Examination of the Persistency of Milk Fatty Acid Composition Responses to Fish Oil and Sunflower Oil in the Diet of Dairy Cows

K. J. Shingfield,^{*1,2} C. K. Reynolds,^{*3} G. Hervás,^{*} J. M. Griinari,[†]
A. S. Grandison,[‡] and D. E. Beever^{*}

^{*}Centre for Dairy Research, Department of Animal Science, The University of Reading,
Earley Gate, Reading, RG6 6AT, UK

[†]Department of Animal Science, University of Helsinki, FIN 00014 Helsinki, Finland

[‡]School of Food Biosciences, The University of Reading, Reading, RG6 6AP, UK

ABSTRACT

Based on the potential benefits of *cis*-9, *trans*-11 conjugated linoleic acid (CLA) for human health, there is a need to develop effective strategies for enhancing milk fat CLA concentrations. Levels of *cis*-9, *trans*-11 CLA in milk can be increased by supplements of fish oil (FO) and sunflower oil (SO), but there is considerable variation in the response. Part of this variance may reflect time-dependent ruminal adaptations to high levels of lipid in the diet, which lead to alterations in the formation of specific biohydrogenation intermediates. To test this hypothesis, 16 late lactation Holstein-British Friesian cows were used in a repeated measures randomized block design to examine milk fatty acid composition responses to FO and SO in the diet over a 28-d period. Cows were allocated at random to corn silage-based rations (8 per treatment) containing 0 (control) or 45 g of oil supplement/kg of dry matter consisting (1:2; wt/wt) of FO and SO (FSO), and milk composition was determined on alternate days from d 1. Compared with the control, the FSO diet decreased mean dry matter intake (21.1 vs. 17.9 kg/d), milk fat (47.7 vs. 32.6 g/kg), and protein content (36.1 vs. 33.3 g/kg), but had no effect on milk yield (27.1 vs. 26.4 kg/d). Reductions in milk fat content relative to the FSO diet were associated with increases in milk *trans*-10 18:1, *trans*-10, *cis*-12 CLA, and *trans*-9, *cis*-11 CLA concentrations ($r^2 = 0.74, 0.57, \text{ and } 0.80$, respectively). Compared with the control, the FSO diet reduced milk 4:0 to 18:0 and *cis* 18:1 content and increased *trans* 18:1, *trans* 18:2, *cis*-9, *trans*-11 CLA, 20:5 n-3, and 22:6 n-3 concentrations. The FSO diet caused a rapid elevation in milk *cis*-9, *trans*-11 CLA content, reaching a

maximum of 5.37 g/100 g of fatty acids on d 5, but these increases were transient, declining to 2.35 g/100 g of fatty acids by d 15. They remained relatively constant thereafter. Even though concentrations of *trans*-11 18:1 followed the same pattern of temporal changes as *cis*-9, *trans*-11 CLA, the total *trans* 18:1 content of FSO milk was unchanged because of the concomitant increases in the concentration of other isomers (Δ^{4-10} and Δ^{12-15}), predominantly *trans*-10 18:1. In conclusion, supplementing diets with FSO enhances milk fat *cis*-9, *trans*-11 CLA content, but the high level of enrichment declines because of changes in ruminal biohydrogenation that result in *trans*-10 replacing *trans*-11 as the major 18:1 biohydrogenation intermediate formed in the rumen.

Key words: *trans* fatty acids, conjugated linoleic acids, polyenoic fatty acid

INTRODUCTION

Conjugated linoleic acid (CLA) is a generic term used to describe single or mixtures of positional and geometric isomers of 18:2 fatty acids containing a conjugated double bond. Supplements of *cis*-9, *trans*-11 CLA have been reported to inhibit the growth of a number of human cancer cell lines and suppress chemically induced tumor development in animal models (Parodi, 1999; Kritchevsky, 2000). In light of the potential benefits to long-term human health, there is considerable interest in developing nutritional strategies to enhance milk fat *cis*-9, *trans*-11 CLA content. Concentrations of *cis*-9, *trans*-11, the major CLA isomer in milk (Sehat et al., 1998), can be increased by feeding whole oilseeds or by supplementing the diet with plant oils or marine lipids (Griinari and Bauman, 1999; Chilliard et al., 2000, 2001). Fish oil (FO) is more effective than plant oils for elevating milk fat *cis*-9, *trans*-11 CLA content (Offer et al., 1999; Chouinard et al., 2001), and these increases can be further enhanced when FO is fed in combination with plant oils or oilseeds rich in 18:2 n-6 (Whitlock et al., 2002; AbuGhazaleh et al., 2003). Even though the

Received February 1, 2005.

Accepted October 4, 2005.

¹Corresponding author: kevin.shingfield@mtt.fi

²Present address: Animal Production Research, MTT Agrifood Research Finland, FIN 31600, Jokioinen, Finland.

³Present address: Department of Animal Sciences, The Ohio State University, OARDC, Wooster 44696-4076.

combined use of marine lipids to modify ruminal biohydrogenation and vegetable oils as a substrate for ruminal *trans*-11 18:1 formation is an established strategy for increasing milk fat *cis*-9, *trans*-11 CLA content, there is considerable variation in the response.

Concentrations of *cis*-9, *trans*-11 CLA in milk from diets containing fish meal and extruded soybeans have been reported to increase until d 21 on the diet, but decline thereafter (AbuGhazaleh et al., 2004). Levels of this CLA isomer in milk were found to decrease after 14 d when FO and extruded soybeans were fed (Whitlock et al., 2002). In earlier studies, milk fat *cis*-9, *trans*-11 CLA responses to sunflower oil (SO; Bauman et al., 2000) or soybean oil (Dhiman et al., 2000) were also reported to be transitory and decreased over time. Although the composition of the basal diet is known to be important (Shingfield et al., 2005), it is probable that part of the variation in milk fat *cis*-9, *trans*-11 CLA content when high levels of FO and plant oils are fed arises from time-dependent adaptations in ruminal biohydrogenation. To test this hypothesis, the fatty acid composition of milk from cows fed corn silage-based diets containing 0 or 45 g of a mixture (1:2, wt/wt) of FO and SO/kg of DM (FSO) was intensively monitored over a 28-d period.

MATERIALS AND METHODS

Experimental Design, Animals, and Management

Sixteen Holstein-British Friesian cows of mean parity 3.7 ± 0.43 , live weight 639 ± 15.6 kg, and 169 ± 6.6 d in lactation were used. Cows were paired on the basis of stage of lactation, milk yield, and live weight and were allocated at random to experimental treatments. Animals were housed in individual tie stalls bedded with sawdust and were offered daily rations as equal meals at 0830 and 1600 h. Cows had continuous access to water and trace-mineralized salt blocks (Baby Red Rockies, Winsford, Cheshire, United Kingdom) and were milked at 0700 and 1600 h.

Experimental Diets

Diets consisted of a TMR based on corn silage (forage:concentrate, 60:40, DM basis) supplemented with 0 (control) or 45 g of a mixture (1:2 wt/wt) of FO and SO/kg of DM (FSO). Oils replaced concentrate ingredients on a proportionate basis, and rations were formulated according to Agricultural and Food Research Council (1993) to meet the nutrient requirements of dairy cows producing 30 kg of milk/d. Ingredient and chemical composition of experimental diets is shown in

Table 1. Formulation and chemical composition of experimental diets (g/kg of DM)

Ingredient	Diet	
	Control	FSO
Corn silage	650.0	650.0
Soybean meal	131.0	114.0
Rapeseed meal ¹	131.0	114.0
Wheat feed (middlings)	55.0	48.0
Blended molasses and urea ²	11.0	10.0
Fish oil ³	0.0	15.0
Sunflower oil ⁴	0.0	30.0
Limestone	8.0	7.1
Minerals and vitamins ⁵	12.7	11.9
Vitamin E premix ⁶	1.0	1.0
Composition, g/kg of DM		
OM	936	941
CP	172	170
NDF	405	387
Starch	167	157
Water-soluble carbohydrate	33.9	29.4
Ether extract	33.5	63.0

¹Solvent extracted rapeseed meal of low glucosinolate content.

²Regumaize 44 (SvG Intermol Limited, Bootle, Merseyside, United Kingdom). Declared composition (g/kg of DM): CP, 440; water-soluble carbohydrate, 550; and metabolizable energy content, 11.8 MJ/kg of DM.

³Ultra-refined herring and mackerel oil (Napros Pharma, AS, Brattvaag, Norway) contained (g/kg): 16:0, 150; *cis*-9 16:1, 75.8; 16:4 n-1, 20.3; 18:0, 27.8; *cis*-9 18:1, 70.5; *cis*-11 18:1, 26.1; 18:2 n-6, 11.3; 18:3 n-3, 7.2; 18:3 n-6, 2.4; 18:4 n-3, 33.9; *cis*-11 20:1, 14.2; 20:4 n-3, 9.1; 20:4 n-6, 8.2; 20:5 n-3, 15.9; 22:4 n-3, 9.1; 22:4 n-6, 8.3; 22:5 n-3, 16.9; 22:6 n-3, 11.3; and total fatty acids, 882.

⁴Sunflower oil (KTC Edibles, Ltd., Wednesbury, UK) contained (g/kg): 16:0, 56.9; 18:0, 29.8; *cis*-9 18:1, 21.9; *cis*-11 18:1, 5.7; 18:2 n-6, 56.8; 18:3 n-3, 0.6; 22:0, 5.2; and total fatty acids, 892.

⁵Proprietary mineral and vitamin supplement (Dairy Direct, Bury St. Edmonds, United Kingdom) declared as containing (g/kg): calcium, 270; magnesium, 60; sodium, 40; phosphorus, 40; zinc, 5.0; manganese, 4.0; and copper, 1.5 as well as (in mg/kg) iodine, 500; cobalt, 50; and selenium, 15; and (in IU/g) retinyl acetate, 500; cholecalciferol, 100; and DL- α -tocopheryl acetate, 0.5.

⁶Premix labeled as containing 500,000 IU of DL- α -tocopheryl acetate/kg (Roche Vitamins Ltd., Welwyn Garden City, United Kingdom).

Table 1. Diets were fed ad libitum as a TMR to avoid selection of dietary components. The control and FSO TMR were prepared at 0730 h on each day of the experiment, and ration mixes were adjusted weekly for changes in component DM content. Ultra-refined herring and mackerel oil (FO; Napros Pharma, AS, Brattvaag, Norway) and SO (KTC Edibles, Ltd., Wednesbury, United Kingdom) were stored in the dark at 4°C until incorporated into daily rations. Oils were mixed with concentrate ingredients in the feed mixer before corn silage was added to optimize oil dispersal. Approximately one-half of the daily control and FSO TMR were stored at 4°C until feeding out at 1630 h.

Measurements, Sample Collection, and Chemical Analysis

Individual animal intakes were recorded daily. Samples of fresh TMR and refused feeds were collected daily during each experimental week and stored at -20°C . At the end of the experiment, feed samples were composited for each experimental week. Samples of corn silage were analyzed for volatile components using accredited and Parliamentary-approved procedures for feedstuff analysis (Statutory Instruments, 1982, 1985) by a commercial laboratory (Natural Resources Management, Bracknell, United Kingdom). Chemical composition of corn silage and concentrates was determined in oven-dried (60°C) ground samples by the same commercial laboratory. Oven DM content was corrected for volatile losses according to Porter et al. (1984). Samples of FO and SO for fatty acid determinations were collected at the end of each experimental week and stored at -20°C before analysis.

Milk yields were recorded daily. Samples of milk for the determination of fat, protein, and lactose content were collected from each cow at 0700 and 1600 h on alternate days, starting on d 1 of the experiment. Milk fat, crude protein, and lactose were determined in samples treated with potassium dichromate preservative (1 mg/mL, Lactabs, Thomson and Capper, Runcorn, United Kingdom) using a Milko-Scan 133B analyzer (Foss Electric Ltd., York, United Kingdom). Near infrared detection of milk constituents was calibrated using milk samples for which reference measurements had previously been made using standard procedures (AOAC, 1990). Estimation of milk CP content by near infrared spectroscopy was based on the assumption that the ratio of true protein:nonprotein nitrogen was constant. Fatty acid composition was determined in untreated samples of milk composited according to yield. Milk samples for fatty acid analysis were stored at -20°C until composited and submitted for fatty acid analysis.

Fatty Acid Analysis

Fatty acid methyl esters (**FAME**) of lipids in FO and SO were prepared in a one-step extraction-transesterification procedure using chloroform according to Sukhija and Palmquist (1988); the one exception was that methanolic sulfuric acid (2%, vol/vol) was used as the methylating reagent and trionadecanoin (T-165, Nu-Chek Prep, Elysian, MN) dissolved in absolute ethanol as an internal standard (Shingfield et al., 2003). For milk fatty acid determinations, lipid in 1-mL samples was extracted using 1 mL of ethanol, 2.5 mL of diethyl ether, and 2.0 mL of hexane (5:4, vol/vol) according to reference procedures (IDF 1C:1987 and IDF 16C:1987,

International Dairy Federation, Brussels, Belgium). This procedure was repeated ($n = 2$) with the exception that 0.5 mL or no ethanol was used during the second and third extractions, respectively. Organic extracts were combined and evaporated to dryness at 60°C under nitrogen for 1 h. Samples were dissolved in hexane and methyl acetate and transesterified to FAME using freshly prepared methanolic sodium methoxide according to Christie (1982). The mixture was neutralized with oxalic acid (1 g of oxalic acid in 30 mL of diethyl ether), centrifuged, and dried using anhydrous calcium chloride. Milk FAME were dissolved (1:3, vol/vol) in hexane before transferring to gas-chromatography vials.

The FAME were separated and quantitated using a gas chromatograph (3400 CX, Varian Instruments, Walnut Creek, CA) 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- μm film of cyanopropyl polysiloxane (CP-SIL 88, Chrompack 7489, Middelburg, The Netherlands) using hydrogen as the carrier and fuel gas. Total FAME profile in a 2- μL sample at a split ratio of 1:50 was determined using a temperature gradient program according to Shingfield et al. (2003). Following sample injection, column temperature was maintained at 70°C for 1 min, increased at a rate of $5^{\circ}\text{C}/\text{min}$ to 100°C , held at 100°C for 2 min, raised to 175°C at a rate of $10^{\circ}\text{C}/\text{min}$, held at 175°C for 34 min, increased at $4^{\circ}\text{C}/\text{min}$ to a final temperature of 225°C that was maintained for 22 min. Peaks were routinely identified by comparison of retention times with authentic FAME standards (GLC #463, special preparation reference standard GLC #606, Nu-Chek Prep Inc.; L-8404, Sigma-Aldrich, Helsinki, Finland) and CRM 164 milk fat reference standard (Community Bureau of Reference, Brussels, Belgium). Identification was confirmed based on electron impact ionization spectra of milk fat FAME obtained by gas chromatography-mass spectrometry (**GC-MS**) using a gas chromatograph (6890, Hewlett-Packard, Wilmington, DE) equipped with a 100-m CP-SIL 88 column and selective quadrupole mass detector (model 5973N, Agilent Technologies Inc., Wilmington, DE) under an ionization voltage of 400 eV, using helium as the carrier gas. Gas chromatography—mass spectrometry analysis was performed using the same temperature gradient applied during routine determinations of milk fat FAME composition.

Isomers of 18:1, 18:2, and CLA methyl esters were further separated under isothermal conditions; column temperature was maintained at 160°C for 75 min, increased to 240°C at a rate of $25^{\circ}\text{C}/\text{min}$, and held at this temperature for 10 min using hydrogen as the carrier gas according to Shingfield et al. (2005). Under these

Figure 1. Partial gas chromatogram indicating the separation of 18:1 and 18:2 isomers obtained under isothermal conditions (160°C) in milk fat from cows fed corn silage-based diets containing fish oil and sunflower oil. The y-axis represents arbitrary response units. Identification was based on electron impact ionization spectra obtained by gas chromatography-mass spectrometry analysis of fatty acid methyl esters and 4,4-dimethyloxaline derivatives and the elution of an authentic standard (L-8404, Sigma-Aldrich, Helsinki, Finland) containing a mixture of geometric isomers of 9,12 18:2. Peak identification: 1 = 18:0; 2 = *trans*-4 18:1; 3 = *trans*-5 18:1; 4 = unresolved *trans*-6, -7, and -8 18:1; 5 = *trans*-9 18:1; 6 = *trans*-10 18:1; 7 = *trans*-11 18:1; 8 = *trans*-12 18:1; 9 = unresolved *trans*-13 and -14 18:1; 10 = *cis*-9 18:1; 11 = *trans*-15 18:1; 12 = *cis*-11 18:1; 13 = *cis*-12 18:1; 14 = *cis*-13 18:1; 15 = unresolved *cis*-14 and *trans*-16 18:1; 16 = 19:0; 17 = *cis*-15 18:1; 18 = *trans*, *trans* 18:2 (double bond position undetermined); 19 = *trans*-11, *trans*-15 18:2; 20 = *trans*-10, *trans*-14 18:2; 21 = *cis*-9, *trans*-13 18:2; 22 = methyl 11-cyclohexyl 11:0; 23 = *cis*-9, *trans*-12 18:2; 24 = *cis*-16 18:1; 25 = *trans*-9, *cis*-12 18:2 (identified based on comparable retention time with an authentic standard); 26 = *cis*-*trans*/*trans*-*cis* 10, 14 18:2; 27 = *cis*-*trans*/*trans*-*cis* 12, 16 18:2; 28 = *trans*-11, *cis*-15 18:2; 29 = methyl 11,12-methylene-18:0; 30 = *cis*-9, *cis*-12 18:2; 31 = *cis*-9, *cis*-15 18:2; 32 = 12, 15 18:2 (double bond geometry undetermined). *Elutes with the same retention time as *trans*-9, *trans*-12 18:2 methyl ester standard.

conditions, several reports have indicated that *cis* and *trans* 18:1 isomers are not completely resolved. Evidence exists that *cis*-6, -7, and -8 coelute with *trans*-13 and -14, *cis*-10 overlaps with *trans*-15, and *cis*-14 and *trans*-16 are detected as a single peak (Molkentin and Precht, 1995; Kramer et al., 2002; Cruz-Hernandez et al., 2004). To establish the extent of overlapping of *cis* and *trans* 18:1 in the current analysis and confirm the identity of other fatty acids not contained in commercially available standards, selected samples of milk fat FAME from both the control and FSO treatments were converted to 4,4-dimethyloxazoline (DMOX) fatty acid derivatives and analyzed by GC-MS. The DMOX derivatives were prepared from milk fat FAME using 2-amino, 2-methyl-1-propanol (500 μ L) by heating overnight under a nitrogen atmosphere according to Fay and Richli (1991) with the exception that a temperature of 150°C was used. Gas-liquid chromatography separation of DMOX derivatives was performed using the same equipment, ionization voltage, temperature gradient, and isothermal conditions applied for GC-MS analysis of FAME. The electron impact ionization spectra obtained was used to locate double bonds 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. Identification was further validated by comparison with an online reference library of DMOX electron impact ionization spectra (www.lipids.co.uk/infores/masspec.html). Mass spectra of the DMOX derivatives gave little indication of significant overlaps of *trans* and *cis* 18:1 isomers with the exception of *cis*-14 18:1 and *trans*-16 18:1, which eluted as a single peak (data not presented). After reanalysis using a temperature program (initial column temperature of 120°C increased at a rate of 0.25°C/min; total run time = 600 min) that allowed for baseline separation of *cis*-14 18:1 and *trans*-16 18:1 DMOX derivatives, it was evident that *trans*-16 was the dominant isomer, accounting for proportionately about 0.80 of the total peak area of unresolved *cis*-14 18:1/*trans*-16 18:1 in the samples analyzed. A partial gas chromatogram indicating typical separation of 18:1 and 18:2 isomers in milk from cows fed the FSO diet under isothermal conditions is shown in Figure 1.

Isomers of CLA were identified by comparison of retention times with an authentic standard (O-5632, Sigma-Aldrich) and chemically synthesized *trans*-9, *cis*-11 CLA (Shingfield et al., 2005) using *cis*-9, *trans*-11 as a landmark isomer. Identification was confirmed based on GC-MS analysis of FAME and DMOX derivatives and cross-referencing with milk samples for which the distribution of CLA isomers was determined by sil-

Figure 2. Partial gas chromatogram indicating the separation of isomers of conjugated linoleic acid (CLA) obtained under isothermal conditions (160°C) in milk fat from cows fed corn silage-based diets containing fish oil and sunflower oil. The y-axis represents arbitrary response units. Identification was based on electron impact ionization spectra obtained by gas chromatography-mass spectrometry analysis of fatty acid methyl esters and 4,4-dimethyloxaline derivatives. Peak identification: 1 = unresolved *trans*-7, *cis*-9 CLA, *trans*-8, *cis*-10 CLA, and *cis*-9, *trans*-11 CLA; 2 = *trans*-9, *cis*-11 CLA; 3 = 21:0; 4 = *trans*-10, *cis*-12 CLA; 5 = unresolved *cis*-9, *cis*-11 CLA and *trans*-11, *cis*-13 CLA; 6 = isomers of 20:2 (double bond position undetermined); 7 = *trans*-11, *trans*-13 CLA; 8 = unresolved *trans*-7, *trans*-9 CLA, *trans*-8, *trans*-10 CLA, *trans*-9, *trans*-11 CLA, and *trans*-10, *trans*-12 CLA; 9 = 10, 14 20:2 (double bond geometry undetermined); 10 = 12, 16 20:2 (double bond geometry undetermined).

ver-ion HPLC (Shingfield et al., 2003, 2005). The GC-MS analysis of DMOX derivatives revealed that under the gas chromatography conditions used, the major CLA peak consisted of *trans*-7, *cis*-9; *trans*-8, *cis*-10; and *cis*-9, *trans*-11 (data not presented). For cows fed comparable diets containing FO and SO, mean concentrations of *trans*-7, *cis*-9 CLA and *trans*-8, *cis*-10 CLA represent proportionately 0.066 ± 0.019 and 0.013 ± 0.002 of milk fat *cis*-9, *trans*-11 CLA content, respectively (Shingfield et al., 2005). Owing to the large number of samples generated in this experiment, it was not possible to separate individual isomers of CLA by silver-ion HPLC; therefore, the major CLA peak determined by gas chromatography was assumed to reflect changes in the predominant *cis*-9, *trans*-11 isomer. A partial gas chromatogram indicating the typical separation of CLA methyl esters obtained under isothermal conditions for milk fat from cows fed the FSO diet is shown in Figure 2.

Statistical Analysis

Measurements of DMI, milk production, and milk fatty acid composition were analyzed by repeated measures ANOVA for a randomized block design using the MIXED procedure of SAS Inst. (2001). The statistical model included the fixed effects of diet, time, and their interaction and the random effects of cow within block, assuming an autoregressive order one covariance structure fitted on the basis of Akaike information and Schwarz Bayesian model fit criteria. Least squares

means are reported, and significance was declared at $P < 0.05$.

Relationships among individual milk fatty acids and those between milk fatty acid composition and milk fat content were examined by regression analysis using the REG procedure of SAS. In cases where close linear or quadratic associations were identified between milk fat content and concentrations of a specific fatty acid in milk, the relationship was further explored with an exponential decay model fitted using the Marquart non-linear algorithm within the NLIN procedure of SAS according to de Veth et al. (2004).

RESULTS

Nutrient Intake and Animal Performance

Inclusion of FO and SO in the diet decreased ($P < 0.05$) DMI intake, milk fat yield, milk protein output, and milk fat content, but had no effect ($P > 0.05$) on milk yield (Table 2). Reductions in DMI of cows fed the FSO treatment were independent ($P > 0.05$) of time on diet (Table 2; Figure 3). Yields of milk, milk protein, and fat for cows on the control diet remained relatively constant throughout the experiment (Figure 3). Compared with the control, the FSO diet resulted in a significant ($P < 0.05$) reduction in the concentration and output of milk protein and fat (Table 2). Changes in milk protein yield were unaffected by time on the FSO diet (Figure 3), but milk protein concentrations (g/kg)

Table 2. Mean effects of fish oil and sunflower oil in the diet on intake, milk yield, and milk composition

	Treatment ¹		SEM	<i>P</i> < ²		<i>D</i> × <i>T</i>
	Control	FSO		<i>D</i>	<i>T</i>	
DMI, kg/d	21.9	17.4	0.45	<0.01	0.04	0.18
Yield						
Milk, kg/d	27.1	26.4	0.85	0.55	0.06	0.71
Fat, g/d	1,242	768	36.6	<0.01	0.01	<0.01
Protein, g/d	975	871	18.4	<0.01	0.27	0.37
Lactose, g/d	1,220	1,208	45.2	0.85	0.02	0.38
Concentration, g/kg						
Fat	46.0	29.0	1.09	<0.01	<0.01	<0.01
Protein	36.1	33.3	0.86	0.04	<0.01	<0.01
Lactose	45.0	45.5	0.56	0.55	<0.01	0.02

¹Refers to corn silage-based diets containing 0 (control) or 45 g of a mixture (1:2, wt/wt) of fish oil and sunflower oil/kg of DM (FSO).

²Probability of significant effects attributable to fish oil and sunflower oil in the diet (*D*), time on diet (*T*), and their interaction (*D* × *T*).

were found to decrease between d 1 and 7 from 34.4 to 31.0, gradually increasing thereafter, reaching a final concentration of 33.8 g/kg on d 27. Reductions in milk fat content and yield for cows on the FSO diet were

time dependent. Both milk fat content and yield from cows fed the FSO diet were progressively decreased from 45.2 g/kg and 1,130 g/d on d 1, reaching a nadir of 22.2 g/kg and 588 g/d on d 19 (Figure 3).

Figure 3. Temporal changes in DMI, milk yield, milk fat yield, and milk protein content in cows fed corn silage-based diets containing 0 (●) or 45 (○) g of a mixture (1:2, wt/wt) of fish oil and sunflower oil/kg of DM. Values represent the means from 8 animals. SEM = 0.45, 0.85, 0.037, and 0.018 kg/d for DMI, milk yield, milk fat yield, and milk protein yield, respectively.

Milk Fatty Acid Composition

Dietary supplementation with FO and SO in the diet resulted in marked alterations in milk fatty acid composition relative to the control diet, changes that were characterized as a reduction ($P < 0.05$) in 4:0 to 18:0 content and an increase in *trans* 18:1, total CLA, C20, and C22 fatty acid concentrations (Table 3).

For most fatty acids, responses to FO and SO in the diet varied according to time on diet, whereas the fatty acid composition of milk from the control diet remained relatively constant throughout the experiment (Figures 4 and 5). Temporal changes in the response to FSO treatment were fatty acid dependent. Concentrations of C4 to C14 fatty acids in milk progressively declined with time on the FSO diet, and concentrations of 16:0 decreased within a few days of supplementation and remained low thereafter (Figure 4). In contrast, the decreases in 18:0 and *cis*-9 18:1 in response to FSO treatment were transient, such that levels of these fatty acids were comparable with concentrations in control milk at the end of the experiment (Figure 4). The FSO diet also increased ($P < 0.001$) milk 20:5 *n*-3 and 22:6 *n*-3 content, but the level of enrichment started to decline after d 5 (Figure 4).

Compared with the control, concentrations of *trans*-4 to *trans*-15, *cis*-11, and *cis*-13 to *cis*-16 18:1 were higher ($P < 0.05$), and that of *cis*-9 and unresolved *cis*-14 and *trans*-16 18:1 were lower ($P < 0.05$), in milk from cows fed the FSO diet (Table 4). Even though total *trans* 18:1 content of FSO milk remained relatively constant after d 7 (data not presented), the distribution of individual isomers varied according to time on diet. Initially, most of the *trans* 18:1 response to FSO in the diet was related to marked increases in *trans*-11 18:1, but after d 5, the levels of this isomer started to decline, an effect that was associated with concomitant increases in several other *trans* 18:1 isomers, the most notable being a rapid elevation in *trans*-10 18:1 content (Figure 5). Temporal changes in response to the FSO diet were not confined to *trans*-10 18:1 and *trans*-11 18:1, and levels of other *trans* 18:1 isomers were also dependent on the duration of feeding (Figure 5). Time-related variations in unresolved *trans*-6+7+8 18:1 concentrations were similar to those of *trans*-9 18:1, and responses of unresolved *trans*-13+14 and *trans*-15 18:1 were comparable with those of *trans*-12 18:1. In marked contrast, concentrations of unresolved *cis*-14 and *trans*-16 18:1 were decreased by the FSO diet, but started to increase after d 9, approaching comparable concentrations to the control after d 15 (data not presented). Feeding the FSO diet enhanced ($P < 0.05$) milk *trans* 18:2 concentrations (Table 5); increases over time to the FSO diet were isomer dependent (data not shown).

For the control diet, concentrations of individual CLA isomers in control milk were relatively constant across sampling times, whereas milk fat CLA responses to FSO were isomer dependent and varied according to time on diet (Figure 5). Increases in unresolved *trans*-7, *cis*-9 CLA, *trans*-8, *cis*-10 CLA, and *cis*-9, *trans*-11 CLA accounted for proportionately 0.97 ± 0.010 of the total milk fat CLA response to FO and SO (data not presented). Concentrations of *cis*-9, *trans*-11 CLA in milk increased immediately on the FSO diet, reaching the highest levels of enrichment on d 5, but declined thereafter (Figure 5). However, there was considerable variation among individual animals; the maximum milk fat *cis*-9, *trans*-11 CLA content for cows fed the FSO diet ranged between 5.34 and 6.76 g/100 g of fatty acids (Figure 6). Across all diets and sampling times ($n = 224$), a close relationship existed between milk *cis*-9, *trans*-11 CLA and *trans*-11 18:1 content [$(\text{cis-9, trans-11 CLA}) = 0.43 \pm 0.043 + 0.35 \pm 0.007 (\text{trans-11 18:1})$; $r^2 = 0.916$; $P < 0.001$].

Feeding the FSO diet caused a significant ($P < 0.01$) reduction in the secretion of total fatty acids in milk because of a substantial decrease ($P < 0.01$) in the amount of short- and medium-chain fatty acids (C4 to C16) secreted in milk, and the total output of long-chain fatty acids (C18 to C26) was higher ($P < 0.05$) for the FSO diet compared with the control (Table 6).

DISCUSSION

Animal Performance

Inclusion of FO and SO in the diet depressed DMI but did not decrease milk yield. Supplementing diets with oils rich in polyunsaturated fatty acids (PUFA) often results in a reduction in nutrient intake and milk production (Chilliard et al., 2001; Lock and Shingfield, 2004). The lower intake on the FSO diet might be related in part to possible negative effects of unsaturated oils in the diet on rumen function (Jenkins, 1993) or to a response to increases in the flow of unsaturated fatty acids at the duodenum. Both FO (Scollan et al., 2001; Shingfield et al., 2003) and SO (Duckett et al., 2002; Sackmann et al., 2003) in the diet increase the flow of biohydrogenation intermediates leaving the rumen. Previous studies have shown that postruminal infusions of unsaturated fatty acids lower DMI in lactating cows (Drackley et al., 1992; Christensen et al., 1994); the extent of that reduction is about 2-fold greater when unsaturated fatty acids are nonesterified as opposed to infusion as triacylglycerides (Litherland et al., 2005).

Decreases in the milk protein content in cows fed the FSO diet are consistent with responses to diets containing FO and 18:2 *n*-6 rich oilseeds (Whitlock et al., 2002; AbuGhazaleh et al., 2002) or fish meal and

Table 3. Mean effects of fish oil and sunflower oil in the diet on milk fatty acid composition (g/100 g of fatty acids)

	Treatment ¹		SEM	P< ²		D × T
	Control	FSO		D	T	
4:0	4.29	3.01	0.101	<0.01	<0.01	<0.01
6:0	2.53	1.41	0.073	<0.01	<0.01	<0.01
8:0	1.48	0.76	0.048	<0.01	<0.01	<0.01
10:0	3.41	1.73	0.115	<0.01	<0.01	<0.01
12:0	4.07	2.26	0.122	<0.01	<0.01	<0.01
13:0 <i>iso</i>	0.04	0.04	0.002	0.75	<0.01	0.31
13:0 <i>ante-iso</i>	0.13	0.06	0.004	<0.01	<0.01	<0.01
14:0	11.42	9.39	0.290	<0.01	<0.01	<0.01
14:1 <i>cis</i> -9	1.32	1.33	0.090	0.94	<0.01	<0.01
15:0	1.50	0.92	0.054	<0.01	0.23	0.23
15:0 <i>iso</i>	0.26	0.17	0.007	<0.01	<0.01	<0.01
15:0 <i>ante-iso</i>	0.46	0.32	0.009	<0.01	<0.01	<0.01
16:0	34.41	27.25	0.829	<0.01	<0.01	<0.01
16:1 <i>cis</i> -9	2.54	2.71	0.169	0.49	<0.01	<0.01
16:1 <i>cis</i> -11	0.03	0.08	0.001	<0.01	<0.01	<0.01
17:0	0.79	0.51	0.014	<0.01	<0.01	<0.01
17:0 <i>iso</i>	0.41	0.82	0.030	<0.01	<0.01	<0.01
17:0 <i>ante-iso</i>	0.15	0.18	0.006	<0.01	<0.01	<0.01
18:0	6.86	4.52	0.236	<0.01	<0.01	<0.01
18:1 <i>cis</i> ³	15.83	13.57	0.464	<0.01	<0.01	<0.01
18:1 <i>trans</i> ³	2.44	18.27	0.525	<0.01	<0.01	<0.01
18:1 total	18.27	31.84	0.724	<0.01	<0.01	<0.01
18:2 ⁴	2.05	2.91	0.094	<0.01	<0.01	<0.01
CLA ⁵	0.50	3.47	0.172	<0.01	<0.01	<0.01
18:3 n-3	0.21	0.23	0.008	0.05	<0.01	0.27
18:3 n-6	0.03	0.01	0.001	<0.01	<0.01	<0.01
18:4 n-3	0.00	0.02	0.001	<0.01	<0.01	<0.01
20:0	0.12	0.12	0.006	0.68	<0.01	0.01
20:1 <i>cis</i> -11	0.04	0.22	0.007	<0.01	<0.01	<0.01
20:1 ⁶	0.13	0.28	0.010	<0.01	<0.01	<0.01
20:2 n-3	0.02	0.05	0.001	<0.01	<0.01	<0.01
20:2 n-6	0.02	0.01	0.001	<0.01	<0.01	<0.01
20:3 n-3	0.00	0.14	0.006	<0.01	<0.01	<0.01
20:3 n-6	0.11	0.12	0.004	0.16	<0.01	<0.01
20:4 n-3	0.01	0.18	0.009	<0.01	<0.01	<0.01
20:4 n-6	0.17	0.12	0.005	<0.01	<0.01	<0.01
20:5 n-3	0.03	0.11	0.005	<0.01	<0.01	<0.01
22:0	0.04	0.06	0.003	<0.01	<0.01	<0.01
22:1 <i>cis</i> -13	0.01	0.02	0.001	<0.01	<0.01	<0.01
22:3 n-3	0.00	0.02	0.002	<0.01	<0.01	<0.01
22:4 n-3	0.00	0.24	0.012	<0.01	<0.01	<0.01
22:4 n-6	0.03	0.02	0.002	<0.01	<0.01	<0.01
22:5 n-3	0.07	0.16	0.005	<0.01	<0.01	<0.01
22:5 n-6	0.00	0.03	0.002	<0.01	<0.01	<0.01
22:6 n-3	0.00	0.07	0.004	<0.01	<0.01	<0.01
24:0	0.02	0.02	0.001	0.49	0.08	0.22
Summary						
Total ≤C14	29.67	20.38	0.624	<0.01	<0.01	<0.01
Total saturates	73.55	54.88	0.800	<0.01	<0.01	<0.01
Total MUFA	22.95	36.87	0.714	<0.01	<0.01	<0.01
Total PUFA	3.47	8.29	0.206	<0.01	<0.01	<0.01

¹Refers to corn silage-based diets containing 0 (control) or 45 g of a mixture (1:2, wt/wt) of fish oil and sunflower oil/kg of DM (FSO).

²Probability of significant effects attributable to fish oil and sunflower oil in the diet (D), time on diet (T), and their interaction (D × T).

³Calculated assuming that *cis*-14 18:1 accounted for proportionately 0.20 of unresolved *cis*-14 18:1 and *trans*-16 18:1.

⁴Sum of 18:2 fatty acids excluding isomers of conjugated linoleic acid.

⁵Conjugated linoleic acid.

⁶Sum of 20:1 isomers (n = 7) excluding *cis*-11 20:1.

Figure 4. Temporal changes in total C4 to C14, 16:0, 18:0, *cis*-9 18:1, 20:5 n-3, and 22:6 n-3 concentrations (g/100 g of fatty acids) in milk from cows fed corn silage-based diets containing 0 (●) or 45 (○) g of a mixture (1:2; wt/wt) of fish oil and sunflower oil/kg of DM. Values represent the means from 8 animals. SEM = 0.62, 0.83, 0.24, 0.44, 0.005, and 0.004 g/100 g of fatty acids for 16:0, 18:0, *cis*-9 18:1, 20:5 n-3, and 22:6 n-3, respectively.

extruded soybeans (AbuGhazaleh et al., 2003). Supplementing diets with oils rich in PUFA generally causes a reduction in milk protein content (Wu and Huber, 1994; Lock and Shingfield, 2004), changes that have

often been attributed to increases in milk yield rather than decreases in milk protein synthesis (DePeters and Cant, 1992). In the current experiment, the FSO diet reduced both milk protein content and output compared

Figure 5. Temporal changes in unresolved *trans*-6+7+8 18:1, *trans*-10 18:1, *trans*-11 18:1, *trans*-12 18:1, *cis*-9, *trans*-11 conjugated linoleic acid (CLA), *trans*-9, *cis*-11 CLA, and *trans*-10, *cis*-12 CLA concentrations (g/100 g of fatty acids) in milk from cows fed corn silage-based diets containing 0 (●) or 45 (○) g of a mixture (1:2, wt/wt) of fish oil and sunflower oil/kg of DM. Values represent the means from 8 animals. SEM = 0.02, 0.73, 0.62, 0.02, 0.17, 0.009, and 0.002 g/100 g of fatty acids, for *trans*-6+7+8 18:1, *trans*-10 18:1, *trans*-11 18:1, *trans*-12 18:1, *cis*-9, *trans*-11 CLA, *trans*-9, *cis*-11 CLA, and *trans*-10, *cis*-12 CLA, respectively.

Table 4. Mean effects of fish oil and sunflower oil in the diet on milk 18:1 content (g/100 g of fatty acids)

	Treatment ¹		SEM	P< ²		
	Control	FSO		D	T	D × T
<i>cis</i> -9	14.89	11.93	0.440	<0.01	<0.01	<0.01
<i>cis</i> -11	0.60	1.16	0.042	<0.01	<0.01	<0.01
<i>cis</i> -12	0.22	0.25	0.019	0.30	<0.01	<0.01
<i>cis</i> -13	0.05	0.12	0.004	<0.01	<0.01	<0.01
<i>cis</i> -15	0.03	0.07	0.002	<0.01	<0.01	<0.01
<i>cis</i> -16	0.06	0.12	0.003	<0.01	<0.01	<0.01
<i>trans</i> -4	0.01	0.03	0.002	<0.01	<0.01	<0.01
<i>trans</i> -5	0.01	0.03	0.002	<0.01	<0.01	0.01
<i>trans</i> -6+7+8	0.22	0.75	0.021	<0.01	<0.01	<0.01
<i>trans</i> -9	0.20	0.70	0.014	<0.01	<0.01	<0.01
<i>trans</i> -10	0.32	7.72	0.732	<0.01	<0.01	<0.01
<i>trans</i> -11	0.69	6.88	0.624	<0.01	<0.01	<0.01
<i>trans</i> -12	0.30	0.92	0.022	<0.01	<0.01	<0.01
<i>trans</i> -13+14	0.37	0.84	0.023	<0.01	<0.01	<0.01
<i>trans</i> -15	0.13	0.26	0.007	<0.01	<0.01	<0.01
<i>trans</i> -16+ <i>cis</i> -14	0.22	0.18	0.006	<0.01	<0.01	<0.01

¹Refers to corn silage-based diets containing 0 (control) or 45 g of a mixture (1:2, wt/wt) of fish oil and sunflower oil/kg of DM (FSO).

²Probability of significant effects attributable to fish oil and sunflower oil in the diet (D), time on diet (T), and their interaction (D × T).

with the control. Energy intake is generally thought to be the major nutritional factor affecting milk protein concentrations (Coulon and Remond, 1991; Lock and Shingfield, 2004), suggesting that the effects of FO and SO on DMI were largely responsible for the reductions in milk protein content in cows fed the FSO diet.

Reductions in milk fat content with the FSO diet are in line with the decreases reported for cows fed fish products (FO or fish meal) and extruded soybeans (Whitlock et al., 2002; AbuGhazaleh et al., 2002, 2004). Both FO (Scollan et al., 2001; Shingfield et al., 2003) and SO (Duckett et al., 2002; Sackmann et al., 2003)

Table 5. Mean effects of fish oil and sunflower oil in the diet on milk 18:2 content (mg/100 g of total fatty acids)

	Treatment ¹		SEM	P< ²		
	Control	FSO		D	F	D × T
Nonconjugated						
<i>cis</i> -9, <i>cis</i> -12	1,679	1,792	68.7	0.26	<0.01	<0.01
<i>cis</i> -9, <i>cis</i> -15	34	22	0.9	<0.01	0.04	0.02
<i>cis</i> -9, <i>trans</i> -12	83	106	3.9	<0.01	<0.01	<0.01
<i>cis</i> -9, <i>trans</i> -13	163	302	11.7	<0.01	<0.01	<0.01
<i>trans</i> -9, <i>cis</i> -12	28	156	7.7	<0.01	<0.01	<0.01
<i>trans</i> -11, <i>cis</i> -15	26	383	9.7	<0.01	<0.01	<0.01
<i>trans</i> -10, <i>trans</i> -14	9	52	2.9	<0.01	<0.01	<0.01
Conjugated						
<i>cis</i> -9, <i>trans</i> -11 ³	440	3,040	173.5	<0.01	<0.01	<0.01
<i>trans</i> -9, <i>cis</i> -11	10	128	8.7	<0.01	<0.01	<0.01
<i>trans</i> -10, <i>cis</i> -12	0.3	74.8	2.35	<0.01	<0.01	<0.01
<i>trans</i> -11, <i>cis</i> -13 ⁴	10	38	1.0	<0.01	<0.01	<0.01
<i>trans</i> -11, <i>trans</i> -13	20	29	1.3	<0.01	0.02	0.03
<i>trans</i> , <i>trans</i> ⁵	18	50	1.3	<0.01	<0.01	<0.01

¹Refers to corn silage-based diets containing 0 (control) or 45 g of a mixture (1:2, wt/wt) of fish oil and sunflower oil/kg of DM (FSO).

²Probability of significant effects attributable to fish oil and sunflower oil in the diet (D), time on diet (T), and their interaction (D × T).

³*cis*-9, *trans*-11 CLA (conjugated linoleic acid) contained *trans*-7, *cis*-9 CLA and *trans*-8, *cis*-10 CLA as minor components.

⁴Contribution of *cis*-9, *cis*-11 CLA assumed to be negligible (Shingfield et al., 2005).

⁵Sum of unresolved *trans*-7, *trans*-9 CLA, *trans*-8, *trans*-10 CLA, *trans*-9, *trans*-11 CLA, and *trans*-10, *trans*-12 CLA.

Figure 6. Temporal changes in milk fat *cis*-9, *trans*-11 conjugated linoleic acid (CLA) content of individual cows fed corn silage-based diets containing 45 g of a mixture (1:2; wt/wt) of fish oil and sunflower oil/kg of DM. Different symbols represent individual animals ($n = 8$).

are known to modify ruminal lipid metabolism, leading to changes in the profile of biohydrogenation intermediates leaving the rumen. It is well established that post-ruminal infusions of *trans*-10, *cis*-12 CLA inhibit milk fat synthesis in dairy cows (Baumgard et al., 2000), and increased formation of this isomer in the rumen has been implicated during dietary induced milk fat depression (MFD; Bauman and Griinari, 2003).

Evaluation of a number of postruminal infusion studies have shown that large reductions in milk fat secretion occur in response to small doses of *trans*-10, *cis*-12 CLA, but increasing the amount of *trans*-10, *cis*-12 CLA infused at >6 g/d elicits relatively minor or no further reductions in milk fat secretion (de Veth et al., 2004). Furthermore, small increases in milk fat *trans*-10, *cis*-12 CLA content following postruminal infusions of this isomer are associated with large decreases in milk fat synthesis; therefore, the use of exponential decay models is an appropriate means to assess the relationship between the reduction in milk fat secretion and milk fat *trans*-10, *cis*-12 CLA content (de Veth et al., 2004). Application of the same nonlinear modeling approach indicated that variations in milk *trans*-10, *cis*-12 CLA content in the present experiment accounted for proportionately 0.57 of the variation in milk fat content on the FSO diet (Figure 7). Assuming that the concentration in milk fat reflects mammary *trans*-10,

cis-12 CLA uptake, this association suggests that at least part of the decrease in milk fat secretion in cows fed FSO can be attributed to increased formation of this intermediate in the rumen.

It is becoming increasingly evident that milk fat *trans*-10, *cis*-12 CLA concentrations are much lower during dietary-induced MFD than are the levels of this isomer in milk when comparable decreases in milk fat synthesis are induced by postruminal *trans*-10, *cis*-12 CLA infusions (Bauman and Griinari, 2003), suggesting that other biohydrogenation intermediates may also contribute to the reduction in the milk fat secretion. Inclusion of marine lipids in the diet can cause a reduction in milk fat yield with no changes or only relatively minor increases in milk fat *trans*-10, *cis*-12 CLA content (Offer et al., 1999, 2001); more recent studies have shown that FO may reduce the flow of *trans*-10, *cis*-12 CLA leaving the rumen (Shingfield et al., 2003). Under these circumstances, decreases in milk fat content in response to FO (Offer et al., 1999; Loor et al., 2005a) or marine algal oil (Offer et al., 2001) are associated with increases in milk fat *trans*-10 18:1 concentrations that arise from increased ruminal formation of this biohydrogenation intermediate (Shingfield et al., 2003; Loor et al., 2005c). In the current study, changes in milk fat *trans*-10 18:1 content accounted for proportionately 0.74 of the variation in milk fat content for the FSO

Table 6. Mean effects of fish oil and sunflower oil in the diet on the secretion of fatty acids in milk (g/d)

	Treatment ¹		SEM	P< ²		
	Control	FSO		D	T	D × T
4:0	37.2	17.7	1.38	<0.01	<0.01	<0.01
6:0	23.9	9.3	0.88	<0.01	<0.01	<0.01
8:0	14.7	5.4	0.57	<0.01	<0.01	<0.01
10:0	34.8	12.5	1.40	<0.01	<0.01	<0.01
12:0	42.7	16.2	1.72	<0.01	<0.01	<0.01
14:0	123	65.8	4.17	<0.01	<0.01	<0.01
14:1 <i>cis</i> -9	14.3	8.6	0.80	<0.01	<0.01	<0.01
16:0	378	186	11.9	<0.01	<0.01	<0.01
16:1 <i>cis</i> -9	28.0	17.3	1.41	<0.01	<0.01	0.08
18:0	75.7	31.4	2.81	<0.01	<0.01	<0.01
18:1 <i>cis</i> ³	175	94.0	5.45	<0.01	<0.01	<0.01
18:1 <i>trans</i> ³	27.3	119	6.06	<0.01	<0.01	<0.01
18:1 total	202	213	9.6	0.45	0.01	<0.01
18:2 ⁴	22.5	19.5	0.72	0.01	<0.01	<0.01
CLA ⁵	5.5	25.2	1.71	<0.01	<0.01	<0.01
18:3 n-3	2.3	1.6	0.07	<0.01	<0.01	<0.01
20:5 n-3	0.36	0.81	0.040	<0.01	<0.01	<0.01
22:6 n-3	0.01	0.50	0.033	<0.01	<0.01	<0.01
Total fatty acids	1073	676	32.5	<0.01	<0.01	<0.01
Summary						
Total ≤C14	318	152	9.5	<0.01	<0.01	<0.01
Total C16 ⁶	410	209	13.0	<0.01	<0.01	<0.01
Total C18-C26 ⁷	243	275	12.1	0.09	<0.01	<0.01
Total saturates	827	410	22.9	<0.01	<0.01	<0.01
Total MUFA ⁸	267	269	9.3	0.90	<0.01	0.02
Total PUFA ⁹	37.9	60.3	2.50	<0.01	<0.01	<0.01

¹Refers to corn silage-based diets containing 0 (control) or 45 g of a mixture (1:2, wt/wt) of fish oil and sunflower oil/kg of DM (FSO).

²Probability of significant effects attributable to fish oil and sunflower oil in the diet (D), time on diet (T), and their interaction (D × T).

³Calculated assuming that *cis*-14 18:1 accounted for proportionately 0.20 of unresolved *cis*-14 18:1 and *trans*-16 18:1.

⁴Sum of 18:2 fatty acids excluding isomers of conjugated linoleic acid.

⁵Conjugated linoleic acid.

⁶Sum of C16 fatty acids.

⁷Sum of C18 to C26 fatty acids.

⁸Monounsaturated fatty acids.

⁹Polyunsaturated fatty acids.

diet (Figure 7). However, a close association between these parameters does not imply a cause and effect in this relationship, but simply indicates that changes in ruminal lipid metabolism causing an increase in *trans*-10 18:1 formation are also conditions in which MFD occurred. Although calcium salts of *trans* 18:1 fatty acids in the diet have recently been shown to reduce milk fat content and yield (Piperova et al., 2004), it remains unclear whether *trans*-10 18:1 is directly involved. Several studies have reported decreases in milk fat content in response to high concentrate diets (Piperova et al., 2002; Peterson et al., 2003) or low forage diets supplemented with PUFA (Griinari et al., 1998; Piperova et al., 2000; Loores et al., 2005b), responses that are also associated with an increase in milk fat *trans*-10 18:1 content. One of the major challenges in interpreting these associations is that changes in ruminal biohydrogenation causing a shift toward *trans*-10 18:1 syn-

thesis may also result in the formation of other intermediates that could also be involved in the regulation of mammary lipid metabolism.

Following the observation that postruminal infusions of CLA isomers devoid of *trans*-10, *cis*-12 CLA decreased milk fat secretion in dairy cows (Chouinard et al., 1999), it has often been speculated that other ruminal biohydrogenation intermediates containing a conjugated bond may also have a role in milk fat synthesis (Bauman and Griinari, 2003; Perfield et al., 2004). In the present study, milk fat concentrations and milk fat yield of cows fed the FSO diet were found to be inversely related with the concentration of several specific fatty acids in milk fat (Table 7). Of these, the strongest correlation existed between milk fat content and *trans*-9, *cis*-11 CLA concentrations ($r = -0.808$, $P < 0.001$; Table 7). Further evaluation of this association indicated that changes in the concentration of this biohydrogenation

Figure 7. Relationship between milk fat content (g/kg) and concentrations of *trans*-10, *cis*-12 conjugated linoleic acid (CLA), *trans*-10 18:1, and *trans*-9, *cis*-11 CLA in milk (g/100 g of fatty acids) from cows fed corn silage-based diets containing 45 g of a mixture (1:2; wt/wt) of fish oil and sunflower oil/kg of DM. Lines fitted represent the following equations: milk fat content (g/kg) = $24.1 \pm 0.54 + 27.8 \pm 3.06 \exp [-0.78 \pm 0.154(\text{trans-10 18:1})]$ ($n = 112$; $r^2 = 0.74$; $P < 0.001$; Panel B); and milk fat content (g/kg) = $21.5 \pm 0.92 + 31.1 \pm 1.69 \exp [-15.7 (\pm 2.07)(\text{trans-9, cis-11 CLA})]$ ($n = 112$; $r^2 = 0.80$; $P < 0.001$; Panel C).

Table 7. Pearson correlation coefficients between fat content (g/kg), fat yield (g/d), and concentrations of specific 18:1 and 18:2 fatty acids in milk (g/100 g of fatty acids) from corn silage-based diets containing fish oil and sunflower oil. Relationships were derived using 112 measurements obtained from 8 animals¹

	Fat content	<i>cis</i> -11	<i>trans</i> -9	<i>trans</i> -10	<i>trans</i> -12	<i>trans</i> -15	<i>trans</i> -10, <i>trans</i> -14	<i>cis</i> -9, <i>trans</i> -13	<i>trans</i> -9, <i>cis</i> -12	<i>trans</i> -9, <i>cis</i> -11	<i>trans</i> -10, <i>cis</i> -12	<i>trans</i> -11, <i>cis</i> -13
<i>cis</i> -11	-0.461***											
<i>trans</i> -9	0.709***	0.579***										
<i>trans</i> -10	0.754***	0.629***	0.708***									
<i>trans</i> -12	0.510***	0.416***		0.396***								
<i>trans</i> -15	0.603***	0.294**	0.793***	0.608***	0.811***							
<i>trans</i> -10, <i>trans</i> -14	0.616***	0.747***	0.628***	0.842***	0.410***	0.476***						
<i>cis</i> -9, <i>trans</i> -13	0.699***	0.242*	0.731***	0.625***	0.650***	0.747***	0.451***					
<i>trans</i> -9, <i>cis</i> -12	0.655***	0.790***	0.717***	0.742***	0.581***	0.490***	0.753***	0.509***				
<i>trans</i> -9, <i>cis</i> -11	0.808***	0.621***	0.776***	0.969***	0.470***	0.617***	0.791***	0.719***	0.790***			
<i>trans</i> -10, <i>cis</i> -12	0.709***	0.262**	0.751***	0.670***	0.762***	0.880***	0.533***	0.754***	0.519***	0.698***		
<i>trans</i> -11, <i>cis</i> -13	-0.569***	0.199*	0.679***	0.412***	0.753***	0.753***	0.294**	0.659***	0.369***	0.499***	0.848***	
Fat yield	0.892***	-0.463***	-0.623***	-0.732***	-0.330**	-0.450***	-0.554***	-0.608***	-0.654***	-0.783***	-0.561***	-0.380***

¹Signs indicate the effect of the variable on the predictor.

* $P < 0.05$, ** $P < 0.01$, and *** $P < 0.001$.

intermediate could account for proportionately 0.80 of the variation in milk fat content observed for cows fed the FSO diet (Figure 7). Although it is not possible from this association to establish whether this isomer is a causative factor contributing to the reduction in milk fat synthesis, it does appear that during MFD induced by diets containing a mixture of FO and SO an increase in milk fat *trans*-9, *cis*-11 CLA can be expected.

Milk Saturated Fatty Acids

Milk from the FSO-fed cows contained lower amounts of total short- and medium-chain fatty acids than milk from the control diet, consistent with the changes in milk fatty acid composition when diets containing a combination of extruded soybeans with fish meal (AbuGhazaleh et al., 2002, 2004) or FO (Whitlock et al., 2002) are fed. Inclusion of unsaturated fatty acids in the diet typically lower short- and medium-chain fatty acid concentrations in milk because of the inhibitory effects of long-chain fatty acids on mammary *de novo* fatty acid synthesis (Grummer, 1991). Postruminal infusions of *trans*-10, *cis*-12 CLA (Baumgard et al., 2000) or calcium salts of *trans* 18:1 in the diet (Piperova et al., 2004) are also known to reduce *de novo* fatty acid synthesis, and it is probable that part of the decrease in endogenous mammary fatty acid synthesis when unsaturated fatty acids are fed is related to increased formation of specific biohydrogenation intermediates in the rumen. Reductions in milk short- and medium-chain fatty acids have also been shown to be greater when lipids rich in 18:2 n-6 are fed compared with supplements predominating in *cis*-9 18:1 or 18:3 n-3 (Kelly et al., 1998; AbuGhazaleh et al., 2003).

The reduction in the concentration and output of 18:0 in milk from the FSO diet can be attributed to both the inhibitory effects of FO (Wonsil et al., 1994; Scollan et al., 2001; Shingfield et al., 2003) and 18:2 n-6 from SO (Polan et al., 1964; Harfoot et al., 1973) on the biohydrogenation of C18 unsaturated fatty acids to 18:0 in the rumen. Milk fat 18:0 concentrations rapidly declined on the FSO diet, reaching levels as low as 2.4 g/100 g of fatty acids on d 5, before gradually increasing (Figure 4). The output of this fatty acid in milk was relatively constant after this period (data not presented), indicating that the changes in 18:0 concentrations reflected the lowered contribution of *de novo* synthesis to total milk fatty acid secretion. It is also notable that the gradual recovery in 18:0 concentrations after d 5 on the FSO diet occurred at the same time when milk fat content and yield declined. There is evidence to suggest that the mammary gland has a stringent requirement for *cis*-9 18:1 (Lor et al., 2005a), a large proportion of which is derived from the action of Δ^9 -

Figure 8. Temporal changes in the apparent transfer of 20:5 n-3 (●) and 22:6 n-3 (○) from the diet into milk (mg/g) of cows fed corn silage-based diets containing 45 g of a mixture (1:2; wt/wt) of fish oil and sunflower oil/kg of DM. Values represent the mean for 8 animals. SEM = 0.89 and 1.07 mg/g for 20:5 n-3 and 22:6 n-3, respectively.

stearoyl-Co A desaturase on 18:0 extracted from circulating blood lipids (Chilliard et al., 2001). It is conceivable that the changes in milk 18:0 content and milk fat secretion on the FSO diet, reflect an adaptation to an acute reduction in mammary 18:0 supply, such that the marked shortage of 18:0 available for endogenous *cis*-9 18:1 synthesis in cows fed a mixture of FO and SO initiated a decrease in mammary lipid synthesis to ensure milk fat fluidity in mammary secretory cells and efficient ejection of milk from the gland.

Milk Long-Chain PUFA

Inclusion of FO in the FSO diet enhanced the concentration and secretion of 20:5 n-3 and 22:6 n-3 in milk, increases that were associated with a mean apparent transfer efficiency of 20:5 n-3 and 22:6 n-3 from the diet into milk of 0.020 and 0.018, respectively, estimates that are in agreement with values reported in the literature (Offer et al., 1999; Chilliard et al., 2001; Shingfield et al., 2003). However, the transfer of long-chain n-3 fatty acids varied according to time on the FSO diet (Figure 8), which may account for the higher 20:5 n-3 and 22:6 n-3 transfer efficiencies of 0.09 and 0.16 reported by Cant et al. (1997). In vitro studies have established that both the type and level of FO modify the extent of 20:5 n-3 and 22:6 n-3 biohydrogenation (Gulati et al., 1999; Dohme et al., 2003), sources of variation that were not altered in this experiment. Overall, the temporal pattern in the apparent transfer of 20:5 n-3 and 22:6 n-3 on the FSO diet suggests that mammary uptake of these fatty acids declined over the course of the experiment, which may be due a progressive in-

crease in the extent of ruminal 20:5 n-3 and 22:6 n-3 metabolism or may reflect a shift in the incorporation of these fatty acids from blood triacylglycerides toward phospholipids.

Milk Trans Fatty Acids and Isomers of CLA

Although *trans*-7, *trans*-11, and *trans*-12 18:1 are substrates for endogenous *trans*-7, *cis*-9 CLA, *cis*-9, *trans*-11 CLA, and *cis*-9, *trans*-12 18:2 synthesis via Δ^9 -stearoyl-Co A desaturase (Griinari et al., 2000; Corl et al., 2001, 2002; Piperova et al., 2002), the occurrence of *trans* 18:1, *trans* 18:2, and isomers of CLA in milk largely reflect the formation of these biohydrogenation intermediates during C18 fatty acid metabolism in the rumen. Reduction of *trans* 18:1 to 18:0 is considered to be the rate-limiting step of biohydrogenation (Griinari and Bauman, 1999); high levels of *trans* 18:1 in milk reflect incomplete metabolism of dietary C18 unsaturated fatty acids in the rumen. The substantial increase in *trans* 18:1 content of milk from the FSO diet would be expected because of the inhibitory effects of FO on the reduction of *trans* 18:1 to 18:0 in the rumen (Wonsil et al., 1994; Scollan et al., 2001; Shingfield et al., 2003) and because lipids rich in 18:2 n-6 are substrates for ruminal *trans* 18:1 synthesis (Kalscheur et al., 1997; Duckett et al., 2002; Sackmann et al., 2003).

The combined use of marine lipids and plant oils or oilseeds rich in 18:2 n-6 for increasing milk fat *cis*-9, *trans*-11 CLA content (Whitlock et al., 2002; AbuGhazaleh et al., 2003) relies, for the most part, on manipulation of ruminal biohydrogenation to enhance the supply of *trans*-11 18:1 available for endogenous conversion in the mammary gland. In the current study, the FSO diet caused a rapid increase in milk fat *trans*-11 18:1 content, but the response was transient with levels of this isomer starting to decline after d 5. Decreases in milk fat *trans*-11 18:1 content were associated with progressive increases in *trans*-10 18:1 concentrations that are indicative of time-dependent changes in biohydrogenation resulting in *trans*-10 replacing *trans*-11 as the predominant *trans* 18:1 leaving the rumen. Shifts in ruminal biohydrogenation toward the formation of *trans*-10 18:1 account for the progressive decrease in milk fat *cis*-9, *trans*-11 CLA content after d 5 on the FSO diet, as endogenous conversion of *trans*-11 18:1 in the mammary gland is the major source of *cis*-9, *trans*-11 CLA (Griinari et al., 2000; Corl et al., 2001, 2002; Piperova et al., 2002), and this isomer is quantitatively the most important in milk fat (Sehat et al., 1998; Piperova et al., 2002; Shingfield et al., 2003). Changes in the predominant biohydrogenation pathways in the rumen also offer an explanation for the decline in milk *cis*-9, *trans*-11 CLA content over time on high-concen-

trate diets supplemented with SO (Bauman et al., 2000) and TMR containing soybean oil (Dhiman et al., 2000) or FO and extruded soybeans (Whitlock et al., 2002).

Reduction of *trans*-10, *cis*-12 CLA produced during 18:2 n-6 metabolism has been considered to be the main source of *trans*-10 18:1 in the rumen (Griinari and Bauman, 1999; Loor and Herbein, 2001), but under certain conditions, isomerization of *cis*-9 18:1 may also contribute (Loor et al., 2002; Mosley et al., 2002; AbuGhazaleh et al., 2005). Milk fat *trans*-10, *cis*-12 CLA concentrations were enhanced by the FSO diet, which could have been expected because of the increased 18:2 n-6 intake (Duckett et al., 2002; Sackmann et al., 2003), but the increases were time dependent. Temporal changes in milk fat *trans*-10, *cis*-12 CLA content were associated with variations in *trans*-10 18:1 content {[*trans*-10, *cis*-12 CLA (mg/100 g of fatty acids)] = $39.3 \pm 4.88 + 4.62 \pm 0.502$ [*trans*-10 18:1 (g/100 g of fatty acids)] (n = 112, $r^2 = 0.444$; $P < 0.001$)}, but a stronger relationship existed between *trans*-10 18:1 and *trans*-9, *cis*-11 CLA concentrations {[*trans*-9, *cis*-11 CLA (mg/100 g of fatty acids)] = $33.0 \pm 3.02 + 12.4 \pm 0.31$ [*trans*-10 C18:1 (g/100 g of fatty acids)] (n = 112, $r^2 = 0.938$; $P < 0.001$)}. Unfortunately, there is no evidence in the literature to reconcile these findings. It is possible that the close association between *trans*-10 18:1 and *trans*-9, *cis*-11 CLA is simply an artefact of little or no biological importance, but this seems unlikely given the strength of this relationship. Furthermore, these relationships are entirely consistent with those derived in an earlier study examining the impact of forage type and level of concentrate in the diet on milk fatty acid composition when FO and SO were fed (Shingfield et al., 2005). One plausible explanation is that *trans*-10 18:1 and *trans*-9, *cis*-11 CLA are produced from a common bacterium or group of bacteria that proliferate in the rumen when diets contain FO and SO.

The underlying causal mechanism for changes in biohydrogenation pathways remains unclear, but it is evident that the marked increases in milk fat *trans*-9, *cis*-11 CLA, *trans*-10, *cis*-12 CLA, and *trans*-10 18:1 content were an adaptation to FO and SO in the diet. Earlier studies have shown that shifts in ruminal biohydrogenation toward *trans*-10 18:1 can occur when low-forage diets are supplemented with PUFA (Griinari et al., 1998; Piperova et al., 2000; Peterson et al., 2003; Loor et al., 2004) in response to increased concentrates in the diet (Kucuk et al., 2001; Piperova et al., 2002; Sackmann et al., 2003; Daniel et al., 2004) or by replacing potato starch with a more rapidly degraded source as wheat starch in the diet (Jurjanz et al., 2004). For diets containing a mixture of FO and SO, levels of *trans*-10 18:1 in milk are higher for corn silage than for grass silage and are enhanced by increases in the proportion

of concentrate in the diet (Shingfield et al., 2005). Often the relative amounts of starch and fiber in the diet have been implicated in inducing changes in the profile of 18:1 intermediates formed in the rumen (Griinari et al., 1998; Griinari and Bauman, 1999; Piperova et al., 2002). However, changes in the carbohydrate composition of the diet do not explain the temporal changes in the concentrations of individual *trans* 18:1 isomers in milk on the FSO diet. In this case, it is probable that the alterations are related to selective changes in the relative number and activity of specific bacterial populations induced by feeding high levels of FO and SO, which are rich in PUFA and are known to be toxic to rumen bacteria (Harfoot and Hazlewood, 1988; Jenkins, 1993).

Supplementing the diet with FO and SO is an effective means of increasing total and *cis*-9, *trans*-11 CLA content in milk. Even though *cis*-9, *trans*-11 CLA enrichment in milk declined after d 5, the levels at the end of the experiment were >5-fold higher than the control. Earlier studies in cows fed the same basal ration offered in this experiment, but containing 12.0 and 18.0 g/kg of DM of FO and SO resulted in total and *cis*-9, *trans*-11 CLA concentrations in milk after 13 d on diet of 3.43 and 3.00 g/100 g of fatty acids, respectively (Shingfield et al., 2005). Corresponding values of 3.41 and 2.88 for milk collected at the same time point on the FSO diet point toward both consistency and predictability in the response, but also indicate that lower amounts of SO than 30 g/kg of DM as fed in this study could be used to attain comparable levels of *cis*-9, *trans*-11 CLA enrichment.

Changes in milk fat *cis*-9, *trans*-11 CLA with time on the FSO diet are consistent with temporal variations in this milk fat constituent reported in earlier studies (Bauman et al., 2000; AbuGhazaleh et al., 2004). Concentrations of *cis*-9, *trans*-11 CLA in milk from diets containing fish meal and extruded soybeans that supplied 5 g of FO and 20 g of soybean oil/kg of DM were found to reach a maximum of 1.48 g/100 g of fatty acids after 21 d on diet but declined to a relatively constant level of 0.78 g/100 g of fatty acids after d 35 (AbuGhazaleh et al., 2004). In contrast, temporal changes in milk fat *cis*-9, *trans*-11 CLA for high-concentrate diets containing 52 g of SO/kg of DM (Bauman et al., 2000) were found to reach a maximum after 7 to 10 d, but this level of enrichment was transient and declined over a 21-d period. Inclusion of 50 g of rapeseed oil/kg in concentrate supplements to cows fed grass silage was shown to enhance milk fat *cis*-9, *trans*-11 CLA content from 0.46 to 1.02 g/100 g of fatty acids, a response that occurred within 7 d, which was maintained for 42 d (Ryhänen et al., 2005). Overall, these findings indicate that both the amount and form of lipid supplement in the

diet, as well as the composition of the basal diet, has a marked effect on the changes in milk fat CLA concentrations that can be expected over an extended period of time.

CONCLUSIONS

Inclusion of FO and SO in the diet decreased DMI, milk protein, and milk fat content, but had no effect on milk yield. Decreases in milk fat content to the FSO diet were associated with an increase in the secretion of several biohydrogenation intermediates in milk, including *trans*-10 18:1, *trans*-10, *cis*-12 CLA, and *trans*-9, *cis*-11 CLA. Compared with the control, the FSO diet reduced milk fat 4:0 to 18:0 and *cis* 18:1 content and increased *trans* 18:1, *trans* 18:2, CLA, 20:5 n-3, and 22:6 n-3 concentrations. Milk fat *cis*-9, *trans*-11 CLA responses to the FSO treatment were extremely rapid, but the levels of enrichment declined after d 5, responses that were associated with concomitant increases in other *trans* fatty acids, predominantly *trans*-10 18:1 and decreases in *trans*-11 18:1 concentrations in milk. The combined use of FO and SO in the diet is an effective strategy for increasing milk fat *cis*-9, *trans*-11 CLA content, but the high level of enrichment declines because of time-dependent modifications in biohydrogenation, causing *trans*-10 to replace *trans*-11 as the major 18:1 intermediate leaving the rumen. Thus, concentrations of the potentially beneficial fatty acids *cis*-9, *trans*-11 CLA and *trans*-11 18:1 decreased over time, while the levels in milk of other *trans* 18:1 and *trans* 18:2 fatty acids of unknown biological efficacy in humans increased. If the mechanisms underlying changes in ruminal lipid metabolism to FO and SO in the diet can be identified and controlled, it could be possible to develop nutritional strategies for long-term production of milk enriched with high levels of *cis*-9, *trans*-11 CLA and *trans*-11 18:1.

ACKNOWLEDGMENTS

Financial support from the Biotechnology and Biological Sciences Research Council, Department for Environment, Food, and Rural Affairs, The Scottish Executive Environment and Rural Affairs Department and the Milk Development Council under the Eating, Food and Health LINK scheme is gratefully acknowledged. The authors thank the staff of the Center for Dairy Research Animal Production Research Unit for diligent care of experimental animals and sample collection and Vesa Toivonen for assistance with the interpretation of mass-spectral data.

REFERENCES

- AbuGhazaleh, A. A., M. B. Riley, E. E. Thies, and T. C. Jenkins. 2005. Dilution rate and pH effects on the conversion of oleic acid to *trans* C18:1 positional isomers in continuous culture. *J. Dairy Sci.* 88:4334–4341.
- AbuGhazaleh, A. A., D. J. Schingoethe, A. R. Hippen, and K. F. Kalscheur. 2003. Milk conjugated linoleic acid response to fish oil supplementation of diets differing in fatty acid profiles. *J. Dairy Sci.* 86:944–953.
- AbuGhazaleh, A. A., D. J. Schingoethe, A. R. Hippen, and K. F. Kalscheur. 2004. Conjugated linoleic acid increases in milk when cows fed fish meal and extruded soybeans for an extended period of time. *J. Dairy Sci.* 87:1758–1766.
- AbuGhazaleh, A. A., D. J. Schingoethe, A. R. Hippen, and L. A. Whitlock. 2002. Feeding fish meal and extruded soybeans enhances the conjugated linoleic acid (CLA) content of milk. *J. Dairy Sci.* 85:624–631.
- Agricultural and Food Research Council. 1993. Energy and Protein Requirements of Ruminants. An Advisory Manual Prepared by the AFRC Technical Committee on Responses to Nutrients. CAB Intl., Wallingford, England.
- Association of Official Analytical Chemists. 1990. Official Methods of Analysis. 15th ed. Assoc. Off. Anal. Chem., Arlington, VA.
- Bauman, D. E., D. M. Barbano, D. A. Dwyer, and J. M. Griinari. 2000. Technical note: Production of butter with enhanced conjugated linoleic acid for use in biomedical studies with animal models. *J. Dairy Sci.* 83:2422–2425.
- Bauman, D. E., and J. M. Griinari. 2003. Nutritional regulation of milk fat synthesis. *Annu. Rev. Nutr.* 23:203–227.
- Baumgard, L. H., B. A. Corl, D. A. Dwyer, and D. E. Bauman. 2000. Identification of the conjugated linoleic isomer that inhibits milk fat synthesis. *Am. J. Physiol.* 278:R179–R184.
- Cant, J. P., A. H. Fredeen, T. MacIntyre, J. Gunn, and N. Crowe. 1997. Effect of fish oil and monensin on milk fat composition in dairy cows. *Can. J. Anim. Sci.* 77:125–131.
- Chilliard, Y., A. Ferlay, and M. Doreau. 2001. Effect of different types of forages, animal fat or marine oils in cow's diet on milk fat secretion and composition, especially conjugated linoleic acid (CLA) and polyunsaturated fatty acids. *Livest. Prod. Sci.* 70:31–48.
- Chilliard, Y., A. Ferlay, R. M. Mansbridge, and M. Doreau. 2000. Ruminant milk fat plasticity: Nutritional control of saturated, polyunsaturated, *trans* and conjugated fatty acids. *Ann. Zootech.* 49:181–205.
- Chouinard, P. Y., L. Corneau, W. R. Butler, Y. Chilliard, J. K. Drackley, and D. E. Bauman. 2001. Effect of dietary lipid source on conjugated linoleic acid concentrations in milk fat. *J. Dairy Sci.* 84:680–690.
- Chouinard, P. Y., L. Corneau, A. Sæbø, and D. E. Bauman. 1999. Milk yield and composition during abomasal infusion of conjugated linoleic acids in dairy cows. *J. Dairy Sci.* 82:2737–2745.
- Christensen, R. A., J. K. Drackley, D. W. LaCount, and J. H. Clark. 1994. Infusion of four long-chain fatty acid mixtures into the abomasum of lactating dairy cows. *J. Dairy Sci.* 77:1052–1069.
- Christie, W. W. 1982. A simple procedure for rapid transmethylation of glycerolipids and cholesteryl esters. *J. Lipid Res.* 23:1072–1075.
- Corl, B. A., L. H. Baumgard, D. A. Dwyer, J. M. Griinari, B. S. Phillips, and D. E. Bauman. 2001. The role of Δ^9 desaturase in the production of *cis*-⁹, *trans*-11 CLA. *J. Nutr. Biochem.* 12:622–630.
- Corl, B. A., L. H. Baumgard, J. M. Griinari, P. Delmonte, K. M. Morehouse, M. P. Yurawecz, and D. E. Bauman. 2002. *Trans*-7, *cis*-9 CLA is synthesized endogenously by Δ^9 desaturase in dairy cows. *Lipids* 37:681–688.
- Coulon, J. B., and B. Remond. 1991. Variations in milk output and milk protein-content in response to the level of energy supply to the dairy-cow—A review. *Livest. Prod. Sci.* 29:31–47.
- Cruz-Hernandez, C., Z. Deng, J. Zhou, A. R. Hill, M. P. Yurawecz, P. Delmonte, M. M. Mossoba, M. E. R. Dugan, and J. K. G. Kramer. 2004. Methods for analysis of conjugated linoleic acids and *trans*-18:1 isomers in dairy fats by using a combination of gas chromatography, silver-ion thin-layer chromatography/gas chromatography, and silver-ion liquid chromatography. *J. AOAC Int.* 87:545–562.
- Daniel, Z. C. T. R., R. J. Wynn, A. M. Salter, and P. J. Buttery. 2004. Differing effects of forage and concentrate diets on the oleic acid and conjugated linoleic acid content of sheep tissues: The role of steroyl-CoA desaturase. *J. Anim. Sci.* 82:747–758.
- DePeters, E. J., and J. P. Cant. 1992. Nutritional factors influencing the nitrogen composition of bovine milk: A review. *J. Dairy Sci.* 75:2043–2070.
- deVeth, M. J., J. M. Griinari, A. M. Pfeiffer, and D. E. Bauman. 2004. Effect of CLA on milk fat synthesis in dairy cows: Comparison of inhibition by methyl esters and free fatty acids, and relationships among studies. *Lipids* 39:365–372.
- Dhiman, T. R., L. D. Satter, M. W. Pariza, M. P. Galli, K. Albright, and M. X. Tolosa. 2000. Conjugated linoleic acid (CLA) content of milk from cows offered diets rich in linoleic and linolenic acid. *J. Dairy Sci.* 83:1016–1027.
- Dohme, F., V. Fievez, K. Raes, and D. I. Demeyer. 2003. Increasing levels of two different fish oils lower ruminal biohydrogenation of eicosapentaenoic and docosahexaenoic acid *in vitro*. *Anim. Res.* 52:309–320.
- Drackley, J. K., T. H. Klusmeyer, A. M. Trusk, and J. H. Clark. 1992. Infusion of long-chain fatty acids varying in saturation and chain length into the abomasum of lactating dairy cows. *J. Dairy Sci.* 75:1517–1526.
- Duckett, S. K., J. G. Andrae, and F. N. Owens. 2002. Effect of high-oil corn or added corn oil on ruminal biohydrogenation of fatty acids and conjugated linoleic acid formation in beef steers fed finishing diets. *J. Anim. Sci.* 81:1251–1261.
- Fay, L., and U. Richli. 1991. Location of double bonds in polyunsaturated fatty acids by gas chromatography-mass spectrometry after 4,4-dimethyloxazoline derivatization. *J. Chromatogr.* 541:89–98.
- Griinari, J. M., and D. E. Bauman. 1999. Biosynthesis of conjugated linoleic acid and its incorporation into meat and milk in ruminants. Pages 180–200 in *Advances in Conjugated Linoleic Acid Research*. Vol. 1. M. P. Yurawecz, M. M. Mossoba, J. K. G. Kramer, M. W. Pariza, and G. J. Nelson, ed. AOCS Press, Champaign, IL.
- Griinari, J. M., B. A. Corl, S. H. Lacy, P. Y. Chouinard, K. V. V. Nurmela, and D. E. Bauman. 2000. Conjugated linoleic acid is synthesized endogenously in lactating dairy cows by Δ^9 desaturase. *J. Nutr.* 130:2285–2291.
- Griinari, J. M., D. A. Dwyer, M. A. McGuire, D. E. Bauman, D. L. Palmquist, and K. V. V. Nurmela. 1998. *Trans*-octadecenoic acids and milk fat depression in lactating dairy cows. *J. Dairy Sci.* 81:1251–1261.
- Grummer, R. R. 1991. Effect of feed on the composition of milk fat. *J. Dairy Sci.* 74:3244–3257.
- Gulati, S. K., J. R. Ashes, and T. W. Scott. 1999. Hydrogenation of eicosapentaenoic and docosahexaenoic acids and their incorporation into milk fat. *Anim. Feed Sci. Technol.* 79:57–64.
- Harfoot, C. G., and G. P. Hazlewood. 1988. Lipid metabolism in the rumen. Pages 285–322 in *The Rumen Microbial Ecosystem*. P. N. Hobson, ed. Elsevier Applied Sci. Publ., London, United Kingdom.
- Harfoot, C. G., R. C. Noble, and J. H. Moore. 1973. Factors influencing the extent of biohydrogenation of linoleic acid by rumen microorganisms *in vitro*. *J. Sci. Food Agric.* 24:961–970.
- Jenkins, T. C. 1993. Lipid metabolism in the rumen. *J. Dairy Sci.* 76:3851–3863.
- Jurjanz, S., V. Monteils, P. Juaneda, and F. Laurent. 2004. Variations of *trans* octadecenoic acid in milk fat induced by feeding different starch-based diets to cows. *Lipids* 39:19–24.
- Kalscheur, K. F., B. B. Teter, L. S. Piperova, and R. A. Erdman. 1997. Effect of fat source on duodenal flow of *trans*-C18:1 fatty acids and milk fat production in dairy cows. *J. Dairy Sci.* 80:2115–2126.
- Kelly, M. L., J. R. Berry, D. A. Dwyer, J. M. Griinari, P. Y. Chouinard, M. E. Van Amburgh, and D. E. Bauman. 1998. Dietary fatty acid sources affect conjugated linoleic acid concentrations in milk from lactating dairy cows. *J. Nutr.* 128:881–885.
- Kritchevsky, D. 2000. Antimutagenic and some other effects of conjugated linoleic acid. *Br. J. Nutr.* 83:459–465.

- Kucuk, O., B. W. Hess, P. A. Ludden, and D. C. Rule. 2001. Effect of forage:concentrate ratio on ruminal digestion and duodenal flow of fatty acids in ewes. *J. Anim. Sci.* 79:2233–2240.
- Litherland, N. B., S. Thire, A. D. Beaulieu, C. K. Reynolds, J. A. Benson, and J. K. Drackley. 2005. Dry matter is decreased more by abomasal infusion of unsaturated free fatty acids than by unsaturated triglycerides. *J. Dairy Sci.* 88:632–643.
- Lock, A. L., and K. J. Shingfield. 2004. Optimising milk composition. Pages 107–188 in *Dairying—Using Science to Meet Consumers' Needs*. Br. Soc. Anim. Sci. Publ. 29. E. Kebreab, J. Mills, and D. E. Beever, ed. Nottingham University Press, Loughborough, United Kingdom.
- Loor, J. J., A. B. P. A. Bandara, and J. H. Herbein. 2002. Characterization of 18:1 and 18:2 isomers produced during microbial biohydrogenation of unsaturated fatty acids from canola and soya bean oil in the rumen of lactating dairy cows. *J. Anim. Nutr. Anim. Physiol.* 86:422–432.
- Loor, J. J., M. Doreau, J. M. Chardigny, A. Ollier, J. L. Sebedio, and Y. Chilliard. 2005a. Effects of ruminal or duodenal supply of fish oil on milk fat secretion and profiles of *trans*-fatty acids and conjugated linoleic acid isomers in dairy cows fed maize silage. *Anim. Feed Sci. Technol.* 119:227–246.
- Loor, J. J., A. Ferlay, A. Ollier, M. Doreau, and Y. Chilliard. 2005b. Relationship among *trans* and conjugated fatty acids and bovine milk fat yield due to dietary concentrate and linseed oil. *J. Dairy Sci.* 88:726–740.
- Loor, J. J., and J. H. Herbein. 2001. Alterations in blood plasma and milk fatty acid profiles of lactating dairy Holstein cows in response to ruminal infusion of a conjugated linoleic acid mixture. *Anim. Res.* 50:463–476.
- Loor, J. J., K. Ueda, A. Ferlay, Y. Chilliard, and M. Doreau. 2004. Biohydrogenation, duodenal flow, and intestinal digestibility of *trans*-fatty acids and conjugated linoleic acids in response to dietary forage:concentrate ratio and linseed oil in dairy cows. *J. Dairy Sci.* 87:2472–2485.
- Loor, J. J., K. Ueda, A. Ferlay, Y. Chilliard, and M. Doreau. 2005c. Intestinal flow and digestibility of *trans*-fatty acids and conjugated linoleic acids (CLA) in dairy cows fed a high-concentrate diet supplemented with fish oil, linseed oil, or sunflower oil. *Anim. Feed Sci. Technol.* 119:203–225.
- Kramer, J. K. G., C. B. Blackadar, and J. Zhou. 2002. Evaluation of two GC columns (60-m SUPELCO WAX 10 and 100-m CP Sil 88) for analysis of milk fat, with emphasis on CLA, 18:1, 18:2 and 18:3 isomers, and short- and long chain FA. *Lipids* 37:823–835.
- Molkentin, J., and D. Pecht. 1995. Optimized analysis of *trans*-octadecenoic acids in edible fats. *Chromatographia* 41:267–272.
- Mosley, E., G. L. Powell, M. B. Riley, and T. C. Jenkins. 2002. Microbial biohydrogenation of oleic acid to *trans* isomers *in vitro*. *J. Lipid Res.* 43:290–296.
- Offer, N. W., M. Marsden, J. Dixon, B. K. Speake, and F. E. Thacker. 1999. Effect of dietary fat supplements on levels of *n*-3 polyunsaturated fatty acids, *trans* acids and conjugated linoleic acid in bovine milk. *Anim. Sci.* 69:613–625.
- Offer, N. W., M. Marsden, and R. H. Phipps. 2001. Effect of oil supplementation of a diet containing a high concentration of starch on levels of *trans* fatty acids and conjugated linoleic acids in bovine milk. *Anim. Sci.* 73:533–540.
- Parodi, P. W. 1999. Conjugated linoleic acid and other anticarcinogenic agents of bovine milk fat. *J. Dairy Sci.* 82:1339–1349.
- Perfield, J. W., A. Sæbø, and D. E. Bauman. 2004. Use of conjugated linoleic acid (CLA) enrichments to examine the effects of *trans*-8, *cis*-10 CLA and *cis*-11, *trans*-13 CLA on milk fat synthesis. *J. Dairy Sci.* 87:1196–1202.
- Peterson, D. G., E. A. Matitashvili, and D. E. Bauman. 2003. Diet-induced milk fat depression results in increased *trans*-10, *cis*-12 CLA in milk fat and coordinate suppression of mRNA abundance for mammary enzymes involved in milk fat synthesis. *J. Nutr.* 133:3098–3102.
- Piperova, L. S., U. Moallem, B. B. Teter, J. Sampugna, M. P. Yurawecz, K. M. Morehouse, D. Luchini, and R. A. Erdman. 2004. Changes in milk fat in response to dietary supplementation with calcium salts of *trans*-18:1 or conjugated linoleic fatty acids in lactating dairy cows. *J. Dairy Sci.* 87:3836–3844.
- Piperova, L. S., L. Sampugna, B. B. Teter, K. F. Kalscheur, M. P. Yurawecz, Y. Ku, K. M. Morehouse, and R. A. Erdman. 2002. Duodenal and milk *trans* octadecanoic acid and conjugated linoleic acid (CLA) isomers indicate that postabsorptive synthesis is the predominant source of *cis*-9-containing CLA in lactating dairy cows. *J. Nutr.* 132:1235–1241.
- Piperova, L. S., B. B. Teter, I. Bruckental, J. Sampugna, S. E. Mills, M. P. Yurawecz, J. Fritsche, K. Ku, and R. A. Erdman. 2000. Mammary lipogenic enzyme activity, *trans* fatty acids and conjugated linoleic acids are altered in lactating dairy cows fed a milk fat-depressing diet. *J. Nutr.* 130:2568–2574.
- Polan, C. E., J. J. McNeill, and S. B. Tove. 1964. Biohydrogenation of unsaturated fatty acids by rumen bacteria. *J. Bacteriol.* 88:1056–1064.
- Porter, M. G., D. C. Patterson, R. W. J. Steen, and F. J. Gordon. 1984. Determination of dry matter and gross energy of grass silage. Pages 89–90 in *Proc. 7th Silage Conference*, The Queen's Univ., Belfast, UK.
- Ryhänen, E.-L., K. Tallavaara, J. M. Griinari, S. Jaakkola, S. Mäntere-Alhonen, and K. J. Shingfield. 2005. Production of conjugated linoleic acid enriched milk and dairy products from cows receiving grass silage supplemented with a cereal-based concentrate containing rapeseed oil. *Int. Dairy J.* 15:207–217.
- Sackmann, J. R., S. K. Duckett, M. H. Gillis, C. E. Realini, A. H. Parks, and R. B. Eggelston. 2003. Effects of forage and sunflower oil levels on ruminal biohydrogenation of fatty acids and conjugated linoleic acid formation in beef steers fed finishing diets. *J. Anim. Sci.* 81:3174–3181.
- SAS Inst. 2001. SAS/STAT Users Guide, Release 8.0. SAS Inst., Inc., Cary, NC.
- Scollan, N. D., M. S. Dhanoa, N. J. Choi, W. J. Maeng, M. Enser, and J. D. Wood. 2001. Biohydrogenation and digestion of long chain fatty acids in steers fed on different sources of lipid. *J. Agric. Sci. (Camb.)* 136:345–355.
- Sehat, N., M. P. Yurawecz, J. A. G. Roach, M. M. Mossoba, J. K. G. Kramer, and Y. Ku. 1998. Silver-ion high-performance liquid chromatographic separation and identification of conjugated linoleic acid isomers. *Lipids* 33:217–221.
- Shingfield, K. J., S. Ahvenjärvi, V. Toivonen, A. Ärolä, K. V. V. Nurmela, P. Huhtanen, and J. M. Griinari. 2003. Effect of fish oil on biohydrogenation of fatty acids and milk fatty acid content in cows. *Anim. Sci.* 77:165–179.
- Shingfield, K. J., C. K. Reynolds, B. Lupoli, V. Toivonen, M. P. Yurawecz, P. Delmonte, J. M. Griinari, A. S. Grandison, and D. E. Beever. 2005. Effect of forage type and proportion of concentrate in the diet on milk fatty acid composition in cows fed sunflower oil and fish oil. *Anim. Sci.* 80:225–238.
- Statutory Instruments. 1982. Number 1144. Agriculture. The Feeding Stuffs (Sampling and Analysis) Regulations. Her Majesty's Stationery Office, London, UK.
- Statutory Instruments. 1985. Number 1119. Agriculture. The Feeding Stuffs (Sampling and Analysis Amendment) Regulations. Her Majesty's Stationery Office, London, UK.
- Sukhija, P. S., and D. L. Palmquist. 1988. Rapid method for determination of total fatty acid content and composition of feedstuffs and feces. *J. Agric. Food Chem.* 36:1202–1206.
- Whitlock, L. A., D. J. Schingoethe, A. R. Hippen, K. F. Kalscheur, R. J. Baer, N. Ramaswamy, and K. M. Kasperon. 2002. Fish oil and extruded soybeans fed in combination increase conjugated linoleic acids in milk of dairy cows more than when fed separately. *J. Dairy Sci.* 85:234–243.
- Wonsil, B. J., J. H. Herbein, and B. A. Watkins. 1994. Dietary and ruminally derived *trans*-18:1 fatty acids alter bovine milk lipids. *J. Nutr.* 124:556–565.
- Wu, Z., and J. T. Huber. 1994. Relationship between dietary-fat supplementation and milk protein-concentration in lactating cows—A review. *Livest. Prod. Sci.* 39:141–155.