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Fat source and dietary forage-to-concentrate ratio influences milk fatty-acid composition in lactating cows

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On the basis of the potential benefits to human health there is an increased interest in producing milk containing lower-saturated fatty acid (SFA) and higher unsaturated fatty acid (FA) concentrations, including cis*-9 18:1 and* cis*-9,* trans*-11-conjugated linoleic acid (CLA). Twenty-four multiparous Holstein cows were used in two experiments according to a completely randomized block design, with 21-day periods to examine the effects of incremental replacement of prilled palm fat (PALM) with sunflower oil (SFO) in high-concentrate diets containing 30 g/kg dry matter (DM) of supplemental fat (Experiment 1) or increases in the forage-to-concentrate (F : C) ratio from 39 : 61 to 48 : 52 of diets containing 30 g/kg DM of SFO (Experiment 2) on milk production, digestibility and milk FA composition. Replacing PALM with SFO had no effect on DM intake, but tended to increase organic matter digestibility, yields of milk, protein and lactose, and decreased linearly milk fat content. Substituting SFO for PALM decreased linearly milk fat 8:0 to 16:0 and* cis*-9 16:1, and increased linearly 18:0,* cis*-9 18:1,* trans*-18:1 (Δ4 to 16), 18:2 and CLA concentrations. Increases in the F : C ratio of diets containing SFO had no effect on intake, yields of milk, milk protein or milk lactose, lowered milk protein content in a quadratic manner, and increased linearly NDF digestion and milk fat secretion. Replacing concentrates with forages in diets containing SFO increased milk fat 4:0 to 10:0 concentrations in a linear or quadratic manner, decreased linearly* cis*-9 16:1,* trans*-6 to -10 18:1, 18:2n-6,* trans*-7,* cis*-9 CLA,* trans*-9,* cis*-11 CLA and* trans*-10,* cis*-12 CLA, without altering milk fat 14:0 to 16:0,* trans*-11 18:1,* cis*-9,* trans*-11 CLA or 18:3n-3 concentrations. In conclusion, replacing prilled palm fat on with SFO in high-concentrate diets had no adverse effects on intake or milk production, other than decreasing milk fat content, but lowered milk fat medium-chain SFA and increased* trans *FA and polyunsaturated FA concentrations. Increases in the proportion of forage in diets containing SFO increased milk fat synthesis, elevated short-chain SFA and lowered* trans *FA concentrations, without altering milk polyunsaturated FA content. Changes in fat yield on high-concentrate diets containing SFO varied between experiments and individual animals, with decreases in milk fat secretion being associated with increases in milk fat* trans*-10 18:1,* trans*-10,* cis*-12 CLA and* trans*-9,* cis*-11 CLA concentrations.*

Keywords: conjugated linoleic acid, dairy cow, milk fat, saturated fatty acid, *trans* fatty acid

Implications

Certain saturated fatty acids (SFA) are thought to increase the risk of cardiovascular diseases and insulin resistance in humans. The present study examined the potential of lowering milk SFA concentrations through altering the diet of dairy cows under two scenarios relevant to commercial milk production. Results demonstrated the feasibility of decreasing SFA and increasing unsaturated fatty acid (FA) in milk, but these were also accompanied by increases in *trans* FA concentrations. Overall, it was possible to alter milk FA composition consistent with the potential to lower the disease risk of consumers, without adverse effects on animal performance.

Introduction

Ruminant milk fat is relatively rich in saturated fatty acids (SFA) and contains low proportions of polyunsaturated fatty acids (PUFA). Clinical and biomedical studies have demonstrated that when consumed in excess, SFA, 12:0, 14:0 and 16:0, in particular, increase cardiovascular disease risk, with evidence to suggest an involvement in the development of

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insulin resistance and dyslipidaemia in humans (WHO, 2003). Milk from ruminants also contains several bioactive lipids including 4:0, several odd- and branched-chain fatty acids (OBCFA), *cis*-9 18:1 and *cis*-9, *trans*-11-conjugated linoleic acid (CLA) with a potential to maintain human health and prevent the onset and development of chronic diseases (Shingfield *et al*., 2008b). In most developed countries, milk and dairy products are the principle source of 12:0, 14:0 and *cis*-9, *trans*-11 CLA in the human diet, and contribute to 16:0 consumption (Shingfield *et al*., 2013). Developing systems for the production of milk containing lower medium-chain SFA and higher OBCFA, *cis*-9 18:1, *cis*-9, *trans*-11 CLA and PUFA concentrations may offer benefits to long-term human health without requiring changes in consumer eating habits.

Fat supplements are often used to increase the energy content of diets fed to lactating cows. Owing to cost and attempts to avoid adverse effects on nutrient digestion in the rumen, palm-based supplements enriched in 16:0 are often fed. Supplements of plant oils or oilseeds rich in 18-carbon unsaturated fatty acids (FA) lower SFA and increase unsaturated FA in bovine milk, with lipid rich in 18:2n-6 being particularly effective for increasing *cis*-9, *trans*-11 CLA concentrations (Palmquist *et al*., 2005; Chilliard *et al*., 2007). However, the magnitude of milk FA composition responses varies according to several factors including the amount and FA profile of lipid supplements and composition of the basal diet (Chilliard *et al*., 2007; Glasser *et al*., 2008a). Furthermore, changes in milk fat composition to increased unsaturated FA supply do not occur in isolation, but may be accompanied by alterations in milk fat secretion. Replacing forages with concentrate ingredients in diets containing plant oils often results in a decrease in milk fat content and secretion (Bauman and Griinari, 2003; Shingfield and Griinari, 2007).

Dietary supplements of plant oils and oilseeds typically increase *trans* FA (TFA) concentrations in bovine milk (Chilliard *et al*., 2007; Shingfield *et al*., 2013). Although the evidence on ruminant-derived TFA on cardiovascular disease risk is inconsistent (Shingfield *et al*., 2008b; Brouwer *et al*., 2010), relatively few studies (Roy *et al*., 2006; Rego *et al*., 2009; Halmemies-Beauchet-Filleau *et al*., 2011) have characterized changes in milk FA composition to plant oils enriched in 18:2n-6 in sufficient detail to allow definitive conclusions on the impact of the distribution of individual TFA isomers to be drawn.

The present study examined the effects of replacing prilled palm fat (PALM) with sunflower oil (SFO) enriched in 18:2n-6 in high-concentrate diets (Experiment 1), and increases in the forage : concentrate (F : C) ratio of diets containing SFO (Experiment 2) under conditions relevant to commercial milk production on animal performance, nutrient digestion and milk fat composition, with specific emphasis on TFA and CLA isomer concentrations. To meet these objectives, the amount of supplemental fat was restricted to 30 g/kg diet DM, whereas differences in the F : C ratio of experimental diets were limited from 39 : 61 to 48 : 52 to resemble the type of diet composition changes that could be expected on-farm.

Material and methods

Experimental design, animals and management

Two experiments were conducted at the University of Tehran Dairy Research Farm (Karaj, Iran). All experimental procedures were in accordance with the guidelines for the use and care of experimental animals and approved by the University of Tehran Animal Ethics Committee. Twenty-four multiparous Holstein cows averaging (mean ± s.e.) 618 ± 9.19 kg live weight, 3.2 ± 0.22 parity and 90 ± 6.04 days in lactation producing 35.8 ± 0.88 kg milk/day were used in Experiment 1. Experiment 2 followed Experiment 1 after a 14-day washout, using 19 of the 24 cows recruited to the first experiment and five additional cows averaging 619 ± 15.1 kg live weight, 3.0 ± 0.24 parity and 107 ± 6.22 days in lactation producing 35.5 ± 0.89 kg milk/day. During the transition between experiments, cows were offered a standard total mixed ration (TMR) that comprised lucerne hay and maize silage (5:4 wt/wt on a dry matter (DM) basis), concentrates (F : C ratio 39 : 61; DM basis) and 18 g/kg diet DM of PALM. Both experiments were conducted according to a completely randomized block design. Within each block, cows were randomly assigned to one of the three treatments. Each experiment comprised 3-day covariate, 4-day adaptation and 21-day interval for experimental measurements and sampling. Cows were housed in individual tie-stalls with continuous access to fresh water and milked three times daily at 0100, 1000 and 1700 h.

Experimental diets

For Experiment 1, treatments comprised TMR containing a mixture of lucerne hay and maize silage (5 : 4 wt/wt; DM basis), concentrates (F : C ratio 39 : 61; DM basis) and 30 g/kg DM of fat prills prepared from fractionated refined palm oil (RumiFat R100, Ecolex, Selangor, Malaysia), refined SFO (Oila-Golrang Pakhsh Co., Tehran, Iran) or a mixture (1 : 1 wt/wt on a fresh weight basis) of PALM and SFO (treatments HPO, HSO and HPS, respectively; Table 1). In the 2nd experiment, treatments comprised TMR based on lucerne hay and maize silage, concentrate ingredients and 30 g/kg DM of SFO with a F : C ratio (on a DM basis) of 39 : 61 (HSO), 44 : 56 (MSO) or 48 : 52 (LSO). The relative proportions of lucerne hay and maize silage in the forage component of each TMR in Experiment 2 were adjusted in accordance with differences in the F : C ratio to ensure that treatments contained the same amount of 18:2n-6 (Table 1). All diets were formulated using the Cornell–Penn–Miner system (CPM-Dairy, version 3.0.7) to meet or exceed the metabolizable energy and protein requirements of cows in mid-lactation producing 40 kg milk/day. Daily rations in both experiments were offered as equal meals at 0800 and 1600 h in amounts to ensure *ad libitum* intakes. Ration mixes were adjusted weekly for changes in component DM content.

Measurements and sampling

Intakes of individual cows were recorded daily. Samples of fresh TMR and refused feeds were collected three times weekly.

Table 1 *Formulation of experimental diets (g/kg DM)*

	Experiment 1[1]			Experiment 2[2]		
Ingredients	HPO	HPS	HSO	HSO	MSO	LSO
Lucerne hay	217	217	217	217	247	276
Maize silage	174	174	174	174	189	205
Ground barley	260	260	260	260	203	146
Ground corn	52	52	52	52	42	31
Molassed beet pulp	69	69	69	69	65	59
Wheat bran	_	_	_	_	28	58
Corn gluten meal	11	11	11	11	7	3
Solvent-extracted soya bean meal	87	87	87	87	77	68
Solvent-extracted rapeseed meal	69	69	69	69	80	92
Prilled palm fat	30	15	_	_	_	_
Sunflower oil	_	15	30	30	30	30
Limestone	10	10	10	10	11	11
Salt	3	3	3	3	3	3
Sodium bicarbonate	13	13	13	13	13	13
Mineral and vitamin premix[3]	4	4	4	4	4	4
Rumen-protected methionine[4]	1	1	1	1	1	1

[1]Treatments consisted of high-concentrate diets containing 30 g/kg DM of supplemental fat in the form of prilled palm fat (HPO), sunflower oil (HSO) or an equal mixture of both fat supplements (HPS).
[2]Treatments consisted of diets containing 30 g/kg dry matter (DM) of sunflower oil with forage-to-concentrate ratio (on a DM basis) of 39 : 61 (HSO), 44 : 56 (MSO) or 48 : 52 (LSO).
[3]Declared as containing (g/kg DM) Ca (180), P (70), Mg (30), Na (50), Fe (4), Cu (3), Zn (3), Mn (5), I (0.1), Co (0.1), Se (0.02), antioxidant (0.4); (IU/g), retinyl acetate (400), cholecalciferol (100) and dl-α-tocopheryl acetate (0.2).
[4]Rumen-protected methionine (Methioplus; Soda Feed Ingredients, Monaco, France).

Once collected, feed samples were dried in a forced-air oven at 60°C for 48 h, milled through a 1 mm screen (Retsch mill; Retsch Co., Haan, Germany) and composited until submitted for chemical analysis. Daily milk yields were recorded throughout both experiments. Milk samples were collected at each milking for all cows on alternate days throughout each experiment, preserved with potassium dichromate (Merck KGaA, Darmstadt, Germany) until analysed for fat, CP, lactose and urea by infrared analysis (MilkoScan 4000; Foss, Hillerød, Denmark). Unpreserved samples of milk were also collected at each milking on days 15, 17 and 19 of each experiment, stored at –20°C, thawed at 38°C, composited according to yield and analysed for FA composition. Spot faecal samples were taken on day 18 of each experiment at 4 h intervals over a 24 h interval, starting at 0830 h and stored at –20°C until submitted for chemical analysis. Apparent whole-tract digestibility was estimated using acid-insoluble ash as an internal marker (Van Keulen and Young, 1977).

Chemical analysis

The DM content of feeds and faeces was determined by oven drying at 105°C for 24 h. Ash was determined by combustion at 550°C for 6 h. Concentrations of acid-insoluble ash were determined following sequential acid hydrolysis (Van Keulen and Young, 1977). Nitrogen (N) was determined by the Kjeldahl method (Kjeltec 1030 Autoanalyzer; Foss Tecator AB, Höganäs, Sweden) and ether extract (EE) using an automated Soxtec System (Soxtec 1043, Foss). NDF was determined using heat-stable α-amylase and sodium sulfite in an Ankom^{200} Fiber Analyzer (Ankom Technology Corp., Fairport, NY, USA) and reported on an ash-free basis. Starch was determined using an amyloglucosidase-based assay (Salo and Salmi, 1968). Concentration of indigestible NDF in samples of TMR and faeces was determined in duplicate following 288-h *in situ* ruminal incubations using 12 μm pore size polyester bags (Saatifil PES 12/6; Saatitech S.p.A., Veniano, Como, Italy) in two non-lactating cows offered grass silage and 1 kg/day of a commercial compound feed (Solid 220; Lantmännen Lantbruk AB, Stockholm, Sweden).

Lipid analysis

Fatty-acid methyl esters (FAME) in dried ground samples of TMR and samples of PALM and SFO lipid supplements were prepared in a one-step extraction–transesterification procedure using 2% (vol/vol) methanolic sulphuric acid as a catalyst and tritridecanoin (T-135; Nu-Check Prep Inc., Elysian, MN, USA) as an internal standard (Halmemies-Beauchet-Filleau *et al.*, 2011). Lipid in 1 ml of milk was extracted in duplicate using a mixture of ammonia, ethanol, diethylether and hexane (0.2 : 1 : 2.5 : 2.5, vol/vol, respectively) and transesterified to FAME using methanolic sodium methoxide (Halmemies-Beauchet-Filleau *et al.*, 2011). The FAME prepared from feeds and milk fat were quantified using a gas chromatograph (model 6890; Hewlett-Packard, Wilmington, DE, USA) equipped with a flame-ionization detector and 100-m fused silica capillary column (CP-SIL; Chromopack 7489, Middelburg, the Netherlands). Total

FAME profile in a 2-μl sample at a split ratio of 1 : 50 was determined using a temperature gradient programme and hydrogen as a carrier gas (Halmemies-Beauchet-Filleau *et al.*, 2011). Isomers of 18:1 were further resolved in a separate analysis under isothermal conditions at 170°C (Halmemies-Beauchet-Filleau *et al.*, 2011). Peaks were identified by comparison of retention times with authentic FAME standards (Halmemies-Beauchet-Filleau *et al.*, 2011). Methyl esters not contained in commercially available standards were identified by GC-MS analysis of 4,4-dimethyloxazoline (DMOX) derivatives prepared from FAME. Preparation of DMOX derivatives, parameters used for GC-MS analysis and interpretation mass spectra were in accordance with earlier reports (Halmemies-Beauchet-Filleau *et al.*, 2011). The distribution of CLA isomers in milk fat was determined by HPLC (model 1090; Hewlett-Packard) using four silver-impregnated silica columns (Chrom-Spher 5 Lipids, 250 × 4.6 mm, 5 μm particle size; Varian Ltd., Walton-on-280, Thames, UK) coupled in series and 0.1% (vol/vol) acetonitrile in heptane as the mobile phase (Halmemies-Beauchet-Filleau *et al.*, 2011). Milk FA composition was expressed as a weight percentage of total FA using theoretical relative response factors to account for the carbon deficiency in the flame-ionization detector response for FAME containing 4- to 10-carbon atoms (Halmemies-Beauchet-Filleau *et al.*, 2011). Concentrations of CLA isomers were calculated on the basis of proportionate peak area responses determined by 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

Intake, milk yield and milk composition data during the covariate period and the last week of each experiment were averaged before analysis by ANOVA for a completely randomized block design, using a mixed model that included the fixed effects of treatment, block and covariate, and cow within treatment and block as a random effect (Statistical Analysis Systems software package version 9.1; SAS Institute, Cary, NC, USA). The effect of block was removed from the model when non-significant ($P > 0.10$). Nutrient digestibility and milk FA composition data were analysed using the same model without covariate. Sums of squares for treatment effects were further separated using orthogonal contrasts into a single degree of freedom comparisons to test for the significance of linear and quadratic components of the response to replacing PALM with SFO or increases in dietary F : C ratio. Least square means are reported and treatment effects were declared significant at $P < 0.05$, with *P*-values between 0.05 and 0.10 considered a trend towards significance.

Associations between milk fat yield and the concentration of specific FA in milk fat based on 48 observations, 24 made during the last week of experiments 1 and 2, respectively, were explored by partial least square regression (PLSR) using the Unscrambler X (version 10.0.1®; Camo, Oslo, Norway). The relationship between the response variables (*Y*-variables; milk fat content and yield as g/kg and g/day, respectively) and explanatory variables (*X*-variables; milk FA composition as g/100 g of FA) was examined by two-block bilinear modelling:

$$X = x + T_A P_A{}' + E_A \text{ and } Y = y + T_A Q_A{}' + F_A$$

where a set of *X*-variables collected in the *X* matrix were decomposed into a *Y*-relevant score matrix for *A* factors, T_A, which were used to model both *X* and *Y* in terms of the loading matrices, $P_A{}'$ and $Q_A{}'$, and their residuals, E_A and F_A. Variables in the PLSR analysis were standardised to a mean of zero and an initial standard deviation of one. The optimal number of factors in the model was defined by the minimum root mean square error in the calibration and cross-validated model. Only variables at $P < 0.05$ were retained in the model, as estimated by jackknifing, and the model was verified by a full cross-validation (Martens and Martens, 2000).

Results

Nutrient intake, digestibility, milk yield and milk composition

The PALM supplement predominated in 16:0 and *cis*-9 18:1, whereas SFO was rich in 18:2n-6 and relatively abundant in *cis*-9 18:1 and 16:0 (Table 2). Substituting PALM with SFO (Experiment 1) progressively increased *cis*-9 18:1, 18:2n-6, monounsaturated FA (MUFA) and PUFA, and decreased 16:0 and SFA in the diet (Table 2). Incremental replacement of PALM with SFO had no effect ($P > 0.10$) on DM, organic matter (OM), CP, starch, metabolizable energy (ME) or total FA ingestion, but increased ($P < 0.05$) NDF and pdNDF intake in a quadratic manner (Table 3). By design, incremental replacement of PALM with SFO progressively decreased ($P < 0.01$) 16:0 and increased ($P < 0.01$) *cis*-9 18:1 and 18:2n-6 intake (Table 3). Substitution of SFO for PALM linearly increased ($P < 0.05$) apparent total-tract EE digestibility, NDF digestibility in a quadratic manner ($P < 0.05$), and tended ($P = 0.09$) to increase OM digestion, but had no effect ($P > 0.10$) on total-tract N or pdNDF digestibility (Table 3). Replacing PALM with SFO tended ($P < 0.09$) to increase the yields of milk, protein and lactose, linearly decreased ($P < 0.01$) milk fat content, but had no effect ($P > 0.10$) on milk urea concentration or milk fat secretion (Table 3).

Increases in the F : C ratio (Experiment 2) resulted in higher dietary NDF and potential digestible NDF (pdNDF) and lower starch concentrations (Table 2). Higher proportions of forage in the diet linearly increased ($P < 0.01$) NDF and pdNDF intake, linearly decreased ($P < 0.01$) starch ingestion, increased ($P < 0.01$) ME intake in a quadratic manner, but had no effect ($P > 0.10$) on DM, OM or total FA consumption (Table 3). Replacing concentrate ingredients with forages linearly increased ($P < 0.01$) 18:3n-3 intake, tended to increase ($P = 0.05$) 18:2n-6 ingestion in a quadratic manner, but had no influence ($P > 0.10$) on the consumption of 16:0 or *cis*-9 18:1 (Table 3). Increases in dietary F : C ratio had no effect ($P > 0.10$) on apparent total-tract OM digestibility, but linearly increased ($P < 0.05$) NDF and EE digestibility and tended ($P < 0.07$) to increase N and pdNDF digestion

Table 2 *Chemical composition of experimental diets and fat supplements (g/kg DM, unless otherwise stated)*

	Experiment 1[1]			Experiment 2[2]			Fat supplements	
Items	HPO	HPS	HSO	HSO	MSO	LSO	Prilled palm fat	Sunflower oil
DM (g/kg as fed)	532	536	523	513	504	492		
OM	911	909	912	919	910	912		
CP	167	169	164	161	160	164		
NDF	293	282	306	304	326	357		
Potentially digestible NDF	185	177	197	189	201	211		
Starch	200	194	193	185	156	141		
Total FA	71.2	73.5	68.0	65.0	65.4	66.3		
FA composition (g/100 g FA)								
12:0	0.17	0.21	0.15	0.13	0.13	0.15	0.09	0.004
14:0	0.88	0.67	0.37	0.33	0.33	0.33	1.26	0.08
16:0	49.3	35.8	18.4	16.2	15.9	15.2	74.4	8.21
cis-9 16:1	0.22	0.20	0.23	0.24	0.25	0.26	0.06	0.08
18:0	4.11	4.23	4.25	4.82	4.63	4.45	4.88	4.04
cis-9 18:1	16.1	18.8	21.6	24.1	23.8	23.8	15.1	23.1
cis-11 18:1	1.26	1.29	1.50	1.51	1.54	1.74	0.13	0.92
18:2n-6	19.5	30.2	43.4	42.5	42.2	43.0	2.82	58.3
18:3n-3	4.63	4.27	4.79	4.53	5.02	5.40	0.11	2.96
20:0	0.43	0.40	0.46	0.48	0.50	0.48	0.33	0.29
cis-9 20:1	nd	nd	nd	nd	nd	nd	0.01	0.12
cis-11 20:1	0.19	0.21	0.26	0.23	0.24	0.23	nd	0.02
22:0	0.35	0.50	0.73	0.81	0.82	0.81	0.05	0.58
cis-13 22:1	0.12	0.12	0.14	0.10	0.10	0.07	nd	nd
24:0	0.32	0.36	0.44	0.45	0.50	0.49	0.06	0.20
Total SFA	56.4	43.1	25.8	24.3	24.5	23.0	81.4	13.6
Total MUFA	18.9	21.7	25.1	27.5	27.4	27.6	15.6	24.4
Total PUFA	24.3	34.8	48.6	47.6	47.5	48.8	3.05	61.9

DM = dry matter; OM = organic matter; FA = fatty acids; MUFA = monounsaturated FA; nd = not detected; PUFA = polyunsaturated FA; SFA = saturated FA.
[1]Treatments consisted of high-concentrate diets containing 30 g/kg DM of supplemental fat in the form of prilled palm fat (HPO), sunflower oil (HSO) or an equal mixture of both fat supplements (HPS).
[2]Treatments consisted of diets containing 30 g/kg DM of sunflower oil with forage-to-concentrate ratio (on a DM basis) of 39 : 61 (HSO), 44 : 56 (MSO) or 48 : 52 (LSO).

(Table 3). Increases in the F : C ratio had no effect ($P > 0.10$) on milk yield or milk protein and lactose output, but linearly increased ($P < 0.05$) milk fat secretion and milk urea concentration and lowered ($P < 0.05$) milk protein content in a quadratic manner (Table 3).

Milk FA composition

Incremental replacement of PALM with SFO linearly decreased ($P < 0.01$) total milk fat SFA and linearly increased ($P < 0.01$) total MUFA, CLA, 18:2 and TFA concentrations (Table 4). Substitution of PALM for SFO increased ($P < 0.05$) 4:0 in a quadratic manner, linearly decreased ($P < 0.05$) 8:0 to 16:0 and linearly increased ($P < 0.01$) 18:0, but had no effect ($P > 0.10$) on 6:0 or 18:3n-3 concentrations (Table 4). Overall, replacing PALM with SFO linearly decreased ($P < 0.01$) the secretion of 4- to 16-carbon FA and linearly increased ($P < 0.01$) the output of ⩾18-carbon FA in milk (Figure 1). Replacing PALM with SFO linearly decreased ($P < 0.01$) milk fat *cis*-9 16:1 and *cis*-13 16:1 and linearly increased ($P < 0.01$) *cis*-16:1 (Δ10 and 12) and *trans*-16:1 (Δ6 to 11 and 13) concentrations (Supplementary Table S1). Furthermore, SFO linearly increased ($P < 0.01$) the relative abundance of *cis*-18:1 (Δ9, 12 to 16) and *trans*-18:1 (Δ4 to 16) in milk fat (Table 5). Substitution of PALM with SFO linearly increased ($P < 0.05$) several PUFA, including Δ9,12 18:2, *cis*-9, *trans*-13 18:2, *cis*-9, *trans*-14 18:2, *cis*-9, *trans*-11 CLA, *cis*-11, *trans*-13 CLA, and several minor *trans*, *cis* (7,9; 9,11; 10,12; 11,13 and 13,15) and *trans*, *trans* (7,9; 8,10; 9,11; 10,12 and 12,14) CLA isomers (Table 6). Replacing PALM with SFO also altered the relative abundance of OBCFA in milk, changes characterized as linear decreases ($P < 0.05$) in 13:0 *anteiso*, 15:0, *cis*-9 15:1, 17:0, *cis*-6 + 7 17:1, *cis*-9 17:1, 11-cyclohexyl 11:0, *cis*-10 19:1 and 23:0, and linear or quadratic increases ($P < 0.05$) in 14:0 *iso* and *trans*-9 15:1 concentrations
(Supplementary Table S2).

Increases in the F : C ratio of diets containing SFO tended ($P < 0.07$) to increase total SFA and decrease total MUFA, but had no effect ($P > 0.10$) on PUFA concentrations (Table 4). Higher proportions of forage increased ($P < 0.05$) 4:0 to 10:0 in a linear or quadratic manner, tended ($P = 0.07$) to enrich 12:0 and 18:0 in a quadratic manner and linearly lowered ($P < 0.05$) TFA, in the absence of changes ($P > 0.10$) in 14:0 to 16:0 or 18:3n-3 concentrations (Table 4). Higher

Table 3 *Effect of incremental replacement of prilled palm fat with sunflower oil in high-concentrate diets (Experiment 1), or increases in the forage-to-concentrate ratio of diets containing sunflower oil (Experiment 2) on intake, apparent total-tract nutrient digestibility, milk production and composition in lactating cows*

	Experiment 1						Experiment 2					
	Treatment[1]				P[2]		Treatment[3]				P[2]	
Items	HPO	HPS	HSO	s.e.m.	L	Q	HSO	MSO	LSO	s.e.m.	L	Q
Intake (kg/day, unless otherwise stated)												
DM	23.8	22.9	24.1	0.68	0.83	0.22	24.4	23.1	24.6	0.70	0.83	0.14
OM	21.7	20.8	21.9	0.62	0.81	0.19	22.4	21.0	22.4	0.64	0.98	0.10
CP	3.99	3.87	3.95	0.113	0.83	0.49	3.92	3.70	4.04	0.113	0.49	0.06
NDF	6.98	6.45	7.36	0.201	0.20	0.009	7.40	7.55	8.78	0.232	<0.001	0.08
Potentially digestible NDF	4.41	4.05	4.74	0.128	0.09	0.003	4.61	4.65	5.19	0.141	0.008	0.17
Starch	4.77	4.44	4.65	0.133	0.51	0.11	4.49	3.61	3.48	0.114	<0.001	0.013
ME (MJ/day)	258	246	267	7.76	0.42	0.10	256	237	267	6.84	0.29	0.009
Fatty acids (g/day)												
16:0	840	602	302	18.2	<0.001	0.18	256	248	248	7.20	0.47	0.62
18:0	69.9	71.2	69.5	2.00	0.91	0.55	75.5	69.4	73.7	2.12	0.56	0.06
cis-9 18:1	273	316	354	9.17	<0.001	0.81	380	361	388	10.9	0.60	0.10
18:2n-6	328	509	709	16.5	<0.001	0.65	672	636	700	19.5	0.32	0.05
18:3n-3	78.7	71.9	78.4	2.20	0.93	0.022	70.6	76.4	88.4	2.31	<0.001	0.28
Total	1698	1683	1636	47.6	0.37	0.79	1583	1513	1629	45.8	0.48	0.11
Apparent digestibility (g/100 g)												
OM	73.2	73.5	75.8	1.02	0.09	0.43	71.4	70.7	74.3	1.36	0.14	0.21
Nitrogen	71.7	73.2	74.0	1.67	0.33	0.90	69.9	69.5	73.3	1.22	0.06	0.16
NDF	54.2	51.7	58.4	1.71	0.09	0.034	51.6	53.7	60.9	2.41	0.013	0.41
pdNDF	68.5	67.0	71.7	1.53	0.14	0.11	68.8	70.4	74.3	2.04	0.07	0.66
EE	79.0	81.4	84.8	1.68	0.024	0.81	81.8	81.7	85.5	1.01	0.017	0.12
Yield												
Milk (kg/day)	36.0	39.5	40.2	1.46	0.06	0.45	39.7	38.1	37.8	1.48	0.38	0.71
ECM (kg/day)	33.5	34.2	33.8	1.39	0.90	0.76	33.7	32.8	32.9	1.27	0.66	0.73
Fat (g/day)	1248	1221	1120	56.2	0.12	0.59	878	1141	1086	67.0	0.041	0.07
Protein (g/day)	1147	1199	1254	43.5	0.09	0.97	1214	1131	1147	37.8	0.23	0.31
Lactose (g/day)	1775	1915	1971	72.1	0.07	0.64	1962	1871	1867	71.1	0.30	0.58
Concentration												
Fat (g/kg)	34.5	31.4	27.7	1.63	0.008	0.89	22.4	30.2	28.6	1.58	0.011	0.024
Protein (g/kg)	31.4	30.3	31.1	0.50	0.74	0.13	31.0	29.6	30.4	0.39	0.29	0.037
Lactose (g/kg)	49.3	48.5	48.5	0.45	0.24	0.49	49.3	49.2	49.3	0.25	0.99	0.73
Urea (mmol/l)	6.07	5.19	5.06	0.502	0.17	0.53	4.40	5.45	5.83	0.387	0.017	0.49

DM = dry matter; OM = organic matter; ME = metabolizable energy; EE = ether extract; ECM = Energy corrected milk.
[1]Treatments consisted of high-concentrate diets containing 30 g/kg DM of supplemental fat in the form of prilled palm fat (HPO), sunflower oil (HSO) or an equal mixture of both fat supplements (HPS).
[2]Significance of linear (L) and quadratic (Q) components of the response to replacing prilled palm fat with sunflower oil in high-concentrate diets (Experiment 1) or increases in the forage-to-concentrate ratio of diets containing sunflower oil (Experiment 2).
[3]Treatments consisted of diets containing 30 g/kg DM of sunflower oil with forage-to-concentrate ratio (on a DM basis) of 39 : 61 (HSO), 44 : 56 (MSO) or 48 : 52 (LSO).

dietary F : C ratios tended ($P < 0.08$) to linearly increase the secretion of ⩽14-, total 16-carbon and ⩾18-carbon FA in milk (Figure 1). Increases in the F : C ratio linearly decreased ($P < 0.05$) *cis*-9 16:1, *trans*-16:1 (Δ6 to 8 and 10), *cis*-11 18:1 and *trans*-6 to -10 18:1, decreased ($P < 0.05$) *cis*-11 16:1 in a quadratic manner, and tended ($P = 0.06$) to increase *cis*-9 18:1 concentration in a quadratic manner (Table 5 and Supplementary Table S1). Higher F : C ratios linearly lowered ($P < 0.05$) milk 18:2n-6, *trans*-7, *cis*-9 CLA, *trans*-7, *trans*-9 CLA, *cis*-9, *cis*-11 CLA, *trans*-9, *cis*-11 CLA and *trans*-10, *cis*-12 CLA, decreased ($P < 0.05$) *cis*-9, *trans*-12 18:2 and *cis*-12, *trans*-14 CLA in a quadratic manner and linearly increased ($P = 0.01$) *trans*-11, *cis*-13 CLA concentration (Table 6). Furthermore, higher proportions of dietary forage linearly increased ($P < 0.01$) 14:0 *iso*, 15:0 *iso* and 29:0 and linearly decreased
($P < 0.05$) *cis*-8 17:1, *cis*-9 17:1 and 18:0 *iso* concentrations (Supplementary Table S2).

The correlation-loading plot derived from PLSR analysis for the first two factors is presented in Supplementary Figure S1. The outer and inner ellipses in loading plot represent proportionately 1.0 and 0.50 of the explained variance of *Y*, respectively. The first two factors accounted for 0.61 of total variance of response variables. Across both experiments,

Table 4 *Effect of incremental replacement of prilled palm fat with sunflower oil in high-concentrate diets (Experiment 1), or increases in the forage-to-concentrate ratio of diets containing sunflower oil (Experiment 2) on milk fatty acid composition (g/100 g fatty acids) in lactating cows*

	Experiment 1						Experiment 2					
	Treatment[1]				*P*[2]		Treatment[3]				*P*[2]	
Fatty acids	HPO	HPS	HSO	s.e.m.	L	Q	HSO	MSO	LSO	s.e.m.	L	Q
4:0	2.68	3.05	2.85	0.094	0.22	0.022	2.50	3.04	2.98	0.132	0.018	0.07
6:0	1.83	1.80	1.72	0.050	0.16	0.74	1.37	1.77	1.66	0.089	0.029	0.028
8:0	1.16	1.06	1.02	0.038	0.020	0.58	0.77	1.03	0.93	0.060	0.07	0.021
10:0	2.88	2.44	2.31	0.107	0.001	0.24	1.78	2.30	2.01	0.133	0.25	0.022
cis-9 10:1	0.29	0.21	0.24	0.014	0.009	0.006	0.16	0.19	0.22	0.014	0.012	0.83
12:0	3.56	2.87	2.76	0.132	<0.001	0.08	2.30	2.63	2.39	0.123	0.61	0.07
cis-9 12:1	0.10	0.06	0.07	0.005	<0.001	0.003	0.05	0.05	0.06	0.004	0.31	0.46
trans-9 12:1	0.09	0.06	0.07	0.005	0.001	0.001	0.06	0.05	0.06	0.004	0.45	0.15
14:0	11.5	10.4	10.1	0.29	0.003	0.23	9.16	9.87	9.43	0.351	0.59	0.19
cis-9 14:1	1.04	0.73	0.92	0.065	0.19	0.004	0.87	0.69	0.93	0.064	0.51	0.015
trans-9 14:1	0.011	0.008	0.011	0.0007	0.97	0.004	0.010	0.008	0.011	0.0009	0.46	0.040
Total 15	2.19	1.87	1.81	0.061	<0.001	0.08	1.78	1.75	1.79	0.042	0.82	0.54
16:0	36.6	29.3	24.0	0.60	<0.001	0.18	20.6	21.8	22.2	0.66	0.16	0.75
Total *cis* 16:1	2.49	1.90	1.70	0.078	<0.001	0.06	1.89	1.64	1.68	0.066	0.037	0.09
Total *trans* 16:1	0.29	0.34	0.47	0.018	<0.001	0.10	0.65	0.46	0.49	0.050	0.038	0.09
Total 16:1	2.78	2.25	2.17	0.077	<0.001	0.024	2.54	2.10	2.17	0.098	0.016	0.047
Total 17	1.15	1.01	1.02	0.035	0.019	0.09	1.11	1.02	1.03	0.032	0.10	0.17
18:0	6.94	10.6	10.4	0.385	<0.001	<0.001	10.2	12.3	11.6	0.595	0.11	0.07
10-*oxo* 18:0	0.019	0.017	0.017	0.0015	0.35	0.52	0.06	0.02	0.02	0.015	0.07	0.24
13-*oxo* 18:0	0.009	0.011	0.011	0.0014	0.30	0.72	0.01	0.01	0.01	0.002	0.67	0.31
Total *cis* 18:1	16.8	20.2	21.6	0.46	<0.001	0.08	23.4	22.1	23.5	0.61	0.87	0.08
Total *trans* 18:1	2.45	4.88	8.21	0.349	<0.001	0.30	12.0	8.93	8.55	1.027	0.026	0.29
Total 18:1	19.2	25.1	29.8	0.54	<0.001	0.39	35.4	31.0	32.1	1.23	0.07	0.08
Total 18:2[4]	2.72	3.78	4.54	0.093	<0.001	0.21	4.86	4.37	4.31	0.168	0.030	0.32
Total CLA	0.44	0.76	1.46	0.055	<0.001	0.009	1.55	1.44	1.57	0.164	0.91	0.54
18:3n-3	0.35	0.35	0.36	0.014	0.67	0.99	0.32	0.33	0.35	0.016	0.19	0.97
18:3n-6	0.04	0.04	0.03	0.002	0.001	0.13	0.03	0.03	0.03	0.002	0.88	0.67
3R,7R,11R,15-tetramethyl-16:0	0.010	0.011	0.008	0.0010	0.14	0.31	0.013	0.009	0.012	0.0018	0.66	0.08
20:0	0.12	0.15	0.13	0.005	0.45	0.004	0.13	0.14	0.14	0.005	0.09	0.08
cis-9 20:1	0.11	0.10	0.11	0.004	0.99	0.92	0.10	0.10	0.11	0.004	0.27	0.07
cis-11 20:1	0.026	0.046	0.056	0.0045	<0.001	0.41	0.062	0.048	0.034	0.0053	0.001	0.99
cis-13 20:1	0.007	0.009	0.006	0.0009	0.38	0.035	0.006	0.005	0.005	0.0006	0.41	0.60
trans-11 20:1	0.010	0.010	0.015	0.003	0.27	0.40	0.016	0.010	0.011	0.0021	0.10	0.17
Total 20:1	0.15	0.17	0.18	0.006	<0.001	0.61	0.19	0.16	0.16	0.008	0.020	0.13
20:2n-6	0.039	0.037	0.044	0.0030	0.24	0.24	0.044	0.040	0.043	0.0031	0.67	0.39
20:3n-3	0.017	0.016	0.016	0.0014	0.73	0.71	0.011	0.009	0.011	0.0009	0.79	0.12
20:3n-6	0.12	0.12	0.11	0.007	0.88	0.66	0.12	0.11	0.10	0.008	0.33	0.83
20:4n-3	0.017	0.015	0.013	0.0010	0.007	0.75	0.012	0.012	0.014	0.0010	0.31	0.40
20:4n-6	0.17	0.16	0.16	0.007	0.17	0.72	0.14	0.16	0.14	0.008	0.79	0.037
20:5n-3[5]	0.042	0.031	0.026	0.0031	0.002	0.41	0.019	0.021	0.023	0.0014	0.09	0.95
22:0	0.055	0.065	0.066	0.0039	0.048	0.36	0.062	0.072	0.068	0.0032	0.20	0.09
cis-13 22:1	0.011	0.012	0.013	0.0008	0.19	0.84	0.010	0.010	0.012	0.0008	0.12	0.37
22:2n-6	0.006	0.003	0.004	0.0007	0.020	0.06	0.004	0.004	0.005	0.0006	0.13	0.81
22:3n-3	0.006	0.006	0.006	0.0004	0.97	0.65	0.005	0.005	0.005	0.0003	0.48	0.70
22:4n-6	0.029	0.030	0.031	0.0025	0.48	0.95	0.031	0.032	0.025	0.0024	0.06	0.15
22:5n-3	0.051	0.051	0.052	0.0022	0.79	0.94	0.044	0.050	0.045	0.0027	0.84	0.11
cis-15 24:1	0.006	0.006	0.007	0.0006	0.13	0.93	0.008	0.005	0.006	0.0006	0.022	0.11
26:0	0.007	0.006	0.005	0.0006	0.08	0.71	0.005	0.005	0.004	0.0004	0.11	0.56
Total others	0.90	0.87	0.81	0.031	0.044	0.64	0.75	0.81	0.82	0.022	0.05	0.43
Total unidentified	0.47	0.45	0.53	0.021	0.06	0.044	0.54	0.46	0.49	0.026	0.19	0.07
Total SFA	71.2	65.2	58.8	0.65	<0.001	0.82	52.5	58.3	56.8	1.56	0.07	0.07
Total MUFA	24.2	28.9	33.8	0.59	<0.001	0.95	39.7	34.6	36.0	1.33	0.07	0.06
Total PUFA	3.99	5.38	6.84	0.143	<0.001	0.81	7.18	6.62	6.67	0.293	0.23	0.41

Table 4 (*Continued*)

	Experiment 1						Experiment 2					
	Treatment[1]				P[2]		Treatment[3]				P[2]	
Fatty acids	HPO	HPS	HSO	s.e.m.	L	Q	HSO	MSO	LSO	s.e.m.	L	Q
Total *trans* FA	2.98	5.43	8.94	0.361	<0.001	0.25	12.9	9.64	9.30	1.080	0.026	0.27
FA (g/100 g fat)	94.0	94.1	94.2	0.027	0.030	0.05	94.1	94.2	94.2	0.037	0.63	0.63

CLA = conjugated linoleic acid; FA = fatty acids; MUFA = monounsaturated FA; PUFA = polyunsaturated FA; SFA = saturated FA.
[1]Treatments consisted of high-concentrate diets containing 30 g/kg dry matter (DM) of supplemental fat in the form of prilled palm fat (HPO), sunflower oil (HSO) or an equal mixture of both fat supplements (HPS).
[2]Significance of linear (L) and quadratic (Q) components of the response to replacing prilled palm fat with sunflower oil in high-concentrate diets (Experiment 1) or increases in the forage-to-concentrate ratio of diets containing sunflower oil (Experiment 2).
[3]Treatments consisted of diets containing 30 g/kg DM of sunflower oil with forage-to-concentrate ratio (on a DM basis) of 39 : 61 (HSO), 44 : 56 (MSO) or 48 : 52 (LSO).
[4]Sum of 18:2 FA excluding isomers of CLA.
[5]Co-elutes with 24:0.

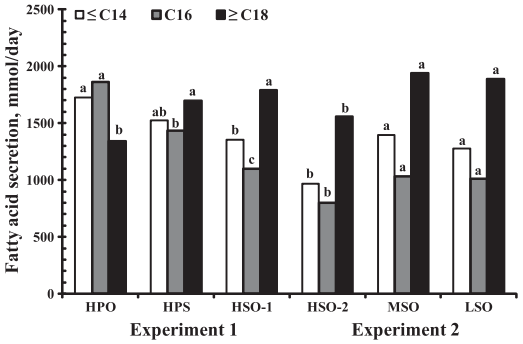

Figure 1 Milk fatty-acid secretion (mmol/day) in cows fed high-concentrate diets containing 30 g/kg (on a dry matter basis) of supplemental fat as prilled palm fat (HPO), sunflower oil (HSO-1) or an equal mixture of both fat supplements (HPS; Experiment 1), or fed diets containing 30 g/kg sunflower oil with forage-to-concentrate ratio (on a dry matter basis) of 39 : 61 (HSO-2), 44 : 56 (MSO) or 48 : 52 (LSO; Experiment 2). Values represent least square means for eight animals during the last week of each experiment. Fatty acids are categorized according to metabolic origin: ⩽C14 synthesized *de novo*, ⩾C18 extracted and incorporated into milk fat from circulating plasma lipids and C16 derived from both sources. [a–c]Different letters within columns indicate differences between treatments within experiment ($P < 0.05$).

65 different FAs were found to be related ($P < 0.05$) with milk fat content and yield. Factor 1 discriminated milk fat content and yield, short- and medium-chain SFA (positive loadings) from 16- and 18-carbon unsaturated FA (negative loadings). Factor 2 discriminated 4:0 and 6:0 (north-east quadrant) from 8:0, 10:0 and 16:0 (south-east quadrant), and *trans*-16:1 (Δ6 + 7, 10, 11 and 13), *cis*-13 18:1, *trans*-18:1 (Δ5, 6 + 7 + 8, 9 and 13 + 14), 18:2n-6, *cis*-9, *trans*-12 18:2, *cis*-9, *trans*-13 18:2, *cis*-9, *trans*-14 18:2, *trans*-7, *cis*-9 CLA, *trans*-10, *trans*-12 CLA and *trans*-11, *trans*-13 CLA (north-west quadrant) from 10-*oxo* 18:0, *trans*-8 16:1, *cis*-11 18:1, *trans*-10 18:1, *trans*-9, *trans*-12 18:2, *trans*-9, *cis*-11 CLA, *trans*-10, *cis*-12 CLA and *trans*-13, *cis*-15 CLA (south-west quadrant). The score plot describes the distribution of all 48 analysed milk samples on the basis of FA composition for cows fed different diets in both experiments along both factors (Supplementary Figure S2). When combined, the correlation loading and score plots provide an additional and complementary interpretation of the two factors in terms of variables and sample distribution in relation to milk fat in the input data. Scores of cows on HPO and HPS treatments showed a high positive correlation with factor 1, whereas three of the eight cows on the HSO treatment experiencing milk fat depression (MFD) in Experiment 2 had a high negative correlation with factor 1.

Discussion

Dietary supplements of plant oils and oilseeds influence the FA composition of bovine milk fat, but there is considerable variation depending on fat source and composition of the basal diet (Chilliard *et al*., 2007; Glasser *et al*., 2008a; Shingfield *et al*., 2013). In the present study, the potential to alter milk fat composition under two scenarios relevant to commercial milk production was examined.

Milk FA composition

Replacing PALM with SFO in high-concentrate diets altered milk fat composition that, to a large extent, reflected differences in the relative intake of 16- and 18-carbon FA. A mean decrease in the relative proportion of 16:0 in milk fat of 0.42 percentage units per g/kg substitution of PALM with SFO in the diet is marginally higher than values of between 0.19 and 0.35 following isolipid replacement of palm-based supplements (40 to 50 g/kg DM) with sunflower seeds (Mohammed *et al*., 2011), milled rapeseeds (Kliem *et al*., 2011), and either maize or safflower oil (He and Armentano, 2011). Changes in milk 16:0 concentration were also accompanied by lowered milk fat content and secretion of short- and medium-chain (8:0 to 14:0) SFA synthesized *de novo* that can, to a large extent, be attributed to a higher availability of 18-carbon FA inhibiting the activity of acetyl-CoA carboxylase in the mammary glands (Shingfield *et al*., 2010). Replacing PALM with SFO in the diet also increased milk 4:0 concentrations

Table 5 *Effect of incremental replacement of prilled palm fat with sunflower oil in high-concentrate diets (Experiment 1), or increases in the forage-to-concentrate ratio of diets containing sunflower oil (Experiment 2) on milk 18:1 composition (g/100 g fatty acids) in lactating cows*

	Experiment 1						Experiment 2					
	Treatment[1]				P^2		Treatment[3]				P^2	
Fatty acids	HPO	HPS	HSO	s.e.m.	L	Q	HSO	MSO	LSO	s.e.m.	L	Q
cis-9 18:1	15.8	18.8	19.3	0.45	<0.001	0.027	21.0	19.7	21.2	0.57	0.76	0.06
cis-11 18:1	0.70	0.64	0.74	0.045	0.54	0.17	0.86	0.66	0.61	0.049	0.002	0.23
cis-12 18:1	0.18	0.53	1.22	0.033	<0.001	<0.001	1.19	1.34	1.35	0.115	0.34	0.65
cis-13 18:1	0.05	0.07	0.10	0.003	<0.001	0.013	0.11	0.10	0.10	0.005	0.10	0.42
cis-15 18:1	0.06	0.08	0.12	0.006	<0.001	0.13	0.13	0.12	0.11	0.007	0.12	0.98
cis-16 18:1	0.04	0.08	0.13	0.003	<0.001	0.06	0.13	0.13	0.13	0.007	0.58	0.71
trans-4 18:1	0.02	0.03	0.04	0.002	<0.001	0.43	0.05	0.05	0.05	0.002	0.53	0.91
trans-5 18:1	0.02	0.03	0.04	0.002	<0.001	0.11	0.05	0.05	0.05	0.003	0.46	0.73
trans-6 + 7 + 8 18:1	0.20	0.38	0.58	0.024	<0.001	0.76	0.91	0.67	0.65	0.078	0.029	0.25
trans-9 18:1	0.18	0.33	0.54	0.013	<0.001	0.12	0.75	0.58	0.60	0.047	0.031	0.13
trans-10 18:1	0.37	0.78	1.20	0.170	0.002	0.97	4.35	1.23	1.09	0.965	0.026	0.22
trans-11 18:1	0.58	1.25	2.60	0.155	<0.001	0.09	2.52	2.94	2.72	0.307	0.65	0.41
trans-12 18:1	0.21	0.52	0.93	0.024	<0.001	0.13	1.03	1.01	1.01	0.044	0.77	0.82
trans-13 + 14 18:1	0.45	0.76	1.13	0.032	<0.001	0.51	1.25	1.21	1.18	0.049	0.36	0.92
trans-15 18:1	0.25	0.45	0.66	0.013	<0.001	0.70	0.65	0.68	0.68	0.025	0.40	0.75
trans-16 18:1	0.18	0.34	0.50	0.010	<0.001	0.93	0.45	0.51	0.50	0.025	0.13	0.26

[1]Treatments consisted of high-concentrate diets containing 30 g/kg dry matter (DM) of supplemental fat in the form of prilled palm fat (HPO), sunflower oil (HSO) or an equal mixture of both fat supplements (HPS).
[2]Significance of linear (L) and quadratic (Q) components of the response to replacing prilled palm fat with sunflower oil in high-concentrate diets (Experiment 1) or increases in the forage-to-concentrate ratio of diets containing sunflower oil (Experiment 2).
[3]Treatments consisted of diets containing 30 g/kg DM of sunflower oil with forage-to-concentrate ratio (on a DM basis) of 39 : 61 (HSO), 44 : 56 (MSO) or 48 : 52 (LSO).

that represent a typical response to dietary plant oil supplements (Shingfield *et al*., 2013), whereas discrimination of 4:0 from longer-chain SFA in the PLSR analysis is consistent with the four-carbon FA being incorporated into milk fat triacylglycerol via two pathways independent of the inhibitable acetyl-CoA carboxylase pathway (Chilliard *et al*., 2007).

Replacing PALM with SFO progressively increased milk fat *cis*-9 18:1 concentrations that reflects a higher ruminal escape of *cis*-9 18:1 and increased availability of 18:0 for desaturation in the mammary glands. On most diets, the extent of biohydrogenation (BH) of *cis*-9 18:1, 18:2n-6 and 18:3n-3 varies between 58% and 87%, 70% and 95%, and 85% and 100%, respectively (Glasser *et al*., 2008b; Shingfield *et al*., 2010). Both an increase in milk 18:0 concentrations, along with the decrease in *cis*-9 18:1/18:0 concentration ratio, suggest that synthesis in the mammary glands was the principle mechanism contributing to higher milk *cis*-9 18:1 concentrations when SFO replaced PALM in the diet. Although 18:0 is the preferred substrate for stearoyl-CoA desaturase in the ruminant mammary glands (Palmquist *et al*., 2005), ca. 2% to 3% of 16:0 extracted from the blood is desaturated to *cis*-9 16:1 (Mosley and McGuire, 2007). A decrease in the availability of 16:0 in the mammary glands when SFO replaced PALM in the diet also offers an explanation for the concomitant decrease in milk *cis*-9 16:1 content.

Substitution of SFO for PALM increased the abundance of numerous TFAs in milk that can be attributed, in the most part, to the formation and accumulation of intermediates during incomplete BH of unsaturated 16- and 18-carbon FA in the rumen (Chilliard *et al*., 2007; Shingfield *et al*., 2010). Earlier studies have also demonstrated that replacing palm-based supplements with milled rapeseeds (Kliem *et al*., 2011), sunflower seeds (Mohammed *et al*., 2011), or maize and safflower oil (He and Armentano, 2011) increase milk TFA content. However, the relative abundance and distribution of TFA isomers is dependent on both the FA composition of fat supplements and the basal diet (Chilliard *et al*., 2007; Shingfield *et al*., 2013). Direct comparisons indicate that plant oils or oilseeds rich in 18:2n-6 can be expected to increase milk fat concentrations of specific *trans*-18:1 (Δ8 to 12) and 8,10; 9,11 and 10,12 geometric isomers of CLA (Roy *et al*., 2006; Rego *et al*., 2009; Halmemies-Beauchet-Filleau *et al*., 2011).

Replacing PALM with SFO increased milk *cis*-9, *trans*-11 CLA concentrations with enrichment on HSO treatment being within the range (0.54 to 1.83 g/100 g FA) reported for cows fed high-concentrate-based diets supplemented with ca. 50 g/kg DM of plant oils rich in 18:2n-6 (Chilliard *et al*., 2007; Shingfield *et al*., 2013). Although most *cis*-9, *trans*-11 CLA secreted in milk is synthesized endogenously from *trans*-11 18:1 in the mammary glands (Palmquist *et al*., 2005), dietary SFO supplements may also increase, in a dose-dependent manner, the amount of *cis*-9, *trans*-11 CLA leaving the rumen in lactating cows (Shingfield *et al*., 2008a). Inclusion of SFO in the diet at the expense of PALM also enriched milk *trans*-7, *cis*-9 CLA, *cis*-9, *trans*-12 18:2, *cis*-9, *trans*-13 18:2 and *cis*-9, *trans*-14 18:2 concentrations that can be explained by

Table 6 *Effect of incremental replacement of prilled palm fat with sunflower oil in high-concentrate diets (Experiment 1), or increases in the forage-to-concentrate ratio of diets containing sunflower oil (Experiment 2) on milk 18:2 composition (mg/100 g fatty acids) in lactating cows*

	Experiment 1						Experiment 2					
	Treatment[1]				P^2		Treatment[3]				P^2	
Fatty acids	HPO	HPS	HSO	s.e.m.	L	Q	HSO	MSO	LSO	s.e.m.	L	Q
cis-9, *cis*-12 18:2	2357	3198	3679	89.4	<0.001	0.12	3837	3502	3359	143	0.028	0.59
cis-12, *cis*-15 18:2	13.2	17.2	28.0	6.32	0.11	0.67	13.9	32.0	27.9	7.19	0.18	0.22
cis-9, *trans*-12 18:2	32.1	53.4	85.1	4.45	<0.001	0.36	109	82.4	92.4	6.95	0.11	0.044
cis-9, *trans*-13 18:2	144	274	422	17.4	<0.001	0.69	487	405	454	29.8	0.44	0.09
cis-9, *trans*-14 18:2	63.3	110	162	5.86	<0.001	0.71	171	159	170	11.4	0.92	0.41
trans-9, *cis*-12 18:2	30.0	50.0	81.1	2.70	<0.001	0.10	96.4	89.4	94.7	5.86	0.84	0.40
trans-11, *cis*-15 18:2	43.4	50.4	48.1	3.65	0.37	0.30	94.9	62.5	65.8	17.5	0.25	0.41
trans-12, *cis*-15 18:2	9.36	9.20	9.90	1.048	0.71	0.74	12.5	11.6	13.4	1.19	0.57	0.37
trans-9, *trans*-12 18:2	10.8	10.1	14.1	0.946	0.021	0.05	21.8	13.9	13.6	3.00	0.07	0.32
trans-11, *trans*-15 18:2	9.09	8.85	11.7	0.912	0.06	0.18	12.3	15.2	14.6	1.31	0.23	0.28
cis-9, *trans*-11 CLA	328	578	1193	45.0	<0.001	0.003	1234	1204	1322	151	0.68	0.69
cis-11, *trans*-13 CLA	1.10	1.36	1.89	0.226	0.023	0.63	2.32	1.62	1.95	0.244	0.31	0.10
cis-12, *trans*-14 CLA	0.58	0.43	0.83	0.108	0.12	0.05	1.13	0.67	0.81	0.097	0.031	0.022
cis-13, *trans*-15 CLA	1.18	1.20	1.44	0.302	0.55	0.77	1.46	1.68	1.27	0.341	0.69	0.45
trans-7, *cis*-9 CLA	34.4	59.5	102	5.39	<0.001	0.21	160	109	115	13.6	0.029	0.09
trans-8, *cis*-10 CLA	3.67	3.67	4.61	0.405	0.12	0.36	5.55	5.57	7.70	1.47	0.31	0.56
trans-9, *cis*-11 CLA	13.9	23.5	40.5	2.97	<0.001	0.32	73.5	34.5	41.4	8.30	0.013	0.035
trans-10, *cis*-12 CLA	5.11	9.64	13.1	1.786	0.005	0.81	34.0	13.0	12.8	5.66	0.015	0.15
trans-11, *cis*-13 CLA	5.28	7.50	7.43	0.692	0.039	0.19	8.74	10.2	13.4	1.14	0.010	0.53
trans-12, *cis*-14 CLA	2.23	2.19	3.50	0.444	0.06	0.22	4.52	2.96	4.03	0.523	0.51	0.05
trans-13, *cis*-15 CLA	0.48	0.47	0.88	0.084	0.003	0.06	1.20	0.78	0.68	0.201	0.08	0.52
trans-7, *trans*-9 CLA	1.82	2.80	2.89	0.295	0.018	0.24	4.03	2.97	2.82	0.306	0.011	0.24
trans-8, *trans*-10 CLA	2.43	5.95	10.6	0.537	<0.001	0.40	10.4	9.27	9.72	0.551	0.41	0.25
trans-9, *trans*-11 CLA	11.8	17.5	23.9	1.39	<0.001	0.81	24.1	24.2	23.1	1.12	0.55	0.65
trans-10, *trans*-12 CLA	4.78	10.5	17.6	1.08	<0.001	0.60	23.1	20.5	20.2	1.33	0.14	0.49
trans-11, *trans*-13 CLA	6.86	7.10	8.84	0.710	0.06	0.40	12.5	10.2	10.2	1.026	0.13	0.35
trans-12, *trans*-14 CLA	0.94	1.15	1.87	0.274	0.026	0.45	1.91	1.57	1.91	0.220	0.99	0.21
trans-13, *trans*-15 CLA	0.38	0.39	0.48	0.059	0.23	0.63	0.61	0.61	0.48	0.087	0.30	0.54
cis-8, *cis*-10 CLA	0.30	0.16	0.89	0.412	0.33	0.40	0.09	0.12	0.05	0.079	0.68	0.59
cis-9, *cis*-11 CLA	1.40	1.53	1.93	0.284	0.19	0.70	3.19	1.87	1.68	0.360	0.007	0.22
cis-10, *cis*-12 CLA	0.29	0.33	0.33	0.124	0.84	0.87	0.71	0.51	0.24	0.160	0.05	0.88

CLA = conjugated linoleic acid.
[1]Treatments consisted of high-concentrate diets containing 30 g/kg dry matter (DM) of supplemental fat in the form of prilled palm fat (HPO), sunflower oil (HSO) or an equal mixture of both fat supplements (HPS).
[2]Significance of linear (L) and quadratic (Q) components of the response to replacing prilled palm fat with sunflower oil in high-concentrate diets (Experiment 1) or increases in the forage-to-concentrate ratio of diets containing sunflower oil (Experiment 2).
[3]Treatments consisted of diets containing 30 g/kg DM of sunflower oil with forage-to-concentrate ratio (on a DM basis) of 39 : 61 (HSO), 44 : 56 (MSO) or 48 : 52 (LSO).

increases in the supply of 18:1 intermediates formed during ruminal BH of unsaturated FA in SFO available for desaturation in the mammary glands (Palmquist *et al.*, 2005; Shingfield *et al.*, 2008b).

Replacing forages with concentrates typically increases the ratio of NDF to starch in the diet and the substitution of 18:3n-3 for *cis*-9 18:1 and 18:2n-6. Both the composition and amount of concentrates in the diet influences the extent of lipolysis and BH in the rumen (Palmquist *et al.*, 2005). At least part of the influence of higher concentrate supplementation on ruminal lipid metabolism is thought to be mediated via decreases in rumen pH (Fuentes *et al.*, 2009) and alterations in microbial communities (Weimer *et al.*, 2010). In the present investigation, increases in the F : C ratio of diets containing SFO resulted in higher milk fat 4:0 to 12:0 concentrations, without altering milk 14:0 and 16:0 content. Relatively minor increases in milk 4:0 to 14:0 concentrations and marginal decreases in 16:0 are in line with the findings from earlier studies examining the influence of forage proportions of diets containing plant oils (Chilliard *et al.*, 2007; Glasser *et al.*, 2008a; Shingfield *et al.*, 2013).

Changes in dietary F : C ratio also influence the complete BH of 18-carbon unsaturated FAs to 18:0 in the rumen leading to changes in the relative abundance of specific *trans* 18:1 and *trans* 18:2 isomers leaving the rumen and incorporated into milk fat (Bauman and Griinari, 2003; Shingfield and Griinari, 2007). Furthermore, the effects of changes in F : C ratio on milk 18:1 composition also differ, depending on the presence or

absence of plant oils in the basal diet (Chilliard *et al*., 2007; Shingfield *et al*., 2013). In the present study, increases in the F : C ratio of diets containing SFO lowered the relative abundance of *trans*-6 to -10 18:1 and increased milk fat 18:0 content. High-concentrate diets containing high amounts of PUFA typically promote *trans*-10, *cis*-12 CLA and *trans*-10 18:1 formation in the rumen (Bauman and Griinari, 2003; Shingfield and Griinari, 2007). Across both experiments, a close association existed between milk fat *trans*-10 18:1 and *trans*-10, *cis*-12 CLA concentrations (Supplementary Figure S1), consistent with *trans*-10 18:1 originating, at least partly, from the reduction of *trans*-10, *cis*-12 CLA, an intermediate formed during the initial isomerization of 18:2n-6 in the rumen (Bauman and Griinari, 2003).

Supplementing high-forage diets with plant oils can be expected to increase milk fat *cis*-9, *trans*-11 CLA concentrations (Palmquist *et al*., 2005; Chilliard *et al*., 2007). However, increases in F : C ratio had no influence on *cis*-9, *trans*-11 CLA or *trans*-11 18:1 enrichment in milk from diets containing SFO. Previous studies suggest that alterations in F : C ratio may have a larger influence on milk *cis*-9, *trans*-11 CLA concentration when higher amounts of plant oil supplements are fed or when differences in the proportion of forage in the diet are more extreme (Shingfield *et al*., 2013).

Animal performance

Replacing PALM with SFO did not influence DM intake, but tended to increase milk yield. Evaluation of published reports indicate that lipid supplements rich in 18:2n-6 generally lower the DM intake and result in numerically higher yields of milk and milk constituents (Rabiee *et al*., 2012). At least part of the increase in milk yield to SFO in the present investigation was associated with improvements in fibre and fat digestibility. In ruminants, the apparent digestibility and availability of 16-carbon FA is ca. 7 percentage units lower compared with 18-carbon FA (Schmidely *et al*., 2008), whereas the absorption of unsaturated FA is generally higher compared with SFA in the small intestine (Glasser *et al*., 2008b).

Milk fat

Replacing PALM with SFO lowered milk fat content, but had no substantial influence on milk fat secretion, consistent with the findings of a recent meta-analysis (Rabiee *et al*., 2012). For diets containing SFO, increases in the F : C ratio resulted in higher milk fat synthesis, in the absence of substantial differences in the intake of total or individual FA, other than a decrease in the ratio of n-6/n-3 PUFA. Increases in the amount of concentrates in diets containing unsaturated FA is known to influence ruminal BH and promote the formation of specific BH intermediates, including *trans*-10 18:1 and *trans*-10, *cis*-12 CLA (Bauman and Griinari, 2003). However, on the basis of the relationship between milk fat yield and milk *trans*-10, *cis*-12 CLA concentrations derived from several post-ruminal infusion studies (Shingfield and Griinari, 2007), increases in the amount of *trans*-10, *cis*-12 CLA at the mammary glands could only account for up to 25% of the difference in milk fat yield between the LSO and HSO treatment.

It is possible that alterations in ruminal lipid metabolism during diet-induced MFD and associated changes in the relative abundance of FA available for milk fat synthesis may also have a direct or indirect effect on the regulation of mammary lipogenesis (Shingfield *et al*., 2010). Examination of changes in milk FA composition and milk fat yield indicated that milk from cows experiencing MFD contained higher concentrations of *trans*-10, *cis*-12 CLA, *trans*-10 18:1, *trans*-9, *cis*-11 CLA, *trans*-9, *trans*-12 18:2, *trans*-8 16:1, 10-*oxo* 18:0, *cis*-11 18:1 and *trans*-13, *cis*-15 CLA (Supplementary Figures S1 and S2). Over a wide range of situations, increases in milk *trans*-10 18:1 concentration is a consistent feature of diet-induced MFD (Bauman and Griinari, 2003; Shingfield and Griinari, 2007). However, reports on the physiological effects of *trans*-10 18:1 in the lactating cow are equivocal. Abomasal infusion of 42.6 g *trans*-10 18:1/day was shown to have no influence on milk fat secretion (Lock *et al*., 2007), whereas post-ruminal infusions of a mixture of 18:1 FAME providing 92.1 g *trans*-10 18:1/day resulted in ca. 20% decrease in milk fat output (Shingfield *et al*., 2009). Across both experiments in the present study, a close association existed between milk fat *trans*-10 18:1 and *trans*-10, *cis*-12 CLA concentration, highlighting the challenges in establishing a direct cause and effect. Limited evidence also indicate that *trans*-9, *cis*-11 CLA inhibits milk fat synthesis in lactating cows (Perfield *et al*., 2007). Over a range of diets causing MFD, a close inverse relationship has been reported between milk *trans*-9, *cis*-11 CLA concentration and milk fat secretion (Roy *et al*., 2006; Shingfield *et al*., 2006; Perfield *et al*., 2007). Even though increases in milk *trans*-6 + 7 + 8 18:1 and *trans*-7, *cis*-9 CLA concentrations have been observed during diet-induced MFD (Kadegowda *et al*., 2008), no causal mechanism has been established. Across experiments in the present investigation, no association was identified between milk fat secretion and *trans*-6 + 7 16:1, *trans*-6 + 7 + 8 18:1 or *trans*-7, *cis*-9 CLA concentrations, whereas *trans*-8 16:1 was located close to *trans*-10, *cis*-12 CLA in the loading plot and opposite to milk fat content and yield (Supplementary Figure S1).

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Supplementary material

For supplementary material/s referred to in this article, please visit http://dx.doi.org/10.1017/S175173111300181X

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