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# Milk fatty acids estimated by mid-infrared spectroscopy and milk yield can predict methane emissions in dairy cows

Stefanie W. Engelke<sup>1</sup> · Gürbüz Daş<sup>1</sup> · Michael Derno<sup>1</sup> · Armin Tuchscherer<sup>2</sup> · Werner Berg<sup>3</sup> · Björn Kuhla<sup>1</sup> · Cornelia C. Metges<sup>1</sup>

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## Abstract

Ruminant enteric methane emission contributes to global warming. Although breeding low methane-emitting cows appears to be possible through genetic selection, doing so requires methane emission quantification by using elaborate instrumentation (respiration chambers, SF<sub>6</sub> technique, GreenFeed) not feasible on a large scale. It has been suggested that milk fatty acids are promising markers of methane production. We hypothesized that methane emission can be predicted from the milk fatty acid concentrations determined by mid-infrared spectroscopy, and the integration of energy-corrected milk yield would improve the prediction. Therefore, we examined relationships between methane emission of cows measured in respiration chambers and milk fatty acids, predicted by mid-infrared spectroscopy, to derive diet-specific and general prediction equations based on milk fatty acid concentrations alone and with the additional consideration of energy-corrected milk yield. Cows were fed diets differing in forage type and linseed supplementation to generate a large variation in both CH<sub>4</sub> emission and milk fatty acids. Depending on the diet, equations derived from regression analysis explained 61 to 96% of variation of methane emission, implying the potential of milk fatty acid data predicted by mid-infrared spectroscopy as novel proxy for direct methane emission measurements. When data from all diets were analyzed collectively, the equation with energy-corrected milk yield ( $\text{CH}_4 \text{ (L/day)} = -1364 + 9.58 \times \text{energy-corrected milk yield} + 18.5 \times \text{saturated fatty acids} + 32.4 \times \text{C18:0}$ ) showed an improved coefficient of determination of cross-validation  $R^2_{\text{CV}} = 0.72$  compared to an equation without energy-corrected milk yield ( $R^2_{\text{CV}} = 0.61$ ). Equations developed for diets supplemented by linseed showed a lower  $R^2_{\text{CV}}$  as compared to diets without linseed (0.39 to 0.58 vs. 0.50 to 0.91). We demonstrate for the first time that milk fatty acid concentrations predicted by mid-infrared spectroscopy together with energy-corrected milk yield can be used to estimate enteric methane emission in dairy cows.

**Keywords** Dairy cows · Enteric methane emission · Methane proxy · Methane prediction equation · Mid-infrared spectroscopy · Milk fatty acids · Linseed supplementation

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## 1 Introduction

Livestock contributes approximately 18% to the global anthropogenic greenhouse gas emission (Hristov et al. 2013). Methane (CH<sub>4</sub>) mitigation through feeding and genetic selection has been reported to range from 2.5 to 19% in cattle (Knapp et al. 2014; Pickering et al. 2015). Genetic selection of low methane-emitting cows requires quantifying individual methane emission in large populations. However, in vivo methane quantification via respiration chambers, the SF<sub>6</sub> technique, and the GreenFeed system are not feasible on a large scale (Hammond et al. 2016). Biochemical relationships

among rumen fermentation pathways, CH<sub>4</sub> production, and milk fatty acid composition suggest the usefulness of milk fatty acid profiles to predict methane emission (liters/day) (Knapp et al. 2014; van Lingen et al. 2014) (Fig. 1). There have been several reports on the relationship between milk fatty acids and methane emission as well as on equations predicting methane yield defined as liters of CH<sub>4</sub> per kilogram dry matter intake and methane intensity defined as liters of CH<sub>4</sub> per kilogram energy-corrected milk yield (Castro-Montoya et al. 2017; van Lingen et al. 2014). Besides dry matter intake, the dietary composition, especially forage type and fat supplements, affects milk fatty acid profile and influences methane prediction equations (Dijkstra et al. 2016; Rico et al. 2016; van Lingen et al. 2014). Thus, diet-specific and general equations for estimation of methane emission are important for different farming conditions. Although gas chromatography is the reference technique to measure milk fatty acids, it is unsuitable for routine milk fatty acid determination. In contrast to gas chromatography, mid-infrared spectroscopy can inexpensively predict major milk fatty acids and groups of fatty acids but prediction quality differs among fatty acids (De Marchi et al. 2014; Soyeurt et al. 2011). A Belgian group predicted CH<sub>4</sub> emission by directly using the global milk mid-infrared spectra with partial least squares regression and compared results with multiple correlations between milk fatty acids and CH<sub>4</sub> (Dehareng et al. 2012). These authors concluded that global milk mid-infrared spectra could be more feasible than predicted milk fatty acids to estimate CH<sub>4</sub> emission (Dehareng et al. 2012). The methane prediction derived from global mid-infrared milk spectra by Dehareng et al. (2012) was further improved by including information on lactation stage (Vanlierde et al. 2015; Vanlierde et al. 2016). Dry

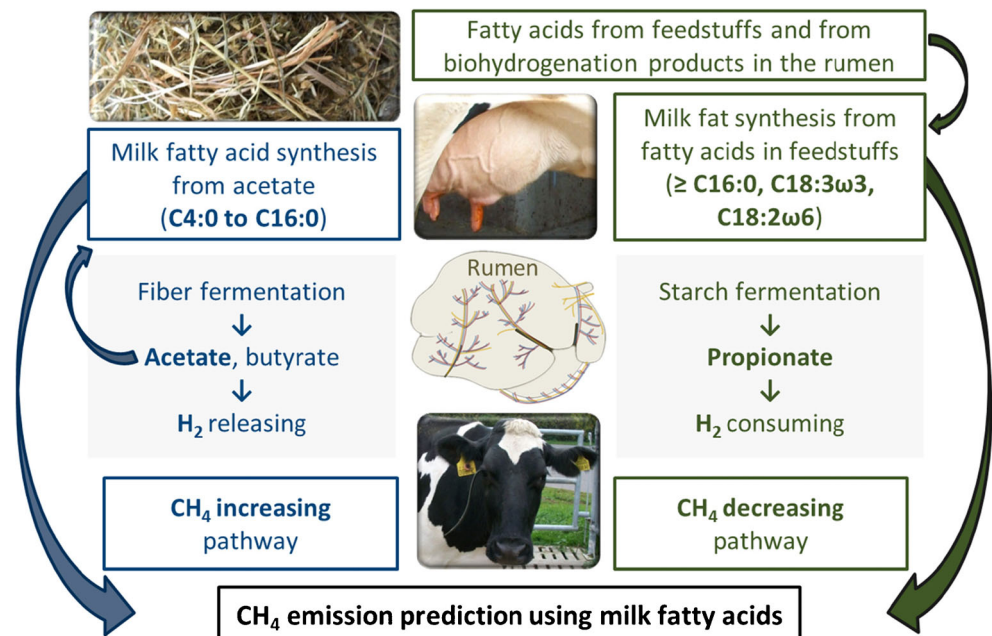
matter intake is the main determinant for CH<sub>4</sub> emission (Hristov et al. 2013; Knapp et al. 2014), but individual cow intake information is not available on farms. In contrast, individual records of energy-corrected milk yield, which is highly related to dry matter intake (Hristov et al. 2013), can readily be calculated from milk testing data. Thus, we hypothesized that CH<sub>4</sub> emission can be predicted from milk fatty acid concentrations determined by mid-infrared spectroscopy, and that the integration of energy-corrected milk yield improves prediction. Our objective was to develop equations that predict CH<sub>4</sub> emission on the basis of milk fatty acid composition estimated by mid-infrared spectroscopy. We further investigated whether inclusion of energy-corrected milk yield, compared with dry matter intake, could improve the prediction. The datasets were based on CH<sub>4</sub> emission of dairy cows measured in respiration chambers fed diets differing in forage type and linseed supplementation to generate a larger variation in both CH<sub>4</sub> emission and milk fatty acids.

## 2 Materials and methods

### 2.1 Animals, experimental design, and diets

Ethical approval for the study was obtained (permission no. 7221.3-1-014/14). Twenty half-sib German Holstein cows (106 ± 28 days in milk, 580 ± 57 kg bodyweight, mean ± standard deviation) in second (15 cows) or third lactation (5 cows) were used. The required number of cows for this study was determined through a power test for crossover experimental designs as described (Rasch et al. 1998) using CADEMO software (Windows ANOV-Version 4.03 (2000); BioMath

**Fig. 1** The physiological relationship between feeding and methane emission and between feeding and milk fatty acid composition. Our aim was to generate regression equations to predict methane emission by using the milk fatty acid composition estimated by mid-infrared spectroscopy



GmbH, Rostock, Germany). Cows were kept in tie stalls and provided with water and total mixed rations for ad libitum intake. Two isoenergetic total mixed rations, in which the major forage component was either corn silage or grass silage both with or without linseed supplementation, were used (7 MJ NE<sub>L</sub>/kg dry matter) to generate a wide range of variation in both methane emissions and milk fatty acid profiles. Diets contained grass and corn silage at dry matter levels of 130 and 450 g/kg (corn silage-based diets) and 360 and 190 g/kg (grass silage-based diets), respectively. Linseed-supplemented diets contained 60 g of fat/kg dry matter compared to 30 g of fat in diets without supplementation. The target nutrient content of the diets was according to recommendations of the German Society of Nutritional Physiology (Gesellschaft für Ernährungsphysiologie 2001). Randomly selected cows ( $n = 10$ ) were fed corn silage-based diets for 5 weeks without linseed supplementation and subsequently for another 5 weeks with linseed supplementation with 1 week in-between for a stepwise change of diets (11 experimental weeks per cow in total) in a randomized order (5 cows started with corn silage without linseed). The other 10 cows were allocated randomly to grass silage diets without or with linseed supplementation for 5 weeks each (5 cows started with grass silage without linseed). Feed intake was recorded daily. Residual feed was removed from the trough before cows were offered fresh feed at 0730 and 1730 h. Cows were milked at 0630 and 1630 h. Three cows feeding on the grass silage-

based diet supplemented with linseed had to be excluded from analysis because of illness or feed refusal.

## 2.2 Determination of methane production and feed and milk composition

Methane emission of each cow was quantified in separate respiration chambers in experimental weeks 5 and 11. Cows entered the chambers 15 h before starting two subsequent 24-h CH<sub>4</sub> measurements (0700 to 0700 h). The average 24-h CH<sub>4</sub> emission was calculated for each cow (Table 1). In the chambers, the temperature and relative humidity were 15 °C and 60%, respectively, and the light was on from 0600 to 1900 h (Demo et al. 2009). The CH<sub>4</sub> recovery in the chambers was 99.9%. Analyses of feed were performed according to the Weender standard analysis (Naumann et al. 1976) with modifications by van Soest et al. (1991) by the accredited feed laboratory Landwirtschaftliche Untersuchungs- und Forschungsanstalt der LMS Agrarberatung GmbH (LUFA, Rostock, Germany). The energy content was calculated as recommended (Gesellschaft für Ernährungsphysiologie 2001). During the respiration measurements, milk aliquots of one evening and the following morning milking were pooled (0.25% of milk yield per milking). Milk fat and protein content was analyzed using mid-infrared spectroscopy (MilkoScan FT6000 and MilkoScan FT+; Foss, Hilleroed, Denmark) by the state control association Mecklenburg-

**Table 1** Performance data and milk fatty acid composition of lactating cows ( $N = 20$ ) receiving rations based on grass silage or corn silage with and without linseed supplementation, and performance of milk fatty acid prediction by mid-infrared spectroscopy. Dry matter intake, energy-corrected milk yield, methane emission, and unsaturated fatty acids are

abbreviated as DMI, ECM, CH<sub>4</sub>, and UFA. Performance of mid-infrared spectroscopy equations to predict milk fatty acids: standard error of calibration ( $SE_C$ ), coefficient of determination of calibration ( $R^2_C$ ), root mean square error (RMSE) of cross-validation, and the cross-validation coefficient of determination ( $R^2_{CV}$ )

Variable	Mean	SD	Minimum	Maximum	$SE_C$	$R^2_C$	RMSE	$R^2_{CV}$
DMI (kg/day)	17.4	3.13	9.3	22.3	—	—	—	—
ECM (kg/day)	26.3	5.36	12.6	36.1	—	—	—	—
Milk fat (g/L)	42.8	6.10	32.1	56.7	—	—	—	—
Methane production								
CH <sub>4</sub> (L/day)	525	108	324	729	—	—	—	—
CH <sub>4</sub> /ECM (L/kg)	20.3	3.66	14.7	34.3	—	—	—	—
CH <sub>4</sub> /DMI (L/kg)	30.5	4.73	20.7	50.4	—	—	—	—
Fatty acid (% of total fat)								
Saturated	66.6	6.69	54.6	78.4	0.06	0.10	0.07	0.99
Unsaturated	33.4	6.69	21.6	45.4	0.05	0.99	0.05	0.99
Mono- (% UFA)	86.8	1.06	84.2	88.9	0.06	0.98	0.06	0.98
Poly- (% UFA)	13.2	1.06	11.1	15.8	0.02	0.83	0.03	0.81
C16:0	28.2	5.03	18.7	37.2	0.09	0.95	0.10	0.94
C18:0	12.5	2.43	8.8	16.7	0.06	0.91	0.06	0.90
C18:1 <i>cis</i>	22.2	4.51	13.9	31.0	0.06	0.97	0.07	0.96
C18:1 <i>trans</i>	3.81	1.39	1.9	6.9	0.03	0.86	0.03	0.85
$\omega$ -3 FA	0.80	0.13	0.57	1.05	0.01	0.76	0.01	0.74

Western Pomerania (Landeskontrollverband für Leistungs- und Qualitätsprüfung Mecklenburg-Vorpommern e.V., Güstrow, Germany). Energy-corrected milk yield (kg/d) was calculated as  $[(0.38 \times \text{milk fat } \%) + (0.21 \times \text{milk protein } \%) + 1.05]/3.28 \times \text{milk yield (kg/d)}$  (Spiekers et al. (2009). Milk fat and fatty acid compositions were analyzed using mid-infrared spectroscopy (MilkoScan FT6000) by a commercial laboratory (Comité du Lait, Battice, Belgium). Multivariate calibration equations using partial least squares regression were used to derive milk fatty acid concentrations. One milk sample (grass silage without linseed group) produced invalid results which were not used in the statistical analysis. Contents of saturated and unsaturated fatty acids, as well as isomers of C18:1 *cis* and *trans* and  $\omega$ -3 fatty acids, were predicted as groups and were expressed as the percentage of total milk fat. Mono- and polyunsaturated fatty acids were expressed as the percentage of unsaturated fatty acids in milk fat. Milk palmitic (C16:0) and stearic acids (C18:0) were estimated individually. Performance data of milk fatty acid prediction are given in Table 1.

## 2.3 Statistical analyses

### 2.3.1 Development of prediction equations and computing of correlations

Methane emission and CH<sub>4</sub> intensity were estimated using multiple linear regression analysis with the stepwise explanatory-independent variable selection method for each diet separately. Methane emission was also predicted based on combined data categorized by basal diet or linseed supplementation, as well as for all available data across four diets to explore diet dependency of methane prediction equations. This was done to test different feeding scenarios and to explore how explanatory milk fatty acids differ in prediction models for various diets. For the regression analysis, three datasets with different independent explanatory variables were made available. The first dataset included only the milk fatty acid concentrations (nine items: groups or individual milk fatty acids; Table 1), whereas the second set additionally included the energy-corrected milk yield, and the third set included dry matter intake instead of energy-corrected milk yield. Prediction models for CH<sub>4</sub> intensity were generated with datasets 1 and 3. The entry and stay levels of explanatory variables for the stepwise (forward) variable selection method were set at  $P \leq 0.15$ . Selection of best models was based on improvement in the multiple fit parameters as described by Kaps and Lamberson (2004). According to this, we selected best models that simultaneously maximized the coefficient of determination ( $R^2_{\text{Model}}$ ) and minimized the conceptual predictive criterion ( $C_p$ ), implying that the lowest number of variables that could explain the highest variation was allowed to enter the equations. In a separate crosscheck, it was also

ensured that best model selection based on the two fit parameters ( $R^2_{\text{Model}}$  and  $C_p$ ) also corresponded to the lowest Akaike's Information Criteria and residual sum of squares, two other commonly used fit parameters. For the sake of clarity, we however report only  $R^2_{\text{Model}}$  as a fit parameter because it is most commonly used and corresponds to the proportion of variation explained. Using the predicted milk fatty acid and measured methane emission data, Pearson correlations were computed. A Spearman rank correlation analysis was also performed to quantify whether the cow rankings for CH<sub>4</sub> emission levels were consistent between consecutive periods, when cows were fed diets with or without linseed in two different periods.

### 2.3.2 Cross-validation of prediction equations

To assess the predictive ability of the developed methane prediction models, an internal cross-validation was performed using SAS software (SAS Institute Inc. 2017). The cross-validation was based on the above-described datasets and run through a stepwise approach. Data sub-sets were generated by omitting data of one animal at a step, and the emission of the omitted animal was predicted using the regression function with the data from remaining animals as described (Moraes et al. 2014). Thus, methane prediction for a specific animal was generated using models fitted without the observation of these specific animals. The root mean square error of cross-validation (RMSE) and the cross-validation coefficient of determination ( $R^2_{\text{CV}}$ ) were then estimated to evaluate the goodness of model fit for the cross-validation.

## 3 Results and discussion

### 3.1 Methane emission and milk fatty acid composition

The dataset included a total of 36 observations from 20 cows to develop methane prediction equations feeding four different diets varying in basal rations (grass silage or corn silage) with or without linseed supplementation. The diets provoked a wide range of methane yield with a minimum of 21 and a maximum of 50 L/kg dry matter intake, and milk fatty acid composition ranged from 55 to 78% saturated fatty acids of total fat (Table 1). Results of studies in which 30 different diets with and without lipid supplements were fed were used in a meta-analysis (van Lingen et al. 2014); the methane yield ( $21.5 \pm 2.46$  g/kg of dry matter intake; mean  $\pm$  SD) was lower than that observed in our study but milk fat levels of C16:0 ( $31.3 \pm 4.91$  g/100 g of total milk fat) and C18:0 ( $9.8 \pm 2.41$  g/100 g of total milk fat) were comparable (Table 1). When diets based on grass and corn silage were fed, mean methane yields ( $24.1 \pm 1.87$  g/kg of dry matter intake) and milk fat levels of C16:0 ( $35.4 \pm 2.72$  g/100 g of total fatty acids) and C18:0 (7.5



$\pm 1.03$  g/100 g of total fatty acids) were reported (van Gastelen et al. 2017) but variability was smaller than in our and the van Lingen et al. (2014) study.

Our results for milk fatty acid composition were largely in line with an earlier study where milk fatty acid contents were also determined by mid-infrared spectroscopy-based predictions (Soyeurt et al. 2011). It has been shown that milk fatty acid composition analyzed by mid-infrared spectroscopy as compared to gas chromatography corresponded well (Soyeurt et al. 2011). Predictions of milk fatty acid contents by mid-infrared spectroscopy validated by gas chromatography showed high accuracies ( $R^2_{CV} \geq 0.88$ ) for individual saturated fatty acids, C16:0, C18:0, C18:1*trans*, and C18:1*cis*, and groups of saturated, unsaturated, and monounsaturated fatty acids, whereas accuracy was  $R^2_{CV} = 0.75$  for  $\omega$ -3 fatty acids, which was similar in our study (Table 1). This agreement between mid-infrared spectroscopy and gas chromatography is the prerequisite to use mid-infrared spectroscopy for routine milk fatty acid recordings.

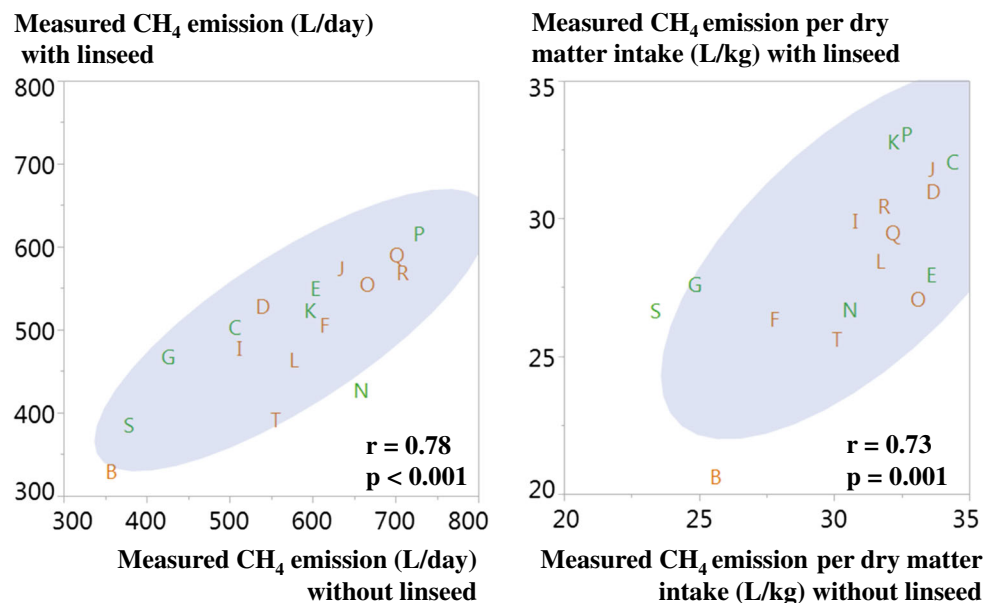
Furthermore, we calculated rank correlation coefficients of  $r = 0.78$  and  $r = 0.73$  ( $P = 0.001$ ) for  $\text{CH}_4$  emission and  $\text{CH}_4$  yield, respectively (Fig. 2). These results indicate replicable responses under the effects of either diet with or without linseed, representing different environmental conditions, and suggested the existence of a genetic component responsible for the individual methane production level.

### 3.2 Predicting methane emission

Most studies reporting prediction equations have been based on milk fatty acid concentrations analyzed by gas chromatography (Castro-Montoya et al. 2017; Rico et al. 2016; van Lingen et al. 2014). Published prediction models based on

global infrared milk spectra have used the model of Dehareng et al. (2012) or further improvement of the model by integrating the lactation stage (Vanlierde et al. 2015; Vanlierde et al. 2016). Dehareng et al. (2012) included methane production data of 11 cows and have reported  $R^2_{CV}$  between 0.68 and 0.79 for grams of  $\text{CH}_4$  per day and grams of  $\text{CH}_4$  per kilogram of milk, respectively. Shetty et al. (2017) developed methane prediction models based on global infrared milk spectra with  $R^2_{\text{validation}} = 0.13$  and concluded that it is not feasible to predict methane emission (liters/day) based on mid-infrared spectra alone. Upon integrating milk yield, the prediction accuracy was improved ( $R^2_{\text{validation}} = 0.35$ ) (Shetty et al. 2017). In the present study, three datasets were used to develop multiple regression equations. When energy-corrected milk yield was additionally included (dataset 2), the goodness of fit was improved by up to 25% relative to the equations based on dataset 1 (Table 2). When energy-corrected milk yield was replaced by dry matter intake in the model (dataset 3; Table 2), the goodness of fit was improved by up to 41% relative to equations derived from dataset 1. Although dry matter intake explains a large part of the variation observed in methane emissions (Knapp et al. 2014), this information is not available on farms. Thus, it cannot be considered as a variable to be implemented in methane prediction equations for on-farm applications. In contrast, energy-corrected milk yield is a readily available cow individual measure reflecting the dry matter intake (Hristov et al. 2013) and consequently can be used as a surrogate for dry matter intake. Using the first dataset, the goodness of the regression fit for  $\text{CH}_4$  emission for individual diets ranged from  $R^2_{\text{Model}} = 0.61$  to 0.94 (Table 2). Using data from all four diets collectively, a coefficient of determination of  $R^2_{\text{Model}} = 0.70$  was obtained.

**Fig. 2** Correlation of measured  $\text{CH}_4$  emission in liters per day and measured  $\text{CH}_4$  emission per kilogram dry matter intake between linseed supplemented and non-supplemented diets considering individual animals (A, B, C, ...), and basal ration (grass silage = green, corn silage = orange) indicating repeatable phenotypes under the effect of either diets with or without linseed. The shaded area depicts the confidence ellipse. The Spearman rank correlation coefficient ( $r$ ) and the probability ( $p$ ) are given



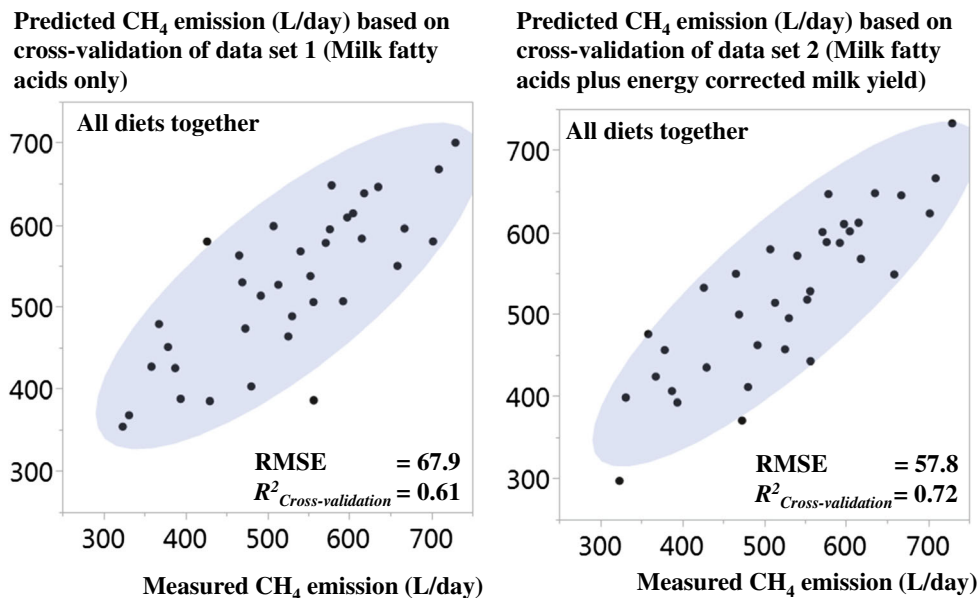
**Table 2** Summary of multiple regression equations predicting methane emission. Dataset 1 included milk fatty acids (saturated, unsaturated, mono- and polyunsaturated fatty acids, and  $\omega$ -3 fatty acids are abbreviated as SFA, UFA, MUFA, PUFA, and  $\omega$ 3, as well as isomers of C18:1*cis* and *trans* and single fatty acids of C16:0 and C18:0), whereas datasets 2 and 3 additionally included energy-corrected milk yield (ECM) or dry matter intake (DMI) as independent variables. Equations were developed separately for experimental diets containing grass silage

without or with linseed supplementation (GS-L0, GS-LS) and corn silage without or with linseed supplementation (CS-L0, CS-LS) as well as for basal rations (GS, CS), rations without or with linseed supplementation (L0, LS), and all experimental diets collectively. The  $R^2_{\text{Model}}$  and the  $P_{\text{Model}}$  value of the model, the root mean square error (RMSE) of cross-validation, and the cross-validation coefficient of determination ( $R^2_{\text{CV}}$ ) are given

CH <sub>4</sub> (L/d)	N	Equation derived from the models ( $Y = a + b_1x_1 + \dots + b_nx_n$ )	$R^2_{\text{Model}}$	$P_{\text{Model}} \leq$	RMSE	$R^2_{\text{CV}}$
<b>Dataset 1</b>						
GS-L0	9	$(-979.3) + 46.2 \times \text{C16:0}$	0.94	0.001	41.91	0.91
GS-LS	7	$1002 + (-19.6) \times \text{C18:1cis}$	0.70	0.019	59.52	0.41
CS-L0	10	$(-702.6) + 40.4 \times \text{C16:0}$	0.68	0.004	71.58	0.51
CS-LS	10	$1233 + (-27.9) \times \text{C18:1cis}$	0.61	0.008	65.69	0.39
GS	16	$(-2217) + 39.7 \times \text{PUFA} + 46.9 \times \text{C16:0} + 70.8 \times \text{C18:0}$	0.82	0.001	65.41	0.68
CS	20	$2351 + (-24.8) \times \text{C16:0} + 71.7 \times \text{C18:0} + (-50.8) \times \text{C18:1cis} + (-1093) \times \omega\text{-3}$	0.75	0.001	74.08	0.50
L0	19	$(-2595) + 22.5 \times \text{SFA} + 27.4 \times \text{C16:0} + 61.5 \times \text{C18:0}$	0.86	0.001	146.9	0.50
LS	17	$1099 + (-23.0) \times \text{C18:1cis}$	0.61	0.001	54.78	0.50
All diets	36	$(-2534) + 33.8 \times \text{SFA} + 55.0 \times \text{C18:0} + 30.9 \times \text{C18:1trans}$	0.70	0.001	67.94	0.61
<b>Dataset 2</b>						
GS-L0	9	$(-979.3) + 46.2 \times \text{C16:0}$	0.94	0.001	41.91	0.91
GS-LS	7	$1002 + (-19.6) \times \text{C18:1cis}$	0.70	0.019	59.52	0.41
CS-L0	10	$(-604.9) + 18.2 \times \text{ECM} + 20.8 \times \text{C16:0}$	0.85	0.001	57.39	0.67
CS-LS	10	$27.42 + 16.7 \times \text{ECM}$	0.66	0.004	59.53	0.47
GS	16	$(-1020) + 10.6 \times \text{ECM} + 27.8 \times \text{C16:0} + 38.8 \times \text{C18:0}$	0.82	0.001	64.67	0.69
CS	20	$284.1 + 16.8 \times \text{ECM} + (-6.58) \times \text{UFA}$	0.77	0.001	59.31	0.66
L0	19	$(-558.4) + 10.0 \times \text{ECM} + 24.3 \times \text{C16:0} + 47.9 \times \text{C18:0} + (-617) \times \omega\text{-3}$	0.91	0.001	49.96	0.84
LS	17	$721.3 + 7.97 \times \text{ECM} + (-16.8) \times \text{C18:1cis}$	0.71	0.001	50.24	0.58
All diets	36	$(-1364) + 9.58 \times \text{ECM} + 18.5 \times \text{SFA} + 32.4 \times \text{C18:0}$	0.79	0.001	57.75	0.72
<b>Dataset 3</b>						
GS-L0	9	$(-979.3) + 46.2 \times \text{C16:0}$	0.94	0.001	41.91	0.91
GS-LS	7	$1002 + (-19.6) \times \text{C18:1cis}$	0.70	0.019	59.52	0.41
CS-L0	10	$153.0 + 33.3 \times \text{DMI} + (-23.1) \times \text{C18:1cis} + 90.3 \times \text{C18:1trans}$	0.96	0.001	34.46	0.88
CS-LS	10	$1233 + (-27.9) \times \text{C18:1cis}$	0.61	0.008	65.69	0.39
GS	16	$(-2217) + 39.7 \times \text{PUFA} + 46.9 \times \text{C16:0} + 70.8 \times \text{C18:0}$	0.82	0.001	65.41	0.68
CS	20	$211.0 + 26.9 \times \text{DMI} + 29.8 \times \text{C18:0} + (-24.1) \times \text{C18:1cis}$	0.90	0.001	40.27	0.84
L0	19	$(-1286) + 12.1 \times \text{DMI} + 37.4 \times \text{C16:0} + 40.4 \times \text{C18:0}$	0.87	0.001	58.13	0.79
LS	17	$292.2 + 21.2 \times \text{DMI} + 25.8 \times \text{C18:0} + (-20.9) \times \text{C18:1cis}$	0.78	0.001	50.95	0.58
All diets	36	$361.4 + 18.9 \times \text{DMI} + 28.5 \times \text{C18:0} + (-23.6) \times \text{C18:1cis}$	0.82	0.001	53.85	0.75

The equations based on datasets 1 to 3 were validated by an internal cross-validation (Table 2). The  $R^2_{\text{CV}}$  ranged between 0.39 and 0.91 with a RMSE between 147 and 35 liters/day and the  $R^2_{\text{CV}}$  was lowest for linseed-supplemented diets. If linseed-supplemented diets were not considered, the  $R^2_{\text{CV}}$  was never lower than 0.50. When coefficients of determination were compared between dataset 1 and datasets 2 and 3 for all four diets collectively, the  $R^2_{\text{CV}}$  was improved from 0.61 to 0.72 by the integration of energy-corrected milk yield (Fig. 3), and to  $R^2_{\text{CV}} = 0.75$  by the integration of dry matter intake as

explanatory variables. In previous studies predicting CH<sub>4</sub> emission (g/day) using gas chromatography to determine milk fatty acid concentrations, improvements of goodness of fit values by 5 to 12% were achieved when dry matter intake was considered in the model (Mohammed et al. 2011; Rico et al. 2016). In a meta-analysis, improved indices of predictive ability were observed when dry matter intake was included (Castro-Montoya et al. (2017)). Nevertheless, a further cross-validation of our prediction equation using an independent validation set of cows fed different diets is desirable.



**Fig. 3** Measured and predicted CH<sub>4</sub> emission derived from the cross-validation of multiple regression equations across all experimental diets (grass silage without linseed, corn silage without linseed, grass silage with linseed and corn silage with linseed) based on dataset 1 (milk fatty acids

only) and dataset 2 (milk fatty acids plus energy-corrected milk yield). The shaded area depicts the confidence ellipse. The cross-validation coefficient of determination ( $R^2_{\text{Cross-validation}}$ ) and the root mean square error (RMSE) of cross-validation are given

A further factor which might be important for methane prediction is stage of lactation, because in early lactation, fatty acids entering milk fat are derived to a larger degree from lipolysis of lipid resources of cows, which can affect explanatory fatty acids in methane prediction models (Vanlierde et al. 2015). The patterns of fatty acids from endogenous lipid depots differ from fatty acids in plant oils (Bayat et al. 2018). Vanlierde et al. (2015) tested the effect of inclusion of days in milk in the prediction model based on milk mid-infrared spectra and showed that lactation stage-dependent prediction is more meaningful compared to lactation stage-independent prediction of methane emission although coefficient of determination did not improve. In a recent study, integrating lactation stage in the model did not improve methane predictive ability ( $R^2_{\text{Validation}} = 0.14$  vs. 0.13) (Shetty et al. 2017). In our study, all cows were in the same lactation stage which might be limiting the validity of our equation to cows in established lactation. This suggests that further research is required on the effect of lactation stage for milk fatty acid-based methane prediction.

Dietary intake of  $\omega$ -3 fatty acids with linseed reduces methane emissions and increases the proportion of polyunsaturated fatty acids in milk whereas it can inhibit mammary lipid synthesis by fatty acid intermediates produced during ruminal bio-hydrogenation (Bauman et al. 2011; Bayat et al. 2018). This complex relationship might play a role in the variability of methane production and milk fatty acid contents. Notably, the predictions obtained in this study with linseed-supplemented diets showed smaller goodness of fit values and cross-validation coefficient of determination values (Table 2) although the RMSE was highest for L0

diets in dataset 1. However, when energy-corrected milk yield or dry matter intake was considered as additional explanatory variable (datasets 2 and 3), also in diets with linseed a larger part of variation could be explained. This implies that inclusion of other parameters explaining additional variation of methane emission should also be considered.

Prediction of CH<sub>4</sub> intensity would be desirable because it considers the product level. However, in our study, CH<sub>4</sub> intensity was not a robust parameter; prediction equations using the first dataset produced largely insignificant weak to moderate fit values. The use of dataset 3 did not generate considerable improvements when compared to dataset 1. In line with this, others found moderate to low  $R^2$  of 0.47 and  $R^2_{\text{Validation}}$  of 0.18 (Castro-Montoya et al. 2017; van Lingen et al. 2014) for predicted CH<sub>4</sub> emission per kilogram milk based on milk fatty acid data only.

The explanatory fatty acids in published prediction equations for methane emission differ considerably between studies (Castro-Montoya et al. 2017; van Lingen et al. 2014). The milk fatty acids C16:0 and C18:1*cis* and *trans* isomers are frequently found in prediction models for CH<sub>4</sub> emission (Castro-Montoya et al. 2017; Chilliard et al. 2009; Rico et al. 2016). A positive relationship with methane emission was found for C16:0 in this ( $r = 0.57$ ,  $P < 0.001$ ) and other studies (Castro-Montoya et al. 2017; Chilliard et al. 2009; van Lingen et al. 2014). Palmitic acid is the saturated fatty acid with the highest concentration in plant feed and is also synthesized de novo from acetate in animal tissues, and acetate concentration in the rumen is directly associated with methane production (van Lingen et al. 2014). In contrast,



C18:0 ( $r = -0.34$ ,  $P = 0.044$ ) and C18:1*cis* isomers ( $r = -0.61$ ,  $P < 0.001$ ) were negatively related to methane emission in our study. Vanrobays et al. (2016) have found weak negative relations for C18:0 and C18:1*cis*9 analyzed with mid-infrared spectroscopy, whereas studies using gas chromatography have reported no correlation for C18:0, but various C18:1*cis* isomers, and the sum of C18 fatty acids was negatively related to methane production (Chilliard et al. 2009; Mohammed et al. 2011; Rico et al. 2016). Stearic acid is an end product of C18:3  $\omega$ -3 bio-hydrogenation and C18:3  $\omega$ -3 is particularly abundant in linseed. Products of bio-hydrogenation and intermediates were negatively related to CH<sub>4</sub> emission (van Gastelen and Dijkstra 2016). In our study, C18:1*trans* isomers were a factor in the model for CH<sub>4</sub> emission in the equation of dataset 1 for all diets. They tended to be negatively related to methane emission ( $r = -0.31$ ,  $P = 0.066$ ), in line with findings from other studies (Mohammed et al. 2011; Rico et al. 2016). Groups of fatty acids appeared to play a minor role as prediction variables in our models, but the group of saturated fatty acids was positively associated with methane emission ( $r = 0.60$ ,  $P < 0.001$ ) as was also found by others (van Gastelen and Dijkstra 2016; Vanrobays et al. 2016). No specific pattern for milk fatty acids as explanatory variables could be identified in the equations. However, it appears that among the milk fatty acids considered in the datasets, C16:0 plays a dominant role in the prediction of methane emission by milk fatty acids.

## 4 Conclusion

The prediction of methane emission using data of all four diets collectively showed moderate predictive accuracies ( $R^2_{CV}$  between 0.61 and 0.75). When energy-corrected milk yield or dry matter intake was additionally considered in the models, prediction accuracy improved by 18 and 23%, respectively. Thus, energy-corrected milk yield in prediction equations is a useful replacement for dry matter intake which cannot be determined on farm. Models developed for diets including linseed seem to be lower in prediction accuracy which deserves further research. We conclude that methane prediction equations based on milk fatty acids estimated by mid-infrared spectroscopy and energy-corrected milk yield could be used as a screening method for individual methane emission of cows.

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## Compliance with ethical standards

Ethical approval for the study was obtained (permission no. 7221.3-1-014/14).

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## Patent

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