Evaluating the Conjugated Linoleic Acid and *Trans* 18:1 Isomers in Milk Fat of Dairy Cows Fed Increasing Amounts of Sunflower Oil and a Constant Level of Fish Oil

C. Cruz-Hernandez,* J. K. G. Kramer,† J. J. Kennelly,* D. R. Glimm,* B. M. Sorensen,* E. K. Okine,* L. A. Goonewardene,* and R. J. Weselake*¹

*Department of Agricultural, Food and Nutritional Science, University of Alberta, Alberta, 4-10 Agriculture/Forestry Centre, Edmonton, Alberta, Canada, T6G-2P5

†Food Research Program, Agriculture and Agri-Food Canada, Guelph, Ontario, Canada

ABSTRACT

The objective was to evaluate different levels of sunflower oil (SFO) in dairy rations to increase vaccenic (*trans*-11-18:1) and rumenic acids (*cis*-9,*trans*-11-18:2) in milk fat, and assess the content and composition of other *trans*-octadecenoic (*trans*-18:1) and conjugated linoleic acids (CLA) isomers. Eighty lactating Holstein cows were fed control diets for 4 wk and then placed on 4 diets for 38 d; milk fat was analyzed after 10 and 38 d. The treatments were: control, 1.5% SFO plus 0.5% fish oil (FO), 3% SFO plus 0.5% FO, and 4.5% SFO plus 0.5% FO. The forage-to-concentrate ratio was 50:50 and consisted of barley/alfalfa/hay silage and corn/barley grain concentrate. There were no differences in milk production. Supplementation of SFO/FO reduced milk fat compared with respective pretreatment periods, but milk protein and lactose levels were not affected. There was a linear decrease in all short- and medium-chain saturated fatty acids (SFA) in milk fat after 10 d (25.5, 24.1, 20.2, and 16.7%) and a corresponding linear increase in total *trans*-18:1 (5.2, 9.1, 14.1, and 21.3%) and total CLA (0.7, 1.9, 2.4, and 3.9%). The other FA in milk fat were not affected. Separation of *trans*-18:1 isomers was achieved by combination of gas chromatography (GC; 100-m highly polar capillary column) and prior separation of *trans* FA by silver ion-thin layer chromatography followed by GC. The CLA isomers were resolved by a combination of GC and silver ion-HPLC. The *trans*-11- and *trans*-10-18:1 isomers accounted for ~50% of the total *trans*-18:1 increase when SFO/FO diets were fed. On continued feeding to 38 d, *trans*-11-18:1 increased with 1.5% SFO/FO, stayed the same with 3%, and declined with 4.5% SFO/FO. Rumenic acid showed a similar pattern on continued feeding as *trans*-

11-18:2; levels increased to 0.43, 1.5, 1.9, and 3.4% at 10 d and to 0.42, 2.15, 2.09, and 2.78% at 38 d. Rumenic acid was the major CLA isomer in all 4 diets: 66, 77, 78 and 85%. The CLA isomers *trans*-7,*cis*-9-, *trans*-9,*cis*-11-, *trans*-10,*cis*-12-, *trans*-11,*trans*-13-, and *trans*-9,*trans*-11-/*trans*-10,*trans*-12-18:2 also increased from 0.18 (control) to 0.52% (4.5% SFO/FO). Milk fat produced from 3% SFO/FO appeared most promising: *trans*-11-18:1 and *cis*-9,*trans*-11-18:2 increased 4.5-fold, total SFA reduced 18%, and moderate levels of *trans*-10-18:1 (3.2%), other *trans*-18:1 (6.6%) and CLA isomers (0.5%) were observed, and that composition remained unchanged to 38 d. The 4.5% SFO/FO diet produced higher levels of *trans*-11-18:1 and *cis*-9,*trans*-11-18:2, a 28% reduction in SFA, and similar levels of other *trans*-18:1 (9.2%) and CLA isomers (0.52%), but the higher levels of *trans*-11-18:1 and *cis*-9,*trans*-11-18:2 were not sustained. A stable milk fat quality was achieved by feeding moderate amounts of SFO (3% of DM) in the presence of 0.5% FO that had 4% vaccenic and 2% rumenic acids.

Key words: conjugated linoleic acid, *trans*-18:1, dairy fat, gas chromatography

INTRODUCTION

Due to increasing demand for value-added foods with health benefits, strategies have been initiated to produce dairy products enriched with rumenic [*cis*(*c*)-9, *trans*(*t*)-11-18:2] and vaccenic acid (*t*11-18:1). Rumenic acid, the major conjugated linoleic acid (CLA) isomer present in milk and meat fats of ruminants, has been reported to protect and moderate against certain cancers, diabetes, immunity, atherosclerosis, bone growth and obesity (Belury, 2002; Ip et al., 2003). Vaccenic acid is produced in rumen bacteria by the action of isomerases and reductases from dietary polyunsaturated fatty acids (PUFA), and is subsequently converted to *c*9,*t*11-18:2 by Δ^9 -desaturase in the tissues of ruminants (Griinari et al., 2000). Vaccenic acid has

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¹Corresponding author: Randall.Weselake@ualberta.ca

been shown to be equally effective in suppressing premalignant lesions in rat mammary glands (Banni et al., 2001; Lock et al., 2004) and the growth of human mammary and colon cancer cell lines (Ip et al., 1999; Miller et al., 2003) because of its conversion to *c9,t11-18:2*. The beneficial effects of *t11-18:1* and *c9,t11-18:2* have generally been demonstrated in animal and in vitro cell culture models. Conjugated linoleic acid studies in humans have shown generally little or no response when a butter enriched in *c9,t11 CLA* was fed (Desroches et al., 2005), or when a commercially produced CLA supplement was provided for up to 2 yr (Gaulhier et al., 2002, 2005). A number of studies have, however, reported adverse effects in humans when the *t10,c12 CLA* isomer was consumed at high levels, such as with commercially produced CLA supplements (Larsen et al., 2003; Tricon et al., 2004). Only trace amounts of the *t10,c12 CLA* isomers are found in the fats of ruminants. This compares to consumption of dairy and beef fat that appear to be associated with reduced risk factors of cardiovascular disease (Parodi 2004; Warensjö et al., 2004), mammary cancer (Aro et al., 2000; Ip et al., 2003) and colorectal cancer (Larssen et al., 2005), and growth inhibition or proliferation of certain human cancer cells, or both (Banni et al., 2003; De La Torre et al., 2006). These results require an explanation, and until such time provide an impetus for continued enrichment of these fatty acid (**FA**) isomers in ruminant products.

Enrichment of milk and meat fats of ruminants with *trans-18:1* and CLA isomers depends on a number of factors including the forage-to-concentrate ratio (Griinari et al., 1998; Loores et al., 2005; Shingfield et al., 2005), the type of forage (Bradford and Allen, 2004; Shingfield et al., 2005), the starch source in the concentrate (Jurjanz et al., 2004), the plant oil added and its PUFA content and composition (Lock and Bauman, 2004; Chilliard and Ferlay, 2004), and inclusion of marine oil (Lee et al., 2005; Whitlock et al., 2006). Diets high in concentrates consisting mainly of highly digestible starch sources showed increased levels of *t10-18:1* in the duodenal flow (Sackmann et al., 2003; Lee et al., 2005), in milk fat (Griinari et al., 1998; Piperova et al., 2000; Loores et al., 2005; Shingfield et al., 2005), and in the tissue lipids of ruminants (Daniel et al., 2004; Bessa et al., 2005). The inclusion of vegetable oils high in PUFA into concentrate diets raised the *t11-18:1* and *c9,t11-18:2* content in milk fat, but the initial increase within the first week was shown to be transient (Bauman et al., 2000; AbuGhazaleh et al., 2004; Shingfield et al., 2006). This is in contrast to cows fed grass silage supplemented with a concentrate containing rapeseed oil, which increased the levels of *t11-18:1* and *c9,t11-18:2* that appeared to be retained for 7 wk (Ryhänen

et al., 2005). Furthermore, 2 recent reports have shown that diets with a higher forage-to-concentrate ratio of 60:40 (Bell et al., 2006) or 73:27 (Roy et al., 2006) and containing safflower oil (6%) or linseed oil (5%), respectively, also retained the increased high levels of *t11-18:1* and *c9,t11-18:2*.

Fish oil (**FO**), fish meal, or algae have been included in diets of ruminants to increase the content of long-chain n-3 PUFA for health reasons, such as docosahexaenoic (**DHA**; 22:6n-3) and eicosapentaenoic acids (**EPA**; 20:5n-3). Even though the transfer efficiencies of DHA (3%) and EPA (4%) into milk fats are very low (Chilliard et al., 2001), DHA has been shown to have an added benefit of increasing the level of *t11-18:1* by inhibiting the reduction of *t11-18:1* to 18:0 in rumen bacteria (AbuGhazaleh and Jenkins, 2004; Lee et al., 2005). Increased rumen production of *t11-18:1* resulted in increased synthesis of *c9,t11-18:2* catalyzed by Δ^9 -desaturase in the tissues of ruminants (Whitlock et al., 2006). The DHA might also inhibit the reduction, however, of other *trans-18:1* isomers to 18:0 in the rumen, and the milk and tissue lipids would reflect what was produced in the modified rumen environment.

The present study was undertaken to investigate 3 lower levels (1.5, 3, and 4.5%) of sunflower oil supplementations compared with the 6% safflower oil addition previously reported (Bell et al., 2006). The barley/corn basal diet was the same but the forage-to-concentrate ratio was reduced from 60:40 to 50:50. In addition, 0.5% FO was included in each of the diets to increase the *trans-18:1* isomer content. All cows were fed the control diet for a 4-wk conditioning period followed by the test diets for 38 d. Milk fat was analyzed after 10 and 38 d on diet to evaluate maximum enrichments of *t11-18:1* and *c9,t11-18:2* during the early (10 d) and later period (38 d). In this study complementary GC and argentation thin layer chromatography and HPLC methods were used for the analysis of all the *trans-18:1* and CLA isomers as described previously (Cruz-Hernandez et al., 2004).

MATERIALS AND METHODS

Animals, Design, and Treatments

The Faculty Animal Policy and Welfare Committee at the University of Alberta approved all animal procedures. Eighty lactating Holstein cows (32 primiparous, 48 multiparous) were housed and managed at the Dairy Research and Technology Center at the University of Alberta in tie-stall facilities with water available at all times. The basal diets (Table 1) were prepared using commercial facilities, and FO (Peruvian anchovy) was added just prior to feeding to prevent oxidation. The animals averaged 297 ± 52 DIM. Cows were blocked

Table 1. Ingredients of experimental diets for lactating dairy cows

Ingredient	Diet ¹ (% of DM)			
	Control	1.5% SFO	3% SFO	4.5% SFO
Barley silage	20.3	20.3	20.3	20.3
Alfalfa silage	19.86	19.86	19.86	19.86
Alfalfa hay	7.91	7.91	7.91	7.91
Processed barley grain	30.88	30.08	28.56	27.08
Ground corn grain	11.3	11.3	11.3	11.3
Sunflower oil	0	1.5	3.0	4.5
Fish oil ²	0	0.5	0.5	0.5
Soybean meal	2.5	2.5	2.5	2.5
Corn gluten meal	3.62	3.62	3.62	3.62
Enertia ³	1.2	0	0	0
Limestone	0.6	0.6	0.6	0.6
Dicalcium phosphate	0.75	0.75	0.75	0.75
Mineral salts ⁴	0.40	0.40	0.40	0.40
Salt	0.3	0.3	0.3	0.3
Magnesium oxide	0.23	0.23	0.23	0.23
Sodium bicarbonate	0.15	0.15	0.15	0.15
Vitamin A, D, and E ⁵	0.08	0.08	0.08	0.08

¹Diets: control; 1.5% SFO = 1.5% sunflower oil; 3% SFO = 3% sunflower oil; 4.5% SFO = 4.5% sunflower oil.

²The fish oil (Peruvian anchovy) was included prior to feeding and contained 18.2% eicosapentaenoic acid (EPA) and 10.9% docosahexaenoic acid (DHA).

³Same as Megalac.

⁴Mineral salt (minimum 95%), iodine (150 mg/kg), cobalt (50 mg/kg), copper (3,500 mg/kg), manganese (10,000 mg/kg), zinc (9,000 mg/kg), and selenium (75 mg/kg).

⁵Vitamin A (minimum 10,000,000 IU/kg); vitamin D (minimum 1,000,000 IU/kg); vitamin E (minimum 10,000 IU/kg).

according to parity, milk yield and DIM and randomly placed into 1 of the 4 groups and fed a high barley and corn grain control diet for 4 wk to condition the cows for subsequent enriched of this basal diet with SFO and FO. After the 4-wk conditioning period, each group was fed 1 of the following 4 diets for a 10-d treatment period: 1) control; 2) 1.5% of DM sunflower oil plus 0.5% fish oil (1.5% SFO); 3) 3% of DM sunflower oil plus 0.5% fish oil (3% SFO); or 4) 4.5% of DM sunflower oil plus 0.5% fish oil (4.5% SFO). After 10 d, half of the cows in each group were supplemented with monensin (results to be reported separately), and the remaining 10 cows on each group continued to receive the same diet. During the 4-wk conditioning period milk production and milk fat percentage were monitored. All diets were formulated to meet or exceed NRC (1989) recommendations. The diets were fed once per day at 0900 h as a TMR consisting of 48% forage and 52% concentrate (Table 1). Feed intake and DMI (kg/d) were recorded daily.

Sampling and Analysis of Feed and Milk

Feed was sampled and ground. A portion of the feed was extracted with chloroform/methanol (1:1), and the total lipids were methylated with NaOCH₃/methanol

(#33080, Supelco Inc., Bellefonte, PA). The fatty acid methyl esters (**FAME**) were purified by thin layer chromatography using the solvent hexane/diethyl ether/acetic acid (85:15:1) and then analyzed by GC; see GC conditions below.

Milk production (kg/d) was measured and an aliquot of milk was sampled from each cow at the a.m. and p.m. milking before and after the 4-wk pretreatment period, and after 10 and 38 d of the experimental period. Milk samples were analyzed for protein, lactose, and fat by near infrared spectroscopy using a FOSS 6000 instrument (Foss North America Inc., Eden Prairie, MN; Kohn et al., 2004) at the Alberta Agriculture, Food and Rural Development Central Milk Testing Laboratory (Edmonton, Alberta, Canada). Milk FA determinations were conducted using the combined a.m. and p.m. samples that were stored at -20°C until analyzed.

Fatty Acid Analysis of Milk

Total milk lipids were extracted using a chloroform/methanol/water (2:2:1.8; vol/vol/vol) mixture at a 20:1 solvent to sample volume ratio (Cruz-Hernandez et al., 2004). Total FAME of milk lipids were prepared using NaOCH₃/methanol and analyzed by GC (Cruz-Hernandez et al., 2004). The GC instrument (Hewlett-Packard model 5890 Series II, Palo Alto, CA) was equipped with a splitless injection port that was flushed after 0.3 min, a flame-ionization detector, an autosampler (Hewlett-Packard, model 7673), a 100-m CP-Sil 88 fused-silica capillary column (100 m × 0.25 mm i.d. × 0.2 µm film thickness; Varian Inc., Mississauga, ON), and a Hewlett-Packard ChemStation software system (Version A.10). Operating conditions included injector and detector temperatures both at 250°C, H₂ as carrier gas (1 mL/min) and for the flame-ionization detector (35 mL/min), N₂ as makeup gas (30 mL/min), and purified air (286 mL/min). A temperature program was: initial temperature of 45°C and held for 4 min, increased by 13°C/min to 175°C and held for 27 min, increased by 4°C/min to 215°C and held for 35 min (Kramer et al., 2001). The FAME were identified by comparison with a GC reference FAME standard (#463) to which a CLA mixture consisting of 4 positional isomers (#UC-59M) and long-chain saturated FAME (21:0, 23:0, and 26:0) were added (Nu-Chek Prep Inc., Elysian, MN). The short-chain FAME were corrected for mass discrepancy using the correction factors published by Wolff et al. (1995). All chemicals and solvents were of analytical grade.

Analysis of Trans-18:1 FAME Isomers Combining Ag⁺-TLC and GC

The *trans*-18:1 isomers were analyzed by GC after a prior separation of the total FAME by silver ion-thin

layer chromatography (**Ag⁺-TLC**; Precht and Molketin, 1996; Kramer et al., 2001; Cruz-Hernandez et al., 2004). The bands were identified under UV light after spraying the plates with 2',7'-dichlorofluorescein, scraped off, collected, and extracted with hexane from the silica. Samples were dissolved in hexane and analyzed by GC using a stepwise temperature program starting at 120°C (120°C held for 200 min, increased 15°C/min to 150°C and held for 70 min, increased 15°C/min to 175°C and held for 60 min, and finally increased 15°C/min to 220°C and held for 50 min). All the *trans*-18:1 isomers from *t*4- to *t*16-18:1 were quantified using the results of both GC determinations (Cruz-Hernandez et al., 2004).

Separation and Identification of CLA Isomers Using GC and Ag⁺-HPLC

All the CLA isomers were analyzed using a combination of GC and Ag⁺-HPLC separations, and the mixture of 4 positional CLA isomers and 21:0 (Nu-Chek Prep) were used to identify the CLA isomers (Cruz-Hernandez et al., 2004). Analysis of the CLA isomers by Ag⁺-HPLC involved an HPLC (model 1100, Agilent Technologies, Palo Alto, CA) equipped with a quaternary pump (G1311A), autosampler (G1313A), diode array detector (G1315A), and a Hewlett-Packard ChemStation software system (Version A.10). Three ChromSpher 5 Lipids analytical silver ion-impregnated columns (4.6 mm i.d. × 250 mm stainless steel; 5-μm particle size; Varian Inc., Mississauga, ON) were used in series. The CLA isomers were quantified based on the GC results that were complemented by Ag⁺-HPLC separations of most of the CLA isomers (Cruz-Hernandez et al., 2004).

Statistical Analyses

Milk composition data were analyzed as a one-way ANOVA with diet (control, 1.5% SFO, 3% SFO, and 4.5% SFO) as the fixed effect using the GLM procedure of SAS (2002) and reported as least square means and standard errors. Milk production and fat percentage were analyzed as a repeated measures design using the MIXED procedure in SAS with diet, lactation number (1, 2 and 3), DIM (1 to 120 d, 121 to 200 d, and <201 d) and 2-way interactions as fixed effects and period (pre- and posttreatment) as the repeated effect. The relationship between milk yield and percentage milk fat with other milk components was determined by regression using the REG procedure in SAS. Statistical significance was declared at $P < 0.05$.

RESULTS

Feed Composition, Milk Yield, and Milk Fat Content

The diets were formulated to be isocaloric and SFO and a small constant amount of FO were added at the

Table 2. Fatty acid (FA) composition of the diets without fish oil (relative %)

FA ¹	Diet ²			
	Control	1.5% SFO	3% SFO	4.5% SFO
8:0	0.02	0.02	0.08	0.09
10:0	0.09	0.07	0.11	0.05
12:0	0.76	0.46	0.55	0.23
14:0	2.23	3.02	3.21	2.21
<i>c</i> 9-14:1	0.09	0.1	0.11	0.05
15:0	0.82	1.06	1.53	0.65
16:0	46.94	33.02	28.9	23.17
Σ <i>trans</i> -16:1	0.05	0.16	0.14	0.12
<i>c</i> 9-16:1	1.3	2.52	2.26	1.67
17:0	0.29	0.39	0.29	0.25
<i>c</i> 9-17:1	0.08	0.11	0.11	0.08
18:0	3.7	4.47	4.14	5.25
Σ <i>trans</i> -18:1	0.27	0.16	0.2	0.15
<i>c</i> 9-18:1	20.51	19.78	24.81	28.98
<i>c</i> 11-18:1	0.73	1.25	1.20	1.07
<i>c</i> 12-18:1	0.02	0.03	0.03	0.03
<i>c</i> 13-18:1	0.03	0.04	0.05	0.04
Σ <i>c/t</i> -18:2	0.24	0.39	0.37	0.35
18:2n-6	14.45	22.59	24.54	28.65
20:0	0.36	0.44	0.33	0.38
Σ <i>c/c/t</i> -18:3	0.10	0.15	0.11	0.09
<i>c</i> 11-20:1	0.2	0.34	0.32	0.29
18:3n-3	5.24	7.6	3.5	4.09
20:2n-6	0.03	0.04	0.03	0.03
22:0	0.23	0.39	0.3	0.46
24:0	0.13	0.18	0.11	0.16
Σ SFA	55.57	43.52	39.55	32.90
Σ <i>trans</i> FA	0.65	0.85	0.78	0.70

¹*c* = *cis*; *t* = *trans*; SFA = saturated fatty acids.

²Diets: control; 1.5% SFO = 1.5% sunflower oil; 3% SFO = 3% sunflower oil; 4.5% SFO = 4.5% sunflower oil.

expense of Enertia and processed barley grain (Table 1). The inclusion of SFO into the diets decreased total dietary saturated fatty acids (SFA), notably palmitic acid (16:0), and increased the level of 18:2n-6 and oleic acid (9*c*-18:1; Table 2). Linolenic acid (18:3n-3), derived mainly from the barley and alfalfa, decreased with the addition of SFO that contained only trace amounts of 18:3n-3. Every effort was made to prevent deterioration of the SFO and FO incorporated into the diet by preparing just the necessary amount of diet each time. The lipids extracted from the feed had a low content of total *trans* FA (0.65 to 0.85%), and a FA composition typically associated with processed vegetable oils [i.e., 9*t*- and 10*t*-18:1, 9*c*12*t*- and 9*t*12*c*-18:2, and 9*c*12*c*15*t*- and 9*t*12*c*15*c*-18:3 (Table 2)]. The fish oil added contained 18.2% EPA and 10.9% DHA.

There were no significant differences in milk production between diets after the 4-wk pretreatment period and after the 10-d treatment study (Table 3); the values after 38 d are not included. The apparent difference in milk fat between the SFO groups and control was not significant ($P = 0.16$). However, the addition of SFO significantly lowered milk fat in each diet group com-

Table 3. Milk yield and composition of lactating dairy cows fed the different levels of sunflower oil (SFO)

Item		Diet				SEM
		Control	1.5% SFO	3% SFO	4.5% SFO	
Milk yield (kg/d)	Pretreatment	28.29 ^{a,x}	32.20 ^{a,x}	28.77 ^{a,x}	30.63 ^{a,x}	1.46
	Treatment	27.26 ^{a,x}	31.31 ^{a,x}	26.55 ^{a,x}	29.51 ^{a,x}	1.42
Fat (%)	Pretreatment	3.93 ^{a,x}	3.93 ^{a,x}	4.10 ^{a,x}	3.92 ^{a,x}	0.145
	Treatment	3.81 ^{a,x}	3.50 ^{a,y}	3.63 ^{a,y}	3.39 ^{a,y}	0.147
Protein (%)	Treatment	3.16 ^a	3.08 ^a	3.17 ^a	3.06 ^a	0.112
Lactose (%)	Treatment	4.65 ^a	4.68 ^a	4.75 ^a	4.61 ^a	0.172

^{a,b,x,y}Values within rows with different superscript letters (a,b) are significantly different ($P < 0.05$); values between the pretreatment and treatment rows with different superscript letters (x,y) are significantly different ($P < 0.05$).

pared with its pretreatment period ($P < 0.05$; Table 3). This would suggest that assignment of cows after, rather than before, the pretreatment period would have resulted in significant MFD with the addition of the SFO diets. There were no significant diet differences in the protein and lactose content during the pretreatment or the treatment period, and therefore, these pretreatment values are not included in Table 3.

Milk Fatty Acid Composition

SFA. The inclusion of increasing amounts of SFO into the dairy rations linearly decreased the milk fat content of most short- and medium-chain fatty acids (SCFA and MCFA) including 16:0 that were synthesized de novo in the mammary glands, except 4:0 and 11:0 (Table 4). Only minor differences were observed in the content of the longer chain SFA greater than 20:0. On the other hand, the increased content of 18:0 reflected decreased reduction of the *trans*-18:1 isomers; the highest level of 18:0 was found in the 3% SFO group. The relative content of the *iso* and *anteiso* FA in milk fat was not affected by the addition of SFO in the diet, except at the highest level (4.5% SFO) that had significant decreased levels of the branch-chain FA.

PUFA and Their Metabolites. The higher levels of SFO addition increased 18:2n-6 and its metabolites formed by rumen bacteria or subsequently in the mammary tissue (Table 4). These metabolites include most of the *trans*-18:1, *c/t*-18:2, and CLA isomers. The total *trans*-18:1 content increased from about 5% on the control diet to 21% on the 4.5% SFO group. There was also a 6-fold increase in the total *trans*-16:1 content in milk fat from about 0.11 to 0.64%. The major *c/t*-18:2 isomers included *t9t12*-, *c9t13*- plus *t8c12*-, *t8c13*- plus *c9t12*-, *t9c12*- and *t11c15*-18:2 (Table 4) that are metabolites of 18:2n-6, 18:3n-3, or both, by rumen bacteria. The relative concentration of most of these *c/t*-18:2 isomers increased with added SFO in the diets. The total CLA content linearly increased from 0.7% on the control diet to 3.9% on the 4.5% SFO group. The barley and alfalfa

in the diet contributed to the 18:3n-3 content in milk fat and decreased with increased levels of SFO in the diet (Table 4).

The long-chain PUFA metabolites of 18:2n-6 and 18:3n-3 generally remained the same in all 4 diets, except for a decrease in arachidonic acid (20:4n-6) at the higher SFO additions, possibly the result of dietary DHA and EPA (Table 4). The relative concentration of the n-3 long-chain PUFA in the milk fat were slightly elevated compared with general commercial milk because small amounts of FO (0.5%) were included in all diets.

Cis and Trans Monounsaturated Fatty Acids. The extent of the overlap of the *cis* and *trans*-18:1 isomers even when using 100-m highly polar capillary columns was exacerbated when milk fats were analyzed that had an increased *trans*-18:1 content (Figure 1A). Complete analysis of the 18:1 isomers required prior separation of the *cis* and *trans* monounsaturated FA by Ag⁺-TLC followed by GC analysis at 120°C to resolve all the *trans*-18:1 isomers (Figure 1B). Using these combination of methods, all the individual *trans*-18:1 isomers were analyzed with the exception of *t6-t8*-18:1.

There was a 4-fold increase in total *trans*-18:1 content from 5.1% (control) to over 20% after 10 d of feeding the 4.5% SFO diet (Table 4). There was also an increase of the total *trans*-16:1 isomers from 0.1 to 0.6% of total lipids. All *trans*-18:1 isomers increased with increased supplementation of SFO/FO, but there were isomer differences (Figure 2). There was a 10-fold increase of *t10*- and an 8-fold increase of *t11*-18:1 in the total milk lipids of the 4.5% SFO compared with the control diet. This compared with a 4-fold increase of *t6-t8* and *t9*, a 3-fold of *t5* and *t12*, and a doubling of the remaining isomers (*t4* and *t13* to *t16*). When the *trans*-18:1 isomers were expressed as percentage of total *trans*-18:1, the relative concentration of the 2 major isomers increased, *t11*-18:1 from 19% to 33%, and *t10*-18:1 from 10 to 22% (Table 5). On the other hand, the relative concentrations of *t4* to *t9* and *t12* were not affected by diet, whereas *t13* to *t16* decreased relative to control. Vaccenic

Table 4. Fatty acid (FA) composition of milk fat for lactating dairy cows fed the different levels of sunflower oil (SFO)

FA ¹ for 10 d	Diet				SEM
	Control	1.5% SFO	3% SFO	4.5% SFO	
	g/100 g of total FA				
4:0	2.57	3.96	2.57	2.23	0.38
6:0	2.14 ^a	1.99 ^{ba}	1.82 ^b	1.46 ^c	0.15
8:0	1.34 ^a	1.20 ^a	0.91 ^b	0.75 ^b	0.14
10:0	3.14 ^a	2.59 ^b	2.11 ^c	1.62 ^d	0.32
11:0	0.39	0.34	0.32	0.34	0.02
12:0	3.61 ^a	2.95 ^b	2.41 ^c	1.88 ^d	0.37
13:0	0.22 ^a	0.20 ^b	0.15 ^c	0.12 ^d	0.02
14:0	10.76 ^a	9.62 ^b	8.72 ^c	7.34 ^d	0.72
15 <i>iso</i>	0.21 ^a	0.21 ^a	0.20 ^a	0.17 ^b	0.01
15 <i>anteiso</i>	0.53 ^a	0.49 ^{ba}	0.45 ^b	0.36 ^c	0.04
c9-14:1	0.94 ^a	0.97 ^a	0.87 ^a	0.72 ^b	0.06
15:0	1.22 ^a	1.14 ^b	1.03 ^c	0.87 ^d	0.07
16 <i>iso</i>	0.33 ^a	0.33 ^a	0.31 ^a	0.26 ^b	0.02
16:0	31.02 ^a	26.12 ^b	22.25 ^c	19.51 ^d	2.50
Σ <i>trans</i> -16:1	0.11 ^c	0.31 ^b	0.40 ^b	0.64 ^a	0.17
c7-16:1	0.19	0.16	0.22	0.17	0.01
c9-16:1	1.57	1.59	1.15	1.05	0.14
c11-16:1	0.04	0.04	0.04	0.03	0.00
17 <i>iso</i>	0.32	0.37	0.42	0.42	0.02
17 <i>anteiso</i>	0.58 ^a	0.55 ^a	0.58 ^a	0.44 ^b	0.03
17:0	0.54 ^a	0.56 ^a	0.54 ^a	0.46 ^b	0.02
c9-17:1	0.25 ^a	0.24 ^a	0.20 ^{ba}	0.16 ^b	0.02
18 <i>iso</i>	0.08	0.08	0.08	0.07	0.00
18:0	8.49 ^d	8.57 ^c	10.21 ^a	9.79 ^b	0.43
Σ <i>trans</i> -18:1	5.10 ^d	9.27 ^c	13.70 ^b	20.69 ^a	3.34
Σ <i>cis</i> -18:1	20.87	19.92	21.43	20.33	0.16
c9-18:1	17.29	15.94	17.57	16.31	0.24
c11-18:1	0.58	0.59	0.63	0.65	0.03
c12-18:1	0.33 ^c	0.67 ^b	0.88 ^a	0.97 ^a	0.14
c13-18:1	0.10 ^c	0.14 ^b	0.15 ^b	0.18 ^a	0.04
17 <i>cyclo</i>	0.07	0.07	0.07	0.07	0.00
t9t12-18:2	0.03 ^b	0.05 ^{ba}	0.06 ^a	0.07 ^a	0.01
c9t13/t8c12-18:2	0.25 ^c	0.44 ^b	0.51 ^a	0.52 ^a	0.06
t8c13-18:2	0.12 ^b	0.20 ^a	0.22 ^a	0.21 ^a	0.02
c9t12-18:2	0.11 ^c	0.16 ^b	0.18 ^a	0.20 ^a	0.02
t9c12-18:2	0.04 ^d	0.08 ^c	0.11 ^b	0.13 ^a	0.02
t11c15-18:2	0.12 ^c	0.26 ^b	0.33 ^{ba}	0.39 ^a	0.06
c11c15-18:2	0.06	0.06	0.05	0.06	0.00
18:2n-6	2.34 ^b	2.62 ^{ba}	2.66 ^{ba}	2.75 ^a	0.09
20:0	0.19 ^b	0.22 ^{ba}	0.23 ^a	0.20 ^{ba}	0.01
18:3n-6	0.04 ^a	0.04 ^{ba}	0.03 ^{bc}	0.03 ^c	0.00
c/c/t-18:3	0.02 ^b	0.05 ^a	0.04 ^a	0.05 ^a	0.29
c9-20:1/t,c,c-18:3	0.16 ^c	0.23 ^a	0.22 ^a	0.19 ^b	0.02
c11-20:1	0.08 ^b	0.15 ^a	0.15 ^a	0.16 ^a	0.02
18:3n-3	0.44 ^a	0.41 ^a	0.36 ^b	0.31 ^c	0.03
Σ CLA	0.66 ^c	1.90 ^b	2.36 ^b	3.87 ^a	0.67
21:0	0.02	0.02	0.02	0.02	0.00
20:2n-6	0.04 ^b	0.05 ^a	0.05 ^a	0.05 ^a	0.00
22:0	0.06 ^b	0.09 ^a	0.09 ^a	0.08 ^a	0.01
20:3n-6	0.12	0.12	0.11	0.10	0.00
20:4n-6	0.15 ^a	0.14 ^b	0.10 ^c	0.09 ^c	0.01
23:0	0.04	0.08	0.03	0.03	0.01
20:5n-3	0.05 ^b	0.06 ^a	0.04 ^b	0.04 ^b	0.00
24:0	0.03	0.03	0.03	0.03	0.00
26:0	0.02	0.02	0.01	0.01	0.00
22:5n-3	0.08 ^a	0.08 ^a	0.07 ^b	0.06 ^b	0.00
22:6n-3	0.03 ^b	0.04 ^a	0.03 ^b	0.03 ^b	0.00
Summaries					
Σ SFA	68.90 ^a	62.80 ^b	56.56 ^c	49.29 ^d	4.00
Σ SCFA (4:0-15:0)	25.52 ^a	24.15 ^a	20.22 ^b	16.71 ^c	1.92
Σ <i>iso</i> + <i>anteiso</i> FA	2.045 ^a	2.018 ^a	2.038 ^a	1.72 ^b	0.07
Σ <i>trans</i> MUFA	5.20 ^d	9.14 ^c	14.10 ^b	21.33 ^a	3.29
Σ <i>cis</i> MUFA	20.87	19.92	21.43	20.33	0.16

Continued

Table 4 (Continued). Fatty acid (FA) composition of milk fat for lactating dairy cows fed the different levels of sunflower oil (SFO)

FA	Diet				SEM
	Control	1.5% SFO	3% SFO	4.5% SFO	
Σ <i>c/t</i> -18:2	0.66 ^c	1.19 ^b	1.41 ^a	1.52 ^a	0.26
Σ n-6 PUFA	2.39 ^b	2.63 ^{ba}	2.68 ^{ba}	2.78 ^a	0.08
Σ n-3 PUFA	0.46 ^a	0.45 ^a	0.40 ^b	0.36 ^b	0.25
Σ n-6 LC PUFA	0.30 ^a	0.29 ^a	0.24 ^b	0.22 ^b	0.02
Σ n-3 LC PUFA	0.11 ^b	0.15 ^a	0.12 ^b	0.13 ^b	0.01
Desaturation index					
14:0/ <i>c</i> 9-14:1	11.94	10.81	10.75	11.26	0.37
16:0/ <i>c</i> 9-16:1	20.27	19.69	18.66	19.03	0.57
17:0/ <i>c</i> 9-17:1	2.23	2.60	3.53	4.28	0.46
18:0/ <i>c</i> 9-18:1	0.50 ^b	0.53 ^{ba}	0.59 ^a	0.61 ^a	0.03
<i>t</i> 11/ <i>c</i> 9 <i>t</i> 11-18:2	1.88	1.99	2.13	1.96	0.06

^{a-d}Values within rows with different superscript letters are significantly different ($P < 0.05$).

¹*c* = *cis*; *t* = *trans*; CLA = conjugated linoleic acid; SFA = saturated fatty acid; LC = long-chain; MUFA = monounsaturated fatty acid; PUFA = polyunsaturated fatty acid; SCFA = short-chain fatty acid.

acid remained the major *trans*-18:1 isomers after 10 d of feeding, and there were no significant differences in the relative concentration of *t*11-18:1 to total *trans*-18:1 among the 3 SFO groups (Table 5). Feeding the SFO/FO diets for an additional 28 d (38 d in total) resulted in a further significant increase of *t*10-18:1 compared with 10 d in the milk fat of all diet groups (Figure 3). On the other hand, the response of *t*11-18:1 to continued feeding depended on the level of SFO/FO included; at 1.5% it continued to increase, at 3% it stayed the same, whereas at 4.5% it declined. After 38 d of feeding the SFO/FO diets the relative abundance of *t*10- and *t*11-18:1 was similar in the milk fat, and often *t*10-18:1 was the predominant *trans*-18:1 isomers as shown in the representative milk fat shown in Figure 1.

The total content of *cis*-monounsaturated FA was not significantly different between diets (Table 4) despite differences in the dietary levels of the major 18:1 isomers. The desaturation index was similar among the SFO diets except for the low SFO group (1.5% SFO) that appeared to show the greatest Δ^9 -desaturase activity (Table 4).

CLA. The CLA isomer composition was determined using a combination of GC (Figure 4A) and Ag⁺-HPLC (Figure 4B) techniques and the isomers were identified using published elution principles (Sehat et al., 1999; Kramer et al., 1999; Delmonte et al., 2005). The GC separation resulted in the resolution of *t*9*c*11-, *t*10*c*12-, and *t*11*t*13-18:2 and provided the sum of *c*9*t*11- plus *t*7*c*9-18:2, *t*11*c*13- plus *c*9*c*11-18:2, and *t*9*t*11- plus *t*10*t*12-18:2 (Figure 4A). In the GC chromatogram 21:0 was identified using an internal standard, but it caused no interference using Ag⁺-HPLC separations because the CLA isomers were detected at 233 nm. Using Ag⁺-HPLC, *t*7*c*9-, *t*11*c*13-, *c*9*c*11-18:2 and all conjugated *tt*-

18:2 isomers were resolved, but not *c*9*t*11- and *t*9*c*11-18:2 (Figure 4B).

The most abundant CLA isomer present in all milk fat was *c*9*t*11-18:2, and it increased significantly from 0.43% on the control diet to 3.28% of total milk fat on the 4.5% SFO diet (Table 6). The relative proportion of *c*9*t*11-18:2 to the total CLA isomers also increased with the addition of SFO from 65% in the control diet to over 77% on all the SFO diets. Continued feeding of the diets for an additional 28 d increased the *c*9*t*11-18:2 content but only at the lowest level of SFO/FO inclusion, and significantly decreased it at the highest level (Figure 3). This pattern was similar to that observed with vaccenic acid, which is not surprising because vaccenic acid serves as precursor for most of the rumenic acid in the mammary tissue. The content of several minor CLA isomers not generally associated with milk fat depression, such as *t*7*c*9-, *t*9*c*11-, *t*10*c*12- and the conjugated *tt*-18:2 isomers, all increased linearly, except *t*11*t*13-18:2 (Figure 5). The total of these CLA isomers increased from 0.18 to 0.59% of total milk fat with the inclusion of SFO, but their relative proportion among the total CLA isomers declined from 27% in the control diet to 13% on the 4.5% SFO/FO diet (Table 6).

DISCUSSION

Due to the increasing demand for value-added foods with health benefits, developing strategies to produce dairy products with enriched *c*9*t*11-18:2, *t*11-18:1 and DHA are of particular interest to researchers and industry alike. This study was designed to evaluate the addition of different levels of SFO to determine at what dietary level of SFO/FO the desired FA isomers could be maximized and maintained in milk fat. The content

Figure 1. Partial GC chromatogram from 18:0 to 18:2n-6 obtained using a 100-m CP Sil 88 capillary column and a temperature program described in the materials and methods section (A). A milk fat was selected from a cow fed the 4% sunflower oil/fish oil diet that contained more *trans*-10- than *trans*-11-18:1 to demonstrate the lack of separation among the *trans*-18:1 isomers. Separation of the *trans*-18:1 fraction by GC operated at 120°C is shown in B. The *trans* fraction was obtained by prior silver ion-thin layer chromatography separation. *c* = *cis*; *t* = *trans*.

and composition of the desired FA was assessed using appropriate methods to resolve in particular eluting *trans* FA and CLA isomers.

The barley/corn-based diet consisting of a 50:50 forage-to-concentrate ratio supplemented with up to 5% SFO plus FO for up to 38 d produced no differences in

Figure 2. The *trans*-18:1 isomer composition expressed as percentage of total milk fat of all 4 diets after 10 d feeding. SFO = sunflower oil; FO = fish oil; *t* = *trans*.

milk yield and composition, and only a moderate milk fat depression, despite marked changes in milk fat composition. Greater milk fat depressions are generally associated when diets with much lower forage content (Griinari et al., 1998; Piperova et al., 2000; Loores et al., 2005) or higher amounts of FO are included in dairy rations (Whitlock et al., 2002; Shingfield et al., 2003).

In this study the greatest change in milk fat composition was the marked increase in total *trans*-18:1 and CLA content at the expense of SCFA including 16:0 that are *de novo* synthesized FA in the mammary tissue. The total *trans*-18:1 content reached levels of 15 to 21% of

total milk fat with the inclusion of 3 and 4.5% SFO plus 0.5% FO in each diet (Table 4). To our knowledge, these results demonstrate for the first time that the increase in total *trans*-18:1 was linear in response to increased levels of SFO, when the other factors in the diet were kept constant, such as forage-to-concentrate ratio, basal diet ingredients, and FO supplementation. These very high levels of total *trans*-18:1 (9 to 24%) in milk fat were obtained by a combination of dietary factors, apart from known genetic, age, and animal to animal variations. These factors include feeding cereal silages (i.e., corn > grass; Shingfield et al., 2005), higher

Table 5. The *trans*-18:1 isomer composition of milk fat (relative % of total *trans*-18:1) from cows fed the different levels of sunflower oil (SFO)

Isomer ¹	Diet				SEM
	Control	1.5% SFO	3% SFO	4.5% SFO	
<i>t</i> 4-18:1	0.61 ^a	0.47 ^b	0.37 ^b	0.34 ^b	0.059
<i>t</i> 5-18:1	0.54 ^a	0.43 ^b	0.38 ^{cb}	0.32 ^c	0.05
<i>t</i> 6- <i>t</i> 8-18:1	5.71	5.91	6.18	5.52	0.14
<i>t</i> 9-18:1	6.46	5.70	6.28	5.89	0.18
<i>t</i> 10-18:1	10.33 ^b	12.54 ^b	20.10 ^a	22.20 ^a	2.87
<i>t</i> 11-18:1	18.53 ^b	32.16 ^a	29.61 ^a	33.23 ^a	3.37
<i>t</i> 12-18:1	8.33 ^a	9.27 ^a	8.28 ^a	6.12 ^b	0.67
<i>t</i> 13-18:1	12.54 ^a	8.32 ^b	7.10 ^b	6.58 ^b	1.35
<i>t</i> 14-18:1	15.33 ^a	10.17 ^b	8.65 ^b	8.04 ^b	1.66
<i>t</i> 15-18:1	13.01 ^a	8.96 ^b	7.76 ^b	7.36 ^b	1.29
<i>t</i> 16-18:1	8.62 ^a	6.07 ^b	5.30 ^{cb}	4.41 ^c	0.91
Total <i>trans</i> -18:1	5.10 ^d	9.27 ^c	13.70 ^b	20.69 ^a	3.34

^{a-d}Values within a row with different superscript letters are significantly different ($P < 0.05$).

¹*t* = *trans*.

Figure 3. The increase of *t*10-18:1, *t*11-18:1, and *c*9*t*11-18:2 in total milk fat over the 38-d feeding period in the 3 sunflower oil (SFO)/fish oil diets. *c* = *cis*; *t* = *trans*.

amounts of concentrate (i.e., 65 > 35%, Loor et al., 2005; Shingfield et al., 2005; Roy et al., 2006), readily fermentable carbohydrates (i.e., wheat > potatoes, Jurjanz et al., 2004), vegetable oil high in PUFA (Bell et al., 2006), and FO (Shingfield et al., 2003; Table 7). The present study includes increased amounts of vegetable oils as a factor contributing to increased levels of total *trans*-18:1. An increase in total *trans*-18:1 was also observed in pasture-fed ruminants supplemented with small amounts of oilseeds, but these increases in total *trans*-18:1 rarely exceed 7% of total milk fat (Fearon et

al., 2004; Rego et al., 2005; Cruz-Hernandez et al., 2006).

However, a high *trans*-18:1 content does not necessarily guarantee a milk fat high in vaccenic and rumenic acid (Table 7). Although there was an 8-fold increase in vaccenic acid to 6.6% of total milk fat after 10 d of feeding the 4.5% SFO/FO diet in the present study, this level could not be sustained on continued feeding (Figure 3). This transient increase of *t*11-18:1 was similar to that reported by Bauman et al. (2000), AbuGhazaleh et al. (2004) and Shingfield et al. (2006). The increase in vaccenic acid was accompanied by a 10-fold increase of *t*10-18:1 in milk fat in the present study, and whereas the content of *t*11-18:1 decreased on continued feeding at the highest addition of SFO/FO, *t*10-18:1 continued to increase (Figure 3). The large increase in total *trans*-18:1 was accounted for by the increase of these 2 isomers that increased to 11.5% of total milk fat, or 55% among the total *trans*-18:1 isomers (Table 5). Similar results were obtained by others showing that *t*10- plus *t*11-18:1 accounted for 51% (Bell et al., 2006) to 84% (Roy et al., 2006) of the total *trans*-18:1 isomers (Table 7). In some cases the relative content of these 2 isomers was inflated because not all the *trans*-18:1 isomers were reported. In this study, the remaining *trans*-18:1 isomers also increased in the milk fat from 3.8 to 9.2%, but their relative proportion among the *trans*-18:1 isomers decreased from 27 to 13% (Table 6).

The levels of vaccenic acid were sustained on continued feeding, but only when lower amounts of the SFO/FO mixture were fed (Figure 3). The levels of vaccenic acid attained were 3.1% of total milk fat with the 1.5% SFO/FO diet and 4% with the 3% SFO/FO diet (Figure 2). These results may explain why higher forage containing diets maintained their high vaccenic acid content on continued feeding (Bell et al., 2006; Roy et al., 2006). The latter produced these conditions by increasing the forage component in the diet and maintaining the same level of dietary oil (Roy et al., 2006). In this study, the dietary oil content was reduced, which effectively decreased the available PUFA and retained the same forage-to-concentrate ratio.

The effect of adding FO to increase the total *trans*-18:1 content in milk fat could not be assessed independently in this study because all 3 test treatments included the same amount of FO at 0.5% DM. Previous in vitro studies showed that the reduction of *t*11-18:1 to 18:0 in rumen bacteria was inhibited by DHA resulting in increased accumulation of *t*11-18:1 (AbuGhazaleh and Jenkins, 2004). This explanation was provided to account for the high levels of *t*11-18:1 in milk fat when FO was included in the diet (Lee et al., 2005; Loor et al., 2005). However, DHA might equally inhibit

Figure 4. A partial GC (A) and Ag⁺-HPLC (B) chromatogram of the CLA region taken from a cow fed the 4.5% sunflower oil/fish oil diet. For the GC separation, a 100-m CP Sil 88 capillary column was used and a temperature program described in the materials and methods section. For the Ag⁺-HPLC separation, 3 ChromSpher 5 Lipids columns were used in series and UV detection was at 233 nm; for more details see the materials and methods section. *c* = *cis*; *t* = *trans*.

the reduction of other *trans*-18:1 isomers formed by rumen bacteria, which would explain the enrichment of many *trans*-18:1 isomers other than *t11*-18:1 in milk fat, in this and other studies as shown in Table 7. The inhibition of this reduction by DHA has not been demon-

strated for the different *trans*-18:1 isomers or in any bacteria. The relative rate of hydrogenation of the different *trans*-18:1 isomers to 18:0 has only been determined for one of the rumen bacteria; that is, *Fusocillus* species (Kemp et al., 1984). The inclusion of a small

Table 6. The conjugated linoleic acid (CLA) isomer composition of milk fat (relative % of total CLA) from cows fed diets containing different levels of sunflower oil (SFO)

Isomer ¹	Diet				SEM
	Control	1.5% SFO	3% SFO	4.5% SFO	
<i>c9t11</i> -CLA	65.97 ^c	76.99 ^b	78.29 ^b	84.69 ^a	3.8
<i>t7c9</i> -CLA	7.80 ^a	5.80 ^{ab}	6.32 ^b	4.81 ^c	0.60
<i>t9c11</i> -CLA	2.85	2.65	3.15	2.48	0.14
<i>t10c12</i> -CLA	6.10 ^a	3.70 ^b	3.36 ^b	2.44 ^b	0.76
<i>t11c13/c9c11</i> -CLA	3.46 ^a	2.70 ^b	2.03 ^c	1.22 ^d	0.48
<i>c10c12</i> -CLA	1.50 ^a	0.96 ^b	0.85 ^b	0.34 ^c	0.23
<i>c11c13</i> -CLA	1.85 ^a	1.63 ^{ba}	1.10 ^{bc}	0.54 ^c	0.30
<i>t11t13</i> -CLA	3.86 ^a	1.61 ^b	1.22 ^b	0.73 ^c	0.69
<i>t9t11/t10t12/t7t9</i> -CLA	6.62 ^a	4.00 ^b	3.70 ^b	2.76 ^c	0.82
Σ <i>t9c11</i> , <i>t10c12</i> , <i>t7c9</i> , and <i>tt</i> -CLA	27.23 ^a	17.73 ^b	17.73 ^b	13.22 ^c	2.9
Σ CLA (% of total milk fat)	0.66 ^c	1.90 ^b	2.36 ^b	3.87 ^a	0.67

^{a-d}Values within a row with different superscript letters are significantly different ($P < 0.05$).

¹*c* = *cis*; *t* = *trans*.

amount of FO in the diet had the additional benefit of slightly increasing the amounts of the long-chain n-3 PUFA in these milk fats (0.12 to 0.15%). Small increases were expected because the transfer efficiencies of DHA (3%) and EPA (4%) into milk fats in known to be low (Chilliard et al., 2001).

Increasing the SFO content in the barley/corn basal diet to 4.5% also resulted in an 8-fold increase of total CLA (0.43 to 3.28%) and a significant improvement in the relative abundance of the desired *c9t11*-18:2 isomer from 65.9 to 84.7% of total CLA after 10 d on diet (Table 6). The levels of *c9t11*-18:2 in total milk fat paralleled the increase of its precursor FA (*t11*-18:1) over the same

time period (Figure 3). The increased levels of rumenic acid were, however, not sustained on continued feeding the 4.5% SFO/FO diet, which dropped significantly by d 38 on diet compared with 10 d (Figure 3). On the other hand, continued feeding the 1.5% SFO/FO diet further increased the rumenic acid content, whereas the 3% SFO/FO diet retained that level after d 10 (Figure 3). These findings help to clarify the apparent inconsistencies previously observed. Some diets significantly increased the rumenic acid content in milk fat, but the high levels could not be sustained on continued feeding (Bauman et al., 2000; AbuGhazaleh et al., 2004; Shingfield et al., 2006; this study diet 4.5% SFO/FO), except 2 recent studies (Bell et al., 2006; Roy et al., 2006). In these 2 studies, the high rumenic acid levels were maintained by feeding higher levels of fiber [i.e., 60% (Bell et al., 2006) and 64% (Roy et al., 2006)]. Our present study showed that the level of rumenic acid could be retained, but only when the dietary oil in the diet was 3% or less of the DM content (Figure 3).

A note of caution is appropriate in assessing the content of vaccenic (*t11*-18:1) and rumenic acids (*c9t11*-18:2) in milk fats. Using only GC techniques provides at best an estimate of the 2 major *trans*-18:1 isomers *t10*- and *t11*-18:1 (Figure 1) and no resolution of the 2 CLA isomers *c9t11*-18:2 and *t7c9*-18:2 (Figure 4). Resolution of these pairs of FA is critical because in each case these FA represent key metabolite of 2 rather different rumen bacteria populations that produce very different end products of PUFA metabolism. Feeding high forage diets supplemented with small amounts of oilseeds rich in PUFA produces mainly vaccenic and rumenic acids (Kraft et al., 2003; Cruz-Hernandez et al., 2004, 2006). On the other hand, increasing the amount of concentrate in the diets that includes readily degradable carbohydrates and oilseeds rich in PUFA causes a shift in rumen bacterial population (Tajima et al., 2001; Klieve

Figure 5. The effect of increased levels of dietary sunflower oil (SFO) on the content of several minor conjugated linoleic acid (CLA) isomers in milk fat. The CLA isomers included *t7c9*-, *t9c11*-, *t10c12*-, *t11c13*-, and the sum of *t7t9*- to *t10t12*-18:2. FO = fish oil; *c* = *cis*; *t* = *trans*.

Table 7. Comparison of the total *trans*-18:1 and conjugated linoleic acid (CLA) isomer content and selected fatty acid isomers in milk fat of cows fed vegetable oils high in 18:2n-6¹

Author	F:C ratio	Oil		Time on diet ³	Fatty acids (% of total milk fat)						Other CLA ⁷
		Type of oil added ²	Amount added, g/kg		Σt -18:1 ⁴	Σt -18:1	<i>t</i> 11-18:1	<i>t</i> 10-18:1	% <i>t</i> 10& <i>t</i> 11 ⁵	<i>c</i> 9 <i>t</i> 11-18:2 ⁶	
Griinari et al. (1998)	50:50	Corn oil	40	2 wk	4 <i>t</i> -14 <i>t</i>	7.0	4.53	0.7		1.98**	ND
Griinari et al. (1998)	20:80	Corn oil	40	2 wk	4 <i>t</i> -14 <i>t</i>	7.2	2.52	2.9		1.10**	ND
Bauman et al. (2000)	50:50	SFO	52	3 wk	4 <i>t</i> -16 <i>t</i>	15.04	7.26	2.77	67	3.70	0.37
Piperova et al. (2000)	25:70	SBO	50	3 wk	6 <i>t</i> -16 <i>t</i>	15.6	1.7	9.2	70	0.55	0.41
Shingfield et al. (2003)	60:40	Fish oil	250 g/d	14 d	4 <i>t</i> -16 <i>t</i>	14.4	9.39	1.01	72	1.66	0.19
AbuGhazaleh et al. (2004)	50:50	2% SBO/0.5% FO	25	0-10 wk*	6 <i>t</i> -11 <i>t</i>	3.5	2.1	0.75		1.16**	(0.15)
Loor et al. (2005)	65:35	Linseed oil	30	4 wk	4 <i>t</i> -16 <i>t</i>	9.02	3.23	0.52	42	1.34	0.48
Loor et al. (2005)	35:65	Linseed oil	30	4 wk	4 <i>t</i> -16 <i>t</i>	12.10	4.53	2.84	61	2.54	0.46
Shingfield et al. (2005)	CS, 65:35	3% SFO/2% FO	30	3 wk	4 <i>t</i> -16 <i>t</i>	21.23	8.04	7.84	75	3.00	0.43
Shingfield et al. (2005)	CS, 35:65	3% SFO/2% FO	30	3 wk	4 <i>t</i> -16 <i>t</i>	20.76	3.48	12.73	78	1.41	0.50
Shingfield et al. (2005)	GS, 65:35	3% SFO/2% FO	30	3 wk	4 <i>t</i> -16 <i>t</i>	12.01	6.26	0.39	55	2.84	0.31
Shingfield et al. (2005)	GS, 35:65	3% SFO/2% FO	30	3 wk	4 <i>t</i> -16 <i>t</i>	17.31	4.48	7.81	71	1.97	0.46
Shingfield et al. (2006)	60:40	2% SFO/1% FO	45	0-28 d*	4 <i>t</i> -16 <i>t</i>	18.27	6.88	7.72	80	3.04	0.43
Bell et al. (2006)	60:40	Safflower oil	60	2,4,8 wk*	6 <i>t</i> -16 <i>t</i>	17.64	10.72	1.40	69	4.12**	(0.04)
Bell et al. (2006)	60:40	Flaxseed oil	60	2,4,8 wk*	6 <i>t</i> -16 <i>t</i>	14.29	6.67	0.63	51	2.80**	(0.0)
Roy et al. (2006)	27:73	SFO	50	(6 d) 18 d	4 <i>t</i> -14 <i>t</i>	23.68	1.36	18.62	84	1.17**	0.24
Roy et al. (2006)	48:52	SFO	50	(6 d) 18 d	4 <i>t</i> -14 <i>t</i>	11.47	1.44	7.22	76	0.93**	Trace
Roy et al. (2006)	64:36	Linseed oil	50	(6 d) 18 d	4 <i>t</i> -14 <i>t</i>	12.21	7.46	0.70	67	2.89**	Trace
Present study	50:50	1.5% SFO/0.5% FO	20	10 d (38 d)	4 <i>t</i> -16 <i>t</i>	9.27	3.08	1.22	46	1.50	0.40
Present study	50:50	3% SFO/0.5% FO	35	10 d (38 d)	4 <i>t</i> -16 <i>t</i>	13.70	3.95	3.18	52	1.88	0.48
Present study	50:50	4.5% SFO/0.5% FO	50	10 d (38 d)	4 <i>t</i> -16 <i>t</i>	20.69	6.62	4.86	55	3.28	0.59

¹Results taken from studies in which these fatty acid isomers were determined. F:C = forage:concentrate; CS = corn silage; GS = grass silage; *c* = *cis*; *t* = *trans*.²SFO = sunflower oil; SBO = soybean oil; FO = fish oil.³The times shown in brackets are not included. *The fatty acid results appear to be averages at all these time periods.⁴*Trans*-18:1 isomers reported.⁵Percentage of *t*10- plus *t*11-18:1 of total *trans*-18:1. No calculations provided when many isomers were not included.⁶Values of *c*9*t*11-18:2 labeled with (**) included unknown amounts of *t*7*c*9- and *t*8*c*10-18:2.⁷CLA isomers other than *c*9*t*11-18:2; ND = not determined; values in brackets are amounts of selected CLA isomers.

et al., 2003) that produces significant amounts of *t*10-18:1 (Grinari et al., 1998; Piperova et al., 2000; Kim et al., 2002) and *t*7c9-18:2 (Yurawecz et al., 1998; Piperova et al., 2000), and correspondingly less vaccenic and rumenic acids. Therefore, a lack of separation of these 2 FA pairs leads to an overestimation of the beneficial FA in milk fat if both pairs are assumed to be vaccenic and rumenic acids. It should be noted that *t*10-18:1 and *t*7c9-18:2 are not the only FA isomers that increased with the feeding of high concentrate diets, but it includes most of the *trans*-18:1 isomers (Figure 2) and the CLA isomers *t*9c11-, *t*10c12-, and *tt*-18:2 (Figure 5). Therefore, a reliable assessment of milk fat requires in addition to GC the use of complimentary argmentation techniques to accurately determine the *trans*-18:1 and CLA isomers; see reviews by Cruz-Hernandez et al. (2004, 2006).

A relatively quick method to assess the effects of dietary manipulations to increase the content of vaccenic and rumenic acids in milk fat is to measure the ratio of *t*10-18:1 to *t*11-18:1. However, it should be kept in mind that this ratio can be somewhat distorted because *t*10-18:1 produced by certain rumen bacteria (Kim et al., 2002; Wallace et al., 2006) and transferred into milk fat is not further desaturated in the mammary tissue (Kramer et al., 2004). On the other hand, the content of *t*11-18:1 is actively reduced by Δ^9 -desaturase to *c*9*t*11-18:2 in the mammary tissue (Corl et al., 2002). Based on the *t*10-18:1 to *t*11-18:1 ratio in milk fat, it would appear that cows fed the 1.5 and 3% SFO/FO diets supported a rumen population dominated by *t*11 producing bacteria, and a *t*10 shift was evident when the 4.5% SFO/FO diet was fed (Figure 3). The *t*10 shift was even greater when higher levels of concentrates and more oil were included in the diets (Table 7).

CONCLUSIONS

It is becoming clear that the key to increasing the vaccenic and rumenic acid content in milk fat involves not only balancing the dietary forage-to-concentrate ratio and selecting carbohydrate sources, but also choosing appropriate oils and the amount of oil, as well as including a small amount of FO. The results of this study showed that a desirable milk fat quality can be achieved and maintained using moderate levels of SFO (i.e., 3% of DM intake) to obtain a stable milk fat composition containing about 4% vaccenic acid and 2% rumenic acid. At the same time efforts should be made to reduce the level of the remaining *trans*-18:1 and CLA isomers because no beneficial effects have so far been attributed to these isomers. In this study, the other *trans*-18:1 and CLA isomers accounted for 9.6 and 0.5% of total milk fat, respectively, when the 3% SFO/FO diet

was fed. Finally, the importance of using appropriate methods to resolve all the *trans*-18:1 and CLA isomers should be stressed because only then can the true content of the desired beneficial FA be assessed.

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