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Milk fatty acids in dairy cows fed whole crude linseed, extruded linseed, or linseed oil, and their relationship with methane output[1]

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ABSTRACT

This experiment studied the effect of 3 different physical forms of linseed fatty acids (FA) on cow dairy performance, milk FA secretion and composition, and their relationship with methane output. Eight multiparous, lactating Holstein cows were assigned to 1 of 4 dietary treatments in a replicated 4 × 4 Latin square design: a control diet (C) based on corn silage (59%) and concentrate (35%), and the same diet supplemented with whole crude linseed (CLS), extruded linseed (ELS), or linseed oil (LSO) at the same FA level (5% of dietary dry matter). Each experimental period lasted 4 wk. Dry matter intake was not modified with CLS but was lowered with both ELS and LSO (−3.1 and −5.1 kg/d, respectively) compared with C. Milk yield and milk fat content were similar for LSO and ELS but lower than for C and CLS (19.9 vs. 22.3 kg/d and 33.8 vs. 43.2 g/kg, on average, respectively). Compared with diet C, CLS changed the concentrations of a small number of FA; the main effects were decreases in 8:0 to 16:0 and increases in 18:0 and *cis*-9 18:1. Compared with diet C (and CLS in most cases), LSO appreciably changed the concentrations of almost all the FA measured; the main effects were decreases in FA from 4:0 to 16:0 and increases in 18:0, *trans*-11 16:1, all *cis* and *trans* 18:1 (except *trans*-11 18:1), and nonconjugated *trans* 18:2 isomers. The effect of ELS was either intermediate between those of CLS and LSO or similar to LSO with a few significant exceptions: increases in 17:0 *iso*; 18:3n-3; *trans*-11 18:1; *cis*-9, *trans*-11 conjugated linoleic acid; and *trans*-11, *trans*-13 conjugated linoleic acid and a smaller increase in *cis*-9 18:1. The most positive correlations (r = 0.87 to 0.91) between milk FA concentrations and methane output were observed for saturated FA from 6:0 to 16:0 and for 10:1, and the most negative correlations (r = −0.86 to −0.90) were observed for *trans*-16+*cis*-14 18:1; *cis*-9, *trans*-13 18:2; *trans*-11 16:1; and *trans*-12 18:1. Thus, milk FA profile can be considered a potential indicator of in vivo methane output in ruminants.

Key words: dairy cow, linseed oil, linseed, milk fatty acid

INTRODUCTION

Milk fat is an important determinant of milk nutritional quality. The saturated fatty acids (**FA**; mainly 12:0, 14:0, and 16:0) are considered to produce negative effects when consumed in excess, whereas others (4:0, *anteiso*-15:0, *cis*-9 18:1, 18:3n-3) have well-known or potential positive effects on human health (Parodi, 2005). In addition, *cis*-9,*trans*-11 18:2, the major isomer of conjugated linoleic acids (**CLA**) in ruminant milk, is anticarcinogenic and antiatherogenic in experimental animal models (Huth et al., 2006). In addition, ruminant milk fat content and composition can be extensively modified by nutritional factors, in particular fat supplementation of the diet (Shingfield et al., 2008).

There is growing interest in feeding linseed to dairy cows because of its FA profile; linolenic acid contributes to dietary n-3 FA and promotes increased CLA content while decreasing the saturated FA content of ruminant milk (review by Chilliard et al., 2007). The effects of linseed supplementation on milk yield and composition have often been studied (review by Glasser et al., 2008a). Many studies have used whole, rolled, crushed, or ground crude linseed (e.g., Kennelly, 1996; Collomb et al., 2004); linseed oil (e.g., Dhiman et al., 2000; Loor et al., 2005); and either extruded or micronized linseed (e.g., Gonthier et al., 2005; Akraim et al., 2007). However, only a few studies have directly compared different physical forms of linseed: whole versus rolled crude linseed (Kennelly, 1996) or ground crude versus extruded linseed (Gonthier et al., 2005; Akraim et al., 2007). There has been no direct comparison between supplementation with linseed oil in free form versus oil contained in linseed. Feeding linseed FA also decreases daily methane (CH_4) emissions in dairy cows (Martin et al., 2007, 2008; Beauchemin et al., 2009), which is of

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major interest given current concern about the effect of greenhouse gases on climate change and because CH_4 emission from ruminants represents a loss of productive energy for the animals (Martin et al., 2009).

The objectives of this study were 1) to compare the effects on cow dairy performance and milk fatty acid secretion and composition of 4 diets based on corn silage and containing either no linseed (control) or high doses of linseed oil in 3 physical forms (whole crude linseed, extruded linseeds, or linseed oil plus linseed meal) and 2) to study the relationship between individual milk FA composition and CH_4 output measured on the same cows and diets (see companion paper, Martin et al., 2008). Several studies have reported simultaneous changes in CH_4 output and milk FA composition after lipid supplementation of dairy cow diet (e.g., Sauer et al., 1998; Johnson et al., 2002; Odongo et al., 2007), but no work has evaluated the use of milk FA composition to predict changes in CH_4 output.

MATERIALS AND METHODS

Experimental details have been presented in a companion paper (Martin et al., 2008) and are summarized in the following.

Animals, Experimental Design, and Diets

Eight lactating, multiparous Holstein cows (213 ± 40 DIM) with an average milk yield of 23.4 ± 2.2 kg/d and an average BW of 672 ± 54 kg at the beginning of the experiment were used. Animals were blocked according to their physiological stage (4 nonpregnant cows and 4 pregnant cows, 161 ± 99 d pregnant at the end of the trial) and assigned to 4 dietary treatments in a replicated 4 × 4 Latin square design. Each experimental period lasted 4 wk.

The treatments were 1) control diet (**C**), 2) diet C with whole crude linseed (**CLS**), 3) diet C with extruded linseed (**ELS**), and 4) diet C with linseed oil (**LSO**). The control diet consisted of 58.7% corn silage, 6.4% grass hay, and 34.9% concentrates, on a DM basis. Linseed oil was added to achieve a theoretical oil level of 5% of dietary DM and replaced part of the concentrate portion of the basal diet to obtain isoenergetic diets on an NE_L basis (target value 7.1 MJ/kg of DM). In the CLS and ELS diets, proportions of whole crude and extruded linseed were calculated so that the mean oil content of these diets was similar to that of the LSO diet. Crude linseed was given as unprocessed whole seeds. Extruded linseed (INZO, Château-Thierry, France) consisted of an extruded mixture of 70% linseed and 30% wheat. In addition, 200 g/d of a commercial mineral–vitamin premix (Galaphos, Centraliment, Aurillac, France) was added to all diets. Diets were formulated to meet the cows' requirements for maintenance and milk production (INRA, 1989) and to contain the same quantity of limiting intestinal-digestible protein (PDI system; INRA, 1989) supplied by all the feedstuffs containing linseed (linseed meal and whole crude and extruded linseeds).

Forages (hay and corn silage) were offered once daily at 0900 h with ad libitum access to corn silage (10% refusals). Concentrates were allocated separately from forages in 2 equal portions at 0900 and 1600 h using a bucket to ensure complete consumption of the linseed. Ingredients and chemical composition of the experimental diets as consumed are given in Table 1.

Cows had free access to water throughout the experiment and were milked twice daily at 0630 and 1630 h. All experimental procedures were conducted in accordance with French guidelines for the use of experimental animals and animal welfare (Fixant les conditions d'attribution de l'autorisation d'expérimenter, 1988).

Measurements and Analyses

Intake and Diet Composition. Feed intake and orts were measured and recorded on 5 consecutive days each week throughout the experiment to calculate DMI. Dry feed samples were pooled at the end of each experimental period for corn silage and the end of the experiment for the other feeds. These samples were ground (0.8-mm screen) and analyzed for OM, N, NDF, ADF, starch, ether extract, and total FA. Fresh samples of each feed (1 kg for corn silage, 100–200 g for other feeds) were also taken at wk 4 and stored (−20°C for corn silage and 4°C for other feeds) before pooling at the end of the experiment. These samples were freeze-dried, ground (0.8-mm screen), and analyzed for FA content.

Organic matter content of feeds was determined by ashing at 550°C for 6 h (AOAC, 1990). Nitrogen was analyzed by the Kjeldahl procedure (AOAC, 1990). Contents of NDF and ADF were determined by sequential procedures (Van Soest et al., 1991) after pretreatment with amylase and were expressed inclusive of residual ash. Starch was analyzed by a polarimetric method (Association Française de Normalisation, 1985). Determination of ether extract was performed after acid hydrolysis, according to AOAC (1990). Fatty acids from linseed oil were directly methylated with 2 mL of 0.5 *N* $NaOCH_3$ in methanol at room temperature for 20 min, followed by 1 mL of 5% HCl in methanol at room temperature for 20 min. Fatty acids in feedstuffs were extracted using a 2:1 chloroform:methanol mixture. Fatty acid methyl esters were recovered in 1 mL of hexane. Tricosanoate (Sigma, Saint-Quentin-Fallavier, France)

Table 1. Ingredients and chemical composition of consumed experimental diets

	Diet[1]				
Item	C	CLS	ELS	LSO	SEM
Ingredient (% of DM)					
Corn silage	58.7	59.6	54.1	51.3	1.09
Grass hay	6.4	6.7	7.8	8.9	0.23
Concentrates	34.9	33.8	38.2	39.8	0.94
Concentrate mixture[2]	11.5	1.9	8.8	4.2	0.44
Extruded wheat	5.2	5.5	0.0	6.9	0.19
Soybean meal	7.6	8.3	8.1	8.6	0.29
Linseed meal	10.6	5.7	0.0	14.3	0.39
Crude linseed	0.0	12.4	0.0	0.0	0.19
Extruded linseed + wheat	0.0	0.0	21.2	0.0	0.37
Linseed oil	0.0	0.0	0.0	5.8	0.16
Mineral–vitamin mix[3]	1.0	1.0	1.2	1.4	0.02
Chemical composition (% of DM)					
OM	95.3	95.5	95.3	89.9	0.15
CP	14.5	14.9	14.6	14.6	0.19
NDF	32.9	32.0	30.8	31.4	0.16
ADF	17.5	16.9	16.7	16.6	0.10
Starch	26.5	24.8	21.2	23.2	0.30
Ether extract	2.6	6.8	7.0	8.4	0.16
Fatty acids (FA)	2.3	5.2	5.7	8.0	0.15
FA profile (% of total FA)					
14:0	0.39	0.20	0.16	0.15	0.003
16:0	15.15	9.74	8.61	8.17	0.065
18:0	2.49	2.83	2.88	2.49	0.073
cis-9 18:1	19.85	16.43	15.45	15.09	0.040
trans-11 18:1	0.91	0.67	0.64	0.70	0.066
cis-9, *cis*-12 18:2	41.34	27.70	24.21	21.32	0.193
20:0	0.38	0.24	0.10	0.11	0.002
18:3n-3	16.20	40.34	46.40	49.15	0.297
22:0	0.28	0.18	0.14	0.08	0.002
24:0	0.31	0.18	0.15	0.71	0.006
Others	2.52	1.39	1.18	1.09	0.023

[1]C = control; CLS = diet C including crude linseed; ELS = diet C including extruded linseed; LSO = diet C including linseed oil.

[2]Composition (g/kg): dehydrated beet pulp, 300; wheat, 200; barley, 200; rapeseed meal, 150; soybean meal, 70; beet molasses, 50; limestone, 10; dicalcium phosphate, 10; magnesium oxide, 5; sodium chloride, 5.

[3]Mineral–vitamin mix = Galaphos, manufactured by Centraliment (Aurillac, France). Composition (g/kg): Ca, 200; P, 45; Mg, 45; Na, 50; Cu, 1.3; Zn, 6.0; Mn, 3.5; I, 0.08; Co, 0.032; Se, 0.020; vitamin A, 600,000 IU; vitamin D_3, 120,000 IU; vitamin E, 1,300 IU.

was added as internal standard. Methyl esters were injected into a Trace-GC 2000 Series gas chromatograph equipped with a flame ionization detector (Thermo Finnigan, Les Ulis, France). Methyl esters were separated using a fused silica capillary column (100 m × 0.25 mm i.d.; CP-Sil 88, Chrompack, Middelburg, the Netherlands). Conditions for chromatographic analysis were as described in Loor et al. (2005).

Milk Yield and Fatty Acids. Milk yield was determined on the same 5 consecutive days as for intake from wk 1 to wk 4. On wk 4, milk samples were taken at each milking on d 2 and 4. One 50-mL aliquot of milk containing potassium bichromate (Merck, Fontenay-sous-Bois, France) was stored at 4°C until analysis for fat, protein, and lactose by infrared analysis with a 3-channel spectrophotometer (MilkoScan, Foss Electric, Hillerød, Denmark; AOAC, 1997).

Aliquots (3 mL) from 2 consecutive milkings were collected and stored at −20°C before lyophilization (Thermovac TM-20, Froilabo, Ozoir-la-Ferriere, France) and FA analysis (Chilliard et al., 2006). These samples were composited based on the a.m. and p.m. milk production. Hexane (2 mL, HPLC grade) was added to 130 mg of lyophilized milk followed by 2 mL of 0.5 *M* sodium methylate. This mixture was vortexed and heated at 50°C for 15 min; 75 μL of HCl 12 *N* was then added and allowed to react for 15 min at ambient temperature. Hexane (3 mL) and deionized water (3 mL) were added, and the mixture was centrifuged (1,160 × *g*, 5 min, 5°C). Fatty acid methyl esters were then separated on a 100 m × 0.25 mm i.d. fused silica capillary column (CP-Sil 88, Chrompack). Samples were injected (0.5 to 1 μL of methyl esters in hexane injected at a 50:1 split ratio) by auto-sampler into a Trace-GC 2000 Series gas

Table 2. Silage, concentrate, and total DM intake; milk production and composition; and BW in cows receiving diets supplemented or not supplemented with linseed

	Diet[1]					
Item	C	CLS	ELS	LSO	SEM	$P <$
DMI (kg/d)						
Silage	11.7[a]	11.7[a]	9.0[b]	7.7[c]	0.30	0.001
Concentrate	6.8[a]	6.6[a]	6.4[a]	5.8[b]	0.16	0.01
Total	19.8[a]	19.5[a]	16.7[b]	14.7[c]	0.31	0.001
Milk (kg/d)	23.0[a]	21.5[a]	20.8[ab]	18.9[b]	0.74	0.05
Composition (g/kg)						
Fat	41.1[a]	45.4[a]	35.3[b]	32.3[b]	1.48	0.001
Protein	34.0	34.6	33.3	34.7	0.71	NS[2]
Lactose	48.3	48.2	48.0	48.6	0.25	NS
Yield (g/d)						
Fat	950[a]	965[a]	709[b]	622[b]	39.1	0.001
Protein	776[a]	733[ab]	680[bc]	644[c]	24.8	0.01
Lactose	1,111[a]	1,033[ab]	1,000[a,b]	922[b]	36.9	0.05
BW (kg)	717	714	708	708	5.4	NS

[a–c]Within a row, means without a common superscript differ ($P < 0.05$).

[1]C = control; CLS = diet C including crude linseed; ELS = diet C including extruded linseed; LSO = diet C including linseed oil.

[2]NS: $P > 0.05$.

chromatograph equipped with a flame-ionization detector (Thermo Finnigan). The injector temperature was maintained at 255°C and the detector temperature at 260°C. The initial oven temperature was held at 70°C for 1 min, increased by 5°C/min to 100°C (held for 2 min), and then increased by 10°C/min to 175°C (held for 40 min) and by 5°C/min to a final temperature of 225°C (held for 15 min). The carrier gas was hydrogen, and the injector pressure was held constant at 158.6 kPa. Satisfactory separations of *cis*- and *trans*-18:1, nonconjugated 18:2, and CLA isomers were obtained with a single chromatographic run. Identification of 18:1, 18:2, CLA, and 18:3 isomers and odd- and branched-chain FA was as described in Loor et al. (2005). Peaks were routinely identified by comparison of retention times with FAME standards (GLC 463, Nu-Chek Prep Inc., Elysian, MN; reference mixture 47885, Supelco, Bellefonte, PA; and custom preparation of C18:1, C18:2, and CLA isomers, kindly donated by J. H. Herbein, Virginia Tech, Blacksburg, VA). Correction factors for C4:0 to C10:0 were determined on each day of analysis using a butter oil reference standard (CRM 164, Community Bureau of Reference, Brussels, Belgium). Peak integration used ChromQuest software (ChromQuest Version 3.0, ThermoQuest Corporation, San Jose, CA).

Statistical Analyses. Data on DMI and milk production were averaged on the first 5 d of wk 4 before statistical analysis. All data from the experiment were analyzed as a replicated 4 × 4 Latin square using the MIXED procedure of SAS (SAS Institute, 2000). The statistical model included cow, period, treatment (4 diets), square (one square for pregnant cows and one square for nonpregnant cows), square × treatment interaction, and residual error. Fixed effects included period, treatment, and square. Cow within square was the random effect. Overall differences between treatment means were considered significant when $P < 0.05$. Differences between diets were determined using the procedure of least squares means. Correlations between milk FA profile, milk fat content, and CH_4 output (measured in companion paper, Martin et al., 2008) were calculated on individual values (n = 32). The PROC CORR and GLM procedures of SAS (SAS Institute, 2000) were used for correlation analysis. Multiple regressions to improve the prediction of CH_4 output from dietary intake components, milk yield and composition, and milk FA concentrations (except 5 minor FA with mean concentrations lower than 0.02% of total FA: 22:0; 22:4n-6; *trans*-4 18:1; *trans*-5 18:1; and *trans*-11, *trans*-13 CLA) were performed using the STEPWISE procedure of SAS.

RESULTS

Dairy Performance

Compared with diet C, diet CLS had no effect on silage and concentrate intakes or total DMI ($P > 0.05$), whereas diets ELS and LSO decreased total DMI (−3.1 and −5.1 kg/d, respectively; $P < 0.001$) through a decrease in corn silage intake (−2.7 and −4.0 kg/d, respectively; $P < 0.001$) (Table 2). The negative effect on DMI was greater for LSO than for ELS ($P < 0.01$) because of combined decreases in forage and concentrate intakes.

Milk yield for LSO (18.9 kg/d) was lower than for C and CLS (22.3 kg/d on average; $P < 0.01$), with ELS taking an intermediate value (Table 2). Compared with that for diet C, milk fat content tended ($P = 0.09$) to be higher for CLS (+4.3 g/kg) and was lower for ELS and LSO (−7.3 g/kg on average; $P < 0.001$). Protein and lactose contents did not vary among diets. The yield of milk fat was lower for ELS and LSO than for C and CLS ($P < 0.001$). The yields of protein and lactose were lower for ELS and LSO than for C ($P < 0.05$). Body weight did not change across diets.

Milk Fatty Acid Composition

Compared with diet C, CLS changed the concentrations of a small number of FA, 8:0 to 16:0 decreased, whereas odd- and branched-chain FA did not change, except for a small decrease in 17:0 (Table 3). For the CLS diet, large increases in 18:0 and *cis*-9 18:1 and small increases in 20:0 and *trans*-16+*cis*-14 18:1 were observed, whereas small decreases were observed in *cis*-9,*cis*-12 18:2; *cis*-9,*cis*-11 CLA (Table 4); 22:5n-3; sum of CLA; and sum of saturated and polyunsaturated FA (Table 3).

Compared with diet C (and CLS in most cases), LSO appreciably changed the concentrations of almost all the FA measured (Tables 3 and 4). Diet LSO, compared with diet C, decreased all FA (except 5:0) from 4:0 to 16:0; *cis*-9,*cis*-12 18:2; and long-chain polyunsaturated FA from 20:3n-6 to 22:5n-3. Diet LSO increased 18:0, *trans*-11 16:1, all *cis* and *trans* 18:1 (except for *trans*-11 18:1), and nonconjugated *trans*-18:2 isomers. However, LSO did not change CLA isomers, with the exception of a slight decrease in *cis*-9,*cis*-11 CLA (Table 4). Overall, LSO very strongly decreased saturated FA and strongly increased unsaturated FA including *trans* FA (Tables 3 and 4).

The effect of ELS was either intermediate between those of CLS and LSO or similar to LSO with a few significant exceptions: increases in 17:0 *iso*; 18:3n-3; *trans*-11 18:1; *cis*-9,*trans*-11 CLA; and *trans*-11,*trans*-13 CLA, and a smaller increase in *cis*-9 18:1.

Milk Fatty Acid Secretion and Desaturation Ratios

The daily secretion of FA was not changed by CLS, except for decreases in 10:0 to 14:0, odd- and branched-chain FA, and sum of 18:1 *trans* and 18:2 *trans* (except *trans*-11 18:1 and *cis*-9,*trans*-11 CLA) and sharp increases in 18:0 and *cis*-9 18:1 (Table 5). Diet LSO, compared with diet C, appreciably decreased the secretion of short- and medium-chain FA, slightly decreased 18:3n-3, appreciably increased *cis* and *trans* isomers of 18:1, except for *cis*-9 18:1 and *trans*-11 18:1, and did not change *cis*-9,*trans*-11 CLA. The effect of ELS was either intermediate between those of CLS and LSO or similar to LSO, except for an increase in the secretion of 18:3n-3.

The transfer rates from diet to milk of 18:3n-3 and C18-FA were lower with lipid-supplemented diets than with diet C (Table 5). The Δ^9-desaturation ratios [product/(substrate + product)] of 5 FA were higher with LSO than with the other diets (except for 18:0 with diet C). There were few differences between diets C, CLS, and ELS: an increase in the 17:0 ratio for ELS and a decrease in the 18:0 ratio for CLS (Table 5).

Correlations Among Milk Fatty Acid Composition, Methane Output, and Milk Fat Content

The most positive correlations (r = 0.87 to 0.91) among individual milk FA concentrations and CH_4 output were observed for saturated FA from 6:0 to 16:0 and for 10:1, whereas r values between 0.83 and 0.71 were observed for 20:4n-6, 11:0, 12:1, 9:0, 15:0, 17:0, and 4:0 (Table 6). The most negative correlations (r = −0.86 to −0.90) with CH_4 output were observed for *trans*-16+*cis*-14 18:1; *cis*-9,*trans*-13 18:2; *trans*-11 16:1; and *trans*-12 18:1, whereas r values between −0.84 and −0.72 were observed for several *cis* and *trans* isomers of 18:1, including *cis*-9 18:1 and *trans*-11,*cis*-15 18:2. When using sums of related FA, the correlation extremes (Figure 1) were observed for 8:0 to 16:0 (r = 0.94) and for the sum of C18-FA (r = −0.94). Almost all the correlations studied were linear, except for *trans*-16+*cis*-14 18:1 and *cis*-9, *trans*-13 18:2 [r = −0.94 and −0.91, respectively, with ln(CH_4 output); Figure 1]. Correlations between CH_4 output and milk FA secretions were lower than correlations between CH_4 output and milk FA concentrations.

Multiple regression analysis (stepwise approach) was performed to describe CH_4 output from a combination of dietary intake components, milk yield and composition, and milk FA concentrations. The best equation included 5 parameters (each significant at $P < 0.01$):

$$\begin{aligned} CH_4 \text{ output (g/d)} = {} & 9.46\ (\pm 1.68) \times \text{milk 16:0} \\ & (\%\text{ of total FA}) - 97.6\ (\pm 19.0) \times \text{milk } \textit{trans}\text{-16} \\ & +\textit{cis}\text{-14 18:1 (\% of total FA)} + 13.3\ (\pm 3.43) \\ & \times \text{forage intake (kg of DM/d)} - 78.3\ (\pm 23.4) \\ & \times \text{milk } \textit{cis}\text{-9 14:1 (\% of total FA)} + 77.4\ (\pm 26.6) \\ & \times \text{milk 18:2n-6 (\% of total FA)} - 21.2\ (\pm 72.6) \end{aligned}$$

$$(n = 32,\ R^2 = 0.953,\ \text{root mean square error} = 28.8\ \text{g/d}).$$

Table 3. Milk fatty acid (FA) composition in cows receiving diets supplemented or not supplemented with linseed

	Diet[1]					
Fatty acid (% of total FA)	C	CLS	ELS	LSO	SEM	$P <$
4:0	3.13[a]	3.11[a]	2.78[a]	2.05[b]	0.125	0.001
5:0	0.02	0.03	0.03	0.02	0.006	NS
6:0	2.24[a]	2.14[a]	1.64[b]	1.06[c]	0.069	0.001
7:0	0.036[a]	0.031[a]	0.026[ab]	0.013[b]	0.005	0.05
8:0	1.41[a]	1.24[b]	0.89[c]	0.54[d]	0.047	0.001
9:0	0.039[a]	0.034[ab]	0.024[bc]	0.013[c]	0.004	0.01
10:0	3.37[a]	2.74[b]	1.89[c]	1.09[d]	0.129	0.001
10:1	0.38[a]	0.30[b]	0.20[c]	0.12[d]	0.012	0.001
11:0	0.07[a]	0.06[a]	0.04[b]	0.03[b]	0.005	0.001
12:0	4.22[a]	3.22[b]	2.36[c]	1.52[d]	0.182	0.001
12:1	0.13[a]	0.09[b]	0.07[bc]	0.05[c]	0.010	0.001
13:0	0.12[a]	0.11[a]	0.09[b]	0.08[b]	0.006	0.001
14:0 *iso*	0.15[a]	0.15[a]	0.10[ab]	0.04[b]	0.026	0.05
14:0	12.59[a]	10.80[b]	8.83[c]	5.88[d]	0.303	0.001
15:0 *iso*	0.29[a]	0.32[a]	0.26[a]	0.15[b]	0.024	0.001
15:0 *anteiso*	0.64[a]	0.60[ab]	0.53[b]	0.38[c]	0.035	0.001
cis-9 14:1	1.33[a]	0.95[b]	0.97[b]	0.89[b]	0.071	0.01
15:0	1.16[a]	1.06[a]	0.92[b]	0.78[c]	0.032	0.001
16:0 *iso*	0.31[a]	0.34[a]	0.27[a]	0.12[b]	0.036	0.01
16:0	29.06[a]	25.00[b]	19.62[c]	15.94[d]	0.685	0.001
17:0 *iso*	0.50[b]	0.47[b]	0.67[a]	0.47[b]	0.035	0.01
trans-11 16:1	0.03[c]	0.03[c]	0.11[b]	0.20[a]	0.010	0.001
17:0 *anteiso*	0.50[a]	0.40[ab]	0.42[ab]	0.34[b]	0.033	0.05
cis-9 16:1	1.60	1.18	1.28	1.48	0.124	NS
cis-11 16:1	0.03	0.02	0.02	0.03	0.004	NS
17:0	0.67[a]	0.57[b]	0.51[c]	0.50[c]	0.012	0.001
18:0 *iso*	0.03	0.02	0.05	0.03	0.011	NS
cis-9 17:1	0.19[ab]	0.17[b]	0.18[ab]	0.20[a]	0.008	0.05
18:0	8.32[c]	13.72[a]	11.74[ab]	11.31[b]	0.730	0.001
Sum of 18:1 *trans*	3.49[b]	2.13[b]	9.95[a]	10.63[a]	0.571	0.001
Sum of 18:1 *cis*	18.73[c]	24.84[b]	25.28[b]	34.22[a]	0.856	0.001
Sum of 18:2[2]	2.59[c]	2.05[c]	4.21[b]	7.17[a]	0.320	0.001
20:0	0.06[b]	0.09[a]	0.06[b]	0.05[b]	0.007	0.05
18:3n-3	0.67[b]	0.65[b]	1.20[a]	0.54[b]	0.062	0.001
Sum of CLA[3]	0.84[b]	0.48[c]	1.33[a]	0.66[bc]	0.108	0.001
22:0	0.02	0.02	0.01	0.01	0.005	NS
20:3n-6	0.06[a]	0.05[a]	0.03[b]	0.03[b]	0.004	0.001
20:4n-6	0.09[a]	0.07[a]	0.06[b]	0.03[c]	0.005	0.001
20:5n-3	0.06[a]	0.05[a]	0.04[a]	0.02[b]	0.004	0.001
22:4n-6	0.01[a]	0.01[a]	0.001[b]	0.001[b]	0.001	0.01
22:5n-3	0.09[a]	0.07[b]	0.06[c]	0.03[d]	0.005	0.001
Other FA	0.76[b]	0.56[b]	1.22[a]	1.27[a]	0.059	0.001
Saturated	68.95[a]	66.27[b]	53.74[c]	42.38[d]	0.794	0.001
Monounsaturated	26.14[d]	29.89[c]	38.61[b]	48.48[a]	0.667	0.001
Polyunsaturated	4.42[c]	3.45[d]	6.94[b]	8.48[a]	0.352	0.001

[a–d]Within a row, means without a common superscript differ ($P < 0.05$).

[1]C = control; CLS = diet C including crude linseed; ELS = diet C including extruded linseed; LSO = diet C including linseed oil.

[2]Nonconjugated 18:2.

[3]CLA = conjugated linoleic acid.

A simpler but very good equation was also obtained when including only the first 3 predictive parameters (each significant at $P < 0.002$):

$$\text{CH}_4 \text{ output (g/d)} = -100.8\ (\pm 22.0) \times \text{milk } \textit{trans}\text{-16+}\textit{cis}\text{-14 18:1 (\% of total FA)} + 6.78\ (\pm 1.75) \times \text{milk 16:0 (\% of total FA)} + 13.1\ (\pm 3.86) \times \text{forage intake (kg of DM/d)} - 80.1\ (\pm 60.9)$$

(n = 32, $R^2 = 0.931$, root mean square error = 37.8 g/d).

Correlations of milk FA concentrations with milk fat content were lower than with CH_4 output (absolute r values between 0.71 and 0.78), positive with 6:0 and 8:0, and negative with *cis*-13 18:1; *cis*-9, *trans*-13 18:2; *trans*-11 16:1; *trans*-12 18:1; and *trans*-10 18:1 (Table 6). The correlation between milk fat content and CH_4 output was −0.72.

Table 4. Milk 18:1 and 18:2 composition in cows receiving diets supplemented or not supplemented with linseed

	Diet[1]					
Fatty acid (% of total fatty acids)	C	CLS	ELS	LSO	SEM	$P <$
trans-4 18:1	0.013[b]	0.006[b]	0.031[a]	0.034[a]	0.004	0.001
trans-5 18:1	0.009[b]	0.004[b]	0.031[a]	0.043[a]	0.005	0.001
trans-6+7+8 18:1	0.25[b]	0.23[b]	0.63[a]	0.69[a]	0.028	0.001
trans-9 18:1	0.22[b]	0.18[b]	0.49[a]	0.43[a]	0.025	0.001
trans-10 18:1	0.39[b]	0.25[b]	2.80[a]	2.69[a]	0.282	0.001
trans-11 18:1	1.49[b]	0.98[b]	2.75[a]	1.08[b]	0.301	0.01
trans-12 18:1	0.36[c]	0.31[c]	0.91[b]	1.11[a]	0.026	0.001
trans-13+14 18:1	0.76[c]	0.18[c]	2.32[b]	4.56[a]	0.342	0.001
trans-16+*cis*-14 18:1	0.35[d]	0.43[c]	0.93[b]	1.52[a]	0.025	0.001
cis-9 18:1	17.40[c]	23.51[ab]	22.41[b]	26.26[a]	1.016	0.001
cis-10 18:1	0.001[b]	0.001[b]	0.30[b]	1.44[a]	0.205	0.001
cis-11 18:1	0.41[c]	0.40[c]	0.50[b]	0.59[a]	0.017	0.001
cis-12 18:1	0.28[b]	0.22[b]	0.25[b]	0.68[a]	0.047	0.001
cis-13 18:1	0.07[c]	0.04[c]	0.15[b]	0.24[a]	0.009	0.001
cis-15+*trans*-17 18:1	0.23[b]	0.24[b]	0.74[b]	3.50[a]	0.263	0.001
trans-9, *trans*-12 18:2	0.001[b]	0.001[b]	0.07[a]	0.07[a]	0.013	0.001
cis-9, *trans*-13 18:2	0.28[c]	0.23[c]	0.79[b]	1.62[a]	0.073	0.001
trans-11, *cis*-15 18:2	0.32[c]	0.20[c]	1.05[b]	1.54[a]	0.122	0.001
cis-9, *cis*-12 18:2	1.69[a]	1.28[c]	1.61[ab]	1.53[b]	0.042	0.001
Other nonconjugated 18:2	0.30[b]	0.34[b]	0.69[b]	2.42[a]	0.163	0.001
cis-9, *trans*-11 CLA[2]	0.77[b]	0.44[b]	1.27[a]	0.65[b]	0.105	0.001
cis-9, *cis*-11 CLA	0.06[a]	0.02[b]	0.03[b]	0.01[c]	0.004	0.001
trans-11, *trans*-13 CLA	0.006[b]	0.010[b]	0.019[a]	0.001[b]	0.003	0.01
Sum of *trans* C18[3]	4.87[b]	3.02[b]	13.16[a]	14.51[a]	0.687	0.001
Sum of non-VA/RA t18[4]	2.62[c]	1.60[c]	9.14[b]	12.78[a]	0.546	0.001

[a–d]Within a row, means without a common superscript differ ($P < 0.05$).

[1]C = control; CLS = diet C including crude linseed; ELS = diet C including extruded linseed; LSO = diet C including linseed oil.

[2]CLA = conjugated linoleic acid.

[3]Sum of 18:1 *trans*, 18:2 *trans*, and CLA *trans*.

[4]Sum of 18:1 *trans* and 18:2 *trans*, except *trans*-11 18:1 (VA) and *cis*-9, *trans*-11 CLA (RA).

DISCUSSION

Dairy Performance

An increase in milk yield with linseed oil supplementation has been reported [Loor et al., 2005, using 3% oil with hay-based diet; Bu et al., 2007, using 4% oil with hay/corn silage (61/39)-based diet], whereas a decrease in milk yield has been observed with extruded linseeds [Gonthier et al., 2005, using 13% linseeds with grass/corn (60/40) silage-based diet; Akraim et al., 2007, using 17% linseed with corn silage-based diet]. The decrease in milk yield observed in our study with both ELS and LSO was probably caused by the decreased DMI and the decreased digestibility of fiber (Martin et al., 2008) because of the high level of oil (5% DMI) or extruded linseed (15% DMI) intake. However, no decrease in DMI with ELS or LSO was observed in earlier studies (Gonthier et al., 2005; Loor et al., 2005; Bu et al., 2007).

The lack of negative effect of feeding CLS on DMI and milk yield is consistent with previous studies on ground crude linseed (Gonthier et al., 2005; Beauchemin et al., 2009), although a decrease in DMI was reported with crushed linseed (Offer et al., 2001) and a decrease in milk yield was observed in 1 of 2 trials using whole crude linseed (Kennelly, 1996). The lack of negative effects with CLS in the current trial is probably because whole crude linseeds did not release FA in the rumen fluid as rapidly as ELS and LSO, and thus, rumen function was not disturbed.

Decreases in milk fat content and yield have been reported with ELS supplementation to diets with relatively high NDF contents (42–43% of DM; Gonthier et al., 2005; Akraim et al., 2007) and were less marked than in the current study with a relatively low NDF content (31%). Similarly, LSO supplementation decreased milk fat content and yield when added to low-NDF (as in the current study) but not to high-NDF diets (Dhiman et al., 2000; Loor et al., 2005; Flachowsky et al., 2006). A slower rate of mammary lipogenesis might have occurred as a result of adding polyunsaturated oil to a starch-rich diet (Chilliard et al., 2007; Harvatine et al., 2009), consistent with observed changes in milk FA profile, in particular the large increases in a range of *trans* isomers (see the following).

Table 5. Milk fatty acid secretion, transfer rate and Δ^9-desaturation ratio in cows receiving diets supplemented or not supplemented with linseed

	Diet[1]					
Item	C	CLS	ELS	LSO	SEM	$P <$
Secretion (g/d)						
4:0 + 6:0 + 8:0	61.1[a]	59.8[a]	36.1[b]	22.9[c]	3.09	0.001
10:0 + 12:0 + 14:0	181.8[a]	153.3[b]	88.8[c]	51.0[d]	9.42	0.001
16:0	264.9[a]	234.4[a]	132.3[b]	93.0[b]	14.07	0.001
Odd and branched chain	70.7[a]	61.0[b]	43.7[c]	31.8[d]	2.15	0.001
18:0	72.0[b]	120.1[a]	76.6[b]	69.3[b]	8.61	0.001
cis-9 18:1	147.7[b]	204.6[a]	146.8[b]	151.9[b]	12.43	0.05
trans-11 18:1	12.8[ab]	8.6[b]	17.6[a]	6.9[b]	2.60	0.05
trans-10 18:1	3.3[b]	2.1[b]	17.8[a]	16.1[a]	1.78	0.001
trans-13+14 18:1	6.9[bc]	1.2[c]	14.6[b]	26.4[a]	2.95	0.001
Sum of other *cis* 18:1	11.3[c]	11.5[c]	18.3[b]	41.8[a]	1.63	0.001
18:3n-3	5.6[b]	5.7[b]	7.8[a]	3.1[c]	0.57	0.001
cis-9, *trans*-11 CLA[2]	6.5[ab]	3.8[b]	8.2[a]	3.8[b]	0.95	0.05
Sum of *trans* C18[3]	42.1[b]	25.9[b]	84.2[a]	84.5[a]	7.20	0.001
Sum of non-RA/VA t18[4]	22.8[b]	13.5[c]	58.3[a]	73.8[a]	4.91	0.001
Sum of C18 fatty acids	297.0[b]	382.7[a]	350.2[ab]	372.6[a]	25.11	0.09
Transfer rate[5]						
18:3n-3	7.7[a]	1.4[bc]	1.9[b]	0.5[c]	0.4	0.001
Sum of C18 fatty acids	81.9[a]	43.0[b]	41.9[b]	35.4[b]	4.0	0.001
Ratio						
cis-9 14:1/(14:0 + *cis*-9 14:1)	0.10[b]	0.08[b]	0.10[b]	0.13[a]	0.01	0.001
cis-9 16:1/(16:0 + *cis*-9 16:1)	0.05[b]	0.05[b]	0.06[b]	0.08[a]	0.004	0.001
cis-9 17:1/(17:0 + *cis*-9 17:1)	0.22[c]	0.22[c]	0.26[b]	0.29[a]	0.01	0.001
cis-9 18:1/(18:0 + *cis*-9 18:1)	0.68[ab]	0.63[c]	0.66[bc]	0.70[a]	0.01	0.01
cis-9, *trans*-11 CLA/(*trans*-11 18:1 + *cis*-9, *trans*-11 CLA)	0.34[b]	0.31[b]	0.33[b]	0.40[a]	0.001	0.01

[a–d]Within a row, means without a common superscript differ ($P < 0.05$).

[1]C = control; CLS = diet C including crude linseed; ELS = diet C including extruded linseed; LSO = diet C including linseed oil.

[2]CLA = conjugated linoleic acid.

[3]Sum of 18:1 *trans*, 18:2 *trans*, and CLA *trans*.

[4]Sum of 18:1 *trans* and 18:2 *trans*, except *trans*-11 18:1 (VA) and *cis*-9, *trans*-11 CLA (RA).

[5]Transfer rate from diet to milk, % [100 × (milk fatty acids, g/d)/(fatty acid intake, g/d)].

In contrast to ELS and LSO effects, CLS did not decrease milk fat content or yield, consistent with previous studies (Kennelly, 1996; Collomb et al., 2004; Gonthier et al., 2005). The lack of negative effects with CLS is probably because CLS did not release FA in the rumen fluid as rapidly as ELS and LSO, and thus, rumen function was not disturbed, as is strongly suggested by the specific changes in milk FA profile with CLS, with increases in 18:0 and *cis*-9 18:1 without any major increase in *trans* isomers (see the following). Furthermore, the high transfer rate to milk of dietary C18-FA from the CLS diet (Table 5) suggests that FA contained in whole crude linseed were equally or even more efficiently digested than those of ELS and LSO diets.

Milk Fatty Acid Composition and Secretion

The marked effect of LSO on milk FA composition (decrease in 4:0–16:0 and increase in *trans* 18:1 and 18:2 isomers) in the current study is consistent with other studies in which LSO was added to high-concentrate, hay-based diets (Loor et al., 2005; Flachowsky et al., 2006). In the 3 studies, a significant increase in *trans*-10 18:1 concentration was observed. Furthermore, *trans*-11 18:1 and *cis*-9,*trans*-11 CLA concentrations remained low in the current study, as in the study by Flachowsky et al. (2006), although these 2 FA increased in the study by Loor et al. (2005). The reason for this discrepancy is not clear and does not appear to be linked to corn silage, diet NDF content, or LSO dose used in the last study compared with the other two studies. A specific response to LSO in the current study (corn-silage diets) was the strong increase in milk *cis*-9 18:1 concentration, which was not observed in the studies with high-concentrate, hay-based diets (Loor et al., 2005; Flachowsky et al., 2006). Large increases with LSO and ELS supplementation in milk concentrations of *trans*-13+14 18:1; *cis*-9,*trans*-13 18:2; and *trans*-11,*cis*-15 18:2 are typical from diets rich in 18:3n-3 (review by Chilliard et al., 2007). Surprisingly, the Δ^9-desaturation ratios of milk FA were increased only by LSO (Table 5), which could reflect an adaptation mechanism of the mammary gland to compensate for a putative increase in the melting

Table 6. Correlations[1] between milk fatty acid concentrations (% of total fatty acids) and either methane output (CH_4, g/d) or milk fat content (MFC, g/kg) in cows receiving diets supplemented or not supplemented with linseed (n = 32)

Positive correlations			Negative correlations		
Item	CH_4	MFC	Item	CH_4	MFC
Sum 8:0−16:0[2]	0.94		Sum of C18	−0.94	
16:0	0.91		*trans*-16+*cis*-14 18:1	−0.90	
Sum 8:0–14:0	0.90		Sum of 18:1 *cis*	−0.88	
8:0	0.90	0.71	*cis*-9, *trans*-13 18:2	−0.87	−0.74
Sum 10:0–14:0	0.90		*trans*-11 16:1	−0.86	−0.74
10:0	0.90		*trans*-12 18:1	−0.86	−0.74
14:0	0.89		Sum of non-RA/VA t18[3]	−0.84	−0.76
6:0	0.88	0.71	*cis*-13 18:1	−0.84	−0.78
12:0	0.88		Sum of *trans* C18	−0.81	−0.72
10:1	0.87		*trans*-13+14 18:1	−0.80	
Sum 4:0–8:0	0.85	0.72	Sum of 18:1 *trans*	−0.79	
20:4n-6	0.83		*trans*-6+7+8 18:1	−0.79	
11:0	0.78		*cis*-15+*trans*-17 18:1	−0.76	
12:1	0.76		*trans*-11, *cis*-15 18:2	−0.75	
9:0	0.75		*cis*-9 18:1	−0.72	
15:0	0.74		*cis*-10 18:1	−0.72	
17:0	0.73		*trans*-10 18:1	−0.66	−0.72
4:0	0.71				

[1]Only correlations >0.7 or <−0.7 ($P < 0.001$) with either CH_4 or MFC are reported.

[2]8:0 + 10:0 + 12:0 + 14:0 + 16:0.

[3]Sum of 18:1 *trans* and 18:2 *trans*, except *trans*-11 18:1 (VA) and *cis*-9, *trans*-11 conjugated linoleic acid (RA).

point of FA available for triglyceride synthesis because of the simultaneous decrease in short- and medium-chain saturated FA and increase in 18:0, *trans* 18:1, and *trans* 18:2 concentrations.

The main effect of whole CLS supplementation was to appreciably increase milk 18:0 and *cis*-9 18:1 concentrations at the expense of 8:0 to 16:0 (Tables 3 and 4) consistent with previous studies using ground or rolled CLS (Kennelly, 1996; Offer et al., 2001; Collomb et al., 2004; Gonthier et al., 2005; Akraim et al., 2007). The only published study comparing whole and rolled CLS showed that whole CLS did not increase *trans* 18:1, whereas rolled CLS did so slightly (Kennelly, 1996), consistent with the weak effects of whole CLS on *trans* 18:1 in the current study. These results confirm that the distribution of whole raw seeds could allow a more complete biohydrogenation of unsaturated FA than giving free oil to dairy ruminants (Chilliard et al., 2003).

Large increases in milk concentrations of 18:3n-3; *trans*-10 to *trans*-16 18:1; and *cis*-9,*trans*-11 CLA with ELS supplementation were reported in cows receiving a corn silage-based diet (Akraim et al., 2007), as in the current study. We observed that for most FA, the effect of ELS was intermediate between those of whole CLS and LSO diets or similar to LSO (Tables 3 and 4), probably because extrusion increases the rate of oil release from the seeds into rumen fluid compared with intact seeds. However, there were some exceptions, in particular for larger-than-expected increases in milk concentrations and secretions (Table 5) of 18:3n-3; *trans*-11 18:1; and *cis*-9, *trans*-11 CLA. This suggests that, alongside the abundant formation of *trans* FA resulting from a rapid oil release from extruded seeds, some 18:3n-3 might have been protected from rumen biohydrogenation or rapidly bypassed from the rumen to the duodenum (Doreau et al., 2009), and for unknown reasons, more *trans*-11 18:1 than *trans*-13+14 18:1 was produced with ELS than with LSO (Table 5).

Relationship Between Milk Fatty Acid Composition and Methane Output

Milk FA arise from both preformed FA absorbed in the intestine and de novo synthesis from rumen acetate and butyrate, so there could be a relationship between methane and milk FA because of 1) the common biochemical pathways between methane, acetate, and butyrate in the rumen and 2) the action of dietary lipids on methane production. The conversion of pyruvate into acetate, butyrate, propionate, carbon dioxide, and hydrogen, which is in turn converted in methane, obeys a stoichiometric relationship: methane = 0.45 acetate − 0.275 propionate + 0.40 butyrate (Demeyer and Van Nevel, 1975; Moss et al., 2000). Dietary FA have a negative action on methane production, especially short- and medium-chain FA and polyunsaturated 18C-FA,

by decreasing protozoa, cellulolytic bacteria, or archaea methanogens (Martin et al., 2009). Despite this relation, the prediction of methane production through milk FA has been seldom explored, and very few experiments have measured both milk FA and methane. Vlaeminck et al. (2006) explored relationships between milk odd- and branched-chain FA, used as microbial markers, and rumen volatile FA and proposed a theoretical model of prediction of methane by these FA (Vlaeminck and Fievez, 2005) using the previously mentioned stoichiometric equation. However, in our experiment, these FA exhibited less strong correlations with methane: r = 0.74 and 0.73 for 15:0 and 17:0, respectively, and r = 0.40 to 0.50 for branched-chain FA (data not shown).

Among the 9 FA that had the strongest negative simple correlations with cow CH_4 output (Table 6), 7 (*trans*-16 18:1; *cis*-9,*trans*-13 18:2; *trans*-12 18:1; *trans*-13+14 18:1; *trans*-6+7+8 18:1; *cis*-15 18:1; *trans*-11,*cis*-15 18:2) were biohydrogenation intermediates known to belong to a cluster of duodenal FA linked

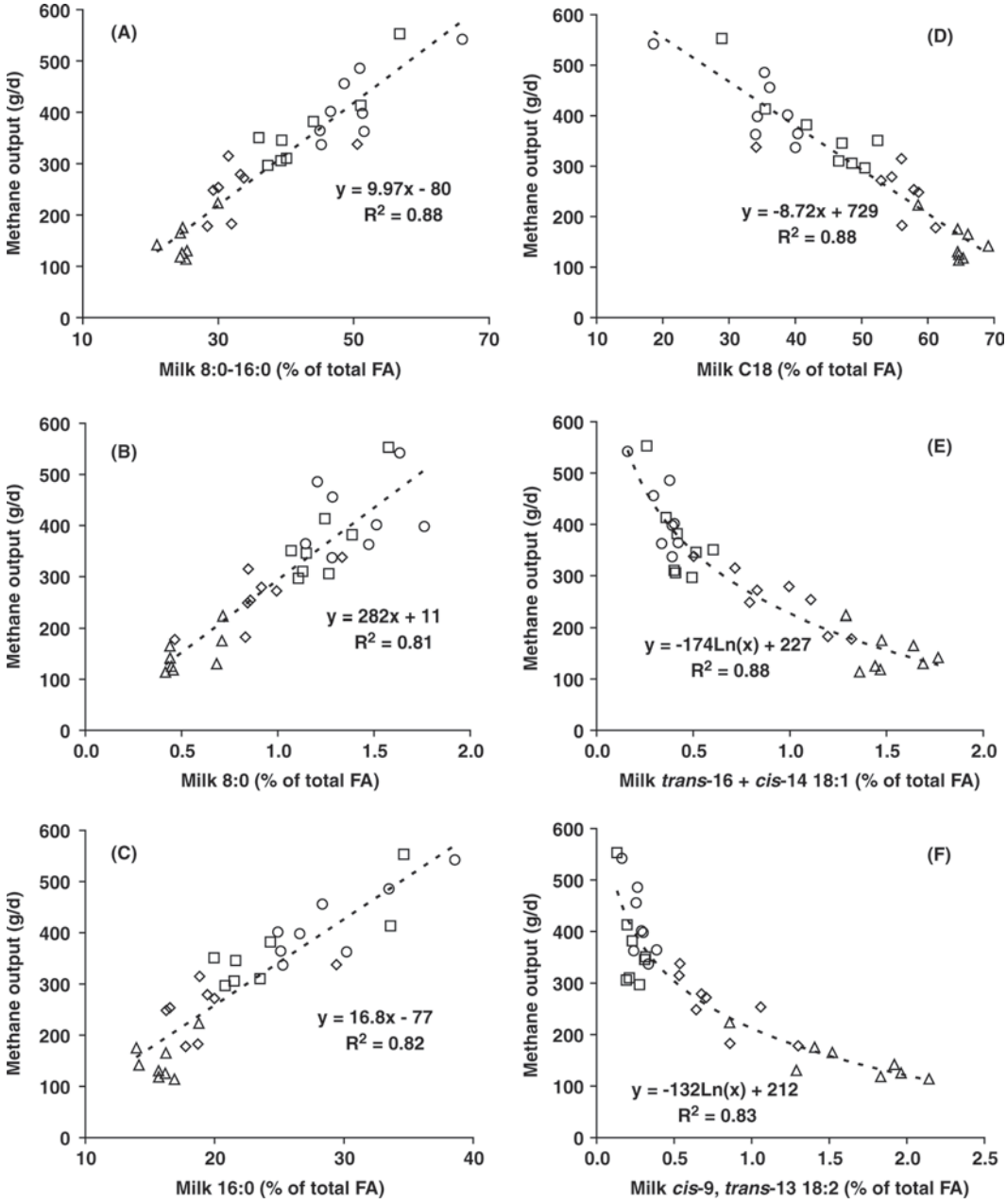

Figure 1. Relationships between methane output and selected milk fatty acid (FA) concentrations (A–F) for lactating dairy cows fed diets supplemented or not supplemented with linseed. ○, control; □, crude linseed; ◊, extruded linseed; Δ, linseed oil.

to dietary 18:3n-3 (Glasser et al., 2008b). Diets rich in polyunsaturated FA such as 18:3n-3 contribute to a decrease in CH_4 through a toxic effect on protozoa and cellulolytic bacteria involved in fiber digestion and hydrogen production and possibly through a direct toxic effect on methanogens that use hydrogen for CH_4 output (review by Martin et al., 2009). However, there was no correlation between dietary 18:3n-3 intake or milk 18:3n-3 concentration or secretion and CH_4 output, which suggests that the effect of dietary 18:3n-3 on CH_4 output is not strongly linked to the flow of 18:3n-3 entering or leaving the rumen. It suggests that the action of linolenic acid on microbes might be due to biohydrogenation intermediates.

In other respects, CH_4 output was positively and strongly correlated to milk 6:0 to16:0, which results mainly from mammary de novo FA synthesis, based primarily on the use of acetate produced in the rumen during fiber digestion. This positive correlation could be related directly to dietary 18:3n-3, which simultaneously decreases CH_4 output and fiber digestion and acetate production in the rumen (see above), possibly decreasing acetate availability at the mammary level and, thus, milk FA synthesis and fat secretion with LSO and ELS diets. Furthermore, an indirect relationship could be the result of the inhibiting effects on de novo FA synthesis in mammary cells by 18:3n-3 or specific *trans* and conjugated FA resulting from biohydrogenation of dietary 18:3n-3 or 18:2n-6 present in the basal diet (Shingfield and Griinari, 2007; Harvatine et al., 2009).

With our database, the best prediction of methane by multiple regression is excellent ($R^2 = 0.95$ vs. $R^2 = 0.88$ for simple regression). Among the 5 predictive variables, 3 of them could be expected: forage intake, which is an estimate of the part of the organic fermented matter that follows the acetate–methane pathway; palmitic acid, which is related to de novo synthesis, although less strongly than shorter chain FA; and *trans*-16+*cis*-14 18:1, which comprises one intermediate of linolenic acid hydrogenation. More surprising are the other 2 FA. The *cis*-9 14:1 isomer, which arises mainly from 14:0 desaturation (review by Bernard et al., 2008), is negatively related to CH_4 output in the multiple regression. Because linseed supplementations sharply decreased 14:0 concentration (which was, thus, positively correlated to CH_4 output; Table 6), it is likely that the increase in the *cis*-9 14:1/(14:0 + *cis*-9 14:1) ratio with LSO diet (Table 5) explains the entry of *cis*-9 14:1 in the stepwise regression. Linoleic acid is positively related to CH_4 output in the multiple regression, likely as a consequence of the negative correlation between milk linoleic and linolenic acids, which is often observed with diets varying in linolenic acid intake (e.g., Loor et al., 2005). It should be emphasized, however, that the entry of *cis*-9 14:1 and 18:2n-6 in the regression only slightly increased the R^2 value of the prediction compared with the equation obtained with the first 3 parameters only (from 0.931 to 0.953).

The observed correlations between milk FA profile and cow CH_4 output do not prove any causal relationship but probably reflect a chain of responses that are maximal with LSO and, to a lesser extent, ELS; that is, with diets that maximize the availability of linseed oil in the rumen and the availability of *trans* 18:1 and 18:2 isomers in the mammary gland and probably minimize acetate production and availability in both organs. Thus, milk FA profile can be considered to be a potential indicator of in vivo CH_4 output, at least to compare different diets with large differences in 18:3n-3 content (amount consumed) and availability for the rumen ecosystem (physical form) and without other lipid sources or additives that decrease methane. It should be emphasized, however, that previous studies using other dietary supplements reported contrasting results. When feeding soybean oil, CH_4 output did not change, whereas milk *trans* 18:1 concentration increased and 16:0 decreased (Sauer et al., 1998). When feeding monensin, CH_4 output decreased, whereas milk *trans* 18:1 concentration increased and 16:0 decreased (Sauer et al., 1998). When feeding a mixture of cottonseed and rapeseed, CH_4 output did not change, whereas milk *trans* 18:1 concentration increased and 16:0 decreased (Johnson et al., 2002). When feeding myristic acid, CH_4 output decreased, whereas milk *trans* 18:1 concentration did not change and 16:0 decreased (Odongo et al., 2007). Thus, further work is needed, using a wide range of basal diets with and without a wide range of lipid supplements, to determine in what dietary conditions milk FA could be used to predict CH_4 output, first in experimental and then in field conditions.

In conclusion, this study shows that a 5% supplementation of lipids from linseed to a corn silage-based diet appreciably changes cow milk FA composition, with a marked effect of the physical form of linseed oil. Inhibition of milk fat secretion, simultaneously with decreased cow CH_4 output, increases with the theoretical availability of linseed oil in the rumen and the appearance of high concentrations of several *trans* FA in milk fat. Thus, milk FA profile can be considered a potential indicator of in vivo CH_4 output; however, the validity area of the correlations that have been established is limited to diets varying in linolenic acid supply and availability. The evaluation of putative effects of using different forms of linseeds in dairy cow diets requires more long-term research on the sustainability of dairy performance, cow health and reproduction, milk quality (FA profile, micronutrients, and sensorial quality), and

CH_4 output. Further work should also consider lower levels of linseed supply and the interaction with the nature of the basal diet (pasture, grass silage, hay, or corn silage).

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