0), whereas other wavenumbers were clearly positively or negatively related with CH_4 emissions. Both the strength and the direction (positive or negative) of the correlations as well as the correlated wavenumbers differed between the different units of CH_4 emission (i.e., production, yield, and intensity; Figure 1).

The evaluation results (i.e., R^2, RMSEP, and CCC analysis) of the GC-determined MFA-based and FTIR-based CH_4 prediction models are shown in Table 4. The observed versus predicted CH_4 production, yield, and intensity plots of the GC-determined MFA-based and

FTIR-based CH_4 prediction models are shown in Figures 2A and 3A, respectively. The residual (i.e., observed minus predicted) versus predicted CH_4 production, yield, and intensity plots of the GC-determined MFA-based and FTIR-based CH_4 prediction models are shown in Figures 2B and 3B, respectively. The R^2, RMSEP (%), and CCC of the GC-determined MFA-based CH_4 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 R^2, RMSEP (%), and CCC of the FTIR-based CH_4 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 CH_4 yield had the lowest prediction potential (moderate predicting ability for both MFA and FTIR based models) and the prediction model for CH_4 intensity had the highest prediction potential (substantial predicting ability for both MFA and FTIR based models, respectively). The MFA and FTIR based prediction models for CH_4 production had substantial and moderate predicting ability, respectively. The variation in predicted CH_4 emission was smaller than that in the observed CH_4 emission, in particular for CH_4 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 CH_4 prediction models and smaller than 1.39 for the FTIR-based CH_4 prediction models (Table 4), suggesting unsatisfactory prediction ability. The Pearson correlations between GC-determined MFA predicted and FTIR predicted CH_4 production, CH_4 yield, and CH_4 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 CH_4 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 R^2CV and the RMSECV of the GC-determined MFA-based CH_4 prediction models ranged from 0.38 to 0.63 and from 8.1% to 11.6%, respectively. The R^2CV and the RMSECV of the FTIR-based CH_4 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 CH_4 prediction potential of GC-determined MFA and milk FTIR spectra for CH_4 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 CH_4 mitigating additives. Our results indicate that the GC-determined MFA-based prediction models had a higher prediction potential than the FTIR-based models and described a larger amount of the observed variation in CH_4 emission.

GC-determined MFA-based CH_4 prediction models

All CH_4 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 CH_4 prediction models, despite the fact that these are synthesized *de novo* in the mammary gland from acetate and β-hydroxybutyrate produced in the rumen, which are both reported to be positively associated with CH_4 emission (Ellis et al., 2008). As reviewed by Van Gastelen and Dijkstra et al. (2016), these short- and medium-straight, even-chain fatty acids were usually not included in the GC-determined MFA-based CH_4 prediction equations (n = 6) previously developed, except for C4:0 and C16:0 that were

included in 1 equation each. The association between CH_4 emissions and both *iso* and *anteiso* OBCFA in the current 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 CH_4 emissions, which is in disagreement with Vlaeminck et al. (2006) and Rico et al. (2016), but in agreement with Chilliard et al. (2009), Dijkstra et al. (2011) and Van Lingen et al. (2014). The negative relations between C18:1, C18:2, and C18:3 isomers in milk and CH_4 emission are in agreement with several other studies (e.g., Van Lingen et al., 2014 and Rico et al., 2016). The associations between CH_4 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 CH_4 prediction.

In general, the prediction potential of the GC-determined MFA-based CH_4 prediction models appears to be moderate to substantial, with the CCC ranging from 0.40 to 0.77. The observed R^2 values ranged from 0.40 to 0.62 and are lower than the ones reported by Dijkstra et al. (2011) for CH_4 yield, and by Chilliard et al. (2009), Mohammed et al. (2011), and Rico et al. (2016) for CH_4 production, but of similar magnitude as Van Lingen et al. (2014) and Van Gastelen et al. (2017a). The recent research, including the present study, on the relationship between GC-determined MFA and CH_4 emission gives inconsistent results. Where some studies found a clear and strong relation between GC-determined MFA and CH_4 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 CH_4 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 CH_4 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 CH_4 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). There are many factors that can influence GC-determined MFA concentrations and therefore the relation between GC-determined MFA and CH_4 emissions (Gengler et al., 2016), such as dietary composition (e.g., Mohammed et al., 2011 and 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 R^2 and R^2CV for the GC-determined MFA-based CH_4 prediction models was small (0.07 for CH_4 production, 0.02 for CH_4 yield, and 0.01 for CH_4 intensity; Table 4). These small differences indicate that all GC-determined MFA-based CH_4 prediction models are robust in terms of CH_4 prediction. The GC-determined MFA-based CH_4 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 CH_4 intensity (Table 3) were also selected in the prediction models developed in the 10-fold cross validation (results not shown). Three of the 4 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*-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 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 to 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 appears 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 – 1,150 cm^{-1}, 1,450 cm^{-1}, 1,500 – 1,575 cm^{-1}, 1,750 cm^{-1}, and 2,850 – 3,000 cm^{-1} are important for the prediction of CH_4 emissions. The latter region, and the bands around 1,175 cm^{-1} and 1,750 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 cm-1 (Osborn and Fearn, 1986; McQueen et al., 1995; Dufour et al., 1998), with the bands around 1,500 – 1,575 cm^{-1} coinciding with the amide II band (Etzion et al., 2004). Additionally, the infrared region between 1,000 – 1,100 cm^{-1} provides information on sugar molecules (Hashimoto and Kameoka, 2008). This suggests that the bands of the FTIR spectra which 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 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^2CV 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^2CV 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 both CH_4 production and CH_4 intensity (g/kg 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^2CV ranging from 0.68 to 0.79. Additionally, Vanlierde et al. (2015) developed both lactation stage independent (i.e., including only FTIR spectra) and lactation stage dependent (i.e., including FTIR spectra and days in milk) 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 same lactation stage and parity and received the same dietary treatment. The

study of 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 of Vanlierde et al. (2015) involved 142 dairy cows, while 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, as repeated measures are generally more closely related than independent observations. 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 CH_4 emissions measured using the SF_6-tracer technique might have contributed to the high prediction potentials found in both studies. In Dehareng et al. (2012) CH_4 production ranged from 218 to 653 g/d and CH_4 intensity ranged from 10.2 to 47.1 g/kg milk, and in Vanlierde et al. (2015) CH_4 production ranged from approximately 180 to 950 g/d, which are not within the range of CH_4 measurements reported in literature (Appuhamy et al., 2016).

More recently, Shetty et al. (2017) concluded that it is not feasible to predict CH_4 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, there is a considerable discrepancy between the results obtained in the present study and the three aforementioned studies. This discrepancy might be the results of different CH_4 emission measurement techniques (i.e., climate respiration chambers, SF_6-tracer technique, and the sniffer technique), the size as well as the structure of the population [i.e., ranging from 11 dairy cows in Dehareng et al. (2012) to 490 dairy cows in Shetty et al. (2017)], the prediction and validation methods

393 (i.e., internal cross validation and external validation), and the duration of measurement and the
394 time between CH_4 measurements and milk FTIR sampling (Shetty et al., 2017).
395
396 ***Comparison of GC-determined MFA-based and FTIR-based CH_4 prediction models***
397 For all CH_4 emission units, but particularly for CH_4 production and CH_4 yield, GC-
398 determined MFA-based prediction models had a higher prediction potential than the FTIR-
399 based models. This is evident by the lower RMSEP values and higher R^2 and CCC values. The
400 higher CCC values are caused by the higher accuracy (C_b) and, in particular, higher precision
401 (r) of the GC-determined MFA-based CH_4 prediction models (Table 4). The relatively larger
402 differences between the GC-determined MFA-based and FTIR-based prediction models for
403 CH_4 production and CH_4 yield might be explained by GC-determined MFA being more closely
404 linked to the ruminal CH_4 production pathways than FTIR spectra. It is known that GC-
405 determined MFA are related to CH_4 production because of the common biochemical pathway
406 between CH_4 and fatty acids in the rumen (Chilliard et al., 2009; Ellis et al., 2008). As discussed
407 above, the FTIR spectra represent the absorbed light by vibrations at several wavelengths of
408 many milk components, including GC-determined MFA, urea, citrate, free fatty acids, and fat,
409 protein, and lactose content. The latter 3 solid major milk components have a low CH_4
410 prediction potential (Negussie et al., 2017) and do not seem to be directly linked with ruminal
411 CH_4 pathways. The relatively small difference between the GC-determined MFA-based and
412 FTIR based prediction models for CH_4 intensity might be explained by the fact that CH_4
413 intensity takes milk yield into account, which is directly associated with enteric CH_4 production
414 by cows and reflected by both the FTIR spectral data and the GC-determined MFA profile, due
415 to dilution effects (Dehareng et al., 2012). This is also illustrated by the somewhat stronger
416 correlation between GC-determined MFA predicted CH_4 intensity and FTIR predicted CH_4

intensity (r = 0.69), compared with the correlation between both methods for CH_4 production (r = 0.62) and CH_4 yield (r = 0.51).

All CH_4 prediction models, both GC-determined MFA-based and FTIR-based, had a scale shift which was different from 1 ($v > 1.26$). This indicates that there is a change in standard deviation between predicted and observed CH_4 values for all CH_4 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 CH_4 values was clearly smaller than that in observed CH_4 values for all CH_4 prediction models. However, the scale shift was greater for all the FTIR-based CH_4 prediction models (v ranges from 1.33 to 2.00) than for the GC-determined MFA-based CH_4 prediction models (v ranges from 1.26 to 1.55), which indicates that GC-determined MFA-based CH_4 prediction models have the ability to describe more of the observed variation in CH_4 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 CH_4 prediction models and < 1.39 for the FTIR-based CH_4 prediction models), suggest that the prediction ability of these models can be regarded as poor (Williams et al., 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 CH_4 intensity (17.9%) and the models for CH_4 intensity had relatively the best RPD. The lowest coefficient of variation is for CH_4 yield (9.3%) and the models for CH_4 yield had the smallest RPD values. Moreover, although the respiration chamber method is generally considered to be the golden standard for CH_4 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

442 reducing prediction accuracy of the prediction methods. The RPD values would suggest that
443 the CH_4 prediction models presented in the current study, both GC-determined MFA-based and
444 FTIR-based, would not be able to classify dairy cows from populations with low variation in
445 CH_4 emission into low and high CH_4 producers. More variation in the dairy population under
446 evaluation, such as greater variation in animal genetics, in dietary composition, and in
447 production management, could potentially improve the ability of the models to predict CH_4
448 emission (Dehareng et al., 2012).

449 It is important to note though, that the present study did not take lactation stage into
450 account. Although lactation stage is a poor CH_4 proxy when considered alone (Negussie et al.,
451 2017), Vanlierde et al. (2015) demonstrated that lactation stage in combination with FTIR
452 improved the CH_4 prediction model. Vanlierde et al. (2015) developed both lactation stage-
453 independent and lactation stage-dependent CH_4 prediction models. The average CH_4 production
454 (g/d) predicted by both models was similar (416 ± 63 g/d). However, in contrast to the lactation
455 stage-independent prediction model, the lactation stage-dependent prediction model showed
456 biologically meaningful behavior throughout lactation: an increase in CH_4 production (g/d)
457 after calving up to approximately 100 DIM, followed by a gradual decline towards the end of
458 lactation (Vanlierde et al., 2015). This effect of lactation stage could also be important for the
459 MFA-based CH_4 prediction models, because Vanrobays et al. (2016) clearly demonstrated that
460 the correlations between GC-determined MFA and CH_4 production in dairy cows vary
461 according to lactation stage. We therefore acknowledge that the CH_4 prediction models of the
462 present study may be improved in terms of predictive power and robustness, when combining
463 GC-determined MFA or FTIR with lactation stage. We were, however, not able to confirm this,
464 because differences in lactation stage were confounded by differences in dietary composition
465 in the dataset used in the present study. Additionally, it should be noted that this study was
466 based on 9 experiments with forage-based diets only (forage varied between 700 and 850 g/kg

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 CH_4 production. Hence, the area of validity of the CH_4 prediction models that have been established in this study, is limited to these conditions.

Application of CH_4 prediction models in practice

In the present study, we show that GC-determined MFA have a higher prediction potential for CH_4 emissions than FTIR spectra. However, the gas chromatography procedure required to obtain the GC-determined MFA profile is unsuitable for routine milk recording, whereas the prediction of CH_4 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 CH_4 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 CH_4 mitigation strategies, and (3) as a tool to breed for dairy cows with lower CH_4 emissions (Cottle et al., 2011). When a dietary strategy is applied in practice, the proxy for CH_4 emission should be able to evaluate whether CH_4 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 CH_4 prediction models were able to estimate the same difference in CH_4 emission as measured in the climate respiration chambers, by comparing CH_4 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, all CH_4 prediction models predicted a difference in CH_4 emission similar to the climate respiration chambers in terms of trend (i.e., increase or decrease). There were only a few exceptions, viz. two for the GC-determined MFA-based and

five for the FTIR-based CH_4 prediction models. Furthermore, the differences in CH_4 emission between the two diets as estimated by the GC-determined MFA-based CH_4 prediction models were generally more in line with the observed differences as measured in the climate respiration chambers, than that of the FTIR-based CH_4 prediction models compared with the difference measured in climate respiration chambers. This suggests that the FTIR-based CH_4 prediction models might have less accuracy relative to the GC-determined MFA-based CH_4 prediction models, both based on a single FTIR or a single GC measurement to determine the MFA profile of a 4-day combined milk sample, to evaluate the effect of forage level and quality on CH_4 emission of dairy cattle.

Breeding for reduced CH_4 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 CH_4 production (Wall et al., 2010). Several studies have shown that CH_4 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 CH_4 emission based on feed intake; Lassen and Løvendahl, 2016 for CH_4 emission measured with a portable air-sampler), indicating that breeding for dairy cows with lower CH_4 emission may be possible. Recently, Vanlierde et al. (2016) reported that FTIR can distinguish cows with low or high daily CH_4 emissions. Direct breeding for lower enteric CH_4 production requires CH_4 production measurements of a large number of individual dairy cows to determine the genetic component of the CH_4 phenotype as well as to determine the genetic correlations of CH_4 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 CH_4 emission as it can be used routinely to estimate CH_4 on commercial dairy farms.

CONCLUSIONS

This study is the first to assess and compare the CH_4 emission prediction potential of both GC-determined MFA profiles and FTIR spectra based on CH_4 emission data obtained in climate respiration chambers and for three different units of CH_4 emission, viz. CH_4 production, yield, and intensity. For both GC-determined MFA and FTIR, the prediction model for CH_4 yield had the lowest prediction potential and the prediction model for CH_4 intensity had the highest prediction potential. For all CH_4 emission units, but particularly for CH_4 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 CH_4 emission than FTIR-based models. Results indicate that the current GC-determined MFA-based and FTIR-based CH_4 prediction models have potential, but have limited current applicability. Additional CH_4 measurements are needed to improve prediction models in terms of accuracy and robustness of both GC-determined MFA and FTIR spectra for CH_4 prediction.

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Table 1. Data sources and characteristics of included studies

Study	Reference	n[1]	No. of treatments	Diet composition / treatments[2]
1	Warner et al. (2015)	25	4	15% concentrate, 85% grass herbage. Grass herbage was cut after 3 vs. 5 weeks of regrowth, after receiving 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 low (65 kg N/ha) vs. high (150 kg N/ha) fertilization rate preceding growth period 28 d vs. 41 d 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 four growth stages (leafy vs. boot vs. early heading vs. late heading) and fed at two intake levels (15.5 kg/d vs. 16.6 kg/d DMI[3]).
6	Hatew et al. (2016)	25	4	20% concentrate, 5% wheat straw, 75% corn silage. Whole-plant corn was harvested at 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 *DGAT1* KK vs. *DGAT1* AA genotype.

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

[2] Proportion (%) on DM basis.

[3] Dry matter intake (kg/d).

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

Variable	Mean	Median	SD	Minimum	Maximum
Animal performance					
Body weight	617	617	59.7	462	817
Parity	2.7	3.0	1.38	1.0	7.0
Days in milk	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 crude protein 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[2] (kg/d)	16.3	16.1	2.18	10.8	24.5
Dietary characteristics (in g/kg DM, unless stated otherwise)					
Dry matter (g/kg)	502	502	101.5	306	797
Ash	77	79	13.5	53	103
Crude protein	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
GE (MJ/kg 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 DMI)	22.5	22.6	2.10	17.2	28.0

Intensity (g/kg FPCM)	14.4	14.4	2.58	8.5	24.8
Milk fatty acids (g/100 g fatty acids) determined with gas chromatography					
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
iso C14:0	0.08	0.08	0.017	0.04	0.13
C14:1 *cis*-9	0.99	0.97	0.238	0.47	1.95
C15:0	0.97	0.97	0.168	0.53	1.56
iso C15:0	0.23	0.23	0.041	0.13	0.37
anteiso C15:0	0.40	0.40	0.068	0.24	0.62
C16:0	31.7	31.7	3.35	24.6	42.3
iso C16:0	0.18	0.18	0.035	0.12	0.34
C16:1 *trans*-9	0.21	0.21	0.037	0.13	0.35
C16:1 *cis*-9	1.9	1.8	0.38	1.0	3.0
C17:0	0.65	0.64	0.099	0.44	0.96
iso C17:0	0.40	0.39	0.060	0.25	0.63
anteiso C17:0	0.42	0.41	0.056	0.32	0.61
C17:1 *cis*-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 *cis*-9[3]	21.0	20.7	3.83	12.3	30.5
C18:1 *cis*-12	0.18	0.15	0.075	0.07	0.47
C18:1 *cis*-13	0.13	0.13	0.037	0.05	0.27
C18:1 *trans*-6	0.20	0.19	0.051	0.06	0.42
C18:1 *trans*-9	0.15	0.14	0.026	0.08	0.25
C18:1 *trans*-10	0.19	0.16	0.091	0.00	0.65
C18:1 *trans*-11	0.89	0.88	0.221	0.17	2.18
C18:1 *trans*-15 + C18:1 *cis*-11	0.77	0.75	0.171	0.33	1.23

C18:2 *cis*-9, *trans*-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 *cis*-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

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

[2] Dry matter intake (kg/d).

[3] C18:1 *cis*-9 represents the sum of C18:1 *cis*-9 and C18:1 *trans*-12, as these 2 FA could not be separated in the analysis. The portion of C18:1 *trans*-12 is considered to be negligible, as this FA is always present in small amounts.

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Table 3. The prediction model developed for methane production (g/d), yield (g/kg DMI[1]), and intensity (g/kg FPCM[2]) based on milk fatty acids determined with gas chromatography

Methane emission	Milk fatty acids	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 *cis*-9	-240.6	32.29	0.007
	C18:1 *trans*-10	-202.8	47.75	0.010
	C18:1 *trans*-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
Methane yield (g/kg DMI)	Intercept	22.9	1.27	< 0.001
	iso C15:0	20.9	4.17	0.003
	anteiso C15:0	-9.6	2.34	< 0.001
	C17:0	7.6	1.26	< 0.001
	C18:1 *trans*-11	-2.4	0.52	< 0.001
	C18:1 *trans*-15 + C18:1 *cis*-11	-2.7	0.84	< 0.001
	C18:3n-3	-4.4	0.81	< 0.001
Methane intensity(g/kg FPCM)	Intercept	8.0	1.13	< 0.001
	iso C15:0	24.8	3.66	< 0.001
	iso C17:0	10.3	2.30	< 0.001
	C18:1 *trans*-15 + C18:1 *cis*-11	-6.6	0.95	< 0.001
	C22:5n-3	22.7	6.61	< 0.001

[1] Dry matter intake (kg/d)

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

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Table 4. The coefficient of determination (R^2) and concordance correlation coefficient (CCC) analysis of the prediction equations and the 10-fold cross validation results

	Overall									10-fold cross validation		
Methane emission	Adjusted R^2	RMSEP RMSEP[5]	RMSEP %[6]	CCC[7]	r[8]	C_b[9]	v[10]	μ[11]	RPD[12]	Number of LV or MFA[13]	R^2CV	RMSECV %
Methane production (g/d)												
GC-determined MFA[1]	0.54	35.7	9.8	0.72	0.75	0.96	1.34	0	1.27	4	0.47	11.6
FTIR[2]	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 DMI[3])												
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 FPCM[4])												
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

[1] Milk fatty acids in g/100 g fatty acids determined with gas chromatography.

[2] Fourier-transform infrared spectra.

[3] Dry matter intake (kg/d)

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

[5] Root mean squared error of prediction expressed in g/d, g/kg DMI, and g/kg FPCM for methane production, yield, and intensity, respectively.

[6] Root mean squared error of prediction expressed as a percentage of the observed mean.

[7] Concordance correlation coefficient, where CCC = $r \times C_b$.

[8] Pearson correlation coefficient; a measure of precision.

[9] Bias correction factor; a measure of accuracy.

[10] Scale shift; change in standard deviation between predicted and observed methane emission.

[11] Location shift; if positive under prediction, if negative over prediction.

[12] Ratio of performance to deviation.

[13] Number of latent variables included in the Fourier-transform infrared based models or the number of milk fatty acids included in the milk fatty acid based models.

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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]	Difference estimated	
					MFA[2]	FTIR[3]
1	Warner et al. (2015)	Grass herbage 5 weeks of regrowth receiving high fertilization compared with grass herbage 3 weeks of regrowth receiving low fertilization	Production (g/d)	+31	+36	+8
			Yield (g/kg DMI)	+2.0	+1.9	+0.4
			Intensity (g/kg FPCM)	+1.3	+1.3	+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)	-12	-13	-15
			Yield (g/kg DMI)	-2.6	-2.9	-0.9
			Intensity (g/kg FPCM)	-1.3	-3.3	-0.8
3	Warner et al. (2016)	Grass silage 62 d of regrowth and high fertilization rate compared with grass silage 28 d of regrowth and low fertilization rate	Production (g/d)	-39	-4	+8
			Yield (g/kg DMI)	+1.6	+1.5	0.0
			Intensity (g/kg FPCM)	+4.6	+2.6	+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)	+24	+32	-1
			Yield (g/kg DMI)	+5.0	+2.8	+1.5
			Intensity (g/kg FPCM)	+4.0	+3.1	+2.6
6	Hatew et al. (2016)	Late harvested whole-plant corn silage compared with early harvested whole-plant corn silage	Production (g/d)	-29	+13	-0.4
			Yield (g/kg DMI)	-1.6	0.0	+0.3

	Intensity (g/kg FPCM)	-0.9	-1.1	+0.4

[1] Climate respiration chambers.

[2] Milk fatty acids in g/100 g fatty acids determined with gas chromatography.

[3] Fourier-transform infrared spectra.

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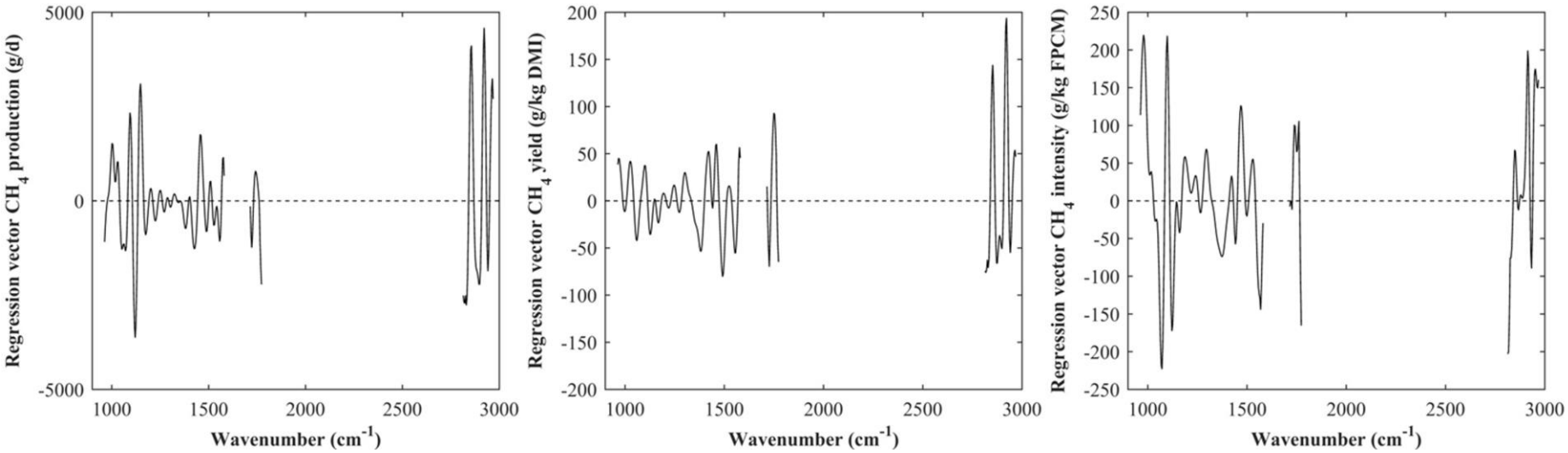
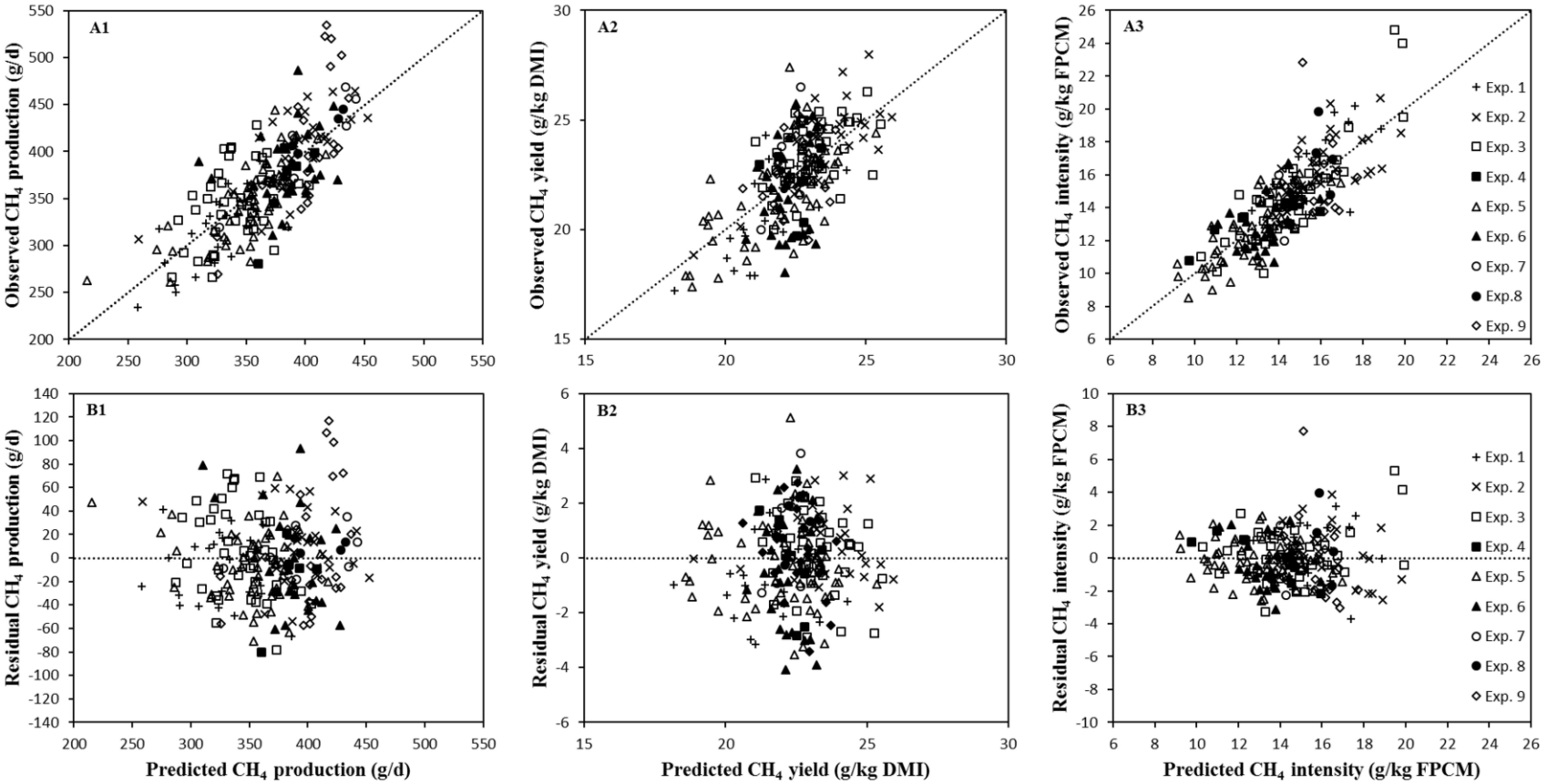


Figure 1. The regression vectors of the PLS models for methane production (g/d), yield (g/kg dry matter intake), and intensity (g/kg fat- and protein-corrected milk) plotted against wavenumbers (cm^{-1}).



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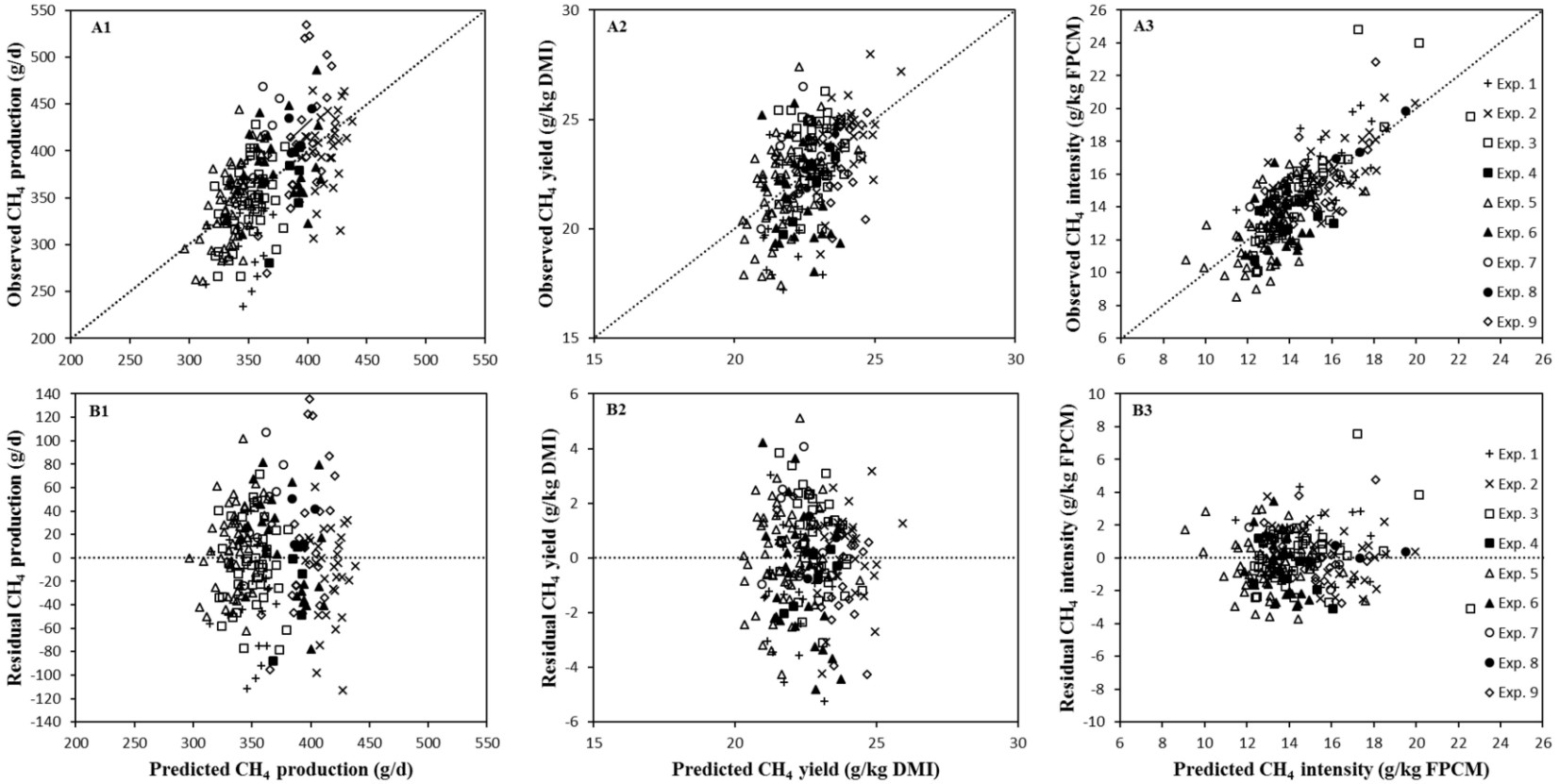
722 **Figure 2.** (A) Observed and predicted, and (B) residual (i.e., observed – predicted) (1) methane production (g/d), (2) methane yield (g/kg dry matter
723 intake), and (3) methane intensity (g/kg fat- and protein-corrected milk) from the regression analyses based on milk fatty acid profiles (g/100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



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727 **Figure 3.** (A) Observed and predicted, and (B) residual (i.e., observed – predicted) (1) methane production (g/d), (2) methane yield (g/kg dry matter
728 intake), and (3) methane intensity (g/kg fat- and protein-corrected milk) from the PLS regression analyses based on Fourier-transform infrared
729 wavenumbers (cm^{-1}). The slope of residuals regressed on predicted values did not differ significantly from zero. The different symbols identify the
730 9 individual experiments described in Table 1.

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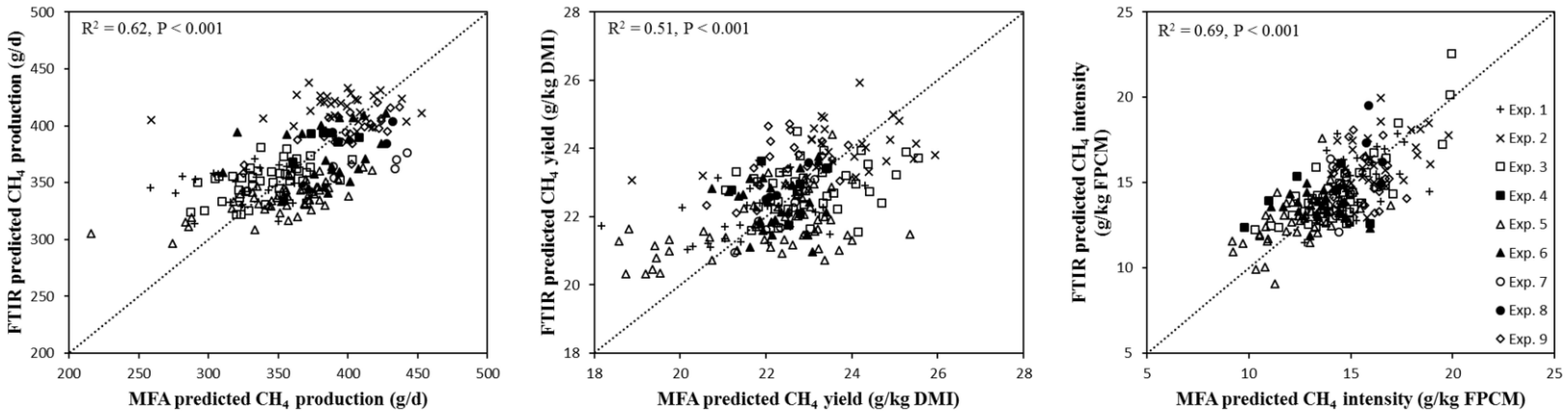

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733 **Figure 4.** The relationship between methane production (g/d), yield (g/kg dry matter intake), and intensity (g/kg fat- and protein-corrected milk)
734 predicted with milk fatty acid profiles determined with gas chromatography and predicted with milk Fourier-transform infrared spectra. The
735 different symbols identify the 9 individual experiments described in Table 1.