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Effect of dietary fat source and concentration on feed intake, enteric methane, and milk production in dairy cows

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ABSTRACT

Dietary fat can be used in dairy cow nutrition to reduce enteric methane (CH_4), but studies with multiple dietary fat concentrations are scarce. Among fat sources, rapeseed is easily accessible in Europe and North America, and palm kernel fat has been shown to be a potent inhibitor of ruminal methanogenesis. Forty-eight cows (half primiparous and half multiparous) were used in a 6 × 6 Latin square design, with 6 periods of 21 d each. Six treatments were used: a control, 3 fat concentrations (low, medium, and high) of rapeseed (RS), and 2 fat concentrations (low and medium) of palm kernel fatty acids (PK). The total crude fat concentrations ranged from 3% to 7% of DM. The cows were fed the treatments as a partial mixed ration, and they received additional concentrate from the GreenFeed units (1 unit for 12 cows) used to measure CH_4 production. Increased dietary crude fat concentration of both RS and PK reduced DMI. The reduction in DMI was stronger in cows fed medium concentration of PK than for any RS concentration, which was comparable to previous studies for both RS and PK. Digestibility of OM was highest at low fat concentration of both fat sources, and lowest at high RS concentration. Digestibility of NDF was reduced by 2 percentage units when cows were fed medium PK concentration instead of the control treatment. Rapeseed supplementation with dietary crude fat up to 5.7% of DM increased milk and ECM yields, but the equivalent PK concentration reduced ECM. Increased fat supplementation decreased CH_4 yield (g CH_4/kg of DMI) linearly when RS was used, and quadratically when PK was used. The medium PK concentration reduced CH_4 yield more than medium RS concentration, but there was no difference for CH_4 intensity (g CH_4/kg of ECM). Rapeseed fat supplementation with dietary crude fat above 5.7% of DM could reduce further CH_4 yield, but fat supplementation was not accompanied by an increase in productivity. The fat source must be accounted for when considering enteric methane reduction, as the PK provided stronger effect than RS, but the associated reduction in milk production did not support the use of PK for methane reduction.

Key words: rapeseed, palm kernel, GreenFeed, fatty acid

INTRODUCTION

Emission of anthropogenic greenhouse gases has to be reduced to slow down climate change (IPCC, 2023). Dairy cows are contributing a significant share of the emissions from agriculture and food production, especially due to CH_4 from enteric fermentation (FAO, 2020). Nutritional strategies can be effective in reducing CH_4 emission from ruminants, and supplementation of fat is one of the most potent feeding strategies (Beauchemin et al., 2022). Common feed ingredients used in dairy cow nutrition supply fat in the form of triglycerides, which are hydrolyzed in rumen into nonesterified fatty acids (**FA**) and glycerol (Moate et al., 2008). Fat and FA reduce enteric CH_4 by multiple mechanisms: (1) FA are not fermented in the rumen, and substitution of fermentable OM therefore reduces enteric CH_4 (Beauchemin et al., 2022); (2) FA are toxic to certain rumen microbes, especially fiber-fermenting bacteria, protozoa, and methanogens (Hristov et al., 2011; Zhou et al., 2015; Silva et al., 2016); (3) UFA are biohydrogenated in the rumen, removing H_2 otherwise available to reduce CO_2 to CH_4 (Beauchemin et al., 2022); and (4) triglycerides can change rumen fermentation toward propionate (Beauchemin et al., 2022). Whereas the replacement of rumen fermentable OM is applicable for all FA, the effect on rumen fiber-degrading microorganisms and methanogens, as well as the effect of biohydrogenation, are highly dependent upon type of FA (Bayat et al., 2018). For instance, only the supplementation of UFA can promote biohydrogenation, due to the presence of double bond(s) in the FA chain (Palmquist and Jenkins, 2017). Also, the toxicity for rumen microorganisms depends on the type of FA. Toxicity is higher in UFA, and especially PUFA, compared with the corresponding SFA. Also, medium-chain FA (**MCFA**), C12

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The list of standard abbreviations for JDS is available at adsa.org/jds-abbreviations-24. Nonstandard abbreviations are available in the Notes.

Table 1. Ingredients and nutrient composition of the treatment PMR (g/kg of DM, average ± SD, n = 6 unless otherwise stated); bait concentrate from the GreenFeed is not included in the PMR

	Treatment[1]					
		Cracked rapeseed			Palm kernel fatty acids	
Ingredient	CO	LR	MR	HR	LP	MP
Rapeseed meal	220	203	186	170	220	220
Rapeseed[2]	0	29.7	59.3	89.0	0	0
Palm kernel[2]	0	0	0	0	12.7	25.4
Basal diet[3]	780	767	754	742	767	754
Nutrient						
Ash	68.9 ± 1.5	68.1 ± 2.2	67 ± 1.5	65.8 ± 1.2	68.4 ± 0.98	67.4 ± 0.71
NDF	321 ± 8.4	321 ± 13	324 ± 5.3	324 ± 14	322 ± 7.3	319 ± 6.6
CP	169 ± 5.9	164 ± 3.3	163 ± 3.7	160 ± 2.1	166 ± 4.9	164 ± 2.5
Starch[4]	182 ± 20	179 ± 20	176 ± 20	173 ± 19	180 ± 20	176 ± 20
Crude fat[4]	29.8 ± 0.63	43.3 ± 0.53	56.8 ± 0.43	70.3 ± 0.33	42.8 ± 0.65	56.2 ± 0.62
Fatty acids[4]	20.4 ± 0.37	32.7 ± 0.60	45.0 ± 0.99	57.3 ± 1.42	32.2 ± 0.37	44.2 ± 0.36

[1] CO = control; LR = low rapeseed; MR = medium rapeseed; HR = high rapeseed; LP = low palm kernel; MP = medium palm kernel.

[2] Cracked rapeseed and palm kernel fatty acids.

[3] Basal diet composition (g/kg of DM): 171 rolled spring barley, 121 rolled dried beet pulp, 181 primary growth grass-clover silage, 121 first regrowth grass-clover silage, 383 corn silage, 16.1 sodium bicarbonate, 5.0 minerals, 1.0 vitamins, and 1.5 TiO_2.

[4] Analyzed on included feedstuffs and calculated according to PMR composition, where each feedstuff is composed of the samples of 2 consecutive periods (n = 3).

in particular, are more toxic compared with long-chain FA (**LCFA**), such as C16 and C18 (Patra, 2013). The toxicity effect has been shown on fiber degradation, with reduced in vitro NDF digestibility of over 50% for C12:0 and C14:0 (Weisbjerg and Børsting, 1989), and reduced protozoa count of over 95% with C12:0 (Hristov et al., 2011). The high toxicity of such MCFA, especially C12:0, could impair DMI, rumen digestibility, and milk production, making the reduction in enteric CH_4 emission counterproductive when evaluated based on methane yield and intensity (Weisbjerg et al., 2008; Hristov et al., 2011; Klop et al., 2017). In contrast, MCFA have been found to reduce enteric CH_4 yield and intensity (g CH_4/kg of DMI and g CH_4/kg of ECM, respectively) and could represent a promising dietary intervention to reduce enteric CH_4 emission from dairy cows (Machmüller et al., 2001). There is a general lack of studies with multiple dietary fat concentrations (Knapp et al., 2014; Beauchemin et al., 2022), which is an issue when aiming for extrapolating, modeling, and optimizing the dietary fat concentration effect in relation to both enteric emissions and productivity. Linear reduction of CH_4 production with increasing dietary fat and FA groups have been shown via meta-analytic approach (Grainger and Beauchemin, 2011; de Ondarza et al., 2024), but fat source–specific studies with multiple dietary fat concentrations, whether present, have not investigated enteric CH_4 emission (Faciola and Broderick, 2013). Supplementation of dietary fat is an important part of dairy cow nutrition primarily due to the potential to increase milk productivity (Palmquist and Jenkins, 2017), and secondarily to increase the biogas energy yield from manure anaerobic digestion (Møller et al., 2014). Negative effects of fat supplementation like milk fat depression are also well known (Dewanckele et al., 2020). Rapeseed is a common oilseed crop in Europe and North America, which is high in LCFA and UFA, especially in the C18 forms. Palm kernel FA, a by-product of palm oil production, is a source of MCFA high in C12:0 and C14:0 and is already available on the poultry feed market.

The aim of this experiment was to quantify the effect on enteric methane, milk production, and feed efficiency of 2 different FA sources, cracked rapeseed and palm kernel FA, fed at different concentrations to lactating dairy cows. It was hypothesized that (1) at a given fat concentration, cows fed palm kernel FA have lower enteric CH_4 production, yield, and intensity than cows fed cracked rapeseed, due to the prevalence of MCFA in palm kernel; and (2) the reduction in enteric CH_4 production, yield, and intensity is linear for both fat sources as FA supplementation increases, according to the findings of previous studies.

MATERIALS AND METHODS

The experiment took place at the research barn at Aarhus University (AU Viborg - Research Centre Foulum, Tjele, Denmark) and complied with the guidelines set by the Danish Ministry of Environment and Food (act 474, May 15, 2014; executive order 2028, December 14, 2020) regarding animal experimentation and care for animals used for scientific purposes. The experiment followed ARRIVE guidelines (Percie du Sert et al., 2020). A license was obtained from the Danish Animal Experiments Inspectorate.

Animals, Experimental Design, and Feedstuffs

A total of 48 lactating Danish Holstein dairy cows, 24 primiparous and 24 multiparous, were used in the trial. Cows were divided in 8 blocks of 6 cows each, according to parity (primiparous and multiparous), DIM (77.3 ± 44 and 73.0 ± 32 d at start of experiment for primiparous and multiparous, respectively, average ± SD), and by genetic yield index, above or below population mean (NAV, 2016). At the beginning of the experiment, primiparous cows weighed 636 ± 66 kg, and multiparous cows weighed 671 ± 75 kg (average ± SD). The experimental design was a replicated 6 × 6 Latin square design, with 6 treatments and 6 periods of 21 d each. Each cow was considered as an experimental unit, on which period and treatment specific measures were taken, and considered as the observational units. Six treatment sequences were generated within each Latin square replica, with the condition of balanced treatments for first order carry-over effects. The sequences were then randomly allocated to the 6 cows within the block. No Latin square could be identical to another. The experiment ran for 6 consecutive periods of 21 d each (126 d in total), and every cow received all treatments (with exception of 7 cows that were replaced, and the 7 cows replacing them, during the trial). The 6 treatments used in the Latin square were 6 partial mixed rations (**PMR**). In addition to the PMR, the cows received concentrate pellets during gas measurement (see the "Gas Exchange" section). The PMR could include 2 possible fat sources: either rapeseed (**RS**) provided as cracked seeds, or palm kernel FA (**PK**) provided as pure FA (Grolux, Cargill, Amsterdam, the Netherlands), which were supplemented at low, medium, and in the case of RS, high dose.

The treatments were as follows: control treatment (**CO**) without added fat; low, medium, and high RS (**LR**, **MR**, and **HR**, respectively); and low and medium PK (**LP** and **MP**, respectively, Table 1). The concentration of fat was planned to be 32, 44, 56, and 68 g crude fat/kg DM, and 19, 30, 41, and 52 g FA/kg DM for control, low, medium, and high concentration, respectively, according to NorFor feed tables (NorFor, 2021). The treatments were formulated according to the NorFor feeding system (Volden, 2011), taking into account the additional 900 g of DM extra concentrate used as bait in the GreenFeed unit. The basal diet included 2 grass-clover silages, corn silage, barley, dried beet pulp, rapeseed meal, minerals, vitamins, and TiO_2 as external digestibility marker. Nutrient composition of the ingredients is reported in Table 2. Increasing fat concentration was obtained by diluting the basal diet (Table 1). For treatments with RS, the inclusion of the rapeseed meal fraction was reduced equivalently with nonfat part of rapeseed supplementation so that all treatments had the same amount of nonfat rapeseed. For treatments with PK, the PK was mixed together with rapeseed meal to reach the required concentration of FA (5.6% and 11% of DM for LP and MP, respectively). For mixing the PK, the product was melted (melting point 28°C), and thereafter it was mixed with the rapeseed meal in a paddle mixer. The PK mixtures were prepared to last 2 consecutive periods and stored in a pile in a barn without insulation or heating. The basal diet was mixed every morning for 15 min in a vertical mixer. Afterward, the basal diet, rapeseed meal, and fat source (if required) were mixed in a horizontal mixer for 8 min.

Housing, Feeding, and Milking

The cows were housed in a loose housing barn in 4 separate sections. Within parity, 2 blocks were randomly selected to be housed together in 1 of 2 separate sections of the barn, each designed for 12 cows. The section was allocated a priori to a parity. Within section, each block was randomly assigned to either the first or last 6 Insentec feeding bins (Marknesse, the Netherlands), and then within block, each cow was assigned randomly and individually to 1 of the 6 bins for the entire duration of the experiment. The feeding bins recorded time stamps and weight changes of the feed when cows had access. Two water bins (Insentec) were available for the cows within each section for ad libitum intake of water. Cows were fed ad libitum, with 2 daily feedings at 1030 and 2000 h. In the first feeding, 35 kg of PMR was fed, whereas the amount provided in the second feeding differed according to the average individual feed intake of the 3 previous days and the amount of feed already provided, to reach the target of 10% residue PMR. If the PMR residue of a given bin was less than 2 kg at 0500 h, an additional 5 kg of PMR was added to the feed bin. Cows were milked twice a day at 0530 h and 1630 h in a side-by-side milking parlor.

Gas Exchange

One GreenFeed emission monitoring unit (**GF**; C-Lock Technology Inc., Rapid City, SD) was available in each section for gas exchange measurements, so 1 GF unit served 12 cows. Spot samples of individual cows' breath and belches were collected and analyzed. Each GF was fitted with sensors for air flow and CH_4, CO_2, and H_2 concentrations. A pelleted concentrate bait (PrimaKalv Opdræt VLOG, DLG, Denmark; Table 2) was used to promote sufficient visits to the GF unit. The bait was dropped when the unit read the cow's eartag. Each

Table 2. Nutrient composition of the ingredients (g/kg of DM or g/kg of total fatty acids for the individual fatty acids; average ± SD; n = 3, except for PK, where n = 1)[1]

Nutrient	RS	PK mix, 5.6%	PK mix, 11%	Rapeseed meal	Barley	Dried beet pulp	Grass-clover silage, primary growth	Grass-clover silage, first regrowth	Corn silage	Pellets[2]	PK
Ash	44.4 ± 0.6	83.3 ± 2.8	78.3 ± 2.2	84.3 ± 2.4	19.9 ± 1.1	71.8 ± 1.4	89.5 ± 9.5	104 ± 2.1	29.0 ± 0.2	69.7 ± 3.5	
NDF	167 ± 3.1	264 ± 2.3	261 ± 7.5	279 ± 3.8	169 ± 1.0	350 ± 5.0	379 ± 1.9	421 ± 8.7	340 ± 12	283 ± 1.4	
CP	188 ± 4.3	374 ± 2.6	361 ± 5.1	391 ± 5.4	93.1 ± 2.3	88.1 ± 2.3	171 ± 0.63	206 ± 0.6	81.9 ± 1.3	206 ± 5.9	
Starch	5.00 ± 1.2	8.00 ± 0.50	4.43 ± 0.60	5.30 ± 0.70	575 ± 14				349 ± 64	144 ± 22	
Crude fat	492 ± 2.4	82.0 ± 3.6	113 ± 5.9	45.0 ± 2.4	30.0 ± 0.9		32.3 ± 2.1	39.3 ± 1.9	25.7 ± 1.9	50.3 ± 2.7	1,000
Fatty acids	442 ± 17			36.9 ± 0.73	25.1 ± 0.83		13.9 ± 0.55	16.9 ± 0.75	18.1 ± 0.54	42.7 ± 6.6	895
C12:0	0.138 ± 0.061			0.696 ± 0.19	0.326 ± 0.048		1.73 ± 0.14	1.44 ± 0.28	2.12 ± 0.19	3.69 ± 2.3	490
C14:0	0.532 ± 0.0099			1.69 ± 0.13	3.17 ± 0.25		4.52 ± 0.12	3.51 ± 0.13	3.63 ± 0.78	3.64 ± 1.1	168
C16:0	47.4 ± 0.68			86.3 ± 1.1	252 ± 0.86		190 ± 2.8	177 ± 1.7	201 ± 12	194 ± 12	90.9
C18:0	17.1 ± 0.76			19.3 ± 0.49	16 ± 0.26		25.2 ± 0.84	22.2 ± 0.57	24.7 ± 0.55	23.6 ± 0.54	25.7
C18:1n7	32.9 ± 1.9			113 ± 5.8	7.41 ± 0.21		2.16 ± 0.28	2.56 ± 0.17	8.50 ± 0.74	23.4 ± 0.88	1.05
C18:1n9	590 ± 12			417 ± 11	125 ± 4.7		15.6 ± 0.57	14.4 ± 0.19	176 ± 9.8	291 ± 7.3	139
C18:2n6	200 ± 6.7			259 ± 1.8	529 ± 2.4		136 ± 0.87	133 ± 1.5	476 ± 34	388 ± 17	4.03
C18:3n3	84.4 ± 3.9			59.2 ± 3.9	45.7 ± 0.46		529 ± 3.1	555 ± 6.2	67.5 ± 12	45.4 ± 3.7	LOD

[1] RS is cracked rapeseed; PK is palm kernel fatty acids; PK mix, 5.6% and PK mix, 11% consist of rapeseed meal with inclusion of PK of 5.6% and 11% of DM, respectively. Empty cells indicate item was not analyzed. LOD = limit of detection.

[2] PrimaKalv Opdræt VLOG, DLG, Denmark, for use as bait concentrate for GreenFeed.

cow was allowed to receive a maximum of 6 bait drops per visit, with a 40 s interval between bait drops, in order to increase time with measurable gas flux. The number of drops and average drop weight were recorded from the units. Each cow was allowed up to 5 visits each day, with a 4 h minimum interval between visits to promote distribution of visits over the day. If cows used all daily bait drops available, a total of 900 g/cow per day of DM of bait was provided. Visits below 2 min were discarded, as were observations without an acceptable head position. Gas concentration sensors were auto-calibrated every third day with zero (20% O_2, balanced with N_2) and span gases (10, 500, 5,000 ppm of H_2, CH_4 and CO_2, 20% O_2, balanced with N_2). Triplicate recovery tests with CO_2 were performed for each unit on d 13 in each period, and before and after the experiment, to check air flow accuracy. From the 4 respective units, 99.5 ± 1.62%, 101 ± 3.4%, 101 ± 1.2%, and 99.3 ± 1.7% of CO_2 was recovered across all recovery tests (average ± SD).

Sampling and Measurements

A sample of each feedstuff was collected on d 6, 13, and 20 in each period, a fraction of which was used for determining DM concentration in an air-forced drying oven at 60°C for 48 h, and another fraction was frozen (−20°C). Once the experiment was completed, all frozen feedstuff subsamples were thawed and 6 sequential samples were pooled (adjacent weeks and periods), resulting in 3 samples for each feedstuff. Silage samples were then freeze-dried, whereas concentrates were oven-dried (60°C for 48 h) before chemical analyses. A subsample of each PMR was collected daily just after mixing on d 19 to 21 in each period, and subsamples were used for either determining DM concentration (60°C for 48 h) or frozen (−20°C). All frozen PMR subsamples were thawed, pooled by PMR and period, and oven-dried for chemical analyses (60°C for 48 h).

Fecal samples were collected from the 24 multiparous cows on d 19 at h 1400, on d 20 at h 0800 and 1400 and on d 21 at h 0800 in every period according to Giagnoni et al. (2021). Samples were frozen immediately after collection (−20°C), thawed and pooled by cow and period at the end of the experiment, and dried for determining DM concentration (60°C for 48 h) and for chemical analyses. Milk yield was recorded individually at every milking, and milk samples were collected at every milking from d 19 to d 21, for a total of 6 milkings. Body weight of cows was measured individually and automatically twice a day by a platform scale as cows left the milking parlor. Rumen samples (20 mL) were collected on d 21 with the ororuminal FLORA sampling device (Profs Products, Wittibreut, Germany; Larsen et al., 2020) on the 24 multiparous cows. The tubes were immediately put on ice before the contents were filtered with cheesecloths and stored at −20°C.

Laboratory Analyses

All feedstuff, PMR and, fecal samples were grinded using a 1 mm screen (ZM 200 mill, Retsch GmbH, Haan, Germany). The samples were analyzed for ash, determined by combustion in a muffle furnace at 525°C for 6 h; for N, determined using the Dumas method (Hansen, 1989) with a Vario MAX CN (Elementar Analysesysteme GmbH, Hanau, Germany); for NDF, using heat-stable amylase and correction for residual ash (Mertens et al., 2002) with an Ankom 200 fiber analyzer. Starch was analyzed in feedstuff samples using a YSI model 2900 analyzer (YSI Inc., Yellow Springs, OH) and heat-stable α-amylase and amyloglucosidase (Kristensen et al., 2007). Crude fat was analyzed in feedstuff samples by petroleum ether extraction using a Soxtec 2050 (Stoldt, 1952) after digestion with HCl at Eurofins Steins Laboratorium (Vejen, Denmark). Fatty acid concentrations were analyzed in feedstuff samples and according to procedures previously reported (Panah et al., 2020). The digestibility marker, TiO_2, was analyzed by spectrophotometry (Myers et al., 2004), with modifications (Wang et al., 2022). Milk samples were analyzed for fat, protein, lactose monohydrate and urea concentration with a MilkoScan 7RM FT+ infrared analyzer (Foss) at Eurofins (Vejen, Denmark). The rumen fluid samples were thawed and analyzed for concentrations of VFA using GC (Kristensen et al., 1996).

Calculations

Intake of DM was calculated as the sum between PMR and GF bait DMI. The PMR DMI was calculated individually from the average feed intake over the last 7 d of each period (sum of feed disappeared from the feeding bin between consecutive morning feedings), and the average DM of the PMR over the last 3 d in the corresponding period. The GF bait DMI was calculated individually from the number of bait drops received over the last 7 d of each period, the average weight of the drop for the specific GF unit (10 drops) in the period, and the DM of the pelleted feed for the period. The apparent total-tract digestibility of nutrients, herein referred to as digestibility, was calculated from the concentration of the nutrient (PMR and bait), the calculated concentration of TiO_2 on the total DMI (assuming 0 TiO_2 concentration in the GF bait concentrate), the concentration of TiO_2 in the feces, and the concentration of the nutrient in the feces. Gas exchange was averaged over the last 7 d of each period. Daily milk protein, fat, and lactose yields (kg/d) were calculated as the sum of milk yield multiplied with nutrient concentration for afternoon and morning milking, respectively, and the average over 3 d. Milk yield was averaged over the last 7 d of each period. Percentages of milk protein, fat, and lactose monohydrate were calculated based on nutrient yield and milk yield in the last 3 d of each period. The ECM yield (3.14 MJ/kg) was calculated according to Sjaunja et al. (1991) as follows: ECM yield (kg/d) = milk yield (kg/d) × [(38.3 × milk fat (g/kg) + 24.2 × milk protein (g/kg) + 15.71 × milk lactose monohydrate (g/kg) + 20.7)/3,140].

Statistical Analyses

The final dataset was analyzed with R 4.2.2 (R Core Team, 2022), with a linear mixed model using the *lmer* function from the *lme4* package (Bates et al., 2015). The model used was

$$Y_{tpjk} = \mu + Tr_t + Pa_p + Tr_tPa_p + Pe_j + \theta_k + \varepsilon_{tpjk},$$

where Y_{tpjk} is the dependent variable (n = 281); μ is the overall intercept; Tr_t is the fixed effect of treatment t (CO, LR, MR, HR, LP and MP); Pa_p is the effect of parity parity p (primiparous and multiparous); Tr_tPa_p is the interaction between treatments and parity; and Pe_j is the fixed effect of period j (1 to 6); θ_k is the random intercept effect for cow k (1 to 48); and ε_{tpjk} is the residual error, checked to be independent, normally distributed, and with homogeneous variance by graphical visualization. For all values involving feces (i.e., digestibility) or rumen VFA analyses, no parity effect was included, and 24 cows were in the random effect and the Pa_p effect was removed (n = 140). Estimated marginal means (**EMM**) and SEM obtained using the *emmeans* package (Lenth et al., 2023) are presented in the tables for the treatment and parity effect, and contrast for linear and quadratic effects were evaluated on the EMM for RS treatments (CO, LR, MR, HR) and PK treatments (CO, LP, MP) within parity. Because it was not possible to fit more than a second order polynomial effect with the PK treatments, the cubic effect was not investigated. Therefore, the conclusions on significant quadratic effects are warranted by the potential presence of a cubic or higher effect, and should be ultimately interpreted as nonlinear responses. The coefficients for the linear and quadratic responses were estimated on the EMM for the analyzed FA concentrations and reported for the selected variable in Figure 1. We removed 7 observations (4 primiparous and 3 multiparous) due to cows becoming unfit for the experiment (drop in milk yield, lameness, or sickness). However, the cows were substituted, with cows fitting the blocking criteria, and using the random effect of the cow to account for difference between cow using the same sequence of treatments in different period. Therefore, the highest SEM among treatments is reported in tables. The *P*-values reported are the result of an F-test from a type II ANOVA on the model specified, where $0.05 < P \leq 0.10$ are considered as tendencies and $P \leq 0.05$ are considered as significant. The model was also tested for the interaction between diet and period, which was not found significant, except for NDF intake and rumen caproic acid, and it was tested for the interaction between diet, period, and parity, which was never found significant. Therefore, these interactions were dropped from the main model.

RESULTS

Feedstuffs and Rations

Nutrient composition of the feedstuffs is presented in Table 2. Crude fat concentration of rapeseed was 492 g/kg of DM. The PK mixtures with rapeseed meal used for LP and MP treatments had a crude fat concentration of 82.0 and 113 g/kg of DM, respectively. The main FA in RS were C18:1n9, C18:2n6, and C18:3n3 (590, 200, and 84.4 g/kg of FA), and in PK the main FA were C12:0, C14:0, and C18:1n9 (490, 168, and 139 g/kg of FA). The nutrient composition of the treatments is presented in Table 1. Ash, NDF, CP, and starch concentrations were 67.6 ± 1.7, 322 ± 9.1, 164 ± 4.6, and 178 ± 18.7 g/kg of DM, respectively, across all treatments (average ± SD). The lowest and highest crude fat concentrations were 29.8 ± 0.63 and 70.3 ± 0.33 g/kg of DM in CO and HR, respectively. For diets with similar fat inclusion, at a low level the crude fat concentrations were 45.0 and 44.2 g/kg of DM for LR and LP, whereas at a medium level, the crude fat concentrations were 56.8 and 56.2 g/kg of DM for MR and MP, respectively.

Feed Intake and Digestibility

Results for feed intake are presented in Table 3. Compared with CO, HR and MP decreased DMI by 5% and 15%, respectively, and the decreases in DMI were similar across parities. However, the decrease in DMI and nutrient intake was linear for primiparous cows fed RS and for multiparous cows fed PK ($P < 0.001$), whereas the decrease in DMI was quadratic in primiparous cows fed PK and in multiparous cows fed RS ($P = 0.005$ and $P = 0.006$, respectively). Intake of NDF, CP, and starch were affected by increased dietary crude fat concentration in a similar way as DMI. Increased dietary crude fat concentration increased the crude fat intake linearly in primiparous cows fed RS treatments and quadratically (with diminishing increase) in primiparous cows fed PK treatments, respectively ($P < 0.001$ and $P = 0.002$), whereas a diminishing quadratic increase was observed in multiparous cows for both fat sources ($P = 0.001$ and $P = 0.02$, for RS and PK, respectively). Digestibility of OM tended to first increase and then decrease with a quadratic trend, peaking at low fat concentration, for cows fed RS ($P = 0.06$). Cows fed different PK concentrations tended to show a similar quadratic response as cows fed RS for OM digestibility ($P = 0.10$ and 0.06, respectively). Digestibility of NDF was lowest in cows fed MP and highest in cows fed RS (54.8% vs. 57.6%, 57.8%, and 57.8%, for CO vs. LR, MR, and HR treatments, respectively; $P = 0.02$). Digestibility of CP was highest in cows fed MP ($P < 0.001$), and it increased in a linear way when crude fat concentration was increased in cows fed PK ($P < 0.001$).

Methane and Rumen VFA

Gas exchange results are presented in Table 4. Methane production, yield, and intensity were decreased by 15%, 9%, and 14%, respectively, for cows fed HR compared with cows fed CO, and the decrease was linear for increasing dietary crude fat concentration ($P < 0.001$). For cows fed PK, increasing dietary crude fat concentration resulted in a quadratic decrease, with increasing decline, in CH_4 production (for both parities, $P < 0.001$) and in CH_4 yield (for multiparous cows, $P = 0.041$). A linear decrease was observed in CH_4 yield for primiparous cows and in CH_4 intensity for both primiparous and multiparous cows ($P < 0.001$). Increasing dietary crude fat concentration decreased CO_2 production linearly for cows fed RS treatments and quadratically for PK treatments ($P < 0.001$). Production of H_2 decreased linearly with increasing crude fat concentration for cows fed PK, but it was not affected when RS diets were fed. The proportion of rumen VFA is shown in Table 5. The acetic acid proportion decreased linearly, and propionic acid proportion increased linearly with increasing dietary crude fat concentration irrespective of fat source. With increasing dietary crude fat concentration, the proportion of valeric and isovaleric acid increased linearly, and the proportion of isobutyric acid decreased linearly in cows fed PK.

Milk Yield and Composition

Milk and milk composition are presented in Table 6. For multiparous cows fed RS treatments, ECM yield tended to be higher in cows fed LR and MR than in cows fed CO and HR treatments, as shown by the quadratic effect ($P = 0.01$; Figure 1). For primiparous cows fed RS no difference in ECM was observed. Both primiparous and multiparous cows decreased ECM yield quadratically with increasing decline when fed PK, with a 17% decrease in ECM yield in cows fed MP compared with cows fed CO. The response in milk yield was similar to the response in ECM yield. Milk fat concentration decreased

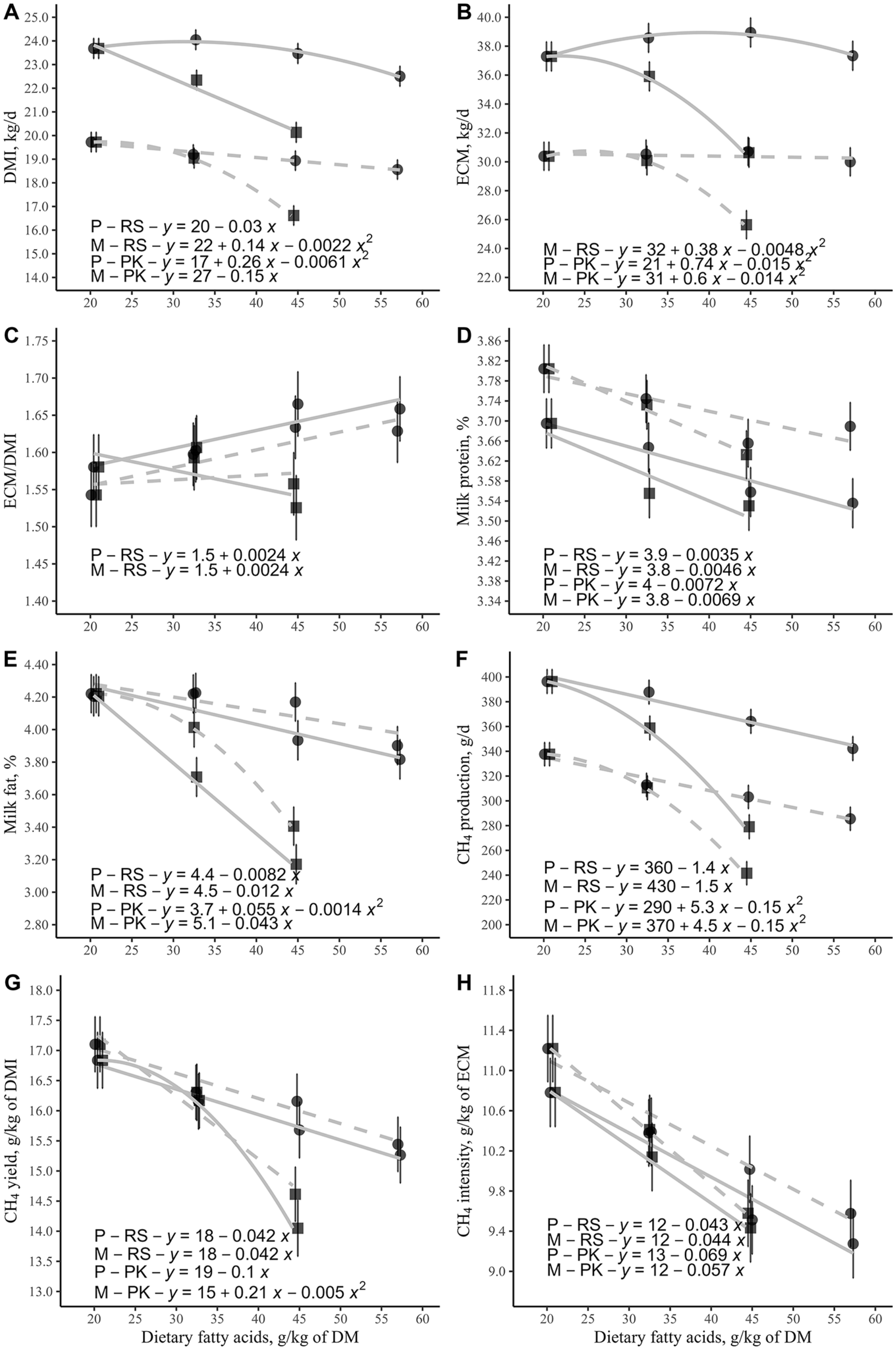

Figure 1. Least squares means and SE for the different concentrations of rapeseed (RS, circles) and palm kernel fatty acids (PK, squares), in primiparous (P, dashed line) and multiparous (M, solid line) dairy cows. Concentrations of fatty acids (x-axis) are for the diet across parities, also visible in Table 1. A slight horizontal shift between points is artificially made to improve the readability of the figure. Estimated coefficients are reported for the resulting equations; when the line and formula are not present, no linear or quadratic effect was found.

Table 3. Intake as the sum of PMR and bait concentrate from GreenFeed units (kg/d) and apparent total-tract digestibility of nutrients (%)

		Treatment[1]							P-value[2]							
Response	Pa[3]	CO	LR	MR	HR	LP	MP	SEM[4]	Tr	Pa	Tr:Pa	RS.L	RS.Q	PK.L	PK.Q
Intake															
DMI	P	19.7^{c}	19.2^{bc}	18.9^{bc}	18.6^{b}	19.0^{bc}	16.6^{a}	0.42	<0.001	<0.001	0.01	<0.001	0.76	<0.001	0.005
	M	23.7^{f}	24.0^{f}	23.5^{ef}	22.5^{de}	22.3^{d}	20.1^{bc}					<0.001	0.006	<0.001	0.13
OM	P	18.4^{b}	17.9^{b}	17.7^{b}	17.3^{b}	17.7^{b}	15.5^{a}	0.40	<0.001	<0.001	0.01	0.001	0.75	<0.001	0.005
	M	22.0^{de}	22.4^{e}	21.9^{de}	21.0^{cd}	20.8^{c}	18.8^{b}					<0.001	0.006	<0.001	0.13
NDF	P	6.30^{b}	6.14^{b}	6.13^{b}	6.00^{b}	6.09^{b}	5.27^{a}	0.14	<0.001	<0.001	0.03	0.018	0.86	<0.001	0.004
	M	7.58^{de}	7.69^{e}	7.59^{de}	7.28^{cd}	7.17^{c}	6.40^{b}					0.007	0.008	<0.001	0.06
CP	P	3.35^{de}	3.16^{bcd}	3.11^{bc}	2.99^{b}	3.17^{bcd}	2.75^{a}	0.070	<0.001	<0.001	0.02	<0.001	0.40	<0.001	0.02
	M	4.01^{h}	3.96^{h}	3.85^{gh}	3.62^{ef}	3.72^{fg}	3.33^{cd}					<0.001	0.031	<0.001	0.249
Starch	P	3.57^{de}	3.43^{cd}	3.33^{bc}	3.20^{b}	3.42^{cd}	2.91^{a}	0.075	<0.001	<0.001	0.009	<0.001	0.87	<0.001	0.001
	M	4.30^{h}	4.28^{h}	4.11^{gh}	3.88^{ef}	3.99^{fg}	3.51^{bcd}					<0.001	0.011	<0.001	0.11
Fat	P	0.597^{a}	0.836^{c}	1.07^{e}	1.29^{f}	0.743^{b}	0.784^{bc}	0.021	<0.001	<0.001	<0.001	<0.001	0.50	<0.001	0.002
	M	0.714^{b}	1.05^{e}	1.33^{f}	1.58^{g}	0.870^{c}	0.952^{d}					<0.001	0.001	<0.001	0.02
Digestibility[5]															
DM		70.7^{ab}	71.0^{b}	70.9^{ab}	69.9^{a}	71.5^{b}	70.9^{ab}	0.32	0.003			0.03	0.02	0.70	0.04
OM		72.4^{ab}	72.7^{b}	72.4^{ab}	71.5^{a}	73.1^{b}	72.6^{ab}	0.34	0.006			0.02	0.06	0.72	0.10
NDF		56.6^{ab}	57.6^{b}	57.8^{b}	57.8^{b}	56.7^{ab}	54.8^{a}	0.76	0.02			0.23	0.42	0.07	0.23
CP		66.7^{ab}	66.4^{ab}	67.3^{abc}	66.0^{a}	67.5^{bc}	68.3^{c}	0.37	<0.001			0.58	0.10	<0.001	0.95

[a–h] Letters for group contrasts reported across treatments and parities ($P < 0.05$).

[1] CO = control; LR = low rapeseed; MR = medium rapeseed; HR = high rapeseed; LP = low palm kernel; MP = medium palm kernel.

[2] Tr = treatment; Pa = parity; RS.L = linear effect of rapeseed supplementation; RS.Q = quadratic effect of rapeseed supplementation; PK.L = linear effect of palm kernel supplementation; PK.Q = quadratic effect of palm kernel supplementation.

[3] Pa = parity; M = multiparous; P = primiparous.

[4] SEM from group contrast across parities, the highest SEM is selected.

[5] Apparent total-tract digestibility measured for 24 multiparous cows with TiO_2 as digestibility marker.

Table 4. Gas production (g/d), yield (g/kg of DMI), and intensity (g/kg of ECM) for CH_4, and gas production of CO_2 and H_2

		Treatment[1]							*P*-value[2]						
Response	Pa[3]	CO	LR	MR	HR	LP	MP	SEM[4]	Tr	Pa	Tr:Pa	RS.L	RS.Q	PK.L	PK.Q
CH_4 (g/d)	P	338^{e}	313^{cd}	303^{bcd}	286^{b}	311^{bcd}	242^{a}	9.7	<0.001	<0.001	0.02	<0.001	0.50	<0.001	0.003
	M	396^{g}	388^{fg}	364^{ef}	342de	359^{e}	279^{abc}					<0.001	0.21	<0.001	0.001
CH_4 (g/kg of DMI)	P	17.1^{eg}	16.3^{defg}	16.2^{bdefg}	15.4^{abcdf}	16.3^{defg}	14.6^{ac}	0.47	<0.001	0.58	0.96	<0.001	0.80	<0.001	0.29
	M	16.8^{fg}	16.2^{cdefg}	15.7^{cdefg}	15.3^{abcde}	16.2^{cdefg}	14.0^{ab}					<0.001	0.65	<0.001	0.04
CH_4 (g/kg of ECM)	P	11.2^{d}	10.4^{abcd}	10.0^{abc}	9.58^{abc}	10.4^{abcd}	9.58^{abc}	0.34	<0.001	0.49	0.82	<0.001	0.33	<0.001	0.959
	M	10.8^{cd}	10.4^{bcd}	9.51^{ab}	9.27^{a}	10.1^{abcd}	9.43^{a}					<0.001	0.70	<0.001	0.90
CO_2 (g/d)	P	11,731^{cd}	11,403^{bc}	11,205^{bc}	11,188^{b}	11,543^{bcd}	10,475^{a}	207	<0.001	<0.001	0.13	<0.001	0.17	<0.001	0.003
	M	13,794^{e}	13,772^{e}	13,552^{e}	13,265^{e}	13,410^{e}	12,384^{d}					<0.001	0.24	<0.001	0.02
H_2 (g/d)	P	1.83^{cdef}	1.67^{bcde}	1.84^{cdef}	1.58^{bcd}	1.24^{ab}	0.940^{a}	0.17	<0.001	<0.001	0.59	0.36	0.69	<0.001	0.33
	M	2.31^{def}	2.52^{f}	2.37^{ef}	2.32^{def}	1.87^{bcde}	1.68^{abc}					0.73	0.25	<0.001	0.37
H_2 (g/kg of DMI)	P	0.101^{bc}	0.107^{bc}	0.104^{bc}	0.106^{bc}	0.0876^{abc}	0.0860^{abc}	0.0088	<0.001	0.10	0.35	0.644	0.71	0.089	0.42
	M	0.0965^{bc}	0.0887^{bc}	0.101^{c}	0.0891^{bc}	0.0683^{ab}	0.0591^{a}					0.954	0.624	<0.001	0.174

[a–g] Letters for group contrasts reported across treatments and parities ($P < 0.05$).

[1] CO = control; LR = low rapeseed; MR = medium rapeseed; HR = high rapeseed; LP = low palm kernel; MP = medium palm kernel.

[2] Tr = treatment; Pa = parity; RS.L = linear effect of rapeseed supplementation; RS.Q = quadratic effect of rapeseed supplementation; PK.L = linear effect of palm kernel supplementation; PK.Q = quadratic effect of palm kernel supplementation.

[3] Pa = parity; M = multiparous; P = primiparous.

[4] SEM from group contrast across parities, the highest SEM is selected.

Table 5. Rumen VFA, as a molar proportion of total VFA (% of total rumen VFA)

	Treatment[1]							*P*-value[2]				
Response	CO	LR	MR	HR	LP	MP	SEM[3]	Tr	RS.L	RS.Q	PK.L	PK.Q
Acetic acid	62.3^{b}	61.8^{b}	61.4^{b}	60.7^{ab}	61.4^{b}	59.1^{a}	0.55	<0.001	0.01	0.80	<0.001	0.15
Propionic acid	22.1^{a}	23.0^{ab}	23.2^{ab}	23.8^{ab}	22.5^{ab}	23.9^{b}	0.55	0.02	0.008	0.64	0.003	0.36
Butyric acid	11.5^{a}	11.1^{a}	11.2^{a}	11.3^{a}	11.6^{a}	11.7^{a}	0.26	0.26	0.63	0.27	0.57	0.79
Isobutyric acid	0.843^{ab}	0.824^{ab}	0.851^{b}	0.864^{b}	0.826^{ab}	0.779^{a}	0.019	0.01	0.19	0.34	0.008	0.45
Valeric acid	1.65^{a}	1.65^{a}	1.67^{a}	1.72^{a}	1.79^{a}	2.17^{b}	0.081	<0.001	0.37	0.67	<0.001	0.06
Isovaleric acid	1.19^{a}	1.19^{a}	1.24^{a}	1.29^{a}	1.38^{a}	2.02^{b}	0.093	<0.001	0.32	0.75	<0.001	0.02
Caproic acid	0.435^{a}	0.415^{a}	0.377^{a}	0.371^{a}	0.407^{a}	0.440^{a}	0.029	0.36	0.06	0.81	0.89	0.36

[a,b] Letters for group contrasts by row ($P < 0.05$).

[1] CO = control; LR = low rapeseed; MR = medium rapeseed; HR = high rapeseed; LP = low palm kernel; MP = medium palm kernel.

[2] Tr = treatment; Pa = parity; RS.L = linear effect of rapeseed supplementation; RS.Q = quadratic effect of rapeseed supplementation; PK.L = linear effect of palm kernel supplementation; PK.Q = quadratic effect of palm kernel supplementation.

[3] The highest SEM is selected across treatments.

linearly with increasing dietary crude fat concentration irrespective of fat source in multiparous cows, but the effect was quadratic in primiparous cows fed PK, with 4.22%, 4.01%, and 3.41% of milk fat for CO, LP and MP, respectively. Increasing dietary crude fat concentration also decreased milk protein concentration in a linear way, irrespective of fat source, whereas milk lactose concentration was increased with increasing dietary crude fat concentration for cows fed RS, and decreased for cows fed PK. Both RS and PK reduce milk urea linearly.

DISCUSSION

Dietary crude fat can play a significant role in reducing enteric CH_4 emission from dairy cows; however, the mitigation potential can depend on dietary crude fat concentration and source. This study provides a comparison between the use of rapeseed and palm kernel FA as fat sources fed at different concentrations.

Feedstuffs and Rations

The fat and FA concentration of the treatments was similar for LR and LP treatments, as well as for MR and MP treatments. No high fat supplementation was included for PK, but the effects on DMI and milk production observed on MP treatment, and discussed in the following sections, were sufficient to make conclusions regarding the mitigation potential of this fat source. As PK was supplied as free FA, a higher reactivity can be expected in the rumen compared with RS (Moate et al., 2008); therefore, the fat form was confounded with the dietary treatment. This choice was made as the fat sources had to be suitable for an actual production farm rather than being fully comparable between them.

Feed Intake and Digestibility

The reduction in feed intake is partially explained by an increase in the gross energy concentration of the treatment by increasing dietary crude fat concentration. Brask et al. (2013a,b) did not find an effect on DMI when a diet based on cracked rapeseed was fed at a FA concentration close to HR, and some studies have reported an increase in DMI due to fat supplements, at least at lower dietary crude fat concentrations (Johnson et al., 2002; Patra, 2013). Weisbjerg et al. (2008) found a linear decrease of 0.42 kg/d of DMI for 10 g FA/kg of DM with fat supplementation of palm FA distillate, rich in C16:0 and C18:1. This decrease is similar to what we observed for primiparous cows fed RS, but it is a smaller decrease compared with the observation for cows fed PK. Weld and Armentano (2017) reported a decrease of 0.60 kg/d in DMI per 10 g FA/kg of DM with supplementation of C18:1 and C18:2 FA and a decrease of 2.1 kg/d in DMI per 10 g FA/kg of DM with supplementation of MCFA, which is similar to our results for RS and PK, respectively. However, we found a tendency for a linear decrease ($P = 0.07$) when increasing the concentration of MCFA, and not a clear reduction in NDF digestibility. Whether the DMI affected the NDF digestibility, or vice versa, goes beyond the design of our experiment. Hristov et al. (2011) reported a strong DMI reduction for cows supplemented with C12:0, but not for cows supplemented with C14:0, when fed as sole FA sources. This suggests that the effect of PK is mostly attributable to C12:0. Faciola and Broderick (2013) reported a linear but not a quadratic effect on DMI from feeding increasing concentrations of C12:0 in multiparous cows. Nevertheless, we only observed a quadratic reduction of DMI with increasing MCFA concentration in primiparous cows, which might indicate an effect of size or productivity of the cow.

The effect of fat supplementation on OM digestibility was significant, however, the reduction was quantitatively minor (1 percentage unit), similar to what observed by Brask et al. (2013a), but in contrast with the increased OM digestibility observed by Hristov et al. (2011), when supplementing C12:0 and C14:0 separately. Faciola and Broderick (2013) found a larger decrease in OM digestibility than we did, probably due to the higher fat concentration of pure C12:0 that they used. Digestibility of NDF was not affected by RS, as also found by Brask et al. (2013a) and by the meta-analysis from Weld and Armentano (2017) for oilseeds. Pronounced reductions in in vitro NDF digestibility compared with control have been reported for MCFA (73%, 57%, and 50% for C12:0, C14:0, and palm kernel oil, respectively; Børsting and Weisbjerg, 1989; Dohme et al., 2000). Within the meta-analysis of Weld and Armentano (2017), 2 experiments used MCFA, and they estimated a linear decrease 2.65 percentage units of NDF digestibility per percentage unit on DM of MCFA added. However, we found a quadratic effect for increasing MCFA supplementation, which was not observed by Weld and Armentano (2017) or Faciola and Broderick (2013). Therefore, the linear prediction used by Weld and Armentano (2017) might result in prediction bias of NDF digestibility with MCFA supplementation when considering our study. We observed a milder reduction in NDF digestibility compared with Faciola and Broderick (2013) for a comparable fat supplementation as in MP. These differences can be in part explained by the difference in C12:0 concentration in the supplemented fat, which was 41% of the total FA provided from PK, whereas Faciola and Broderick (2013) included it as a pure FA and expected to have a stronger effect on the rumen environment than C14:0.

As mentioned, the fat source was confounded with the physical form of fat (RS: cracked seed; PK: free

Table 6. Milk yield (kg/d), ECM yield (kg/d), and milk fat, protein, and lactose concentration (%) and yield (kg/d)

		Treatment[1]							*P*-value[2]						
Response	Pa[3]	CO	LR	MR	HR	LP	MP	SEM[4]	Tr	Pa	Tr:Pa	RS.L	RS.Q	PK.L	PK.Q
Milk yield	P	28.6^{ab}	29.0^{abc}	29.5^{bc}	29.5^{bc}	29.3^{abc}	27.2^{a}	1.2	<0.001	<0.001	0.22	0.13	0.74	0.033	0.03
	M	36.1^{de}	37.3^{ef}	39.1^{f}	37.9^{ef}	37.3^{ef}	34.7^{cd}					<0.001	0.01	0.036	<0.001
ECM yield	P	30.4^{b}	30.5^{b}	30.7^{b}	30.0^{b}	30.1^{b}	25.6^{a}	1.0	<0.001	<0.001	0.004	0.69	0.37	<0.001	<0.001
	M	37.3^{cd}	38.6^{d}	38.9^{d}	37.3^{cd}	35.9^{c}	30.6^{b}					0.76	0.003	<0.001	<0.001
Fat, %	P	4.22^{cd}	4.22^{cd}	4.17^{cd}	3.90^{cd}	4.01^{cd}	3.41^{ab}	0.12	<0.001	0.31	0.17	0.003	0.07	<0.001	0.03
	M	4.20^{d}	4.23^{d}	3.93^{bcd}	3.82^{bc}	3.71^{bc}	3.17^{a}					<0.001	0.34	<0.001	0.82
Protein, %	P	3.80^{d}	3.74^{abcd}	3.66^{abc}	3.69^{abc}	3.73^{abcd}	3.63^{abc}	0.049	<0.001	0.05	0.46	<0.001	0.06	<0.001	0.67
	M	3.70^{cd}	3.65^{bcd}	3.56^{ab}	3.54^{ab}	3.56^{ab}	3.53^{a}					<0.001	0.60	<0.001	0.06
Lactose, %	P	4.98^{efg}	5.01^{fgh}	5.03^{gh}	5.05^{h}	4.96^{def}	4.92^{cd}	0.021	<0.001	<0.001	0.002	<0.001	0.83	<0.001	0.50
	M	4.84^{bc}	4.91^{de}	4.92^{def}	4.95^{defg}	4.81^{b}	4.74^{a}					<0.001	0.08	<0.001	0.26
Fat yield	P	1.21^{bc}	1.21^{bc}	1.22^{bc}	1.15^{b}	1.16^{bc}	0.919^{a}	0.044	<0.001	<0.001	0.003	0.18	0.16	<0.001	0.003
	M	1.49^{e}	1.55^{e}	1.51^{e}	1.43^{de}	1.36^{cd}	1.06^{ab}					0.07	0.01	<0.001	0.01
Protein yield	P	1.09^{b}	1.08^{b}	1.07^{b}	1.09^{b}	1.09^{b}	0.982^{a}	0.037	<0.001	<0.001	0.06	0.93	0.55	<0.001	0.004
	M	1.31^{c}	1.34^{c}	1.38^{c}	1.33^{c}	1.31^{c}	1.20^{b}					0.12	0.02	<0.001	0.003
Lactose yield	P	1.43^{ab}	1.45^{ab}	1.48^{bc}	1.49^{bc}	1.45^{ab}	1.34^{a}	0.061	<0.001	<0.001	0.06	0.04	0.72	0.011	0.03
	M	1.75^{cd}	1.83^{def}	1.92^{f}	1.87^{ef}	1.80^{de}	1.64^{bc}					<0.001	0.006	0.002	<0.001
Urea, mmol/L	P	3.88^{ef}	3.56^{cde}	3.27^{bcd}	2.74^{a}	3.62^{cde}	3.43^{cd}	0.11	<0.001	0.29	0.26	<0.001	0.163	<0.001	0.746
	M	4.24^{f}	3.68^{de}	3.27^{c}	2.83^{ab}	3.69^{de}	3.57^{cde}					<0.001	0.443	<0.001	0.023

[a–h] Letters for group contrasts reported across treatments and parities ($P < 0.05$).

[1] CO = control; LR = low rapeseed; MR = medium rapeseed; HR = high rapeseed; LP = low palm kernel; MP = medium palm kernel.

[2] Tr = treatment; Pa = parity; RS.L = linear effect of rapeseed supplementation; RS.Q = quadratic effect of rapeseed supplementation; PK.L = linear effect of palm kernel supplementation; PK.Q = quadratic effect of palm kernel supplementation.

[3] Pa = parity; M = multiparous; *P* = primiparous.

[4] SEM from group contrast across parities, the highest SEM is selected.

FA), but given the findings from other studies, the effect of the source should have been minor. Hoffmann et al. (2016) also did not find a difference in DMI when feeding rapeseed either as cracked seeds or as oil, and Arndt et al. (2022) concluded that there was no effect on reduction in CH_4 intensity when comparing oilseeds and oil supplements. Brask et al. (2013b) found a numerical, but not significant, decrease in DMI of 2% and 14% when cracked rapeseed and rapeseed oil were supplemented, respectively, at similar FA concentration as HR, and with similar response on DMI as we found for cracked rapeseed.

Methane and Rumen VFA

Grainger and Beauchemin (2011) and Patra (2013) reported reductions of 1 and 0.66 g CH_4/kg of DMI, respectively, by increasing dietary fat of 10 g/kg of DM. The reductions reported are higher than what we observed for increasing RS supplementation (0.40 g CH_4/kg of DMI by increasing dietary fat of 10 g/kg of DM), but lower than what we observed for increasing PK supplementation (1.1 g CH_4/kg of DMI by increasing dietary fat of 10 g/kg of DM). However, reduction from PK is not fully comparable because we found a quadratic effect, with increasing CH_4 yield reduction, when increasing fat supplementation of PK on CH_4 yield. Grainger and Beauchemin (2011) also reported a quadratic effect, but in their case with diminishing decrease, for increasing fat concentration on CH_4 yield. However, the diets included in their analysis reached a higher crude fat concentration than normal (>10% of DM), and when the analysis was repeated with dietary treatments having a crude fat concentration below 8% of DM, they found a linear, but not a quadratic, decrease in CH_4 yield. This is in contrast with our trial, and might depend on the increased variation between treatments when merging multiple studies for a meta-analysis, compared with use a single response-trial as in our case. Another meta-analysis found a reduction of CH_4 production of 3.77% per percentage unit of added fat (de Ondarza et al., 2024), which corresponds to a reduction of 1.3 g CH_4/d for primiparous cows and 1.5 g CH_4/d for multiparous cows, for every gram of FA added per kilogram of DM. These values reflect the slope coefficients estimated in Figure 1F for RS (1.4 for primiparous and 1.5 for multiparous), but not for PK when fed at medium concentration. Furthermore, de Ondarza et al. (2024) found that the reduction in CH_4 production was higher for UFA and PUFA supplementation, but this was not reflected in our RS treatments, high in C18:1 and C18:2.

Hristov et al. (2011), in contrast to our experiment, did not observe differences in CH_4 production when feeding cows different FA (C12:0, C14:0, and C18:0). They suggest a possible additive effect between the C12:0 and C14:0, as they found no effect of the FA individually. However, they only measured the gas concentration directly from the rumen cannula, and they observed a lower DMI and ECM when C12:0 was added to the diet, which might indicate an increase in CH_4 yield and intensity, if not accompanied by a proportional reduction in CH_4 production. In our case, the CH_4 yield decreased linearly by increasing RS, and decreased quadratically by increasing PK concentration (Figure 1G). For CH_4 intensity, a similar linear decrease was observed for both fat sources (Figure 1H), and to our knowledge, no single trials have tested such responses. Dohme et al. (2001) observed a reduction of 20% of CH_4 yield in vitro with C12:0 addition, which is close to the reduction of 15% of CH_4 yield that we observed when feeding cows MP instead of CO. We observed a linear decrease of H_2 production with increasing concentration of PK but not RS, which was also reflected in a stronger reduction of CH_4 production (g/d). However, the decrease in H_2 production was followed by a decrease in DMI and ECM, resulting in similar responses for both H_2 production and yield. Whether there is a causation link with H_2 production is not clear.

Hristov et al. (2011) observed a decrease in rumen acetate proportion and an increase in valerate and isovalerate proportions when supplementing C12:0, but not with C14:0. These changes in VFA proportions when supplementing C12:0 were similar to what was observed in our study when comparing cows fed CO and MP. For both fat sources, the linear decrease and increase in acetate and propionate proportion, respectively, are also in agreement with a previous meta-analysis (Patra, 2013); however, butyrate proportion was not affect by fat supplementation in our study. The higher propionate observed in cows fed MP compared with cows fed CO seems sufficient to reduce CH yield also by affecting the rumen H_2 balance (Ungerfeld, 2020) and thereby reducing CH_4 yield and intensity more than the equivalent MR treatment. In fact, CH_4 yield was lower in cows fed MP than cows fed MR, but the 2 treatments had similar CH_4 intensity. Therefore, feeding MP instead of MR does not provide an advantage in term CH_4 emission per unit of product, and it is necessary to discuss the milk production to assess the potential benefits of the treatments.

Milk Yield and Milk Nutrients

Supplementation of oilseeds has been reported to increase milk yield, but decrease milk fat and protein concentrations (Rabiee et al., 2012). We observed maximum milk yield for cows fed 5.7% of DM fat with RS supplementation, and linear decreases in milk fat and protein concentration. The ECM and milk yield observed in our study for cows fed RS reflected previous findings from

Patra (2013), where milk yield peaked with a dietary fat concentration between 4% and 6% of DM. Milk fat concentration and yield were found to decrease with dietary fat supplementation in a meta-analysis (de Ondarza et al., 2024) when considering multiple fat sources. In contrast, we found that using RS as fat source did not decrease milk fat yield, but we also found a slight decrease in milk fat concentration (around 0.01% decrease per gram of added FA per kilogram of DM). For cows fed PK, the relationship followed a similar trend, but milk yield decreased already when fat concentration approached 5% of DM, indicating that the drop in yield occurs earlier compared with cows fed RS. The fat source affected the milk composition to such an extent that cows fed MP decreased ECM yield compared with cows fed CO, showing different responses in milk nutrient composition between RS and PK. Hristov et al. (2011) also observed a decrease in ECM when supplementing C12:0, but they found no effect of C14:0.

CONCLUSIONS

Increasing dietary fat supplementation decreased CH_4 production and yield linearly in cows fed cracked rapeseed, and it quadratically decreased CH_4 production and yield, with increasing decline, in cows fed palm kernel FA. Therefore, at low fat supplementation the reduction in CH_4 was similar in the 2 fat sources, but cows fed palm kernel FA had lower CH_4 production and yield at medium fat supplementation. Cracked rapeseed supplementation with dietary crude fat up to 5.7% of DM increased milk and ECM production and decreased CH_4 production, yield, and intensity. In addition, supplementation above 5.7% of DM reduced further CH_4 production, yield, and intensity, but was not accompanied by increases in milk and ECM production. Palm kernel FA supplementation at or above 2 percentage units of DM had a detrimental effect on milk productivity and a reduction potential for CH_4 intensity equivalent as cracked rapeseed.

NOTES

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Nonstandard abbreviations used: CO = control; EMM = estimated marginal means; FA = fatty acid; GF = GreenFeed emission monitoring unit; HR = high RS; LCFA = long-chain FA;LOD = limit of detection; LP = low PK; LR = low RS; MCFA = medium-chain FA; MP = medium PK; MR = medium RS; Pa = parity; PK = palm kernel fatty acids; PK.L = linear effect of palm kernel supplementation; PK.Q = quadratic effect of palm kernel supplementation; PMR = partial mixed ration; RS = rapeseed; RS.L = linear effect of rapeseed supplementation; RS.Q = quadratic effect of rapeseed supplementation; Tr = treatment.

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