Prediction of enteric methane output from milk fatty acid concentrations and rumen fermentation parameters in dairy cows fed sunflower, flax, or canola seeds

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ABSTRACT

Milk fatty acid (FA) composition has been suggested as a means of predicting enteric methane (CH_4) output in lactating dairy cattle because of the common biochemical pathways among CH_4 , acetate, and butyrate in the rumen. Sixteen lactating Holstein cows were used in a Latin square design with four 28-d periods. All diets contained steam-rolled barley, a pelleted supplement, barley silage [45% of dietary dry matter (DM)] and 3.3% added fat (DM basis) from 1 of 4 sources: calcium salts of long-chain FA (palm oil; control) or crushed oilseeds from sunflower, flax, or canola. The objectives of this study were to (1) compare the effect of diets on milk FA profile; (2) model CH_4 production from milk FA composition, intake, and rumen fermentation variables; and (3) test the applicability of CH_4 prediction equations reported in previous studies. Methane (g/d) was measured in chambers (2 animals/chamber) on 3 consecutive days (d 21–23). The test variables included total DM intake (DMI, kg/d; d 21–23), forage DMI (kg/d; d 21–23), milk yield (kg/d; d 21–23), milk components (d 18–21), milk FA composition (% total FA methyl esters; d 18–21), rumen volatile FA (mol/100 mol; d 19–21), and protozoal counts (d 19–21), and were averaged by chamber and period to determine relationships between CH_4 and the test variables. Milk *trans*(t)10-, t11-18:1, and *cis*(c)9t11-18:2 were greater for sunflower seeds compared with the other diets. Forage DMI (correlation coefficient, $r = 0.52$; $n = 32$), DMI ($r = 0.52$; $n = 32$), and rumen acetate + butyrate:propionate ($r = 0.72$; $n = 16$) were positively related to CH_4 (g/d), whereas rumen propionate ($r = 0.63$; $n = 16$), milk c9-17:1 ($r = 0.64$; $n = 32$), and c11-18:1 ($r = 0.64$; $n = 32$) were negatively related to CH_4 . The best regression equation (coefficient of determination = 0.90; $n = 16$) was CH_4 (g/d) = $-910.8 (\pm 156.7) \times \text{milk c9-17:1} + 331.2 (\pm 88.8) \times \text{milk 16:0 iso} + 0.0001 (\pm 0.00) \times \text{total entodiniomorphs} + 242.5 (\pm 39.7)$. Removing

rumen parameters from the model also resulted in a reasonably good estimate (coefficient of determination = 0.83; $n = 32$) of CH_4 . Stepwise regression analysis within diets resulted in greater coefficient of determination and lower standard error values. Predictions of CH_4 , using equations from previous studies for the data set from this study, resulted in a mean overestimation ranging from 19 to 61% across studies. Thus, milk FA alone may not be suitable for developing universal CH_4 prediction equations.

Key words: methane, milk fatty acid composition, prediction equation

INTRODUCTION

Enteric methane (CH_4) produced by ruminants represents an energy loss to the animal (Johnson and Johnson, 1995). It also contributes to the enhanced greenhouse gas effect and global warming (IPCC, 2006). A need exists for simple, inexpensive measurement techniques to estimate CH_4 emissions from dairy cows in research settings and under field conditions. Current measurement techniques, such as whole-animal chambers and tracer gas techniques, require complex instrumentation and, thus, are limited in their use (Johnson and Johnson, 1995).

Decades ago, a stoichiometric relationship between CH_4 and ruminal acetate, propionate, and butyrate was proposed (Demeyer and Van Nevel, 1975). These short-chain fatty acids (FA) formed in the rumen in particular act as precursors for the de novo synthesis of milk FA in the mammary tissue. Because the precursors for the synthesis of CH_4 and de novo synthesis of milk FA arise in the rumen, a relationship between CH_4 and milk FA was proposed (Chilliard et al., 2009). Chilliard et al. (2009) developed CH_4 predictive equations from milk FA composition in dairy cows offered 3 different physical forms of linseed (crude, extruded, and linseed oil). Their best equation predicted CH_4 ($R^2 = 0.95$) with the milk FA 16:0, *trans*(t)16/*cis*(c)14-18:1, c9-14:1, 18:2n-6, and forage intake. More recently, Dijkstra et al. (2011) developed CH_4 predictive equations from

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milk FA using a data set containing 50 observations from 3 different experiments. However, their best equation contained a different set of variables ($17:0$ anteiso, $t10+t11-18:1$, $c11-18:1$, and $c13-18:1$) with a coefficient of determination (R^2) value of 0.73. Yet another study (Montoya et al., 2011) reported that milk odd- and branched-chain fatty acids (**OBCFA**) hold potential as predictors of rumen methanogenesis. In the above studies, the variables contributing to CH_4 were not the same, suggesting that the applicability of these equations across varied feeding conditions may be limited.

In a previous study, Beauchemin et al. (2009) investigated the effect of crushed sunflower seed (**SS**), canola seed (**CS**), flax seed (**FS**), and a control diet [**CTL**; calcium salts of long-chain FA (palm oil)] on CH_4 emissions. They found that the oilseed-supplemented diets decreased CH_4 production (g/d) by an average of 13% compared with the CTL. The various sources of long-chain FA used in their study differed in degree of saturation and rumen availability and, hence, provided an opportunity to compare their effects on milk FA composition and evaluate the potential of predicting CH_4 production within and across diets. Thus, the objectives of the present study were to expand the study presented by Beauchemin et al. (2009) to (1) determine the effects of CTL, SS, FS, and CS diets on milk FA profile; (2) model the relationship between CH_4 production and milk FA concentrations, intake, milk production, and rumen fermentation variables; and (3) test the CH_4 prediction equations reported by Chilliard et al. (2009) and Dijkstra et al. (2011).

MATERIALS AND METHODS

Experimental Design

Experimental details were reported in a companion paper (Beauchemin et al., 2009). In brief, 16 lactating Holstein cows maintained in 2 groups (8 primiparous ruminally cannulated and 8 multiparous intact cows) were offered 4 diets in four 28-d periods in a Latin square design. The experimental diets were SS, FS, CS, and CTL. Dietary forage:concentrate ratio was 45:54, with barley silage as the sole forage component. All diets contained steam-rolled barley (~32.5% DM basis) and a pelleted supplement containing ground barley, protein sources, vitamins, and minerals. Oilseeds were crushed in a roller mill to ensure the hulls were cracked and added to the diets to provide 3.3% added fat on a DM basis. Detailed ingredients and chemical composition of the diets are reported in Table 1.

Dry matter intakes and milk production (a.m. and p.m.) were recorded daily throughout the experiment. However, for this study, only DMI and milk produced

while the animals were in the chamber (d 21–23) were considered. Total lipids from the TMR were extracted with chloroform:methanol (1:1) along with an internal standard (23:0; Mohammed et al., 2009). The TMR fat was methylated with methanolic HCl and the total dietary FA content was determined relative to the response factors for a known amount of the internal standard (23:0). The total FA intake was determined as the product of DMI and the total FA content of the diet. Intakes of individual FA for each cow were determined by multiplying the proportion of the particular FA with the total FA consumed.

Milk sampled (a.m. and p.m.) on 3 consecutive days (d 18–20) was used for component analysis (reported in Beauchemin et al., 2009). Milk FA composition was determined from the pooled milk samples (d 18–20) as described by Mohammed et al. (2009). In brief, milk fat was extracted using a chloroform:methanol:water (2:2:1.8) mixture and methylated with 0.5 *N* methanolic base (Supelco Inc., Bellefonte, PA). The FA methyl esters (**FAME**) were analyzed on a Varian 3600 gas chromatograph (Varian Chromatography Systems, Walnut Creek, CA) with a temperature-programmable on-column injector and flame ionization detector using the 175°C temperature program described by Kramer et al. (2008). Injector and detector temperatures were maintained at 250°C and helium was used as the carrier gas at a flow rate of 1 mL/min. The FAME were identified by comparison with the standards GLC 463 and UC-59M (both from Nu-Chek prep Inc., Elysian, MN). Individual FAME were reported as a percentage of total FAME. The short-chain FAME were corrected for their flame ionization detector deficiency based on theoretical correction factors reported by Wolff et al. (1995).

Ruminal contents were collected only from primiparous cows at 0 and 4 h after the morning feeding on d 19 to 21. Rumen fermentation parameters (pH, VFA, and NH_3-N) were analyzed before (0 h) and after (4 h) feeding, whereas protozoal counts were determined in the samples after feeding as described by Beauchemin et al. (2009). Readers are referred to Beauchemin et al. (2009) for the effect of diets on rumen fermentation variables.

Enteric CH_4 was measured in environmental chambers (2 animals/chamber) on 3 consecutive days in the last week of each period (d 21–23). Cows fed the same diet were paired and housed in each chamber in 2 individual stanchions equipped with feeders. The detailed procedure for recording CH_4 in the chambers was described in Beauchemin et al. (2007, 2009). In brief, cows were conditioned to the chambers before the start of the experiment. Chambers were calibrated at the beginning of each period by releasing a known amount of

Table 1. Ingredient and chemical composition of the diets¹ (DM basis; Beauchemin et al., 2009)

Ingredient, %	Diet			
	CTL	SS	FS	CS
Barley silage	45.0	45.0	45.0	45.0
Barley grain, steam-rolled	32.5	32.5	32.5	32.5
Sunflower seeds, crushed	0	10.55	0	0
Flaxseed, crushed	0	0	9.32	0
Canola seed, crushed	0	0	0	9.32
Pelleted supplement				
Beet pulp	4.65	0	0	0
Barley grain, ground	3.23	1.24	3.22	3.22
Ca salts of long-chain fatty acids (palm oil) ²	3.95	0	0	0
Canola meal, high RUP ³	3.02	2.88	2.52	2.52
Soybean meal, high RUP ⁴	3.09	2.60	2.56	2.56
Corn gluten meal	3.00	2.60	2.52	2.52
Dicalcium phosphorous	0.16	0.09	0	0
Urea	0.56	0.56	0.21	0.21
Calcium carbonate	0	1.14	1.19	1.19
Na bicarbonate	0.42	0.42	0.42	0.42
NaCl	0.25	0.25	0.25	0.25
Vitamin-mineral mix ⁵	0.17	0.17	0.17	0.17
OM	93.3	93.7	93.7	93.4
CP	16.8	16.4	16.4	16.9
NDF	28.8	30.6	28.8	29.9
ADF	18.3	19.6	18.4	19.6
NFC ⁶	42.7	39.4	41.6	39.9
Crude fat	5.0	7.3	6.9	6.7

¹Diets were control [CTL; calcium salts of long-chain fatty acids (palm oil)], sunflower seed (SS), flax seed (FS), and canola seed (CS).

²Enertia (ADM Alliance Nutrition, Quincy, IL).

³Alberta Gold (Canbra Foods Ltd., Lethbridge, AB, Canada).

⁴Soy Pass (Borregaard LignoTech USA Inc., Rothschild, WI).

⁵Added to supply per kilogram of dietary DM: S, 150 mg; K, 120 mg; Mg, 70 mg; Zn, 30 mg; Cu, 8.5 mg; Co, 0.12 mg; Se, 0.2 mg; vitamin A, 8,400 IU; vitamin D, 840 IU; and vitamin E, 8 IU.

⁶NFC = 100 – (CP + NDF + ether extract + ash).

CH₄ gas, and the measuring procedures were adjusted to obtain 100% recovery. Air flow rates in the intake and exhaust ducts were measured and CH₄ concentrations in these ducts were monitored (Beauchemin et al., 2007). The amount of CH₄ produced within a chamber by the 2 cows was determined by calculating the difference between the incoming and outgoing mass of CH₄.

Statistical Analysis

The milk FAME and intake of FA were analyzed by the Mixed procedure of SAS (version 9.1; SAS Institute Inc., Cary, NC) using data from individual cows (n = 64). Cow within diet was the experimental unit, with diet designated as fixed factor and cow and period as random factors. For all correlations and stepwise regression analyses, intake (total DMI, kg/d; forage DMI, kg/d; FA intake, g/d) and milk production (milk yield, kg/d; milk components, percentage and kg/d) parameters, rumen fermentation parameters (VFA, mol/100 mol; pH; protozoal counts), milk FA composition (% of

total FAME) and CH₄ (g/d) were averaged by chamber (experimental unit) and period before including them in the model. It should be noted that milk FA variables ≤0.02% [13:0 *iso*; 21:0; *t*5- and *t*10-16:1; *c*7-17:1; *c*9- and *c*10-16:1; *t*4- and *t*5-18:1; *t*6/*t*11-, *t*12-, and *t*13-20:1; *c*13- and *c*15-22:1; *t*9,*t*12-18:2; 18:3n-6; 20:2n-6; 22:2n-6; EPA; DHA; and conjugated linoleic acid (**CLA**) isomers such as *t*9,*c*11-, *c*10,*c*12-, *c*11,*c*13-, *t*12,*t*14-, and *t*11,*t*13-CLA] were not included in the regression analysis. Correlations between CH₄ and the test variables (intake, milk production, rumen fermentation, and milk FA composition) were determined using the PROC CORR procedure of SAS. Stepwise regression analysis (PROC STEPWISE procedure of SAS) was done using CH₄ (g/d) as the dependent variable and the test variables as independent variables using a threshold of *P* < 0.05. Correlations and stepwise regression analyses were done using data from all diets (n = 32 for intake variables and milk FA profile; n = 16 for rumen parameters) as well as within diets (n = 8 for intake variables and milk FA profile; n = 4 for rumen parameters).

RESULTS

Intakes

Dry matter intakes were greater for the CS diet compared with the SS, FS, and CTL diets (Table 2). Intakes of 12:0, 14:0, 16:0, and saturated fatty acids (SFA) were greater for the CTL diet compared with the oilseed-supplemented diets. Intake of oleic acid was greatest for the CS diet, followed by the CTL, FS, and SS diets, with no difference between the latter 2. Linoleic acid (LeA) intake was greatest for the SS diet and linolenic acid (LN) intake was greatest for the FS diet. However, intake of LeA + LN was greatest for the FS diet, followed by the SS, CS and CTL diets.

Milk FA Composition

Total milk SFA were greater for the CTL diet compared with other diets (Table 3). Milk 16:0 was greater for the CTL diet compared with the other diets. Among the *t*-18:1 isomers, *t*10- and *t*11-18:1 concentrations were greater for the SS diet compared with the CS, FS, and CTL diets (Table 4). Consistent with this finding, total *t*-18:1 and total *t*-monounsaturated FA were also greater for the SS diet compared with the CS, FS, and CTL diets. Milk *c*9*t*11-18:2 (rumenic acid, RA) content and yield were greater for the SS diet and least for the CTL diet, but lower for the FS and CS diets compared with the SS diet, with no difference between the two. However, milk *t*10,*c*12-CLA was not different among the diets. Among the 4 desaturase indices calculated,

only *c*9-18:1/18:0 differed among treatments with greater values observed for the CTL diet compared with other diets.

Correlations Between CH₄ and the Test Variables

The significant positive and negative correlations between CH₄ and the test variables from all diets ($n = 32$ for milk FA, intake and production variables; $n = 16$ for rumen variables) are reported in Table 5. Among intake variables, CH₄ was positively related to DMI and forage DMI ($r = 0.52$ for both). Enteric CH₄ was positively related to acetate + butyrate:propionate ($r = 0.72$), acetate:propionate ($r = 0.66$), and total entodiniomorphs ($r = 0.58$), and negatively related to rumen propionate ($r = 0.63$) and valerate ($r = 0.60$) among the rumen variables. Correlations of CH₄ with milk FA revealed that CH₄ was negatively related to *c*9-17:1 ($r = 0.65$) and *c*11-18:1 ($r = 0.64$) and positively related to total SFA ($r = 0.39$). When correlations were repeated within diets ($n = 8$ for milk FA, intake and production variables and $n = 4$ for rumen variables), the correlation coefficient values were greater (Table 6) compared with the correlations among diets (Table 5). For the flax diet, CH₄ was strongly and positively related to rumen acetate, butyrate, and milk 14:0 *iso* and negatively related to milk *c*9-17:1, *c*11-18:1, and *c*13-18:1 (Table 6). The correlation coefficient values for the FS diet were relatively higher than for the other diets. Milk 9:0, Σ 8:0 to 14:0, and Σ SFA were positively related and milk *c*9-17:1 was negatively related to CH₄ for the SS diet. Enteric CH₄ for the CTL diet was posi-

Table 2. Effect of diets¹ on DMI, fatty acid intake, milk production, and methane emissions

Variable ²	Diet				SEM	P-value
	CTL	SS	FS	CS		
Total DMI, kg/d	18.1 ^b	18.2 ^b	18.0 ^b	19.4 ^a	0.54	0.03
Forage DMI, kg/d	8.2	8.2	8.1	8.7	0.29	0.12
Milk yield, kg/d	27.0	26.1	25.7	26.4	1.77	0.76
Milk fat, %	3.2	3.0	3.3	3.1	0.18	0.19
Milk fat, kg/d	0.84	0.77	0.81	0.80	0.06	0.38
Methane, g/d	293 ^a	264 ^b	241 ^b	265 ^b	19.3	<0.01
Total FA intake, g/d	1,352 ^b	1,267 ^b	1,298 ^b	1,590 ^a	56.8	<0.01
12:0	5.07 ^a	0.73 ^b	0.61 ^b	0.83 ^b	0.22	<0.01
14:0	20.6 ^a	6.8 ^b	6.2 ^b	7.1 ^b	0.40	<0.01
16:0	635 ^a	201 ^b	191 ^b	191 ^b	12.8	<0.01
18:0	57.6 ^a	60.9 ^a	55.9 ^a	32.5 ^b	2.36	<0.01
SFA	803 ^a	352 ^b	317 ^b	323 ^b	16.9	<0.01
Oleic acid	273 ^b	226 ^c	231 ^c	614 ^a	16.7	<0.01
LeA	138 ^d	559 ^a	330 ^c	402 ^b	17.6	<0.01
LN	11.6 ^c	19.6 ^c	326 ^a	89.6 ^b	10.2	<0.01
LeA + LN	150 ^d	579 ^b	656 ^a	491 ^c	24.8	<0.01

^{a-d}Within a row, means bearing different superscript letters are significantly different ($P < 0.05$).

¹Diets were control [CTL; calcium salts of long-chain fatty acids (palm oil)], sunflower seed (SS), flax seed (FS), and canola seed (CS).

²FA = fatty acids; SFA = saturated FA; LeA = linoleic acid; LN = linolenic acid.

Table 3. Effect of diets¹ on milk saturated fatty acid (SFA) composition

Variable ²	Diet				SEM	P-value
	CTL	SS	FS	CS		
4:0	0.64	0.48	0.58	0.48	0.08	NS
5:0	0.08	0.07	0.07	0.06	0.01	NS
6:0	1.00	1.05	1.15	1.20	0.09	0.01
7:0	0.20	0.18	0.16	0.17	0.02	NS
8:0	0.77	0.83	0.91	0.93	0.06	<0.01
9:0	0.25	0.22	0.20	0.30	0.04	NS
10:0	1.57	1.69	1.91	2.05	0.15	<0.01
11:0	0.28 ^c	0.30 ^{bc}	0.35 ^{ab}	0.37 ^a	0.03	0.01
12:0	2.07 ^b	2.19 ^b	2.43 ^a	2.59 ^a	0.15	<0.01
13:0	0.08 ^c	0.10 ^b	0.12 ^{ab}	0.13 ^a	0.01	<0.01
14:0 <i>iso</i>	0.12	0.14	0.12	0.11	0.02	NS
14:0	8.59 ^b	8.65 ^b	8.88 ^b	9.75 ^a	0.37	<0.01
15:0 <i>iso</i>	0.11 ^b	0.13 ^a	0.13 ^a	0.13 ^a	0.01	<0.01
15:0 <i>anteiso</i>	0.36 ^b	0.35 ^b	0.40 ^a	0.42 ^a	0.02	<0.01
15:0	0.86 ^b	0.86 ^b	0.91 ^{ab}	0.98 ^a	0.04	<0.01
16:0 <i>iso</i>	0.22	0.24	0.26	0.21	0.03	NS
16:0	36.18 ^a	22.33 ^b	22.62 ^b	22.76 ^b	0.76	<0.01
17:0	0.35 ^b	0.36 ^b	0.43 ^a	0.41 ^a	0.02	<0.01
17:0 <i>anteiso</i>	0.34 ^a	0.27 ^b	0.36 ^a	0.34 ^a	0.02	<0.01
18:0 <i>iso</i>	0.03	0.03	0.04	0.03	0.00	NS
18:0	8.76 ^b	14.32 ^a	13.54 ^a	13.94 ^a	0.73	<0.01
20:0	0.10 ^b	0.12 ^b	0.10 ^b	0.21 ^a	0.01	<0.01
24:0	0.02 ^b	0.02 ^b	0.03 ^a	0.02 ^b	0.00	<0.01
SFA	62.82 ^a	54.90 ^c	55.53 ^c	57.53 ^b	1.14	<0.01
<i>Iso</i> FA	0.69	0.77	0.72	0.70	0.05	NS
<i>Anteiso</i> FA	0.75 ^b	0.68 ^c	0.82 ^a	0.83 ^a	0.04	<0.01
Branched FA	1.45	1.45	1.54	1.52	0.07	NS

^{a-c}Within a row, means bearing different superscript letters are different ($P < 0.05$).

¹Diets were control [CTL; calcium salts of long-chain fatty acids (palm oil)], sunflower seed (SS), flax seed (FS), and canola seed (CS).

²FA = fatty acid; expressed as % total fatty acid methyl esters.

tively related to total entodiniomorphs and negatively related to milk *c*15-18:1 and *c*11-18:1. In the case of the CS diet, however, only DMI and forage DMI were positively related to CH₄ and 15:0 tended to be negatively related ($r = 0.65$; $P = 0.08$) to CH₄.

Regression Analysis

Stepwise regression analysis done with each group of test variables using data from all diets is presented in Table 7. The best equation resulted when all of the test variables (intake, milk FA, and rumen fermentation variables) were included in the model. According to this equation (equation 5 in Table 7), milk *c*9-17:1, 16:0 *iso* and total entodiniomorphs contributed 64, 18, and 8% of the variability in enteric CH₄ ($R^2 = 0.90$; $n = 16$; $P < 0.0001$). A reasonably good prediction equation resulted with the inclusion of milk FA and intake parameters (equation 4 in Table 7; $R^2 = 0.83$; $n = 32$). Variables that contributed to CH₄ were *c*9-17:1 (42%), *c*11-18:1 (14%), *t,t*-CLA (7%), total *t*-18:1 (6%), 15:0 *anteiso* (5%), 17:0 (5%), and DMI (6%). Milk FA alone predicted 75% of the variability in CH₄, with the major contributors being *c*9-17:1 (42%) and *c*11-18:1

(14%; equation 3 in Table 7). When only intake and production variables were included in the model, DMI, milk yield, and intake of SFA predicted 27, 15, and 16% of the variability in CH₄ (equation 2 in Table 7). The simplest equation resulted with the model containing only the rumen variable acetate:propionate ratio explaining 44% of the variability in enteric CH₄ (equation 1 in Table 7). Repeating the regression analysis within diets revealed that coefficient of determination values were greater and standard error values lower (Table 8). However, the variables contributing to CH₄ were not all the same from one diet to another.

Enteric CH₄ Predicted from Equations Reported in Previous Studies

Enteric CH₄ (g/d) estimated using equation 1 and 2 reported by Chilliard et al. (2009) resulted in over-prediction of CH₄ (Table 9). The average percentage over-prediction for the FS, SS, CS, and CTL diets was 12, 14, 20, and 41%, respectively, for equation 2, and 54, 64, 48, and 79%, respectively, for equation 1. Enteric CH₄ per kilogram of DM (g/d), predicted using the equation reported by Dijkstra et al. (2011), also re-

Table 4. Effect of diets¹ on milk monounsaturated fatty acids (MUFA), polyunsaturated fatty acids (PUFA), conjugated linoleic acid (CLA) isomers, and desaturase indices

Variable ²	Diet				SEM	P-value
	CTL	SS	FS	CS		
c9-14:1	0.92	0.91	0.90	0.94	0.07	NS
t9-16:1	0.19	0.23	0.25	0.20	0.02	NS
c9-16:1	1.87 ^a	1.18 ^b	1.20 ^b	1.11 ^b	0.10	<0.01
c9-17:1	0.13	0.14	0.15	0.12	0.01	NS
t6/t8-18:1	0.24	0.22	0.14	0.23	0.03	0.06
t9-18:1	0.33	0.39	0.30	0.36	0.03	0.07
t10-18:1	0.91 ^b	1.47 ^a	1.03 ^b	0.91 ^b	0.16	<0.01
t11-18:1	0.84 ^b	1.69 ^a	1.07 ^b	0.92 ^b	0.14	<0.01
t12-18:1	0.31 ^b	0.49 ^a	0.43 ^a	0.36 ^b	0.03	<0.01
t13/t14/c6-8	0.82 ^b	0.92 ^b	1.36 ^a	0.80 ^{bc}	0.10	<0.01
c9/c10/t15-18:1	22.61 ^c	28.02 ^a	26.62 ^b	28.04 ^a	0.77	<0.01
c11-18:1	0.63 ^b	0.68 ^b	0.65 ^b	0.78 ^a	0.05	<0.01
c12-18:1	0.20 ^c	0.43 ^a	0.32 ^b	0.18 ^c	0.02	<0.01
c13-18:1	0.06 ^a	0.04 ^b	0.06 ^a	0.05 ^{ab}	0.01	0.01
c14/t16-18:1	0.20 ^c	0.36 ^b	0.69 ^a	0.29 ^b	0.03	<0.01
c15-18:1/19:0	0.11 ^b	0.10 ^b	0.49 ^a	0.14 ^b	0.04	<0.01
c16-18:1	0.06 ^b	0.07 ^b	0.13 ^a	0.06 ^b	0.01	<0.01
Σ t-16:1	0.58	0.59	0.57	0.56	0.03	NS
Σ c-16:1	1.92 ^a	1.23 ^b	1.24 ^b	1.16 ^b	0.10	<0.01
Σ t-18:1	3.49 ^{bc}	5.20 ^a	4.36 ^{ab}	3.62 ^b	0.41	<0.01
Σ c-18:1	23.87 ^b	29.71 ^a	28.97 ^a	29.53 ^a	0.81	<0.01
Σ t-MUFA	4.08 ^b	5.80 ^a	4.94 ^{ab}	4.20 ^b	0.42	<0.01
Σ c-MUFA	27.01 ^b	32.16 ^a	31.42 ^a	32.05 ^a	0.81	<0.01
Σ MUFA	31.09 ^c	37.96 ^a	36.36 ^{ab}	36.25 ^b	0.98	<0.01
t9/t12-18:2	0.01	0.01	0.01	0.01	0.00	NS
c9/t12-18:2	0.11 ^b	0.14 ^b	0.28 ^a	0.12 ^b	0.02	<0.01
c9,t13/t8,c12-18:2	0.21 ^b	0.29 ^b	0.59 ^a	0.25 ^b	0.05	<0.01
t8,c13-18:2	0.03 ^c	0.05 ^a	0.04 ^b	0.03 ^{bc}	0.00	<0.01
t11,c15-18:2	0.08 ^{bc}	0.06 ^{bc}	0.38 ^a	0.10 ^b	0.03	<0.01
c9/t11-CLA	0.51 ^c	0.97 ^a	0.68 ^b	0.61 ^{bc}	0.07	<0.01
t10c12-CLA	0.02	0.02	0.02	0.02	0.00	NS
18:2n-6	2.15 ^c	2.82 ^a	2.42 ^b	2.14 ^c	0.11	<0.01
18:3n-6	0.03 ^a	0.03 ^a	0.01 ^b	0.02 ^a	0.00	<0.01
18:3n-3	0.19 ^c	0.18 ^c	1.01 ^a	0.30 ^b	0.04	<0.01
20:2n-6	0.02	0.02	0.02	0.02	0.00	NS
20:4n-6	0.09 ^b	0.11 ^a	0.10 ^{ab}	0.11 ^a	0.01	0.01
22:2n-6	0.01	0.01	0.01	0.01	0.00	0.08
22:4n-3	0.04	0.03	0.03	0.03	0.01	NS
DPA	0.03 ^b	0.03 ^b	0.05 ^a	0.04 ^{ab}	0.00	0.01
DHA	0.00	0.00	0.01	0.00	0.00	NS
Σ n-3 PUFA	0.27 ^c	0.25 ^c	1.11 ^a	0.38 ^b	0.04	<0.01
Σ n-6 PUFA	2.41 ^c	3.11 ^a	2.65 ^b	2.42 ^c	0.11	<0.01
Σ PUFA	2.68 ^c	3.35 ^b	3.76 ^a	2.80 ^c	0.14	<0.01
RA yield, g/d	4.24 ^b	7.41 ^a	5.30 ^b	4.99 ^b	0.65	<0.01
c9-14:1/14:0	0.11	0.11	0.10	0.10	0.01	NS
c9-16:1/16:0	0.05	0.05	0.05	0.05	0.00	NS
c9-18:1/18:0	2.71 ^a	2.03 ^b	2.03 ^b	2.04 ^b	0.11	<0.01
RA/t11-18:1	0.71	0.65	0.78	0.74	0.06	NS

^{a-c}Within a row, means bearing different superscript letters are different ($P < 0.05$).

¹Diets were control [CTL; calcium salts of long-chain fatty acids (palm oil)], sunflower seed (SS), flax seed (FS), and canola seed (CS).

²c = *cis*; t = *trans*; DPA = docosapentaenoic acid; DHA = docosahexaenoic acid; RA = rumenic acid (c9,t11-18:2); expressed as % of total fatty acid methyl esters.

sulted in over-prediction (equation 3 in Table 9; 34.5% for FS; 2% for SS; 26% for CS; and 15% for CTL).

DISCUSSION

Greater concentrations of milk SFA for the CTL diet compared with the SS, FS, and CS diets (Table

3) could be attributed mainly to the greater intakes of 16:0 for the CTL diet than for the SS, FS and CS diets (Table 2). Consistent with this observation, milk 16:0 was also greater for the CTL diet than the SS, FS and CS diets (Table 3). Greater concentrations of LeA in milk for the SS diet (Table 4) was consistent with the greater intakes of LeA for the SS diet (Table 2).

Table 5. Correlations between average methane (g/d) and milk fatty acid concentrations (% total fatty acid methyl esters) or rumen parameters (mol/100 mol) or intake parameters (kg/d) in cows supplemented with various sources of long-chain fatty acids¹

Variable ²	Negative correlation			Variable ³	Positive correlation		
	r	P-value	n		r	P-value	n
Milk fatty acid				Milk fatty acid			
c9-17:1	−0.65	<0.01	32	Σ SFA	0.39	0.03	32
c11-18:1	−0.64	<0.01	32	8:0	0.37	0.03	32
Σ PUFA	−0.48	0.01	32	Milk 16:0 <i>iso</i>	0.32	0.07	32
c13-18:1	−0.43	0.01	32				
t6/t7/t8-18:1	−0.40	0.02	32				
18:2n-6	−0.39	0.03	32				
t6/t7/t8-16:1/17:0 <i>iso</i>	−0.38	0.03	32				
c16-18:1	−0.38	0.03	32				
c9t13/t8c12-18:2	−0.38	0.03	32				
18:3n-3	−0.36	0.04	32				
Σ n-6 PUFA	−0.36	0.04	32				
t10-18:1	−0.34	0.05	32				
c14t16-18:1	−0.30	0.09	32				
15:0	−0.30	0.09	32				
Rumen parameter				Rumen parameter			
Propionate	−0.63	0.01	16	Ac + Bu:Pr	0.72	<0.01	16
Valerate	−0.60	0.02	16	Ac:Pr	0.66	0.01	16
				Σ Entodiniomorphs	0.58	0.02	16
				Acetate	0.56	0.02	16
				Intake parameter			
				DMI	0.52	<0.01	32
				Forage DMI	0.52	<0.01	32
				Milk protein %	0.42	0.02	32
				Intake 12:0	0.39	0.03	32
				Intake SFA	0.34	0.05	32
				Intake 14:0	0.34	0.06	32
				Intake 16:0	0.31	0.08	32

¹Diets were control [calcium salts of long-chain fatty acids (palm oil)], sunflower seed, flax seed, and canola seed.

²c = *cis*; t = *trans*; PUFA = polyunsaturated fatty acids.

³SFA = saturated fatty acids; Ac = acetate; Bu = butyrate; Pr = propionate.

Similarly, greater milk LN for the FS diet (Table 4) was consistent with greater intakes of LN for the FS diet (Table 2). Greater polyunsaturated FA (PUFA) intake often results in inhibition of biohydrogenation efficiency (Harfoot et al., 1973; Beam et al., 2000). Greater milk 18:0 for the CS, FS, and SS diets compared with the CTL diet was consistent with the greater PUFA intakes for these diets. To evaluate biohydrogenation efficiency, milk 18:0 per gram of LeA + LN intake was determined (CTL = 0.06%; SS = 0.02%; FS = 0.02%; and CS = 0.02%). The greater values of milk 18:0 per gram of LeA + LN intake for the CTL diet than for the SS, FS, and CS diets suggest that the CTL diet was less inhibitory to biohydrogenation compared with the other diets.

Greater t11-18:1 for the SS diet compared with the FS diet was anticipated based on previous studies in which diets supplemented with a lipid source rich in LeA resulted in greater t11-18:1 compared with a lipid source rich in LN (Bell et al., 2006; Bu et al., 2007). However, a lack of difference in t11-18:1 between the CTL diet and FS or CS diets was surprising. As expected, milk t10-18:1 was greater for the SS diet compared

with the other diets. Several studies demonstrated that diets rich in SS or oil invariably cause a shift to t10-18:1 (Bell et al., 2006; Shingfield et al., 2006; Mohammed et al., 2011). Greater milk CLA for the SS diet compared with the other diets could be attributed to the greater proportion of t11-18:1 for the SS diet than for the CS, FS, and CTL diets. It should be noted that despite the greater intake of total PUFA substrate (LeA + LN) for the FS diet, milk CLA was greater for the SS diet and not for the FS diet, reflecting greater efficiency of SS in increasing milk CLA. This observation is supported by the greater RA yield for the SS diet compared with the other diets.

Relationship Between CH₄ and Test Variables

It should be noted that the observed relationships between CH₄ production and test variables does not imply a cause and effect relationship. However, a strong and consistent relationship between a test variable and CH₄ production would be suspected of having some biological relevance. One of our objectives was to model the relationship between CH₄ and various test variables

Table 6. Treatment-wise correlations between average methane (g/d) and milk fatty acid concentrations (% total fatty acid methyl esters), rumen parameters (mol/100 mol), or intake parameters (kg/d) in cows supplemented with various sources of long-chain fatty acids¹

Variable ²	Negative correlation			Variable ³	Positive correlation		
	r	P-value	n		r	P-value	n
Control				Control			
Milk c15-18:1/19:0	-0.84	0.01	8	Σ Entodiniomorphs	0.99	0.01	4
Milk c11-18:1	-0.73	0.04	8				
Milk t6-t8-18:1	-0.73	0.04	8				
Sunflower				Sunflower			
Milk c9-17:1	-0.80	0.02	8	Milk 9:0	0.81	0.01	8
Milk Σ c-MUFA	-0.79	0.02	8	Milk Σ 8:0-14:0	0.79	0.02	8
Milk Σ c-18:1	-0.79	0.02	8	Milk Σ SFA	0.76	0.03	8
Milk Σ MUFA	-0.75	0.03	8	De novo FA	0.76	0.03	8
Milk c9/c10/t15-18:1	-0.74	0.04	8	Milk Σ 10:0-14:0	0.75	0.03	8
Milk c11-18:1	-0.71	0.04	8	Milk 12:0	0.75	0.03	8
Preformed FA	-0.71	0.05	8	Milk 15:0 <i>anteiso</i>	0.65	0.08	8
Flax				Flax			
Milk c9-17:1	-0.83	0.01	8	Rumen acetate	0.98	0.02	4
Milk c11-18:1	-0.83	0.01	8	Rumen butyrate	0.96	0.04	4
Milk c13-18:1	-0.81	0.01	8	Milk 14:0 <i>iso</i>	0.94	<0.01	8
Milk LeA	-0.64	0.09	8	Total FA intake	0.74	0.03	8
				Milk 8:0	0.74	0.04	8
				Milk 15:0 <i>iso</i>	0.63	0.08	8
Canola				Canola			
15:0	-0.65	0.08	8	DMI	0.73	0.04	8
				Forage DMI	0.73	0.04	8

¹Diets were control [calcium salts of long-chain fatty acids (palm oil)], sunflower seed, flax seed, and canola seed.

²c = *cis*; t = *trans*; MUFA = monounsaturated fatty acids; FA = fatty acid; LeA = linoleic acid.

³SFA = saturated fatty acids.

to determine if it would be possible to predict CH₄ with reasonable accuracy.

Correlations between CH₄ and the test variables were stronger within diet (Table 6) compared with those obtained with all diets (Table 5) in the model. This finding is consistent with the strong correlations observed in the study of Chilliard et al. (2009), wherein diets used were not very diverse and differed only in the physical form of linseed supplemented. Lower correlation coefficient values observed when all diets were included in the model (Table 5) reflect the weakness of the model in predicting CH₄ for diverse diets. Similarly, Dijkstra et al. (2011) reported relatively weaker correlations between CH₄ and milk FA in a meta-analysis of data from 3 different experiments.

It is well known that CH₄ output is proportional to DMI (Giger-Reverdin et al., 2003; Grainger et al., 2007). Consistent with this, total DMI and forage DMI were positively related to CH₄ (Table 5). Supplementing diets with oilseeds rich in unsaturated FA has been recommended as a strategy for inhibiting CH₄ emissions (Boadi et al., 2004; Beauchemin et al., 2007). It was anticipated that intake of oleic acid, LeA, LN, or LeA + LN would, therefore, be negatively related to CH₄ production. However, none of these FA was related to CH₄ production, indicating that the CH₄-suppressing effects observed with the supplementation of oilseeds is

not due to the direct action of the unsaturated FA but possibly due to the intermediary metabolic products resulting from the biohydrogenation of these FA in the rumen. This effect is supported by the negative correlations observed between CH₄ and the isomers of 18:1 and 18:2 in milk, which is consistent with the findings of Chilliard et al. (2009; Table 5).

Negative correlations of CH₄ with rumen propionate and positive correlations with acetate, acetate:propionate, and the ratio acetate + butyrate:propionate (Table 5) are consistent with the stoichiometric relationship CH₄ = 0.45 acetate - 0.275 propionate + 0.40 butyrate (Demeyer and Van Nevel, 1975; Moss et al., 2000). The conversion of pyruvate to acetate in the rumen results in the formation of H, whereas the conversion of pyruvate to propionate involves utilization of H (Moss et al., 2000). This fermentation pathway explains the positive and negative correlations of acetate and propionate, respectively, with CH₄. The positive correlations observed between CH₄ production and total entodiniomorphs could be explained by the symbiotic relationship between methanogens and protozoa (Vogels et al., 1980; Finlay et al., 1994). As much as 37% of methanogenesis in the rumen is estimated to be contributed by methanogens associated with protozoa (Finlay et al., 1994). The strong positive correlation between CH₄ production and total entodiniomorphs for the CTL

Table 7. Stepwise regression analysis of various parameters with methane (CH₄) output using data from all diets¹

Variable ²	Partial R ²	Model R ²	F-value	P-value
With rumen parameters (n = 16; P = 0.005)				
Acetate:propionate (a), mol/100 mol	0.44	0.44	10.92	0.01
Equation 1		CH ₄ (g/d) = 75.34a (±22.8) + 59.41 (±58.2)		
With intake and production parameters (n = 32; P < 0.001)				
DMI (a), kg/d	0.27	0.27	11.09	<0.01
Milk yield (b), kg/d	0.15	0.42	7.54	0.01
Intake of SFA (c), g/d	0.16	0.58	10.68	<0.01
Equation 2		CH ₄ (g/d) = 19.1a (±3.71) - 6.35b (±1.65) + 0.11c (±0.03) + 34.43 (±65.8)		
With milk fatty acid concentrations (% FAME) (n = 32; P < 0.0001)				
c9-17:1 (a)	0.42	0.42	21.45	<0.01
c11-18:1 (b)	0.14	0.56	9.08	0.01
t,t-CLA (c)	0.07	0.63	5.67	0.02
Σ t-18:1 (d)	0.06	0.69	5.71	0.02
15:0 anteiso (e)	0.05	0.74	5.27	0.03
Equation 3		CH ₄ (g/d) = -486.2a (±191.8) - 122.7b (±44.12) + 2,220c (±515.6) - 11.76d (±4.66) + 260.1e (±113.3) + 272.4 (±67.8)		
With milk fatty acids (% FAME), intake and production parameters (kg/d) (n = 32; P < 0.0001)				
c9-17:1 (a)	0.42	0.42	21.45	<0.01
c11-18:1 (b)	0.14	0.56	9.08	<0.01
t,t-CLA (c)	0.07	0.63	5.67	0.02
Σ t-18:1 (d)	0.06	0.69	5.71	0.02
15:0 anteiso (e)	0.05	0.74	5.27	0.03
17:0 (f)	0.05	0.79	5.89	0.02
c9-17:1 (removed)	0.02	0.77	2.24	0.15
DMI (g)	0.06	0.83	9.57	<0.01
Equation 4		CH ₄ (g/d) = -124.9b (±33.66) + 2,115.9c (±432.6) - 10.04d (±3.84) + 455.5e (±98.9) - 305.9f (±80.7) + 7.83g (±2.53) + 104.8 (±61.9)		
With milk fatty acids (% FAME), intake, production and rumen parameters (n = 16; P < 0.0001)				
c9-17:1 (a)	0.64	0.64	25.13	<0.01
16:0 iso (b)	0.18	0.82	13.23	<0.01
Σ Entodiniomorphs (c)	0.08	0.90	10.36	0.01
Equation 5		CH ₄ (g/d) = -910.8a (±156.7) + 331.2b (±88.8) + 0.0001c (±0.00) + 242.5 (±39.7)		

¹Diets were control [calcium salts of long-chain fatty acids (palm oil)], sunflower seed, flax seed, and canola seed.²c = *cis*; t = *trans*; SFA = saturated fatty acids; FAME = fatty acid methyl esters; CLA = conjugated linoleic acid.

diet is consistent with the greater CH₄ emissions and total protozoa observed for the CTL diet compared with the other diets (Beauchemin et al., 2009).

The best equation in this study predicted CH₄ with the variables milk c9-17:1 and 16:0 *iso* and total entodiniomorphs. It is well known that milk 17:0 has microbial origin and c9-17:1 is a production of desaturation of 17:0 by the mammary desaturase system (Fievez et al., 2003). However, the biological relevance of 17:0 in connection with methanogenesis is not known and is worth exploring. The role of 16:0 *iso* in relation to methanogenesis is not known except that it has microbial origin. Prediction of CH₄ with only milk FA and intake variables also resulted in c9-17:1 being the major contributor, followed by c11-18:1; t,t-CLA; total t-18:1; 15:0 *anteiso*; 17:0; and DMI. Variables in this equation were only partly similar to the equations reported in previous studies (c11-18:1; Dijkstra et al., 2011). The major contributors predicting CH₄ (g/kg of DM) in the study of Dijkstra et al. (2011) were 17:0 *anteiso*, fol-

lowed by t10 + t11-18:1; c11-18:1; and c13-18:1 and those in the study of Chilliard et al. (2009) were milk 14c/16t-18:1, 16:0, and forage intake. The lack of similarity in the equations could be due to differences in the diets as well as analytical procedures among the studies. Even the prediction equation developed for the FS diet in the current study was different from the equations for linseed-supplemented diets reported by Chilliard et al. (2009). Diets used in their study were corn silage based (unlike barley silage in the present study) and contained a greater mean proportion of forage (63 vs. 45%) than in the current study. Differences in the equations could, therefore, be due to differences in the basal diet and proportion of forages between the studies. In the current study, forage DMI was included in the model for stepwise regression analyses. However, it did not contribute to the variability in CH₄, unlike in the study of Chilliard et al. (2009). The lack of contribution of forage DMI to CH₄ in this study could be because forage DMI was estimated from TMR DMI

Table 8. Stepwise regression analysis of milk fatty acids, intake, and production parameters with methane output using data from each diet¹

Variable ²	Partial R ²	Model R ²	F-value	P-value
Control (n = 8; P = 0.0004)				
c15-18:1 (a)	0.71	0.71	14.57	0.01
t10,c12-CLA (b)	0.25	0.96	33.09	<0.01
11:0 (c)	0.031	0.991	16.45	0.02
c9-20:1 (d)	0.0060	0.997	12.38	0.04
Σ fatty acid intake (e)	0.0020	0.999	18.64	0.04
10:0 (f)	0.0001	1.000	9,547.95	0.01
Equation	CH ₄ (g/d) = -3,798.2a (±0.65) - 3,927.4b (±1.46) - 79.7c (±0.11) + 116.3d (±0.28) - 0.02e (±0.00) - 4.67f (±0.05) + 806.2 (±0.16)			
Sunflower (n = 8; P < 0.0001)				
c9-17:1 (a)	0.65	0.65	11.04	0.01
c14/t16-18:1 (b)	0.28	0.93	19.26	0.01
DMI (c)	0.06	0.99	24.86	0.01
13:0 (d)	0.009	0.999	81.13	<0.01
t,t diene (e)	0.001	1.000	384.5	<0.01
Equation	CH ₄ (g/d) = -1,587.5a (±0.03) - 696.9b (±0.02) - 7.34c (±0.00) - 210.2d (±0.06) + 76.5e (±0.15) + 881.9 (±0.02)			
Flax (n = 8; P < 0.0001)				
Intake of LN (a)	0.77	0.77	20.22	<0.01
c9-16:1 (b)	0.15	0.92	9.31	0.034
c9-17:1 (c)	0.067	0.987	21.48	0.01
9:0 (d)	0.012	0.999	88.29	<0.01
c11-18:1 (e)	0.001	1.000	87.20	0.01
Equation	CH ₄ (g/d) = 0.86a (±0.00) - 247.6b (±0.00) - 283.5c (±0.01) + 155.7d (±0.01) - 13.83e (±0.00) + 278.2 (±0.01)			
Canola (n = 8; P < 0.0001)				
DMI (a)	0.54	0.54	6.93	0.04
Σ n-3 PUFA (b)	0.34	0.88	13.88	0.01
c11-20:1 (c)	0.09	0.97	13.69	0.02
Intake of LN (d)	0.02	0.99	11.45	0.04
13:0 anteiso (e)	0.01	1.00	983.01	<0.01
Equation	CH ₄ (g/d) = 35.5a (±0.10) + 634.9b (±2.85) - 783.2c (±7.07) - 0.43d (±0.01) - 564.1e (±18.0) - 530.5 (±2.28)			

¹Diets were control [calcium salts of long-chain fatty acids (palm oil)], sunflower seed, flax seed, and canola seed.²c = *cis*; t = *trans*; CLA = conjugated linoleic acid; LN = linolenic acid; PUFA = polyunsaturated fatty acids.**Table 9.** Prediction of methane (CH₄) output from equations reported in the literature

Diet ¹	Mean predicted CH ₄	Mean actual CH ₄	% over predicted
Equation 1 (Chilliard et al., 2009) ²			
CTL	526.0	293.3	79.4
SS	431.8	264.0	63.6
FS	369.9	240.4	53.9
CS	392.2	265.1	48.0
Equation 2 (Chilliard et al., 2009) ³			
CTL	412.1	293.3	40.5
SS	302.1	264.0	14.4
FS	269.0	240.4	11.9
CS	319.3	265.1	20.4
Equation 3 (Dijkstra et al., 2011) ⁴			
CTL	18.69	16.20	15.35
SS	14.82	14.53	1.99
FS	18.01	13.39	34.50
CS	17.18	13.64	25.98

¹Diets were control [CTL; calcium salts of long-chain fatty acids (palm oil)], sunflower seed (SS), flax seed (FS), and canola seed (CS).²CH₄ output (g/d) = 9.46 × milk 16:0 (% of total FAME) - 97.6 × milk t16,c14-18:1 + 13.3 × forage intake (kg of DM/d) - 78.3 × milk c9-14:1 + 77.4 × milk 18:2n-6 - 21.2; t = *trans*; c = *cis*; FAME = fatty acid methyl esters.³CH₄ output (g/d) = -100.8 (±22.0) × milk t16/c14-18:1 (% of total FAME) + 6.78 (±1.75) × milk 16:0 (% of total FAME) + 13.1 (±3.86) × forage intake (kg of DM/d) + 80.1 (±60.9).⁴CH₄ (g/kg of DM) = 24.6 + 8.74 × 17:0 anteiso (% of total FAME) - 1.97 × t10+t11-18:1 - 9.09 × c11-18:1 + 5.07 × c13-18:1.

based on the proportion of forage in the diet, whereas forage DMI in the study of Chilliard et al. (2009) was recorded, as it involved feeding forage and grain separately.

Based on the stoichiometric relationship between CH₄ and rumen VFA (Demeyer and Van Nevel, 1975; Moss et al., 2000) and the role of milk OBCFA as markers for predicting rumen fermentation pattern (Vlaeminck et al., 2006), Montoya et al. (2011) reported that milk OBCFA hold potential as predictors of rumen methanogenesis. In the current study, CH₄ production was related positively to milk 14:0 *iso* (FS diet, Table 6) and negatively to c9-17:1 (Tables 5 and 6), consistent with the findings of Montoya et al. (2011). Among the different OBCFA, c9-17:1 contributed most in the CH₄ prediction equations developed in this study.

The best equation described by Chilliard et al. (2009) involved the variables milk 16:0, t16/c14-18:1, forage intake, c9-14:1, and milk 18:2. Estimating CH₄ using both equations 1 and 2 reported by Chilliard et al. (2009) resulted in over-prediction of CH₄ (Table 9). This could be attributed to the lack of correlations between CH₄ and the variables described above (except for forage DMI and milk 18:2) in the present study. However, it should be noted that equation 2 described by Chilliard et al. (2009) involving only 2 milk FA and forage DMI resulted in lesser over-estimation of CH₄ (mean value for all 4 diets 22%), in contrast to equation 1 involving 4 milk FA and forage DMI (mean value for all 4 diets 61%). This result suggests that milk FA variables may be less suitable for predicting methane compared to DMI variables. The CH₄ prediction equation developed by Dijkstra et al. (2011) involving the variables 17:0 *anteiso*, t10- + t11-18:1, c11-18:1, and c13-18:1 also resulted in over-prediction. This could be attributed to the lack of correlations between CH₄ and the variables described above (except the variables c11- and c13-18:1) in the current study.

CONCLUSIONS

A data set from dairy cows fed diets supplemented with oilseeds was used to evaluate the effects of fat-supplemented diets on milk FA profile; model CH₄ production from milk FA composition, intake, and rumen fermentation variables; and test the applicability of CH₄ prediction equations reported in previous studies. Milk FA composition for cows fed diets supplemented with various sources of long-chain FA revealed that t11-18:1, RA, and RA yield was greater for the SS diet compared with FS, CS, and CTL diets. Methane output (g/d) was positively related to total DMI (kg/d), forage DMI (kg/d), and the ratio ruminal acetate + butyrate:propionate (mol/100 mol) and negatively re-

lated to rumen propionate concentration, milk c9-17:1, and c11-18:1 (% of total FAME). The best equation predicted CH₄ with a correlation coefficient of 0.90 and included milk c9-17:1, 16:0 *iso*, and total entodiniomorphs. Using only milk FA, intake and production variables also provided a good estimate of CH₄ (R² = 0.83). More accurate predictions were developed when specific to individual diets, but the data set was limited in size. Previously published equations over-predicted CH₄ production by 19 to 61%. Overall, this body of research demonstrates that the scope for developing universal methane prediction equations from milk FA composition alone appears to be limited.

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