High-Concentrate Diets and Polyunsaturated Oils Alter *Trans* and Conjugated Isomers in Bovine Rumen, Blood, and Milk

J. J. Loor,* A. Ferlay, A. Ollier, K. Ueda,† M. Doreau, and Y. Chilliard

Herbivore Research Unit INRA-Theix, 63122 St-Genès Champanelle, France

ABSTRACT

Three Holstein cows were fed a high-concentrate diet (65:35 concentrate to forage) supplemented with either 5% sunflower oil (SO), 5% linseed oil (LO), or 2.5% fish oil (FO) to examine effects on biohydrogenation and fatty acid profiles in rumen, blood plasma, and milk. Diets were fed in a 3 × 3 Latin square with 4-wk periods with grass hay as the forage. Milk yield, dry matter intake, and percentages of milk fat (2.64) and protein (3.22) did not differ. All diets resulted in incomplete hydrogenation of unsaturated fatty acids as indicated by the profiles of 18:1 isomers, conjugated 18:2 isomers, nonconjugated 18:2 isomers, and 18:0 in ruminal fluid. Percentages of 8:0-14:0 and 16:0 in milk fat were greater with FO. Percentage and yield of *trans*10,*cis*12-18:2 were small and greater in cows fed SO (0.14%, 0.57 g/d) than FO (0.03%, 0.15 g/d) or LO (0.04%, 0.12 g/d). Percentage and yield of *trans*10-18:1, however, increased with FO (6.16%) and SO (6.47%) compared with LO (1.65%). Dietary FO doubled percentage of *cis*11-18:1 in rumen, plasma, and milk fat. Despite a lack of difference in ruminal percentage of *trans*11-18:1 (10.5%), cows fed FO had greater plasma *trans*11-18:1 (116 vs. 61.5 µg/mL) but this response did not result in greater *trans*11-18:1 percentage in milk fat, which averaged 5.41% across diets. Percentage (2.2%) and yield (14.3 g/d) of *cis*9,*trans*11-18:2 in milk fat did not differ due to oils. Unique responses to feeding LO included greater than 2-fold increases in percentages of *trans*13+14-18:1, *trans*15-18:1, *trans*16-18:1, *cis*15-18:1, *cis*9,*trans*12-18:2 and *trans*11,*cis*15-18:2 in rumen, plasma, and milk, and *cis*9,*trans*13-18:2 in plasma and milk. Ruminal 18:0 percentage had the highest positive correlation with milk fat content ($r = 0.82$) across all diets. When compared with previous data with cows

fed high-concentrate diets without oil supplementation, results suggest that greater production of *trans*10-18:1, *cis*11-18:1, and *trans*11,*cis*15-18:2 coupled with low production of 18:0 in the rumen may be associated with low milk fat content when feeding high-concentrate diets and fish oil. In contrast, SO or LO could lead to low milk fat content by increasing ruminal *trans*10-18:1 (SO) or *trans*11,*cis*15-18:2 and *trans*9,*trans*12-18:2 (LO) along with a reduction in mammary synthesis of 8:0-16:0. Simultaneous increases in ruminal *trans*11-18:1 with fish oil, at a fraction of sunflower oil supplementation, may represent an effective strategy to maintain *cis*9,*trans*11-18:2 synthesis in mammary while reducing milk fat output and sparing energy.

(Key words: high-concentrate diet, polyunsaturated oil, *trans* fatty acid, milk conjugated linoleic acid)

Abbreviation key: CLA = conjugated linoleic acids, DHA = docosahexaenoic acid, EPA = eicosapentaenoic acid, FO = high-concentrate diet plus 2.5% fish oil, LO = high-concentrate diet plus 5% linseed oil, SO = high-concentrate diet plus 5% sunflower oil.

INTRODUCTION

Extensive literature over the past few decades suggests that milk fat depression is a multifactorial phenomenon (see review by Bauman and Griinari, 2003). Acetate and butyrate production (mol/d) are decreased by high dietary concentrate (Sutton et al., 2003), partly accounting for lower jugular plasma concentration and jugular-mammary venous difference for acetate and BHBA (Loor et al., 2005a). These changes may contribute to the effect of dietary concentrate level on milk fat yield. Others, however, have recently shown that despite an estimated 34% more acetate and 35% more butyrate produced in the rumen of cows fed high-concentrate diets there was a significant depression in milk fat yield (Oba and Allen, 2003). Recent studies clearly suggest that unique fatty acid intermediates produced by altered ruminal biohydrogenation are causally related to diet-induced milk fat depression (Bauman and Griinari, 2003).

Some of the greatest reductions in milk fat yield have been observed with high-concentrate diets supple-

Received May 4, 2005.

Accepted July 14, 2005.

Corresponding author: Yves Chilliard; e-mail: yves.chilliard@clermont.inra.fr.

*Current address: Department of Animal Sciences, University of Illinois, 206 ERML, Urbana, IL, 61801 (E-mail: jloor@uiuc.edu).

†Current address: Graduate School of Agriculture, Hokkaido University, Sapporo, 060-8589, Japan (E-mail: ueko@anim.agr.hokudai.ac.jp).

mented with vegetable or marine oils (Griinari et al., 1998; Piperova et al., 2000; Peterson et al., 2003; Loor et al., 2005a,b). Complete ruminal biohydrogenation of polyunsaturated fatty acids is reduced under these circumstances resulting in higher intestinal flows of *trans*-18:1 isomers, conjugated linoleic acids (**CLA**), and other nonconjugated 18:2 isomers (Piperova et al., 2002; Shingfield et al., 2003; Loor et al., 2004b). Vegetable oils rich in linoleic acid fed with a high-concentrate diet resulted in higher percentages of *trans*10-18:1 in milk fat (Griinari et al., 1998; Piperova et al., 2000; Peterson et al., 2003). *Cis*9,*trans*11-18:2, the primary CLA in milk fat, also increased along with small significant increases in *trans*7,*cis*9-18:2 (Piperova et al., 2000) and *trans*10,*cis*12-18:2 (Piperova et al., 2000; Peterson et al., 2003).

*Cis*9,*trans*11-CLA and vaccenic acid (its precursor in tissues) in meat and milk are examples of biohydrogenation intermediates that may have beneficial implications for human health (Corl et al., 2003). Alterations in biohydrogenation pathways due to diet are important because increased ruminal production of *trans*10-18:1 and *trans*10,*cis*12-18:2 have been associated with milk fat depression (Bauman and Griinari, 2003), and because a reduction in *trans*11-18:1 production in the rumen may decrease endogenous synthesis of *cis*9-*trans*11-18:2 in tissues. Furthermore, *trans*11-18:1 in milk fat was transiently increased for 1 wk after addition of polyunsaturated oils to high-concentrate or corn silage-based diets, after which it decreased along with a simultaneous increase in *trans*10-18:1 (Chilliard and Ferlay, 2004; Roy et al., 2005).

Previous studies compared effects of TMR containing mixtures of fish oil and different sources of unsaturated fatty acids (AbuGhazaleh et al., 2003), or concentrate:forage ratio and linseed oil (Loor et al., 2005a) fed to dairy cows on blood plasma metabolites, milk composition, and profiles of *trans*-18:1 and CLA isomers in blood plasma and milk fat. Furthermore, previous results suggest that the nature of supplemental polyunsaturated fatty acids added to high-concentrate diets likely alters the profile and amount of hydrogenation intermediates available for secretion in milk. Thus, the primary objective of this study was to evaluate the effectiveness of high-concentrate diets in combination with fish oil [20 g/100 g of total fatty acids 20:5n-3 (eicosapentaenoic acid, **EPA**) and 7 g/100 g of 22:6n-3 (docosahexaenoic acid, **DHA**)], linseed oil (51 g/100 g 18:3n-3), or sunflower oil (69 g/100 g 18:2n-6) in modifying ruminal, blood plasma, and milk fat *cis* and *trans* isomers of 18:1, nonconjugated 18:2, and conjugated 18:2 isomers. In addition, we studied the correlations between these isomers in ruminal fluid and milk fat content.

MATERIALS AND METHODS

Animals and Diets

Three lactating multiparous Holstein cows, 84 ± 21 DIM, and cannulated in the rumen and duodenum were used in a 3×3 Latin square design during three 4-wk periods to evaluate responses to feeding diets with a high (65:35) concentrate to forage (grass hay) ratio supplemented (on a DM basis) with 2.5% fish oil (**FO**), 5.0% linseed oil (**LO**), or 5.0% sunflower oil (**SO**) (Loor et al., 2005c). The level of fish oil was chosen for the following reasons: 1) to avoid possible detrimental effects on DMI, which are proportional to the amount fed (Doreau and Chilliard, 1997; Donovan et al., 2000); 2) to avoid excessive decreases in milk fat yield (Chilliard et al., 2001); and 3) to allow intake of EPA plus DHA at less than 25% the amount of linoleic plus linolenic acid in cows receiving SO or LO, which in a previous *in vitro* study was as effective in elevating *trans*11-18:1 as linoleic acid (AbuGhazaleh and Jenkins, 2004). To allow cows to adapt to oil-supplemented diets, all cows were fed a diet supplemented with an oil mixture comprising sunflower oil, linseed oil, plus fish oil (40 g/d + 40 g/d + 20 g/d, respectively) for 3 wk before the first experimental period. Cows were housed in a tie-stall barn during the experiment. The concentrate mixture with oils (homogeneously mixed) was prepared daily and, along with the forage, was offered in equal amounts at 0900, 1330, and 1700 h for *ad libitum* consumption. Hay and concentrate were given separately. The desired forage:concentrate ratio was maintained by daily adjustment of forage and concentrate amounts offered, depending on the refusals of the previous day.

The concentrate mixture contained (DM basis) on average 28.2% beet pulp, 18.9% ground wheat, 18.9% barley, 14.2% rapeseed meal, 13.7% soybean meal, 2.0% wet beet molasses, 3.1% buffers, 0.5% binding agent, and 0.5% mineral-vitamin mix. Cows were milked at 0600 and 1700 h.

Sampling, Measurements, and Analyses

Ruminal fluid (300 mL) was collected during wk 4 by suction from the ventral sac via the ruminal cannula at 0900, 1100, 1300, 1500, 1700, 2000, and 0000 h (Loor et al., 2004b). Milk production and DMI were recorded daily throughout the experiment. Milk was sampled at each milking during the last 5 d of wk 4. One 50-mL aliquot from each of these milkings containing potassium bichromate (Merck, Fontenay-Sus-Bois, France) was stored at 4°C until analyzed for fat, protein, and lactose by infrared analysis with a 3-channel spectrophotometer (AOAC, 1997; CILAL, Theix, France). Additional 3-mL aliquots from 2 consecutive milkings

on the last day of wk 4 were also collected and stored at -20°C before isolation of milk fat and fatty acid analysis. These samples were composited based on a.m. and p.m. milk production. Data on milk production and DMI were averaged over the last 5 d of wk 4 before statistical analysis.

For plasma total fatty acid analysis, blood samples (50 mL) were collected in EDTA-containing Vacutainer tubes (CML, Nemours, France) from the jugular vein at 0830 h on the last day of wk 4. For plasma metabolite analysis, an additional 10 mL of blood was collected from the jugular and abdominal mammary vein by venipuncture using EDTA-containing (0.47 mol/L) Vacutainers. Blood was centrifuged at $3000 \times g$ for 15 min for harvesting plasma. Plasma was stored at -20°C until analyzed for fatty acids and metabolites. Plasma concentrations of metabolites were determined as described by Ferlay and Chilliard (1999) with an ELAN autoanalyzer using the same kits and procedures as in Loor et al. (2005a). Insulin and leptin were determined by radioimmunoassay as described in Delavaud et al. (2002).

Plasma total lipids were extracted with chloroform/methanol (2:1, vol/vol; Loor et al., 2005a). Fatty acids in plasma lipids were methylated with 2 mL of 0.5 N NaOCH_3 at 50°C for 30 min, followed by 2 mL of 14% boron trifluoride in methanol at 50°C for 30 min (Loor et al., 2005a). Tricosanoate (Sigma, Saint-Quentin Fallavier, France) was used as the internal standard. Fatty acids in milk fat were directly methylated with 1 mL of 2 N methanolic NaOCH_3 at room temperature for 20 min, followed by 1 mL of 14% boron trifluoride in methanol at room temperature for 20 min (Loor et al., 2005a). Fatty acids in mixed ruminal fluid were methylated with the same protocol as for duodenal fatty acids (Loor et al., 2004b, 2005c). In all cases, fatty acid methyl esters were recovered in 1 mL of hexane. Samples were injected by autosampler into a Trace-GC 2000 Series gas chromatograph equipped with a flame-ionization detector (Thermo Finnigan, Les Ulis, France). Methyl esters from all samples were separated on a $100 \text{ m} \times 0.25 \text{ mm}$ i.d. fused silica capillary column (CP-Sil 88, Chrompack, Middelburg, The Netherlands). Identification of 18:1, 18:2, CLA, and 18:3 isomers and odd and branched-chain fatty acids was as described in Loor et al. (2004b). A butter reference standard (BCR 164; Commission of the European Communities, Community Bureau of Reference, Brussels, Belgium) was used to estimate correction factors for short-chain (4:0-10:0) fatty acids.

For fatty acid analysis (0.5 to 1 μL of methyl esters in hexane injected at a 50:1 split ratio), the injector temperature was maintained at 250°C and the detector temperature was maintained at 255°C . The oven tem-

perature was held at 70°C for 1 min, increased at $5^{\circ}\text{C}/\text{min}$ to 100°C (held for 2 min), then increased at $10^{\circ}\text{C}/\text{min}$ to 175°C (held for 40 min), and increased at $5^{\circ}\text{C}/\text{min}$ to a final temperature of 225°C (held for 15 min). Hydrogen was the carrier gas. Injector pressure was held constant at 158.6 kPa. For all samples, satisfactory separations of *cis*- and *trans*-18:1 and nonconjugated 18:2 isomers were obtained with a single chromatographic run (see Figure 1 in Loor et al., 2004b). Separation of CLA isomers in duodenal lipids was shown in Loor et al. (2005c). Diurnal profiles of *trans*10-18:1, *trans*11-18:1, *cis*9,*trans*11-18:2, *trans*10,*cis*12-18:2, *trans*11,*cis*15-18:2, and 18:0 in ruminal fluid were presented in Loor et al. (2004a). Intake and duodenal flows of fatty acids were presented in Loor et al. (2005c).

Statistical Analyses

Data for DMI, milk production and composition, fatty acids in plasma, ruminal fluid, and milk fat are reported as least squares means $\pm$ SEM. Data were analyzed as a Latin square using the MIXED procedure of SAS (SAS Inst., Inc., Cary, NC). The statistical model included: cow, period, oil type, and residual error. Fixed effects included period and oil type. Cow was the random effect. Compound symmetry was the covariate structure used. Overall differences between treatment means were considered significant when $P \leq 0.05$.

RESULTS

Production Variables and Blood Plasma Metabolites

Average DMI and milk, fat, protein, and lactose concentration and yields did not differ in response to oil type (Table 1). In cows fed LO, triglyceride concentration in jugular plasma and jugular-mammary venous difference were higher compared with other treatments (Table 2). In contrast, feeding SO resulted in higher jugular concentrations of glucose. The jugular-mammary venous difference for insulin was greater with FO compared with other treatments. Across diets there appeared to be net production of leptin by the mammary gland (i.e., significantly negative jugular-mammary venous difference), in agreement with data on leptin expression in this tissue (Bonnet et al., 2002).

Fatty Acid Composition of Ruminal Fluid

Among 18:1 isomers, percentage of *trans*10-18:1 was similar between FO and SO, but FO increased *cis*11-18:1 to the largest extent (Table 3). Among 18:2 isomers, both FO and LO resulted in greater *trans*11,*cis*15-18:2

Table 1. Dry matter intake and milk production and composition in Holstein cows fed a high-concentrate diet supplemented with fish oil (FO, 2.5% of DM), linseed oil (LO, 5%), or sunflower oil (SO, 5%).

	Supplement			SEM	P =
	FO	LO	SO		
DMI, kg/d	17.1	17.2	19.3	2.4	0.78
Milk, kg/d	27.2	24.4	26.5	6.9	0.70
Composition, %					
Fat	2.56	2.75	2.62	0.36	0.57
Protein	2.97	3.18	3.50	0.28	0.10
Lactose	4.77	4.71	4.68	0.10	0.72
Yield, kg/d					
Fat	0.69	0.71	0.72	0.24	0.98
Protein	0.79	0.75	0.86	0.14	0.39
Lactose	1.30	1.14	1.26	0.33	0.71

than did SO. There also were unique responses for individual oils. Feeding LO resulted in lower *trans*10-18:1 and in greater percentages of *trans*13+14-18:1, *trans*15-18:1, *trans*16-18:1, *cis*12-18:1, and *cis*15-18:1. Feeding SO, however, resulted in higher percentages of *cis*9,*cis*12-18:2, *cis*9,*trans*11-18:2, and *trans*10,*cis*12-18:2, and decreased *trans*11,*trans*13-18:2. Feeding LO increased percentages of *trans*9,*cis*12-18:2, *cis*9-*trans*13-18:2, *trans*9,*trans*12-18:2, *trans*11,*cis*15-18:2, and *trans*11,*cis*13 + *cis*9,*cis*11-18:2, and decreased *trans*,*trans*-18:2. Feeding LO also resulted in greater

percentages of *cis*9,*cis*12,*cis*15-18:3 and *trans*9-*trans*12,*trans*15-18:3. Dietary FO resulted in markedly lower 18:0, and increased percentages of *cis*11-18:1, *trans*8,*cis*13-18:2, *trans*9,*cis*11-18:2, *cis*10,*trans*12-18:2, *cis*11,*trans*13-18:2, 20:2, 20:3n-3, 20:3n-6, 20:4n-6, 20:5n-3, 21:0, 22:1n-9, 22:5n-3, and 22:6n-3.

Plasma Fatty Acid Concentrations

Despite differences in the amount of supplemental oil fed, concentration of total fatty acids in plasma did

Table 2. Plasma metabolites and hormones, and apparent mammary uptake in Holstein cows fed a high-concentrate diet supplemented with fish oil (FO, 2.5% of DM), linseed oil (LO, 5%), or sunflower oil (SO, 5%).

	Supplement			SEM	P =
	FO	LO	SO		
Jugular concentration, mmol/L					
Acetate	0.463	0.596	0.413	0.103	0.49
BHBA	0.434	0.475	0.398	0.058	0.66
Lactate	1.189	0.739	1.237	0.307	0.51
Glucose	3.55 ^b	3.62 ^{ab}	3.80 ^a	0.09	0.05
Total cholesterol	6.23	6.78	7.64	1.14	0.57
Phospholipid	3.14	3.17	3.48	0.46	0.78
Triglyceride	0.100 ^b	0.154 ^a	0.133 ^{ab}	0.01	0.05
Free glycerol	0.185	0.258	0.230	0.047	0.58
NEFA	0.129	0.235	0.177	0.044	0.11
Urea	4.59	4.29	4.28	0.43	0.84
Insulin, μ IU/mL	22.1	16.6	22.7	2.0	0.12
Leptin, ng/mL	6.22	6.15	6.28	1.78	0.99
Jugular-mammary venous difference					
Acetate	0.331	0.444	0.262	0.063	0.11
BHBA	0.121	0.174	0.108	0.038	0.50
Lactate	0.379	0.075	0.119	0.224	0.62
Glucose	0.760	0.906	0.859	0.229	0.56
Total cholesterol	-1.24	-0.23	-0.17	0.76	0.57
Phospholipid	-0.36	-0.12	-0.12	0.25	0.75
Triglyceride	0.062 ^b	0.093 ^a	0.066 ^b	0.010	0.05
Free glycerol	0.025	0.064	0.045	0.068	0.92
NEFA	-0.053	-0.179	-0.105	0.064	0.45
Urea	-0.045 ^b	0.080 ^a	-0.035 ^b	0.038	0.05
Insulin, μ IU/mL	5.85 ^a	-7.45 ^b	-4.62 ^b	3.96	0.05
Leptin, ng/mL	-0.55	-0.17	-0.31	0.28	0.52

^{a,b}Means with unlike superscripts differ significantly ($P < 0.05$).

Table 3. Percentage of selected fatty acids in ruminal fluid from Holstein cows fed a high-concentrate diet supplemented with fish oil (FO, 2.5% of DM), linseed oil (LO, 5%), or sunflower oil (SO, 5%).

Fatty acid	Supplement			SEM	P =
	FO	LO	SO		
	g/100 g of total fatty acids				
16:0	24.5 ^a	13.9 ^c	17.5 ^b	0.66	0.001
18:0	11.9 ^b	25.6 ^a	24.3 ^a	6.4	0.001
18:1 <i>trans</i>					
4	0.18	0.16	0.17	0.03	0.66
5	0.10	0.11	0.12	0.01	0.69
6 + 7 + 8	0.76	0.99	0.99	0.11	0.09
9	0.49	0.58	0.66	0.04	0.13
10	8.25 ^a	3.06 ^b	8.35 ^a	3.11	0.05
11	9.20	11.4	11.0	2.00	0.30
12	1.14	1.19	1.19	0.08	0.86
13 + 14	1.46 ^c	4.16 ^a	1.80 ^b	0.19	0.001
15	0.68 ^b	1.85 ^a	0.78 ^b	0.08	0.001
16	0.37 ^b	1.15 ^a	0.52 ^b	0.08	0.001
18:1 <i>cis</i>					
9	5.20	5.49	5.63	0.87	0.74
11	2.19 ^a	0.97 ^b	1.13 ^b	0.29	0.001
12	0.32 ^c	1.04 ^a	0.87 ^b	0.10	0.01
13	0.15	0.20	0.11	0.01	0.07
15	0.41 ^b	1.58 ^a	0.19 ^b	0.20	0.001
Nonconjugated 18:2					
<i>cis</i> 9, <i>cis</i> 12	3.65 ^b	3.36 ^b	9.48 ^a	1.69	0.05
<i>cis</i> 9, <i>trans</i> 12	0.14 ^b	0.22 ^a	0.13 ^b	0.01	0.001
<i>trans</i> 8, <i>cis</i> 13	0.57 ^a	0.22 ^b	0.32 ^b	0.10	0.04
<i>trans</i> 9, <i>cis</i> 12	0.19 ^b	0.35 ^a	0.16 ^b	0.07	0.01
<i>cis</i> 9, <i>trans</i> 13	0.20 ^b	0.28 ^a	0.12 ^c	0.03	0.001
<i>trans</i> 9, <i>trans</i> 12	0.22 ^b	0.39 ^a	0.10 ^b	0.13	0.02
<i>trans</i> 11, <i>cis</i> 15	2.93 ^b	8.56 ^a	0.95 ^c	1.29	0.001
Conjugated 18:2					
<i>cis</i> 9, <i>trans</i> 11	0.73 ^b	0.70 ^b	1.46 ^a	0.13	0.04
<i>trans</i> 9, <i>cis</i> 11	0.07 ^a	0.01 ^b	0.01 ^b	0.02	0.004
<i>trans</i> 8, <i>cis</i> 10	0.08	0.05	0.05	0.01	0.16
<i>trans</i> 10, <i>cis</i> 12	0.07 ^b	0.06 ^b	0.23 ^a	0.04	0.01
<i>cis</i> 10, <i>trans</i> 12	0.04 ^a	0.002 ^b	0.02 ^b	0.01	0.05
<i>cis</i> 11, <i>trans</i> 13	0.05 ^a	0.02 ^b	0.01 ^b	0.01	0.05
<i>trans</i> 11, <i>cis</i> 13 + <i>cis</i> 9, <i>cis</i> 11	0.08 ^b	0.15 ^a	0.09 ^b	0.01	0.05
<i>trans</i> 11, <i>trans</i> 13	0.18 ^a	0.23 ^a	0.09 ^b	0.03	0.02
<i>trans,trans</i>	0.33 ^a	0.18 ^b	0.30 ^a	0.03	0.05
18:3					
<i>cis</i> 9, <i>cis</i> 12, <i>cis</i> 15	1.20 ^b	2.46 ^a	0.88 ^c	0.25	0.04
<i>trans</i> 9, <i>trans</i> 12, <i>trans</i> 15	0.24 ^b	0.56 ^a	0.09 ^c	0.08	0.001
20:2	0.13 ^a	0.07 ^b	0.07 ^b	0.01	0.04
20:3n-3	0.31 ^a	0.02 ^b	0.05 ^b	0.05	0.01
20:3n-6	0.08 ^a	0.004 ^b	0.02 ^b	0.02	0.02
20:4n-6	0.06 ^a	0.001 ^b	0.01 ^b	0.01	0.03
20:5n-3	1.77 ^a	0.01 ^b	0.10 ^b	0.20	0.001
21:0	0.32 ^a	0.05 ^b	0.08 ^b	0.06	0.01
22:0	0.54 ^b	0.30 ^c	0.59 ^a	0.02	0.001
22:1n-9	0.15 ^a	0.05 ^b	0.06 ^b	0.01	0.001
22:5n-3	0.54 ^a	0.01 ^b	0.07 ^b	0.09	0.001
22:6n-3	0.86 ^a	0.04 ^b	0.12 ^b	0.09	0.001
Total, ¹ mg/g of DM	29.9	38.6	34.9	3.28	0.10

^{a,b,c}Means with unlike superscripts differ significantly ($P < 0.05$).

¹Sum of fatty acids with 12- to 24-carbons including 18:1, 18:2, and 18:3 isomers.

not differ due to treatments (Table 4). Among oils, feeding FO resulted in greater concentrations of 14:0, 16:0, most odd- and branched-chain fatty acids, and nearly all 20-carbon, 22-carbon, and 24-carbon fatty acids. In contrast, FO decreased concentration of 18:0 dramatically compared with LO and SO.

Plasma 18:1 Isomer Concentrations

Total *trans*-18:1 in plasma was greater with FO than other treatments (Table 5). Among individual isomers, the higher concentration of *trans*11-18:1 primarily, but also *trans*10-18:1, accounted for the greater response

Table 4. Concentration of 12- to 22-carbon fatty acids (not including 18:1, 18:2, or 18:3 isomers) in jugular blood plasma from Holstein cows fed a high-concentrate diet supplemented with fish oil (FO, 2.5% of DM), linseed oil (LO, 5%), or sunflower oil (SO, 5%).

Fatty acid	Supplement			SEM	P =
	FO	LO	SO		
	$\mu\text{g/mL}$				
12:0	1.95	1.30	1.24	0.32	0.31
14:0	18.6 ^a	12.0 ^b	12.5 ^b	1.5	0.05
<i>iso</i> -14:0	1.06	1.18	1.31	0.08	0.24
<i>cis</i> 9-14:1	1.22	1.04	1.23	0.17	0.69
15:0	24.1 ^a	12.8 ^b	13.6 ^b	3.1	0.04
<i>cis</i> 10-15:1	3.18	1.88	2.59	0.97	0.67
<i>iso</i> -15:0	10.5 ^a	6.60 ^b	6.43 ^b	1.37	0.05
<i>anteiso</i> -15:0	12.7	13.7	11.5	2.3	0.62
16:0	332.4 ^a	197.8 ^b	221.8 ^b	48.0	0.02
<i>iso</i> -16:0	10.1 ^a	5.66 ^b	6.78 ^b	1.38	0.05
<i>trans</i> 9-16:1 + <i>iso</i> -17:0	18.5 ^a	8.45 ^b	12.2 ^b	3.86	0.02
<i>cis</i> 9-16:1	67.4 ^a	25.7 ^b	19.7 ^b	8.7	0.03
17:0	25.4 ^a	14.3 ^b	17.7 ^b	3.7	0.001
<i>cis</i> 10-17:1	4.40	5.00	3.66	0.78	0.10
18:0	228.3 ^b	354.5 ^a	384.9 ^a	32.1	0.05
19:0	5.13	3.48	4.23	1.14	0.63
20:0	4.89 ^a	3.72 ^b	2.99 ^b	0.54	0.05
<i>cis</i> 11-20:1	4.44 ^a	2.98 ^b	3.06 ^b	0.33	0.05
20:2	7.32 ^a	4.29 ^b	4.71 ^b	0.58	0.05
20:3n-3	5.71 ^a	0.82 ^b	0.98 ^b	1.20	0.05
20:3n-6	24.4	22.2	23.1	4.9	0.95
20:4n-6	57.6 ^a	17.4 ^b	26.4 ^b	8.5	0.05
20:5n-3	352.1 ^a	42.2 ^b	77.2 ^b	88.0	0.05
22:0	4.26 ^a	2.51 ^b	2.27 ^b	0.49	0.05
22:1n-9	32.2	13.6	9.02	20.3	0.72
22:2	1.38	0.64	1.03	0.41	0.50
22:4n-6	1.24	1.92	1.46	0.30	0.37
22:5n-3	38.0 ^a	24.4 ^b	32.0 ^a	6.3	0.05
22:6n-3	30.8 ^a	8.37 ^b	15.3 ^b	5.54	0.002
24:0	6.92 ^a	2.10 ^b	2.62 ^b	1.30	0.05
24:1n-9	0.68	0.60	0.48	0.17	0.71
Total ¹	2823	2630	2914	415	0.49

^{a,b}Means with unlike superscripts differ significantly ($P < 0.05$).

¹Total = sum of 12:0 to 24:1n-9 including 18:1, 18:2, and 18:3 isomers.

with FO. Feeding SO also increased *trans*10-18:1 concentration compared with LO. In contrast, LO supplementation resulted in greater concentrations of *trans*13+14-18:1, *trans*15-18:1, and *trans*16-18:1 compared with FO or LO. Contrary to results for *trans*-18:1, LO increased total *cis*-18:1 to the largest extent, and this response was due primarily to greater *cis*9-18:1 and *cis*15-18:1. FO resulted in greater *cis*11-18:1 compared with LO or SO.

Plasma 18:2 and 18:3 Isomer Concentrations

Total concentration of 18:3 isomers was greater with LO, which resulted from higher *cis*9,*cis*12,*cis*15-18:3 and *trans*9,*trans*12,*trans*15-18:3 (Table 6). Interestingly, concentration of total conjugated 18:2 isomers was greater when FO was fed compared with SO and intermediate with LO. Among isomers that increased with FO were *trans*9,*cis*11-18:2, *cis*10,*trans*12-18:2,

and *trans,trans*-18:2, and nonconjugated *trans*11,*cis*15-18:2. Circulating concentration of *cis*9,*trans*11-18:2 was higher when LO was fed compared with FO, and intermediate with SO. Feeding LO also increased concentration of *trans*11,*cis*13- + *cis*9,*cis*11-18:2 compared with other treatments. Feeding SO resulted in greater concentrations of *trans*10,*cis*12-18:2 and lower *trans*8,*cis*10 compared with FO and LO. Concentration of total nonconjugated 18:2 isomers was greater with SO than other treatments, primarily due to a substantial increase in *cis*9,*cis*12-18:2. Among other isomers, *cis*9,*trans*12-18:2, *cis*9,*trans*13-18:2, and *trans*11,*cis*15-18:2 were greater in response to LO compared with other treatments.

Percentage and Yield of Selected Fatty Acids in Milk

Cows fed FO had higher percentages of 8:0, 10:0, *cis*9-10:1, 12:0, 13:0, 14:0, 15:0, 16:0, most odd- and

Table 5. Concentration of 18:1 isomers in blood plasma from Holstein cows fed a high-concentrate diet supplemented with fish oil (FO, 2.5% of DM), linseed oil (LO, 5%), or sunflower oil (SO, 5%).

Isomer	Supplement			SEM	P =
	FO	LO	SO		
	$\mu\text{g/mL}$				
18:1 <i>trans</i>					
4	3.55	3.69	3.72	0.51	0.59
5	2.51	3.02	2.90	0.74	0.88
6 + 7 + 8	5.98	5.84	6.13	1.27	0.98
9	7.07	5.42	6.14	1.10	0.49
10	41.1 ^a	11.3 ^b	31.1 ^a	10.7	0.05
11	115.6 ^a	64.5 ^b	58.4 ^b	15.8	0.05
12	14.3	16.1	16.4	3.0	0.49
13 + 14	16.8 ^b	41.1 ^a	18.0 ^b	7.3	0.05
15	5.00 ^b	14.4 ^a	5.53 ^b	2.29	0.05
16	3.15 ^b	11.7 ^a	5.22 ^b	1.31	0.04
Total	210.1 ^a	162.6 ^b	148.2 ^b	23.1	0.04
18:1 <i>cis</i>					
9	90.2 ^b	152.6 ^a	110.8 ^b	14.8	0.05
11	30.3 ^a	14.3 ^b	14.8 ^b	3.6	0.01
12	16.6	35.2	25.5	7.9	0.19
13	2.85	4.56	3.19	0.72	0.19
15	4.15 ^b	14.0 ^a	3.06 ^b	3.22	0.04
Total	149.0 ^b	235.0 ^a	162.9 ^b	20.2	0.05

^{a,b}Means with unlike superscripts differ significantly ($P < 0.05$).

branched-chain fatty acids, all 20-carbon fatty acids, and most 22-carbon fatty acids (Table 7). However, similar to ruminal fluid and plasma, the percentage and yield of 18:0 when FO was fed were much lower com-

pared with LO and SO. Despite greater percentages in milk in cows fed FO, yields of saturated fatty acids with 8- to 14-carbons did not differ across treatments. Yields of total 20-carbon and 20:5n-3, 22:5n-3, and 22:6n-3

Table 6. Concentration of 18:2 and 18:3 isomers in blood plasma from Holstein cows fed a high-concentrate diet supplemented with fish oil (FO, 2.5% of DM), linseed oil (LO, 5%), or sunflower oil (SO, 5%).

Isomer	Supplement			SEM	P =
	FO	LO	SO		
	$\mu\text{g/mL}$				
18:3					
<i>cis</i> 9, <i>cis</i> 12, <i>cis</i> 15	150.0 ^b	353.1 ^a	140.7 ^b	41.4	0.01
<i>trans</i> 9, <i>trans</i> 12, <i>trans</i> 15	5.10 ^b	16.0 ^a	3.54 ^b	2.23	0.05
Total	154.9 ^b	369.1 ^a	144.3 ^b	44.3	0.01
18:2 conjugated					
<i>cis</i> 9, <i>trans</i> 11	6.90 ^b	10.4 ^a	8.36 ^{ab}	0.76	0.05
<i>trans</i> 9, <i>cis</i> 11	3.23 ^a	0.00 ^b	0.54 ^b	0.66	0.05
<i>trans</i> 8, <i>cis</i> 10	1.28 ^a	1.40 ^a	0.82 ^b	0.13	0.05
<i>trans</i> 10, <i>cis</i> 12	2.42 ^b	1.87 ^b	3.26 ^a	0.30	0.05
<i>cis</i> 10, <i>trans</i> 12	5.57 ^a	0.39 ^c	1.10 ^b	0.30	0.01
<i>cis</i> 11, <i>trans</i> 13	0.00	0.50	0.56	0.33	0.43
<i>trans</i> 11, <i>cis</i> 13 + <i>cis</i> 9, <i>cis</i> 11	0.00 ^b	0.86 ^a	0.00 ^b	0.29	0.05
<i>trans</i> 11, <i>trans</i> 13	2.51	4.61	2.42	1.12	0.38
<i>trans</i> , <i>trans</i>	12.4 ^a	7.31 ^b	5.26 ^b	0.84	0.01
Total	34.3 ^a	27.3 ^{ab}	22.3 ^b	2.43	0.05
18:2 nonconjugated					
<i>cis</i> 9, <i>cis</i> 12	900.4 ^b	969.1 ^b	1483 ^a	148	0.02
<i>cis</i> 9, <i>trans</i> 12	2.38 ^b	4.91 ^a	2.39 ^b	0.59	0.05
<i>trans</i> 8, <i>cis</i> 13	7.46	9.33	8.47	1.32	0.64
<i>trans</i> 9, <i>cis</i> 12	6.81	4.60	5.70	1.00	0.11
<i>cis</i> 9, <i>trans</i> 13	4.06 ^b	7.80 ^a	4.65 ^b	1.00	0.05
<i>trans</i> 9, <i>trans</i> 12	1.71	2.60	1.72	0.62	0.47
<i>trans</i> 11, <i>cis</i> 15	13.5 ^b	24.1 ^a	4.69 ^c	2.77	0.02
Total	936.4 ^b	1022 ^b	1511 ^a	154.9	0.03

^{a,b,c}Means with unlike superscripts differ significantly ($P < 0.05$).

Table 7. Percentage of 4- to 24-carbon (not including 18:1, 18:2, or 18:3 isomers) fatty acids and yield of selected fatty acids in milk from Holstein cows fed a high-concentrate diet supplemented with fish oil (FO, 2.5% of DM), linseed oil (LO, 5%), or sunflower oil (SO, 5%).

Fatty acid	Supplement			SEM	P =
	FO	LO	SO		
	g/100 g of total fatty acids				
4:0	2.99	2.27	2.21	0.48	0.51
6:0	1.66	1.17	1.14	0.34	0.36
8:0	1.10 ^a	0.69 ^b	0.64 ^b	0.10	0.05
10:0	2.81 ^a	1.61 ^b	1.54 ^b	0.39	0.05
<i>cis</i> 9-10:1	0.22 ^a	0.15 ^b	0.13 ^b	0.03	0.05
11:0	0.05	0.02	0.02	0.01	0.10
12:0	3.43 ^a	1.98 ^b	2.03 ^b	0.36	0.05
13:0	0.18 ^a	0.12 ^b	0.12 ^b	0.02	0.05
14:0	12.5 ^a	8.46 ^b	8.99 ^b	1.02	0.05
8:0-14:0	19.8 ^a	12.7 ^b	13.2 ^b	1.78	0.05
<i>iso</i> -14:0	0.09	0.07	0.08	0.01	0.22
<i>cis</i> 9-14:1	0.99	0.76	0.86	0.14	0.59
15:0	1.29 ^a	0.84 ^b	0.87 ^b	0.07	0.01
<i>cis</i> 10-15:1	0.02	0.02	0.02	0.01	0.96
<i>iso</i> -15:0	0.32 ^a	0.16 ^b	0.19 ^b	0.04	0.05
<i>anteiso</i> -15:0	0.76 ^a	0.46 ^b	0.51 ^b	0.06	0.04
16:0	27.5 ^a	20.7 ^b	21.2 ^b	2.0	0.05
<i>iso</i> -16:0	0.33 ^a	0.20 ^b	0.26 ^b	0.03	0.05
<i>trans</i> 9-16:1 + <i>iso</i> -17:0	0.83 ^a	0.43 ^b	0.67 ^b	0.07	0.04
<i>cis</i> 9-16:1	2.00 ^a	1.18 ^b	1.41 ^b	0.23	0.05
17:0	0.68 ^a	0.50 ^b	0.49 ^b	0.04	0.02
<i>cis</i> 10-17:1	0.25	0.20	0.19	0.03	0.43
18:0	3.49 ^b	10.1 ^a	11.2 ^a	1.50	0.03
19:0	0.15	0.16	0.16	0.03	0.98
20:0	0.21	0.15	0.19	0.03	0.47
<i>cis</i> 11-20:1	0.54 ^a	0.14 ^b	0.18 ^b	0.04	0.01
20:2	0.10	0.06	0.08	0.02	0.45
20:3n-3	0.45 ^a	0.02 ^b	0.02 ^b	0.10	0.05
20:3n-6	0.11 ^a	0.04 ^b	0.03 ^b	0.01	0.05
20:4n-6	0.14 ^a	0.07 ^b	0.05 ^b	0.01	0.05
20:5n-3	0.48 ^a	0.03 ^b	0.07 ^b	0.08	0.03
21:0	0.14 ^a	0.05 ^b	0.06 ^b	0.02	0.05
22:0	0.10 ^a	0.05 ^c	0.08 ^b	0.001	0.02
22:1n-9	0.05 ^a	0.01 ^b	0.02 ^b	0.007	0.01
22:2	0.01	0.01	0.01	0.004	0.45
22:5n-3	0.39 ^a	0.12 ^b	0.15 ^b	0.07	0.05
22:6n-3	0.19 ^a	0.04 ^b	0.06 ^b	0.03	0.05
24:0	0.04	0.06	0.05	0.01	0.54
24:1n-9	0.02	0.01	0.02	0.003	0.22
Yield, g/d					
8:0-14:0	126.0	88.5	99.1	37.6	0.21
Total odd + branched-chain	30.2 ^a	19.6 ^b	21.6 ^b	5.9	0.01
16:0	173.1	130.2	136.5	49.1	0.36
18:0	22.3 ^b	70.0 ^a	78.0 ^a	19.5	0.05
Total 20-carbon	17.2 ^a	4.36 ^b	4.98 ^b	2.61	0.01
20:5n-3	2.81 ^a	0.25 ^b	0.27 ^b	0.43	0.01
22:5n-3	2.14 ^a	0.63 ^b	0.72 ^b	0.27	0.02
22:6n-3	0.95 ^a	0.19 ^b	0.29 ^b	0.15	0.05

^{a,b,c}Means with unlike superscripts differ significantly ($P < 0.05$).

were greater in response to FO compared with other treatments, in agreement with their higher percentage in milk fat.

Percentage and Yield of 18:1 Isomers

Percentage and yield of total *trans*-18:1 isomers did not differ between treatments (Table 8). However, feed-

ing LO resulted in higher percentage of *trans*13+14-18:1, *trans*15-18:1, and *trans*16-18:1 compared with other treatments. Yield of *trans*13+14 was more than 10 g/d higher in response to LO compared with FO and SO. Both FO and SO increased percentage and yield of *trans*10-18:1 compared with LO. Feeding SO also resulted in higher percentage of *trans*6+7+8-18:1 and *trans*9-18:1. Contrary to total *trans*-18:1, percentage

Table 8. Percentage of all 18:1 isomers and yield of selected isomers in milk fat from Holstein cows fed a high-concentrate diet supplemented with fish oil (FO, 2.5% of DM), linseed oil (LO, 5%), or sunflower oil (SO, 5%).

Isomer	Supplement			SEM	P =
	FO	LO	SO		
	g/100 g of total fatty acids				
18:1 <i>trans</i>					
4	0.05	0.06	0.10	0.02	0.53
5	0.05	0.07	0.11	0.03	0.25
6 + 7 + 8	0.51 ^b	0.73 ^{ab}	0.96 ^a	0.15	0.05
9	0.49 ^b	0.56 ^{ab}	0.69 ^a	0.06	0.05
10	6.16 ^a	1.65 ^b	6.47 ^a	1.35	0.05
11	6.58	5.43	4.22	0.80	0.20
12	0.77	0.96	0.98	0.13	0.50
13 + 14	0.88 ^b	3.00 ^a	1.27 ^b	0.41	0.05
15	0.33 ^b	1.27 ^a	0.46 ^b	0.35	0.05
16	0.16 ^c	0.97 ^a	0.43 ^b	0.12	0.03
Total	15.7	13.4	15.2	2.6	0.77
18:1 <i>cis</i>					
9	7.03 ^b	17.7 ^a	17.9 ^a	1.29	0.01
11	1.64 ^a	0.75 ^b	0.82 ^b	0.20	0.01
12	0.26 ^b	0.74 ^a	0.87 ^a	0.15	0.05
13	0.12 ^b	0.23 ^a	0.14 ^b	0.02	0.05
15	0.29 ^b	1.43 ^a	0.17 ^b	0.20	0.04
Total	9.68 ^b	22.1 ^a	20.4 ^a	1.96	0.01
Yield, g/d					
Total <i>trans</i> -18:1	89.9	77.9	80.6	21.1	0.85
<i>trans</i> 10-18:1	31.9 ^a	6.96 ^b	26.4 ^a	7.1	0.05
<i>trans</i> 11-18:1	40.3	39.4	31.4	14.4	0.54
<i>trans</i> 13+14-18:1	5.78 ^b	17.1 ^a	7.41 ^b	2.9	0.05
Total <i>cis</i> -18:1	59.0 ^b	132.2 ^a	130.2 ^a	31.5	0.05
<i>cis</i> 9-18:1	42.6 ^b	108.7 ^a	116.6 ^a	28.1	0.05
<i>cis</i> 11-18:1	9.67 ^a	4.09 ^b	4.32 ^b	0.95	0.01
<i>cis</i> 15-18:1	1.96 ^b	7.68 ^a	0.94 ^b	1.35	0.03

^{a,b,c}Means with unlike superscripts differ significantly ($P < 0.05$).

and yield of total *cis*-18:1 isomers was greater when LO and SO were fed compared with FO. This response was due primarily to a 2.5-fold decrease in the percentage and yield of *cis*9-18:1. In contrast, FO more than doubled the percentage and yield of *cis*11-18:1 and decreased *cis*12-18:1 compared with other treatments. Feeding LO resulted in a substantial increase in percentage and yield of *cis*15-18:1 compared with FO and SO.

Percentage and Yield of 18:2 and 18:3 Isomers

Similar to rumen fluid and plasma, dietary LO increased percentage and yield of total 18:3 isomers to the largest extent, primarily due to greater percentage and yield of *cis*9,*cis*12,*cis*15-18:3 (Table 9). Percentage and yield of total conjugated 18:2 isomers did not differ among treatments. Among conjugated 18:2 isomers, however, percentage of *trans*11,*cis*13 + *cis*9,*cis*11-18:2 and *trans*11,*trans*13-18:2 were greater in response to LO compared with other treatments. Dietary SO increased percentage and yield of *trans*10,*cis*12-18:2. Percentage and yield of *cis*9,*trans*11-18:2 did not differ due

to treatment. Percentage and yield of nonconjugated 18:2 isomers increased when cows were fed LO compared with FO and SO. This response was primarily associated with greater percentage and yield of *trans*11,*cis*15-18:2, but also greater percentage of *cis*9,*trans*12-18:2 and *cis*9,*trans*13-18:2. Feeding FO also resulted in greater percentage and yield of *trans*11,*cis*15-18:2 compared with SO.

DISCUSSION

Milk Fat Content, Saturated Fatty Acids, and Oleic Acid

High dietary concentrate:forage ratio and unsaturated vegetable oils (e.g., corn, soybean, linseed) reduce milk fat content and yield substantially compared with typical diets (Griinari et al., 1998; Piperova et al., 2000; Peterson et al., 2003; Loor et al., 2005a). In the present study, we did not observe a significant oil effect on milk fat content or yield despite differences, according to oil used, in percentages and yields of fatty acids synthesized de novo: (8:0-14:0), 16:0 (increased with FO), or

Table 9. Percentage of all 18:2 and 18:3 isomers and yield of selected isomers in milk fat from Holstein cows fed a high-concentrate diet supplemented with fish oil (FO, 2.5% of DM), linseed oil (LO, 5%), or sunflower oil (SO, 5%).

Isomer	Supplement			SEM	P =
	FO	LO	SO		
	g/100 g of total fatty acids				
18:3					
<i>cis</i> 9, <i>cis</i> 12, <i>cis</i> 15	0.60 ^b	1.18 ^a	0.66 ^b	0.08	0.01
<i>trans</i> 9, <i>trans</i> 12, <i>trans</i> 15	0.27 ^a	0.27 ^a	0.17 ^b	0.02	0.05
Total	0.94 ^b	1.50 ^a	0.89 ^b	0.13	0.05
18:2 conjugated					
<i>cis</i> 9, <i>trans</i> 11	2.29	2.36	1.83	0.25	0.26
<i>trans</i> 9, <i>cis</i> 11	0.09	0.06	0.12	0.03	0.40
<i>trans</i> 10, <i>cis</i> 12	0.03 ^b	0.04 ^b	0.14 ^a	0.03	0.06
<i>cis</i> 10, <i>trans</i> 12	0.05	0.02	0.03	0.01	0.49
<i>cis</i> 11, <i>trans</i> 13	0.04	0.02	0.03	0.02	0.91
<i>trans</i> 11, <i>cis</i> 13 + <i>cis</i> 9, <i>cis</i> 11	0.05 ^b	0.10 ^a	0.06 ^b	0.02	0.05
<i>trans</i> 11, <i>trans</i> 13	0.08 ^b	0.15 ^a	0.08 ^b	0.03	0.05
<i>trans</i> , <i>trans</i>	0.11	0.09	0.13	0.04	0.90
Total	2.73	2.84	2.40	0.21	0.40
18:2 nonconjugated					
<i>cis</i> 9, <i>cis</i> 12	1.64 ^b	2.10 ^b	3.57 ^a	0.24	0.01
<i>cis</i> 9, <i>trans</i> 12	0.07 ^c	0.24 ^a	0.16 ^b	0.03	0.05
<i>trans</i> 8, <i>cis</i> 13	0.15	0.16	0.16	0.03	0.99
<i>trans</i> 9, <i>cis</i> 12	0.16	0.22	0.10	0.04	0.19
<i>cis</i> 9, <i>trans</i> 13	0.32 ^b	0.99 ^a	0.41 ^b	0.13	0.05
<i>trans</i> 9, <i>trans</i> 12	0.15	0.16	0.07	0.05	0.21
<i>trans</i> 11, <i>cis</i> 15	1.75 ^b	2.87 ^a	0.41 ^c	0.17	0.01
Total	4.33 ^b	6.82 ^a	4.98 ^b	0.60	0.05
Yield, g/d					
Total nonconjugated 18:2	26.0 ^b	40.0 ^a	28.3 ^b	6.6	0.05
<i>trans</i> 11, <i>cis</i> 15-18:2	10.2 ^b	17.6 ^a	1.82 ^c	2.77	0.05
Total conjugated 18:2	17.0	17.9	15.6	5.8	0.74
<i>cis</i> 9, <i>trans</i> 11-18:2	14.2	15.6	13.0	5.7	0.83
<i>trans</i> 10, <i>cis</i> 12-18:2	0.15 ^b	0.12 ^b	0.57 ^a	0.10	0.05
Total 18:3	6.06 ^{ab}	8.34 ^a	5.03 ^b	2.05	0.05
18:3n-3	3.74 ^b	7.04 ^a	3.77 ^b	1.29	0.05

^{a,b,c}Means with unlike superscripts differ significantly ($P < 0.05$).

taken up from the blood: 18:0, and *cis*9-18:1 (decreased with FO). However, the low level of milk fat content observed with LO in this study is in agreement with previous results (Loor et al., 2005a).

The marked reduction in milk stearic acid yield with fish oil was associated with an apparent reduction in biohydrogenation of *trans*-18:1 to 18:0 as shown by lower ruminal 18:0 percentage (Table 3) and duodenal flow (Loor et al., 2005c). Indeed, ruminal concentrations of *trans*-18:1 (Table 3), although numerically lower, did not differ significantly between FO (22.6%), LO (24.7%), or SO (25.6%), despite more than 2-fold lower intake of 18:2n-6 and 18:3n-3 in cows fed FO (Loor et al., 2005b). Intake of 18:0 across diets was low, and with FO was ~18 g/d lower (19 vs. 37 g/d; Loor et al., 2005b). Thus, it is likely that dietary 18:0 intakes had a modest contribution to its ruminal concentration. Reduced ruminal outflow of 18:0 derived from biohydrogenation of unsaturated fatty acids likely reduced availability of 18:0 (low plasma concentration; Table 4) for desaturation, resulting in lower milk yield of *cis*9-18:1. Based on ex-

tensive literature and current results, availability of 18:0 for endogenous *cis*9-18:1 production seems to be crucial for milk fat synthesis (see also Donovan et al., 2000; Chilliard et al., 2003; Loor et al., 2005b). Furthermore, the reduction in 18:0 availability seems to be the strongest link among nearly all previous studies dealing with milk fat depression induced by fish oil supplementation (see review by Chilliard et al., 2001; Donovan et al., 2000; Shingfield et al., 2003; Loor et al., 2005b).

Interestingly, we observed a significant negative correlation ($r = -0.73$, $P = 0.02$) between ruminal fluid *cis*9-18:1 and milk fat content (Figure 1C). This negative relationship could be due in part to the fact that oleic acid in the rumen can be isomerized to *trans*10-18:1 (Mosley et al., 2002) [which tended ($P = 0.12$) to be negatively correlated ($r = -0.55$) with milk fat content], and is supported by a positive correlation ($r = 0.60$, $P = 0.08$) between both fatty acids in the rumen (Figure 1F). Ruminal oleic acid also was positively correlated ($r = 0.63$, $P = 0.07$) with ruminal *trans*9,*trans*12-18:2,

Figure 1. Correlation analysis among ruminal fluid concentration of selected fatty acids and milk fat content or among ruminal concentration of *cis*9-18:1 and *trans*10-18:1. Correlation values were -0.55 ($P = 0.12$), -0.61 ($P < 0.05$), -0.73 ($P < 0.01$), -0.70 ($P < 0.01$), $+0.82$ ($P < 0.01$), and $+0.60$ ($P < 0.05$) for panels A through F.

which, based on our previous analysis (Loor et al., 2005a) and current data (Figure 1D), is negatively associated with milk fat content. Intake of oleic acid in this study was 124 g/d lower in cows fed FO compared with SO and LO (Loor et al., 2005c), but there were no differences in ruminal fluid *cis*9-18:1 percentage across diets (Table 2), which raises the possibility that, in cows fed FO, other 18:1 and/or 18:2 intermediates gave rise to oleic acid. In this context, the negative correlation ($r = 0.71$, $P < 0.05$) between ruminal oleic acid and *trans*11-18:1 suggests the latter could be isomerized to *cis*9-18:1 (Mosley et al., 2002). Despite the negative correlation between ruminal oleic acid and milk fat content, data

do not lend support to this fatty acid as being involved in the low-fat milk syndrome because 1) there was no correlation ($r = -0.08$) between milk fat *cis*9-18:1 and milk fat content and 2) ruminal *cis*9-18:1 was not significantly correlated with its percentage in milk fat ($r = 0.20$).

Data from this study suggest that feeding high-concentrate diets with linseed oil and sunflower oil may result in a more pronounced decrease in de novo fatty acid synthesis than fish oil, which would partly account for the observation that the 3 oils resulted in milk with low fat content. Thus, a decrease in synthesis of 8:0-16:0 (resulting in their lower percentage in milk fat

with both LO and SO, Table 7) could be a more important cause of milk fat depression with either 18:3- (Loor et al., 2005a) or 18:2-rich oil supplementation, than with FO supplementation.

***Trans*10-18:1, *Cis*11-18:1, Nonconjugated Isomers, and Milk Fat Depression**

We previously compiled literature data confirming (Griinari et al., 1998; Bauman and Griinari, 2003) that increased percentage of *trans*10-18:1 in milk fat is positively correlated with milk fat depression in cows fed high-concentrate diets with or without unsaturated oils, or mixed diets with various levels of fish oil (Loor et al., 2005a). In the present study, we found that FO and SO compared with LO, resulted in 2.7-fold increase in *trans*10-18:1 in rumen, a 3-fold increase in plasma, and a 4-fold increase in milk. Despite the similar low level of milk fat content and yield among the 3 diets, ruminal *trans*10-18:1 tended ($P = 0.12$) to be negatively correlated with milk fat content. Our data indicate that low milk fat content when feeding FO is associated with appreciable increases in *cis*11-18:1 in ruminal fluid (2.1-fold), plasma (2.1-fold), and milk fat (2- to 3-fold). We had previously observed a 2-fold increase in *cis*11-18:1 percentage in milk fat along with a 28% reduction in milk fat yield in cows receiving a 300-mL infusion of fish oil into the rumen compared with controls (Loor et al., 2005b). Data from Offer et al. (1999), Donovan et al. (2000), Whitlock et al. (2002), and Shingfield et al. (2003) provide other evidence for a link between *cis*11-18:1 and FO-induced milk fat depression. Dietary FO resulted in a 7-fold increase in percentage of *trans*9,*cis*11-18:2 in rumen (Table 3), suggesting this fatty acid may be a ruminal precursor of *cis*11-18:1. Interestingly, concentration of *trans*9,*cis*11-18:2 in plasma was 12-fold greater with FO (Table 5) compared with LO or SO, but its concentration in milk fat did not differ (Table 8). The relatively high concentrations of *trans*11,*cis*15-18:2 in rumen, plasma, and milk with FO could also contribute to low milk fat yield and content (see Loor et al., 2005a).

Ruminal percentage of *trans*9,*trans*12-18:2 and *trans*9,*cis*12-18:2 were negatively correlated with milk fat content, which agrees with our previous study with LO supplementation (Loor et al., 2005a). Although their concentrations in plasma and percentages in milk fat did not differ due to oils in the present study, both isomers increased in ruminal fluid with LO (Table 2). The negative relationship between rumen percentage and milk fat content (Figure 1D) for *trans*9,*trans*12-18:2 ($r = -0.69$; $P = 0.05$) suggests that this isomer might be a component of the mechanisms whereby 18:3-rich oils cause milk fat depression. Responses to feeding

LO were marked also for percentages of several other 18:3n-3 biohydrogenation intermediates: *trans*13+14-18:1, *trans*15-18:1, *trans*16-18:1, *cis*15-18:1, *cis*9,*trans*12-18:2, and *trans*11,*cis*15-18:2 in rumen, plasma, and milk fat, and *cis*9,*trans*13-18:2 in plasma and milk (likely arising from desaturation of *trans*13-18:1). In a previous study, milk fat *cis*15-18:1, *cis*9,*trans*12-18:2, *cis*9,*trans*13-18:2, and *trans*11,*cis*15-18:2 were negatively correlated with milk fat 4:0-16:0 percentage (Loor et al., 2005a), and are other candidates to explain the decrease in de novo lipogenesis with LO.

Although abomasal infusions of *trans*10,*cis*12-18:2 or dietary rumen-inert mixtures of CLA have caused milk fat depression (Baumgard et al., 2000; Loor and Herbstein, 2003; Perfield et al., 2004), feeding studies are inconclusive on the putative role of nondietary, endogenously synthesized *trans*10,*cis*12-18:2. Increases in milk fat *trans*10,*cis*12-18:2 in cows with diet-induced milk fat depression were very modest (less than 0.10 to 0.15% of total milk fatty acids; Piperova et al., 2000; Whitlock et al., 2002; Peterson et al., 2003; present study). Even with polyunsaturated fatty acid supplementation, results show discrepancies between *trans*10,*cis*12-18:2 and milk fat yield (AbuGhazaleh et al., 2003; Shingfield et al., 2003; Loor et al., 2005a,b) or even no correlation between milk *trans*10,*cis*12-18:2 and milk fat content (Precht et al., 2002; Bradford and Allen, 2004; Loor et al., 2005a; present study). It appears likely that other biohydrogenation intermediates in addition to *trans*10,*cis*12-18:2 are involved in diet-induced milk fat depression (Bauman and Griinari, 2003; Peterson et al., 2003; Loor et al., 2005a,b).

Biohydrogenation Intermediates with Practical Applications

Responses in the percentage and yield of certain fatty acids with potential applications in human health and energy sparing in the cow (Bauman and Griinari, 2003) differed according to polyunsaturated fatty acid supply in the diet. Although we did not include a control diet for comparison, all oils resulted in high *trans*11-18:1 and *cis*9,*trans*11-18:2 in milk fat, which are far above typical values in milk from cows receiving nonlipid-supplemented diets. Comparison of our measurements of duodenal flow of *trans*11-18:1 and *cis*9,*trans*11-18:2 (Loor et al., 2004b; 2005c) with their yield in milk fat across diets confirmed (Piperova et al., 2002; Shingfield et al., 2003) that endogenous synthesis is the primary source of *cis*9,*trans*11-18:2. Even if all of the duodenal *cis*9,*trans*11-18:2 was secreted in milk fat, we observed that when feeding FO, LO, or SO, at least 97, 90, or 82%, respectively, of milk *cis*9,*trans*11-18:2 was synthesized via *trans*11-18:1 desaturation. These estimates are

lower than previous ones (Piperova et al., 2002) and suggest for the first time that endogenous synthesis of *cis9,trans11-18:2* may decrease as duodenal flow of this isomer increases. More importantly, we present evidence that supplementation of a high-concentrate diet with fish oil providing <25% EPA and DHA relative to linoleic acid from sunflower oil was effective in enhancing flow of *trans11-18:1* to maintain synthesis of *cis9,trans11-18:1* in the mammary gland. This result confirms in vitro data showing similar accumulation of *trans-18:1* due to DHA compared with soybean oil at 6 times the level of DHA supplementation (AbuGhazaleh and Jenkins, 2004).

Previous studies have observed increases in milk *trans10-18:1* with fish oil supplementation to TMR (Donovan et al., 2000; Whitlock et al., 2002; Shingfield et al., 2003) or fish oil infusion into the rumen in cows fed corn silage diets (Lor et al., 2005b). Our data suggest that it is feasible to enhance ruminal outflow of *trans11-18:1* and simultaneously *trans10-18:1*, *cis11-18:1*, or *trans11,cis15-18:2* by supplementing fish oil at a fraction of linoleic acid-rich oils when fed in combination with high-concentrate diets. The net result is greater *trans11-18:1* and *cis9,trans11-18:2* in milk and potentially more *trans10-18:1*, *cis11-18:1*, or *trans11,cis15-18:2* to reduce milk fat synthesis. This could be an effective strategy to reduce milk fat output and spare energy, which may be useful in certain situations in high-yielding dairy cows (Bauman and Griinari, 2003), while enhancing concentration of the anticarcinogenic *cis9,trans11-18:2* (Corl et al., 2003). However, there is a need to evaluate how the increase in several other *trans* isomers of 18:1, 18:2, or 18:3 (Tables 8 and 9) could positively or negatively affect the nutritional value of milk fat.

In summary, milk fat content did not differ among oil treatments but was clearly lower when compared with feeding high- or low-concentrate diets alone (Lor et al., 2005a). *Trans10,cis12-18:2* increased in rumen or milk fat with sunflower oil, but percentage and yield of *trans10-18:1* was similar among fish oil and sunflower oil. Only feeding SO led to changes typical for classical diet-induced milk fat depression, i.e., increased *trans10-18:1* and *trans10,cis12-18:2* and decreased de novo fatty acid synthesis. Fish oil resulted in a marked increase in percentage of *cis11-18:1* in rumen, plasma, and milk fat. The effects of FO, compared with LO and SO, on milk fat were not mediated via inhibition of de novo fatty acid synthesis. Results with LO confirmed previous data (Lor et al., 2005a) for higher *trans13+14-18:1*, *trans15-18:1*, *trans16-18:1*, *cis15-18:1*, *cis9,trans12-18:2*, *cis9,trans13-18:2* and *trans11,cis15-18:2*. Although persistent, increases in *cis11-18:1*, *trans11,cis15-18:2*, and *trans9,trans12-18:2*

with both LO and FO occurred along with the increase in other isomers in ruminal fluid and milk fat, making it difficult to differentiate their respective roles in diet-induced milk fat depression. Taken together, our data indicates that milk fat depression typically observed with fish oil supplementation could be linked to reduced availability of stearic acid and a potential antilipogenic effect of *trans10-18:1*, *cis11-18:1*, or *trans11,cis15-18:2*. Simultaneous increases in ruminal *trans11-18:1*, with fish oil at a fraction of sunflower oil supplementation, may represent an effective strategy to maintain *cis9,trans11-18:2* synthesis in mammary while reducing milk fat output and sparing energy. Feeding studies are still inconclusive on the role of ruminally-derived *trans10,cis12-18:2* and milk fat depression but highlight the possibility that other biohydrogenation intermediates may also play a role in this phenomenon.

ACKNOWLEDGMENTS

Authors acknowledge the assistance of J. P. Pezant and the team of Les Cèdres Experimental Unit for feeding, milking, blood sampling, and caring for cows, as well as M. Tourret, and C. Delavaud for help in laboratory analyses. This study was partly funded by the INRA Programme "Micronutrients in animal products, and their health value for consumers."

REFERENCES

- AbuGhazaleh, A. A., and T. C. Jenkins. 2004. Short communication: Docosahexaenoic acid promotes vaccenic acid accumulation in mixed ruminal cultures incubated with linoleic acid. *J. Dairy Sci.* 87:645–651.
- 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.
- Association of Official Analytical Chemists. 1997. Official Methods of Analysis. 16th ed. AOAC Int., Gaithersburg, MD.
- 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, A. Sæbø, and D. E. Bauman. 2000. Identification of the conjugated linoleic acid isomer that inhibits milk fat synthesis. *Am. J. Physiol.* 278:R179–R184.
- Bonnet, M., C. Delavaud, K. Laud, I. Gourdou, C. Leroux, J. Djiane, and Y. Chilliard. 2002. Mammary leptin synthesis, milk leptin and their putative physiological roles. *Reprod. Nutr. Dev.* 42:399–413.
- Bradford, B. J., and M. S. Allen. 2004. Milk fat responses to a change in diet fermentability vary by production level in dairy cattle. *J. Dairy Sci.* 87:3800–3807.
- Chilliard, Y., and A. Ferlay. 2004. Dietary lipids and forages interactions on cow and goat milk fatty acid composition and sensory properties. *Reprod. Nutr. Dev.* 44:467–492.
- 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., J. Rouel, P. Capitan, J. M. Chabosseau, K. Raynal-Ljutovac, and A. Ferlay. 2003. Correlations between milk fat

- content and fatty acid composition in goats receiving different combinations of forages and lipid supplements. Page 343 in Book of Abstracts, 54th Annu. Meeting EAAP, Rome, Italy. Y. van der Honing, ed. Wageningen Acad. Publ., Wageningen, The Netherlands.
- Corl, B. A., D. M. Barbano, D. E. Bauman, and C. Ip. 2003. Cis-9, trans-11 CLA derived endogenously from trans-11 18:1 reduces cancer risk in rats. *J. Nutr.* 133:2893–2900.
- Delavaud, C., A. Ferlay, Y. Faulconnier, F. Bocquier, G. Kann, and Y. Chilliard. 2002. Plasma leptin concentration in adult cattle: Effects of breed, adiposity, feeding level, and meal intake. *J. Anim. Sci.* 80:1317–1328.
- Donovan, D. C., D. J. Schingoethe, R. J. Baer, J. Ryali, A. R. Hippen, and S. T. Franklin. 2000. Influence of dietary fish oil on conjugated linoleic acid and other fatty acids in milk fat from lactating dairy cows. *J. Dairy Sci.* 83:2620–2628.
- Doreau, M., and Y. Chilliard. 1997. Effects of ruminal or post-ruminal fish oil supplementation on intake and digestion in dairy cows. *Reprod. Nutr. Dev.* 37:113–124.
- Ferlay, A., and Y. Chilliard. 1999. Effects of the infusion of non-selective beta-, and selective beta1- or beta2-adrenergic agonists, on body fat mobilisation in underfed or overfed non-pregnant heifers. *Reprod. Nutr. Dev.* 39:409–421.
- 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 cows. *J. Dairy Sci.* 81:1251–1261.
- Loor, J. J., M. Doreau, J. M. Chardigny, A. Ollier, J. L. Sebedio, and Y. Chilliard. 2005b. 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. 2005a. 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. 2003. Reduced fatty acid synthesis and desaturation due to exogenous trans10,cis12-CLA in cows fed oleic or linoleic oil. *J. Dairy Sci.* 86:1354–1369.
- Loor, J. J., K. Ueda, A. Ferlay, Y. Chilliard, and M. Doreau. 2004a. Short communication: Diurnal profiles of conjugated linoleic acids and trans fatty acids in ruminal fluid from cows fed a high-concentrate diet supplemented with fish oil, linseed oil, or sunflower oil. *J. Dairy Sci.* 87:2468–2471.
- Loor, J. J., K. Ueda, A. Ferlay, Y. Chilliard, and M. Doreau. 2004b. Biohydrogenation, duodenal flows, and intestinal digestion 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.
- Mosley, E. E., G. Powell, M. Riley, and T. C. Jenkins. 2002. Microbial biohydrogenation of oleic acid to trans isomers in vitro. *J. Lipid Res.* 43:290–296.
- Oba, M., and M. S. Allen. 2003. Effects of corn grain conservation method on feeding behavior and productivity of lactating dairy cows at two dietary starch concentrations. *J. Dairy Sci.* 86:174–183.
- 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.
- Perfield, J. W., II, A. L. Lock, A. M. Pfeiffer, and D. E. Bauman. 2004. Effects of amide-protected and lipid-encapsulated conjugated linoleic acid supplements on milk fat synthesis. *J. Dairy Sci.* 87:3010–3016.
- Peterson, D. G., E. A. Matitashvili, and D. E. Bauman. 2003. Diet-induced milk fat depression in dairy cows 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.
- Pipero, L. S., J. Sampugna, B. B. Teter, K. Kalscheur, M. Yurawecz, Y. Ku, K. Morehouse, and R. A. Erdman. 2002. Duodenal and milk trans octadecenoic acid and conjugated linoleic acid (CLA) isomers indicate postabsorptive synthesis is the predominant source of cis9-containing CLA in lactating dairy cows. *J. Nutr.* 132:1235–1241.
- Pipero, 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 related in lactating dairy cows fed a milk fat-depressing diet. *J. Nutr.* 130:2568–2574.
- Precht, D., H. Hagemeyer, W. Kanitz, and J. Voigt. 2002. Milk fat depression and the role of trans and CLA fatty acid isomers by feeding a high fiber diet with calcium soaps of fatty acids in early lactating dairy cows. *Milchwissenschaft* 57:518–522.
- Roy, A., A. Ferlay, A. Ollier, and Y. Chilliard. 2005. Kinetics of trans and conjugated fatty acids (FA) concentrations in cow milk after addition of plant oils to different basal diets. Page 136 in Book of Abstracts, 56th Annu. Meeting EAAP, Uppsala, Sweden. Wageningen Acad. Publ., Wageningen, The Netherlands.
- Shingfield, K., S. Ahvenjärvi, V. Toivonen, A. Ärölä, K. V. V. Nurmela, P. Huhtanen, and J. Griinari. 2003. Effect of dietary fish oil on biohydrogenation of fatty acids and milk fatty acid content in cows. *Anim. Sci.* 77:165–179.
- Sutton, J. D., M. S. Dhanoa, S. V. Morant, J. France, D. J. Napper, and E. Schuller. 2003. Rates of production of acetate, propionate, and butyrate in the rumen of lactating dairy cows given normal and low-roughage diets. *J. Dairy Sci.* 86:3620–3633.
- Whitlock, L. A., D. J. Schingoethe, A. R. Hippen, K. F. Kalscheur, R. J. Baer, N. Ramaswamy, and K. M. Kasperson. 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.