Relationship Among *Trans* and Conjugated Fatty Acids and Bovine Milk Fat Yield Due to Dietary Concentrate and Linseed Oil

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

Herbivore Research Unit INRA-Theix, 63122 St.-Genes Champanelle, France

ABSTRACT

Effects on fatty acid profiles and milk fat yield due to dietary concentrate and supplemental 18:3n-3 were evaluated in 4 lactating Holstein cows fed a low- (35:65 concentrate:forage; L) or high- (65:35; H) concentrate diet without (LC, HC) added oil or with linseed oil (LCO, HCO) at 3% of DM. A 4 × 4 Latin square with four 4-wk periods was used. Milk yield and dry matter intake averaged 26.7 and 20.2 kg/d, respectively, across treatments. Plasma acetate and β -hydroxybutyrate decreased, whereas glucose, nonesterified fatty acids, and leptin increased with high-concentrate diets. Milk fat percentage was lower in cows fed high-concentrate diets (2.31 vs. 3.38), resulting in decreases in yield of 11 (HC) and 42% (HCO). Reduced yields of 8:0-16:0 and *cis*9-18:1 fatty acids accounted for 69 and 17%, respectively, of the decrease in milk fat yield with HC vs. LC (–90 g/d), and for 26 and 33%, respectively, of the decrease with HCO vs. LCO (–400 g/d). Total *trans*-18:1 yield increased by 25 (HCO) and 59 (LCO) g/d with oil addition. *Trans*10-18:1 yield was 5-fold greater with high-concentrate diets. *Trans*11-18:1 increased by 13 (HCO) and 19 (LCO) g/d with oil addition. *Trans*13+14-18:1 yield increased by 9 (HCO) and 18 (LCO) g/d with linseed oil. Yield of total conjugated linoleic acids (CLA) in milk averaged 6 g/d with LC or HC compared with 14 g/d with LCO or HCO. *Cis*9,*trans*11-CLA yield was not affected by concentrate level but increased by 147% in response to oil. Feeding oil increased yields of *trans*11,*cis*13-, *trans*11,*trans*13-, and *trans*,*trans*-CLA, primarily with LCO. *Trans*10,*cis*12-CLA yield (average of 0.08 g/d) was not affected by treatments. Yield of *trans*11,*cis*15-18:2 was 1 g/d in cows fed LC or HC and 10 g/d with LCO or HCO. Yields of *cis*9,*trans*11-18:2, *cis*9,*trans*12-18:2, and *cis*9,*trans*13-18:2 were positively correlated ($r = 0.74$ to 0.94) with yields of *trans*11-18:1, *trans*12-18:1, and *trans*13+14-18:1, respectively.

Plasma concentrations of biohydrogenation intermediates with concentrate or linseed oil level followed similar changes as those in milk fat. Milk fat depression was observed when HC induced an increase in *trans*10-18:1 yield. A correlation of 0.84 across 31 comparisons from 13 published studies, including the present one, was found among the increase in percentage of *trans*10-18:1 in milk fat and decreased milk fat yield. We observed, however, more drastic milk fat depression when HCO increased yields of total *trans*-18:1, *trans*11,*cis*15-18:2, *trans* isomers of 18:3, and reduced yields of 18:0 plus *cis*9-18:1.

(**Key words:** high concentrate, linseed oil, milk conjugated linoleic acids, *trans* fatty acids)

Abbreviation key: LC = low-concentrate diet without linseed oil, HC = high-concentrate diet without linseed oil, LCO = low-concentrate diet with linseed oil, HCO = high-concentrate diet with linseed oil, CLA = conjugated linoleic acids.

INTRODUCTION

Diets with a high concentrate:forage ratio fed with unsaturated oils reduce milk fat production to the largest extent (review by Bauman and Griinari, 2003). Biohydrogenation of polyunsaturated fatty acids in the rumen is reduced with high-concentrate diets causing accumulation of *trans*-18:1 isomers (Kalscheur et al., 1997; Doreau et al., 1999). In cows fed high-concentrate diets without (Piperova et al., 2002) or with (Griinari et al., 1998; Piperova et al., 2000; Peterson et al., 2003) vegetable oils rich in linoleic acid, *trans*10-18:1 percentage in milk fat was as high or higher than *trans*11-18:1. *Trans*7,*cis*9-18:2 and *trans*10,*cis*12-18:2, along with *cis*9,*trans*11-18:2, also increased primarily when the high-concentrate diet was supplemented with oil high in linoleic acid (Piperova et al., 2000).

Experiments assessing the effects of concentrate:forage ratio on milk fat composition have used corn silage alone, alfalfa hay alone, corn plus alfalfa or grass silage, or corn plus mixtures of small cereal grain silages (e.g., barley and triticale) as the forage source, with corn grain being the primary source of starch in the concentrate mixture (Kalscheur et al., 1997; Griinari et al.,

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

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

1998; Piperova et al., 2000; Peterson et al., 2003). High-linoleic acid oils (e.g., corn or soybean oil) were the preferred substrate for biohydrogenation. Type of forage and supplemental unsaturated fatty acids alter *trans*-18:1 and conjugated linoleic acid (CLA) isomers in milk fat to varying extents in cows (Chilliard et al., 2001) or goats (Chilliard et al., 2003a). Differences in starch source may account for some of the observed variation in the milk fat depression response in cows fed high-concentrate diets (Bauman and Griinari, 2003).

To our knowledge, there are no published studies comparing level of concentrate:forage ratio and linseed oil (a source of α -linolenic acid) on milk composition. The present study examined the effects of feeding high-concentrate diets containing wheat as the primary starch source fed alone or in combination with linseed oil and grass hay as the sole forage on milk fat yield, blood plasma metabolites, and profiles of *trans*-18:1 and CLA isomers in blood plasma and milk fat. Results pertaining to ruminal digestion (Ueda et al., 2003) and duodenal flows and digestibility of fatty acids (Loor et al., 2004) have already been reported.

MATERIALS AND METHODS

Animals and Diets

Four peak-lactation multiparous Holstein cows with cannulas in the rumen and duodenum were used in a 4 × 4 Latin square design with factorial arrangement of treatments during four 4-wk periods to evaluate responses to feeding diets with a low (35:65) or high (65:35) concentrate to forage ratio without (LC, HC) added oil or with linseed oil (LCO, HCO) supplemented at 3% of DM (Ueda et al., 2003; Loor et al., 2004). Average BW and DIM for cows at the onset of the experiment was 658 ± 13 kg and 71 ± 16 d. The sole forage was long-cut grass hay and the concentrate mixture was based primarily on ground wheat, rapeseed, sunflower meal, and wheat bran. Details of ingredient, chemical composition, and fatty acid profiles of the diet have been presented previously (Loor et al., 2004). By design, diets were not isoenergetic but were close to being isonitrogenous. Cows were housed in a tie-stall barn during the experiment. The concentrate mixture with or without linseed oil was prepared daily and, along with the forage, was offered in equal amounts at 0900, 1330, and 1700 h for ad libitum consumption. The first 5 d of each experimental period were used as a transition between treatments. Cows were milked at 0600 and 1700 h.

Sampling, Measurements, and Analyses

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 collected and stored at -20°C until the end of the experiment before lyophilization (Thermovac TM-20, Froilabo, Ozoir-la-Ferrière, France) 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 obtained with heparinized (150 USP) 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 a Vacutainer tube containing EDTA (0.47 mol/L). Blood was centrifuged at 3000 × 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 (Merck-Clevenot S.A., Nogent-sur-Marne, France), by spectrophotometric assays using specific kits (Glucose RTU, BioMerieux, Marcy-l'Étoile, France; Urea, kit no. 489 620, Boehringer-Mannheim, Meylan, France; nonesterified fatty acids, NEFA C, WAKO, Unipath S.A., Dardilly, France; free and esterified cholesterol kits, Biotrol Diagnostic, Chennevières-les-Louvres, France; phospholipids PAP 150, BioMerieux; triglycerides PAP 150, BioMerieux; acetate, kit no. 0 148 261, Boehringer-Mannheim; lactate PAP, BioMerieux). β -Hydroxybutyrate was analyzed with an automated micromethod (Ferlay and Chilliard, 1999). Insulin was determined by radioimmunoassay using a commercial kit (INSI-PR RIA, CIS Bio International, Gif-sur-Yvette, France). Leptin was determined using a specific radioimmunoassay, as described by Delavaud et al. (2002). Intraassay CV for BHBA, lactate, glucose, free glycerol, NEFA, total cholesterol, free cholesterol, phospholipid, triglyceride, insulin, and leptin were 4.1, 0.5, 0.5, 1.7, 0.5, 1.7, 2.4, 2, 2.4, 6.6, and 6%, respectively. Interassay CV for BHBA, lactate, glucose, free glycerol, NEFA, total cholesterol, free cholesterol, phospholipid, triglyceride, insulin, and leptin were 4, 8, 2, 4, 7, 2.3, 2.5, 3.2, 5, 11.2, and 9%, respectively.

Plasma total lipids were extracted with chloroform/methanol (2:1, vol/vol; Loor et al., 2002a,b). Fatty acids in lyophilized milk were directly methylated with 1 mL

of 2 *N* methanolic NaOCH₃ at room temperature for 20 min, followed by 1 mL of 14% boron trifluoride in methanol at room temperature for 20 min (Christie et al., 2001). Fatty acids in plasma lipids were methylated with 2 mL of 0.5 *N* NaOCH₃ 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., 2002a,b). In all cases, fatty acid methyl esters were recovered in 1 mL of hexane. Tricosanoate (Sigma, Saint-Quentin Fallavier, France) was used as the internal standard. 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 m × 0.25 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. (2004). A butter reference standard (CRM 164; Commission of the European Communities, Community Bureau of Reference, Brussels, Belgium) was used to estimate correction factors for short-chain (4:0 to 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 initial oven temperature was held at 70°C for 1 min, increased 5°C/min to 100°C (held for 2 min), then increased at 10°C/min to 175°C (held for 40 min), and 5°C/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. Satisfactory separations of *cis*- and *trans*-18:1, nonconjugated 18:2, and CLA isomers were obtained with a single chromatographic run (see Figure 1 in Loor et al., 2004).

Statistical Analysis

Data for forage and concentrate intake, milk production and composition, fatty acids in plasma, and fatty acids in milk fat are reported as least squares means ± SEM. Data were analyzed as a Latin square with factorial arrangement of treatments using the MIXED procedure of SAS (SAS Inst., Inc., Cary, NC). The statistical model included: cow, period, forage level, oil supplementation level, forage × oil interaction, and residual error. Fixed effects included period, forage level, oil supplementation level, and forage × oil interaction. Cow was the random effect. Compound symmetry was the covariate structure used. Nonlinear regression analysis and ANOVA were used to examine relationships among selected fatty acids, or between selected fatty acids and milk fat percentage.

Overall differences between treatment means were considered to be significant when $P \leq 0.05$. Interactions for level of concentrate and oil were considered significant at $P \leq 0.10$ to guard against Type II error. This was deemed appropriate because of the low power of the experimental design to test for interactions rather than main effects, combined with the a priori expectation for interactions.

RESULTS

DMI and Milk Production and Composition

Average DMI and milk, protein, and lactose yields during the last 5 d of wk 4 did not differ in response to concentrate or oil level (Table 1). Protein content decreased with LCO compared to LC but increased with HCO compared to HC (concentrate × oil interaction, $P < 0.05$). Feeding high-concentrate diets substantially reduced milk fat percentage and yield (−28%). The reduction in milk fat yield was less pronounced in cows fed HC compared to LC (−11%) than HCO compared to LCO (−42%) (concentrate × oil interaction, $P = 0.07$).

Blood Plasma Metabolite Concentrations

In cows fed high-concentrate diets, acetate and BHBA concentrations in jugular plasma were lower ($P < 0.05$) compared with low-concentrate diets (Table 2). In contrast, feeding high-concentrate diets increased ($P < 0.05$) concentrations of glucose, glycerol, and NEFA. Concentration of leptin tended ($P = 0.10$) to be greater in cows fed the high-concentrate diets. Linseed oil supplementation resulted in concentrations of glucose, total cholesterol, free cholesterol, phospholipid, and urea that were 4, 27, 24, 24, and 23%, respectively, greater ($P < 0.05$).

Jugular-mammary venous differences for acetate and BHBA decreased ($P < 0.05$) in response to feeding high-concentrate diets, but the jugular-mammary venous difference for glucose was increased (Table 2). Jugular-mammary venous difference for NEFA was near zero when HC or LC were fed but was negative ($P < 0.05$) when HCO or LCO were fed. Jugular-mammary venous difference for insulin tended ($P = 0.07$) to be greater in response to feeding high-concentrate diets.

Plasma Fatty Acid Concentrations

Concentration of total fatty acids in jugular blood plasma averaged 2883 µg/mL in response to linseed oil ($P < 0.05$) compared with 2114 µg/mL for unsupplemented diets (Table 3). When cows were fed high-concentrate diets, concentration of *iso*-15, *anteiso*-15, *iso*-16, and *cis*10-17:1 was lower ($P < 0.05$). Cows fed linseed

Figure 1. Secretion response of selected fatty acid isomers in response to feeding the high-concentrate vs. low-concentrate (HC-LC), high-concentrate plus linseed oil vs. low-concentrate plus linseed oil (HCO-LCO), high-concentrate plus linseed oil vs. high-concentrate (HCO-HC), or the low-concentrate plus linseed oil diet vs. low-concentrate diet (LCO-LC).

Table 1. Dry matter intake and milk production and composition in Holstein cows fed a high- (H) or low- (L) concentrate diet without supplemental oil (HC, LC), or supplemented at 3% of DM with linseed oil (HCO, LCO).

Variable	Diets				SEM	Effect ¹		
	LC	LCO	HC	HCO		Conc	Oil	Conc × Oil
DMI, kg/d								
Forage	13.0	12.4	6.9	6.4	0.5	0.01	0.23	0.98
Concentrate	7.4	7.2	13.5	14.0	0.6	0.01	0.75	0.48
Total	20.4	19.6	20.4	20.4	1.0	0.65	0.64	0.65
Milk, kg/d	24.2	27.7	28.8	26.2	2.6	0.50	0.85	0.20
Composition, %								
Fat	3.30	3.45	2.40	2.22	0.25	0.01	0.95	0.52
Protein	3.10	2.95	3.03	3.21	0.13	0.12	0.81	0.01
Lactose	4.50	4.83	4.76	4.78	0.13	0.42	0.19	0.24
Yield, kg/d								
Fat	0.79	0.95	0.70	0.55	0.08	0.01	0.91	0.07
Protein	0.75	0.81	0.88	0.84	0.09	0.29	0.85	0.49
Lactose	1.09	1.34	1.38	1.25	0.13	0.41	0.64	0.14

¹P value for the effect of concentrate level (Conc), oil, and the interaction of concentrate and oil.

oil had greater concentrations of 15:0, *anteiso*-15:0, *cis*9-16:1, *cis*10-17:1, 19:0, and 20:5n-3 ($P < 0.05$). A significant concentrate × oil interaction ($P < 0.05$) was found for the concentration of 14:0 and 18:0 in plasma.

Plasma 18:1 Isomer Concentrations

Total *trans*-18:1 in plasma increased ($P = 0.01$) substantially with linseed oil, and more markedly when it

Table 2. Plasma metabolite (mmol/L) and hormone concentrations, and apparent mammary uptake in Holstein cows fed a high- (H) or low- (L) concentrate diet without supplemental oil (HC, LC), or supplemented at 3% of DM with linseed oil (HCO, LCO).

Variable	Diets				SEM	Effect ¹		
	LC	LCO	HC	HCO		Conc	Oil	Conc × Oil
Jugular concentration								
Acetate	0.983	0.783	0.433	0.416	0.150	0.01	0.45	0.49
β -hydroxybutyrate	0.373	0.340	0.235	0.217	0.040	0.01	0.49	0.74
Lactate	0.964	0.815	0.991	0.626	0.203	0.63	0.15	0.53
Glucose	3.25	3.40	3.55	3.70	0.07	0.01	0.05	0.91
Total cholesterol	4.39	5.69	3.93	4.86	0.41	0.14	0.02	0.66
Free cholesterol	0.943	1.21	0.852	1.02	0.08	0.12	0.02	0.59
Phospholipid	2.11	2.73	2.05	2.42	0.18	0.32	0.01	0.51
Triglyceride	0.073	0.091	0.081	0.079	0.012	0.90	0.53	0.44
Free glycerol	0.222	0.206	0.356	0.304	0.035	0.01	0.36	0.63
NEFA	0.082	0.091	0.144	0.141	0.026	0.05	0.92	0.82
Urea	6.06	7.89	5.94	6.88	0.68	0.30	0.03	0.40
Insulin, μ IU/mL	16.3	12.4	16.9	17.0	2.10	0.20	0.37	0.34
Leptin, ng/mL	2.95	2.81	3.60	3.54	0.68	0.10	0.81	0.92
Jugular-mammary venous difference								
Acetate	0.631	0.428	0.247	0.272	0.118	0.05	0.48	0.40
β -hydroxybutyrate	0.151	0.118	0.076	0.081	0.021	0.05	0.66	0.63
Lactate	0.521	0.378	0.585	0.19	0.153	0.70	0.11	0.43
Glucose	0.70	0.64	0.91	0.88	0.11	0.05	0.65	0.89
Total cholesterol	-0.09	-0.14	-0.05	-0.50	0.16	0.33	0.14	0.21
Free cholesterol	-0.010	-0.010	0.016	-0.081	0.036	0.51	0.18	0.18
Phospholipid	-0.07	-0.07	-0.02	-0.21	0.08	0.56	0.22	0.22
Triglyceride	0.030	0.045	0.050	0.051	0.009	0.16	0.41	0.44
Free glycerol	0.040	0.011	-0.055	0.040	0.093	0.73	0.73	0.52
NEFA	-0.002	-0.053	0.011	-0.037	0.020	0.50	0.04	0.96
Urea	-0.050	0.003	0.001	-0.0003	0.003	0.57	0.06	0.02
Insulin	-0.983	-0.299	3.667	2.237	1.810	0.07	0.84	0.57
Leptin	0.124	-0.154	0.562	-0.126	0.401	0.58	0.26	0.62

¹P value for the effect of concentrate level (Conc), oil, and the interaction of concentrate and oil.

Table 3. 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- (H) or low- (L) concentrate diet without supplemental oil (HC, LC), or supplemented at 3% of DM with linseed oil (HCO, LCO).

Fatty Acid	Diets				SEM	Effect ¹		
	LC	LCO	HC	HCO		Conc	Oil	Conc × Oil
	$\mu\text{g/mL}$							
12:0	1.10	0.95	1.17	1.37	0.27	0.36	0.94	0.52
14:0	14.3	10.7	12.6	15.7	1.3	0.22	0.86	0.02
<i>cis</i> 9-14:1	0.48	0.34	0.44	0.44	0.09	0.77	0.44	0.41
15:0	15.4	16.8	13.9	18.0	1.2	0.86	0.02	0.19
<i>iso</i> -15:0	0.4	0.4	0.2	0.2	0.02	0.01	0.28	0.55
<i>anteiso</i> -15:0	19.0	24.4	12.6	15.4	1.3	0.01	0.01	0.26
16:0	202	207	204	232	12	0.30	0.22	0.37
<i>iso</i> -16:0	7.79	7.45	5.73	5.87	0.55	0.01	0.75	0.39
<i>trans</i> 9-16:1 + <i>iso</i> -17:0	7.90	5.02	5.78	5.28	0.74	0.23	0.04	0.14
<i>cis</i> 9-16:1	24.3	60.1	24.4	42.6	5.8	0.07	0.01	0.07
17:0	17.5	16.2	18.6	19.0	1.2	0.13	0.71	0.48
<i>cis</i> 10-17:1	1.37	1.84	0.91	1.02	0.11	0.01	0.02	0.14
18:0	308	451	301	364	20	0.04	0.01	0.05
19:0	0.43	0.67	0.52	1.02	0.17	0.15	0.03	0.36
20:0	6.95	1.27	4.44	1.03	3.57	0.71	0.23	0.75
<i>cis</i> 11-20:1	0.09	0.08	0.41	0.06	0.07	0.05	0.02	0.03
20:3n-3	43.6	45.5	45.1	44.9	3.0	0.84	0.71	0.65
20:3n-6	48.3	35.7	45.5	35.2	5.1	0.57	0.01	0.69
20:4n-6	16.9	16.2	20.7	24.7	1.4	0.01	0.24	0.10
20:5n-3	1.31	1.71	1.21	1.77	0.19	0.94	0.03	0.67
22:5n-3	0.09	0.03	0.13	0.08	0.01	0.01	0.01	0.64
Total ²	2108	2810	2122	2960	113	0.49	0.01	0.57

¹*P* value for the effect of concentrate level (Conc), oil, and the interaction of concentrate and oil.²Total = sum of 12:0 to 22:5n-3 (including 18:1, 18:2, and 18:3 isomers).

was added to the high-concentrate diet (Table 4). Among individual isomers, concentration of *trans*-18:1 with double bonds in positions 5 through 11 and 13

through 15 of the carbon chain were greater ($P < 0.05$) in response to high-concentrate diets (Table 4). Linseed oil supplementation resulted in greater ($P < 0.05$) con-

Table 4. Concentration of 18:1 isomers in jugular blood plasma from Holstein cows fed a high- (H) or low- (L) concentrate diet without supplemental oil (HC, LC), or supplemented at 3% of DM with linseed oil (HCO, LCO).

Isomer	Diets				SEM	Effect ¹		
	LC	LCO	HC	HCO		Conc	Oil	Conc × Oil
	$\mu\text{g/mL}$							
18:1 <i>trans</i>								
4	1.33	1.49	1.39	2.02	0.21	0.11	0.04	0.19
5	0.28	0.59	0.57	1.87	0.33	0.04	0.03	0.16
6+7+8	1.40	3.66	3.33	6.92	0.74	0.01	0.01	0.39
9	1.22	3.27	2.94	5.74	0.64	0.01	0.01	0.56
10	1.43	3.93	8.44	19.3	4.2	0.01	0.10	0.31
11	13.7	35.9	19.2	91.7	8.0	0.05	0.01	0.12
12	2.06	7.53	4.12	8.51	1.00	0.15	0.01	0.59
13+14	9.31	22.3	12.1	29.9	1.42	0.01	0.01	0.08
15	1.57	7.10	3.26	9.29	0.72	0.02	0.01	0.71
16	1.79	6.43	2.80	6.19	0.66	0.53	0.01	0.32
Total	34.1	92.1	58.3	181	19.2	0.01	0.01	0.10
18:1 <i>cis</i>								
9	153	212	146	176	20	0.28	0.04	0.46
11	13.4	11.4	17.3	23.1	2.3	0.01	0.40	0.09
12	4.31	13.7	9.78	27.8	5.30	0.06	0.01	0.38
13	0.91	1.19	1.18	2.23	0.35	0.08	0.08	0.29
15	3.02	4.95	3.57	10.8	1.04	0.01	0.01	0.01
Total	174	242	178	239	21.1	0.82	0.01	0.78

¹*P* value for the effect of concentrate level (Conc), oil, and the interaction of concentrate and oil.

Table 5. Concentration of 18:2 and 18:3 isomers in jugular blood plasma from Holstein cows fed a high- (H) or low- (L) concentrate diet without supplemental oil (HC, LC), or supplemented at 3% of DM with linseed oil (HCO, LCO).

Isomer ²	Diets				SEM	Effect ¹		
	LC	LCO	HC	HCO		Conc	Oil	Conc × Oil
	$\mu\text{g/mL}$							
18:2 nonconjugated								
<i>cis</i> 9, <i>cis</i> 12	925	1060	1029	1263	54	0.01	0.01	0.37
<i>cis</i> 9, <i>trans</i> 12	0.98	1.71	0.88	1.70	0.28	0.80	0.01	0.85
<i>cis</i> 9, <i>trans</i> 13	6.89	7.89	5.45	4.12	2.09	0.24	0.94	0.59
<i>trans</i> 9, <i>cis</i> 12	0.13	0.92	0.84	2.34	0.34	0.01	0.01	0.27
<i>trans</i> 9, <i>trans</i> 12	0.12	0.22	0.31	1.45	0.39	0.10	0.14	0.22
<i>trans</i> 11, <i>cis</i> 15	1.36	3.92	1.56	28.1	6.57	0.08	0.04	0.08
Total	935	1078	1039	1305	54	0.01	0.01	0.28
18:2 conjugated								
<i>cis</i> 9, <i>trans</i> 11	1.84	4.83	2.06	9.48	1.49	0.10	0.01	0.14
<i>trans</i> 11, <i>cis</i> 13 + <i>cis</i> 9, <i>cis</i> 11	0.10	0.09	0.19	0.05	0.03	0.40	0.03	0.05
<i>trans</i> 9, <i>cis</i> 11	0.07	0.08	0.00	0.03	0.03	0.08	0.61	0.83
<i>cis</i> 10, <i>cis</i> 12	0.04	0.18	0.00	0.17	0.02	0.28	0.01	0.51
<i>trans</i> 11, <i>trans</i> 13	7.03	13.8	3.34	7.62	0.87	0.01	0.01	0.17
<i>trans</i> , <i>trans</i>	0.17	0.17	0.19	0.30	0.10	0.44	0.54	0.55
Total	9.24	19.2	5.78	17.6	2.01	0.22	0.01	0.62
18:3								
<i>cis</i> 9, <i>cis</i> 12, <i>cis</i> 15	184	432	99	359	25	0.01	0.01	0.80
<i>cis</i> 9, <i>trans</i> 12, <i>cis</i> 15	2.14	15.5	4.66	6.00	3.07	0.28	0.03	0.07
<i>cis</i> 9, <i>trans</i> ,12, <i>trans</i> 15	0.37	1.96	0.41	2.88	0.41	0.26	0.01	0.30
<i>trans</i> 9, <i>trans</i> 12, <i>trans</i> 15	7.45	18.8	5.67	10.78	5.37	0.35	0.13	0.55
Total	206	462	115	379	25.7	0.01	0.01	0.89

¹P value for the effect of concentrate level (Conc), oil, and the interaction of concentrate and oil.²*Cis*9,*cis*15-18:2, *cis*11,*cis*13-18:2, and *trans*10,*cis*12-18:2 were not detectable in plasma.

centrations of *trans*4-18:1 through *trans*9-18:1, and *trans*11-18:1 through *trans*16-18:1. Feeding linseed oil increased ($P < 0.05$) total *cis*-18:1 concentration by an average of 40%, and this response was mostly due to increases in oleic acid.

Plasma 18:2 and 18:3 Isomer Concentrations

Linoleic acid (97 to 99% of total nonconjugated 18:2) concentration was greater ($P < 0.05$) in cows fed high-concentrate diets and it increased ($P < 0.05$) further with linseed oil (Table 5). *Trans*11,*cis*15-18:2 increased (concentrate × oil interaction, $P = 0.08$) drastically with the high-concentrate diet plus linseed oil. Linseed oil resulted in greater total CLA (+10.9 $\mu\text{g/mL}$). Concentration of *cis*9,*trans*11-18:2 increased ($P < 0.05$) when linseed oil was fed. Feeding low-concentrate diets resulted in greater ($P < 0.05$) concentrations of *trans*11,*trans*13-18:2 (the main CLA isomer in plasma), which were increased ($P < 0.05$) further with linseed oil. Total concentration of 18:3 isomers was greater ($P < 0.05$) in cows fed low-concentrate diets. Linseed oil supplementation increased ($P < 0.05$) 18:3 isomers 2 (LCO) to 3 (HCO) times. α -Linolenic acid was the primary 18:3 isomer. *Cis*9,*cis*15-18:2, *cis*11,*cis*13-18:2, and *trans*10,*cis*12-18:2 were not detectable in plasma.

Milk Total Fatty Acid Yields

Cows fed high-concentrate diets had lower yields of 4:0, 6:0, 8:0, 14:0, and 16:0 (Table 6). Yields of 8:0, 10:0, 12:0, 14:0, and 16:0 decreased ($P < 0.05$) in response to feeding linseed oil. Reductions in yield of 4:0 to 16:0 (mostly synthesized de novo) accounted for 76 (without oil) to 34% (with oil) of the decrease in total milk fatty acid yield in response to feeding high-concentrate diets. Stearic acid yield increased (concentrate × oil interaction, $P < 0.05$) more markedly with the low-concentrate diet plus linseed oil. Yields of *cis*9-10:1, *cis*9-14:1, and *cis*9-16:1 (products of Δ^9 -desaturation) decreased ($P < 0.05$) with supplemental linseed oil.

Yields of 18:1 Isomers

Oleic acid accounted for 85 to 94% of total *cis*-18:1 isomers (Table 7). Responses to linseed oil varied with concentrate level. Whereas feeding LCO vs. LC increased total *cis*-18:1 by 110%, feeding HCO vs. HC reduced yield by 27% (concentrate × oil interaction, $P < 0.05$). Total *trans*-18:1 yield increased (concentrate × oil interaction, $P < 0.05$) more markedly with the low-concentrate diet plus linseed oil (Table 7). Yields of *trans*4-18:1, *trans*5-18:1, *trans*6+7+8-18:1, *trans*12-18:1, *trans*13+14-18:1, and *trans*16-18:1 were greater

Table 6. Yield of 4- to 22-carbon [not including 18:1, 18:2, or 18:3 isomers] fatty acids in milk fat from Holstein cows fed a high- (H) or low- (L) concentrate diet without supplemental oil (HC, LC), or supplemented at 3% of DM with linseed oil (HCO, LCO).

Fatty acid	Diets				SEM	Effect ¹		
	LC	LCO	HC	HCO		Conc	Oil	Conc × Oil
	g/d							
4:0	22.2	28.0	20.4	14.3	2.37	0.01	0.94	0.01
6:0	17.3	16.4	16.0	7.83	2.06	0.01	0.12	0.04
8:0	11.3	10.2	10.8	4.68	1.49	0.06	0.03	0.11
10:0	25.8	17.4	24.5	11.5	3.42	0.21	0.02	0.33
<i>cis</i> 9-10:1	2.47	1.72	2.14	0.78	0.28	0.02	0.01	0.22
11:0	0.55	0.31	0.95	0.34	0.23	0.33	0.08	0.42
12:0	29.2	18.1	27.2	13.5	4.02	0.29	0.02	0.53
13:0	1.80	1.16	2.08	0.98	0.39	0.89	0.03	0.51
14:0	85.7	70.1	72.4	42.3	9.85	0.05	0.05	0.42
8:0 to 14:0 ²	152	138	135	70.0	22	0.11	0.04	0.39
<i>iso</i> -14:0	0.94	1.03	0.53	0.35	0.08	0.01	0.55	0.11
<i>cis</i> 9-14:1	7.36	4.73	6.39	3.76	0.87	0.18	0.01	0.99
15:0	9.13	7.47	9.09	6.57	1.31	0.28	0.14	0.30
<i>iso</i> -15:0	2.52	2.98	1.39	0.94	0.16	0.01	0.98	0.02
<i>anteiso</i> -15:0	5.30	5.76	3.79	2.57	0.34	0.01	0.28	0.03
16:0	192	124	147	90.2	10.6	0.02	0.01	0.90
<i>iso</i> -16:0	2.11	2.30	1.57	0.95	0.20	0.01	0.27	0.05
<i>trans</i> 9-16:1 + <i>iso</i> -17:0	3.5	4.0	3.5	3.7	0.5	0.14	0.05	0.17
<i>cis</i> 9-16:1	9.26	6.02	8.44	5.27	1.47	0.37	0.01	0.93
17:0	5.09	4.01	4.78	2.80	0.57	0.10	0.05	0.25
<i>cis</i> 10-17:1	1.45	1.24	1.47	0.95	0.24	0.49	0.09	0.43
18:0	45.3	110	33.9	38.7	9.37	0.01	0.01	0.01
19:0	0.66	1.74	0.54	0.47	0.42	0.12	0.25	0.19
20:0	0.82	0.35	0.60	0.22	0.10	0.10	0.01	0.66
<i>cis</i> 11-20:1	0.64	0.78	0.49	0.39	0.07	0.01	0.81	0.11
21:0	0.31	0.54	0.21	0.15	0.10	0.03	0.39	0.16
20:3n-3	0.61	0.40	0.65	0.29	0.10	0.62	0.01	0.32
20:3n-6	0.39	0.49	0.25	0.18	0.04	0.01	0.64	0.06
20:4n-6	0.85	0.54	0.93	0.36	0.11	0.60	0.01	0.21
20:5n-3	0.44	0.42	0.31	0.23	0.05	0.01	0.15	0.35
<i>cis</i> 13, <i>cis</i> 16-22:2	0.043	0.031	0.035	0.018	0.004	0.04	0.01	0.35
22:4n-6	0.09	0.05	0.08	0.15	0.06	0.44	0.85	0.37
22:5n-3	0.57	0.21	0.72	0.36	0.09	0.11	0.01	0.98
22:6n-3	0.05	0.46	0.04	0.59	0.29	0.82	0.13	0.81
Total ³	707	843	622	481	60	0.01	0.71	0.07

¹*P* value for the effect of concentrate level (Conc), oil, and the interaction of concentrate and oil.²Sum of even-numbered saturated fatty acids.³Total = sum of 4:0 to 22:6n-3 (including unidentified fatty acids, 18:1, 18:2, and 18:3 isomers).

(concentrate × oil interaction, $P < 0.10$) with linseed oil addition primarily to the low-concentrate diet. Yield of *trans*10-18:1 was 3 to 5 times greater in response to high-concentrate diets. Linseed oil resulted in greater ($P < 0.05$) yields of *trans*9-18:1, *trans*11-18:1, and *trans*15-18:1.

Yields of 18:2 and 18:3 Isomers

Feeding linseed oil resulted in increases ($P < 0.05$) of 51 (HCO) and 80% (LCO) in the yield of nonconjugated isomers of 18:2, except for *cis*9,*cis*12-18:2 (Table 8). *Trans*11,*cis*15-18:2 alone accounted for 20 (LCO) and 35% (HCO) of the increase in total yield of nonconjugated 18:2 isomers in response to linseed oil. Yields of *cis*9,*trans*12-18:2 and *cis*9,*trans*13-18:2 were substan-

tially increased (concentrate × oil interaction, $P < 0.05$) with linseed oil addition to the low-concentrate diet primarily. Feeding linseed oil increased yields of total CLA by 120 to 169% (Table 8). *Cis*9,*trans*11-18:2 accounted for 80 to 91% of total conjugated 18:2 isomers regardless of diet. Its yield, however, increased by an average of 147% with linseed oil. *Trans*11,*cis*13- + *cis*9,*cis*11-18:2 and *trans*11,*trans*13-18:2 yields were increased to a greater extent (concentrate × oil interaction, $P < 0.05$) with the low-concentrate diet plus linseed oil.

Feeding linseed oil increased ($P < 0.05$) yields of total 18:3 isomers by 63%. As expected, this response was largely due to the greater ($P < 0.05$) yield of α -linolenic acid provided in the diet, which accounted for 88% of total 18:3 in milk fat. Yield of *trans*9,*trans*12,*trans*15-

Table 7. Yield of 18:1 isomers in milk fat from Holstein cows fed a high- (H) or low- (L) concentrate diet without supplemental oil (HC, LC), or supplemented at 3% of DM with linseed oil (HCO, LCO).

Isomer	Diets				SEM	Effect ¹		
	LC	LCO	HC	HCO		Conc	Oil	Conc × Oil
	g/d							
18:1 <i>trans</i>								
4	0.10	0.36	0.19	0.22	0.04	0.45	0.01	0.01
5	0.07	0.25	0.17	0.22	0.03	0.34	0.01	0.06
6+7+8	1.34	4.48	2.51	3.50	0.40	0.81	0.01	0.02
9	0.97	2.42	1.42	2.81	0.35	0.22	0.01	0.94
10	2.00	4.41	10.3	13.0	3.15	0.01	0.37	0.95
11	7.93	27.2	8.18	21.3	2.80	0.33	0.01	0.29
12	1.44	5.33	2.10	2.99	0.40	0.05	0.01	0.01
13+14	2.43	20.4	3.80	12.30	3.05	0.10	0.01	0.05
15	0.78	6.44	1.15	2.02	1.38	0.17	0.04	0.11
16	1.16	5.81	1.39	2.37	0.38	0.01	0.01	0.01
Total	18.2	77.1	31.2	55.7	7.16	0.53	0.01	0.02
18:1 <i>cis</i>								
9	108	201	92.8	69.1	22.4	0.01	0.11	0.02
11	4.79	5.51	6.44	5.89	0.71	0.18	0.91	0.38
12	1.01	2.49	2.51	2.94	0.55	0.09	0.10	0.34
13	0.49	2.31	0.65	0.57	0.74	0.30	0.26	0.22
15	0.45	9.98	1.41	2.86	3.63	0.40	0.15	0.28
Total	115	241	104	76.3	31.8	0.02	0.15	0.04

¹P value for the effect of concentrate level (Conc), oil, and the interaction of concentrate and oil.**Table 8.** Yield of 18:2 and 18:3 isomers in milk fat from Holstein cows fed a high- (H) or low- (L) concentrate diet without supplemental oil (HC, LC), or supplemented at 3% of DM with linseed oil (HCO, LCO).

Isomer	Diets				SEM	Effect ¹		
	LC	LCO	HC	HCO		Conc	Oil	Conc × Oil
	g/d							
18:2 nonconjugated								
<i>cis</i> 9, <i>cis</i> 12	11.4	11.7	15.4	11.1	1.7	0.28	0.21	0.17
<i>cis</i> 9, <i>cis</i> 15	1.02	1.20	0.91	0.79	0.12	0.05	0.81	0.28
<i>cis</i> 9, <i>trans</i> 12	0.41	1.69	0.78	1.23	0.19	0.78	0.01	0.02
<i>cis</i> 9, <i>trans</i> 13	0.87	4.29	1.44	3.08	0.43	0.38	0.01	0.03
<i>trans</i> 9, <i>cis</i> 12	0.19	0.56	0.33	0.99	0.15	0.07	0.01	0.31
<i>trans</i> 9, <i>trans</i> 12	0.02	0.27	0.19	0.85	0.18	0.05	0.02	0.25
<i>trans</i> 11, <i>cis</i> 15	0.76	6.71	1.41	12.9	2.34	0.16	0.01	0.25
Total	14.7	26.4	20.5	31.0	3.61	0.14	0.01	0.86
18:2 conjugated								
<i>cis</i> 9, <i>trans</i> 11	4.39	11.3	4.98	11.8	1.76	0.73	0.01	0.98
<i>trans</i> 11, <i>cis</i> 13 + <i>cis</i> 9, <i>cis</i> 11	0.07	0.58	0.12	0.26	0.05	0.01	0.01	0.01
<i>trans</i> 9, <i>cis</i> 11	0.15	0.78	0.25	0.18	0.29	0.41	0.36	0.26
<i>cis</i> 10, <i>cis</i> 12	0.19	0.27	0.16	0.12	0.05	0.06	0.69	0.21
<i>cis</i> 11, <i>cis</i> 13	0.10	0.06	0.00	0.00	0.04	0.03	0.48	0.48
<i>trans</i> 10, <i>cis</i> 12	0.05	0.14	0.07	0.05	0.04	0.42	0.39	0.18
<i>trans</i> 11, <i>trans</i> 13	0.16	0.75	0.13	0.33	0.06	0.01	0.01	0.01
<i>trans</i> , <i>trans</i>	0.17	0.30	0.21	0.26	0.04	0.97	0.01	0.15
Total	5.28	14.2	5.92	13.0	1.77	0.86	0.01	0.57
18:3								
<i>cis</i> 9, <i>cis</i> 12, <i>cis</i> 15	5.53	8.42	4.72	7.63	0.84	0.35	0.01	0.99
<i>cis</i> 9, <i>trans</i> 12, <i>cis</i> 15	0.13	0.16	0.01	0.04	0.02	0.01	0.25	0.96
<i>cis</i> 9, <i>trans</i> ,12, <i>trans</i> 15	0.11	0.18	0.04	0.10	0.02	0.01	0.01	0.61
<i>trans</i> 9, <i>trans</i> 12, <i>trans</i> 15	0.23	1.28	0.19	0.53	0.08	0.01	0.01	0.01
Total	6.25	10.2	5.17	8.35	0.86	0.10	0.01	0.63

¹P value for the effect of concentrate level (Conc), oil, and the interaction of concentrate and oil.

Table 9. Percentages of selected fatty acids in milk fat from Holstein cows fed a high- (H) or low- (L) concentrate diet without supplemental oil (HC, LC), or supplemented at 3% of DM with linseed oil (HCO, LCO).

Fatty acids	Diets				SEM	Effect ¹		
	LC	LCO	HC	HCO		Conc	Oil	Conc × Oil
	— g/100 g of total fatty acids —							
8:0-14:0 ²	23.3	15.4	22.8	15.1	1.0	0.73	0.01	0.92
16:0	29.4	17.2	25.7	18.7	0.9	0.08	0.01	0.04
18:0	7.05	14.8	6.17	8.07	1.0	0.01	0.01	0.01
total <i>trans</i> -18:1	2.69	9.02	5.00	12.1	1.21	0.02	0.01	0.69
<i>trans</i> 10-18:1	0.28	0.52	1.66	2.84	0.46	0.01	0.07	0.10
<i>trans</i> 11-18:1	1.12	3.23	1.32	4.53	0.53	0.27	0.01	0.09
<i>trans</i> 13+14-18:1	0.34	2.42	0.61	2.56	0.50	0.42	0.01	0.21
total <i>cis</i> -18:1	17.7	31.8	18.3	16.7	4.5	0.14	0.20	0.11
<i>cis</i> 9-18:1	15.3	23.8	14.9	14.4	2.8	0.55	0.26	0.04
total nonconjugated 18:2	2.25	3.37	3.54	7.06	0.84	0.01	0.01	0.15
<i>trans</i> 11, <i>cis</i> 15-18:2	0.11	0.80	0.23	2.84	0.50	0.08	0.01	0.05
total conjugated 18:2	0.82	1.82	1.04	3.00	0.36	0.15	0.01	0.31
<i>cis</i> 9, <i>trans</i> 11-18:2	0.62	1.34	0.81	2.54	0.36	0.13	0.02	0.12
total 18:3	0.96	1.30	0.91	1.86	0.18	0.14	0.01	0.09

¹P value for the effect of concentrate level (Conc), oil, and the interaction of concentrate and oil.²Sum of even-numbered saturated fatty acids.

18:3 was more than 6 times greater due to feeding LCO compared with HC (concentrate × oil interaction, $P = 0.01$).

Percentage of Selected Fatty Acids in Milk Fat

Significant concentrate × oil interactions were found for the percentage of a number of fatty acids (Table 9). A more pronounced decrease ($P < 0.05$) in percentage of 16:0 was observed with LCO vs. LC. Percentage of 18:0 and *cis*9-18:1 increased markedly with LCO ($P < 0.05$); whereas percentages of *trans*10-18:1, *trans*11-18:1, *trans*11,*cis*15-18:2, and total 18:3 isomers was increased ($P < 0.10$) to a greater extent with HCO. *Cis*9-*trans*11-18:2 percentage also tended ($P = 0.12$) to increase more markedly when HCO was fed.

Correlation Analysis

Iso-14:0, *iso*-15:0, and 18:0 percentages had the highest positive correlations with milk fat percentage (Table 10). *Trans*9-18:1 and *trans*10-18:1 had the most negative correlations with milk fat percentage among *trans*-18:1, but *cis*11-18:1, *trans*9,*cis*11-CLA, and 18:2n-6 had the highest overall negative correlations. Percentages of *cis*15-18:1, *trans*6+7+8-18:1, *trans*9-18:1, *trans*11-18:1, and *trans*12-18:1 (also *trans*4-18:1, *trans*5-18:1, *cis*13-18:1, data not shown, and total *trans*-18:1, $r = -0.97$) in milk fat were highly negatively correlated with the percentage of even-numbered 4:0 to 16:0 in milk fat. Among all 18:2 isomers, *cis*9,*trans*11-18:2, *cis*9,*trans*13-18:2, *cis*9,*trans*12-18:2, and *trans*11,*cis*13- +

*cis*9,*cis*11-18:2 had the most negative correlations with percentage of 4:0 to 16:0 in milk fat.

DISCUSSION

Plasma Metabolites and Hormones

In the present study, reduced plasma acetate concentration in cows fed high-concentrate diets was associated with lower molar concentration of acetate in ruminal fluid (Ueda et al., 2003). Although ruminal butyrate concentration was not affected by concentrate level (Ueda et al., 2003), recent data suggest that acetate and butyrate production (mol/d) may be decreased by high dietary concentrate (Sutton et al., 2003). Such responses could account for the observed lower jugular plasma concentration and jugular-mammary venous difference for acetate and BHBA (Table 3). These changes may partly contribute to the effect of dietary concentrate level on milk fat yield. Others have 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). In the present study, the absence of an interaction effect on plasma acetate and BHBA and the low correlation between jugular-mammary venous differences for those metabolites and either milk fat percentage ($r = 0.12$ to 0.24) or percentage of 4:0-16:0 ($r = 0.28$ to 0.33) clearly suggests that other factors are needed to fully explain milk fat depression due to high-concentrate diets.

Responses in plasma glucose with high-concentrate diets, but not NEFA or free glycerol, were consistent

Table 10. Correlations ($n = 16$; $P \leq 0.05$) among the percentage of 4:0-16:0¹ or milk fat and selected milk fatty acids.

Fatty acid	Fat	Fatty acid	4:0-16:0 ²
4:0	0.55	17:0	0.71
<i>iso</i> -14:0	0.82	20:0	0.69
<i>iso</i> -15:0	0.78	<i>trans</i> 6+7+8-18:1	-0.89
18:0	0.66	<i>trans</i> 9-18:1	-0.74
<i>trans</i> 9-18:1	-0.65	<i>trans</i> 11-18:1	-0.84
<i>trans</i> 10-18:1	-0.70	<i>trans</i> 12-18:1	-0.77
<i>cis</i> 11-18:1	-0.85	<i>cis</i> 15-18:1	-0.81
<i>cis</i> 12-18:1	-0.62	<i>cis</i> 9, <i>trans</i> 13-18:2	-0.90
18:2n-6	-0.82	<i>cis</i> 9, <i>trans</i> 12-18:2	-0.89
<i>trans</i> 9, <i>trans</i> 12-18:2	-0.63	<i>trans</i> 11, <i>cis</i> 15-18:2	-0.73
<i>trans</i> 9, <i>cis</i> 12-18:2	-0.65	<i>cis</i> 9, <i>trans</i> 11-18:2	-0.80
<i>cis</i> 9, <i>trans</i> 11-18:2	-0.56	<i>trans</i> 11, <i>cis</i> 13-18:2	-0.89
<i>trans</i> 9, <i>cis</i> 11-18:2	-0.73	<i>trans</i> 11, <i>trans</i> 13-18:2	-0.69
<i>trans</i> , <i>trans</i> -CLA	-0.58	<i>trans</i> , <i>trans</i> -CLA	-0.68
18:3n-3	-0.53	18:3n-3	-0.70
		20:4n-6	0.78

¹Correlations were very similar if using concentrations of 8:0-14:0, which more closely reflects de novo fatty acid synthesis.

²Sum of even-numbered saturated fatty acids.

with previous results (Gaynor et al., 1995; Griinari et al., 1998). As in the present study, Gagliostro et al. (1991) reported greater concentrations of phospholipids and cholesterol in blood plasma from cows infused with rapeseed oil into the duodenum. Dietary unsaturated oils also increase concentrations of all plasma lipid fractions in blood (Loor et al., 2002b). It was previously shown that dry nonpregnant cows fed high levels of concentrate compared with underfed cows (Delavaud et al., 2002) had greater concentrations of plasma leptin, in agreement with the present study.

Yields of Milk Fat, Stearic, and Oleic Acids

High dietary concentrate:forage reduced milk fat percentage and yield (Table 1). Gaynor et al. (1995) and Kalscheur et al. (1997) found similar responses in cows fed 75% concentrate (DM basis) without added lipid. Others reported more pronounced reductions in milk fat percentage and yield when feeding high-concentrate diets plus corn or soybean oil (Griinari et al., 1998; Piperova et al., 2000; Peterson et al., 2003). We found a significant oil $\times$ concentrate interaction effect for milk fat yield. The positive response (+20%) in cows fed LCO vs. LC was due to higher yields of 18:0 (+65 g/d) and *cis*9-18:1 (+93 g/d) in milk (oil effect, Figure 1), which more than compensated for the decrease in yield of 8:0 to 14:0 (-14 g/d) plus 16:0 (-68 g/d) (oil effect, Figure 1). The negative response (-21%) to linseed oil on milk fat yield in cows fed HCO vs. HC was due to lower yields of 8:0 to 14:0 (-65 g/d), 16:0 (-57 g/d), and *cis*9-18:1 (-24 g/d) (oil effect, Figure 1).

The marked increase in milk oleic acid yield with LCO compared with HCO may have been associated

with greater duodenal flow of 18:0 (+258 g/d) (Loor et al., 2004) and plasma availability for desaturation in the mammary gland. The higher concentration of 18:0 in plasma (Table 3) and yield in milk fat with LCO vs. LC (Table 6; oil effect, Figure 1) support this suggestion. In contrast, yield and percentage of 18:0 increased very little when feeding HCO (oil effect, Figure 1; Table 9), which was partly associated with a smaller increase (+112 g/d) in duodenal flow of 18:0 compared with LCO (Loor et al., 2004). Reduced milk fat output with HCO may have been associated with a lack of endogenously synthesized oleic acid for triglyceride formation (Loor and Herbein, 2003). Alternatively, substitution of *trans*-18:1 for oleic acid could increase the melting point of milk fat and inhibit its secretion from mammary cells (Chilliard et al., 2000). The importance of 18:0 in the process of milk fat secretion is supported by a relatively high correlation between 18:0 and milk fat content (Table 10) and the coordinated responses in 18:0 and oleic acid yields (Figure 1; see also Loor and Herbein, 2003 and Chilliard et al., 2003b).

*Trans*10-18:1 and Milk Fat Yield

Addition of high-linoleic acid oil (1 to 4% of DM) to high-concentrate diets resulted in pronounced milk fat depression along with increased *trans*10-18:1 in milk fat (Griinari et al., 1998; Peterson et al., 2003). Across a substantial number of published studies (Figure 2), data clearly suggests 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. Greater *trans*10-18:1 percent-

Figure 2. Relationship ($n = 31$ comparisons) among the change in percentage of *trans*10-18:1 in milk fat with the corresponding change in fat yield observed in the current and other published studies. Responses were calculated by comparison with unsupplemented control diets (Offer et al., 1999, 2001; Piperova et al., 2000; Piperova et al., 2004 only for control vs. Ca-salts of *trans*-18:1 treatments; Peterson et al., 2003; Donovan et al., 2000; Whitlock et al., 2002; AbuGhazaleh et al., 2003a,b; AbuGhazaleh et al., 2004; Onetti et al., 2004 only for corn silage vs. corn silage + tallow) or by comparing the treatment with highest milk fat yield against the remaining 3 in factorial designs (current study and Griinari et al., 1998). Fitting a linear equation ($y = -5.5312 \times -8.7709$) through the data resulted in a value for $R^2 = 0.63$.

age and reduced mRNA abundance for a number of lipogenic enzymes were observed due to feeding a high-concentrate diet plus linoleic acid rich oils (1 to 5% of DM) (Piperova et al., 2000; Peterson et al., 2003). Our results show that feeding high-concentrate diets without linseed oil essentially did not change uptake of 18-carbon fatty acids (e.g., 18:0, total *trans*-18:1, total *cis*-18:1, total 18:2, and total 18:3; Tables 6, 7, and 8; concentrate effect for 18:0 in Figure 1), but it decreased synthesis of 14:0 (Table 6) and probably 16:0 (concentrate effect, Figure 1). The responses in 14:0 and 16:0 (Table 6, oil effect in Figure 1), but also 4:0, 6:0, and 8:0 (Table 6), to high-concentrate diets were more marked with linseed oil. Although abomasal infusions of *trans*10,*cis*12-18:2 caused milk fat depression (Baumgard et al., 2000; Loor and Herbein, 2003), few feeding studies have shown increases in *trans*10,*cis*12-18:2 in cows with depressed milk fat and they were very modest (less than 0.10% of total milk fatty acids; Piperova et al., 2000; Peterson et al., 2003). We speculate that *trans*10,*cis*12-18:2 is likely not the only fatty acid capable of inhibiting milk fat synthesis.

*Trans*10-18:1 in response to high-concentrate plus unsaturated oil accounted for 36% of total *trans*-18:1 in the study of Griinari et al. (1998), for 59% in Piperova et al. (2000), for 67% in Offer et al. (2001), for 43% in

Peterson et al. (2003), or for 24% in the present study. Piperova et al. (2000) used corn silage as the sole forage compared with alfalfa hay by Griinari et al. (1998) or grass hay in the present study. Different proportions of *trans*10-18:1 in milk fat may be associated with higher dietary starch content (32 to 45%) in those studies compared with ours (23%), and the fact that they fed linoleic acid-rich oils or fish oil. We previously speculated (Loor et al., 2004) that changes in ruminal microbial populations, buffering capacity, or increased passage rate with higher dietary starch intake may play a role in enhancing *trans*-18:1 production in the rumen. In goats, feeding linseed oil or high-oleic sunflower oil increased *trans*10-18:1 when the basal diet was corn silage (20% starch content in DMI) but not with alfalfa hay (16% starch content in DMI) (Chilliard et al., 2003a). Although Figure 2 clearly suggests that *trans*10-18:1 is a good candidate to explain milk fat depression in cows, it should be noted that, in the present study, several other isomers of 18:1 and 18:2 were more closely related to either milk fat percentage and 4:0-16:0 percentage (Table 10 and next section).

Rumen-Derived *Trans* Fatty Acids and De Novo Fatty Acid Synthesis

Compared with the level of dietary concentrate, which decreased yields of 4:0, 6:0, 14:0, 16:0, odd, and

branched-chain fatty acids with 14 to 16 carbons mainly (Table 6), linseed oil alone caused greater reductions in the yield (Table 6) and percentage (Table 9) of most fatty acids (8:0 to 16:0) synthesized de novo in mammary. Sunflower oil supplementation to grazing cows resulted in a comparable response (Kay et al., 2004). Chilliard et al. (2000) discussed how dietary manipulation could reduce the percentage of saturated fatty acids synthesized de novo. More recently, it was shown that pure *trans*11-18:1 compared with oleic acid reduced activity of acetyl-CoA carboxylase and fatty acid synthase (EC 2.3.1.85) in bovine mammary cell cultures (Jayan and Herbein, 2000). Although speculative given the small number of observations, data from correlations (Table 10) raise the possibility that *cis*11-18:1, *cis*15-18:1, and several *trans*-18:1 isomers may be good candidates (in addition to *trans*10-18:1) as potential inhibitors of milk fat synthesis. Among octadecadienoic acids, those that may have been in part synthesized via desaturation of their corresponding 18:1 isomers (*cis*11-, *trans*11-, *trans*12-, and *trans*13-), could be involved in the reduction of de novo fatty acid synthesis and contribute to an overall decrease in fat synthesis. Good candidates also could be among those fatty acids that increased the most in blood plasma with HCO (for example, *trans*6-18:1 through *trans*11-18:1, *trans*13+14-18:1, and *trans*11,*cis*15-18:2; Tables 4 and 5). Further studies with purified preparations are needed to unravel the role of the different isomers in the regulation of milk fat secretion in ruminants.

Linolenic Acid Intake and Biohydrogenation Intermediates in Plasma and Milk

In terms of actual amounts, responses in *trans*11-18:1, *trans*13+14-18:1, *cis*11-18:1, *cis*15-18:1, *trans*11,*cis*15-18:2, and *cis*9,*trans*11-18:2 due to feeding HCO were more pronounced in plasma (Tables 4 and 5) than in milk (Tables 7 and 8). The major isomers secreted in milk fat were *trans*11-18:1 (up to 27 g/d), *trans*13+14-18:1 (up to 20 g/d), *cis*9,*trans*13-18:2 (up to 4 g/d), and *trans*11,*cis*15-18:2 (up to 13 g/d), whereas the yield of α -linolenic acid remained below 9 g/d. These data are in agreement with duodenal flows of 18:3n-3 and its biohydrogenation intermediates (Loor et al., 2004), and with milk fatty acid responses to increased 18:3n-3 intake (Loor et al., 2002a, 2003; Chilliard et al., 2003c; Kay et al., 2004).

The increase in milk total CLA yield (Table 8) and percentage (Table 9) in response to linseed oil is in agreement with previous milk data (Chilliard et al., 2000) and with their greater duodenal flow plus that of *trans*11-18:1 (Loor et al., 2004). In blood plasma, *trans*11,*trans*13-18:2 was the major CLA followed by

*cis*9,*trans*11-18:2. *Trans*11,*trans*13-18:2 in milk is likely derived from the circulation and its low concentration in milk fat, coupled with the low correlation between plasma concentration and percentage in milk fat (data not shown), may indicate that it was not readily taken up by the mammary gland (Loor and Herbein, 2003).

No changes were found in the percentage or yield of *trans*10,*cis*12-CLA, and it was not correlated to changes in *trans*10-18:1 seen due to concentrate-linseed oil interactions. Thus, contrary to previous studies (Piperova et al., 2000; Peterson et al., 2003) but, in agreement with correlation analyses by Precht et al. (2002) and Chilliard et al. (2003b), this CLA isomer was not associated with changes in milk fat content ($r = 0.23$) or yield in our study.

Apparent Desaturation of Fatty Acids via Δ^9 -Desaturase

Correlation among percentage of *trans*11-18:1 ($r = 0.94$) and percentage of *cis*9,*trans*11-18:2 ($r = 0.97$) in plasma with their respective percentages in milk fat was high as previously shown (Loor et al., 2002b). Basal activity of Δ^9 -desaturase was not affected (e.g., no effect on desaturation ratios of *cis*9-14:1/14:0 and *cis*9-16:1/16:0). A role for Δ^9 -desaturase in desaturation of *trans*-18:1 isomers (Bauman and Griinari, 2003) is supported by the close associations we observed among increases of substrates and products in plasma and milk fat. Yield of *cis*9,*trans*11-18:2, *cis*9,*trans*12-18:2, *cis*9,*trans*13-18:2 in milk increased by up to 2.6, 4, and 4.9 times, respectively, in response to linseed oil, which corresponded with greater duodenal flow (Loor et al., 2004) and availability of *trans*11-18:1, *trans*12-18:1, and *trans*13+14-18:1 in blood plasma (Table 4). From current and previous results (Chilliard et al., 2003ac), it appears that *cis*9,*trans*13-18:2 may be formed via desaturation of *trans*13+14-18:1 which, based on its duodenal flow (Loor et al., 2004), is derived primarily during biohydrogenation of dietary 18:3n-3.

CONCLUSIONS

Milk fat yield decreased by feeding the high-concentrate diet but was much lower with the combination of linseed oil and high dietary concentrate. Yields of 4:0, 6:0, branched-chain fatty acids, 18:0, and *cis*9-18:1 in milk fat were drastically reduced but yields of various *trans*- and *cis*-18:1 isomers (not including *trans*10-18:1), *trans*11,*cis*15-18:2, and *trans* isomers of 18:3 increased when linseed oil was fed in combination with high dietary concentrate. Results, however, indicate that influx of *trans*10-18:1 may affect key regulatory steps in

the pathway for de novo lipogenesis in mammary tissue when cows are fed high-concentrate diets without unsaturated oils. Other *trans*-fatty acids and a decrease in oleic acid secretion, however, must be considered to fully explain the marked depression in milk fat yield with high-concentrate diets supplemented with unsaturated oils. Lower 18:0 availability for endogenous synthesis of *cis*9-18:1 appears to be a primary factor reducing milk fat synthesis when feeding high-concentrate diets and unsaturated oils. It remains to be determined whether a single isomer or a combination of isomers is directly responsible for reduced mammary lipogenesis. The changes in milk fatty acid profile due to oil supplementation, especially when fed with high-concentrate diets, should modify the nutritional value of dairy products.

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