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Prediction of enteric methane emissions from Holstein dairy cows fed various forage sources

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Milk fatty acid (FA) profile has been previously used as a predictor of enteric CH_4 output in dairy cows fed diets supplemented with plant oils, which can potentially impact ruminal fermentation. The objective of this study was to investigate the relationships between milk FA and enteric CH_4 emissions in lactating dairy cows fed different types of forages in the context of commonly fed diets. A total of 81 observations from three separate 3 × 3 Latin square design (32-day periods) experiments including a total of 27 lactating cows (96 ± 27 days in milk; mean ± SD) were used. Dietary forages were included at 60% of ration dry matter and were as follows: (1) 100% corn silage, (2) 100% alfalfa silage, (3) 100% barley silage, (4) 100% timothy silage, (5) 50 : 50 mix of corn and alfalfa silages, (6) 50 : 50 mix of barley and corn silages and (7) 50 : 50 mix of timothy and alfalfa silages. Enteric CH_4 output was measured using respiration chambers during 3 consecutive days. Milk was sampled during the last 7 days of each period and analyzed for components and FA profile. Test variables included dry matter intake (DMI; kg/day), NDF (%), ether extract (%), milk yield (kg/day), milk components (%) and individual milk FA (% of total FA). Candidate multivariate models were obtained using the Least Absolute Shrinkage and Selection Operator and Least-Angle Regression methods based on the Schwarz Bayesian Criterion. Data were then fitted into a random regression using the MIXED procedure including the random effects of cow, period and study. A positive correlation was observed between CH_4 and DMI ($r = 0.59$, $P < 0.001$), whereas negative associations were observed between CH_4 and cis*9-17:1 ($r = -0.58$, $P < 0.001$), and* trans*8,* cis*13-18:2 ($r = -0.51$, $P < 0.001$). Three different candidate models were selected and the best fit candidate model predicted CH_4 with a coefficient of determination of 0.84 after correction for cow, period and study effects and was: CH_4 (g/day) = 319.7 − 57.4 × 15:0 − 13.8 ×* cis*9-17:1 − 39.5 ×* trans*10-18:1 − 59.9 ×* cis*11-18:1 − 253.1 ×* trans*8,* cis*12-18:2 − 642.7 ×* trans*8,* cis*13-18:2 − 195.7 ×* trans*11,* cis*15-18:2 + 16.5 × DMI. Overall and linear prediction biases of all models were not significant ($P > 0.19$). Milk FA profile and DMI can be used to predict CH_4 emissions in dairy cows across a wide range of dietary forage sources.*

Keywords: dairy cow, fatty acid, forage, methane emission

Implications

Given the importance of CH_4 as an anthropogenic greenhouse gas, the quantification of emissions is important to better understand and mitigate them. The present study investigated the relationships between milk fatty acids and enteric CH_4 emissions in lactating dairy cows fed different types of forage. The wide range of forage species used in the present data set is expected to represent equally diverse feeding conditions present in dairy farms. Thus, the CH_4 prediction models here proposed could be applicable to commercial conditions and aid in the effort to estimate CH_4 emissions.

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Introduction

In addition to its relevance in terms of environmental impact, CH_4 resulting from digestive processes in ruminants represents important dietary energy losses (Kebreab *et al.*, 2008), which can vary depending on several dietary factors (Ramin and Huhtanen, 2013). However, direct measurements of CH_4 are difficult to perform under regular farm conditions, and therefore, the development of prediction equations to estimate CH_4 output has garnered considerable interest over the course of more than one decade (Benchaar *et al.*, 1998; Vlaeminck and Fievez, 2005; Ramin and Huhtanen, 2013).

Several factors are known to affect CH_4 output, including dietary constituents such as carbohydrates, lipids and ionophores, all of which can potentially affect the predominant

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ruminal microbial population (Johnson and Johnson, 1995; Sauer *et al*., 1998; Weimer *et al*., 2010). However, dry matter intake (DMI) is considered to be a major factor explaining CH_4 emissions, and equations often include it as a predictor (Benchaar *et al*., 1998; Ramin and Huhtanen, 2013). Milk fatty acid (FA) profile can reflect changes in absorbed FA composition, which in turn is affected by ruminal metabolism of lipids, including lipolysis, biohydrogenation (BH) and microbial synthesis (Fievez *et al*., 2012), and may thus predict important changes in the ruminal environment. Therefore, several studies have reported prediction models for CH_4 output using milk FA profile. However, prediction equations are often obtained from experiments in which dietary components such as oils are evaluated (Chilliard *et al*., 2009; Mohammed *et al*., 2011), which may not be representative of commonly fed forage-based diets. Importantly, Chung *et al*. (2011) reported that dietary inclusion of linseed as a source of polyunsaturated fatty acid (PUFA) was associated with decreased CH_4 output in dairy cows when fed barley silage-, but not when fed grass hay-based diets, suggesting an important interaction with forage type, which may make prediction equations only relevant in certain scenarios. Others have reported the associations between CH_4 and milk FA across experiments using different dietary fat sources with potential negative effects on ruminal microorganisms, such as PUFA and medium-chain fatty acid (MCFA), or across different forages (Castro-Montoya *et al*., 2011; Dijkstra *et al*., 2011; van Lingen *et al*., 2014). It is therefore possible that these associations between CH_4 and milk FA profile might not apply when diets do not include such dietary fat supplements. Moreover, Mohammed *et al*. (2011) evaluated a set of previously published models (Chilliard *et al*., 2009; Dijkstra *et al*., 2011) and reported a mean overestimation of CH_4 ranging from 19% to 61%, thus evidencing the difficulty in the application of some models to predict CH_4 emissions.

Although the number of different FA present in milk has been estimated to be >400, routine separation of all these FA is technically demanding and most analyses only identify a small fraction of them (Jensen, 2002). Consequently, it is likely that different prediction models would result from the association of CH_4 to other milk FA, which are not commonly identified.

The objective of the present study was to investigate the relationships between a wide range of milk FA and enteric CH_4 emissions in lactating dairy cows using individual observations from cows fed different forage species.

Material and methods

Data set description

A total of 81 observations from three 3 × 3 Latin square design (32-day periods) experiments (Hassanat *et al*., 2013 and 2014; Benchaar *et al*., 2014) using a total of 27 (n = 9/experiment) lactating cows (96 ± 27 days in milk; mean ± SD) were used. All experiments were conducted at the Dairy and Swine Research and Development Centre, Sherbrooke, Quebec, Canada, and all animal procedures were approved by the local Animal Care Committee in agreement with the guidelines of the Canadian Council on Animal Care (1993).

Dietary forages included at 60% of ration dry matter (DM) were as follows: (1) 100% corn silage (n = 18), (2) 100% alfalfa silage (n = 18), (3) 100% barley silage (n = 9), (4) 100% timothy silage (n = 9), (5) 50 : 50 mix of corn and alfalfa silages (n = 9), (6) 50 : 50 mix of barley and corn silages (n = 9) and (7) 50 : 50 mix of timothy and alfalfa silages (n = 9). All cows were housed in a tie-stall barn, had continuous access to water and were fed *ad libitum* in two equal servings at 0900 and 1930 h.

Experimental measurements

All feed analyses and experimental measurements were performed after a 2-week adaptation period to diet as previously described (Hassanat *et al*., 2013 and 2014; Benchaar *et al*., 2014). Enteric CH_4 output was measured for each cow during 3 consecutive days using individual respiration chambers as described by Hassanat *et al*. (2013). Cows were milked twice daily at 0700 and 1900 h in their respective stalls and milk was sampled from each cow at each milking and stored at −20°C without preservative until analyzed for FA composition. Milk lipids were extracted and methylated according to the study by Chouinard *et al*. (1997). FAs were quantified in a gas chromatograph (7890 A GC; Agilent Technologies Canada, Inc., Mississauga, ON, Canada) equipped with a 100-m CP-Sil 88 capillary column (0.25-mm i.d., 0.20-μm film thickness; Agilent Technologies Canada Inc.) and a flame ionization detector. Co-eluting peaks were separated and identified using a set of three different temperature programs as described by Kramer *et al*. (2008) with modifications (Boivin *et al*., 2013). Most FA peaks were identified and quantified using either a quantitative mixture or pure methyl ester standards (Larodan Fine Chemicals, Solna, Sweden; Sigma-Aldrich Canada Ltd, Oakville, ON, Canada; Matreya LLC, Pleasant Gap, PA, USA; Nu Chek Prep, Elysian, MN, USA). *cis*9, *trans*11, *cis*15-18:3 was identified using a qualitative standard obtained from Naturia, Inc. (Sherbrooke, QC, Canada). Given no quantitative standard was available, the peak response factor for *cis*9, 12, 15-18:3 was used to adjust the peak areas of this isomer. The 18:1 and 18:2 isomers for which standards were not commercially available were identified by order of elution according to the studies by Precht *et al*. (2001) and Kramer *et al*. (2008), and the response factors for *cis*9-18:1 and *cis*9, 12-18:2 were used to quantify these peaks, respectively. A partial chromatogram of this region is shown in Supplementary Figure S1.

Statistical analysis

Test variables included the chemical composition (CP, NDF, starch and ether extract (EE); % of DM) of dietary treatments, daily DMI, daily milk yield (Table 1), and 83 individual milk FA and FA sums (Table 2). Associations between test variables and CH_4 were analyzed using the CORR procedure of SAS (The SAS Institute, Inc., Cary, NC, USA). The GLMSELECT procedure was used to identify a set of candidate models using the Least Absolute Shrinkage and Selection Operator (LASSO; Tibshirani, 1996) and Least Angle Regression (LARS; Efron *et al*., 2004)

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Table 1 *Descriptive statistics of diet composition, production variables and CH_4 output of a data set including 81 observations from 27 lactating Holstein dairy cows fed various forage sources*

Variables	Mean	SD	Minimum	Maximum
% of DM				
CP	16.3	0.9	13.7	18.6
NDF	32.8	3.6	26.8	40.8
Starch	20.0	5.2	10.9	31.0
Ether extract	5.3	1.2	2.9	7.5
kg/day				
DMI	24.1	2.4	17.9	32.2
Forage intake	14.5	1.5	10.7	19.3
Milk yield	35.6	5.2	20.0	46.0
Milk fat (%)	3.79	0.55	1.88	5.37
Milk protein (%)	3.23	0.26	2.74	4.01
CH_4 (g/day)	484	58	315	633

DM = dry matter; DMI = dry matter intake.

methods by the Schwarz Bayesian Criterion (SBC). Three sets of variables were used for model selection. The first set included individual milk FA, milk yield, DMI and dietary components, whereas the second set included the same variables in addition to FA sums. The last set included dietary components and milk FA, but not milk yield or DMI. For highly correlated test variables, the variable with the highest association with CH_4 was kept in the data set (Supplementary Table S1). Selected models were initially evaluated in Prog REG in terms of multicollinearity (variance inflation factor >10), high influence and leverage observations by DFFITS ($>2\sqrt{p/n}$; where p is the number of parameters estimated in the model and n the number of observations) and Cook's distance ($>4/n$), and homoscedasticity by the normality of the residuals. Selected models were then fitted into separate random regressions using the MIXED procedure of SAS accounting for the random effects of cow, period and study. In addition to the random effects, each model included the selected test variables as fixed effects. Cow was the subject of the repeated statement, and the model included a random intercept. The VC and UN covariance structures provided best fit for the random and repeated statements, respectively. Denominator degrees of freedom were calculated by the Satterthwaite equation. The associations between observed CH_4 output and the values predicted by each model were evaluated by linear regression. In addition, prediction bias was assessed by regressing residuals against predicted values (St-Pierre, 2003). For each model, predicted values were centered around their mean value and used as an independent variable as provided in the following equation:

$$e_i = b_0 + b_1\left(X_i - \overline{X}\right) + \check{e}_i$$

where e_i is the model estimated residual, b_0, b_1 the intercept and the slope, respectively, X_i the i^{th} predicted value of CH_4, $\overline{X}$ the mean of all predicted values and $\check{e}_i$ the error term.

Table 2 *Descriptive statistics of milk fatty acids of a data set including 81 observations from 27 lactating Holstein dairy cows fed various forage sources*

Fatty acid (% of total fatty acids)	Mean	SD	Minimum	Maximum
4:0	4.493	1.039	2.652	7.057
6:0	2.508	0.440	1.867	3.418
8:0	1.322	0.203	0.956	1.714
10:0	2.947	0.458	2.033	4.158
*cis*9-10:1	0.292	0.043	0.174	0.367
11:0	0.082	0.044	0.034	0.207
12:0	3.402	0.612	2.241	5.481
iso-13:0	0.031	0.007	0.017	0.055
anteiso-13:0	0.017	0.005	0.010	0.033
*cis*9-12:1	0.093	0.022	0.046	0.157
13:0	0.132	0.051	0.068	0.297
iso-14:0	0.122	0.038	0.051	0.217
14:0	12.311	1.120	9.470	14.750
iso-15:0	0.217	0.032	0.161	0.295
anteiso-15:0	0.440	0.052	0.300	0.576
*cis*9-14:1	1.094	0.237	0.590	1.644
*cis*11-14:1	0.042	0.016	0.017	0.109
15:0	1.329	0.294	0.897	2.300
iso-16:0	0.255	0.075	0.126	0.557
16:0	34.990	2.720	29.280	40.547
*trans*9-16:1	0.038	0.009	0.023	0.064
iso-17:0[1]	0.279	0.048	0.186	0.410
*cis*9-16:1[2]	1.574	0.348	0.865	2.592
anteiso-17:0[3]	0.461	0.065	0.324	0.656
*cis*11-16:1	0.030	0.012	0.013	0.096
*cis*13-16:1	0.157	0.043	0.084	0.266
17:0	0.587	0.087	0.440	0.827
iso-18:0	0.041	0.016	0.020	0.080
*cis*7-17:1	0.033	0.007	0.017	0.049
*cis*8-17:1	0.018	0.012	0.005	0.067
*cis*9-17:1	0.228	0.047	0.148	0.417
18:0	7.863	1.507	5.183	10.688
*trans*4-18:1	0.016	0.007	0.005	0.034
*trans*5-18:1	0.013	0.005	0.004	0.026
*trans*6–8-18:1	0.198	0.054	0.119	0.348
*trans*9-18:1	0.179	0.049	0.103	0.276
*trans*10-18:1	0.320	0.177	0.174	1.554
*trans*11-18:1	0.619	0.166	0.200	1.195
*trans*12-18:1	0.190	0.055	0.087	0.346
*cis*6–8-18:1	0.119	0.058	0.003	0.266
*trans*13–14-18:1	0.247	0.131	<0.001	0.598
*cis*9-18:1[4]	15.650	2.420	11.076	22.651
*trans*15-18:1	0.513	0.281	0.127	1.098
*cis*11-18:1	0.702	0.213	0.297	1.270
*cis*12-18:1	0.287	0.097	0.135	0.676
*cis*13-18:1	0.065	0.040	0.025	0.198
*cis*14-18:1	0.037	0.011	0.023	0.073
*trans*16-18:1	0.166	0.054	0.078	0.356
*cis*15-18:1	0.027	0.008	0.010	0.046
19:0	0.035	0.009	0.020	0.058
*trans*9, 12-18:2	0.017	0.010	0.005	0.047
*cis*9, *trans*13-18:2	0.091	0.020	0.045	0.153
*trans*8, *cis*12-18:2	0.145	0.035	0.070	0.238
*trans*8, *cis*13-18:2	0.078	0.022	0.034	0.141
*cis*9, *trans*12-18:2	0.044	0.012	0.024	0.080
*trans*9, *cis*12-18:2	0.029	0.014	0.013	0.080

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Table 2 (*Continued*)

Fatty acid (% of total fatty acids)	Mean	SD	Minimum	Maximum
*trans*11, *cis*15-18:2	0.063	0.040	0.013	0.173
*cis*9, 12-18:2	1.432	0.340	0.852	2.512
20:0	0.100	0.053	0.012	0.194
*cis*6, 9, 12-18:3	0.023	0.008	0.008	0.039
*cis*9-20:1	0.047	0.047	<0.001	0.135
*cis*11-20:1	0.049	0.050	<0.001	0.165
*cis*9, 12, 15-18:3	0.331	0.143	0.172	0.860
*cis*9, *trans*11-18:2[5]	0.298	0.094	0.165	0.648
*trans*10, *cis*12-18:2	0.022	0.009	0.006	0.048
*cis*6, 9, 12, 15-18:4	0.015	0.008	0.005	0.046
*cis*11, 14-20:2	0.025	0.010	0.006	0.043
*cis*9, *trans*11, *cis*15-18:3	0.023	0.007	0.010	0.047
22:0	0.031	0.021	0.003	0.079
*cis*8, 11, 14-20:3	0.071	0.035	0.019	0.155
*cis*13-22:1	0.010	0.005	0.003	0.022
*cis*11, 14, 17-20:3	0.007	0.004	0.001	0.018
*cis*5, 8, 11, 14-20:4	0.090	0.046	0.019	0.185
*cis*8, 11, 14, 17-20:4	0.010	0.005	0.002	0.030
*cis*13, 16-22:2	0.012	0.005	0.005	0.028
*cis*5, 8, 11, 14, 17-20:5	0.022	0.012	0.004	0.057
24:0	0.010	0.007	0.003	0.031
*cis*9-24:1	0.013	0.013	<0.001	0.054
*cis*13, 16, 19-22:3	0.004	0.005	<0.001	0.018
*cis*7, 10, 13, 16-22:4	0.019	0.010	0.004	0.046
*cis*4, 7, 10, 13, 16-22:5	0.007	0.007	<0.001	0.025
*cis*7, 10, 13, 16, 19-22:5	0.045	0.030	0.002	0.110
*cis*4, 7, 10, 13, 16, 19-22:6	0.010	0.006	0.003	0.029

[1]Co-elution with minor concentration of *trans*10-16:1.
[2]Co-elution with minor concentration of *trans*13-16:1.
[3]Co-elution with minor concentration of *cis*10-16:1.
[4]Co-elution with minor concentration of *cis*10-18:1.
[5]Co-elution with minor concentration of *trans*7, *cis*9-18:2.

The intercept, at the mean value of the regressor, was used to asses overall model bias, whereas the slope was used to determine the linear bias. The significance of both measures of model bias was determined by their respective *t* tests. The maximum bias for each model was determined using equation (1) with the minimum and maximum model predicted values as inputs and judged relative to the standard error (St-Pierre, 2003). In addition, the root mean squared prediction error (RMSPE) estimated from the difference between observed and model predicted values was used to further evaluate candidate models. Decomposition of mean squared prediction error (MSPE) into ECT (error due to overall prediction bias), ER (error due to linear bias) and ED (error due to random variation) was performed according to the study by Bibby and Toutenburg (1977).

Results and discussion

The data set used in the present study comprised individual cow observations from three separate Latin square design experiments feeding a range of forages including corn, alfalfa, barley, or timothy silages or 50 : 50 mixes of corn and alfalfa, barley and corn, or timothy and alfalfa silages, reflecting a wide range of dietary conditions even though all forages were included at 60% of ration DM. The dietary concentrations of CP, NDF, starch and EE were (% of DM; mean ± SD) 16.3 ± 0.9%, 32.8 ± 3.6%, 20.0 ± 5.2% and 5.3 ± 1.2%, respectively (Table 1). The small SD around the mean indicated most of the data were within a small range, despite the wide spread of values (i.e. minimum and maximum values).

Similarly, the data set also included a wide range of values for DMI, forage intake, milk yield, milk fat concentration, milk protein concentration and CH_4 output, averaging 24.1 ± 2.4 kg/day, 14.5 ± 1.5 kg/day, 35.6 ± 5.2 kg/day, 3.79 ± 0.55%, 3.23 ± 0.26% and 484 ± 58 g/day, respectively. In addition, the concentration of 83 individual milk FA and several groups of FA, which were also evaluated as predictors of CH_4, exhibited a great range of values, probably reflecting the differences in diet composition and individual cow variation (Table 2). In contrast to the current data set where dietary treatments included a range of forage types and no oil supplementation, previous studies that have evaluated the associations between similar types of test variables and CH_4 comprised observations from experiments in which FA with potent effects on ruminal flora and fermentation (e.g. MCFA or PUFA) were used (Chilliard *et al.*, 2009; Dijkstra *et al.*, 2011; Mohammed *et al.*, 2011; van Lingen *et al.*, 2014).

Associations between test variables and CH_4 output

Previous studies reported stronger correlations between CH_4 output and milk FA within subsets (i.e. within dietary treatments) compared with complete data sets (i.e. across treatments), suggesting an important effect of specific dietary nutrients on CH_4 predictors (Chilliard *et al.*, 2009; Mohammed *et al.*, 2011). The approach of the current study aimed at describing the associations between milk FA and enteric CH_4 across several dietary forages or forage combinations, thus expecting applicability in a wide range of dairy farms. The nature of the associations between CH_4 and specific milk FA, or FA groups, are expected to reflect the characteristics of ruminal fermentation, as affected by dietary ingredients or nutrients. Similarly to the studies by Mohammed *et al.* (2011) and Castro-Montoya *et al.* (2011), we observed moderate negative associations between CH_4 output and *cis*9-17:1, 17:0 and 15:0 in milk fat (Table 3). Compared with acetate production, which liberates hydrogen, ruminal synthesis of propionate uses hydrogen, thus reducing availability of hydrogen for CH_4 synthesis (Moss *et al.*, 2000). Accordingly, as both 15:0 and 17:0 originate *de novo* from ruminal propionate (French *et al.*, 2012), and *cis*9-17:1 from mammary desaturation of 17:0 (Fievez *et al.*, 2003), these FA are expected to be negatively correlated with CH_4 output. In contrast to our observations, Dijkstra *et al.* (2011) and van Lingen *et al.* (2014) found no significant association between CH_4 and 15:0 or 17:0. In addition, when feeding cows with linseed supplemented diets, Chilliard *et al.*

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Table 3 *Correlations between CH_4 (g/day) and test variables (% of total fatty acid (FA) unless otherwise specified)[1] from a data set including 81 observations from 27 lactating Holstein dairy cows fed various forage sources*

Test variables	*r*	*P*-value
∑ 17:1	−0.60	<0.001
*cis*9-17:1	−0.58	<0.001
*trans*8, *cis*13-18:2	−0.51	<0.001
*cis*4, 7, 10, 13, 16, 19-22:6	−0.48	<0.001
*cis*11, 14, 17-20:3	−0.46	<0.001
*cis*6, 9, 12, 15-18:4	−0.45	<0.001
*cis*4, 7, 10, 13, 16-22:5	−0.44	<0.001
*cis*9-20:1	−0.44	<0.001
17:0	−0.42	<0.001
*trans*10-18:1 to *trans*11-18:1 ratio	−0.42	<0.001
*trans*10-18:1	−0.41	<0.001
*cis*13, 16, 19-22:3	−0.41	<0.001
∑ Odd- and branched-chain FA	−0.39	<0.001
∑ Odd-chain FA	−0.39	<0.001
15:0	−0.31	0.006
∑ FA > 16C[2]	−0.14	0.21
∑ FA = 16C[3]	−0.04	0.71
*trans*11-18:1	0.09	0.42
∑ Branched-chain FA	0.10	0.39
∑ FA <16C[4]	0.34	<0.001
Forage intake (kg/day)	0.59	<0.001
DMI (kg/day)	0.59	<0.001

[1]Correlations >0.4 or <−0.4, and those most biologically relevant are shown. A full list of correlations is shown in Supplementary Table S1.
[2]From circulation (preformed).
[3]Both from circulation and from *de novo* mammary synthesis.
[4]From *de novo* mammary synthesis.

(2009) reported strong positive correlations between 15:0 and 17:0 milk FA and CH_4.

The predominant FA BH pathways can serve as indicators of the effects of diet on ruminal microbes (Lourenço *et al.*, 2010). Importantly, shifts in ruminal bacteria and BH intermediates (i.e. elevations in the concentration of milk *trans*10-18:1 at the expense of *trans*11-18:1) are caused by low ruminal pH (Qiu *et al.*, 2004; Fuentes *et al.*, 2009), or by a direct toxic effect of PUFA on bacteria (Bauman and Griinari, 2001; Maia *et al.*, 2007). In agreement with previous studies (Chilliard *et al.*, 2009; Mohammed *et al.*, 2011), *trans*10-18:1 was negatively associated with CH_4 in the present study, whereas *trans*11-18:1 was not correlated (Table 3). Interestingly, when this association was measured as the ratio of *trans*10-18:1 : *trans*11-18:1, there was no meaningful change in the correlation coefficient. Others have also reported the sum of *trans*10-18:1 and *trans*11-18:1 to be inversely associated with CH_4 output (Dijkstra *et al.*, 2011; van Lingen *et al.*, 2014), although this may preclude from evidencing the effect of shifts in ruminal BH pathways, when related to ruminal fermentation and CH_4 formation.

The concentrations of several n-3 FA were negatively correlated with CH_4 output, including *cis*4, 7, 10, 13, 16, 19-22:6, *cis*11, 14, 17-20:3, *cis*6, 9, 12, 15-18:4, *cis*4, 7, 10, 13, 16-22:5 and *cis*13, 16, 19-22:3 (Table 3). In addition, *trans*8, *cis*13-18:2, *trans*8, *cis*12-18:2 and *cis*9-20:1 were also negatively correlated with CH_4 output. In general, dietary PUFA are expected to be negatively associated with CH_4, as high concentrations of these FA reduce fiber digestibility and thus do not favor CH_4 formation (Patra, 2013). This could be the result of the inhibition of growth of more sensitive species of ruminal bacteria when exposed to PUFA (Maia *et al.*, 2007). The forementioned milk long-chain n-3 FA could originate from post-absorptive elongation of 18:3 n-3 present in feeds, although this process is thought to be very inefficient in dairy cows (Hagemeister *et al.*, 1991).

Milk fat concentration of *trans*8, *cis*13-18:2 presented one of the strongest negative correlations with CH_4 in the current data set. However, this FA is not routinely measured, and reports about its link with CH_4 are non-existent to our knowledge. Possibly downstream in the same BH pathway, *cis*13-18:1 or *trans*8-18:1 could be indicators of ruminal BH of this FA. In line with this, we observed negative associations between both *cis*13-18:1 and *trans*6–8-18:1 with CH_4 output (Supplementary Table S1), as have others (Chilliard *et al.*, 2009; Mohammed *et al.*, 2011; van Lingen *et al.*, 2014).

Only few milk FA were positively correlated with CH_4, including 10:0, *cis*11-14:1, 12:0 and total *de novo* synthesized ($r = 0.34$ to 0.38; Supplementary Table S1). In agreement, positive associations between CH_4 and 10:0, 12:0 and FA <16C have been reported previously (Chilliard *et al.*, 2009; van Lingen *et al.*, 2014). On the contrary, *cis*11-14:1 is not commonly reported, and its association with CH_4 is largely unknown. Castro-Montoya *et al.* (2011) reported that iso-14:0, iso-15:0 and iso-16:0 FA, which are more abundant in fibrolytic bacteria (Vlaeminck *et al.*, 2006) were positively related to estimated CH_4 output. In the present study, only iso-16:0 was positively correlated to CH_4 output, whereas iso-14:0 tended to be positively correlated ($P = 0.07$; Supplementary Table S1). In addition, total branched-chain FA were not significantly correlated with CH_4. Others have also reported positive associations between CH_4 and iso-16:0 (Chilliard *et al.*, 2009; van Lingen *et al.*, 2014) and between CH_4 and iso-14:0 (van Lingen *et al.*, 2014). CH_4 output is known to increase with DMI, and in agreement with previous reports, both forage intake and DMI were the test variables more strongly and positively correlated to CH_4 (Mohammed *et al.*, 2011; Ramin and Huhtanen, 2013).

Regression analyses

Using three sets of test variables, three candidate models were obtained by LASSO and LARS selection methods based on the SBC (Table 4). The first set included the concentrations of all individual milk FA, milk yield, and DMI, and the selected variables were 15:0, *cis*9-17:1, *trans*10-18:1, *cis*11-18:1, *trans*8, *cis*12-18:2, *trans*8, *cis*13-18:2, *trans*11, *cis*15-18:2 and DMI, where all variables except for DMI were negatively related to CH_4 output. The second set included the concentrations of milk FA groups, in addition to those included in the first set, and selected variables were *cis*9-17:1, *cis*11-18:1, *trans*8, *cis*13-18:2, odd-chain FA and DMI, and similarly to model

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no. 1, only DMI was positively associated to CH_4. The third data set included only the concentrations of individual milk FA, without DMI or milk yield, and predictor variables selected were *cis*11-14:1, *cis*9-17:1, *cis*11-18:1 and *trans*8, *cis*13-18:2, where all selected FA were negatively associated with CH_4 except for *cis*11-14:1, which was positively related with CH_4.

Table 4 *Selected candidate models for prediction of CH_4 (g/day) output obtained by Least Absolute Shrinkage and Selection Operator and Least-Angle Regression methods based on the Schwarz Bayesian Criterion (SBC) from a data set including 81 observations from 27 lactating Holstein dairy cows fed various forage sources*

Models no.[1]	Estimate	SE	*P*-value
1 Including diet components, individual milk FA, milk yield and DMI in data set			
Intercept	319.7	52.8	<0.001
15:0	−57.4	13.9	<0.001
*cis*9-17:1	−13.8	99.9	0.89
*trans*10-18:1	−39.5	24.06	0.11
*cis*11-18:1	−59.9	15.9	<0.001
*trans*8, *cis*12-18:2	−253.1	123.4	0.04
*trans*8, *cis*13-18:2	−642.7	189.2	0.002
*trans*11, *cis*15-18:2	−195.7	90.3	0.03
DMI	16.5	1.75	<0.001
2 Including diet components, individual milk FA, FA sums, milk yield and DMI in data set			
Intercept	441.5	45.2	<0.001
*cis*9-17:1	25.05	95.2	0.79
*cis*11-18:1	−79.2	15.6	<0.001
*trans*8, *cis*13-18:2	−1118.9	153.03	<0.001
Odd-chain FA	−46.2	9.6	<0.001
DMI	14.9	1.7	<0.001
3 Including diet components, individual milk FA, but not DMI or milk yield in data set			
Intercept	669.1	32.8	<0.001
*cis*11-14:1	838.7	287.6	0.01
*cis*9-17:1	−493.2	106.0	<0.001
*cis*11-18:1	−44.2	21.4	0.04
*trans*8, *cis*13-18:2	−963.7	214.9	<0.001

FA = fatty acid; DMI = dry matter intake.
[1]Regression estimates, standard errors and *P*-values from the mixed model including the random effects of cow, period and study. Root mean squared error, R^2 and SBC score obtained by Prog REG for models nos 1 to 3 were 27.2, 0.80 and 565.2, 29.3, 0.76 and 567.4, and 38.6, 0.58 and 608.8, respectively.

Milk fat concentrations of *cis*9-17:1,*cis*11-18:1 and *trans*8, *cis*13-18:2 were all negatively related to CH_4 emissions and were common to all candidate models. A few of these predictors have been previously included in regression equations estimating CH_4 output. Similarly to models nos 1 to 3 in the present study, *cis*9-17:1 was included in the best fit equation by Mohammed *et al.* (2011), and *cis*11-18:1 was included in that reported by Dijkstra *et al.* (2011). To our knowledge, no previous studies have reported prediction models including *trans*8, *cis*13-18:2, and its selection in the current candidate models suggests an important role in the prediction of CH_4, which should be further studied. Milk fat concentration of *trans*10-18:1 was selected in model no. 2, and given its known link to altered ruminal BH pathways as a result of low dietary fiber and excess PUFA (Rico and Harvatine, 2013), this FA could be an important predictor of CH_4. Previous equations have included the sum of *trans*10-18:1 and *trans*11-18:1 as a predictor of CH_4 (Dijkstra *et al.*, 2011; van Lingen *et al.*, 2014).

Previous studies have predicted CH_4 output per kilogram of DMI (Dijkstra *et al.*, 2011; van Lingen *et al.*, 2014), or using DMI or forage intake as predictors (Chilliard *et al.*, 2009; Mohammed *et al.*, 2011). Although DMI is a major determinant of CH_4 emissions (Ramin and Huhtanen, 2013), this parameter is difficult to assess in dairy farms, in particular individual DMI values, which are harder to determine compared with group level intakes. Therefore, model no. 3, which predicts CH_4 without the use of DMI measurements could potentially be of higher practical value.

Based on the highest coefficients of determination, and lowest RMSE and SBC values for the three candidate models before correction for random effects (Table 4), the best fit was observed for model no. 1, followed by model no. 2 and then model no. 3. Using the observations from a single experiment, Chilliard *et al.* (2009) reported an R^2 of 0.95 for the best fit equation. In the present study, the R^2 after correction for cow, period and study effects in the mixed model were 0.84, 0.83 and 0.80, respectively (predicted *v.* observed values; Figures 1 to 3, panel a). Interestingly,

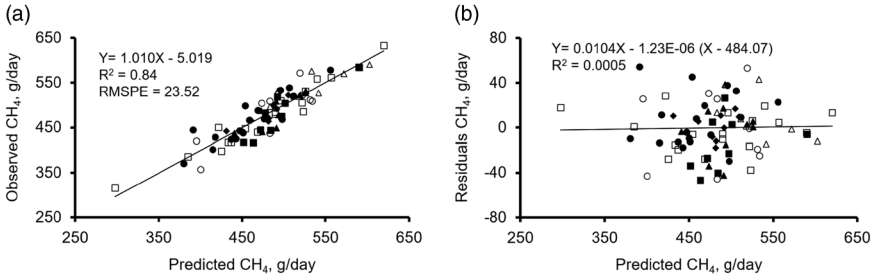


Figure 1 Associations between observed and predicted CH_4 output accounting for cow, period and study variation for model no. 1, obtained from a data set including 81 observations from 27 lactating Holstein dairy cows fed various forage sources. Forages were corn silage (□), alfalfa silage (●), barley silage (▲), timothy silage (■), corn–alfalfa silages mix (○), corn–barley silages mix (Δ) and timothy–alfalfa silages mix (♦). (a) The association between model predicted and observed values. (b) the residual by predicted plot. The equation in (b) was obtained from the association between residuals and predicted CH_4 centered around the mean of predicted values. The mean (−1.23E − 06 g/day) and linear (0.0104) biases were not different from 0 ($P > 0.84$). RMSPE = root mean squared prediction error.

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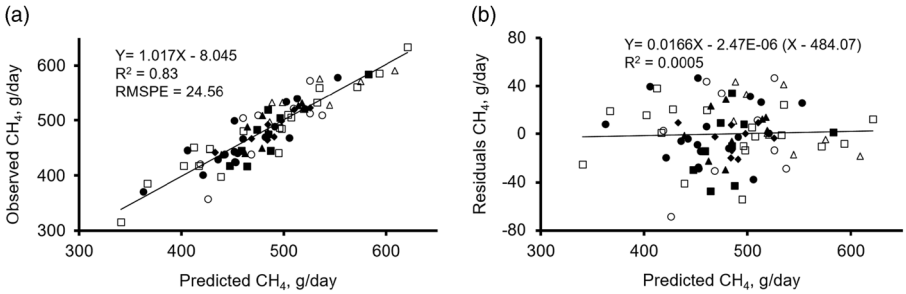


Figure 2 Associations between observed and predicted CH_4 output accounting for cow, period and study variation for model no. 2, obtained from a data set including 81 observations from 27 lactating Holstein dairy cows fed various forage sources. Forages were corn silage (□), alfalfa silage (●), barley silage (▲), timothy silage (■), corn–alfalfa silages mix (○), corn–barley silages mix (Δ) and timothy–alfalfa silages mix (♦). (a) The association between model predicted and observed values. (b) The residual by predicted plot. The equation in (b) was obtained from the association between residuals and predicted CH_4 centered around the mean of predicted values. The mean (−2.47E − 06 g/day) and linear (0.0166) biases were not different from 0 ($P > 0.75$). RMSPE = root mean squared prediction error.

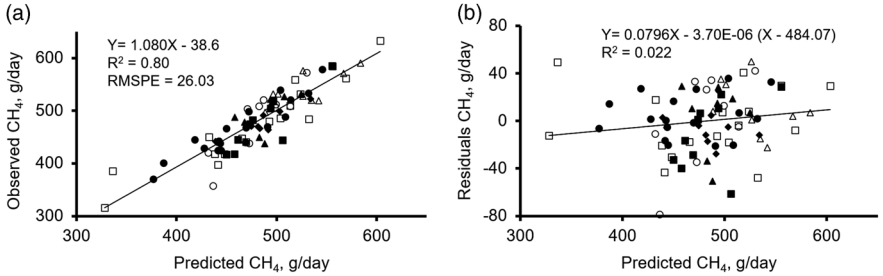


Figure 3 Associations between observed and predicted CH_4 output accounting for cow, period and study variation for model no. 3, obtained from a data set including 81 observations from 27 lactating Holstein dairy cows fed various forage sources. Forages were corn silage (□), alfalfa silage (●), barley silage (▲), timothy silage (■), corn–alfalfa silages mix (○), corn–barley silages mix (Δ), timothy–alfalfa silages mix (♦). (a) The association between model predicted and observed values. (b) The residual by predicted plot. The equation in (b) was obtained from the association between residuals and predicted CH_4 centered around the mean of predicted values. The mean (−3.7E − 06 g/day) and linear (0.0796) biases were not different from 0 ($P > 0.19$). RMSPE = root mean squared prediction error.

Table 5 *Regression estimates for the association between residuals and model predicted CH_4 (g/day) values centered around the mean for all candidate models*

					RMSPE[1]				
	Estimate	SE	*P*-value	R^2	g/day	%	ECT (%)	ER (%)	ED (%)
Model no. 1									
Intercept	−1.23E − 06	2.61	1.00	0.0005	23.52	4.9	0	0.06	99.94
Predicted CH_4[2]	1.04E − 02	0.05	0.84						
Model no. 2									
Intercept	2.47E − 06	2.73	1.00	0.0013	24.56	5.1	0	0.13	99.87
Predicted CH_4[2]	1.66E − 02	0.05	0.75						
Model no. 3									
Intercept	3.70E − 06	2.89	1.00	0.0217	26.03	5.4	0	2.17	97.83
Predicted CH_4[2]	7.96E −02	0.06	0.19						

Data set included 81 observations from 27 lactating Holstein dairy cows fed various forage sources.
[1]Root mean squared prediction error (RMSPE) expressed as g/day and percentage of the observed mean. Mean squared prediction error was decomposed into ECT (error due to overall prediction bias), ER (error due to deviation of the regression slope) and ED (error due to random variation).
[2]Regressor shifted to its mean value.

R^2 values for the same models before correcting for cow, period and study effects were 0.80, 0.76 and 0.58, respectively (Table 4). This suggests that, particularly for model no. 3, a great portion of the variation was explained by these random effects. The origin of this variation is unknown. However, it could be related to the predominant microbial population of each animal, which would influence the ruminal fermentation processes. The R^2 of all three models

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are similar to those previously reported by Mohammed *et al*. (2011) from a single experiment, and higher than those reported by Dijkstra *et al*. (2011) and van Lingen *et al*. (2014) using observations from multiple experiments. In agreement with the highest R^2 observed for model no. 1, RMSPE was the lowest for this model, indicating a smaller difference between observed and model predicted values compared with models nos 2 and 3. For all candidate models, the plots of the residual by predicted values (centered around the mean) were used as indicators of prediction bias (Figures 1 to 3, panel b; St-Pierre, 2003). Based on equation (1), the intercepts of these plots were not different from 0 ($P = 1.0$; Table 5), indicating absence of overall prediction bias. Similarly, based on the slopes, the maximum linear bias was calculated to be <13 g/day across all models, but was not significant ($P > 0.19$ for all slopes). The lack of prediction bias and high association between predicted and observed values suggest high performance and validity of the models here proposed to predict CH_4 emissions in dairy cows. In addition, decomposition of the MSPE indicated that most of the error (>97% of total MSPE in all models) was associated to random variation and not to mean or regression bias. However, further validation against independent data sets is encouraged, in particular during early lactation, given that the increased adipose tissue mobilization expected during this period could potentially alter milk FA profile and thus the relationships here proposed.

Conclusions

Using a data set from diets including a range of forage species, *cis*9-17:1, *cis*11-18:1 and *trans*8, *cis*13-18:2 were the most common FA across candidate models and were all negatively associated to CH_4 output. Conversely, *cis*11-14:1, which was positively correlated with CH_4, was selected only when DMI was not present in the data set. From a mechanistic point of view, the nature of the associations between these FA and CH_4 is not well understood, and future research efforts should be made to elucidate them.

Across diets differing in forage source, milk FA were good predictors of CH_4 output. In agreement with previous findings, DMI was a main determinant of CH_4 production. However, considering the difficulty in measuring DMI under practical conditions, a model is proposed (model no. 3) as a practical tool to estimate on-farm CH_4 output based on milk FA profile.

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Supplementary material

To view supplementary material for this article, please visit http://dx.doi.org/10.1017/S1751731115001949

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