Methane production and digestion of different physical forms of rapeseed as fat supplements in dairy cows

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ABSTRACT

The purpose of this experiment was to study the effect of the physical form of rapeseed fat on methane (CH_4) mitigation properties, feed digestion, and rumen fermentation. Four lactating ruminal-, duodenal-, and ileal-cannulated Danish Holstein dairy cows (143 d in milk, milk yield of 34.3 kg) were submitted to a 4×4 Latin square design with 4 rations: 1 control with rapeseed meal (low-fat, CON) and 3 fat-supplemented rations with either rapeseed cake (RSC), whole cracked rapeseed (WCR), or rapeseed oil (RSO). Dietary fat concentrations were 3.5 in CON, 5.5 in RSC, 6.2 in WCR, and 6.5% in RSO. The amount of fat-free rapeseed was kept constant for all rations. The forage consisted of corn silage and grass silage and the forage to concentrate ratio was 50:50 on a dry matter basis. Diurnal samples of duodenal and ileal digesta and feces were compiled. The methane production was measured for 4 d in open-circuit respiration chambers. Additional fat reduced the CH_4 production per kilogram of dry matter intake and as a proportion of the gross energy intake by 11 and 14%, respectively. Neither the total tract nor the rumen digestibility of organic matter (OM) or neutral detergent fiber were significantly affected by the treatment. Relating the CH_4 production to the total-tract digested OM showed a tendency to decrease CH_4 per kilogram of digested OM for fat-supplemented rations versus CON. The acetate to propionate ratio was not affected for RSC and WCR but was increased for RSO compared with CON. The rumen ammonia concentration was not affected by the ration. The milk and energy-corrected milk yields were unaffected by the fat supplementation. In conclusion, rapeseed is an appropriate fat source to reduce the enteric CH_4 production without affecting neutral detergent fiber digestion or milk production. The physical form of fat did not influence the CH_4 -reducing effect of rapeseed fat.

However, differences in the volatile fatty acid pattern indicate that different mechanisms may be involved.

Key words: canola, fiber digestion, cattle

INTRODUCTION

Globally, agriculture accounts for 47% of total anthropogenic methane (CH_4) emissions, with enteric fermentation contributing 32% of the total non- CO_2 emissions from agriculture in 2005 (Smith et al., 2007). The CH_4 production per animal varies depending on the feed composition, feed quality, and production level from 2 to 12% of the gross energy (GE) intake (Johnson and Johnson, 1995) under extreme circumstances, but values between 3 and 7% (Martin et al., 2008) are more realistic in intensive dairy production.

Numerous studies have discussed nutritional possibilities to reduce the enteric CH_4 production (Boadi et al., 2004; Beauchemin et al., 2008), and fat supplementation is among the most promising tools to depress CH_4 production from ruminants (Martin et al., 2008). Furthermore, fat is fed to dairy cows to increase the energy density of the ration or to alter the product quality (Beauchemin et al., 2007). Several oils and oil seeds have been tested for their potential to reduce the CH_4 production, and effects of chain length and saturation have been reported. The degree of saturation is important, as the negative effect on bacterial growth increases with the degree of unsaturation, inhibiting both fibrolytic bacteria and methanogens (Giger-Reverdin et al., 2003). With reduced fiber digestibility and a shift in fermentation pattern, less hydrogen arises and, thus, less CH_4 (Boadi et al., 2004). Additionally, fat often replaces carbohydrates in the ration, thereby directly reducing rumen fermentation. Reduced fiber digestibility is associated with reduced DMI and milk production; therefore it has to be considered whether the overall reduction of CH_4 production from the animal due to the addition of fat is accompanied by a reduction per kilogram of product or per kilogram of feed digested.

Feeding whole seeds or cake, a by-product from the plant oil production, as well as pure oil, are tools to increase the dietary fat concentration, but the difference

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in physical form might influence the effect of fat in the rumen. Czerkawski et al. (1966) showed that the effect of FA on CH₄ production was stronger when the same amount was infused to the rumen once daily, compared with continuous infusion. Similarly, Machmüller et al. (2000) concluded that this could be of importance to achieve momentarily high fat concentrations in the rumen rather than a constant presence at a lower level. Oil in seeds is stored intracellularly, and the fat release depends on the digestion and breakdown of the cell wall, which leads to a slower release compared with feeding oil directly (Steele et al., 1971). This indicates that pure oil may increase the rumen FA concentration faster and reduce the CH₄ production more effectively compared with seeds or cake (Martin et al., 2008).

Oilseed rape (*Brassica napus*) is widely grown in many countries. The by-products, rapeseed meal and cake, remaining after oil extraction are common feed components in dairy cow rations. Rapeseed meal has a low crude fat concentration (about 4%) compared with rapeseed cake (10–20%) and whole seeds (approximately 50%). The aim of the current experiment was to study the effect of rapeseed fat and the physical form in which it was fed on enteric CH₄ production, rumen fermentation, and digestion.

MATERIALS AND METHODS

Animals and Rations

The experiment complied with the guidelines of the Danish Ministry of Justice with respect to animal experimentation and care of animals under study. Four lactating Danish Holstein dairy cows (1 primiparous and 3 multiparous) were assigned to 1 of 4 rations over 4 periods according to a balanced Latin square design; each period consisted of 4 wk. One cow was omitted from the last period due to disease.

The cows were 143 DIM (SD = 74 d), had a milk yield of 34.3 kg (SD = 8.6 kg), and a BW of 592 kg (SD = 81 kg) at the beginning of the experiment. All animals were fitted with a ruminal cannula (#1C, Bar Diamond Inc., Parma, ID), a duodenal cannula (open T-piece placed 60 cm caudal to pylorus), and an ileal cannula (open T-piece placed 20 cm cranial to the cecum). The cows were housed in a tie stall with rubber mats and sawdust as bedding and had free access to water. They were milked and fed twice daily at 0500 and 1700 h. Total mixed rations were prepared once a day and fed to the cows on an ad libitum basis after milking. The feed intake was recorded on a daily basis. The animals were weighed at the start of the experiment as well as just before and after the respiration chamber measurements (the last week of each period).

The rations were a control ration (**CON**) and 3 high-fat rations with fat supplemented as either rapeseed cake (**RSC**), whole cracked rapeseed (**WCR**), or rapeseed oil (**RSO**), respectively. The amount of fat-free rapeseed was equal for all rations, as the basic rapeseed meal content in the CON was reduced according to the fat-free rapeseed which was supplemented with either cake or seed in the treatment rations. Rapeseed cake, whole rapeseed, and rapeseed oil were obtained from Danraps (DLG Food Oil, Dronninglund, Denmark). The rapeseed used in this study was double-00 rape, equivalent to what is known as canola in North America.

The chemical composition of ingredients is shown in Table 1. All rations were fed as TMR with a forage to concentrate ratio of 50:50 (Table 2). The forage consisted of 54% corn silage and 46% prewilted perennial ryegrass silage (on DM basis). The corn silage was stored in a bunker silo and the grass silage in bales.

Measurements

The milk production and composition were measured once a week during morning and evening milkings. Weekly samples of the feed ingredients were stored (−20°C) and pooled during the whole experiment. Samples of TMR and refusals were taken daily in connection with the afternoon feeding, stored (−20°C), and pooled for each period from d 15 to 20.

Chromic oxide was used as a flow marker, and 10 g was administered to the rumen via the ruminal cannula during each of the 2 daily feedings, except when the cows were in the respiration chambers.

Samples of duodenal chyme (600 mL), ileal chyme (300 mL), and feces (350 mL) were taken from d 15 to 19 at 1000, 1800 (d 15), 0200, 1200, 2000 (d 16), 0400, 1400, 2200 (d 17), 0600, 1600, 2400 (d 18), and 0800 h (d 19; 12 samples, representing every second hour of the day). Samples from the duodenum and ileum were taken in tube-formed plastic bags which were mounted to the cannulas with plastic knees. Duodenal, ileal, and fecal samples were added to the frozen pooled sample from previous samples at each sampling time. At the end of the period, representative subsamples from thawed material were taken and freeze-dried for chemical analysis. At the 12 sampling times, rumen liquid was sampled from the ventral ruminal sac with a collection tube (#RT, Bar Diamond Inc.). The rumen liquid pH was measured immediately, and two 8-mL samples were taken and frozen (−20°C) immediately for VFA and ammonia (NH₃) analysis at each sampling time.

Chemical Analysis

Ash was determined by combustion at 525°C for 6 h. Nitrogen was determined by the Dumas principle

Table 1. Chemical composition (g/kg of DM unless otherwise noted) of feedstuffs

Item	Barley	Beet pulp	RS ¹ meal	RS cake	Whole RS	RS oil	Grass silage	Corn silage
DM, g/kg of fresh matter	878	903	904	968	940		424	295
OM	980	958	922	937	960		896	966
CP	115	102	381	300	192		163	99
Crude fat	30.0	4.8	54.7	173	479	1,000	30.8	30.0
NDF	161	408	260	201	150		420	392
INDF ²	36.4	28.2	96.7	90.0	53.6		56.8	92.1
Gross energy, MJ/kg of DM	18.2	17.5	19.6	22.3	28.7	39.6	17.8	18.7
NE _L , MJ/kg of DM	9.03	7.90	8.68	9.99	14.35	22.3	6.55	7.00
OMD, ³ %							75.2	72.7
Fatty acids, g/kg of DM								
C16:0	6.30	1.99	3.53	8.44	19.0	43.7	2.93	3.73
C18:0	4.20	0.06	0.74	2.46	6.35	15.5	0.30	3.60
C18:1	2.98	0.82	20.2	80.3	218	537	0.85	4.28
C18:2	10.8	1.19	11.7	33.4	80.4	191	2.72	9.10
C18:3	0.96	0.23	3.46	13.7	38.2	91.4	10.4	2.39
Total FA ⁴	22.4	4.60	46.2	151	385	930	18.6	21.0

¹RS = rapeseed.²INDF = indigestible NDF.³OMD = in vitro OM digestibility.⁴Sum of fatty acids includes, beside those shown, C16:1, C20:0, C20:1, C20:2, and C22:0.

(Hansen, 1989), and CP was calculated as $N \times 6.25$. Crude fat was analyzed as Soxhlet extraction with petroleum ether (Soxtec 2050, Foss Analytical, Hillerød, Denmark) after hydrolyzing with HCl (Stoldt, 1952). The NDF content was analyzed by neutral detergent extraction according to Mertens (2002) with a Fibertec M6 System (Foss Analytical) using heat-stable amylase and corrected for ash. The indigestible NDF in freeze-dried ground (1.5mm) feed samples was determined as residual NDF after 288 h (12 d) of Dacron bag incubation in the rumen of 3 heifers fed a standard ration (Åkerlind et al., 2011). The GE was determined by

adiabatic bomb calorimeter (Parr 6300 Oxygen Bomb Calorimeter, Parr Instrument Company, Moline, IL).

The concentrations of VFA were analyzed according to the method described by Canibe et al. (2007) using a Hewlett Packard gas chromatograph (model 6890; Agilent Technologies Inc., Wilmington, DE) equipped with a flame ionization detector and a 30-m SGE BP1 column (Scientific Instrument Services, NJ). Fatty acids in feed were analyzed by GC after an acidic Bligh and Dyer extraction with hydrochloric acid-water-chloroform and methanol, and subsequent methylation as described by Jensen (2008). For determination of NH₃,

Table 2. Ration ingredients and chemical composition (g/kg of DM unless otherwise noted)

Item	Ration ¹			
	CON	RSC	WCR	RSO
Barley	143	137	137	138
Beet pulp dried	143	137	137	138
Rapeseed meal, 4% fat	190	62	149	184
Rapeseed cake, 17% fat	0	156	0	0
Rapeseed, cracked	0	0	69	0
Rapeseed oil	0	0	0	33
Corn silage	238	232	232	232
Grass silage	286	275	275	275
DM, g/kg of fresh matter	479	500	492	494
OM	934	937	939	939
CP	169	171	168	171
Crude fat	35	55	62	65
Fatty acids ²	26	43	50	53
NDF	332	328	326	322
Gross energy, MJ/kg of DM	18.4	18.9	19.1	19.1
NE _L , MJ/kg of DM	7.6	7.8	8.0	8.1

¹CON = control, RSC = rapeseed cake, WCR = whole cracked rapeseed, RSO = rapeseed oil.²Calculated values based on analysis of ingredients.

the rumen fluid was made alkaline with KOH, and NH_3 was determined by titration after distillation.

Chromic oxide was determined by colorimetry after oxidation to chromate (Schürch et al., 1950). The OM digestibility was determined in vitro for grass and corn silage as described by Tilley and Terry (1963). Milk concentrations of fat, protein, and lactose were analyzed by a Milkoscan Msc4000 infrared analyzer (Foss Analytical).

Methane Measurements

During the fourth week of each period, the CH_4 production was measured for 2×48 h in four 17-m³ open-circuit respiration chambers (Hellwing et al., 2012). The animals were housed individually. The chambers were covered with transparent polycarbonate and placed in a square so that the cows faced each other. The chambers were located in the barn where the cows were usually housed to minimize changes in the environment and the daily routines during the CH_4 measurements were identical to the period outside the chambers. The mean ambient temperature in the chambers was 21.1°C, ranging from 15.6 to 29.5°C.

The cows changed chambers diagonally after the first 48 h to balance out any differences in background levels of CH_4 and CO_2 . Cow and chamber were confounded over periods and, therefore, every ration was tested in every chamber.

The chambers were opened twice daily, at 0500 and 1700 h, for about 20 min during milking and subsequent feeding. Methane was measured as the accumulated amount (L) over 24 h and is reported under standard conditions (0°C, 101.325 kPa). The measurements during the openings of the chambers for milking and feeding were deleted (about 60 min/d). The CH_4 production during this period was assumed to correspond to the mean of the rest of the day.

The air flow was measured by a HFM-200 flow meter with a laminar flow element from Teledyne Hastings Instruments (Hampton, VA). The background (inlet air), as well as the chamber outlet air concentration of CH_4 , was measured every 12 1/2 min with an infrared analyzer. All instruments were from Columbus Instruments (Columbus, OH). The air flow was adjusted individually for every animal depending on the BW and milk yield to obtain a CO_2 concentration in the chamber below, but close to 9,000 ppm. The instruments were calibrated every second day with zero gas (nitrogen) and a span gas with nitrogen and 20.55% O_2 , 5,000 ppm CO_2 , and 800 ppm CH_4 (Yara Praxair AS, Oslo, Norway). The temperature, humidity, and CO_2 concentration of the chamber air were monitored

with independent sensors (Veng System A/S, Roslev, Denmark) for alarm purposes.

Calculations and Statistical Analysis

The net energy content in feeds was calculated in Scandinavian Feed Units (SFU; Weisbjerg and Hvelplund, 1993) and presented in megajoules of NE_L by using a fixed conversion factor of 7.89 MJ of NE_L /SFU, as described by Hvelplund et al. (2007). Content of SFU is calculated based on content of ash, CP, crude fiber, crude fat, and in vitro OM digestibility.

The apparent rumen digestibility was calculated as the feed intake minus the duodenal flow divided by feed intake. Apparent small intestine digestibility was calculated as the duodenal flow minus ileal flow divided by duodenal flow and, accordingly, apparent large intestine digestibility as ileal flow minus fecal flow divided by ileal flow. Apparent total-tract digestibility was calculated as the feed intake minus fecal flow divided by feed intake.

Average ECM yield for each cow per period was calculated according to Sjaunja et al. (1991) as follows: $\text{ECM} = \text{milk yield} \times (383 \times \text{fat \%} + 242 \times \text{protein \%} + 783.2)/3,140$. For samples with repeated measures (VFA, NH_3 , and pH), an average for each cow-period was calculated before the statistical analysis.

The data was evaluated with the MIXED procedure (SAS 9.2 version, SAS Institute Inc., Cary, NC) with treatment and period as fixed effects and cow as random effect.

The results are reported as LSM and SEM for each treatment. The SEM was different for RSO because one cow receiving RSO was omitted. Therefore, the RSO SEM is reported and a factor for the SEM of the other treatments is noted under each table. Apart from the mean treatment effect, the significance of orthogonal contrasts were calculated for CON versus fat supplement, RSC and WCR versus RSO, and RSC versus WCR. *P*-values < 0.05 were regarded as significant and *P* < 0.1 as a tendency.

RESULTS

Milk Production

Average milk production and ECM were not significantly affected by the treatment (Table 3). The milk fat content was numerically lower in fat supplemented rations compared with CON. Milk protein content was numerically higher on RSC and WCR than on RSO (*P* = 0.71). Daily milk fat production in g/d was 1,187, 1,170, 1,164, and 968 for CON, RSC, WCR, and RSO,

Table 3. Milk production and composition

Item	Rations ¹				SEM ²	<i>P</i> -value ³	Contrast (<i>P</i> -value) ⁴		
	CON	RSC	WCR	RSO			CON vs. Fat	RSC and WCR vs. RSO	RSC vs. WCR
Milk, kg/d	27.4	31.2	28.1	26.3	4.61	0.23	0.52	0.16	0.18
Milk fat, g/kg	40.4	39.1	41.2	37.9	4.39	0.90	0.78	0.62	0.64
Milk protein, g/kg	33.5	33.1	32.9	32.4	1.13	0.71	0.35	0.51	0.83
Milk lactose, g/kg	46.4	46.8	46.1	46.4	1.28	0.26	0.80	0.99	0.07
ECM, kg/d	27.2	30.2	25.1	28.4	4.37	0.12	0.61	0.04	0.29

¹CON = control, RSC = rapeseed cake, WCR = whole cracked rapeseed, RSO = rapeseed oil.

²SEM for RSO; the SEM for the other treatments is the presented SEM $\times$ 0.93.

³Fixed effect of treatment.

⁴Linear orthogonal contrasts of treatment.

respectively. Daily milk protein production was 915, 1,030, 928, and 849 g/d for CON, RSC, WCR, and RSO. The yield of milk solids per day was not affected by the ration.

Feed Intake

The DMI was not affected by the treatments. The intake was numerically greater for cows consuming CON and RSC than WCR and RSO. The higher GE content in the 3 fat-supplemented rations compensated for the numerically lower DMI, and the GE intake was similar between CON and fat-supplemented rations. As planned, fat intake increased in the fat-supplemented rations, and NDF and CP intakes were not affected, as shown in Table 4. Adding rapeseed to the ration increased the intake of total FA from 471 g/d in CON to 804, 835, and 892 g/d for RSC, WCR, and RSO, respectively. Compared with CON, adding fat almost doubled the absolute intake of C18:0 ($P = 0.001$) and tripled the intake of C18:1 ($P < 0.003$).

Digestibility

Duodenal, ileal, and fecal flows were unaffected by the treatment, except for an increased fat flow in the fat-supplemented rations (data not shown). Neither total-tract nor ruminal apparent digestibility was affected by the treatment for any measured nutrient (Table 4). The flow of crude fat to the duodenum was higher than intake by an average of 210 g/d, or 22.7%; no difference was observed between rations in ruminal fat digestibility ($P = 0.46$). The NDF digestibility in the hind gut was close to zero for all rations except RSC.

VFA, Ammonia, and Rumen pH

Fat supplementation did not affect VFA concentrations in the rumen ($P = 0.24$). Bound-fat supplements

(rapeseed cake, whole rapeseed) resulted in higher propionic acid proportion ($P = 0.005$), and tended to reduce the acetic acid proportion ($P = 0.07$) compared with free supplement (oil). Consequently, the acetate to propionate ratio was 2.95 for RSO and 2.76, 2.73, and 2.74 for CON, RSC, and WCR, respectively, resulting in a significant difference ($P = 0.01$) between the 2 bound-fat supplements and RSO (Table 5).

The ammonia concentration in the rumen fluid was not influenced by the ration ($P = 0.19$), but a tendency for a higher concentration in RSC than in WCR ($P = 0.06$) was noted. The rumen pH was within the normal physiological range: an average of 6.29 for the 16 observations ranging from 5.60 to 6.91.

Methane Production

Adding rapeseed fat to the ration reduced the enteric CH₄ production significantly in liters per day and also related to the feed intake and ECM yield (Table 6). The average reduction (L of CH₄ per kg of DMI per percent fat added) was 4.6, 4.8, and 3.8% for RSC, WCR, and RSO, respectively. A tendency was observed for a reduction in CH₄ per kilogram of OM digested in the whole digestive tract ($P = 0.08$) when fat was added, but the ration had no effect on CH₄ per kilogram of totally digested carbohydrate or NDF and per kilogram of ruminally digested OM and NDF.

DISCUSSION

Intake and Milk Production

The rations were calculated to have identical crude fat content. However, the fat content in the rapeseed cake was not as high as planned, which resulted in a lower fat content in RSC.

We hypothesized that fat supplementation at the present levels would only reduce CH₄ production with-

Table 4. Intake and apparent digestibility of nutrients

Item	Ration ¹				SEM ²	P-value ³	Contrast (P-value) ⁴		
	CON	RSC	WCR	RSO			CON vs. Fat	RSC and WCR vs. RSO	RSC vs. WCR
DM									
Intake, kg/d	18.3	18.9	17.9	15.8	2.07	0.54	0.62	0.22	0.58
OM									
Intake, kg/d	17.1	17.7	16.8	14.9	1.96	0.56	0.64	0.23	0.61
Duodenal flow, kg/d	9.84	9.81	10.3	9.19	1.30	0.85	0.94	0.48	0.67
Rumen digestibility, %	42.5	44.6	38.1	40.6	2.34	0.23	0.57	0.79	0.06
Total-tract digestibility, %	73.2	74.3	72.0	71.9	1.36	0.41	0.71	0.41	0.16
NDF									
Intake, kg/d	6.10	6.20	5.83	5.18	0.74	0.59	0.54	0.27	0.60
Duodenal flow, kg/d	2.35	2.16	2.17	1.93	0.32	0.43	0.18	0.31	0.96
Rumen digestibility, %	61.7	65.5	62.7	63.9	2.57	0.22	0.14	0.93	0.13
Hindgut digestibility, %	1.55	9.64	1.28	1.83	6.39	0.61	0.66	0.63	0.29
Total-tract digestibility, %	61.7	63.3	60.4	61.1	2.37	0.60	0.98	0.72	0.23
Crude fat									
Intake, kg/d	0.65	1.03	1.11	1.00	0.10	0.01	0.003	0.48	0.38
Duodenal flow, kg/d	0.83	1.18	1.35	1.27	0.12	0.01	0.004	0.98	0.15
Rumen digestibility, %	-27.5	-15.7	-22.5	-26.4	6.21	0.46	0.37	0.36	0.39
Small intestine digestibility, %	75.0	75.0	75.0	69.9	3.18	0.33	0.44	0.10	0.99
Total-tract digestibility, %	65.4	67.8	65.1	58.0	4.29	0.25	0.59	0.07	0.50
FA intake, g/d	471	804	835	892	89.1	0.009	0.002	0.88	0.30
CP									
Intake, kg/d	3.09	3.23	3.00	2.70	0.35	0.53	0.68	0.23	0.48
Duodenal flow, kg/d	3.66	3.46	3.61	3.26	0.45	0.78	0.51	0.50	0.69
Ruminal digestibility, %	-19.0	-6.88	-21.3	-16.3	5.16	0.21	0.44	0.73	0.06
Total-tract digestibility, %	65.1	66.9	64.5	64.5	1.63	0.52	0.85	0.52	0.22

¹CON = control, RSC = rapeseed cake, WCR = whole cracked rapeseed, RSO = rapeseed oil.

²SEM for RSO; the SEM for the other treatments is the presented SEM $\times$ 0.86.

³Fixed effect of treatment.

⁴Linear orthogonal contrasts of treatment.

out affecting feed energy intake and milk yield. Negative effects of fat supplementation on DMI have been reported in some (Harvatine and Allen, 2006; Martin et al., 2008) but not all previous studies (Johnson et al., 2002; Moate et al., 2011). The effect of fat on DMI and GE intake depends on the level of supplementa-

tion. When cows are fed a low level of supplementation, DMI remains unaffected and GE intake increases, due to a higher energy density. When a moderate amount of fat is supplemented, DMI is reduced but GE intake is unchanged, whereas a further increase of the fat concentration in the ration can reduce the DMI to an

Table 5. Rumen VFA, ammonia, and pH (mean of 12 diurnal samples)

Item	Ration ¹				SEM ²	P-value ³	Contrast (P-value) ⁴		
	CON	RSC	WCR	RSO			CON vs. Fat	RSC and WCR vs. RSO	RSC vs. WCR
Total VFA, mmol/L	109	105	103	98.6	3.52	0.24	0.10	0.20	0.63
VFA, % of total VFA									
Acetic acid (A)	60.5	59.8	60.1	61.0	0.67	0.22	0.68	0.07	0.50
Butyric acid	15.1	15.7	15.4	15.8	0.52	0.21	0.08	0.40	0.34
Isobutyric acid	0.74	0.78	0.72	0.84	0.06	0.32	0.39	0.17	0.29
Propionic acid (P)	22.1	22.1	22.1	20.8	0.93	0.02	0.10	0.005	0.91
Valeric acid	1.64	1.67	1.70	1.62	0.06	0.44	0.51	0.22	0.58
A:P ratio	2.76	2.73	2.74	2.95	0.15	0.05	0.34	0.01	0.89
NH ₃ -N, mg/100 g	14.8	18.8	14.5	16.1	1.92	0.19	0.33	0.78	0.06
Average pH	6.32	6.28	6.26	6.28	0.05	0.73	0.31	0.75	0.73

¹CON = control, RSC = rapeseed cake, WCR = whole cracked rapeseed, RSO = rapeseed oil.

²SEM for RSO; the SEM for the other treatments is the presented SEM $\times$ 0.91.

³Fixed effect of treatment.

⁴Linear orthogonal contrasts of treatment.

Table 6. Methane production

CH ₄ production	Ration ¹				SEM ²	<i>P</i> -value ³	Contrasts (<i>P</i> -value) ⁴		
	CON	RSC	WCR	RSO			CON vs. Fat	RSC and WCR vs. Oil	RSC vs. WCR
L/d	569	531	478	462	51.0	0.04	0.02	0.18	0.10
L/kg of ECM	20.4	19.0	16.9	16.7	2.03	0.008	0.003	0.11	0.02
L/kg of DMI ⁵	29.6	26.9	25.8	26.4	1.78	0.07	0.02	0.99	0.37
% of GE ⁶ intake	6.32	5.60	5.31	5.40	0.03	0.002	<0.001	0.04	0.02
L/kg of total digested OM	46.1	40.3	39.8	43.0	3.10	0.22	0.08	0.36	0.87
L/kg of total digested NDF	154	135	137	146	10.3	0.20	0.18	0.39	0.85
L/kg of total digested CHO ⁷	57.2	51.4	51.1	54.9	3.68	0.33	0.16	0.34	0.92
L/kg of rumen digested OM	79.8	69.9	79.1	79.3	7.49	0.54	0.57	0.55	0.26
L/kg of rumen digested NDF	154	132	132	140	12.3	0.45	0.16	0.60	0.99

¹CON = control, RSC = rapeseed cake, WCR = whole cracked rapeseed, RSO = rapeseed oil.

²SEM for RSO; the SEM for the other treatments is the presented SEM $\times$ 0.88.

³Fixed effect of treatment.

⁴Linear orthogonal contrasts of treatment.

⁵DMI during CH₄ measurements.

⁶GE = gross energy.

⁷CHO = carbohydrate calculated as OM minus fat minus CP.

extent where the GE intake is also reduced (Grainger and Beauchemin, 2011). Neither DMI nor GE intake was affected significantly in the present study, indicating that the fat concentration in the rations (5.5–6.5% of DM) was within the nutritionally acceptable range. The rapeseed oil supplementation numerically reduced the DMI by 2.2 kg compared with CON, but the cows responded differently to the addition of oil, which is illustrated by the higher standard deviation between cows for DMI on RSO (5.3 kg) compared with the other rations (3.1 kg). This was also supported by heterogeneous variance for DMI, as a likelihood ratio test showed. Dry matter intake depression can be expected when the dietary fat concentration exceeds 6 to 7% (Beauchemin et al., 2007). A fat concentration of 6.5% in RSO in the present study may therefore be at a borderline where it affects some, but not necessarily all, animals. This was illustrated by the pronounced depressing effect of oil supplementation on DMI for the 2 cows with the highest feed intake level (per kg of BW) compared with the 2 cows with the lowest feed intake level (values not shown).

NDF Digestibility

Ruminal NDF digestibility was 63% on average, which is in accordance with previous studies with similar ration compositions (Lund et al., 2007). Rapeseed fat includes mainly monounsaturated FA and was presented at moderate concentrations in the present study; therefore, it did not affect NDF digestibility. The same effect has been observed with comparable additions of rapeseed (Chelikani et al., 2004; Beauchemin et al., 2009).

Few studies have compared how different physical forms of the same fat affect digestion. Even though effects of the physical form and processing of whole seeds were reported for linseed (Martin et al., 2008), this might not be valid for rapeseed. Pallister and Smithard (1987) found no effect of whole or extruded rapeseed or rapeseed oil on fiber digestion, using a comparable fat content in the control ration and slightly higher levels in the fat-supplemented rations compared with the present experiment. Ferlay et al. (1992) hypothesized that extrusion ruptures cell membranes and increases the availability of fat. However, in an experiment with feeding raw and extruded rapeseed to dairy cows, no effect on digestion was noted (Ferlay et al., 1992). This might be due to the high fat content in rapeseed compared with other oilseeds (Ferlay et al., 1992).

Total-tract NDF digestibility was lower than the ruminal NDF digestibility for all fat-supplemented rations. That problem has been observed earlier in connection with flow markers (Faichney, 1972; Lund et al., 2007) and different types of duodenal cannulas (Stensig and Robinson, 1997). The hindgut digestibility of NDF was higher for RSC compared with the other rations, but still within the physiological range (Huhtanen et al., 2006).

Fat Digestibility

The fat digestibility in the rumen was negative for all rations. Schmidely et al. (2008) reported, in a meta-analysis, a slightly positive FA balance from feed to duodenum (duodenal flow – intake). However, they found a net disappearance of FA with higher intake (50–120 g of FA/kg of DMI). In the present study,

RSO only slightly exceeded the 50 g of FA/kg of DM, and the other rations had a lower FA content. Scollan et al. (2001) reported a positive net FA flow to the duodenum with an increase in rumen FA balance by 21.8% when steers receive rations with 60 g/kg of DM fat, which fits well with the present experiment where crude fat increased by 22.7% from feed to duodenum. As observed earlier (Doreau and Chilliard, 1997), a net FA synthesis takes place in the rumen at low to moderate fat contents.

The digestibility of fat in the small intestine is highly variable; Doreau and Chilliard (1997) reported variations from 55 to 92% in a literature survey. The digestibility of fat in the small intestine depends on the chain length and degree of unsaturation (Weisbjerg et al., 1992; Doreau and Chilliard, 1997). The present digestibility for fat with mainly C18 FA was between 70 and 75%, which is in agreement with earlier reported values (Schmidely et al., 2008). With increasing fat consumption, the intestinal digestibility decreases because the absorptive capacity is limited, but absorption of FA can be higher than 1 kg/d (Doreau and Chilliard, 1997). As the highest FA intake in the present experiment was 892 g/d, fat absorption was not affected by treatment.

VFA and NH₃

Molar concentration of VFA was in agreement with earlier studies with dairy cows receiving rapeseed fat supplementation (Chelikani et al., 2004; Beauchemin et al., 2009). The higher acetate to propionate ratio for RSO compared with CON is surprising, because fat addition is believed to inhibit NDF but not starch fermentation, and therefore the propionic acid proportion was expected to increase as observed by Harvatine and Allen (2006). According to fermentation stoichiometry, a shift in fermentation pattern toward acetate, as observed for RSO, should result in enhanced CH₄ production (Boadi et al., 2004). However, cows fed RSO produced less CH₄ than on CON. The higher propionate proportion could be due to reduction in total VFA production because less substrate was fermented in the rumen, or because differences were buffered, as the values shown are averages of day and night time measurements.

The ammonia concentration in the rumen is considered to be the balance between entry sources (degradable feed N, N recycling) and outputs (incorporation into microbes, N absorption, ammonia N outflow; Doreau and Ferlay, 1995). In agreement with previous studies (Oldick and Firkins, 2000), fat addition itself did not affect the protein digestion in the rumen.

Methane Production

The CH₄ production in absolute numbers (L/d), as well as in relation to intake, was comparable to previous studies (Beauchemin et al., 2008; Moate et al., 2011). The reduction in CH₄ loss expressed as the proportion of GE intake when rapeseed fat was supplemented was less than found by Beauchemin et al. (2009) with dairy cows (18 vs. 14% in the present study) or Machmüller et al. (2000) with lambs (22%). This was most likely due to a higher fat concentration (Beauchemin et al., 2009) and a higher forage proportion (Machmüller et al., 2000) compared with rations in the present study. The reduction in CH₄ per kg of DMI compared with CON per percentage fat added was highest for WCR (4.8%) followed by 4.6 for RSC and 3.8% for RSO. The present reductions per kg of DMI were not as high as the 5.6% reduction found in a review by Beauchemin et al. (2008), but close to the values presented in the review by Grainger and Beauchemin (2011; between 4.7 and 5.1% depending on fat level in the ration).

Earlier studies with rapeseed found contradictory results; Martin et al. (2011) added 3% fat as extruded rapeseed to a dairy cow ration without finding any effect on CH₄. Beauchemin and McGinn (2006) found a significant reduction in CH₄ loss as proportion of GE when feeding a ration with 4.6% rapeseed oil added to heifers, but no effect per kg of DMI. In studies using dairy cows, Moate et al. (2011) found a significant reduction in CH₄ per kg of DMI when adding 2.6% fat to the ration as a rapeseed-hominy meal mix, and Beauchemin et al. (2009) found a reduction for 1.7% fat added as crushed rapeseed compared with the control ration.

Beauchemin and McGinn (2006) observed a feed intake depression and therefore, no effect on CH₄ per kg of DMI was observed when heifers received rapeseed oil. In a later study, Beauchemin et al. (2009) compared the CH₄-reducing properties of different oil seeds and emphasized that rapeseed did not depress DMI in contrast to other oil seeds. The present results and those of Moate et al. (2011) confirm that rapeseed fat does not always depress feed intake. The depressed feed intake in heifers found by Beauchemin and McGinn (2006) was probably due to the high fat supplementation level.

We hypothesized that fat from seeds is released more slowly in the rumen, and therefore might affect both CH₄ production and digestion differently than oil. Martin et al. (2008) found a stronger effect on CH₄ production the higher the fat content in the supplement (i.e., extruded linseed being less effective than whole linseed and whole linseed less effective than linseed oil). Conversely, Beauchemin et al. (2007) compared sunflower

seeds and sunflower oil and found, in agreement with the present study, no difference in the CH₄-depressing properties for different physical forms of the same fat source. The linseed fat in the study by Martin et al. (2008) was supplemented at a higher level, and linseed FA have a higher degree of unsaturation than rapeseed or sunflower. Furthermore, the different physical forms in the study by Martin et al. (2008) were confounded with fat contents, as the oil ration included 1.4 and 1.6% per kg of DM more fat than extruded and crude seed rations, respectively.

The fat concentration in rapeseed cake was lower than planned, which resulted in a lower fat content in RSC than in WCR and RSO. A meta-analysis by Beauchemin et al. (2008) showed a linear relationship between the percentage of fat added and the reduction in CH₄. A rapeseed cake probably would have been more effective in reducing CH₄ if it had had a higher fat concentration.

Fat supplementation depresses CH₄ production by lowering the quantity of OM degraded in the rumen, by influencing the microbial activity and ecosystem, and, to a very minor extent, by biohydrogenation of unsaturated FA (Johnson and Johnson, 1995). Although rumen digestibilities and VFA composition were not altered, the numeric reduction in CH₄ per kg of digested carbohydrate indicates that fermentation pathways were affected by fat supplementation in addition to the effect of fat being nonfermentable.

An optimal rumen microbial ecosystem is a prerequisite for efficient milk production, and it is well known that methanogens and fibrolytic bacteria may be hampered by addition of fat. The methanogen population of the rumen was studied in parallel to the present study by Poulsen (2012); despite the fact that fat supplementation reduced the CH₄ production, the total abundance of the methanogens in the rumen was unaffected by the ration.

CONCLUSIONS

Supplementation with rapeseed fat up to 6 to 6.5% fat of ration of DM reduced GE loss of enteric CH₄ by 14% without compromising the NDF digestibility or milk production. Adding fat by supplementing rapeseed in different forms decreased CH₄ production and numerically increased milk and ECM yields in the present study, resulting in less CH₄ per kg of product.

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