Effect of plant oils and camelina expeller on milk fatty acid composition in lactating cows fed diets based on red clover silage

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ABSTRACT

Five multiparous Finnish Ayrshire cows fed red clover silage-based diets were used in a 5 × 5 Latin square with 21-d experimental periods to evaluate the effects of various plant oils or camelina expeller on animal performance and milk fatty acid composition. Treatments consisted of 5 concentrate supplements containing no additional lipid (control), or 29 g/kg of lipid from rapeseed oil (RO), sunflower-seed oil (SFO), camelina-seed oil (CO), or camelina expeller (CE). Cows were offered red clover silage ad libitum and 12 kg/d of experimental concentrates. Treatments had no effect on silage or total dry matter intake, whole-tract digestibility coefficients, milk yield, or milk composition. Plant oils in the diet decreased short- and medium-chain saturated fatty acid (6:0–16:0) concentrations, including odd- and branched-chain fatty acids and enhanced milk fat 18:0 and 18-carbon unsaturated fatty acid content. Increases in the relative proportions of *cis* 18:1, *trans* 18:1, nonconjugated 18:2, conjugated linoleic acid (CLA), and polyunsaturated fatty acids in milk fat were dependent on the fatty acid composition of oils in the diet. Rapeseed oil in the diet was associated with the enrichment of *trans* 18:1 (Δ 4, 6, 7, 8, and 9), *cis*-9 18:1, and *trans*-7,*cis*-9 CLA, SFO resulted in the highest concentrations of *trans*-5, *trans*-10, and *trans*-11 18:1, Δ 9,11 CLA, Δ 10,12 CLA, and 18:2n-6, whereas CO enhanced *trans*-13–16 18:1, Δ 11,15 18:2, Δ 12,15 18:2, *cis*-9,*trans*-13 18:2, Δ 11,13 CLA, Δ 12,14 CLA, Δ 13,15 CLA, Δ 9,11,15 18:3, and 18:3n-3. Relative to CO, CE resulted in lower 18:0 and *cis*-9 18:1 concentrations and higher proportions of *trans*-10 18:1, *trans*-11 18:1, *cis*-9,*trans*-11 CLA, *cis*-9,*trans*-13 18:2, and *trans*-11,*cis*-15 18:2. Comparison of milk fat composition responses to CO and CE suggest that the biohydrogenation of unsaturated 18-carbon fatty acids to 18:0 in the rumen

was less complete for camelina lipid supplied as an expeller than as free oil. In conclusion, moderate amounts of plant oils in diets based on red clover silage had no adverse effects on silage dry matter intake, nutrient digestion, or milk production, but altered milk fat composition, with changes characterized as a decrease in saturated fatty acids, an increase in *trans* fatty acids, and enrichment of specific unsaturated fatty acids depending on the fatty acid composition of lipid supplements.

Key words: red clover silage, plant oil, *trans* fatty acid, conjugated linoleic acid

INTRODUCTION

Lipids are an important component of milk, affecting the aroma, flavor, texture, and storage characteristics of milk and dairy products. Milk contains a high proportion of saturated fatty acids (SFA) because of the extensive hydrogenation of dietary unsaturated fatty acids in the rumen and de novo synthesis of short- and medium-chain saturates in the mammary gland (Chilliard et al., 2007; Shingfield et al., 2008b). Due to the incomplete biohydrogenation of unsaturated fatty acids in the rumen, *trans* fatty acid intermediates accumulate, which can be incorporated into milk fat triacylglycerides following digestion and absorption in the small intestine (Chilliard et al., 2007). Clinical and biomedical studies have established that excessive consumption of SFA and *trans* fatty acids is associated with increased cardiovascular disease risk in humans (WHO, 2003). An excessive intake of 12:0, 14:0, and 16:0 fatty acids in the human diet is a known risk factor for cardiovascular disease and may lower insulin sensitivity, whereas 18:0 is considered to be neutral with respect to the effects on circulating plasma cholesterol concentrations (Mensink et al., 2003; WHO, 2003; Shingfield et al., 2008b). Furthermore, milk fat contains 4:0 and several branched-chain SFA (15:0 *iso*, 15:0 *anteiso*, and 16:0 *iso*) that exhibit anticarcinogenic properties, and other bioactive lipids, including *cis*-9

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18:1, *cis*-9,*trans*-11 conjugated linoleic acid (CLA), and 18:3n-3, that are thought to exert beneficial effects with respect to lowering the incidence of chronic disease in humans (Gebauer et al., 2006; Chilliard et al., 2007; Shingfield et al., 2008b).

Milk fatty acid composition is known to be dependent on both the proportion and type of forage in the diet (Dewhurst et al., 2006; Chilliard et al., 2007). Replacing grass silage with red clover silage is known to enhance milk fat polyunsaturated fatty acid (PUFA) content, 18:3n-3 in particular, with minimal increases in *trans* 18:1 content and decreased SFA concentrations (Dewhurst et al., 2006; Vanhatalo et al., 2007). Furthermore, the composition of the basal diet is an important determinant of milk fatty acid composition responses to plant oils and oilseeds (Griinari et al., 1998; Roy et al., 2006; Chilliard et al., 2007), but limited data exist on the effect of lipid supplements on milk fat composition in cows fed diets based on red clover silage. Inclusion of plant oils (rapeseed oil, sunflower-seed oil, soybean oil, and linseed oil) in diets based on grass silage or maize silage typically reduce the proportion of medium-chain and total SFA and increase 18:0, *cis*-9 18:1, *trans* fatty acid, PUFA, and CLA contents of bovine milk fat (Givens and Shingfield, 2006; Chilliard et al., 2007; Shingfield et al., 2008b). Recent evidence suggests that *trans* fatty acids in ruminant-derived foods at current levels of intake in the human diet are not associated with an increase in cardiovascular disease risk (Jakobsen et al., 2006; Chardigny et al., 2008), whereas emerging data from clinical trials tend to implicate *trans* fatty acids with more than one double bond as being particularly harmful (refer to Shingfield et al., 2008b), highlighting the importance of characterizing changes in the relative abundance of specific biohydrogenation intermediates in bovine milk fat.

Camelina (*Camelina sativa* L.) is an ancient oilseed crop rich in 18:3n-3 (Zubr, 2003; Peiretti and Meineri, 2007), but information on its use as a feed ingredient to alter milk fat composition is limited (Hurtaud and Peyraud, 2007). It is known that camelina expeller is relatively abundant in essential amino acids (Zubr, 2003), indicating the potential of this oilseed as a high-quality protein and lipid supplement for ruminants.

In this experiment, we examined the potential of moderate amounts of plant oils or camelina expeller in the diet to alter milk fatty acid composition. The amount of lipid supplied by the experimental treatments was designed to induce changes in milk fat composition but avoid the negative effects on forage and total DMI that are known to occur at high rates of inclusion (Chilliard, 1993). Rapeseed oil, sunflower-seed oil, and camelina-seed oil or expeller were used as sources of *cis*-9 18:1, 18:2n-6, and 18:3n-3, respectively, to investigate the

role of plant oil composition on milk production and milk fat composition in cows fed red clover silage-based diets with specific emphasis on the relative abundance of *trans* fatty acids and isomers of CLA. Furthermore, direct comparison of the effects of camelina-seed oil and camelina expeller were made to provide insight into the effects attributable to the form of lipid in the diet.

MATERIALS AND METHODS

Animals, Experimental Design, and Diets

The experiment was performed at the University of Helsinki research farm in Viikki, Finland. All experimental procedures were approved by the National Ethics Committee in accordance with the *Use of Vertebrates for Scientific Purposes Act* of 1985. Five multiparous Finnish Ayrshire cows averaging 115 ± 5.0 DIM and producing 33.5 ± 1.62 kg of milk/d were allocated at random to experimental diets according to a 5×5 Latin square with 21-d periods. The cows averaged 607 ± 30.3 kg and 637 ± 42.3 kg of BW at the beginning and the end of the experiment, respectively. Treatments consisted of 5 concentrate supplements containing no additional lipid (control), rapeseed oil (RO), sunflower-seed oil (SFO), camelina-seed oil (CO), or camelina expeller (CE). Concentrates (12 kg/d) were fed as 4 equal meals at 0615, 1000, 1645, and 2000 h. Red clover (*Trifolium pratense* 'Ilte') silage was offered ad libitum (to achieve 5 to 10% refusals) 4 times daily at 0700, 1200, 1500, and 1800 h. Experimental diets were designed to meet or exceed requirements for ME and MP (MTT Agrifood Research Finland, 2006). Cows were housed in individual tie stalls equipped with forage intake control feeding stations (Insentec BV, Marknesse, the Netherlands), which were fitted with separate concentrate troughs. Cows had continuous access to water, and they were milked twice daily at 0630 and 1700 h.

Experimental silage was prepared from secondary growth of red clover sward grown at Viikki (60°10'N, 24°56'E), cut with a mower-conditioner at mid-flowering, wilted to 21 to 26% DM, and ensiled with a formic acid based additive (AIV2+, Kemira Ltd., Helsinki, Finland) applied at a rate of 6.5 L/t before baling. Ingredient composition of experimental concentrates containing no additional lipid, RO (Raisio Feed Ltd., Raisio, Finland), SFO (Cargill Nordic Ltd., Espoo, Finland), CO (Raisio Feed Ltd.), and cold-pressed CE (Raisio Feed Ltd.) is shown in Table 1. A small amount of RO was incorporated into the CE concentrate to compensate for the contribution of lipid from the solvent-extracted rapeseed meal included in all other experimental treatments. Concentrates contained iso-

Table 1. Formulation and ingredient composition of experimental concentrates (% on an air-dry basis)

Item	Concentrate ¹				
	Control	RO	SFO	CO	CE
Barley	20.0	20.0	20.0	20.0	20.0
Wheat	20.0	20.0	20.0	20.0	20.0
Rapeseed meal	20.0	20.0	20.0	20.0	—
Camelina expeller	—	—	—	—	20.0
Molassed sugar-beet pulp	20.0	20.0	20.0	20.0	20.0
Cereal bran	11.2	8.3	8.3	8.3	10.6
Sugar-beet molasses	6.0	6.0	6.0	6.0	6.0
Rapeseed oil	—	2.9	—	—	0.6
Sunflower-seed oil	—	—	2.9	—	—
Camelina-seed oil	—	—	—	2.9	—
Calcium carbonate	1.4	1.4	1.4	1.4	1.4
Sodium chloride	0.7	0.7	0.7	0.7	0.7
Magnesium oxide	0.3	0.3	0.3	0.3	0.3
Mineral premix ²	0.2	0.2	0.2	0.2	0.2
Vitamin premix ³	0.2	0.2	0.2	0.2	0.2

¹Refers to experimental concentrates containing no additional lipid (control), rapeseed oil (RO), sunflower-seed oil (SFO), camelina-seed oil (CO), or camelina expeller (CE).

²Declared as containing (g/kg of DM) Zn (37), Cu (6.3), Mn (5.0), I (1.9), Co (0.37), and Se (0.14).

³Declared as containing (mg/kg of DM) retinyl acetate (1,350), cholecalciferol (23), DL- α -tocopheryl acetate (4,500), and choline (800).

nitrogenous amounts of rapeseed or camelina protein (CE treatment) and were formulated assuming no major differences in rumen undegradable protein content between rapeseed meal and CE. Plant oils and CE were included in concentrates to supply an additional 350 g/d of oil, an amount predicted to alter milk fat composition (Givens and Shingfield, 2006; Glasser et al., 2008) but avoid decreases in forage DMI (Huhtanen et al., 2007, 2008).

Measurements and Sampling

Feed intake was determined as the difference between the amounts of silage and concentrates offered and the amount of refused feeds. Daily feed intake and milk yield were recorded throughout the experiment, but only measurements on d 15 to 20 of each period were used for statistical analysis. During this period, representative samples of silage and concentrates fed on each day were collected and combined to provide a composite sample for chemical determinations. Spot fecal samples were obtained from the rectum twice daily (0700 and 1500 h) on d 17 to 21 of each period to estimate nutrient digestibility using acid-insoluble ash as an internal marker (Kokkonen et al., 2000). Feed and fecal samples were stored at -20°C until analyzed for chemical composition. Samples of milk were collected from each cow over 4 consecutive milkings, starting at 1700 h on d 17. Milk samples treated with preservative (Bronopol, Valio Ltd., Helsinki, Finland) were analyzed for milk fat, CP, lactose, and urea. Unpreserved milk samples were composited according to yield and stored

at -20°C until analyzed for fatty acid composition. In addition, samples of milk were collected over 2 consecutive milkings, starting at 1700 h on d 19, placed in an ice-bath, and stored at 4°C until submitted for sensory quality on d 20 and determination of peroxide concentrations on d 21. Blood samples from the coccygeal vessels were collected at 0530, 0900, and 1130 h into evacuated collection tubes (Venoject, Terumo Europe Ltd., Leuven, Belgium) containing potassium EDTA and placed on ice. Once collected, blood samples were centrifuged (15 min at $870 \times g$ at room temperature) and plasma was stored at -20°C pending analysis for glucose and NEFA. Cows were weighed at the beginning and at the end of the experiment.

Chemical Analysis and Calculations

The DM content of silage, concentrates, and feces was determined by oven drying at 102°C for 24 h. Silage DM content was corrected for the loss of volatiles during drying (Kokkonen et al., 2000). Chemical composition of feeds and feces was determined by standard procedures (Kokkonen et al., 2000). Ash was analyzed according to AOAC (1995; method 942.05). Neutral detergent fiber was determined using sodium sulfite and α -amylase and reported on an ash-free basis (VanSoest et al., 1991). Silage in vitro OM digestibility was determined based on pepsin-cellulase solubility (Nousiainen et al., 2003). Diet digestibility was determined using acid insoluble ash as an internal standard (Kokkonen et al., 2000). Plasma glucose (Glucose RTU kit, Bio-Mérieux, Marcy l'Etoile, France) and NEFA (NEFA

C kit, Wako Chemicals GmbH, Neuss, Germany) were measured enzymatically in accordance with the instructions of the manufacturer.

Milk fat, CP, lactose, and urea were determined by infrared analysis (MilkoScan FT6000, Foss Electric, Hillerød, Denmark). Unpasteurized samples of milk were submitted to an experienced 6-member taste panel for the assessment of aroma and flavor. Milk samples were presented to the panel at 15°C and evaluated using a numerical interval scale from 1 (poor) to 5 (excellent) according to International Dairy Federation Standard 99C (IDF, 1997). Milk peroxides were measured according to standard procedures (ISO 3976; ISO, 2006).

Lipid Analysis

Fatty acid methyl esters (**FAME**) in freeze-dried feed samples were prepared in a one-step extraction-trans-esterification procedure using 2% (vol/vol) methanolic sulfuric acid as a catalyst and tritridecanoin (T-135, Nu-Chek Prep, Elysian, MN) as an internal standard according to reference procedures (Shingfield et al., 2003).

Lipid in a 1-mL milk sample was extracted using ammonia, ethanol, diethylether, and hexane (0.2: 1:2.5:2.5 vol:vol). Extracts were combined and evaporated to dryness at 40°C under oxygen-free nitrogen. Samples were dissolved in hexane and methyl acetate and trans-esterified to FAME using freshly prepared methanolic sodium methoxide (Shingfield et al., 2003).

The FAME prepared from samples of feeds and milk fat were quantified using a gas chromatograph (model 6890, Hewlett-Packard, Wilmington, DE) equipped with a flame-ionization detector, automatic injector, split injection port, and a 100-m fused silica capillary column (i.d. 0.25 mm) coated with a 0.2- μ m film of cyanopropyl polysiloxane (CP-SIL, Chromopack 7489, Middelburg, the Netherlands). Total FAME profile in a 2- μ L sample at a split ratio of 1:50 was determined using a temperature gradient program and hydrogen as a carrier gas operated at constant pressure (137.9 kPa) at a flow rate of 0.5 mL/min (Shingfield et al., 2003). Injector and detector temperatures were maintained at 255°C. Isomers of 18:1 were further resolved in a separate analysis under isothermal conditions at 170°C (Shingfield et al., 2003). Peaks were identified by comparison of retention times with authentic FAME standards (GLC 463 and 606, N-21/23/24-M, U-37/39/43/54/64/85/87-M, Nu-Chek Prep; L-8404, H-6389/6639, and O-4129, Sigma-Aldrich, Helsinki, Finland). Methyl esters not contained in commercially available standards were formally identified by GC-MS analysis of 4,4-dimethyloxazoline (**DMOX**) derivatives prepared from FAME by incubation overnight with

2-amino,2-methyl-1-propanol under a nitrogen atmosphere at 170°C (Shingfield et al., 2006). Impact ionization spectra of DMOX derivatives were obtained using an identical gas chromatograph equipped with a selective quadrupole mass detector (model 5973N, Agilent Technologies Inc., Wilmington, DE), operated at 230°C in the electron impact ionization mode, and mass spectra were recorded under an ionization voltage of 70 eV. Chromatography was achieved using the same temperature gradient applied for the analysis of FAME and helium as the carrier gas (Shingfield et al., 2006). Double bond geometry was determined based on atomic mass unit distances, with an interval of 12 atomic mass units between the most intense peaks of clusters of ions containing n and $n - 1$ carbon atoms being interpreted as cleavage of the double bond between carbon n and $n + 1$ in the fatty acid moiety. Odd-numbered fragments at m/z 139, 153, and 167 were used as diagnostic ions to locate double bonds at $\Delta 4$, $\Delta 5$, and $\Delta 6$, respectively (Spitzer, 1996). When available, the deduced fatty acid structure was verified by comparison with the mass spectrum of DMOX derivatives prepared from authentic FAME standards and cross-referencing with an online reference spectra library (<http://lipidlibrary.aocs.org/ms/masspec.html>). Because GC-MS analysis of DMOX derivatives does not discriminate between geometric isomers, the double bond geometry of polyenoic fatty acids was deduced based on the known elution order of geometric isomers of $\Delta 9,12$ 18:2 and $\Delta 9,12,15$ 18:3 methyl esters (L-8404 and L6031, Sigma-Aldrich) of known composition. Partial gas chromatograms indicating the separation of 16:1 and 17:1, nonconjugated 18:2, and 20:1 methyl esters are presented in Figures 1, 2, and 3, respectively.

The distribution of CLA isomers was determined using an HPLC system (model 1090; Hewlett-Packard, Wilmington, DE) equipped with 4 silver-impregnated silica columns (Chrom-Spher 5 Lipids, 250 $\times$ 4.6 mm, 5- μ m particle size; Varian Ltd., Walton-on-Thames, UK) coupled in series. Methyl esters of CLA were separated under isocratic conditions at 22°C using 0.1% (vol/vol) acetonitrile in heptane at a flow rate of 1 mL/min and monitoring column effluent at 233 and 210 nm (Shingfield et al., 2003). Identification of CLA isomers was performed using commercially available CLA methyl ester standards and chemically synthesized *trans*-9,*cis*-11 CLA (Shingfield et al., 2005).

Milk fatty acid composition was expressed as a weight percentage of total fatty acids using theoretical relative response factors (Wolff et al., 1995). Milk fatty acid content was determined using tritridecanoin (Nu-Chek Prep) as an internal standard (Shingfield et al., 2003). Concentrations of CLA isomers were calculated based on proportionate peak area responses determined by

Figure 1. Partial gas chromatogram indicating the separation of 16:1 and 17:1 isomers obtained using a temperature gradient for fatty acid methyl esters (FAME) prepared from milk fat of cows fed red clover-based diets supplemented with plant lipids. Identification was verified based on argentation silver-ion thin-layer chromatography fractionation of FAME and electron impact ionization spectra recorded during GC-MS analysis of methyl esters and corresponding 4,4-dimethyloxazoline (DMOX) derivatives. Peak identification: 1 = 16:0; 2 = unresolved *trans*-6 16:1 and *trans*-7 16:1; 3 = *trans*-8 16:1; 4 = unresolved *iso* 17:0 and *trans*-9 16:1; 5 = *trans*-10 16:1; 6 = *trans*-11 16:1; 7 = *trans*-12 16:1; 8 = *trans*-13 16:1; 9 = unresolved *trans*-14 16:1 and *cis*-8 16:1; 10 = unresolved *cis*-9 16:1 and *anteiso* 17:0; 11 = *cis*-10 16:1; 12 = 3,7,11,15-tetramethyl-16:0; 13 = *cis*-11 16:1; 14 = *cis*-12 16:1; 15 = 17:0; 16 = 7-methyl-hexadecyl-7-enoate; 17 = *trans*-9 17:1; 18 = *iso* 18:0; 19 = unresolved *cis*-6 17:1 and *cis*-7 17:1; 20 = *cis*-8 17:1; 21 = *cis*-9 17:1; 22 = 18:0; * = unidentified.

HPLC and the sum of *trans*-7,*cis*-9 CLA, *trans*-8,*cis*-10 CLA, and *cis*-9,*trans*-11 CLA weight percentage determined by GC analysis.

Statistical Analysis

Experimental data were analyzed by ANOVA using the Mixed procedure of SAS (version 9.1, SAS institute, Cary, NC) with a model that included the random effect of cow and fixed effects of period and treatment (residual effect df = 12). Sums of squares for treatment effects were further separated into single degree of freedom comparisons to test for the significance of preplanned contrasts as follows: (1) plant oils in the diet (control vs. RO + SFO + CO); (2) degree of unsaturation of plant oil supplements [monounsaturated fatty acids (MUFA) vs. PUFA; RO vs. SFO + CO]; (3) comparison of plant oil PUFA sources (18:2 vs. 18:3; SFO vs. CO); and (4) effect of the form of camelina lipid in the diet (CO vs. CE). Arithmetic means are reported and treatment effects declared significant at $P \leq 0.05$. Differences at $P > 0.05$ to 0.10 were considered as a trend toward significance.

RESULTS

Because of an unexpected thunderstorm during harvesting, the DM content of experimental red clover

silage was lower than targeted (Table 2). Silage was of moderate fermentation quality as indicated by a relatively high pH, VFA, and ammonium-N concentrations. Analysis of samples after completion of the experiment indicated that the 18:3n-3 and total fatty acid contents of the CE concentrate were lower than expected because the CE contained lower amounts of oil than indicated based on preliminary analysis made before the start of the trial.

Cows consumed all the concentrates fed. Silage DMI was not affected by treatment ($P > 0.05$; Table 3). However, inclusion of CE rather than CO in the diet tended ($P \leq 0.09$; Table 3) to decrease silage and total DMI. Inclusion of plant oils in concentrate supplements increased ($P < 0.001$; Table 3) fatty acid intake somewhat less than planned, by 261 g/d, on average. Fatty acid intake was 94 g/d lower ($P < 0.001$; Table 3) for the CE compared with the CO treatment. Inclusion of plant oils or CE had no effect ($P > 0.05$) on OM, NDF, or N whole-tract digestibility or on plasma NEFA and glucose concentrations (Table 3).

Daily yields of milk, fat, protein, and lactose were not altered ($P > 0.05$; Table 3) by treatment. Milk peroxide content was higher ($P = 0.012$; Table 3) for the SFO treatment compared with CO, whereas milk organoleptic properties were not affected ($P > 0.05$) by treatment. Overall, taste panel scores indicated that the milk was of good to high organoleptic quality.

Figure 2. Partial gas chromatogram indicating the separation of nonconjugated 18:2 isomers obtained using a temperature gradient for fatty acid methyl esters (FAME) prepared from milk fat of cows fed red clover-based diets supplemented with plant oils. Identification was verified based on argentation silver-ion thin-layer chromatography fractionation of FAME and electron impact ionization spectra recorded during GC-MS analysis of methyl esters and corresponding 4,4-dimethyloxazoline (DMOX) derivatives. Peak identification: 1 = *trans*-9,*trans*-13 18:2; 2 = unresolved *cis*-15 18:1 and 19:0; 3 = *trans*-11,*trans*-15 18:2; 4 = *cis*-9,*trans*-13 18:2; 5 = 11-cyclohexyl-11:0; 6 = *cis*-9,*trans*-14 18:2; 7 = *cis*-9,*trans*-12 18:2; 8 = *cis*-16 18:1; 9 = *trans*-9,*cis*-12 18:2; 10 = *trans*-11,*cis*-15 18:2; 11 = *cis*-7 19:1; 12 = *cis*-9,*cis*-12 18:2 containing *cis*-9,*cis*-15 18:2 as a minor component; 13 = *cis*-10 19:1; 14 = unresolved *trans*-12,*cis*-15 18:2 and *cis*-11 19:1; 15 = *cis*-12 19:1; 16 = *cis*-12,*cis*-15 18:2; 17 = 20:0; * = unidentified.

Treatment effects on milk fatty acid composition are shown in Tables 4, 5, 6, 7, 8, and 9. Supplementing red clover-based diets with plant oils decreased ($P < 0.001$) milk fat SFA concentration by 5.2 percentage units, with the decrease originating almost entirely from decreased ($P < 0.001$) 16:0 and enhanced ($P < 0.001$) MUFA and PUFA concentrations (Table 4). Oil supplementation decreased ($P \leq 0.045$) concentrations of 6- to 14-carbon fatty acids and increased ($P \leq 0.003$) the relative abundance of 18:0, *cis* 18:1, *trans* 18:1, total *trans* fatty acids, and CLA in milk fat (Table 4). Oil supplementation resulted in relatively minor decreases ($P \leq 0.05$) or no changes in the concentrations of odd- and branched-chain fatty acids in milk fat (Table 5).

Inclusion of plant oils and CE in the diet altered the distribution of 18:1 and 18:2 isomers in milk fat depending on the composition of lipid supplement. Supplementing the diet with RO versus SFO and CO tended ($P = 0.08$) to enhance milk fat *cis*-9 18:1 content and markedly increased ($P \leq 0.003$) *trans*-4 and *trans*-6 to *trans*-9 18:1 concentrations (Table 7). Feeding RO

versus oils rich in PUFA lowered ($P \leq 0.011$) milk fat *cis*-12 18:1, *trans*-8,*trans*-10 CLA, *trans*-10,*cis*-12 CLA, *trans*-10,*trans*-12 CLA, 18:2n-6, total nonconjugated 18:2, and PUFA contents (Tables 4, 7, and 8). Relative to SFO and CO, RO in the diet enhanced ($P = 0.012$) milk *trans*-7,*cis*-9 CLA concentrations (Table 8) and resulted in numerically small, but often statistically significant ($P \leq 0.05$), changes in the relative abundance of 17- and 19-carbon fatty acids in milk (Table 5).

Inclusion of SFO instead of CO in concentrates increased ($P \leq 0.044$) the proportion of 18:0, *trans*-4 and *trans*-6 to *trans*-12 18:1 in milk fat and decreased ($P = 0.033$) that of *cis*-15 18:1 (Tables 4 and 7). Treatment SFO enriched ($P < 0.001$) 18:2n-6, *trans*-9,*cis*-12 18:2, *trans*-8,*trans*-10 CLA, *trans*-10,*cis*-12 CLA, and *trans*-10,*trans*-12 CLA in milk fat compared with CO, whereas CO enhanced ($P \leq 0.05$) the concentrations of several other 18:2 isomers, including geometric isomers of Δ 11,15 18:2, Δ 12,15 18:2, Δ 11,13 CLA, and 12,14 CLA (Table 8). Furthermore, milk fat 18:3n-3, Δ 9,11,15 18:3, 20:0, and 20:1 concentrations were higher ($P <$

Figure 3. Partial gas chromatogram indicating the separation of 20:1 isomers obtained using a temperature gradient for fatty acid methyl esters (FAME) prepared from milk fat of cows fed red clover-based diets supplemented with plant oils. Identification was verified based on argention silver-ion thin-layer chromatography fractionation of FAME and electron impact ionization spectra recorded during GC-MS analysis of methyl esters and corresponding 4,4-dimethyloxazoline (DMOX) derivatives. Peak identification: 1 = 20:0; 2 = Δ 7,9 17:2; 3 = *cis*-6,*cis*-9,*cis*-12 18:3; 4 = unresolved *trans*-6 20:1 and *trans*-7 20:1; 5 = *trans*-9 20:1; 6 = *trans*-10 20:1; 7 = *trans*-11 20:1; 8 = *trans*-12 20:1; 9 = *trans*-13 20:1; 10 = *cis*-9 20:1; 11 = *trans*-15 20:1; 12 = *cis*-11 20:1; 13 = *cis*-9,*cis*-12,*cis*-15 18:3; 14 = *cis*-13 20:1; 15 = *cis*-14 20:1; 16 = unresolved *trans*-7,*cis*-9 18:2, *trans*-8,*cis*-10 18:2, and *cis*-9,*trans*-11 18:2; * = unidentified.

0.001; Tables 4 and 9) for CO than SFO, whereas milk fat product:substrate stearoyl-CoA desaturase concentration ratios were enhanced ($P \leq 0.015$) for 10- and 18-carbon fatty acids for CO relative to SFO (Table 4).

Feeding camelina as an expeller rather than an oil decreased ($P < 0.001$) milk fat total SFA concentration and elevated ($P \leq 0.004$) MUFA and PUFA contents

(Table 4). Inclusion of CE also increased ($P \leq 0.05$) the concentrations of 14-carbon fatty acids, *cis* 16:1, *trans* 16:1, and several odd- and branched-chain fatty acids compared with CO (Tables 4, 5, and 6). Relative proportions of 18:0 and *cis*-9 18:1 in milk fat were lower ($P < 0.001$; Tables 4 and 7) and those of *trans* 18:1, *cis*-12 18:1, and *cis*-15 18:1 were higher ($P < 0.001$;

Table 2. Chemical composition of silage and concentrate supplements

Item	Silage ¹	Concentrate ²				
		Control	RO	SFO	CO	CE
DM (% as fed)	23.9	87.4	88.1	88.2	88.6	88.2
OM (% of DM)	88.1	93.0	93.4	93.2	93.2	91.2
CP (% of DM)	18.1	16.5	15.8	15.7	17.0	16.4
NDF (% of DM)	40.0	22.5	21.6	21.7	22.0	22.7
Fatty acids (% of DM)	1.92	3.14	5.72	5.48	5.64	4.90
Fatty acid composition (g/100 g of fatty acids)						
16:0	19.0	15.8	9.85	11.1	13.6	14.0
18:0	3.74	1.48	1.47	2.37	2.01	2.02
<i>cis</i> -9 18:1	3.00	25.3	40.3	25.3	23.5	23.7
<i>cis</i> -11 18:1	0.57	4.24	3.91	2.95	3.27	2.18
18:2n-6	14.9	39.6	31.5	48.4	29.5	31.1
18:3n-3	33.8	6.98	8.13	4.46	15.0	14.3
20:0	5.44	0.41	0.46	0.37	0.85	0.81
<i>cis</i> -11 20:1	0.17	1.31	0.96	1.15	6.14	5.32
Other	19.4	4.88	3.42	3.90	6.13	6.57

¹Mean fermentation characteristics: pH 4.95, in DM (%) lactic acid, 6.74; acetic acid, 2.97; propionic acid, 0.08; butyric acid, 0.76; % of total N ammonium-N, 16.1; soluble N 43.7; in vitro OM digestibility %, 63.4.

²Refers to experimental concentrates containing no additional lipid (control), rapeseed oil (RO), sunflower-seed oil (SFO), camelina-seed oil (CO), or camelina expeller (CE).

Table 3. Effect of plant oils or camelina expeller on nutrient intake, whole-tract apparent nutrient digestibility, plasma metabolites, milk production, and milk taste panel scores in cows fed red clover-based diets

Item	Treatment ¹					SEM	Significance ²			
	Control	RO	SFO	CO	CE		Oil	MUFA vs. PUFA	18:2 vs. 18:3	Form
Intake										
Silage DM (kg/d)	12.9	12.8	12.4	12.7	12.2	0.44	0.35	0.33	0.40	0.09
Total DM (kg/d)	23.3	23.4	23.0	23.3	22.7	0.46	0.66	0.42	0.37	0.06
OM (kg/d)	21.1	21.2	20.8	21.1	20.3	0.37	0.73	0.33	0.34	0.009
NDF (kg/d)	7.49	7.40	7.28	7.40	7.26	0.172	0.18	0.54	0.31	0.25
Nitrogen (g/d)	637	632	651	644	633	16.1	0.78	0.44	0.75	0.65
ME (MJ/d)	244	251	247	250	247	4.8	0.044	0.42	0.37	0.29
16:0 (g/d)	104	110	115	133	121	2.1	<0.001	<0.001	<0.001	<0.001
18:0 (g/d)	14.8	18.7	23.7	22.0	20.0	0.39	<0.001	<0.001	<0.001	<0.001
<i>cis</i> -9 18:1 (g/d)	95.4	256	161	153	134	1.38	<0.001	<0.001	0.001	<0.001
18:2n-6 (g/d)	177	233	330	221	204	1.8	<0.001	<0.001	<0.001	<0.001
18:3n-3 (g/d)	114	138	113	181	161	3.2	<0.001	<0.001	<0.001	<0.001
Sum of fatty acids (g/d)	609	876	857	876	782	11.1	<0.001	0.23	0.036	<0.001
Apparent digestibility (%)										
OM	69.9	71.9	71.4	68.5	68.0	2.00	0.71	0.33	0.22	0.83
NDF	55.0	59.5	58.6	54.6	52.5	2.45	0.31	0.26	0.19	0.47
Nitrogen	67.5	66.4	66.1	63.5	63.1	2.95	0.43	0.59	0.46	0.91
Yield										
Milk (kg/d)	31.1	32.3	32.3	31.2	32.2	2.65	0.31	0.54	0.30	0.33
ECM ³ (kg/d)	30.5	31.3	30.6	30.5	30.4	2.63	0.71	0.41	0.98	0.89
Milk fat (g/d)	1,225	1,255	1,178	1,234	1,192	117.5	0.97	0.41	0.41	0.54
Milk protein (g/d)	1,013	1,027	1,050	992	1,014	71.6	0.69	0.82	0.07	0.45
Milk lactose (g/d)	1,431	1,500	1,502	1,450	1,485	137.5	0.27	0.63	0.37	0.54
Concentration in milk										
Fat (%)	3.96	3.86	3.64	3.93	3.67	0.170	0.44	0.71	0.22	0.26
Protein (%)	3.30	3.20	3.26	3.23	3.15	0.090	0.16	0.38	0.55	0.17
Lactose (%)	4.60	4.60	4.63	4.61	4.61	0.088	0.78	0.74	0.77	0.95
Urea (mmol/L)	6.76	6.07	6.09	6.35	5.69	0.366	0.11	0.70	0.55	0.15
Milk peroxides (mmol of O ₂ /kg of milk fat)	0.14	0.14	0.16	0.11	0.13	0.014	0.97	0.53	0.012	0.33
Milk taste panel score ⁴	4.05	4.13	4.15	4.18	3.82	0.149	0.55	0.86	0.90	0.12
Plasma										
Free fatty acids (mmol/L)	0.155	0.172	0.172	0.180	0.167	0.0113	0.13	0.79	0.58	0.39
Glucose (mmol/L)	3.88	4.00	3.94	3.87	3.77	0.075	0.45	0.20	0.42	0.21

¹Refers to red clover silage-based diets containing no additional lipid (control), rapeseed oil (RO), sunflower-seed oil (SFO), camelina-seed oil (CO), or camelina expeller (CE).

²Significance of effects due to plant oil in the diet (Oil; control vs. RO, SFO, and CO); degree of unsaturation of lipid supplement [monounsaturated fatty acids (MUFA) vs. polyunsaturated fatty acids (PUFA); RO vs. SFO and CO]; source of PUFA in the diet (18:2 vs. 18:3; SFO vs. CO); and form of camelina lipid in the diet (Form; CO vs. CE).

³Calculated according to Sjaunja et al. (1990).

⁴Milk assessed by a trained 6-member panel using a numerical score from 1 (poor) to 5 (excellent).

Tables 4 and 7) for CE compared with CO. Milk fat 18:2 composition was dependent on the form of camelina in the diet, with CE enhancing ($P < 0.001$) the concentrations of almost all measured 18:2 isomers, *cis*-9,*trans*-13 18:2, Δ 11,15 18:2, *cis*-9,*trans*-11 CLA, and *trans*-7,*cis*-9 CLA, in particular (Table 8). Milk fat 18:3n-3 concentration was lower ($P = 0.009$; Table 4) when camelina was fed as an expeller. Compared with CO, CE decreased ($P < 0.001$) milk fat 20:0 and *cis*-9 20:1 concentrations (Table 4) and increased ($P \leq 0.048$) *trans*-9 to *trans*-13 20:1 contents (Table 9). In addition, milk fat desaturase indices for even-numbered fatty acids with 14 to 20 carbon atoms were higher ($P \leq 0.007$; Table 4) for treatment CE than for treatment CO.

DISCUSSION

Feeding red clover silage as the sole forage source in a low concentrate diet is known to enhance milk fat 18:2n-6 and 18:3n-3 concentrations compared with grass silage (Dewhurst et al., 2006; Vanhatalo et al., 2007). In the present study, the intake of red clover silage and total DM was not altered by the relatively moderate inclusion of plant oils in concentrate supplements comprising approximately 46% of the diet DMI. These findings are in accordance with responses predicted by models of intake developed and evaluated using published data (Huhtanen et al., 2007, 2008). At higher inclusion rates, plant oils or oilseeds may, under certain circumstances, induce negative effects on DMI

Table 4. Effect of plant oils or camelina expeller on milk fatty acid (FA) composition in cows fed red clover-based diets

FA (g/100 g of total FA)	Treatment ¹					SEM	Significance ²			
	Control	RO	SFO	CO	CE		Oil	MUFA vs. PUFA	18:2 vs. 18:3	Form
4:0	3.35	3.54	3.58	3.57	3.67	0.085	0.005	0.55	0.90	0.20
6:0	1.76	1.72	1.71	1.69	1.69	0.034	0.045	0.41	0.63	0.92
8:0	1.23	1.14	1.14	1.14	1.09	0.044	<0.001	0.84	0.77	0.019
10:0	3.19	2.75	2.76	2.72	2.57	0.169	<0.001	0.82	0.54	0.06
<i>cis</i> -9 10:1	0.30	0.27	0.26	0.27	0.26	0.013	<0.001	0.16	0.018	0.014
12:0	3.91	3.24	3.24	3.20	3.08	0.214	<0.001	0.84	0.68	0.21
<i>cis</i> -9 12:1	0.09	0.07	0.07	0.07	0.07	0.006	<0.001	0.42	0.43	0.81
<i>trans</i> -9 12:1	0.08	0.07	0.06	0.07	0.07	0.004	<0.001	0.30	0.33	0.55
14:0	13.0	11.8	11.7	11.6	11.9	0.29	<0.001	0.21	0.41	0.049
<i>cis</i> -9 14:1	0.97	0.87	0.82	0.85	0.99	0.061	0.001	0.22	0.36	0.003
<i>trans</i> -9 14:1	0.014	0.012	0.011	0.012	0.014	0.0007	0.002	0.25	0.34	0.006
Sum of (Σ) 15	2.22	2.01	1.98	1.92	1.98	0.077	<0.001	0.020	0.041	0.06
16:0	32.4	27.3	26.5	27.1	26.8	1.38	<0.001	0.24	0.29	0.55
8-0-16:0	0.044	0.037	0.037	0.044	0.033	0.0042	0.09	0.28	0.045	0.006
10-0-16:0	0.009	0.008	0.010	0.008	0.007	0.0007	0.10	0.003	<0.001	0.08
Σ <i>cis</i> 16:1	1.60	1.38	1.34	1.36	1.52	0.032	<0.001	0.43	0.53	0.003
Σ <i>trans</i> 16:1	0.29	0.31	0.28	0.31	0.41	0.020	0.14	0.38	0.045	<0.001
Σ 16:1	1.89	1.68	1.62	1.67	1.93	0.032	<0.001	0.35	0.29	<0.001
Σ 17	1.21	1.11	1.12	1.11	1.23	0.042	<0.001	0.86	0.60	<0.001
18:0	7.63	10.4	10.9	9.86	7.33	0.468	<0.001	0.94	<0.001	<0.001
9-0-18:0	0.006	0.007	0.006	0.005	0.005	0.0011	0.67	0.23	0.71	0.83
10-0-18:0	0.21	0.22	0.22	0.22	0.18	0.016	0.58	0.73	0.95	0.019
13-0-18:0	0.04	0.04	0.04	0.04	0.04	0.003	0.60	0.64	0.11	0.59
15-0-18:0	0.005	0.006	0.007	0.006	0.006	0.001	0.46	0.95	0.55	0.70
Σ <i>cis</i> 18:1	14.5	18.7	18.1	18.1	15.7	0.62	<0.001	0.12	0.84	<0.001
Σ <i>trans</i> 18:1	4.02	5.20	5.55	4.91	8.28	0.267	<0.001	0.91	0.06	<0.001
Σ 18:1	18.5	23.9	23.7	23.0	24.0	0.72	<0.001	0.12	0.10	0.027
Σ 18:2 ³	2.96	2.97	3.50	3.23	4.14	0.128	<0.001	<0.001	<0.001	<0.001
Σ conjugated linoleic acid (CLA)	0.59	0.77	0.83	0.79	1.33	0.052	<0.001	0.35	0.41	<0.001
18:3n-3	1.10	1.02	0.99	1.17	1.06	0.049	0.22	0.07	<0.001	0.009
18:3n-6	0.05	0.05	0.06	0.06	0.05	0.006	0.45	0.08	0.80	0.25
<i>cis</i> -9, <i>trans</i> -11, <i>cis</i> -15 18:3	0.03	0.04	0.03	0.05	0.05	0.005	0.003	0.036	<0.001	0.37
<i>cis</i> -9, <i>trans</i> -11, <i>trans</i> -15 18:3	0.014	0.016	0.012	0.023	0.055	0.0020	0.17	0.47	<0.001	<0.001
18:4n-3	0.02	0.02	0.03	0.02	0.02	0.002	0.46	0.51	0.30	0.33
20:0	0.42	0.40	0.41	0.77	0.57	0.036	0.002	<0.001	<0.001	<0.001
Σ <i>cis</i> 20:1	0.50	0.45	0.45	1.24	1.20	0.052	<0.001	<0.001	<0.001	0.45
Σ <i>trans</i> 20:1	0.08	0.07	0.07	0.23	0.29	0.015	0.018	<0.001	<0.001	0.012
Σ 20:1	0.58	0.52	0.53	1.47	1.49	0.065	0.001	<0.001	<0.001	0.82
20:2n-6	0.045	0.037	0.043	0.073	0.088	0.0037	0.030	<0.001	<0.001	<0.001
20:3n-3	0.020	0.015	0.016	0.037	0.037	0.0019	0.043	<0.001	<0.001	0.86
20:3n-6	0.093	0.089	0.094	0.087	0.073	0.0138	0.66	0.90	0.34	0.08
20:4n-3	0.09	0.08	0.08	0.07	0.08	0.011	0.40	0.37	0.59	0.57
20:4n-6	0.07	0.08	0.09	0.08	0.07	0.011	0.39	0.95	0.59	0.50
20:5n-3 ⁴	0.13	0.12	0.12	0.11	0.10	0.006	<0.001	0.43	0.24	0.011
22:0	0.10	0.10	0.11	0.12	0.10	0.005	0.002	<0.001	0.005	<0.001
Σ 22:1	0.07	0.06	0.06	0.19	0.18	0.008	0.002	<0.001	<0.001	0.21
22:2n-6	0.006	0.005	0.006	0.008	0.012	0.0006	0.50	0.018	0.034	<0.001
22:3n-3	0.003	0.003	0.002	0.012	0.015	0.0009	0.015	<0.001	<0.001	0.010
22:4n-6	0.018	0.018	0.018	0.016	0.015	0.0019	0.42	0.53	0.26	0.17
22:5n-3	0.074	0.068	0.071	0.071	0.060	0.0044	0.09	0.18	0.80	0.002
22:6n-3	0.004	0.003	0.003	0.003	0.003	0.0003	0.22	0.84	0.91	0.51
<i>cis</i> -15 24:1	0.014	0.015	0.012	0.027	0.028	0.0011	<0.001	<0.001	<0.001	0.74
26:0	0.013	0.012	0.012	0.011	0.012	0.0005	0.018	0.12	0.017	0.07
28:0	0.003	0.003	0.004	0.003	0.004	0.0002	0.18	0.39	0.006	0.002
Others	0.60	0.48	0.52	0.57	0.57	0.031	0.006	0.026	0.11	0.94
Summary										
Σ 4- to 14-carbon	28.6	26.2	26.0	25.9	25.8	0.65	<0.001	0.44	0.57	0.78
Σ <i>trans</i> fatty acids	6.56	8.08	8.53	8.47	11.7	0.425	0.003	0.44	0.92	<0.001
Σ Saturated fatty acids	71.0	66.1	65.9	65.4	62.6	1.01	<0.001	0.29	0.38	<0.001
Σ MUFA	23.2	28.1	27.7	28.2	29.7	0.78	<0.001	0.67	0.31	0.004
Σ PUFA	5.33	5.40	5.99	5.93	7.27	0.240	<0.001	<0.001	0.55	<0.001

Continued

Table 4 (Continued). Effect of plant oils or camelina expeller on milk fatty acid (FA) composition in cows fed red clover-based diets

FA (g/100 g of total FA)	Treatment ¹						Significance ²			
	Control	RO	SFO	CO	CE	SEM	Oil	MUFA vs. PUFA	18:2 vs. 18:3	Form
FA (g/100 g of fat)	93.8	94.0	94.1	93.9	93.9	0.810	0.08	0.98	0.14	0.68
Ratio										
<i>cis</i> -9 10:1/10:0	0.096	0.099	0.094	0.101	0.100	0.0069	0.31	0.33	0.015	0.69
<i>cis</i> -9 12:1/12:0	0.024	0.023	0.021	0.023	0.023	0.0011	0.006	0.24	0.12	0.45
<i>cis</i> -9 14:1/14:0	0.075	0.074	0.070	0.073	0.083	0.0051	0.33	0.29	0.25	0.004
<i>cis</i> -9 16:1/16:0	0.047	0.048	0.047	0.047	0.053	0.0021	0.38	0.58	0.74	<0.001
<i>cis</i> -9 18:1/18:0	1.75	1.69	1.54	1.69	1.85	0.079	0.015	0.10	0.011	0.007
<i>cis</i> -9, <i>trans</i> -11 CLA/ <i>trans</i> -11 18:1	0.463	0.441	0.448	0.467	0.467	0.0215	0.50	0.33	0.37	0.99
<i>cis</i> -9 20:1/20:0	0.778	0.754	0.722	0.767	0.860	0.0409	0.15	0.66	0.09	0.002

¹Refers to red clover silage-based diets containing no additional lipid (control), rapeseed oil (RO), sunflower-seed oil (SFO), camelina-seed oil (CO), or camelina expeller (CE).

²Significance of effects due to plant oil in the diet (Oil; control vs. RO, SFO, and CO); degree of unsaturation of lipid supplement [monounsaturated fatty acids (MUFA) vs. polyunsaturated fatty acids (PUFA); RO vs. SFO and CO]; source of PUFA in the diet (18:2 vs. 18:3; SFO vs. CO); and form of camelina lipid in the diet (Form; CO vs. CE).

³Sum of 18:2 fatty acids excluding isomers of CLA.

⁴Co-elutes with 24:0.

Table 5. Effect of plant oils or camelina expeller on milk odd- and branched-chain fatty acid (FA) content in cows fed red clover-based diets

FA (mg/100 g of total FA)	Treatment ¹						Significance ²			
	Control	RO	SFO	CO	CE	SEM	Oil	MUFA vs. PUFA	18:2 vs. 18:3	Form
<i>anteiso</i> 13:0	7.86	7.34	7.22	6.67	6.97	0.400	0.048	0.32	0.24	0.51
<i>iso</i> 13:0	23.2	21.2	20.7	20.0	21.8	1.47	0.011	0.35	0.50	0.11
<i>iso</i> 14:0	117	113	107	106	109	5.5	0.07	0.20	0.85	0.58
15:0	1,368	1,233	1,191	1,688	1,206	31.6	<0.001	0.005	0.24	0.06
<i>anteiso</i> 15:0	479	434	441	423	433	40.1	<0.001	0.84	0.15	0.42
<i>iso</i> 15:0	185	165	162	158	159	8.3	<0.001	0.34	0.42	0.91
<i>cis</i> -9 15:1	14.8	13.3	11.6	12.0	14.2	0.68	0.004	0.07	0.64	0.026
<i>trans</i> -5 15:1	131	124	133	121	118	8.0	0.51	0.75	0.21	0.76
<i>trans</i> -6 15:1	37.8	36.2	34.8	34.2	39.5	2.47	0.023	0.17	0.65	0.002
<i>trans</i> -9 15:1	15.7	15.2	18.9	15.6	22.8	2.00	0.71	0.43	0.27	0.027
<i>trans</i> -10 15:1	6.55	6.12	7.36	5.94	8.08	0.878	0.95	0.63	0.28	0.11
<i>iso</i> 16:0	266	238	269	242	265	17.4	0.22	0.22	0.12	0.18
17:0	649	585	603	583	582	18.9	<0.001	0.28	0.025	0.91
<i>iso</i> 17:0 ³	285	276	285	281	366	16.7	0.61	0.53	0.75	<0.001
<i>cis</i> -6 + 7 17:1	54.3	49.2	46.1	43.3	47.5	2.86	<0.001	0.004	0.08	0.014
<i>cis</i> -8 17:1	23.4	22.0	21.2	23.6	30.7	1.32	0.17	0.69	0.022	<0.001
<i>cis</i> -9 17:1	177	156	145	153	169	5.1	<0.001	0.20	0.23	0.013
<i>trans</i> -9 17:1	5.73	5.44	4.31	6.53	17.3	0.54	0.53	0.98	0.003	<0.001
Δ 7,9 17:2	20.1	20.9	19.0	22.4	23.3	1.73	0.70	0.92	0.16	0.69
<i>iso</i> 18:0	60.4	54.2	58.6	53.9	58.9	2.43	0.005	0.21	0.019	0.014
11-Cyclohexyl 11:0	106	110	109	104	108	8.2	0.81	0.46	0.41	0.43
7-Methyl-hexadecyl-7-enoate	13.6	12.0	11.4	12.7	14.8	0.91	<0.001	0.75	0.008	<0.001
<i>cis</i> -7 19:1	24.0	20.6	21.5	22.6	28.2	1.46	0.029	0.18	0.38	<0.001
<i>cis</i> -10 19:1	21.0	21.4	21.9	21.8	29.6	1.55	0.68	0.77	0.97	0.002
<i>cis</i> -12 19:1	4.98	4.65	5.47	7.29	12.6	0.64	0.18	0.015	0.024	<0.001
3,7,11,15-Tetramethyl-16:0	130	111	124	113	171	8.9	0.19	0.50	0.41	<0.001
21:0	51.7	50.4	54.9	55.5	56.0	2.80	0.22	0.009	0.74	0.76
<i>cis</i> -13 21:1	4.04	3.13	3.17	4.55	5.66	0.387	0.37	0.15	0.025	0.07
23:0	45.9	41.6	43.8	42.6	37.1	3.58	0.10	0.42	0.58	0.028
<i>cis</i> -14 23:1	13.7	12.7	12.5	13.0	17.0	0.69	0.14	0.91	0.54	<0.001

¹Refers to red clover silage-based diets containing no additional lipid (control), rapeseed oil (RO), sunflower-seed oil (SFO), camelina-seed oil (CO), or camelina expeller (CE).

²Significance of effects due to plant oil in the diet (Oil; control vs. RO, SFO, and CO); degree of unsaturation of lipid supplement [monounsaturated fatty acids (MUFA) vs. polyunsaturated fatty acids (PUFA); RO vs. SFO and CO]; source of PUFA in the diet (18:2 vs. 18:3; SFO vs. CO); and form of camelina lipid in the diet (Form; CO vs. CE).

³Co-elutes with *trans*-9 16:1.

Table 6. Effect of plant oils or camelina expeller on milk 16:1 content in cows fed red clover-based diets

Fatty acid (mg/100 g of total fatty acids)	Treatment ¹						Significance ²			
	Control	RO	SFO	CO	CE	SEM	Oil	MUFA vs. PUFA	18:2 vs. 18:3	Form
<i>cis</i> -9 16:1 ³	1,502	1,287	1,234	1,263	1,422	31.6	<0.001	0.29	0.49	0.002
<i>cis</i> -10 16:1	15.2	16.2	19.2	18.9	31.0	1.65	0.06	0.08	0.84	<0.001
<i>cis</i> -11 16:1	58.9	49.1	57.3	52.4	41.9	4.87	0.22	0.27	0.41	0.09
<i>cis</i> -12 16:1	26.1	27.3	25.4	29.1	26.7	1.61	0.27	0.94	0.013	0.08
<i>trans</i> -6 + 7 16:1	43.1	48.7	45.1	45.6	62.0	3.61	0.09	0.10	0.82	<0.001
<i>trans</i> -8 16:1	12.2	16.3	15.3	15.2	32.5	2.09	0.16	0.66	0.99	<0.001
<i>trans</i> -10 16:1	14.6	17.2	17.6	18.0	30.9	1.09	0.004	0.50	0.69	<0.001
<i>trans</i> -11 16:1	23.1	26.6	21.1	26.8	60.1	4.13	0.67	0.56	0.28	<0.001
<i>trans</i> -12 16:1	132	139	134	141	148	9.7	0.19	0.79	0.16	0.23
<i>trans</i> -13 16:1	32.4	29.5	24.8	30.8	32.9	1.87	0.09	0.48	0.043	0.45
<i>trans</i> -14 16:1 ⁴	27.3	28.1	26.6	30.8	44.6	2.01	0.42	0.68	0.032	<0.001

¹Refers to red clover silage-based diets containing no additional lipid (control), rapeseed oil (RO), sunflower-seed oil (SFO), camelina-seed oil (CO), or camelina expeller (CE).

²Significance of effects due to plant oil in the diet (Oil; control vs. RO, SFO, and CO); degree of unsaturation of lipid supplement [monounsaturated fatty acids (MUFA) vs. polyunsaturated fatty acids (PUFA); RO vs. SFO and CO]; source of PUFA in the diet (18:2 vs. 18:3; SFO vs. CO); and form of camelina lipid in the diet (Form; CO vs. CE).

³Co-elutes with *anteiso* 17:0.

⁴Co-elutes with *cis*-8 16:1.

(Roy et al., 2006; Huhtanen et al., 2008), but not in all cases (Loor et al., 2005; Shingfield et al., 2008a). In this experiment, inclusion of camelina as an expeller rather than oil in concentrate supplements tended to decrease silage DMI, consistent with previous findings (Hurtaud and Peyraud, 2007), an effect that is not explained by dietary fatty acid intake.

Decreases in DMI to lipid supplements have often been attributed to the negative effect of unsaturated fatty acids on ruminal OM digestion, ruminal fermentation, and the tendency to shift the site of digestion from the rumen to the intestine (Lock and Shingfield, 2004). In this experiment, moderate amounts of lipid in the diet (on average 10 g of supplemental fatty acids/kg

Table 7. Effect of plant oils or camelina expeller on milk 18:1 content in cows fed red clover-based diets

Fatty acid (g/100 g of total fatty acids)	Treatment ¹						Significance ²			
	Control	RO	SFO	CO	CE	SEM	Oil	MUFA vs. PUFA	18:2 vs. 18:3	Form
<i>cis</i> -9 18:1	13.2	17.3	16.6	16.5	13.5	0.59	<0.001	0.08	0.85	<0.001
<i>cis</i> -11 18:1	0.55	0.60	0.55	0.60	0.55	0.028	0.037	0.09	0.029	0.032
<i>cis</i> -12 18:1	0.33	0.38	0.57	0.46	0.85	0.047	0.006	0.011	0.07	<0.001
<i>cis</i> -13 18:1	0.08	0.10	0.09	0.10	0.16	0.005	0.014	0.13	0.69	<0.001
<i>cis</i> -15 18:1 ³	0.24	0.23	0.21	0.27	0.64	0.021	0.98	0.50	0.033	<0.001
<i>cis</i> -16 18:1	0.08	0.09	0.09	0.09	0.05	0.004	0.007	0.45	0.76	<0.001
<i>trans</i> -4 18:1	0.016	0.044	0.036	0.029	0.041	0.002	<0.001	<0.001	0.007	<0.001
<i>trans</i> -5 18:1	0.05	0.08	0.16	0.06	0.06	0.041	0.33	0.53	0.10	0.96
<i>trans</i> -6 + 7 + 8 18:1	0.24	0.46	0.39	0.34	0.61	0.023	<0.001	<0.001	0.044	<0.001
<i>trans</i> -9 18:1	0.22	0.36	0.33	0.30	0.53	0.014	<0.001	0.003	0.033	<0.001
<i>trans</i> -10 18:1	0.36	0.51	0.56	0.42	0.96	0.045	0.008	0.73	0.017	<0.001
<i>trans</i> -11 18:1	0.96	1.28	1.42	1.21	2.18	0.082	<0.001	0.56	0.022	<0.001
<i>trans</i> -12 18:1	0.43	0.55	0.57	0.53	0.92	0.025	<0.001	0.67	0.015	<0.001
<i>trans</i> -13 + 14 18:1	0.74	0.82	0.82	0.84	1.71	0.077	0.22	0.92	0.78	<0.001
<i>trans</i> -15 18:1	0.48	0.53	0.53	0.57	0.79	0.024	0.002	0.33	0.08	<0.001
<i>trans</i> -16 18:1 ⁴	0.52	0.57	0.58	0.60	0.48	0.034	0.09	0.56	0.74	0.016

¹Refers to red clover silage-based diets containing no additional lipid (control), rapeseed oil (RO), sunflower-seed oil (SFO), camelina-seed oil (CO), or camelina expeller (CE).

²Significance of effects due to plant oil in the diet (Oil; control vs. RO, SFO, and CO); degree of unsaturation of lipid supplement [monounsaturated fatty acids (MUFA) vs. polyunsaturated fatty acids (PUFA); RO vs. SFO and CO]; source of PUFA in the diet (18:2 vs. 18:3; SFO vs. CO); and form of camelina lipid in the diet (Form; CO vs. CE).

³Co-elutes with 19:0.

⁴Contains *cis*-14 18:1 and *trans*-5, *trans*-11 18:2 as minor components.

Table 8. Effect of plant oils or camelina expeller on milk 18:2 content in cows fed red clover-based diets

Fatty acid (mg/100 g of total fatty acids)	Treatment ¹						Significance ²			
	Control	RO	SFO	CO	CE	SEM	Oil	MUFA vs. PUFA	18:2 vs. 18:3	Form
<i>cis</i> -9, <i>cis</i> -12 18:2 ³	2,084	1,985	2,547	2,095	1,978	91.5	0.08	<0.001	<0.001	0.16
<i>cis</i> -12, <i>cis</i> -15 18:2	28.8	35.5	30.1	61.4	53.5	2.89	<0.001	0.003	<0.001	0.026
<i>cis</i> -9, <i>trans</i> -12 18:2	30.1	45.2	49.6	50.4	67.3	6.45	0.016	0.51	0.92	0.06
<i>cis</i> -9, <i>trans</i> -13 18:2	276	303	293	339	650	23.3	0.13	0.58	0.11	<0.001
<i>cis</i> -9, <i>trans</i> -14 18:2	141	141	145	152	267	9.2	0.61	0.41	0.52	<0.001
<i>trans</i> -9, <i>cis</i> -12 18:2	29.3	36.3	52.1	38.6	68.7	2.88	<0.001	0.005	<0.001	<0.001
<i>trans</i> -11, <i>cis</i> -15 18:2	153	161	109	226	618	24.0	0.68	0.82	0.003	<0.001
<i>trans</i> -12, <i>cis</i> -15 18:2 ⁴	70.7	69.7	62.8	82.7	128	3.5	0.69	0.29	<0.001	<0.001
<i>trans</i> -9, <i>trans</i> -13 18:2	35.5	59.0	56.8	50.4	79.0	3.47	<0.001	0.23	0.22	<0.001
<i>trans</i> -11, <i>trans</i> -15 18:2	34.3	39.2	36.9	49.6	102	4.19	0.14	0.43	0.049	<0.001
<i>cis</i> -9, <i>trans</i> -11 CLA ⁵	441	560	636	567	1,020	43.9	0.001	0.28	0.13	<0.001
<i>cis</i> -11, <i>trans</i> -13 CLA	1.58	1.28	1.33	2.20	2.79	0.196	0.84	0.048	0.014	0.042
<i>cis</i> -12, <i>trans</i> -14 CLA	0.80	1.12	1.37	2.28	1.08	0.141	<0.001	<0.001	<0.001	<0.001
<i>cis</i> -13, <i>trans</i> -15 CLA	1.09	1.58	1.38	1.13	1.58	0.214	0.17	0.14	0.30	0.08
<i>trans</i> -7, <i>cis</i> -9 CLA	47.0	78.1	63.6	60.4	125	4.88	0.002	0.012	0.62	<0.001
<i>trans</i> -8, <i>cis</i> -10 CLA	11.5	14.5	14.8	13.4	18.4	1.11	0.005	0.65	0.20	<0.001
<i>trans</i> -9, <i>cis</i> -11 CLA	10.5	11.9	12.6	12.1	20.5	1.22	0.21	0.73	0.77	<0.001
<i>trans</i> -10, <i>cis</i> -12 CLA	2.84	3.79	6.91	3.88	7.03	0.582	0.004	0.009	<0.001	<0.001
<i>trans</i> -11, <i>cis</i> -13 CLA	6.21	11.4	9.90	21.3	6.46	1.133	<0.001	0.001	<0.001	<0.001
<i>trans</i> -12, <i>cis</i> -14 CLA	5.12	6.32	5.36	11.0	11.6	0.69	0.002	0.014	<0.001	0.43
<i>trans</i> -6, <i>trans</i> -8 CLA	0.87	0.81	0.85	0.55	0.93	0.091	0.24	0.36	0.039	0.013
<i>trans</i> -7, <i>trans</i> -9 CLA	1.71	2.13	1.96	1.39	2.26	0.311	0.61	0.07	0.06	0.007
<i>trans</i> -8, <i>trans</i> -10 CLA	3.09	3.81	6.28	3.99	4.31	0.275	<0.001	<0.001	<0.001	0.08
<i>trans</i> -9, <i>trans</i> -11 CLA	25.0	24.7	26.0	23.9	26.5	2.15	0.92	0.86	0.12	0.06
<i>trans</i> -10, <i>trans</i> -12 CLA	4.27	6.18	11.87	6.68	9.34	0.617	<0.001	<0.001	<0.001	<0.001
<i>trans</i> -11, <i>trans</i> -13 CLA	18.1	23.8	19.7	36.1	51.3	2.80	0.005	0.14	<0.001	<0.001
<i>trans</i> -12, <i>trans</i> -14 CLA	9.16	12.1	10.6	18.0	15.9	1.21	<0.001	0.009	<0.001	0.025
<i>trans</i> -13, <i>trans</i> -15 CLA	1.09	1.39	1.29	1.97	0.71	0.130	0.009	0.15	0.003	<0.001

¹Refers to red clover silage-based diets containing no additional lipid (control), rapeseed oil (RO), sunflower-seed oil (SFO), camelina-seed oil (CO), or camelina expeller (CE).

²Significance of effects due to plant oil in the diet (Oil; control vs. RO, SFO, and CO); degree of unsaturation of lipid supplement [monounsaturated fatty acids (MUFA) vs. polyunsaturated fatty acids (PUFA); RO vs. SFO and CO]; source of PUFA in the diet (18:2 vs. 18:3; SFO vs. CO); and form of camelina lipid in the diet (Form; CO vs. CE).

³Contains *cis*-9,*cis*-15 18:2 as a minor component.

⁴Co-elutes with *cis*-11 19:1.

⁵Conjugated linoleic acid.

Table 9. Effect of plant oils or camelina expeller on milk 20:1 content in cows fed red clover-based diets

Fatty acid (mg/100 g of total fatty acids)	Treatment ¹						Significance ²			
	Control	RO	SFO	CO	CE	SEM	Oil	MUFA vs. PUFA	18:2 vs. 18:3	Form
<i>cis</i> -9 20:1	327	297	294	581	490	23.0	<0.001	<0.001	<0.001	<0.001
<i>cis</i> -10 20:1	22.9	18.6	21.6	57.3	56.9	4.20	0.035	<0.001	<0.001	0.95
<i>cis</i> -11 20:1	125	111	115	552	545	27.4	<0.001	<0.001	<0.001	0.83
<i>cis</i> -13 20:1	21.9	17.2	16.2	40.2	75.5	3.19	0.46	0.009	<0.001	<0.001
<i>cis</i> -14 20:1	5.98	4.90	5.38	11.4	32.4	1.45	0.45	0.07	0.010	<0.001
<i>trans</i> -6 + 7 + 8 20:1	14.7	12.8	11.8	17.7	20.7	2.75	0.86	0.58	0.16	0.46
<i>trans</i> -9 20:1	16.0	15.0	15.7	45.9	63.2	4.87	0.12	0.022	<0.001	0.027
<i>trans</i> -10 20:1	7.18	6.58	6.98	23.3	29.5	1.43	0.002	<0.001	<0.001	0.002
<i>trans</i> -11 20:1	11.9	10.7	11.0	48.9	64.8	3.93	0.011	<0.001	<0.001	0.006
<i>trans</i> -12 20:1	14.6	14.8	14.8	48.4	59.0	2.69	<0.001	<0.001	<0.001	0.006
<i>trans</i> -13 20:1 ³	15.4	13.9	14.4	42.2	48.6	2.43	0.005	<0.001	<0.001	0.048

¹Refers to red clover silage-based diets containing no additional lipid (control), rapeseed oil (RO), sunflower-seed oil (SFO), camelina-seed oil (CO), or camelina expeller (CE).

²Significance of effects due to plant oil in the diet (Oil; control vs. RO, SFO, and CO); degree of unsaturation of lipid supplement [monounsaturated fatty acids (MUFA) vs. polyunsaturated fatty acids (PUFA); RO vs. SFO and CO]; source of PUFA in the diet (18:2 vs. 18:3; SFO vs. CO); and form of camelina lipid in the diet (Form; CO vs. CE).

³Co-elutes with *cis*-8 20:1.

of diet DM) had no effect on OM or NDF total-tract digestibility consistent with studies examining the effects of RO (Chelikani et al., 2004), SFO (Shingfield et al., 2008a), and linseed oil (Doreau et al., 2009a) on nutrient digestibility in cattle.

The effects of lipid supplements on milk yield and milk fat are variable and known to be dependent on inclusion rate, degree of unsaturation, physical form, and basal diet composition (Lock and Shingfield, 2004; Roy et al., 2006; Shingfield et al., 2010a). In the current study, inclusion of plant lipids in concentrate supplements had no effect on milk yield or milk composition in cows fed red clover silage, possibly because the intake of DM and ME was similar across treatments. Milk protein content is often decreased by inclusion of plant lipids in the diet, which has been attributed to the effects on energy intake and limitations in glucose supply and microbial protein synthesis (Lock and Shingfield, 2004). In the present study, supplementing the diet with plant oils had no effect on milk protein secretion or on ME intake, and resulted in relatively minor alterations in odd- and branched-chain fatty acids in milk, consistent with treatments having rather minor effects on rumen function and the energetic efficiency of microbial protein synthesis.

Effect of Plant Oil Supplementation

Mammary de novo synthesis accounts for all 4:0 to 12:0, most of the 14:0, and about half of 16:0 secreted in milk, whereas all 18-carbon and longer chain fatty acids are derived from the diet or from adipose tissue (Chilliard et al., 2007). In this experiment, plant oils and CE decreased the relative proportions of 6- to 14-carbon fatty acids and 16:0 in milk fat by, on average, 2.8 and 5.4 percentage units, respectively. Many experiments have shown that plant oils and oilseeds in the diet typically lower the concentration of short- and medium-chain SFA in bovine milk (Givens and Shingfield, 2006; Glasser et al., 2008), responses that can be explained by the inhibitory effects of long-chain fatty acids on acetyl-CoA carboxylase activity and the synthesis of SFA de novo in mammary secretory cells (Shingfield et al., 2010a). Comparisons between lipid treatments indicated that the composition or form of lipid in the diet had no substantive effects on the secretion of fatty acids synthesized de novo, but altered the relative abundance of long-chain fatty acids in milk fat, including *trans*-9,*cis*-11 CLA and *trans*-10,*cis*-12 CLA, isomers that inhibit mammary lipogenesis during postruminal infusions (Shingfield and Griinari, 2007).

Supplementing red clover silage-based diets with plant lipids decreased total SFA by, on average, 0.03 percentage units per gram of supplemental fatty ac-

ids; that is, within the range (0.02–0.03) of responses reported to plant oils in cows fed diets based on maize silage (Jenkins, 1998; Hurtaud and Peyraud, 2007), grass silage (Offer et al., 1999; Ryhänen et al., 2005), or grazed grass (Rego et al., 2005, 2009).

In this experiment, milk fat 18:3n-3 content was relatively high and concentrations of *cis*-9 18:1, 18:2n-6, and CLA were rather low compared with earlier studies examining the effects of RO or SFO in the diet (Dewhurst et al., 2006; Givens and Shingfield, 2006; Glasser et al., 2008). A high enrichment of 18:3n-3 in milk for all treatments can be explained by the use of red clover silage as the basal forage. However, milk fat 18:3n-3 concentrations were marginally lower than or similar to values reported in earlier studies in cows fed high proportions of red clover silage and no additional lipid (Dewhurst et al., 2006; Vanhatalo et al., 2007). Red clover silage fed in the present study was prepared from regrowths harvested at a later stage of maturity compared with previous reports, whereas milk fat 18:3n-3 content is known to decrease with advances in the stage of red clover maturity (Vanhatalo et al., 2007).

Plant oil supplementation increased milk fat 18:0, *trans* 18:1 (Δ 4, 6 to 12, and 15), total *trans* fatty acid, *cis*-12 18:1, *cis*-13 18:1, and *cis*-16 18:1 contents by 36, 35, 27, 42, 21, and 13%, respectively. Including plant oils in the diet increased milk fat proportions of *cis*-9 18:1 by approximately 3.6 percentage units, which can be explained by plant oils increasing ruminal outflow of 18:0 or *cis*-9 18:1 and conversion of 18:0 to *cis*-9 18:1 via stearoyl-CoA desaturase in the mammary gland (Shingfield et al., 2010a). Inclusion of plant oils in the diet also enhanced milk fat *cis*-9,*trans*-11 CLA content by, on average, 33%, in agreement with the magnitude of response reported in previous investigations (Lor et al., 2005; Rego et al., 2005, 2009). Enrichment of *cis*-9,*trans*-11 CLA in milk can be attributed to incomplete biohydrogenation of dietary 18-carbon unsaturated fatty acids in the rumen leading to increases in the supply of *trans*-11 18:1 available for desaturation in the mammary gland (Lor et al., 2004; Shingfield et al., 2008a; Doreau et al., 2009b). Furthermore, incremental amounts of SFO in the diet are known to linearly increase ruminal outflow of *cis*-9,*trans*-11 CLA (Shingfield et al., 2008a), implying that a greater supply of preformed *cis*-9,*trans*-11 CLA available at the mammary gland may account for the higher enrichment of this isomer in milk of cows fed the SFO treatment.

Lower concentrations of most odd- and branched-chain fatty acids in milk in response to plant oils are consistent with previous reports in cows fed diets based on grass hay (Lor et al., 2005; Roy et al., 2006), maize silage (Roy et al., 2006; Chilliard et al., 2009), or grazed

grass (Rego et al., 2005, 2009). Odd- and branched-chain fatty acids in milk are derived principally from the digestion of microbial lipids that are synthesized *de novo* in the rumen using short-chain fatty acid precursors (Vlaeminck et al., 2006), indicating that changes in the appearance of fatty acids in milk fat derived from microbial lipids may, at least in part, be related to alterations in the relative abundance of specific populations of bacteria and protozoa in the rumen.

Monounsaturated Versus Polyunsaturated Plant Oils

Despite extensive hydrogenation, small amounts of dietary unsaturated fatty acids can escape the rumen (Loor et al., 2004; Shingfield et al., 2008a; Doreau et al., 2009b). Higher ingestion of *cis*-9 18:1 would account for the increase in milk fat *cis*-9 18:1 content for the RO treatment. Relative to other plant oils, RO in the diet resulted in lower concentrations of milk fat *cis*-12 18:1, 18:2n-6, nonconjugated 18:2, and geometric isomers of Δ 10,12 CLA, differences that can be attributed to a lower intake of dietary PUFA and decreases in the supply of intermediates formed during ruminal hydrogenation of 18:2n-6 and 18:3n-3 available for incorporation into milk fat triacylglycerides (Chilliard et al., 2007; Shingfield et al., 2010a).

Milk fat on the RO treatment contained higher *trans*-4 18:1, *trans*-6 to *trans*-9 18:1, and *trans*-7,*cis*-9 CLA concentrations compared with PUFA-enriched oil supplements, consistent with recent reports on the effects of RO in the diet on bovine milk fat composition (Shingfield et al., 2008b; Givens et al., 2009; Rego et al., 2009). Changes in the relative abundance of these minor fatty acids in milk reflect increased formation of *trans* 18:1 (Δ 6, 7, 10 to 16) during biohydrogenation of *cis*-9 18:1 in the rumen and conversion of *trans*-7 18:1 to *trans*-7,*cis*-9 CLA via the action of stearoyl-CoA desaturase in the mammary gland (Shingfield et al., 2010a). Overall, the effects of RO on milk fatty acid composition relative to SFO and CO were rather small compared with the differences in 18-carbon unsaturated fatty acid intake between experimental treatments.

Sunflower Versus Camelina Oil

Supplementing red clover silage with SFO rather than CO increased proportions of milk fat 18:2n-6 by 0.45 percentage units to a final concentration of 2.55 g/100 g of fatty acids due to a higher 18:2n-6 intake. Marginal increases in milk fat 18:2n-6 content per gram of additional SFO were similar or marginally higher in the present study compared with previous investigations in cows fed diets based on grass silage (Dewhurst

et al., 2006), maize silage (Roy et al., 2006), grass hay (Collomb et al., 2004), or fresh pasture (Rego et al., 2009).

Concentrations of specific 18:1, CLA, and nonconjugated 18:2 isomers in milk differed between SFO and CO treatments and can be explained by the formation of various intermediates during the biohydrogenation of 18:2n-6 and 18:3n-3 in the rumen (Chilliard et al., 2007; Shingfield et al., 2010a). Inclusion of SFO in the diet was associated with enhanced milk fat *trans*-8,*trans*-10 CLA, *trans*-10,*cis*-12 CLA, and *trans*-10,*trans*-12 CLA concentrations consistent with earlier observations (Chilliard et al., 2007; Shingfield et al., 2008a; Rego et al., 2009). The concentrations of almost all the other 18:2 fatty acids, Δ 11,15 18:2, Δ 12,15 18:2, Δ 11,13 CLA, Δ 12,14 CLA, and Δ 13,15 CLA in particular, were higher in milk from cows fed CO, which is in agreement with the changes in milk fatty acid composition reported in cows fed linseed oil supplements as a source of 18:3n-3 in the diet (Collomb et al., 2004; Shingfield et al., 2008b; Rego et al., 2009). Relative proportions of 18:3n-3 and *cis*-15 18:1 were greater in milk from cows fed diets containing CO that also reflects the higher intake of 18:3n-3.

Supplementing red clover-based diets with CO increased milk fat 18:3n-3 concentrations by, on average, 0.18 percentage units compared with SFO to a final concentration of 1.17 g/100 g of fatty acids. Marginal increases in 18:3n-3 content on the CO treatment are similar to those reported for camelina meal and camelina seeds in cows fed maize silage-based diets (Hurtaud and Peyraud, 2007). However, the absolute concentration of 18:3n-3 in milk on diets camelina containing was considerably lower in earlier investigations (on average 0.34 g/100 g of fatty acids), highlighting the effect of red clover silage on milk 18:3n-3 content across all treatments in this experiment. Furthermore, the relative abundance of milk fat *trans* 18:1, and *trans*-10 in particular, was several-fold lower in the present study compared with that reported previously for milk from cows fed diets containing camelina lipids (Hurtaud and Peyraud, 2007). It is probable that between-study variations in the concentration and distribution of 18:1 isomers are related to differences in the composition of basal diet, including the relative proportions of starch and NDF (Shingfield and Grinari, 2007).

Milk fat concentration ratios of product:substrate for stearoyl-CoA desaturase for fatty acids containing 10- and 18-carbon fatty acids were higher on the CO treatment, in agreement with earlier investigations of camelina lipid in the diet (Hurtaud and Peyraud, 2007), but the reasons for this are not obvious. Increased intakes of *cis*-11 20:1 on the CO diet appear to be a

plausible explanation for the higher concentrations of 20-carbon fatty acids in milk fat relative to other plant oil supplements.

Camelina Oil Versus Camelina Expeller

Direct comparisons of CO and CE treatments are confounded because of differences in the source of protein in the diet. Nevertheless, rapeseed meal and CE could be expected to be of equal biological value, given the similarity in CP content and amino acid composition between these oilseed crops (Zubr, 2003). These considerations are also supported by the lack of differences in milk protein content and daily protein yield for CO and CE.

The form of camelina in the diet had a major effect on the concentration of 18-carbon fatty acids in milk fat, but concentrations of the sum of 4- to 14-carbon fatty acids and that of 16:0 were unaltered. Milk 18:3n-3 content was lower on CE than CO due to a lower 18:3n-3 intake. Concentrations of 18:0 and *cis*-9 18:1 were also lower (−2.53 and −3.00 percentage units, respectively), but those of *cis*-12 18:1, *cis*-13 18:1, *cis*-15 18:1, *trans* 18:1 (Δ 4, 6 to 15), *cis*-9,*trans*-13 18:2, and Δ 11,15 18:2 were higher for CE compared with CO, differences that point toward the biohydrogenation of 18-carbon unsaturated fatty acids to 18:0 in the rumen being less complete when camelina lipid is supplied in the diet as an expeller rather than free oil. The approximately 2-fold increase in *trans*-10 18:1, *trans*-11 18:1, and *cis*-9,*trans*-11 CLA content in milk for the CE than CO diet in spite of the lower PUFA intake is consistent with this suggestion.

The presence of a physically disrupted seed coat in oilseed expellers might afford a degree of protection to unsaturated fatty acids from hydrogenation in the rumen. Direct comparisons of fatty acid flow at the duodenum (Doreau et al., 2009b) or milk fat composition responses to supplements of rapeseed (Givens et al., 2009) and linseed (Chilliard et al., 2009) lipids are consistent with the form of oil in the diet altering the supply of specific long-chain fatty acid precursors available for milk fat synthesis. However, the efficiency of transfer of 18:2n-6 and 18:3n-3 from the diet into milk was comparable between CE and CO (approximately 11.4 and 7.9%, respectively), which would tend to imply no substantive differences in the first committed steps in the hydrogenation of PUFA in CE or CO in the rumen.

One possible explanation for the differences in milk fat composition between CE and CO treatments is that camelina oilseeds contain components that inhibit the complete biohydrogenation of 18-carbon unsaturated fatty acids to 18:0 in the rumen. Previous in-

vestigations have reported similar changes in milk fat 18-carbon fatty acids during comparisons of camelina, rapeseed, and linseed expeller in the diet (Mihhejev et al., 2007). Further studies are required to substantiate these considerations and identify potential compounds in camelina oilseeds with potential bioactivity in the rumen. Lower concentrations of 20:0 and *cis*-9 20:1 in milk fat for CE compared with CO, coupled with the higher *trans* 20:1 enrichment, are also consistent with a higher ruminal outflow of biohydrogenation intermediates in cows fed CE than CO.

Relative to CO, CE increased milk *cis*-10 16:1 and *trans*-6 to *trans*-11 16:1 concentrations. No 16-carbon unsaturated fatty acids other than *cis*-9 16:1 were detected in feed ingredients, indicating that the 16:1 isomers in milk fat arise by the hydrogenation of *cis*-9 16:1 in the rumen, by β -oxidation of 18:1 fatty acids, or via both mechanisms (Destailats et al., 2000). Recent studies have shown that ruminal digesta of sheep (Toral et al., 2010) and duodenal digesta in cattle (Shingfield et al., 2010b) contain several *cis* and *trans* 16:1 isomers. Milk fat also contained 8-O-16:0 and 10-O-16:0. It is possible that 10-O-16:0 in milk is derived from the hydration of *cis*-9 16:1 in the rumen via a mechanism analogous to that reported for *cis*-9 18:1 (Jenkins et al., 2006; McKain et al., 2010), whereas 8-O-16:0 may originate from β -oxidation of 10-O-18:0, an intermediate of ruminal *cis*-9 18:1 metabolism.

Milk Sensory Characteristics and Nutritive Value

Supplementing diets with SFO elevated milk peroxide concentrations compared with CO, but the magnitude of these changes was not associated with detectable differences in taste panel scores. Camelina oil is rich in α - and γ -tocopherols (Zubr and Matthäus, 2002), which may account for the lower concentrations of peroxides in milk fat for CO relative to SFO. Even though peroxides formed during the initial stages of lipid peroxidation can be converted to secondary compounds, along with the relatively short storage of raw milk and anhydrous milk fat in the present study, current data are consistent with milk from cows fed CO being less susceptible to oxidation and development of off-flavors compared with SFO.

Milk and dairy products are a significant source of fatty acids in the Western diet (Givens and Shingfield, 2006) and therefore interest is increasing in developing nutritional strategies to decrease the concentration of medium-chain SFA and enhance *cis*-9 18:1, *cis*-9,*trans*-11 CLA, and 18:3n-3 contents of ruminant-derived foods with the potential to improve long-term human health. Replacing SFA and *trans* unsaturated fats with *cis*-containing MUFA and PUFA is thought to be more effec-

tive in preventing coronary heart disease than reducing overall fat intake (Hu et al., 1997). Replacing 2.2 g of SFA consumption (equivalent to 7.6% of average daily SFA intake in European populations) with MUFA and PUFA is predicted to lower total blood cholesterol by 0.06 mmol/L and thereby result in 3,000 (−0.77%) and 9,800 (−1.67%) fewer deaths to stroke and coronary heart disease, respectively, in the EU-15 member states (Lloyd-Williams et al., 2008). Recent predictions of the effect of decreasing milk fat SFA content from 70 to 55% (−8.8% in daily SFA intake) and increasing *cis* MUFA from 20 to 32% suggest that implementation across the whole food chain may lower deaths from coronary heart disease in Europe by between 1.96 and 3.91% (Givens, 2008). In the current study, inclusion of moderate amounts of plant oils or CE in diets based on red clover silage was associated with decreases in milk fat total SFA, 12:0, 14:0, and 16:0 contents of 8.5, 18.4, 9.6, and 16.9%, respectively. Assuming that the contribution of milk and dairy products to total SFA consumption remains at levels of around 41.0% (Givens and Shingfield, 2006), a larger reduction in total milk SFA of 18.5% would be required to realize the expected benefits on mortality rates in Europe.

In the present experiment, supplementing red clover silage-based diets with CO represented the most effective strategy to enhance milk fat 18:3n-3 content, but the magnitude of increase was relatively minor. Nutritional adequacy of 18:3n-3 in humans is thought to vary between 1.4 to 3.0 g/d (Gebauer et al., 2006), which would correspond to a daily consumption of milk fat from dairy products or fresh milk from cows fed the CO treatment of between 129 and 273 g or 3.2 and 6.9 L, respectively. However, milk and dairy products are not the primary source of 18:3n-3 in the human diet and contribute about 9% to total 18:3n-3 intake in the Western diets (Gebauer et al., 2006), indicating that much lower amounts of dairy products from CO milk would be required to attain nutritional adequacy.

CONCLUSIONS

Moderate inclusion of plant oils or CE in red clover silage-based diets had no negative effect on silage DMI, milk yield, or milk composition, but did alter milk fatty acid composition (decreases in 12:0, 14:0, 16:0, and total SFA concentrations) and enrich the content of unsaturated fatty acids inherent to lipid supplements. For all treatments, milk fat 18:3n-3 content was relatively high due to the use of red clover silage as the basal forage, but further increases to oil supplements or CE were marginal. Rapeseed oil in the diet increased milk fat *trans*-4, *trans*-6 to *trans*-9 18:1, and *trans*-7,*cis*-9 CLA content. Supplements of SFO specifically enriched

trans-8,*trans*-10 and Δ 10,12 CLA in milk, whereas CO enhanced milk fat *cis*-15 18:1, Δ 11,15 18:2, Δ 12,15 18:2, Δ 11,13 CLA, and Δ 12,14 CLA contents. Comparisons of milk fat composition responses between expeller and oil provided evidence to suggest that camelina seeds may contain one or more components that inhibits the complete biohydrogenation of unsaturated fatty acids in the rumen. Feeding camelina as an expeller rather than an oil increased the concentration of numerous biohydrogenation intermediates in milk, including *trans*-11 18:1 and *cis*-9,*trans*-11 CLA.

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