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Milled rapeseeds and oats decrease milk saturated fatty acids and ruminal methane emissions in dairy cows without changes in product sensory quality

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Plant lipids in the diet are known to modify milk fatty acid (FA) composition and mitigate ruminal methane emissions. The objective of the present work was to examine the potential of milled rapeseeds and oats to decrease both milk saturated FAs and ruminal methane emissions in practical farm settings. In the pilot study, six Finnish Ayrshire cows were fed a control diet for 3 weeks, which was then followed by a lipid-rich test diet for 3 weeks. The experimental diets were based on grass silage supplemented with barley and rapeseed meals in the control diet and with oats and milled rapeseeds in the test diet. The lipid inclusion rate was 55 g/kg dry matter (DM). In the main study, the whole Finnish Ayrshire research herd in milk (n = 49–59) was used in a switch-back-designed study. The cows were fed a control diet for 3 weeks, then a test diet for 4 weeks, and, finally, a control diet for 3 weeks. The diets were the same as in the pilot study except for a lower lipid inclusion level of 50 g/kg DM. The test diet decreased DM intake by 15% and energy-corrected milk (ECM) yield by 13% in the pilot study. The adjustment of supplemental lipids from 55 g/kg to 50 g/kg DM was successful, as the DM intake decreased only by 4% relative to the control diet in the main study. Furthermore, the yields of milk, lactose, protein, and fat were also unaffected by dietary lipids in the main study. The milk fat composition was significantly altered in both studies. The milk fat saturated FAs were decreased by 16%–20% in the test diet, mainly due to the *de novo* FAs of 6- to 16-carbons (a reduction of 22%–48%). Milk fat *cis*-9 18:1 was increased by 63%–78% in the test diet relative to the control. Dairy products' (milk, butter, and cheese) organoleptic quality was not compromised by the modified lipid profile. Ruminal methane and hydrogen intensities (n = 23; g or mg/kg ECM) were 20% and 39% lower, respectively, in the test diet than in the control diet. This reduction can be attributed to a lower amount of organic matter fermented in the rumen, as indicated by the lower DM intake and nutrient digestibility.

KEYWORDS

plant lipid, grass silage, milk fat, saturated fatty acid, trans fatty acid, organoleptic quality, methane, hydrogen

1 Introduction

Ruminants are dependent on the anaerobic microbial ecosystem in the rumen to ferment and transform human-indigestible forages into dairy and meat products of high quality. However, due to the microbial metabolism of carbohydrates, ruminants are also significant producers of enteric methane (CH_4). In addition, CH_4 formation represents an unproductive loss of dietary energy to the ruminant animal (Min et al., 2022). Adding plant lipids that are not fermentable in the rumen to dairy cow diets suppresses CH_4 emission intensity [g CH_4/kg energy-corrected milk (ECM)], on average, by 12% (Hristov et al., 2022). Oilseeds have a CH_4 mitigation potential similar to that of pure oils, with the advantage that the lipid may be released at a slower rate in the rumen. Therefore, oilseeds may have a less harmful effect on the rumen function (Hristov et al., 2022) and, in turn, allow further lactational performance at high levels of lipid inclusion. However, practical evidence on the feasibility and effectiveness of feeding milled full-fat oilseeds at the whole-herd level to mitigate ruminant methane emissions is lacking.

Cardiovascular disease (CVD) is the leading cause of morbidity and mortality for humans worldwide (Perna and Hewlings, 2023). Compiled evidence suggests that the replacement of saturated fatty acids (SFAs) with unsaturated ones in dairy products may alleviate human CVD risk (Livingstone et al., 2012; Clifton and Keogh, 2017; Vasilopoulou et al., 2020). The research on the effects of individual SFAs is inconclusive, but most studies indicate that SFAs of 12- to 18-carbons may increase the risk for CVD, whereas shorter-chain SFAs may be beneficial or neutral (Perna and Hewlings, 2023). However, some studies suggest that 18:0 stearic acid (SA) does not increase CVD risk (Briggs et al., 2017). Dietary unsaturated fatty acids (FAs) have great potential to modify the FA composition of ruminant milk by decreasing the proportion of SFAs and increasing that of unsaturated FAs inherent to lipid supplements, such as *cis*-9 18:1 oleic acid (OA) rich in the lipids of rapeseed (*Brassica napus*) and oats (*Avena sativa*) (Collomb et al., 2004; Fant et al., 2023). Furthermore, the ability to increase milk fat monounsaturated FAs through dietary inclusion is much greater in magnitude than with polyunsaturated ones (Kliem and Shingfield, 2016). Beyond a certain threshold of dietary lipid supply, both feed intake and milk yield decline significantly (Drackley et al., 2007; Huhtanen et al., 2008; Vanhatalo and Halmemies-Beauchet-Filleau, 2020). Consequently, this threshold, influenced by various factors, especially the basal diet and the characteristics of the lipid supplement (Benchaar et al., 2015; Halmemies-Beauchet-Filleau et al., 2017), should not be exceeded when adjusting milk fat composition in practical farm settings. Moreover, the form in which lipids are included in the ruminant diet significantly affects their bioavailability and the ultimate composition of the final product. Furthermore, rupture of rapeseed seedcoats is necessary to enhance the availability of lipids within the seeds for absorption (Kairenius et al., 2009).

The milk FA composition affects the texture, flavor, and shelf life of dairy products (Kennelly, 1996; Hillbrick and Augustin, 2002). However, monounsaturated FAs are less prone to oxidation than polyunsaturated ones (Kennelly, 1996), which reduces the risk for off-flavors and shorter shelf lives. Furthermore, dairy products with lipids rich in OA have resulted in products with softer textures, but with similar flavors to standard products (Chen et al., 2004; Ryhänen et al., 2005).

The objective of this study was to examine the potential of the lipid in milled rapeseeds and oats to replace a part of the SFAs in milk fat with monounsaturated ones inherent to these lipid supplements and to mitigate ruminal methane emissions in practical farm settings. We hypothesized that replacing rapeseed meal and barley with milled rapeseed and oats in a dairy cow diet will not impair the lactation performance or sensory quality of the dairy products but will soften the milk fat and mitigate rumen methanogenesis.

2 Materials and methods

The experiments were conducted at the University of Helsinki Viikki research farm (60°13′N, 24°02′E) in Finland. The pilot dairy cow study was conducted in the spring of 2018 and the main dairy cow study in the autumn of 2018. Similar dietary ingredients and the same analytical methods were used in both experiments.

2.1 Pilot dairy cow study

The effects of a tailored test diet elevated in lipids were first studied with a limited number of dairy cows. This pilot study was carried out to ensure maximal changes in milk fat composition, without compromising animal health and performance, when later implementing the test diet for a large number of animals. The pilot study was conducted with six multiparous Finnish Ayrshire cows that weighed (mean ± SD) 711 kg ± 35.3 kg, were of parity 3.0 ± 0.63, were 181 ± 32.5 days in milk, and were producing 36.0 kg/d ± 4.77 kg/d of milk pre-trial. All cows were fed a control diet for 3 weeks (period 1), followed by a lipid-rich test diet for another 3 weeks (period 2). The dietary shift was made gradually over 5 days. The dairy cow partial mixed rations (PMRs) were based on grass silage (Table 1). The prewilted grass silage was prepared from a first cut of mixed timothy (*Phleum pratense*) and meadow fescue (*Festuca pratensis*) sward, which was ensiled with a formic acid-based additive (4–6 L/t feed; AIV®2 Plus Na; Taminco Finland Ltd, Eastman Chemical Company, Oulu, Finland) in big bales. The concentrates in the PMRs comprised home-grown cereals, rapeseed feeds as a protein supplement, molassed sugar-beet pulp (Opti Leike mure; Lantmännen Feed Ltd, Turku, Finland), and vitamins and minerals (Seleeni-E-Melli TMR; Lantmännen Feed Ltd). The rapeseed protein was isonitrogenously supplied either as a lipid-extracted meal (control diet; Farmarin Rypsi Mixer; Hankkija Ltd, Hyvinkää Suomi) or as domestic full-fat seeds (Hauhon Myllärit Ltd, Hauho, Finland), and was milled using a sieve pore size of 6–8 mm (test diet). The cereal in the control diet was barley (*Hordeum vulgare*), and in the test diet, oats (Table 1). The amount of additional plant lipids in the test diet from rapeseeds and oats was adjusted to ca. 55 g/kg diet dry matter (DM). The PMRs were distributed three times per day at 09:00, 15:00, and 20:00, fed freely,

TABLE 1 The ingredients of the partially mixed rations.

	Pilot study		Main study	
Ingredient (g/kg dry matter)	Control diet	Test diet	Control diet	Test diet
Grass silage	600	600	600	600
Barley	194	–	189	–
Oats	–	138	–	136
Rapeseed meal	122	18	120	34
Milled rapeseeds	–	160	–	139
Molassed sugar-beet pulp	69	69	70	70
Minerals and vitamins	15	15	17	17
Propylene glycol	–	–	4	4

and supplemented with 3 kg/d of commercial concentrate (Maituri 10 000; Lantmännen Feed Ltd) at milking times. The main ingredients of the commercial concentrate were rapeseed meal, wheat, barley, molassed sugar-beet pulp, sugar-beet molasses, faba beans, and protected fat. The cows had free access to drinking water.

The cows were kept in tie-stalls equipped with PMR feeding troughs (Insentec RIC, Marknesse, the Netherlands) that registered intakes. They were milked twice a day (Delpro; DeLaval, Tumba, Sweden) starting at 06:00 and 17:00. The samples of feed and feces were collected during the last week of both periods. The fecal spot (1-L) sample was taken from the rectum during five consecutive milkings, starting on the morning milking of day 17. Furthermore, all milk from the cows was collected over these milkings to produce around 350 L of control and modified milk. This milk was analyzed for major constituents (using a 15- to 20-ml sample preserved with Bronopol; lactose, crude fat, crude protein, urea), as well as FAs (using an unpreserved milk sample of 100 ml). In addition, ultra-high temperature (UHT) processed milk, cheese, and butter were prepared from raw milk for sensory analyses. After adjusting the milk fat content to 1.5%, UHT milk was produced at Valio R&D (Helsinki, Finland) by heating the milk to 150°C for 3 s. The butter and semi-hard Dutch-type cheese were produced at Häme University of Applied Sciences' pilot dairy plant (Hämeenlinna, Finland). To produce butter, the cream was pasteurized and churned in two phases at 10°C. Salt was added to achieve a salt content of 1.4%. The test diet butter required a longer churning time than the control diet butter (120 min vs. 240 min). To produce semi-hard Dutch-type cheese, raw milk was standardized to a fat-to-protein ratio of 0.8 and pasteurized (for a minimum of 72°C for 15 s). The DVS CHN-019 starter culture (Chr. Hansen, Hørsholm, Denmark) and the CHY-MAX E (Chr. Hansen) rennet were added. The cheese loaves were ripened for 7 weeks at 11°C. The dry matter and fat contents for the control diet cheese were 53.6% and 19.0%, respectively, and for the test diet cheese 54.0% and 20.0%, respectively. In addition, the samples of milk from individual cows were taken every third day at the morning and evening milking starting from the dietary change. The samples were composited according to milk yield by cow and by day and analyzed in a similar way to the tank milk for the major constituents and FAs.

2.2 Main dairy cow study

The whole Finnish Ayrshire research herd in milk (n = 49–59) was used in a switch-back-designed study. The cows were fed a control diet for 3 weeks (period 1) followed by the lipid-rich test diet for 4 weeks (period 2). After this, all cows were switched back to the control diet (3 weeks; period 3). The dietary shifts were made gradually over 5 days. The last week of all periods was the sampling week. The cows were housed in an insulated free-stall barn equipped with a milking robot (Lely Astronaut A3; Lely, Maassluis, the Netherlands). The dairy herd was predominantly autumn calving and the number of cows in milk was 49, 52, 50, and 59 at the beginning of the experiment and during the sampling weeks of periods 1, 2, and 3, respectively. The number of days in milk was, on average, 176, 153, 141, and 117 at the beginning of the experiment and in the sampling weeks of periods 1, 2, and 3, respectively.

The dietary ingredients were the same as in the pilot study. However, based on the observations of the pilot study, the amount of supplemental lipids in the test diet was adjusted from 55 to 50 g/kg DM in order to promote feed intake, and, in turn, higher milk production while on the test diet. The adjustment was carried out by reducing the proportion of milled rapeseed and, correspondingly, increasing that of rapeseed meal in the test diet PMR (Table 1). In addition, propylene glycol was added to the PMR concentrate mixture to prevent concentrate dusting. The chemical composition of the PMR concentrate ingredients is presented in Table 2. The animals had free access to PMRs that were distributed four times per day at 08:00, 12:00, 18:00, and 22:00. When visiting the milking robot, the cows producing less than 30 kg of milk per day, between 30 kg of milk per day and 40 kg of milk per day and over 40 kg of milk per day at the beginning of the trial received 3 kg/d, 4 kg/d or 5 kg/d of commercial concentrate (Maituri 10000, Lantmännen Feed Ltd), respectively, throughout the experiment.

TABLE 2 The chemical composition of the partial mixed rations (PMRs) concentrate ingredients in the main study.

	Barley	Oats	Rapeseed meal	Milled rapeseeds	Molassed sugar-beet pulp
Dry matter (g/kg)	880	874	880	909	880
In dry matter (g/kg)					
Ash	25.3	39.7	77.0	45.2	39.8
Crude protein	117	158	389	242	106
Starch	607	351	4.60	6.10	229
Neutral detergent fiber	173	317	248	149	338
Total fat	25.2	43.4	43.2	434	38.3
ME (MJ/kg dry matter[1])	13.2	11.5	11.4	19.0	12.2
Fatty acids (FA) (g/100 g FA)					
16:0	22.0	17.9	8.27	4.76	13.4
18:0	1.70	2.09	2.14	1.85	1.50
cis-9 18:1	11.8	34.0	46.1	52.6	38.2
cis-9,*cis*-12 18:2	53.9	39.7	24.6	21.6	35.9
cis-9,*cis*-12,*cis*-15 18:3	6.98	2.50	6.82	12.0	6.22
cis-13 22:1	0.16	0.24	0.30	0.02	0.12
Saturated FAs	25.0	21.3	12.7	8.07	16.1
Monounsaturated FAs	14.0	36.5	55.9	58.2	41.7
Polyunsaturated FAs	61.0	42.3	31.5	33.7	42.2
Total FAs (g/kg dry matter)	10.2	27.5	24.3	373	28.5

[1]Metabolizable energy (ME) calculated according to Luke (2023).

The main ingredients of the commercial concentrate were rapeseed meal, maize, barley, sugar-beet molasses, molassed sugar-beet pulp, and protected fat.

The PMR feeding troughs (Insentec RIC) registered intakes automatically and individually. The milk yield, body weight, and commercial concentrate distribution were individually registered by the milking robot throughout the experiment. The experimental feeds were sampled once a day during the sampling week (n = 7), composited by periods, and stored frozen at −20°C until analysis.

The milk and feces were sampled from 13 multiparous dairy cows that weighed (mean ± SD) 678 kg ± 62.4 kg, were of parity 2.9 ± 1.75, and were producing 32.1 kg/d ± 7.5 kg/d of milk pre-trial. Of these cows, 10 were in late lactation (number of days in milk ranging from 153 to 308 at the beginning of the experiment) and three were in early lactation (number of days in milk ranging from 13 to 27 at the beginning of the experiment). The milk was individually sampled on day 15 of period 1 onwards, via the Lely Shuttle, from the first milking every third day at 09:00 onwards. The milk preserved with Bronopol (15 ml–20 ml) was analyzed for lactose, crude fat, crude protein, and urea, and unpreserved milk (10 ml) for FAs. The milk FA samples were stored frozen at −20°C prior to analysis. In addition, the tank milk was sampled every second day at 09:00 and analyzed for lactose, crude fat, crude protein, and urea throughout the study. The spot fecal samples (1 L) from the rectum were taken every day during the sampling week (n = 7) at 09:00 onwards, composited by cow and period, and frozen at −20°C before the analysis.

All cows freely visited the milking robot equipped with the GreenFeed system (C-Lock Inc., Rapid City, SD, USA) that measures gas exchange (Huhtanen et al., 2015). Automatic gas calibrations using a mixture of nitrogen (N_2) and oxygen (O_2), and a mixture of CH_4, O_2, hydrogen (H_2), and carbon dioxide (CO_2) were performed daily. The CO_2 recovery tests were conducted at the beginning of the experiment and every sampling week. Only the records of cows (n = 23) that were in milk during all three experimental periods and, on average, had 10 or more accepted readings from the GreenFeed system (more than 2 min of uninterrupted gas recordings during a visit) in the last week of each experimental period were used in the statistical analysis. These cows weighed 646 kg ± 72.8 kg, were of parity 2.3 ± 1.70, and produced 30.5 kg± 7.74 kg of milk per day pre-trial. Eight cows were in late lactation (number of days in milk ranging from 155 to 332 at the beginning of the experiment) and 15 were in early lactation (number of days in milk ranging from 0 to 36 at the beginning of the experiment). The energy-corrected milk for the gas intensity data was calculated from the tank milk composition of the sampling week and individual milk yields.

2.3 Sample analysis

The primary DM content of feeds and feces was determined by oven drying at 103°C for 20–24 h. The silage DM content was corrected for volatile losses by Huida et al. (1986). The chemical composition of feeds and feces was analyzed by standard procedures. Prior to the analysis, the dried feed (50°C for 48 h) and fecal (70°C for 48 h) samples were ground to pass through a 1-mm sieve. The ash was determined by ashing at 600°C for 20–24 h (Heraeus Thermicon T; Heraeus, Hanau, Germany). The neutral detergent fiber (NDF) was determined using sodium sulfite (Van Soest et al., 1991) and α-amylase (only concentrates) with an automatic FiberTherm FT12 analyzer (Gerhardt, Königswinter, Germany). The NDF content is reported on an ash-free basis. The crude protein was analyzed, as described by Pitkänen et al. (2023), using undried material for feces. For the analysis of total fat, the samples were hydrolyzed with 800 mL of HCl (4 mol/L) (SoxCap 2047 hydrolysis unit; FOSS Analytical, Hillerød, Denmark) following an extraction with 90 mL of petroleum ether (FOSS Soxtec 8000 extraction unit; FOSS Analytical, Hillerød, Denmark). The starch content was measured by using the amyloglucosidase and α-amylase method with a K-TSTA kit (Megazyme Co., Wickslow, Ireland) and a spectrophotometer (Shimadzu UV-VIS mini1240; Shimadzu Europa GmbH, Duisburg, Germany), according to the manufacturer's instructions (Pitkänen et al., 2023). The silage fermentation quality was determined from undried samples, as described by Pitkänen et al. (2023). The FA analysis of feeds and milk is described in detail by Lamminen et al. (2019). In brief, the lipids in feeds were extracted with a mixture of hexane and isopropanol (3: 2, vol: vol), and the lipids in milk with a mixture of ammonia, ethanol, diethyl ether, and hexane (0.2: 1.0: 2.5: 2.5, vol: vol). The fatty acid methyl esters were prepared and analyzed using a gas chromatograph (GC2010 Plus; Shimadzu, Kyoto, Japan) equipped with a 100-m fused silica capillary column (CP-SIL 88, Agilent J&W, Santa Clara, CA, USA). The milk lactose, crude fat, crude protein, and urea contents were determined by mid-infrared analysis in a commercial laboratory (MilkoScan FT+, Foss Electric A/S, Hillerød, Denmark; Valio Ltd, Seinäjoki, Finland). A trained sensory panel (n = 10) was used to evaluate the test and control UHT milks. Overall liking was rated, and the sensory profile of the milks was studied using the Check-All-That-Apply (CATA) method. Regarding the test and control butter and cheese, both the concept and sensory properties were evaluated by the respondents (n = 151), who were at least monthly users of butter and at least weekly users of cheese. Interest toward the concept, overall liking, product attributes (CATA), preference (which butter/cheese would you prefer), and reasons for preference were studied.

2.4 Calculations and statistical analysis

Energy-corrected milk yield was corrected to an energy content of 3.14 MJ/kg (Luke, 2023). The metabolizable energy (ME), metabolizable protein (MP), and protein balance in the rumen (PBV) were calculated according to the Finnish feed evaluation system (Luke, 2023). The apparent digestibility of nutrients was calculated using acid-insoluble ash as an internal marker in feeds and feces (Van Keulen and Young, 1977).

The data were analyzed using PROC MIXED of the Statistical Analysis System (SAS version 9.4, 2012). In the pilot dairy cow study, the data on nutrient intake and digestibility were analyzed with pairwise t-tests (PDIFF option), with a statistical model containing diet as a fixed effect and cow as a random effect. The time series data on lactational performance and milk composition were analyzed by ANOVA for repeated measures using polynomial contrast (linear, quadratic, cubic), and a model that had the sampling day as the fixed effect with a Satterthwaite correction. The AR(1) covariance structure was applied with a cow as the subject for repeated measures. In the main dairy cow study, only data obtained during sampling weeks were analyzed by ANOVA for linear and quadratic responses. The statistical model contained period as a fixed and cow as a random effect. The RedJade Sensory Software (RedJade Sensory Solutions LLC, Pleasant Hill, CA, USA) was used for the collection and analysis of the sensory data of the UHT milks evaluated by an expert panel. The Z test was used to analyze differences between the milks in overall liking and sensory profile. Data on the sensory evaluation of cheese and butter by a consumer panel was analyzed using Microsoft Excel® (version 2016; Microsoft Corporation, Redmond, WA, USA). The differences in overall liking and product preference were analyzed using the t-test, and the frequencies of the different product attributes and product preferences were calculated. The reasons to prefer a cheese/butter were asked with an open question. In all analyses, the results were considered statistically significant when the p-value was ≤ 0.05. The differences at a p-value > 0.05 to 0.10 were considered as a trend toward significance. The normality of the residuals was tested using a univariate procedure and the Shapiro–Wilk test. If the residuals were not normally distributed, the variables were transformed (log, square, inverse) to obtain a normal distribution of the residuals.

3 Results

3.1 Feed and diet composition

The chemical composition of the experimental feeds is presented in Table 3. The grass silages were of high (main study) or moderate (pilot study) nutritive value in terms of their digestible organic matter contents, which is typical of the early to normal growth stage for silage making. In the main study, the grass silage was restrictively fermented, as indicated by the low levels of fermentation acids and high levels of residual sugars. In the pilot study, the grass silage was more extensively fermented. The forage-to-concentrate ratio of the diets consumed averaged 54: 46 and 51:49 on a DM basis for the pilot and the main dairy cow studies, respectively. The experimental PMR concentrates were isonitrogenous for rapeseed protein, but in the control diet, the concentrate contained more starch and less total fat than that of the test diet. Furthermore, milled rapeseeds contained 10 times more

TABLE 3 The chemical composition of the experimental feeds in the pilot study and in the main study.

	Pilot study				Main study			
	Grass silage[1]	Control diet PMR concentrate	Test diet PMR concentrate	Concentrate at milkings	Grass silage[2]	Control diet PMR concentrate	Test diet PMR concentrate	Concentrate at milking robot
Dry matter (g/kg)	253	863	892	862	411	875	889	878
In dry matter (g/kg)								
Ash	78.7	52.3	76.4	66.9	73.9	93.1	83.0	73.7
Crude protein	146	206	175	191	136	201	195	203
Metabolizable protein[3]	81.2	116	104	119	82.2	93.0	89.3	119
Protein balance in the rumen[3]	24.8	28.2	26.5	38.0	12.9	64.1	63.3	38.0
Starch	–	293	151	283	2.50	328	162	298
Neutral detergent fiber	552	236	248	236	508	219	258	203
Total fat	28.1	35.7	186	42.2	24.9	25.4	170	31.3
Digestible organic matter	675				696			
Metabolizable energy (MJ/kg dry matter[3])	10.8	11.8	14.5	12.8	11.1	12.0	13.8	12.8
Fatty acid (FA) composition (g/100 g FA)								
16:0	17.4	12.3	5.61	33.6	17.9	14.7	5.90	42.3
18:0	1.25	1.68	1.80	2.43	1.60	2.09	1.87	2.36
cis-9 18:1	3.26	37.5	53.6	32.4	3.22	34.5	51.0	23.4
cis-9,*cis*-12 18:2	18.2	33.4	21.9	23.7	29.5	35.6	23.1	24.2
cis-9,*cis*-12,*cis*-15 18:3	54.5	7.18	10.5	3.27	55.0	5.72	11.2	2.91
cis-13 22:1	0.01	0.15	0.03	0.05	–	0.16	0.04	0.07
Saturated FAs	21.2	15.3	8.69	37.4	22.4	18.4	9.23	46.0
Monounsaturated FAs	5.93	44.1	58.9	35.6	6.12	39.3	56.5	26.9
Polyunsaturated FAs	72.8	40.6	32.5	27.0	71.4	41.4	34.3	27.1
Total FAs (g/kg dry matter)	16.5	15.0	179	36.8	14.4	17.4	167	27.8

[1]Fermentation characteristics: pH 4.32; in dry matter (g/kg) water-soluble carbohydrates 83.5, lactic acid 80.5, acetic acid 7.49, propionic acid < 0.01, and butyric acid < 0.01; and ammonium-N of total N (g/kg N) 74.9.
[2]Fermentation characteristics: pH 4.70; in dry matter (g/kg) water-soluble carbohydrates 165, lactic acid 2.48, acetic acid 1.49, propionic acid 1.88, butyric acid 0.09, and ethanol 9.99; and ammonium-N of total N (g/kg N) 31.0.
[3]Calculated according to Luke (2023).

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total fat than rapeseed meal (Table 2). The predominant FA in grass silage was *cis*-9,*cis*-12,*cis*-15 18:3 α-linolenic acid (ALA), whereas for the PMR concentrates *cis*-9,*cis*-12 18:2 linoleic acid (LA) and OA were the most abundant (Table 3). The FA composition of the PMR concentrate ingredients used in the main dairy cow study is presented in Table 2. Of the total FA in the milled rapeseeds, OA formed 53 g/100 g FA followed by 22 g/100 g FA of LA, and 12 g/100 g FA of ALA. The lipids in all the experimental rapeseed feeds were low in *cis*-13 22:1 erucic acid. The oats contained 1.7 times more total fat than barley. Compared with barley lipids, oat lipids contained more OA (12 g/100 g FA vs 34 g/100 g FA) and less LA (54 g/100 g FA vs 40 g/100 g FA).

3.2 Nutrient intake and digestibility

In the pilot dairy cow study, milled rapeseeds together with the oats tended to decrease DM intake by 3.3 kg/d ($p = 0.072$; Table 4) relative to the control diet, but on ME intake the decrease was only numerical ($p > 0.10$). The test diet increased the intake of total fat by 0.78 kg/d ($p < 0.001$). The intake of all FA, OA, LA, and ALA, in particular, was increased by the test diet ($p < 0.001$; Supplemental Table 1). Furthermore, the apparent total tract digestibility of all nutrients was lower for the test than the control diet ($p \leq 0.003$; Table 4; Supplemental Table 1).

In the main dairy cow study, milled rapeseeds together with oats decreased the DM intake by 0.9 kg/d ($p = 0.027$ for quadratic response; Table 5) relative to the control diet; the decrease originating mainly from the lower silage intake ($p = 0.009$). Furthermore, the test diet decreased crude protein and starch intake ($p \leq 0.003$), but increased that of energy-rich total fat by 0.98 kg/d ($p < 0.001$). Of the individual FAs, the consumption of OA, LA, and ALA in particular was increased by the test diet relative to the control ($p < 0.001$). Lipid supplementation had no effect on ME intake ($p > 0.10$). For both diets, the PBV was positive and was, on average, 34 g/kg diet DM. The apparent total tract digestibility of all nutrients was lower for the test than for the control diet ($p < 0.001$).

3.3 Milk production and composition

In the pilot dairy cow study, the milk yield tended to decrease cubically from 26.6 kg/d to 23.3 kg/d ($p = 0.053$) and the ECM yield decreased linearly from 27.6 kg/d to 23.9 kg/d ($p = 0.041$) after switching from the control to the test diet (Supplemental Table 2). However, dietary plant lipids had no effect on milk fat, lactose, protein, and urea concentrations ($p > 0.10$).

TABLE 4 Nutrient intake, apparent total tract digestibility coefficients, and the composition of tank milk used for processing dairy products, and the dairy product sensory quality in the pilot study.

	Control diet	Test diet	SEM	Significance
Intake[1] (kg/d)				
Dry matter	21.9	18.6	1.18	0.072
Starch	2.70	1.93	0.109	< 0.001
Neutral detergent fiber	9.00	7.43	0.539	0.068
Total fat	0.73	1.51	0.073	< 0.001
ME-corrected intake[2] (MJ/d)	233	219	11.7	0.411
Digestibility coefficients[1]				
Organic matter	0.744	0.647	0.0040	< 0.001
Neutral detergent fiber	0.613	0.478	0.0065	< 0.001
Total fat	0.763	0.612	0.0275	0.003
Tank milk composition				
Lactose (g/kg)	41.6	42.3		
Protein (g/kg)	35.7	35.6		
Fat (g/kg)	42.9	43.1		
Fatty acid (FA)[1] (g/100 g FA)				
4- to 14-carbon FAs	29.1	18.7		
16:0	31.4	19.4		
18:0	8.95	17.4		
cis-9 18:1	18.1	31.2		

(Continued)

TABLE 4 Continued

	Control diet	Test diet	SEM	Significance
Saturated FAs	71.8	57.8		
Monounsaturated FAs	24.6	38.9		
Polyunsaturated FAs	2.81	2.43		
Trans FAs	3.55	5.33		
Ultra-high temperature-processed milk				
Overall rating, average[3]	2.9	2.6	0.11	0.081
Attributes	Neutral, musty, old	Old, grainy flavor, pea flavor		
Butter				
Overall rating, average[4]	4.9	5.1	0.19	0.219
Attributes	Difficult to spread, natural taste, hard	Natural taste, low salt, yellow color		
Preference[5]	47%	53%		
Reasons to prefer the product	Better taste, natural taste, optimal saltiness	Better taste, natural taste, better spreadability		
Cheese				
Overall rating, average[4]	4.7	4.7	0.21	0.826
Attributes	Soft, aromatic, full taste	Soft, tasty, optimal saltiness		
Preference[5]	51%	49%		
Reasons to prefer the product	Better taste, stronger taste, better structure	Better taste, better structure, softer		

[1]Nutrient intake, digestibility coefficients, and milk fatty acids are presented in more detail in Supplemental Table 1.
[2]Metabolizable energy intake corrected for the associative effects according to Luke (2023).
[3]Scale 1–4 (1—I do not like it at all; 4,—I like it very much).
[4]Scale 1–7 (1—I do not like it at all; 7—I like it very much).
[5]Percentage of respondents who preferred the product.

In the main dairy cow study, milled rapeseeds together with oats did not affect the yields of milk, ECM, lactose, fat, or protein ($p > 0.10$ for quadratic response; Table 6). However, the yields of milk, ECM, lactose, and protein decreased linearly ($p \leq 0.025$) during the experiment. Dietary plant lipids had no effect on milk fat or lactose concentration ($p > 0.10$), but there was a subtle quadratic increase ($p = 0.010$) in the milk protein concentration, with a concomitant linear decrease ($p = 0.028$) in the milk urea concentration as the experiment progressed.

The milk FA composition was similarly modified by the test diet in both experiments (Table 6; Supplemental Table 2), with the large changes reaching a plateau within 10 days after the dietary changes (Figures 1, 2; Supplemental Table 2). The milled rapeseeds together with oats decreased the total SFA in milk fat by 11.7% to 14.2%-units ($p < 0.001$ for quadratic response; Table 6; Supplemental Table 2) relative to the control diet. The milk fat concentration of all SFAs of 6- to 16-carbon, 16:0 palmitic acid (PA), in particular, was decreased ($p \leq 0.005$) by the test diet. By contrast, it almost doubled the milk fat OA and SA concentrations ($p < 0.001$) relative to the control diet. Furthermore, it resulted in minor decreases in milk fat LA and ALA ($p < 0.001$) concentrations. The test diet increased milk fat concentration of total *trans* FAs ($p < 0.001$), of which *trans*-11 18:1 predominated. However, the increases in milk fat *trans* FAs were rather limited in magnitude.

3.4 Sensory quality of dairy products

No significant differences in sensory characteristics were seen in the UHT milk, butter, and cheese produced from the test milk and the control milk ($p > 0.05$; Table 4). In overall ratings, the new products with less saturated fat got very similar ratings to the control products. There was also no major difference in which products, the test or the control, were preferred. The test butter was considered softer and easier to spread, and more than 40% of respondents found nothing to improve in the butter. The saltiness of the test cheese was better and the taste milder than in the control cheese. Both cheeses were soft, and a slightly harder construction had been hoped for. Regarding the concept of reduced saturated fat dairy products, over half of the respondents considered a good FA composition important. Based on the product description, 70% of respondents would probably buy milk products with a modified FA composition.

3.5 Gas exchange

The dairy cow gas exchange is presented in Table 7. The milled rapeseeds together with the oats decreased ruminal CH_4 and H_2 total emissions (g/d and mg/d, respectively), yields (g/kg DM intake

TABLE 5 Nutrient intake and apparent total tract digestibility coefficients in the main study.

	Diet			Mean response to the test diet[1]	SEM	Significance	
	Control Period 1	Test Period 2	Control Period 3			Linear	Quadratic
Intake, (kg/d)							
Dry matter	21.9	21.2	22.2	−0.9	0.70	0.582	0.027
Silage	11.1	10.6	11.4	−0.7	0.36	0.468	0.009
Organic matter	20.2	19.5	20.4	−0.80	0.64	0.671	0.045
Crude protein	3.80	3.50	3.65	−0.23	0.119	0.034	0.003
MP[2]	2.00	1.92	2.03	−0.10	0.064	0.674	0.008
PBV[2]	0.75	0.72	0.76	−0.04	0.024	0.622	0.013
Starch	3.47	2.21	3.50	−1.28	0.108	0.205	< 0.001
Neutral detergent fiber	8.02	7.81	8.15	−0.28	0.256	0.576	0.073
Total fat	0.56	1.56	0.60	0.98	0.031	< 0.001	< 0.001
ME-corrected intake[3] (MJ/d)	239	240	242	−1	7.2	0.569	0.863
Fatty acid intake (g/d)	380	1,280	409	885.5	24.5	< 0.001	< 0.001
16:0	72.0	125	115	31.5	4.06	< 0.001	< 0.001
18:0	5.05	5.22	4.87	0.26	0.186	0.104	0.003
cis-9 18:1[4]	69.4	558	70.0	488.3			
	(1.84)	(2.74)	(1.84)		0.015	< 0.001	< 0.001
cis-9,*cis*-12 18:2	98.7	290	94.0	193.7	5.60	0.015	< 0.001
cis-9,*cis*-12,*cis*-15 18:3	105	197	99.1	95.0	4.37	0.419	< 0.001
cis-13 22:1	0.23	0.49	0.32	0.22	0.011	< 0.001	< 0.001
Saturated FAs	88.7	153	130	43.7	4.65	< 0.001	< 0.001
Monounsaturated FAs[4]	89.2	641	87.4	552.7			
	(1.95)	(2.80)	(1.94)		0.015	< 0.001	< 0.001
Polyunsaturated FAs	204	488	193	290	9.9	0.063	<0.001
Digestibility coefficients							
Dry matter	0.709	0.608	0.675	−0.084	0.0063	< 0.001	< 0.001
Organic matter	0.721	0.623	0.688	−0.081	0.0065	< 0.001	< 0.001
Crude protein	0.700	0.633	0.655	−0.045	0.0080	< 0.001	< 0.001
Neutral detergent fiber	0.523	0.387	0.463	−0.106	0.0134	< 0.001	< 0.001
Total fat[5]	0.590	0.484	0.631	−0.127			
	(0.348)	(0.247)	(0.399)		0.0180	0.121	< 0.001

[1]Calculated as: test diet; period 2–(control diet; period 1 + control diet; period 3)/2.
[2]Metabolizable protein (MP) and protein balance in the rumen (PBV) were calculated according to Luke (2023).
[3]Metabolizable energy intake corrected for associative effects according to Luke (2023).
[4]Log_{10} conversion is given in parentheses below to obtain normality.
[5]Square conversion is given in parentheses below to obtain normality.

or mg/kg DM intake), and intensities (g/kg ECM or mg/kg ECM; $p < 0.001$ for the quadratic response). Depending on the emission unit, the decrease was 16%–20% for CH_4 and 36%–39% for H_2. However, the effect of plant lipids on CO_2 emissions (decrease of 3%–5%; $p \leq 0.084$) was limited in magnitude. The plant lipids had no major effect on the O_2 consumption of dairy cows ($p > 0.10$).

4 Discussion

The novel feature of this experiment was in assessing the feasibility of simultaneously decreasing both bovine milk fat SFAs and ruminal methane emissions when milled rapeseeds and oats instead of rapeseed meal and barley are fed to animals in practical

TABLE 6 Milk yield and milk composition in the main study.

	Diet			Mean response to the test diet[1]		Significance	
	Control Period 1	Test Period 2	Control Period 3		SEM	Linear	Quadratic
Yield (kg/d)							
Milk	31.4	29.7	27.6	0.2	2.66	< 0.001	0.638
Energy-corrected milk	32.4	30.8	29.0	0.1	2.61	0.025	0.796
Lactose	1.41	1.34	1.23	0.02	0.133	< 0.001	0.418
Fat	1.32	1.27	1.18	0.02	0.113	0.123	0.727
Protein	1.13	1.05	1.03	−0.03	0.083	0.007	0.488
Concentration in milk							
Lactose (g/kg)	44.5	44.7	44.1	0.4	0.60	0.216	0.156
Fat (g/kg)	42.8	43.3	43.6	0.1	2.27	0.726	0.996
Protein (g/kg)	36.4	36.3	38.1	−1.0	1.03	0.002	0.010
Urea (mg/dL)	27.3	27.0	24.9	0.9	1.37	0.028	0.254
Concentration in milk fat [g/100 g fatty acids (FAs)]							
4:0	3.32	3.06	3.12	−0.16	0.085	0.002	0.030
6:0	2.15	1.64	2.03	−0.45	0.047	< 0.001	< 0.001
8:0	1.36	0.91	1.26	−0.40	0.035	< 0.001	< 0.001
10:0	3.25	1.88	2.95	−1.22	0.101	< 0.001	< 0.001
12:0	3.95	2.19	3.63	−1.60	0.131	< 0.001	< 0.001
14:0	12.5	8.91	12.0	−3.34	0.250	0.004	< 0.001
16:0	32.3	21.5	33.1	−11.2	0.48	0.609	< 0.001
18:0	8.55	16.2	8.26	7.80	0.379	0.364	< 0.001
cis-9 18:1	18.0	29.6	18.4	11.4	0.60	0.013	< 0.001
trans-10 18:1[2]	0.14	0.32	0.15	0.18			
	(7.19)	(3.29)	(6.91)		0.209	0.004	< 0.001
trans-11 18:1[2]	0.88	0.92	0.89	0.04			
	(1.18)	(1.11)	(1.78)		0.062	0.950	0.330
cis-9,*cis*-12 18:2	1.29	1.05	1.26	−0.23	0.044	0.187	< 0.001
cis-9,*trans*-11 18:2	0.47	0.50	0.49	0.02	0.035	0.503	0.561
cis-9,*cis*-12,*cis*-15 18:3	0.41	0.35	0.37	−0.04	0.015	0.006	< 0.001
cis-13 22:1	0.007	0.006	0.008	-0.002	0.0005	0.017	< 0.001
Saturated FAs	72.3	60.1	71.2	−11.7	0.69	0.002	< 0.001
Monounsaturated FAs	24.4	36.9	25.4	12.0	0.64	< 0.001	< 0.001
Polyunsaturated FAs	2.69	2.26	2.66	−0.42	0.077	0.351	< 0.001
Trans FAs[2]	3.51	4.53	3.61	1.15			
	(0.32)	(0.23)	(0.28)		0.010	0.004	< 0.001

[1]Calculated as: test diet; period 2–(control diet; period 1 + control diet; period 3)/2.
[2]Inverse conversion is given in parentheses below to obtain normality.

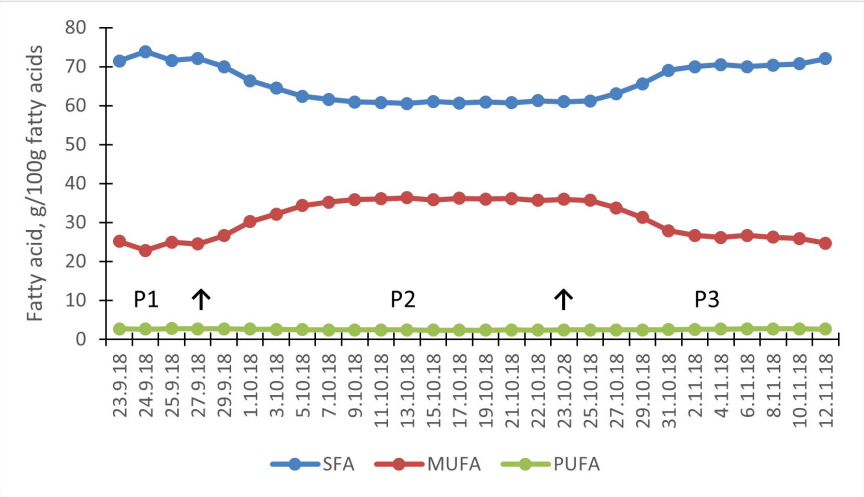

FIGURE 1
Effect of milled rapeseeds and oats on tank milk saturated fatty acid (SFA), monounsaturated fatty acid (MUFA), and polyunsaturated fatty acid (PUFA) concentrations in the main dairy cow study. Arrows represent the dietary change of the whole herd from control diet in period 1 (P1) to lipid supplemented diet in period 2 (P2), and back to control diet for period 3 (P3).

whole-herd conditions. In addition, the milk was processed into several dairy products (e.g., UHT milk, butter, and cheese), of which the sensory quality was evaluated to confirm the applicability up to the final products.

Given the limited number of animals, the results on feed intake and milk yields obtained in the pilot study should be interpreted with some caution. However, it is noteworthy that the results were highly consistent between the pilot and the main dairy cow studies, except for variations in animal performance. Nevertheless, at high lipid inclusion rates, a significant decrease in feed intake and milk yield, as observed in the pilot study, is expected when a situation-specific threshold in lipid supply is surpassed (Drackley et al., 2007; Benchaar et al., 2015; Halmemies-Beauchet-Filleau et al., 2017). This is discussed in more detail later below.

4.1 Feed and diet composition

The main forage component of the diet affects bovine milk FAs (Glasser et al., 2008) and CH_4 response to plant lipids (Vanhatalo and Halmemies-Beauchet-Filleau, 2020). Our experimental diets were based on digestible grass silage that is typical in northern latitudes. The grass silage-rich diets together with using oats instead

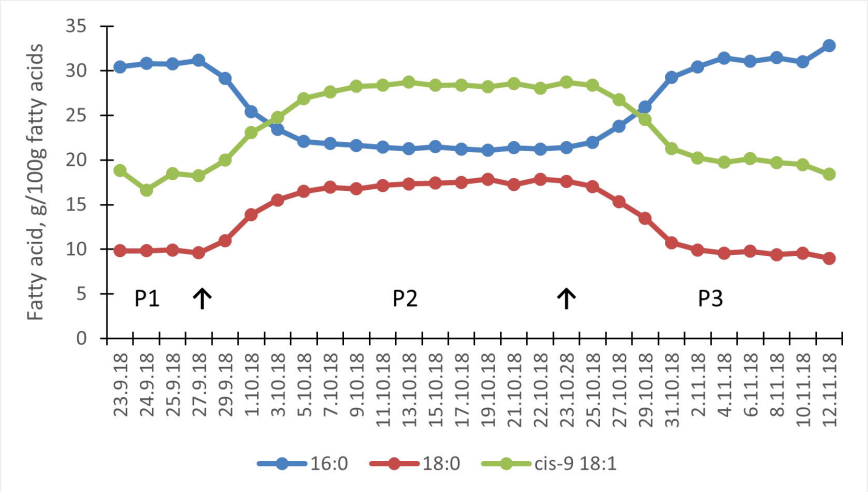

FIGURE 2
Effect of milled rapeseeds and oats on tank milk palmitic acid (PA, 16:0), stearic acid (SA, 18:0), and oleic acid (OA, cis-9 18:1) concentrations in the main dairy cow study. Arrows represent the dietary change of the whole herd from control diet in period 1 (P1) to lipid supplemented diet in period 2 (P2), and back to control diet for period 3 (P3).

TABLE 7 Gas production or consumption (mass per day), gas yield (mass per dry matter intake), and gas intensity (mass per energy-corrected milk production) in the main study.

	Diet			Mean response to the test diet[1]		Significance	
	Control Period 1	Test Period 2	Control Period 3		SEM	Linear	Quadratic
Methane							
g/d	456	378	468	−84	16.9	0.783	< 0.001
g/kg DMI[2]	22.2	18.0	20.7	−3.5	0.66	0.007	< 0.001
g/kg ECM[3]	14.1	12.1	16.0	−3.0	1.09	0.050	< 0.001
Carbon dioxide							
g/d	12,447	11,870	12,575	−641	352.8	0.735	< 0.001
g/kg DMI[2]	605	564	560	−19	13.7	< 0.001	0.084
g/kg ECM[3,4]	376	377	418	−20			
	(2.56)	(2.56)	(2.60)		0.027	0.005	0.035
Hydrogen							
mg/d	653	399	598	−227	49.6	0.157	< 0.001
mg/kg DMI[2]	31.7	18.6	26.7	−10.6	2.23	0.018	< 0.001
mg/kg ECM[3,4]	19.4	12.1	20.3	−7.8			
	(1.25)	(1.05)	(1.23)		0.044	0.318	< 0.001
Oxygen							
g/d	10,592	10,271	10,141	−96	270.0	0.003	0.606
g/kg DMI[2]	516	488	452	4	10.7	< 0.001	0.268
g/kg ECM[3,5]	320	325	337	−4			
	(0.003)	(0.003)	(0.003)		0.0002	0.324	0.778

[1]Calculated as: test diet; period 2–(control diet; period 1 + control diet; period 3)/2.
[2]Dry matter intake.
[3]Energy-corrected milk (ECM) calculated using tank milk composition determined every second day.
[4]Log_{10} conversion is given in parentheses below to obtain normality.
[5]Inverse conversion is given in parentheses below to obtain normality.

of barley as the cereal for the test diet PMR led to moderate starch contents for the control (123–158 g/kg DM) and test diets (104 g/kg DM). The lipid content and the FA composition of milled rapeseeds, oats, and barley were similar to previous reports (Welch, 1975; Brask et al., 2013b), with OA forming a major part of the lipid for both milled rapeseeds (53 g/100 g FA) and oats (34 g/100 g FA) in the present work.

4.2 Nutrient intake and digestibility

The milled rapeseeds together with oats decreased DM intake by 15% relative to the control diet in the pilot study. The fluctuation in the daily feed intake indicated a slight excess in lipid supplementation for efficient rumen function. This was reflected also in the standard error of the mean (SEM), which was moderately high for feed intake. To maintain a higher and more regular feed intake, the lipid supplementation rate was decreased from 55 g/kg to 50 g/kg test diet DM for the main study. The lower rate was successful as DM intake was only decreased by 4% relative to the control diet in the main study. At high inclusion rates (i.e., at 40 g/kg or more in DM), lipid supplementation has often suppressed feed intake (Huhtanen et al., 2008; Bayat et al., 2018; Ramin et al., 2021a), with the decrease being generally more pronounced on starch-rich diets (Benchaar et al., 2015; Vanhatalo and Halmemies-Beauchet-Filleau, 2020). The rather low dietary starch content together with the non-excessive lipid inclusion rate probably explains the limited reduction in the feed intake in the main study. In addition to the decreased DM intake, using oats that contain less starch than barley in the test diet relative to the control diet contributed to 0.77 kg and 1. 28 kg lower daily starch intake by the test diet cows in the pilot and main dairy cow studies, respectively. However, the lower starch intake on the test diet was compensated by energy-rich lipids leading to similar ME intakes between diets in both studies.

The milled rapeseeds together with oats substantially increased OA, LA, and ALA intakes relative to the control diet, thus reflecting the FA content and composition of the dietary feed ingredients. The vast majority of the supplemental OA was derived from milled rapeseeds and to a lesser extent from oats. The increase in OA intake was of similar magnitude to the previous studies using high rapeseed oil supplementation (Ferlay et al., 1993; Bayat et al., 2018). It is noteworthy that on the control diet ALA, inherent to the chloroplasts in forage leaves (Glasser et al., 2013), represented the major FA consumed by cows; therefore, highlighting the importance of the basal forage to FA intake. Though forages have rather low lipid concentrations, lipid intake from forage can be substantial because forage intake is typically high in ruminant diets (Glasser et al., 2013).

In the present study, supplying a 50–55 g/kg diet DM of lipids from milled rapeseeds and oats significantly suppressed organic matter and the fiber total tract digestibility. This may also explain, at least in part, the decrease in DM intake of the test diet relative to the control diet. Jenkins (1993) proposed various explanatory mechanisms for this, including the direct adverse effects of unsaturated FAs on ruminal microbial communities, cellulolytic microbes in particular, and free FAs forming a protective lipid layer over feed particles in the rumen. However, several reports have subsequently challenged these theories on fiber-rich diets based on grass or legume silage that indicate little if any effect of plant oils on fiber digestibility, even at rather high inclusion rates (Benchaar et al., 2015; Halmemies-Beauchet-Filleau et al., 2017; Bayat et al., 2018). In addition to lipid supplementation, switching cereal fiber quality from barley in the control diet to oats in the test diet may have contributed to a lowered fiber digestion of the test diet. Furthermore, replacing barley with oats has previously decreased NDF digestibility (Vanhatalo et al., 2006; Ramin et al., 2021b). This can be attributed to the significantly higher indigestible NDF content of oats relative to barley (Ramin et al., 2021b).

Once the seedcoat is ruptured, the lipid digestibility of whole rapeseeds is similar to pure oil (Brask et al., 2013b). The digestibility of lipids is often increased (Benchaar et al., 2015; Halmemies-Beauchet-Filleau et al., 2017) or unaffected (Ferlay et al., 1993; Brask et al., 2013a; Brask et al., 2013b) by plant lipid supplementation. In the present studies, however, the apparent digestibility of total fat was unexpectedly lower for the lipid-supplemented test diet than for the control diet. In part, this may be attributable to the reduced intestinal absorption of SA at high post-ruminal flows (Glasser et al., 2008). Indeed, the intake of 18-carbon dietary unsaturated FAs was many times higher on the test diet relative to the control diet, with SA being the end-product of their ruminal biohydrogenation (Shingfield et al., 2010). Though the milling of rapeseeds was assessed as being visually successful, it can also be speculated that some seeds may have escaped the milling through the 6- to 8-mm sieves intact.

4.3 Milk production and composition

The linear decrease in the ECM yield in the pilot study after dietary change from the control to the test diet is in line with the concomitant large reduction in feed intake, and a numerical 6% decrease in the ME intake. However, due to the experimental design, the effect of time and diet cannot be separated in the pilot study. Therefore, a part of the linear decline in animal performance can be attributed to the natural and gradual decline in the milk yield of late-lactation cows. However, in the main dairy cow study, the ECM yield was unaffected by dietary plant lipids, which is consistent with similar ME intakes across treatments due to a much more limited decrease in feed intake. Previously milled rapeseeds have neither affected the ECM yields when supplementing diets based on grass silage (Kairenius et al., 2009; Mierlita et al., 2023) nor a mixture of grass and maize silages (Brask et al., 2013b). In addition, replacing barley with oats has not affected the ECM (Ramin et al., 2021b) or slightly increased it (Vanhatalo et al., 2006). Similar to the pilot study, the linear decrease in the ECM, and protein and lactose yields during the main study can be attributed to the advances in the lactation stage of animals, as 10 out of 13 were in late lactation at the beginning of the experiment and thus on a descending lactation curve. It is worth noting, however, that the decline in milk yield was almost twice less rapid in the main study than in the pilot study between the periods. This confirms that the advance in the lactation stage was not the only cause of the decline in the milk yield in the pilot study. Overall, milled rapeseeds together with oats had negligible effects on the production of milk and the major constituents of milk in the main dairy cow study.

The milled rapeseeds together with oats significantly modified milk FA composition. Relative to the control diet, the total SFA content of milk fat on the test diet was 14.2%-units lower in the pilot study and 11.7%-units lower in the main study. Plant lipids decreased total SFA by, on average, 0.013%- to 0.015%-units per g of supplemental FA. This decrease was similar in extent to previous reports for milled rapeseeds (0.013%-units per g of supplemental FA; Collomb et al., 2004), and for pure rapeseed oil supplementation (0.015%- to 0.019%-units per g of supplemental FA; Bayat et al., 2018; Razzaghi et al., 2022) the decrease in SFA being generally lower on diets high in fiber and low in starch (Razzaghi et al., 2022; Mierlita et al., 2023). Moreover, the 6- to 16-carbon SFAs, derived entirely or in the case of PA 50%–80% from mammary *de novo* synthesis (Halmemies-Beauchet-Filleau et al., 2013), were consistently 22% to 48% lower in milk fat from the test diet than from the control diet. This is in good agreement with the increased supply of long-chain FAs known to inhibit mammary *de novo* synthesis of short- and medium-chain SFAs (Shingfield et al., 2010).

The total monounsaturated FA was 48%–59% higher in milk fat from the test diet than the control diet. This increase principally originated from OA (0.013%- to 0.015%-units per g of supplemental FA) which was the predominant FA in the dietary lipid sources rapeseeds and oats. The increase in milk fat OA was similar to previous studies with rapeseed oil (0.010%- to 0.016%-units per g of supplemental FA; Bayat et al., 2018; Razzaghi et al., 2022) or when replacing barley with oats (0.015%-units per g of supplemental FA; Fant et al., 2023). Milk fat OA has a dual origin. Part of it originates from direct mammary uptake, with circulating OA being derived predominantly from the diet (Shingfield et al., 2010) or during a negative energy balance also from adipose tissue mobilization

(Gross et al., 2011; Jorjong et al., 2014). Another part of milk fat OA originates from mammary desaturation of SA, which is the end-product of ruminal biohydrogenation of dietary18-carbon unsaturated FA (Shingfield et al., 2010). Therefore, the significant increase in OA, LA, and ALA intakes for the test diet is directly, and, via SA, also indirectly reflected in the milk OA in the present study. The increase in milk fat SA on the test diet is a typical response to plant lipid supplementation (Bayat et al., 2018; Razzaghi et al., 2022; Fant et al., 2023).

The present increases in milk *trans* FAs were limited for lipid in milled rapeseeds and oats (0.0011%- to 0.0014%-units per g of supplemental FA) compared with previous studies with pure rapeseed oil (0.0046%- to 0.0062%-units per g of supplemental FA; Bayat et al., 2018; Razzaghi et al., 2022). In addition, the major *trans* isomers increased in milk fat by the test diet were *trans*-11 18:1 vaccenic acid and *cis*-9,*trans*-11 18:2 rumenic acid, with potentially beneficial effects on human health (Field et al., 2009; Koba and Yanagita, 2014). The moderate increase in milk fat *trans* FAs was in line with a previous report indicating higher ruminal OA and lower *trans*-FA outflow when milled rapeseeds were used instead of pure rapeseed oil supplementation (Kairenius et al., 2009). This suggests partial protection from the ruptured rapeseed seedcoat against ruminal lipid metabolism.

Despite the higher LA and ALA intakes, their milk fat concentrations were slightly lower on the test diet relative to the control diet. This is consistent with more extensive biohydrogenation of LA and ALA relative to OA in the rumen (Shingfield et al., 2010) and the limited effects on milk LA and ALA, when these polyunsaturated FAs have been supplemented in the form of plant oils (Rego et al., 2009; Halmemies-Beauchet-Filleau et al., 2017). The increase in ALA intake through forage generally results in a higher transfer efficiency from the diet into milk (Kalač and Samková, 2010; Halmemies-Beauchet-Filleau et al., 2013), probably due to the fact that more microbial digestion of surrounding material is needed before forage lipids are exposed to ruminal metabolism. This is supported by the concomitant decrease in milk fat ALA content and grass silage consumption despite higher general ALA intake on the test diet in the present study.

4.4 Dairy products with modified FA composition

The sensory characteristics of the UHT milk, butter, and cheese containing less SFAs were similar to those of the control products and were preferred by an equal percentage of consumers as the control products. In general, the test diet butter and cheese were perceived to be of softer texture relative to the control products. Rapeseed lipid inclusion in the diet resulted in softer textures of dairy products, with acceptable organoleptic quality also previously (Ryhänen et al., 2005; Halmemies-Beauchet-Filleau et al., 2011). Furthermore, no change in the milk sensory quality was observed when oats replaced barley as a cereal in the dairy cow diet (Vanhatalo et al., 2006). The concept of reduced-saturated-fat dairy products was received positively by Finnish consumers, and respondents had a positive view of it. Most consumers considered the products suitable for themselves, and they would be ready to buy them if the product quality is the same as with current products. Some consumers did not entirely understand how the change in FA composition was achieved. This should be taken into account when communicating about these types of products. The consumers' level of acceptance and attitudes toward test butter with low levels of SFAs and a low carbon emission footprint has been reported in a separate paper (Asioli et al., 2023). This complementary study indicated that about one-third of Finnish consumers was willing to pay a premium price for the new type of butter, the consumer attitudes being most promising with young and highly educated consumers.

For a considerable time, many human dietary guidelines recommend that SFA intake should be restricted to reduce the risk of CVD. As dairy foods are often the single greatest dietary source of SFAs, there has been a considerable number of studies examining how dairy cow diets can be modified to reduce the SFA content of milk fat, mainly by replacing them with *cis*-monounsaturated FAs or ALA. There are, however, few detailed human randomized controlled trials (RCTs) examining the chronic impact of such changes on milk FAs on markers of CVD risk. The review of 10 published RCTs, by Livingstone et al. (2012), indicated a tendency toward a believed beneficial lowering effect on fasting serum total and low-density lipoprotein cholesterol (LDL-C) following chronic consumption of modified milk and dairy foods. The recent detailed RESET RCT (Vasilopoulou et al., 2020) used diets containing milk, cheese, and butter with normal (control) or modified FA composition (Kliem et al., 2019), which was similar to the test diet milk in the current dairy cow studies. The study found that in adults at a moderate CVD risk, the consumption of FA-modified dairy foods for 12 weeks significantly moderated the increase in the levels of serum LDL-C seen on the conventional dairy food diet and improved vascular endothelial function. This provides more confidence that milk FA modification, as in the current studies, can provide health benefits. There is, however, increasing uncertainly that the heavy reliance on serum LDL-C as the key risk factor for CVD is too simplistic, in part because it takes no account of the variation in risk linked to the LDL particle size profile (Givens, 2023).

4.5 Gas exchange

The effects of plant lipids on ruminal methanogenesis are dependent on the level of supplementation, the FA profile of the supplements, and the composition of the basal diet (Vanhatalo and Halmemies-Beauchet-Filleau, 2020). Lipids in milled rapeseeds and oats significantly decreased ruminal H_2 load in the main dairy cow study. In addition, CH_4 and H_2 intensities (g or mg gas/kg ECM) were 20% and 39% lower, respectively, on the test diet than on the control diet. For each 1% plant lipid added to the diet, CH_4 intensity was reduced by 4.6%. This agrees well with previous plant lipid data for rapeseed oil (a reduction of 4.5%–5.2% in CH_4 intensity for each

additional 1% in plant lipid; Bayat et al., 2018; Razzaghi et al., 2022) and replacing barley with oats (a reduction of 6.0% in CH_4 intensity for each additional 1% in plant lipid; Fant et al., 2021; Ramin et al., 2021b) on high-grass silage diets. However, it was less effective compared with milled rapeseeds in a diet based on a mixture of grass and maize silage (a reduction of 8.2% in CH_4 intensity for each additional 1% in plant lipid; Brask et al., 2013b).

The reduction of ruminal CH_4 and H_2 production in the present study can be attributed to the lower amount of organic matter fermented in the rumen, as indicated by the lower DM intake and nutrient whole-tract digestibility. Having more organic matter in the feces could be expected to increase CH_4 emissions from manure. However, Ramin et al. (2021a) reported similar total fecal CH_4 emissions *in vitro* (L/d) from feces of cows fed rapeseed lipids compared with unsupplemented ones, despite higher amounts of organic matter being left in the feces. This was due to a significantly lower CH_4 yield (L/kg fecal organic matter) from the feces of cows fed rapeseed lipids. Furthermore, it is possible that the ruminal biohydrogenation of dietary unsaturated FAs served as a minor alternative sink for metabolic H_2 to mitigate CH_4 formation (Beauchemin et al., 2022). Dietary lipid supplementation may also shift rumen fermentation patterns from acetate to propionate (Vanhatalo and Halmemies-Beauchet-Filleau, 2020). However, decreases in methane production due to rapeseed lipids have not been associated with shifts in ruminal fermentation patterns on grass silage-based diets (Brask et al., 2013a; Bayat et al., 2018).

About 155.2 million tons of bovine milk is produced in EU-27 (Eurostat, 2021). If all the dairy cows in EU-27 consumed a diet mitigating CH_4 emissions by 3 g of each kg of milk produced, which is comparable to the decrease observed in the present study, then annual CH_4 emissions would decrease by 465,600 t in the EU-27 area. This decrease would represent about 8.4% of the annual bovine CH_4 emission, 5.6% of the annual agricultural CH_4 emission, and 3.2% of the annual total CH_4 emission in EU-27 (Eurostat, 2021).

5 Conclusion

Replacing rapeseed meal and barley with full-fat milled rapeseed and oats in a whole-dairy-herd diet had no adverse effects on ME intake and milk production at a 50 g/kg lipid supplementation rate in the diet DM, but modified milk fat composition as OA inherent to lipid supplements replaced a substantial proportion of the SFAs in the milk fat. This decrease in milk fat SFAs can be attributed to the lower level of mammary *de novo* synthesis due to the increased supply of OA and its biohydrogenation end-product SA for milk fat synthesis. The dairy products (UHT milk, butter, cheese) with a modified lipid profile were of a similar organoleptic quality to the control products. Further research is needed to assess whether or not the changed milk FA profile has long-lasting health benefits when consumed by humans. The lipids in the milled rapeseeds and oats significantly decreased ruminal H_2 load and further CH_4 emissions, which is consistent with lower DM intake and nutrient digestibility. Therefore, milled rapeseeds and oats as regular dietary ingredients are an efficient means to soften milk fat and mitigate methane emissions at the whole-herd level.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material. Further inquiries can be directed to the corresponding author.

Ethics statement

Ethical approval was not required for the study involving animals in accordance with the local legislation and institutional requirements because no prior authorization is required for projects that are likely to cause a level of harm lower than that caused by the introduction of a needle. Therefore, milk and fecal sampling carried out in this project did not require ethical approval according to national regulations (https://avi.fi/en/services/individuals/licences-notices-and-applications/animals/laboratory-animals).

Author contributions

AH-B-F: Conceptualization, Supervision, Writing – review & editing, Writing – original draft. SJ: Conceptualization, Writing – review & editing, Supervision. TK: Conceptualization, Supervision, Writing – review & editing. AT: Writing – original draft, Writing – review & editing. DG: Conceptualization, Funding acquisition, Project administration, Writing – review & editing. AV: Conceptualization, Funding acquisition, Project administration, Writing – review & editing.

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Conflict of interest

Author AT was employed by the company Valio Ltd.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fanim.2023.1278495/full#supplementary-material

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