Methane output and diet digestibility in response to feeding dairy cows crude linseed, extruded linseed, or linseed oil

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Methane output and diet digestibility in response to feeding dairy cows crude linseed, extruded linseed, or linseed oil[1]

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ABSTRACT: This experiment studied the effect of 3 forms of presentation of linseed fatty acids (FA) on methane output using the sulfur hexafluoride tracer technique, total tract digestibility, and performance of dairy cows. Eight multiparous lactating Holstein cows (initial milk yield 23.4 ± 2.2 kg/d) were assigned to 4 dietary treatments in a replicated 4 × 4 Latin square design: a control diet (C) consisting of corn silage (59%), grass hay (6%), and concentrate (35%) and the same diet with crude linseed (CLS), extruded linseed (ELS), or linseed oil (LSO) at the same FA level (5.7% of dietary DM). Each experimental period lasted 4 wk. All the forms of linseed FA significantly decreased daily CH_4 emissions ($P < 0.001$) but to different extents (−12% with CLS, −38% with ELS, −64% with LSO) compared with C. The same ranking among diets was observed for CH_4 output expressed as a percentage of energy intake ($P < 0.001$) or in grams per kilogram of OM intake ($P < 0.001$). Methane production per unit of digested NDF was similar for C, CLS, and ELS but was less for LSO (138 vs. 68 g/kg of digested NDF, respectively; $P < 0.001$). Measured as grams per kilogram of milk or fat-corrected milk yield, methane emission was similar for C and CLS and was less for ELS and LSO ($P < 0.001$), LSO being less than ELS ($P < 0.01$). Total tract NDF digestibility was significantly less ($P < 0.001$) for the 3 supplemented diets than for C (−6.8% on average; $P < 0.001$). Starch digestibility was similar for all diets (mean 93.5%). Compared with C, DMI was not modified with CLS ($P > 0.05$) but was decreased with ELS and LSO (−3.1 and −5.1 kg/d, respectively; $P < 0.001$). Milk yield and milk fat content were similar for LSO and ELS but less than for C and CLS (19.9 vs. 22.3 kg/d and 33.8 vs. 43.2 g/kg, on average, respectively; $P < 0.01$ and $P < 0.001$). Linseed FA offer a promising dietary means to depress ruminal methanogenesis. The form of presentation of linseed FA greatly influences methane output from dairy cows. The negative effects of linseed on milk production will need to be overcome if it is to be considered as a methane mitigation agent. Optimal conditions for the utilization of linseed FA in ruminant diets need to be determined before recommending its use for the dairy industry.

Key words: dairy cow, digestion, fatty acid, linseed, linseed oil, methane

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INTRODUCTION

A major concern of citizens in many countries today is the increased production of greenhouse gases and their effect on climate change. Methane is the second most problematic greenhouse gas after CO_2. Ruminant livestock are responsible for about 15 to 20% of the total anthropogenic emission of CH_4 (Moss et al., 2000). Methane emissions from ruminants also represent a loss of productive energy for the animal. Thus, the development of feeding strategies to mitigate these methane emissions may bring not only environmental benefits for the planet but also nutritional benefits for the animal. Dietary fatty acids (**FA**), and more particularly PUFA, are among the most promising dietary alternatives able to depress ruminal methanogenesis (Martin et al., 2006). It has been shown that FA from linseed can decrease methane production in vitro (Broudiscou and Lassalas, 1991) as well as in vivo in sheep at maintenance (Czerkawski et al., 1966b) and in growing lambs (Machmüller et al., 2000). However, to our knowledge, this effect has never been confirmed in dairy cows.

Linseed is not frequently used in ruminant feeding, especially because several experiments in which more than 5% linseed oil was supplied to sheep at maintenance have shown a strong negative effect on ruminal digestion (Ikwuegbu and Sutton, 1982). However, re-

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cent data have demonstrated that adding 3% linseed oil to dairy cow diets does not depress ruminal digestion (Ueda et al., 2003). Until now, no experiment has been conducted with dairy cows fed diets containing linseeds at levels above 3%. It is thus unclear whether the lack of negative effect of linseeds on digestion in dairy cows is due to the low level of supplementation. There is increasing interest in feeding linseed to dairy cows because of its FA profile; linolenic acid contributes dietary n-3 FA and promotes increased CLA content of milk from ruminants (Chilliard et al., 2007). Linseed oil was used in our study to examine the effects of linseed FA, but in practical feeding conditions, crude or extruded linseed would likely to be used, because it is more readily available, easy to use, and less costly. Until now, no direct comparison of these 3 physical forms of linseed FA has been made using dairy cows.

The objectives of this trial were 1) to evaluate, in vivo, the effect of lipid supply from linseed on the emission of CH_4 and 2) to assess the consequences of a relatively high level of linseed supplementation on digestive efficiency and performance of dairy cows. Three diets containing crude linseed, extruded linseeds, and linseed oil plus linseed meal were compared with a control diet. Methane production, diet digestibility, and performance of dairy cows were determined, and the relationship between CH_4 production and dietary characteristics and milk yield was evaluated.

MATERIALS AND METHODS

All experimental procedures were conducted in accordance with French guidelines for the use of experimental animals and animal welfare (Anonymous, 1988).

Animals, Experimental Design, and Diets

Eight lactating multiparous Holstein cows (213 ± 40 d in milk) 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) 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 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 (Vandeputte Savonnerie et Huilerie, Mouscron, Belgium) 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 of 7.1 MJ/kg of DM). In the CLS and ELS diets, proportions of crude and extruded linseed were calculated so that the mean oil content of these diets was similar to that of the LSO diet. A level of 5% added lipids was considered desirable to test the effects of lipids on rumen methanogenesis and to evaluate differences due to the form of linseed FA. 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. After a short cooking period (5 min, 110°C, 304 kPa), extrusion was performed using a 1-screw extruder with an output temperature of 130°C. Incorporation of the 3 forms of linseed oil in the diets was achieved during a 3-d transition period. In addition, 200 g/d of a commercial mineral-vitamin premix (Galaphos Midi Duo GR, CCPA, Aurillac, France) was added to all diets. Ingredients and chemical composition of the experimental diets as consumed are given in Table 1. Diets were formulated to meet the requirements for maintenance and milk production of the cow (INRA, 1989). These requirements were calculated at the beginning of the experiment from milk yield at that time and were readjusted each experimental period assuming a monthly decrease in milk production of 10%. Diets were also formulated to contain the same quantity of limiting intestinal digestible protein (PDI system, INRA, 1989) supplied by all feedstuffs containing linseed (linseed meal, crude and extruded linseeds).

Forages (hay and corn silage) were offered once daily at 0900 h with ad libitum access for 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. The forage:concentrate ratio was maintained as close as possible to the targeted ratio by adjusting the amounts of forages and concentrates offered daily based on the composition of the refusals of the previous day. Crude and extruded linseed were mixed manually with the other concentrate ingredients immediately before feeding. Linseed oil was administered twice daily by drenching with the aid of a syringe. This way of distributing the oil was chosen, because in a preexperimental period, mixing oil with the concentrate obstructed the capillary tube used for gas collection using the tracer technique.

Cows were kept in individual stalls in a well-ventilated shed to avoid accumulation of gases eructed by animals in ambient air and had free access to water throughout the experiment. They were milked twice daily at 0630 and 1630 h.

Measurements and Analyses

Intake and Milk Yield. Feed intake and orts were measured and recorded on 5 consecutive days each week throughout the experiment to calculate DMI. Dry matter content in feeds was measured at 60°C for 72 h every day for corn silage and once per week for other feeds. 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, total FA, and GE.

Table 1. Ingredient and chemical composition of the experimental diets as consumed

	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					
OM, % of DM	95.3	95.5	95.3	89.9	0.15
CP, % of DM	14.5	14.9	14.6	14.6	0.19
NDF, % of DM	32.9	32.0	30.8	31.4	0.16
ADF, % of DM	17.5	16.9	16.7	16.6	0.10
Starch, % of DM	26.5	24.8	21.2	23.2	0.30
Ether extract, % of DM	2.6	6.8	7.0	8.4	0.16
GE, MJ/kg of DM	17.4	18.4	18.0	18.8	0.04
Fatty acid profile, % of total fatty acids					
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
18:1 *cis*-9	19.85	16.43	15.45	15.09	0.040
18:1 *trans*-11	0.91	0.67	0.64	0.70	0.066
18:2 *cis*-9, *cis*-12	41.34	27.70	24.21	21.32	0.193
20:0	0.38	0.24	0.10	0.11	0.002
18:3 *cis*-9, *cis*-12, *cis*-15	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]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.

Fresh samples of each feed (1 kg for corn silage, 100 to 200 g for other feeds) were also taken at wk 4 and stored (−25°C for corn silage and 4°C for other feeds) before being pooled 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). The NDF and ADF contents were determined by sequential procedures (Van Soest et al., 1991) after pretreatment with amylase and were expressed inclusive of residual ash. Starch was analyzed using a polarimetric method (AFNOR, 1985). The GE content of feeds was determined using an adiabatic bomb calorimeter (Gallenkamp Autobomb; Loughborough, Leics, UK). Determination of ether extract was performed according to AOAC (1990). Fatty acids from linseed oil were directly methylated with 2 mL of 0.5 *M* $NaOCH_3$ 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) was added as internal standard. Methyl esters were injected into a Trace-GC 2000 Series gas chromatograph equipped with a flame ionization detector (Thermofinnigan, 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 chromatography analysis were as described in Loor et al. (2005).

Milk yield was determined on the same 5 consecutive days as for intake from wk 1 to 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 analyzed for fat, protein, and lactose by infrared analysis with a 3-channel spectrophotometer

(AOAC, 1997). Milk energy was calculated from its fat, protein, and lactose content (Tyrrell and Reid, 1965).

Diet Digestibility. Total tract digestibility was determined from total collection of feces for 5 d in wk 4. Feces were removed once daily for weighing and mixing before sampling a 1% aliquot. After DM determination (60°C for 72 h), dry fecal samples were pooled across days for each cow and each period and then ground (0.8-mm screen) and analyzed for OM, starch, NDF, and ADF as described previously.

Methane Emissions. Methane production was determined during the same 5 d as for digestibility in wk 4, using the SF_6 tracer technique (Johnson et al., 1994) as described by Pinares-Patiño et al. (2003). Brass permeation tubes (12.5 mm × 40 mm i.d.) weighing about 32 g were used. These were loaded with about 600 mg of SF_6 at liquid N_2 temperature (−196°C) and calibrated by regular weighing (twice a week) for an 8-wk period while immersed in a water bath at 39°C. Permeation rate of SF_6 from the tubes was 1.523 ± 0.351 mg/d. A calibrated permeation tube was dosed orally into the rumen of each cow 2 wk before sampling gas in period 1. Representative breath samples from each animal were sampled in preevacuated (91.2 kPa) yoke-shaped polyvinyl chloride collection devices (~2.5 L) by means of capillary and Teflon tubing fitted to a halter. The collection devices were changed every 24 h before the morning feeding. The devices containing the samples were immediately transported to the laboratory and overpressured with N_2 gas to about 142.0 kPa before SF_6 and CH_4 analyses. Background concentrations of these gases were also measured in ambient air samples collected every day in the shed during the same 5-d breath sampling period. Daily CH_4 production from each animal was calculated according to Johnson et al. (1994), using the known permeation rate of SF_6 and the concentrations (above the background) of SF_6 and CH_4 in the breath samples:

$$CH_4\ (g/d) = SF_6 \text{ permeation rate } (g/d) \times [CH_4]/[SF_6].$$

Concentrations of SF_6 and CH_4 in breath and ambient air samples were determined by gas chromatography. A gas chromatograph (CP-9003, Varian-Chrompack, Les Ulis, France) fitted with an electron capture detector (Autosystem XL, Perkin Elmer Instruments, Courtaboeuf, France) or with a flame ionization detector was used to determine the concentrations of SF_6 and CH_4, respectively. The samples were run on chromatographs equipped either with a Molecular Sieve 0.5-nm column (3 m × 3.2 mm i.d; Interchim, Montluçon, France) maintained at 50°C for the SF_6 or with a Porapak N 80–100 mesh column (3 m × 3.2 mm i.d.; Alltech France SARL, Templemars, France) maintained at 40°C for the CH_4. The flow rate of the carrier gas was 30 mL/min of N_2 for the SF_6 and 40 mL/min of He for the CH_4. Chromatographic analyses were performed after calibration with standard gases (Air Liquide, Mitry-Mory, France) for SF_6 (55 and 195 pg/g) and CH_4 (100 µg/g).

Statistical Analyses. Data on CH_4 production, diet digestibility, DMI, and milk production were averaged over the first 5 d of wk 4 before statistical analysis. All data from the experiment were analyzed as a 4 × 4 Latin square using the MIXED procedure (SAS Inst. Inc., Cary, NC). The statistical model included cow, period, treatment, and residual error. Fixed effects included period and treatment. Cow was the random effect. Overall differences between treatment means were considered to be significant when $P < 0.05$.

RESULTS

Feed Intake and Milk Production

Feed intake variables are presented in Table 2. Compared with diet C, diet CLS had no effect on total DMI ($P > 0.05$), but diets ELS and LSO decreased total DMI (−3.1 and −5.1 kg/d, respectively; $P < 0.001$), mainly through a decrease in corn silage intake (−2.7 and −4.0 kg/d, respectively; $P < 0.001$). The negative effect on DMI was greater for LSO than for ELS ($P < 0.01$). As a consequence, GE intake was significantly less for LSO than for ELS diet ($P < 0.01$) and less for ELS than for CLS and C diets ($P < 0.001$).

Milk yield and 4% fat-corrected milk (**FCM**) yield were similar for the LSO and ELS diets, but these were less than the C and CLS diets (Table 2). Compared with diet C, milk fat content tended ($P = 0.09$) to be greater for CLS (+4.3 g/kg) but was less ($P < 0.001$) for ELS (−5.8 g/kg) and LSO (−8.8 g/kg). Protein and lactose contents did not vary among diets. Milk energy output was 72.6 MJ/d on average for diets C and CLS but was less for diets ELS and LSO (−15.3 MJ/d on average; $P < 0.001$).

Diet Digestibility

Dry matter and OM digestibilities were significantly less ($P < 0.01$) for the 3 supplemented diets than for the C diet (−4.0 and −4.2 percentage units on average, respectively, Table 3). This difference was due to a decrease in NDF digestibility ($P < 0.05$), because starch digestibility was similar for all diets (93.5% on average). The decrease in NDF digestibility was numerically greater for the ELS diet (−9.4 percentage units) than for the CLS or LSO diets (−5.5 percentage units on average), but differences among the 3 supplemented diets were not significant ($P > 0.1$). Digestibility of ADF was also less for CLS and ELS than for C and LSO diets ($P < 0.01$).

Methane Output

Daily methane emissions differed ($P < 0.001$) among all the diets (Table 4). The ranking of diets for daily methane production was C > CLS > ELS > LSO. The same ranking was observed for CH_4 output reported as grams per kilogram of OM intake or as a percentage of GE intake ($P < 0.001$). Methane output in grams

Table 2. Intake and milk yield and composition for lactating dairy cows fed diets supplemented with linseed

	Diet[1]					
Item	C	CLS	ELS	LSO	SEM	$P <$
DMI, kg/d						
Total	19.8[a]	19.5[a]	16.7[b]	14.7[c]	0.30	0.001
Silage	11.7[a]	11.7[a]	9.0[b]	7.7[c]	0.29	0.001
Concentrate	6.8[a]	6.6[a]	6.4[a]	5.8[b]	0.15	0.001
OM intake, kg/d	18.9[a]	18.7[a]	15.9[b]	14.2[c]	0.28	0.001
GE intake, MJ/d	344.2[a]	358.1[a]	299.9[b]	275.8[c]	5.32	0.001
Milk yield, kg/d	23.0[a]	21.5[a]	20.8[ab]	18.9[b]	0.71	0.01
4% fat-corrected milk, kg/d	23.4[a]	23.1[a]	18.9[b]	16.9[b]	0.77	0.001
Milk composition, g/kg						
Fat	41.1[a]	45.4[a]	35.3[b]	32.3[b]	1.71	0.001
Protein	34.0	34.6	33.3	34.7	0.67	NS[2]
Lactose	48.3	48.2	48.0	48.6	0.25	NS
Milk energy output, MJ/d	73.4[a]	71.7[a]	60.0[b]	54.6[b]	2.31	0.001

[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 = not significant ($P > 0.05$).

per kilogram of NDF intake as well as in grams per kilogram of digested OM was greatest for C and CLS, intermediate for ELS, and lowest for LSO ($P < 0.001$). Methane production per kilogram of digested NDF was similar ($P > 0.05$) for C, CLS, and ELS diets (138 g/kg of digested NDF on average), but much less for the LSO diet (68 g/kg of digested NDF). Methane production per kilogram of milk or FCM produced was similar for C and CLS diets but less for ELS and LSO diets, with the ELS diet ranked greater than the LSO diet ($P < 0.001$). Energy lost as methane when expressed as a percentage of milk energy output was similar for C, CLS, and ELS diets (28.7% of milk energy on average) but was less for the LSO diet (15.3% of milk energy; $P < 0.001$).

DISCUSSION

Feed Intake and Milk Yield

The lack of effect of CLS on DMI is in agreement with previous findings (Ward et al., 2002; Gonthier et al., 2005). A decrease in DMI with ELS or LSO was not observed in earlier studies (Gonthier et al., 2005; Loor et al., 2005; Bu et al., 2007), except by Offer et al. (2001), who used a diet based on corn silage, as in the present study. The decline in DMI that occurred when LSO was fed cannot be fully explained by disturbances in rumen function, because digestibility was not different among the 3 supplemented diets. It is possible that the FA intake had a direct inhibitory effect on voluntary intake via inhibition of ruminoreticular motility (Chilliard, 1993).

Dietary lipids generally increase milk yield as reviewed by Chilliard and Ferlay (2004). This increase has been reported specifically for linseed oil more (Bu et al., 2007) or less intensely (Loor et al., 2005), whereas a decrease in milk yield has been observed with extruded linseeds (Gonthier et al., 2005; Akraim et al., 2007). The decrease in milk and FCM yield and fat content observed in our study with both ELS and LSO diets was probably caused by the lesser DMI and the lesser digestibility of fiber due to the high level of oil intake (5% of DMI). In addition, a lesser mammary lipogenesis may have occurred as a result of adding polyunsaturated oil to a starch-rich diet (Chilliard et al., 2007). The lack of negative effect of feeding CLS on DMI, milk

Table 3. Total tract digestibility of DM, OM, fiber, and starch in lactating dairy cows fed diets supplemented with linseed

	Diet[1]					
Item	C	CLS	ELS	LSO	SEM	$P <$
DM, %	66.5[a]	62.2[b]	63.5[b]	61.7[b]	0.78	0.01
OM, %	70.0[a]	65.2[b]	66.7[b]	65.4[b]	0.78	0.01
NDF, %	47.5[a]	41.9[b]	38.1[b]	42.2[b]	1.74	0.05
ADF, %	44.7[a]	36.8[b]	34.1[b]	44.0[a]	2.22	0.01
Starch, %	93.4	93.0	93.0	94.7	0.54	NS[2]

[a,b]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 = not significant ($P > 0.05$).

Table 4. Methane emissions in lactating dairy cows fed diets supplemented with linseed

Item	Diet[1]				SEM	$P <$
	C	CLS	ELS	LSO		
CH_4, g/d	418.1[a]	369.4[b]	258.1[c]	149.2[d]	13.64	0.001
CH_4, % GE intake	6.7[a]	5.7[b]	4.8[c]	3.0[d]	0.21	0.001
CH_4, g/kg of OM intake	22.0[a]	19.8[b]	16.3[c]	10.5[d]	0.72	0.001
CH_4, g/kg of NDF intake	63.8[a]	59.3[a]	50.7[b]	27.5[c]	2.19	0.001
CH_4, g/kg of digested OM	31.4[a]	30.2[a]	24.5[b]	16.2[c]	1.08	0.001
CH_4, g/kg of digested NDF	136.2[a]	141.0[a]	135.9[a]	68.1[b]	6.42	0.001
CH_4, g/kg of milk	17.4[a]	17.9[a]	12.2[b]	8.1[c]	0.94	0.001
CH_4, g/kg of 4% fat-corrected milk	19.3[a]	16.4[ab]	14.8[b]	9.3[c]	1.27	0.001
CH_4, % milk energy output	33.8[a]	29.0[a]	25.7[a]	15.7[b]	2.30	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.

yield, and fat content, and 4% FCM, is likely due to the fact that CLS did not release FA in the rumen fluid as rapidly as ELS and LSO did, and thus rumen function was not disturbed.

Diet Digestibility

In this experiment, supplying 5.7% lipids from linseed significantly reduced OM and fiber digestibility of a corn silage-concentrate diet fed to dairy cows. This negative effect has been shown in sheep at maintenance receiving a supplement of 5% (Cottyn et al., 1971) or 7% (Ikwuegbu and Sutton, 1982; Sutton et al., 1983) linseed oil in hay-concentrate diets. By contrast, other experiments in dairy cows [3% linseed oil with either a hay-based diet (Ueda et al., 2003) or a corn silage-based diet (A. Ferlay, INRA, Saint Genès Champanelle, France, and Y. Chilliard, unpublished data)] or dry cows [2.5% of FA from linseed or linseed oil, Doreau et al., in press)], in lambs (6.7% linseed, i.e., 2.5% FA; Machmüller et al., 2000), or in sheep (10.5% linseed, i.e., 4.8% FA given 12 times/d; Wachira et al., 2000) did not show any decrease in cell wall digestibility due to lipids from linseed. Furthermore, Gonthier et al. (2004) showed an increase in total digestibility of OM and fiber with a supplement of 3.5 to 4% FA from extruded linseed added to a grass and corn silage-based diet. From these experiments combined, it can be concluded that the amount of added lipids and their form of presentation (oil vs. seed) are major determining factors for the negative effect of linseed FA on digestibility. Providing linseed twice daily in the present study may have contributed to a high decrease in digestibility, because the effects on digestibility have been less in a study where cows were fed 3 times daily a diet with 3% linseed oil (Ueda et al., 2003). In addition, we speculate that the negative effect of lipids on digestion is more pronounced with corn silage diets than with hay diets, based on results from our study and the study by Ben Salem et al. (1993) in which cows were fed a diet containing 7% rapeseed oil.

In ruminants, about 90% of total digestible fiber is digested in the rumen, although a possible decrease in ruminal fiber digestion can be partially compensated for by digestion in the large intestine. Thus, the 7-percentage unit decrease in NDF digestibility in the digestive tract observed in the present trial probably resulted from an even larger decrease in ruminal digestion (Ikwuegbu and Sutton, 1982; Sutton et al., 1983). Starch digestion was not altered by the 3 linseed FA supplements. This is consistent with previous data on different sources of lipids, in particular with linseed oil in cows (Ueda et al., 2003) and sheep (Ikwuegbu and Sutton, 1982) and linseed in lambs (Machmüller et al., 2000).

The absence of any differences in digestibility between CLS, ELS, and LSO diets was unexpected. It is generally thought that the inclusion of oil in seeds gives a partial protection against microbial attack or limits the effects of oil on ruminal microbes or both. For linseed, the present results suggest that linseed hulls did not prevent FA release in the rumen. Very few experiments have compared the effect of different forms of oleaginous seeds on digestion in ruminants. Gonthier et al. (2004), comparing crude and extruded linseed, found no evidence for any difference between forms, in agreement with the present experiment. A similar absence of difference between crude and extruded oleaginous seeds has been shown by others (Ferlay et al., 1992; Petit et al., 1997) with soybean or rapeseed. Only a few comparisons between seeds and oils have been published. Pallister and Smithard (1987) reported a trend toward a lesser ruminal OM digestibility with extruded rapeseed than with crude rapeseed or rapeseed oil, as observed in our study for fiber digestibility with ELS compared with CLS and LSO ($P = 0.11$). Had we used more animals in our study, we might have detected the small differences among linseed treatments. According to the literature and the present data, the form of lipid supplementation does not seem to significantly modify diet digestibility, but more research is needed to conclude on this point.

Methane Emissions

Methane emissions obtained for the control diet (418 g/d and 17.4 g/kg of milk) are in agreement with those

reported in the literature (392 to 464 g/d and 14.3 to 19.6 g/kg of milk) with the tracer method (Lovett et al., 2005) and in respiratory chambers (Kinsman et al., 1995; Vermorel, 1995; Sauer et al., 1998) for dairy cows at a similar level of milk production (20 to 30 kg of milk/d). In our experiment, cows lost 6.7% of GE intake as eructed methane with the control diet, which was similar to values (6.2 to 6.7%) reported by Vermorel (1995) for dairy cows of similar breed and physiological and nutritional conditions and for small dairy ruminants such as ewes and goats (6.2 to 6.3%).

Supply of lipids from linseed significantly decreased the amount of CH_4 emitted by dairy cows, with a marked effect of the different forms of linseed FA (−12% with CLS, −38% with ELS, −64% with LSO compared with the C diet). Thus, inhibition of the ruminant methanogenesis may increase with the theoretical availability or release pattern of linseed FA (LSO > ELS > CLS) in the rumen, whereas no such difference was observed for digestibility. The decrease in methane emission with linseed oil in dairy cows confirms in vitro data (Broudiscou and Lassalas, 1991). A depressive effect of linseed FA on in vivo CH_4 emissions, quantified in respiratory chambers, has been shown in growing lambs supplemented with 6.7% of crushed whole linseed (i.e., 2.5% of oil; Machmüller et al., 2000) or in sheep at maintenance receiving 5% of linseed oil in intraruminal continuous infusion (Czerkawski et al., 1966a). In this last trial, the decrease in methane (−38%) was less than in the present study (−64%) with a similar level of linseed oil supplementation. However, the distribution pattern of oil differed between these 2 studies (continuous vs. twice daily). The negative effect of linseed oil FA on methanogenesis has been shown to be smaller when the same quantity of FA is distributed continuously compared with once (Czerkawski et al., 1966b).

The reduction in methanogenesis with added linseed FA cannot be explained by the reduction in intake. When methane emission is expressed per kilogram of OM or NDF intake, the same ranking between diets occurred in terms of their reduction in methane (LSO > ELS > CLS > C). However, when methane production was expressed per kilogram of digested NDF, it was similar for C, CLS, and ELS diets but was less for the LSO diet. Thus, the reduced fiber digestibility explained the decrease in methane production that occurred when diets were supplemented with CLS and ELS. The PUFA in free oil probably interact more rapidly with microorganisms in the rumen than FA in seeds. This is evidenced by a more pronounced shift of the VFA pattern toward propionate for oils than for seeds (Jouany et al., 2000). This effect may be emphasized by the mode of dispensing of the oil used in this study (twice daily by oral dosing) for the LSO diet. Thus, a shift in fiber digestion from the rumen to the large intestine may have occurred for the LSO diet, and, as a consequence, less methane was produced per unit of digested NDF. The omission of the hindgut methane by the SF_6 technique probably resulted in an underestimation of methane production for the LSO diet compared with the other diets. We can assume that differences among diets in fiber digested in the rumen are greater than differences in the total tract. This has been shown by Sutton et al. (1983), who observed a larger decrease in OM digestion in the rumen (−19 points) than in the total tract (−3 points) in sheep supplemented with 7% linseed oil. Thus, had fiber digestion in the rumen been measured, it may have explained the differences in methanogenesis between the 3 diets containing FA from linseed.

Polyunsaturated FA decrease methane through a toxic effect on microorganisms involved in fiber digestion and hydrogen production such as protozoa (Doreau and Ferlay, 1995) and cellulolytic bacteria (Nagaraja et al., 1997). This effect, observed with all long-chain FA, is probably through an action on the cell membrane particularly of gram-positive bacteria. It has been shown in vitro that linolenic acid is particularly toxic for the 3 cellulolytic bacterial species (*Fibrobacter succinogenes, Ruminococcus albus*, and *Ruminococcus flavefaciens*), because it disrupts cell integrity (Maia et al., 2006). In addition, a direct toxic effect of PUFA on methanogens that use hydrogen for methane production may have occurred, as shown in vitro with linseed oil hydrolysate (Prins et al., 1972). In this case, free hydrogen may accumulate in the gas mixture, resulting in growth inhibition of cellulolytic bacteria (Wolin et al., 1997), and fiber digestibility may be impaired as observed in the present experiment.

The effects of FA from linseed on methanogenesis were observed in our study for cows fed the different diets for 4 wk, but these results need to be confirmed in a longer-term study. An adaptation of the rumen microflora to oil supplementation over the long term may be possible, and the long-term persistence of methane-suppressing feed manipulations has been recognized as an important issue (Woodward et al., 2006; Grainger et al., 2008).

This study demonstrates that a 5.7% supply of lipids from linseed significantly decreases the quantity of CH_4 emitted daily by dairy cows, with a marked effect of the physical form of linseed FA. Inhibition of rumen methanogenesis appears to increase with the theoretical availability of linseed FA in the rumen. The use of linseeds in dairy cow diets may result in positive environmental effects. However, their use as a mitigating agent requires sustained long-terms effect on methane without causing negative effects on animal performance. Effect of the different forms of linseeds or oil on milk quality in terms of FA profiles (increase in n-3 FA, CLA, *trans* FA, etc.) also needs to be assessed. Optimal conditions for the utilization of linseed FA in ruminant nutrition thus remains to be determined before recommending their use in commercial dairy production. Further work should consider lesser levels of linseed supply, the form of adding the linseed lipids to the diet (distribution pattern, variations in processing techniques), and the interaction with the nature of the basal diet (pasture, grass silage, hay, or corn silage).

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