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Reduced Fatty Acid Synthesis and Desaturation Due to Exogenous *trans*10,*cis*12-CLA in Cows Fed Oleic or Linoleic Oil[1]

J. J. Loor[2] and J. H. Herbein
Department of Dairy Science
Virginia Tech
Blacksburg, VA 24061-0315

ABSTRACT

To determine effects of an elevated supply of *cis*9, *trans*11-18:2 (9/11CLA) or *trans*10,*cis*12-18:2 (10/12CLA) on de novo synthesis and desaturation of long-chain fatty acids, four Holstein cows fed high-oleic sunflower (OLE) or high-linoleic safflower oil (LIN) at 2.5% of DM were infused (0.625 g/h) with 9/11CLA or 10/12CLA for 48 h via the abomasum. Treatments were assigned in a 2 × 2 factorial design. The assigned diets were fed for 11 d before each 48-h infusion period. Milk samples were obtained at 12 and 0 h before infusion and at 12-h intervals from 0 to 96 h. Concentrations of *trans*11-18:1 and 18:2n-6 in arterial plasma phospholipid, triglyceride, and FFA fractions were greater due to feeding LIN compared with OLE. Infused 9/11CLA and 10/12CLA were incorporated into plasma triglycerides and FFA primarily. Exogenous 10/12CLA also was found in plasma phospholipids. Milk yield and DMI were not affected by treatments. Percentages and yields of protein, lactose, and SNF in milk also were not affected by treatments. Milk fat percentage and yield, however, decreased 25% from 0 to 96 h in response to infusion of 10/12CLA compared with 9/11CLA. Yields of *trans*11-18:1, 9/11CLA and 18:2n-6 in milk fat before infusion were higher when LIN was fed compared with OLE. Infusion of 9/11CLA, regardless of diet, increased 9/11CLA in milk fat by 44%. Although 10/12CLA was not detectable in milk fat before infusion, it averaged 6 mg/g of total fatty acids and 2 g/d after 48 h. At 48 h, recovery in milk of infused 9/11CLA was 16% compared with 8% for 10/12CLA. Yields of saturated 6:0 to 16:0, *cis*9-18:1, 9/11CLA, and 20:4n-6 were reduced by 10/12CLA infusion. Due to a 40% increase in the concentration of 18:0 by 48 h of 10/12CLA infusion, however, yield of 18:0 was not affected. Ratios of *cis*9-18:1/18:0, 9/11CLA/*trans*11-18:1, and 20:4n-6/18:2n-6 in milk fat decreased in response to infusion of 10/12CLA, regardless of diet. At peak concentration of 10/12CLA, reductions in *cis*9-18:1 and saturated 4:0-16:0 yields accounted for 36% and 53% of the decrease in total fatty acid yield. Results indicated 10/12CLA alters lipid metabolism in the bovine mammary gland by simultaneously reducing de novo synthesis and desaturation. Furthermore, milk triglyceride synthesis may have a stringent requirement for endogenously synthesized oleic acid.
(**Key words:** rumenic acid, biohydrogenation, milk fat, unsaturated oil)

Abbreviation key: CLA = conjugated linoleic acid, **OLE** = high-oleic sunflower oil, **LIN** = high-linoleic safflower oil, **9/11CLA** = *cis*9,*trans*11-18:2 infusion, **10/12CLA** = *trans*10,*cis*12-18:2 infusion, **TG+FFA** = fatty acids in blood plasma triglycerides plus FFA fractions.

INTRODUCTION

Dairy products are the primary natural source of conjugated linoleic acid (**CLA**) isomers in the food chain. The CLA isomers originate from partial hydrogenation of 18:2n-6 in the rumen (Kepler and Tove, 1967; Kemp et al., 1975). Under most dietary conditions, *cis*9,-*trans*11-18:2 is the primary CLA produced (see review by Chilliard et al., 2000). Isomers of CLA are transient intermediates of the hydrogenation process, which leads to preferential accumulation of *trans*11-18:1 and 18:0 (Kepler and Tove, 1967; Kemp et al., 1975). After absorption from the digestive tract, *trans*11-18:1 can be used as a substrate for endogenous synthesis of *cis*9,-*trans*11-18:2, via Δ^9 desaturase (EC 1.14.99.5), in the mammary gland of the cow (see review by Bauman et al., 2001) or human tissues (see review by Pariza et al., 2001). Concentrations of *trans*11-18:1 and *cis*9,*trans*11-18:2 in milk fat can be enhanced by feeding diets containing unsaturated oil with a high linoleic acid content (Chilliard et al., 2000; Bauman et al., 2001). Feeding greater amounts of grain or grain plus unsaturated oil in place of forage causes production of milk fat with

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Corresponding author: J. H. Herbein; e-mail: herbeinj@vt.edu.
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[2]Current address: Department of Animal Sciences, University of Illinois, 206 ERML, Urbana, IL, 61801; E-mail: jloor@uiuc.edu.

lower concentrations of *trans*11-18:1 and greater concentrations of total *trans*-18:1, due to a substantial increase in *trans*10-18:1 primarily (Piperova et al., 2000; Piperova et al., 2002; Loor et al., 2002b). In some cases *trans*10,*cis*12-18:2 also increased by feeding high-grain diets with (Piperova et al., 2000) or without high-linoleic oil (Piperova et al., 2002).

A review of initial studies using dietary CLA mixtures (primarily *cis*9,*trans*11-18:2 plus *trans*10,*cis*12-18:2) indicated CLA isomers had potential anticarcinogenic, antidiabetic, and antilipogenic properties in laboratory animals (Pariza et al., 2001). Subsequent work indicated the *cis*9,*trans*11-18:2 in bovine milk fat was more effective for inhibition of growth of human mammary cancer cells than synthetic *trans*10,*cis*12-18:2 (O'Shea et al., 2000), whereas *trans*10,*cis*12-18:2 was identified as the isomer responsible for reduced lipogenesis (Pariza et al., 2001).

Administration of mixtures of CLA isomers to lactating cows via abomasal infusion reduced de novo fatty acid synthesis and fat yield (Loor and Herbein, 1998; Chouinard et al., 1999a). Reduced concentrations of products of desaturation reactions, however, indicated the CLA mixtures also tended to reduce desaturation of long-chain fatty acids. Subsequently, relatively pure (>90%) sources of CLA isomers allowed Baumgard et al. (2000) to determine that *trans*10,cis12-18:2 was responsible for reduced milk fat percentage and reduced concentrations of saturated medium-chain fatty acids in milk fat. Baumgard et al. (2000, 2001) also found an increase in the ratios (substrate to product) of fatty acid pairs associated with Δ^9 desaturase activity in response to abomasal infusion of *trans*10,*cis*12-18:2. Infused *cis*9, *trans*11-18:2 increased the ratio of 18:0 to *cis*9-18:1 compared with basal (Baumgard et al., 2000). In rat liver homogenates *trans*10,*cis*12-18:2 decreased Δ^9 desaturase activity, whereas *cis*9,*trans*11-18:2 decreased Δ^6 desaturase (EC 1.14.99.25) activity (Pariza et al., 2001). In the mammary gland, portions of diet- or rumen-derived 18:0, *trans*11-18:1, 18:2n-6, and 18:3n-3 may be used as substrates for endogenous synthesis of *cis*9-18:1, *cis*9,*trans*11-18:2, 20:4n-6, and 20:5n-3, respectively, via Δ^9, Δ^5, and Δ^6 desaturase activity (Hermansen et al., 1995; Enjalbert et al., 1998; Bauman et al., 2001).

As indicated above, diets containing an unsaturated oil as a source of linoleic acid can be used as a practical means for enhancing the *cis*9,*trans*11-18:2 content of bovine milk fat. However, the addition of a rumen-protected CLA mixture (Giesy et al., 2002) or purified *trans*10,*cis*12-18:2 to the diet appears to be the only practical method for substantial enhancement of the *trans*10,*cis*12-18:2 content of milk fat. The concentration of *trans*10,*cis*12-18:2 in milk fat was proportional to the daily quantity of *trans*10,*cis*12-18:2 entering the rumen of cows fed a typical TMR (Loor and Herbein, 2001). As expected, however, milk fat yield and apparent desaturation of fatty acids were inversely proportional to *trans*10,*cis*12-18:2 input. It is not known whether a supplemental supply of unsaturated fatty acids in the diet would alleviate the inhibitory effect of *trans*10,*cis*12-18:2 on desaturation of fatty acids, such as 18:0, *trans*11-18:1, 18:2n-6, or 18:3n-3, in the mammary gland. Thus, the objective of this study was to evaluate desaturation of these fatty acids in lactating cows fed high-oleic oil or high-linoleic oil and infused with *cis*9,*trans*11-18:2 or *trans*10,*cis*12-18:2 via the abomasum.

MATERIALS AND METHODS

Animals and Diets

Four midlactation primiparous Holstein cows (126 to 138 DIM) were used in a 2×2 factorial design with four 15-d periods to evaluate responses to a diet containing (2.5% of DM) high-oleic sunflower oil (**OLE**) or high-linoleic safflower oil (**LIN**) combined with abomasal infusion (48 h) of *cis*9,*trans*11-18:2 (**9/11CLA**) or *trans*10,*cis*12-18:2 (**10/12CLA**). Cows were housed in tie stalls and milked at 0100 and 1300 h throughout the study. Diets were formulated using Dair4 (Stallings et al., 1985) to meet the requirements of cows producing 30 kg milk and consuming 19 kg DM daily (NRC, 1989). Diets were fed as a TMR (Table 1) in equal amounts at 1400 and 0200 h. The amount of TMR offered was enough to allow 5 to 10% feed refusal, which was weighed at 1400 h. Cows initially were fed a basal diet (similar to OLE and LIN, but without oil), which was replaced incrementally (0, 25, 50, 75, then 100%) with a mixture of equal parts OLE and LIN to allow cows to adapt to a diet containing oil. During 4 d before the start of the first period and 4 d between each of 15-d periods, the incremental replacement procedure was used to provide a transition from the previous diet (equal parts OLE and LIN for all cows before the first period) to the assigned diet for the next period (OLE or LIN in the following periods). Intake of DM was measured every 12 h during d 11 through d 15. Continuous infusion of 9/11CLA or 10/12CLA via the abomasum began at 1400 h on d 11 and continued for 48 h. Milk samples were obtained at each milking from d 11 through 15 (–12 to 96 h relative to the start of the 48-h infusion). Milk was collected in a stainless steel bucket, weighed, and thoroughly mixed before sampling. The experimental protocol was reviewed and approved by the Virginia Polytechnic Institute and State University Animal Care Committee.

Table 1. Ingredient, chemical composition, and fatty acid profiles of diets supplemented at 2.5% of DM with high-oleic sunflower oil (OLE) or high-linoleic safflower oil (LIN).[1]

	OLE	LIN
Ingredients	% of DM	
Alfalfa silage	28.5	28.5
Corn silage	13.5	13.5
Orchardgrass hay	7.0	7.0
Ground corn	35.7	35.7
Soybean meal, 48% CP	7.6	7.6
SoyPlus[2]	3.5	3.5
Sunflower oil[3]	2.5	0.0
Safflower oil	0.0	2.5
Mineral/vitamin mix[4]	1.1	1.1
Limestone	0.4	0.4
Dicalcium phosphate	0.2	0.2
Chemical composition		
NDF	34.4	34.4
ADF	22.1	22.3
CP	16.0	16.0
Total fatty acids[5]	5.3	5.3
	-mg/g of Total fatty acids -	
14:0	1	2
16:0	91	117
*cis*9-16:1	2	2
18:0	29	34
*cis*9-18:1	555	242
18:2n-6	292	573
18:3n-3	30	30

[1]Four samples (collected in each period) of forages and supplements were composited and analyzed in duplicate.

[2]SoyPlus (West Central Cooperative, Ralston, IA) g/kg: CP = 483, RUP = 600 (g/kg CP), fatty acids = 49, and NE_L = 2.10 Mcal/kg.

[3]OLE or LIN (Columbus Foods Co., North Albany, IL).

[4]Mineral/vitamin mix (Southern States Cooperative, Richmond, VA) g/kg: salt (38-48), $NaHCO_3$ (180), Ca (145-174), P (65), Cl (58), S (32), Mg (22), K (35), Mn (1), Zn (1), Fe (0.3), Cu (0.1), I (0.02), Co (0.003), Se (0.005), F (0.65), retinyl acetate (0.36), cholecalciferol (0.01), dl-α-tocopherol acetate (0.59).

[5]Total fatty acids = 14:0 + 16:0 + *cis*9-16:1 + 18:0 + *cis*9-18:1 + 18:2n-6 + 18:3n-3.

Infusion Procedures

Before 48-h infusion of 9/11CLA or 10/12CLA (Natural Lipids, Norway) the CLA mixtures (Table 2), were emulsified in skim milk. Due to differences in purity of CLA mixtures, it required 16.5 g of 9/11CLA and 15.6 g of 10/12CLA to obtain a dose of 15 g of each isomer. Emulsions were prepared the day before an infusion by combining 15 g of CLA with 3.5 g of glycerol (Eastman Kodak Co., Rochester, NY) and 1.8 g of soy lecithin powder (Refined, Alfa, Ward Hill, MA) in 975 ml of skim milk at room temperature. The mixture was homogenized at 12,000 rpm for 2 min with a Polytron homogenizer (PT 10/35, Brinkmann Instruments, Westbury, NY). Emulsions were dispensed into 1 L Viaflex plastic bags (Baxter Corporation, Deerfield, IL) and stored at 4°C. During infusion, bags were attached to a platform on a wrist-action shaker (Burrell Corporation, Pittsburgh, PA) set at low speed. Emulsions flowed through Tygon tubing (1.6 mm i.d., 0.8-mm wall; Fisher Scientific Co., Pittsburgh, PA) to a Harvard Peristaltic pump (55-1762; Harvard Apparatus, South Natick, MA). Flow from the pump was via Tygon tubing (3.2 mm i.d., 1.6-mm wall) passed through a rumen cannula, rumen, and omasum before terminating in the abomasum. A perforated Nalgene plastic bottle (60 ml) was attached to the end of the tubing to secure it in the abomasum. The tubing was primed with 15 ml of emulsion at the start of infusion, and flow rate was set at 41.7 ml/h. After 24 h, the empty bag was replaced with another bag containing 1 L of emulsion with 15 g of CLA.

Sampling, Measurements, and Analysis

Forages and the concentrate portion of each TMR were sampled on the last day of each period. Samples were dried in a forced-air oven at 60°C and stored in sealed containers at room temperature until analyzed. Equal amounts of samples from each period were combined to determine chemical composition (Table 1). In preparation for analyses, dried forages and concentrates were ground first through a 2-mm screen (Thomas-Wiley Laboratory Mill, Arthur H. Thomas, Philadelphia, PA), then through a 1-mm screen in a

Table 2. Fatty acid composition of dietary oils and CLA mixtures.

	High-oleic sunflower oil	High-linoleic safflower oil	*cis*9,*trans*11-CLA (9/11CLA)	*trans*10, *cis*12-CLA (10/12CLA)
Fatty acid	mg/g of Total fatty acids			
14:0	1	1	0	0
16:0	46	59	0	11
*cis*9-16:1	1	1	0	0
18:0	23	24	0	5
*cis*9-18:1	793	152	83	4
18:2 isomers				
*cis*9,*cis*12	132	760	0	0
*cis*9,*trans*11	0	0	907	18
*trans*10,*cis*12	0	0	10	962
18:3n-3	4	3	0	0

Cyclotec mill (Tecator 1093, Hoganas, Sweden). Ground samples were analyzed for ADF and NDF (Van Soest et al., 1991), total N (AOAC, 1990), and fatty acid content.

Two 50-ml aliquots of milk were collected at –12, 0, 12, 24, 36, 48, 60, 72, 84, and 96 h relative to the start of infusion. The first aliquot containing Bronopol (D & F Control Systems, San Ramon, CA) was stored at 4°C until analyzed for fat, protein, SNF, and lactose (AOAC, 1990) by infrared analysis with a four-channel spectrophotometer (Multispec, Foss Food Technology Corp., Eden Prairie, MN) at the Virginia Dairy Herd Improvement Association laboratory. The second aliquot was stored at –20°C until the end of the study, thawed, and centrifuged at 10,000 × *g* for 1 h to harvest milk fat for fatty acid analysis.

Plasma total fatty acid profiles were determined using blood samples (10 ml) obtained from the coccygeal artery/vein after collection of milk samples. Profiles of fatty acids in arterial blood plasma lipid fractions and estimated fatty acid extraction ratios were determined using coccygeal artery and subcutaneous mammary vein samples (10 ml) obtained at 2-h intervals from –12 to 0 h and 36 to 48 h relative to the start of a CLA infusion. Blood was transferred to tubes containing 286 IU of heparin in 100 μl of sterile saline and centrifuged at 3,000 × *g* for 15 min for harvesting plasma. An equal volume of plasma from each of the six arterial or six venous samples obtained before (–12 to 0 h) and during (36 to 48 h) CLA infusion was pooled for isolation of plasma lipid fractions. Plasma was stored at –20°C until lipid extraction.

Total lipids were extracted from all plasma samples with chloroform/methanol (2:1, vol/vol). Lipid fractions (FFA, phospholipids, cholesterol esters, and triglycerides) in arterial and venous samples obtained before and during CLA infusion were isolated (Ågren et al., 1992) using 500 mg of Bond Elut aminopropyl columns (Varian, Walnut Creek, CA). Fatty acids in forages, concentrates, milk fat, blood plasma (total fatty acid profiles), and plasma lipid fractions were methylated by in situ transesterification with 0.5 N methanolic NaOH in methanol followed by 14% boron trifluoride in methanol (Loor and Herbein, 2001). Undecenoate (Nu-Check Prep, Elysian, MN) was used as the internal standard. Samples were injected by an autosampler into a Hewlett-Packard 5890A gas chromatograph equipped with a flame ionization detector (Hewlett-Packard, Sunnyvale, CA). Methyl esters were separated on a 100 m × 0.25 mm i.d. fused silica capillary column (CP-Sil 88,Chrompack, Middleburg, The Netherlands).

To identify peaks and determine response factors for individual fatty acids, known quantities of pure methyl esters were combined to obtain a calibration standard mixture with a total of 52 fatty acids. A custom preparation (Virginia Tech DaSc479, Nu-Check Prep, Elysian, MN), designed to resemble a typical milk fat, containing a total of 25 pure methyl esters (4:0 to 22:5n-3) was used as a base to which individual 18:1 and 18:2 isomers were added. Pure *trans*9-18:1, *trans*11-18:1, *cis*9-18:1, and *cis*11-18:1 methyl esters were purchased from Nu-Check Prep (Elysian, MN). *Trans*6-18:1, *trans*7-18:1, *trans*12-18:1, *cis*12-18:1, and *cis*13-18:1 were purchased from Sigma Chemical Co. (St. Louis, MO). *Trans*13-18:1 and *cis*15-18:1 were purchased from Supelco Inc. (Bellefonte, PA). The nonconjugated 18:2 isomer mixture was purchased from Sigma Chemical Co. (St. Louis, MO), and contained *trans*9,*trans*12-18:2, *cis*9,-*trans*12-18:2, *trans*9,*cis*12-18:2, and *cis*9,*cis*12-18:2. The conjugated linoleic acid mixture (Nu-Check Prep, Elysian, MN) contained *cis*9,*trans*11-18:2, *trans*8,*cis*10-18:2, *cis*11,*trans*13-18:2, *trans*10,*cis*12-18:2, *cis*9,*cis*11-18:2, *cis*10,*cis*12-18:2, *cis*11,*cis*13-18:2, *trans*11, *trans*13-18:2, and *trans*,*trans*-18:2. *Trans*10-18:1, *trans*16-18:1, and *trans*11,*cis*15-18:2 were not available commercially. They were identified by order of elution as described in Griinari et al. (1998) and Ulberth and Henninger (1994). The response factor for 18:0 was used to quantify these fatty acids.

An 80 to 1 split ratio was used for injection of 0.5 μl hexane containing methyl esters of fatty acids from forage, concentrate, or milk fat samples. The carrier gas was ultrapure hydrogen, and inlet pressure was maintained at 23.1 psi. Injector temperature was maintained at 250°C, and detector temperature was maintained at 255°C. The initial oven temperature was 70°C (held for 1 min), increased 5°C/min to 100°C (held for 2 min), increased 10°C/min to 175°C (held for 40 min), and increased 5°C/min to 225°C (held for 15 min).

Injections of 0.5 μl methyl esters in hexane (splitless) were used for arterial plasma (total fatty acid profiles). The purge valve was closed for 0.8 min after injection. Injections of 2.5 μl were used for arterial and venous plasma lipid fractions, and the purge valve was closed for 1.5 min after injection. For both analyses, the injector and detector temperatures were 250 and 275°C. The initial column temperature was 40°C (held for 1.5 min), increased 40°C/min to 100°C (held for 10 min), increased 20°C/min to 175°C (held for 45 min), and increased 10°C/min to 220°C (held for 25 min).

Mammary gland extraction ratios for individual fatty acids were estimated using the sum of the amount of a fatty acid in the triglyceride fraction plus the amount in the FFA fraction (**TG**+**FFA**) of arterial and venous samples (Enjalbert et al., 1998). Extraction (%) of fatty acids from arterial plasma was calculated as [(arterial - venous concentration)/arterial concentration] × 100.

Statistical Analysis

Data for DMI, milk production, milk composition, fatty acid intake, milk fatty acids, and ratios of milk fatty acids were analyzed as a Latin square with factorial arrangement of treatments and repeated measures using the MIXED procedure of SAS (2000). Compound symmetry was the covariate structure used for all repeated measures analysis. The statistical model included cow, period, oil supplement, CLA isomer, time, CLA isomer × oil interaction, oil × time interaction, CLA isomer × time interaction, oil × CLA isomer × time interaction, and residual error. Fixed effects in the model included period, oil supplement, CLA isomer, time, oil × isomer interaction, and oil × isomer × time interaction. Cow was the random effect. Data for fatty acid profiles in blood plasma and mammary fatty acid extraction were analyzed as a Latin square with factorial arrangement of treatments without repeated measures using the MIXED procedure of SAS (2000). The statistical model included cow, period, oil supplement, CLA isomer, oil × isomer interaction, and residual error. Fixed effects in the model included: period, oil supplement, CLA isomer, and oil × isomer interaction. Cow was the random effect. One cow in the high-oleic oil group receiving 9/11CLA was omitted from all statistical analyses from that period, because the infusion line inadvertently was dislodged from the abomasum into the rumen. Overall differences between treatment least squares means were considered significant at $P \leq 0.05$, but all P values are presented in tables.

RESULTS

Dry Matter Intake and Milk Production and Composition

Overall, dry matter intake and milk production throughout the 96-h sampling period did not differ in response to diet or CLA isomer (Table 3). Percentages (data not shown) and yields (Table 3) of protein, lactose, and SNF in milk also did not differ. Milk fat percentage from 24 to 96 h, however, was substantially reduced by infusion of 10/12CLA, regardless of diet (Figure 1). The lower overall fat concentration in response to 10/12CLA reduced overall milk fat yield (Table 3) by 25% compared with 9/11CLA.

Fatty Acid Intake and Total Plasma Fatty Acid Concentrations

Estimated total fatty acid intake (g/d) from oil-supplemented diets was similar for all treatments and averaged 1071 g/d (Table 4). Intakes of 14:0, 16:0, *cis*9-16:1, and 18:0 were slightly, but significantly, higher when LIN was fed. As expected, the primary fatty acid in the DM was *cis*9-18:1 when OLE was fed and 18:2n-6 when LIN was fed. Concentration of total fatty acids in blood plasma at the end of the 48-h infusion period (1,957 μg/ml) was similar for all treatments (Table 5). When cows were fed OLE, concentrations of *cis*9-18:1, *trans*6/7/8-18:1, *trans*9-18:1, and 18:3n-3 were greater compared with feeding LIN. When cows were fed LIN, concentrations of 18:2n-6 and the primary biohydrogenation intermediates, *cis*9,*trans*11-18:2 and *trans*11-18:1 were elevated in plasma. In addition, concentrations of *cis*12-18:1, *trans*12-18:1, and *trans*16-18:1 were elevated when LIN was fed. Concentrations of *cis*9,-*trans*11-18:2 in plasma increased in response to 9/11CLA infusion, whereas *trans*10-18:1 and *trans*10,*cis*12-18:2 were elevated in response to 10/12CLA infusion.

Fatty Acid Distribution in Blood Plasma Lipid Fractions

Samples obtained between 36 and 48 h were used to determine the distribution of *cis*9-18:1, 18:2n-6, *trans*11-18:1, and CLA isomers in blood plasma lipid fractions for transport to the mammary gland (Figure 2). Concentrations (milligrams per gram total fatty acids in each lipid fraction) of *cis*9-18:1 and 18:2n-6 in all lipid fractions reflected the amount of each fatty acid contained in OLE or LIN. Oleic acid concentration in phospholipids, cholesteryl esters, triglycerides, and FFA when OLE was fed averaged 100, 32, 97, and 96 mg/g, respectively, compared with 53, 19, 50, and 53 mg/g when LIN was fed. In contrast, 18:2n-6 concentration in phospholipids, cholesteryl esters, triglycerides, and FFA due to feeding LIN averaged 450, 880, 100, and 100 mg/g, respectively, compared with 370, 850, 50, and 50 mg/g when OLE was fed.

The concentration of *trans*11-18:1 in phospholipids, triglycerides, and FFA was elevated when cows were fed LIN compared with OLE regardless of isomer (Figure 2). Overall, the concentrations of individual CLA isomers in triglycerides and FFA increased in proportion to the amount of isomer infused. *Trans*10,*cis*12-18:2 was detectable only when 10/12CLA was infused, and averaged 2, 11, and 6 mg/g, respectively, in phospholipids, triglycerides, and FFA. The elevated concentrations of CLA isomers in blood plasma lipids at 36 to 48 h corresponded with the peak in their concentrations in milk fat (data not shown).

Fatty Acids in Arterial Plasma Triglycerides plus Free Fatty Acids

To estimate the primary pool of fatty acids available to the mammary gland for uptake and incorporation into

Table 3. Dry matter intake, milk production, and milk component yields by cows fed diets containing (2.5% of DM) high-oleic sunflower oil (OLE) or high-linoleic safflower oil (LIN) and infused into the abomasum for 2 d with 15 g/d *cis*9,*trans*11-CLA (9/11) or *trans*10,*cis*12-CLA (10/12).[1]

		OLE			LIN			P^2		
	Basal[3]	9/11	10/12	Basal	9/11	10/12	SEM	O	I	O × I
Variable	kg/d									
DMI	19.6	20.4	19.8	19.8	19.8	19.6	0.5	0.38	0.46	0.69
Milk yield	28.8	30.4	29.6	28.8	29.8	30.0	1.1	0.86	0.47	0.18
Fat	0.98	1.04	0.78	0.98	1.00	0.76	0.04	0.07	0.01	0.35
Protein	0.86	0.92	0.90	0.86	0.90	0.88	0.03	0.30	0.15	0.56
Lactose	1.34	1.40	1.36	1.34	1.38	1.40	0.20	0.77	0.52	0.19
SNF	2.40	2.54	2.50	2.42	2.54	2.52	0.20	0.60	0.26	0.59

[1]Values are the average of means ± pooled SEM for four cows, except for OLE-9/11CLA with three cows, obtained from 12 through 96 h of infusion.

[2]Probability of difference between treatment means due to oil (O), CLA isomer (I), and their interaction (O × I).

[3]Basal = the means of observations at -12 and 0 h before infusion are shown for comparison only.

milk fat, the sum of individual fatty acids in plasma TG+FFA was determined. Concentration of total TG+FFA in coccygeal blood plasma at 48 h averaged 99 μg/ml and did not differ across treatments (Table 6). When cows were fed OLE concentrations of *cis*9-18:1 and 18:3n-3 in TG+FFA were 76 and 38% greater compared with feeding LIN. In contrast, feeding LIN resulted in elevated concentrations of 18:2n-6 and *cis*12-, *cis*15-, *trans*10-, *trans*11-, *trans*12-, *trans*13/14-, and *trans*16-18:1. Infusing 10/12CLA, regardless of diet, elevated concentrations of *cis*11-, *cis*12-, *cis*13-, *trans*6/7/8-, *trans*9-, *trans*10-, and *trans*12-18:1. Concentrations of 14:0 and 18:3n-3 in TG+FFA also were elevated by 10/12CLA infusion. Greater concentrations of *cis*9,*trans*11-18:2 or *trans*10,*cis*12-18:2 in TG+FFA was expected in response to infusion of either CLA isomer, but alterations in concentrations of the *cis*- and *trans*-isomers of 18:1, 14:0, or 18:3n-3 were not. The cause(s) of the response may be due to reduced need of exogenous fatty acids for milk fat synthesis or the effects of 10/12CLA on tissues other than the mammary gland.

Extraction Ratios of Fatty Acids by the Mammary Gland

Mammary gland extraction of total fatty acids from TG+FFA did not differ due to treatments and averaged 39% (Table 7). Despite greater concentrations of several fatty acids in TG+FFA in response to OLE or LIN, extraction ratios for most fatty acids did not differ due to treatment. The exception was extraction of 18:2n-6, which was greater when cows were fed LIN compared with OLE. Infusion of 10/12CLA resulted in lower extraction of 18:0 and higher extraction of *cis*9,*trans*11-18:2, regardless of diet. Extraction of *trans*10,*cis*12-18:2 during 10/12CLA infusion averaged 81%, which was numerically higher than the extraction ratios for all other fatty acids flowing to the mammary gland in blood plasma.

Milk Fatty Acid Yields

Concentration of saturated fatty acids with 6 to 16 carbons (Figure 1), regardless of diet, decreased 19% from 0 to 60 h in response to 10/12CLA then remained low until 96 h. Overall yields of fatty acids with 4 to 16 carbons were reduced when 10/12CLA was infused (Table 8). Reductions in the yields of the short- and medium-chain fatty acids were the primary cause of the 19% reduction in the yield of total fatty acids in response to 10/12CLA.

*Trans*10,*cis*12-18:2 was not detectable in milk fat before infusion, but its concentration peaked (6 mg/g total fatty acids) at 48 h then remained elevated in milk fat when cows were infused with 10/12CLA (data not shown). Yield of *trans*10,*cis*12-18:2 averaged 2 g/d from 0 to 96 h. The basal concentration before infusion of *cis*9,*trans*11-18:2 (10 mg/g) was greater due to feeding LIN compared with OLE and accounted for the effect of diet on its yield in milk fat (Table 8). Similarly, the basal concentration of *trans*11-18:1 was greater when LIN was fed compared with OLE (Figure 3). During 10/12CLA infusion, concentration of *cis*9,*trans*11-18:2 in milk fat was reduced by 40% when cows were fed LIN. As a result, yield of *cis*9,*trans*11-18:2 decreased with 10/12CLA infusion.

The basal concentration of 18:0 in milk fat did not differ due to diet (144 mg/g), but the 18:0 concentration increased by approximately 40% during 10/12CLA infusion (Figure 3). As a result of the lower yield of milk fat in response to 10/12CLA infusion, however, the yield of 18:0 from 0 to 60 h did not differ due to type of infusion (Table 8). The concentration of *cis*9-18:1 in milk fat did not change during infusion of 9/11CLA

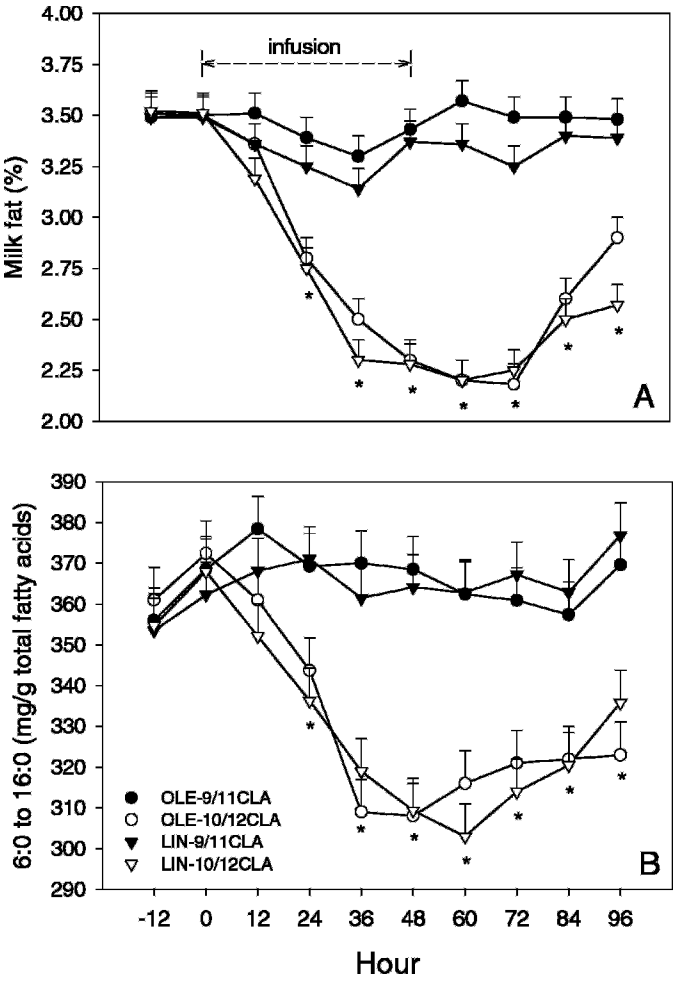

Figure 1. Milk fat percentage (panel A) and concentration of 6:0 to 16:0 saturated fatty acids (panel B) in milk fat from cows fed high-oleic (OLE) or high-linoleic (LIN) oil, and infused into the abomasum with *cis*9,*trans*11-18:2 (9/11CLA) or *trans*10,*cis*12-18:2 (10/12CLA) for 48 h. Values are means plus pooled SEM for four cows, except for OLE-9/11CLA with three cows, at each 12-h interval. Asterisks indicate a significant ($P < 0.05$) time by CLA isomer interaction.

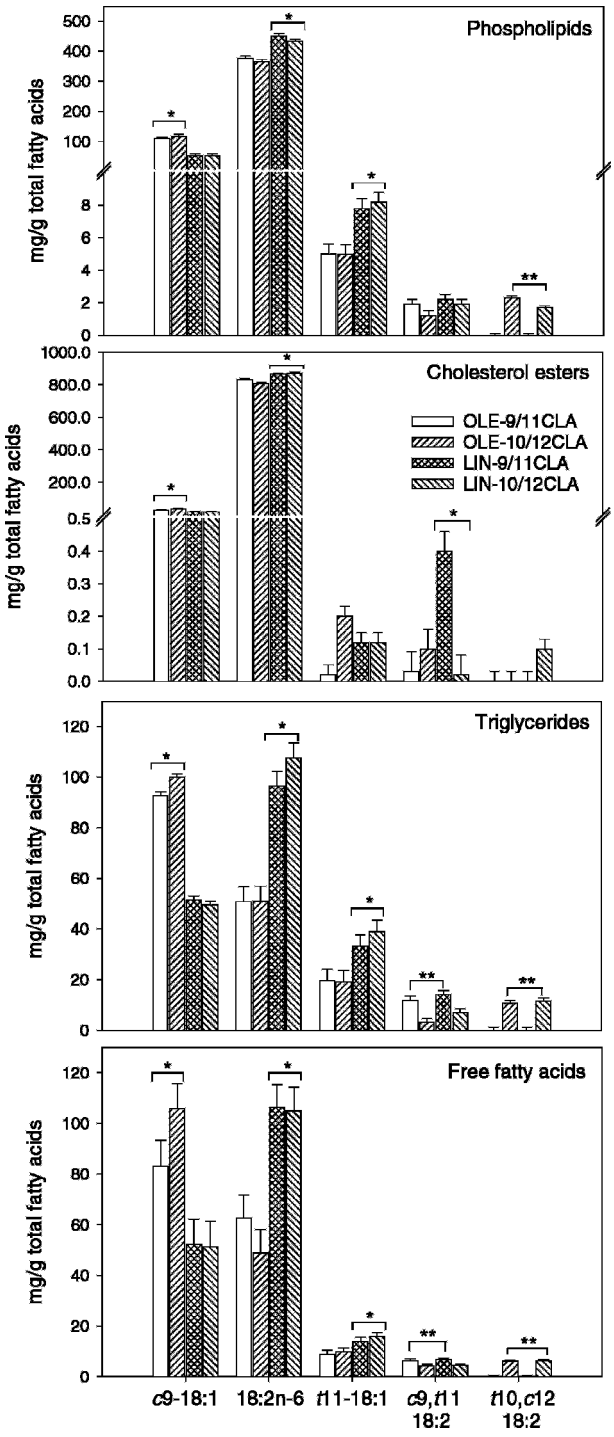

Figure 2. Distribution (mg/g total fatty acids in each fraction) of *cis*9-18:1, 18:2n-6, *trans*11-18:1, *cis*9,*trans*11-18:2, and *trans*10,*cis*12-18:2 at 48 h in blood plasma phospholipids, cholesterol esters, triglycerides, or free fatty acids from cows fed high-oleic (OLE) or high-linoleic (LIN) oil, and infused into the abomasum with *cis*9,*trans*11-18:2 (9/11CLA) or *trans*10,*cis*12-18:2 (10/12CLA) for 48 h. Values are means plus pooled SEM for four cows, except for OLE-9/11CLA with three cows. Asterisks denote differences ($P < 0.05$) due to oil (*) or isomer (**).

when cows were fed OLE or LIN (data not shown). However, when LIN was fed and 10/12CLA was infused, concentration of *cis*9-18:1 was 12% lower from 24 to 60 h (significant diet × isomer × time interaction). The greater availability of dietary *cis*9-18:1 when OLE was fed, compared with LIN, probably masked the potential effects of 10/12CLA infusion on desaturation of 18:0. Indicators of inhibition of desaturase activity are discussed in the "normalized ratios" section below.

Cows fed LIN had higher basal concentrations of 18:2n-6 (Figure 3) and 20:4n-6 (16 vs. 13 mg/g) (data not shown). Similar to the response in 18:0 concentration, infusion of 10/12CLA increased the concentration of 18:2n-6 and decreased 20:4n-6 concentration (data not shown). As noted above, however, the depressed yield

Table 4. Fatty acid intake by cows fed diets containing (2.5% of DM) high-oleic sunflower oil (OLE) or high-linoleic safflower oil (LIN) and infused into the abomasum for 2 d with 15 g/d *cis*9,*trans*11-CLA (9/11) or *trans*10,*cis*12-CLA (10/12).[1]

		OLE			LIN			P^2		
	Basal[3]	9/11	10/12	Basal	9/11	10/12	SEM	O	I	O × I
Fatty acid	g/d									
14:0	1	1	1	2	2	2	0.1	0.01	0.40	0.78
16:0	99	100	97	125	125	124	6	0.01	0.39	0.74
*cis*9-16:1	1	2	2	2	2	2	0.1	0.01	0.39	0.72
18:0	32	32	31	36	36	36	2	0.01	0.39	0.72
*cis*9-18:1	598	603	591	257	258	255	20	0.01	0.49	0.67
18:2n-6	318	324	312	599	611	504	23	0.01	0.42	0.81
18:3n-3	33	33	32	32	32	32	2	0.69	0.39	0.71
Total	1082	1096	1067	1063	1066	1054	52	0.37	0.39	0.70

[1]Values are the average of means ± pooled SEM for four cows, except for OLE-9/11CLA with three cows, obtained from 12 through 96 h of infusion.

[2]Probability of difference between treatment means due to oils (O), CLA isomer (I), and their interaction (O × I).

[3]Basal = the means of observations at -12 and 0 h before infusion are shown for comparison only.

of nearly all fatty acids (Table 8) masked the divergent changes in concentrations of substrate/product fatty acid pairs for desaturase reactions.

Normalized Ratios of Milk Fatty Acids

Normalized ratios (mg/g product ([mg/g substrate + mg/g product]) were estimated to assess the extent of desaturation of specific fatty acids during milk fat synthesis (Sol-Morales et al., 2000). The basal ratios of *cis*9-14:1/14:0, *cis*9-16:1/16:0, *cis*9-18:1/18:0, *cis*9,-*trans*11-18:2/*trans*11-18:1, and 20:4n-6/18:2n-6 were higher when cows were fed OLE compared with LIN (Table 9). Higher ratios indicated cows desaturated more of the substrate fatty acids when they were fed OLE compared with LIN. Compared with 9/11CLA, however, infusion of 10/12CLA decreased the above ratios, regardless of diet. Lower ratios were evident after only 24 h of 10/12CLA infusion, regardless of diet, and were maintained until 72 h (data not shown). The decline in the ratios suggested exogenous *trans*10,*cis*12-18:2 reduced the amount and(or) activity of Δ^6, Δ^5, and Δ^9 desaturases in the mammary gland.

DISCUSSION

Regardless of CLA isomer infused, feeding high-oleic or high-linoleic oil did not affect total fatty acid intake but more than doubled intakes of *cis*9-18:1 or 18:2n-6 (Table 4). Differences in intakes of *cis*9-18:1 and 18:2n-6 due to type of oil fed led to major changes in the profiles of most fatty acids in blood plasma lipids. Oleic acid accounted for 10% of total fatty acids in triglycerides, FFA, and phospholipids when high-oleic oil was fed (Figure 2). Linoleic acid also accounted for 10% of triglycerides and FFA but was 42% of total fatty acids in phospholipids when high-linoleic oil was fed. These three lipid fractions contained 53% of total fatty acids in blood plasma. Plasma cholesteryl esters also contained more *cis*9-18:1 or 18:2n-6 in response to intake of high-oleic or high-linoleic oil, but 18:2n-6 averaged 85% of total fatty acids regardless of oil type.

Concentrations of isomers derived from isomerization and hydrogenation of dietary *cis*9-18:1 or 18:2n-6 also were proportional to intake of both fatty acids from the diet. For example, feeding high-oleic oil increased concentrations of *trans*6/7/8-18:1 and *trans*9-18:1 by 86 and 57% in blood plasma compared with feeding high-linoleic oil. Concentrations of *cis*12-18:1 and *trans*10-, *trans*11-, *trans*12-, or *trans*16-18:1, however, were 69, 31, 82, 42, and 60%, respectively, greater in response to feeding high-linoleic oil compared with high-oleic oil. *Trans*11-18:1 was the primary *trans*-18:1 isomer in blood plasma, and it accounted for 42 or 55% of total *trans*-18:1 when cows were fed high-oleic or high-linoleic oil, respectively.

Among lipid fractions, triglycerides and FFA contained 2 to 4% *trans*11-18:1, whereas phospholipids contained 0.8% *trans*11-18:1 (Figure 2). Overall, *trans* isomers of 18:1 were primarily found in triglycerides (7% of total fatty acids), FFA (5%), and phospholipids (2%). Greater flow of *trans*-18:1 isomers to the duodenum during hydrogenation of supplemental 18:2n-6 in the rumen increased absorption and incorporation of 18:1 and 18:2 isomers into blood plasma triglycerides and phospholipids (Bickerstaffe et al., 1972; Loor et al., 2002c). Thus, upon hydrolysis of triglycerides and phospholipids, the plasma FFA pool also contains more *trans*-18:1 isomers. Our results confirmed previous in vivo and in vitro observations, indicating ruminal hy-

Table 5. Concentrations of fatty acids in blood plasma from cows fed diets containing (2.5% of DM) high-oleic sunflower oil (OLE) or high-linoleic safflower oil (LIN) and infused into the abomasum for 2 d with 15 g/d *cis*9,*trans*11-CLA (9/11) or *trans*10,*cis*12-CLA (10/12).[1]

		OLE			LIN			P[2]		
	Basal[3]	9/11	10/12	Basal	9/11	10/12	SEM	O	I	O × I
Fatty acid	μg/ml									
14:0	5.3	4.9	6.8	5.7	5.8	6.6	0.1	0.55	0.06	0.41
*cis*9-14:1	0.1	0.1	0.1	0.1	0.1	0.1	0.0	0.17	0.06	0.40
16:0	111	92	110	107	108	118	13	0.25	0.18	0.70
*cis*9-16:1	7.4	6.2	7.7	7.1	5.8	6.4	0.8	0.30	0.21	0.59
18:0	201	170	192	191	194	202	22	0.35	0.41	0.69
Cis 18:1										
9	113	95	106	62	59	63	6	0.01	0.16	0.46
11	2.8	2.8	3.4	2.6	2.5	3.0	0.3	0.22	0.06	0.76
12	6.7	4.5	6.1	9.5	8.7	9.2	1.2	0.02	0.35	0.66
13	0.7	0.4	0.5	0.8	0.3	0.4	0.1	0.24	0.11	0.93
15	0.8	0.7	0.7	0.7	0.9	1.0	0.1	0.10	0.60	0.54
Trans 18:1										
6 + 7 + 8	1.8	1.2	1.5	0.9	0.7	0.8	0.1	0.01	0.07	0.43
9	0.9	1.0	1.1	0.6	0.7	0.8	0.1	0.01	0.12	0.60
10	1.7	1.0	1.5	1.8	1.3	2.0	0.1	0.01	0.01	0.53
11	4.8	4.0	4.7	7.8	7.6	8.3	1.0	0.01	0.44	0.99
12	1.9	1.6	2.2	2.6	2.5	2.9	0.3	0.03	0.09	0.67
13 + 14	4.7	3.6	3.9	4.9	4.4	4.6	0.4	0.07	0.47	0.90
16	0.7	0.6	0.5	0.9	0.6	0.9	0.1	0.02	0.07	0.01
Nonconjugated 18:2										
*cis*9,*cis*12	1006	844	972	1091	1089	1136	120	0.05	0.40	0.70
*trans*9,*trans*12	0.2	0.1	0.4	0.2	0.2	0.2	0.2	0.66	0.44	0.44
*cis*9,*trans*12	0	0.8	0.2	0.1	0.1	0.1	0.3	0.21	0.31	0.39
*trans*9,*cis*12	0.1	0.1	0	0.2	0.6	0	0.2	0.22	0.14	0.22
*trans*11,*cis*15	1.7	1.1	1.4	0.8	1.2	1.3	0.2	0.56	0.06	0.27
Conjugated 18:2										
*cis*9,*trans*11	1.6	2.7	1.5	1.9	3.8	2.5	0.7	0.05	0.03	0.91
*trans*10,*cis*12	0	0	3.0	0	0	3.0	0.3	0.98	0.01	0.61
18:3n-3	51	44	53	33	32	35	5	0.01	0.18	0.53
20:3n-6	27	25	29	26	26	29	4	0.72	0.18	0.81
20:4n-6	30	28	33	31	29	35	6	0.51	0.05	0.91
20:5n-3	8.2	8.1	11	9.8	8.2	11	2	0.90	0.06	0.62
Total	1870	1922	2169	2093	1920	1818	144	0.08	0.40	0.08

[1]Values are the average of means ± pooled SEM for four cows, except for OLE-9/11CLA with three cows, obtained at the end of CLA infusion (48 h).

[2]Probability of difference between treatment means due to oil (O), CLA isomer (I), and their interaction (O × I).

[3]Basal = the means of observations at -12 and 0 h before infusion are shown for comparison only.

drogenation of *cis*9-18:1 and 18:2n-6 gives rise to geometrical isomers of 18:1 with double bonds at positions 6 through 16 of the carbon chain (Bickerstaffe et al., 1972; Kemp et al., 1975; Loor et al., 2002a). Ruminal isomerization of oleic acid was associated with production of several *trans*18:1 but primarily *trans*6/7/8-18:1, *trans*9-18:1, and *trans*10-18:1 (Loor et al., 2002a; Mosley et al., 2002). Under normal circumstances, however, *trans*11-18:1 is by far the predominant *trans*-18:1 isomer resulting from hydrogenation of 18:2n-6 in the rumen.

Whereas *cis*9,*trans*11-18:2 in plasma was proportional to dietary 18:2n-6 intake, *trans*10,*cis*12-18:2 was detectable only after 10/12CLA was infused (Table 5). Infused 9/11CLA was primarily incorporated into plasma FFA and triglycerides, where it averaged 7 to 13 mg/g of total fatty acids. During infusion of 10/12CLA, regardless of diet, concentration of *trans*10,*cis*12-18:2 increased from nondetectable levels to 15 mg/g in total blood plasma (Table 5). Its concentration in triglycerides, FFA, and phospholipids averaged 11, 6, and 2 mg/g after infusion of 10/12CLA (Figure 2). The triglyceride and FFA fractions contained approximately 3% of total fatty acids in plasma, whereas the phospholipid fraction contained approximately 48% of total plasma fatty acids. However, absolute amounts of *cis*9,*trans*11-18:2 or *trans*10,*cis*12-18:2 in the three fractions during infusion of 9/11CLA or 10/12CLA was similar. To our knowledge, these are the first data available on incorporation of *trans*10,*cis*12-18:2 in bovine plasma lipids. The increase in the proportions of *cis*9,*trans*11-18:2 or *trans*10,*cis*12-18:2 in triglycerides and FFA indicated

Table 6. Arterial concentrations of fatty acids in blood plasma triglycerides plus FFA in cows fed diets containing (2.5% of dry matter) high-oleic sunflower oil (OLE) or high-linoleic safflower oil (LIN) and infused into the abomasum for 2 d with 15 g/d *cis*9,*trans*11-CLA (9/11) or *trans*10,*cis*12-CLA (10/12).[1]

		OLE			LIN			P[2]		
	Basal[3]	9/11	10/12	Basal	9/11	10/12	SEM	O	I	O × I
Fatty acid	μg/ml									
14:0	2.1	2.1	2.5	1.8	2.1	2.6	0.2	0.74	0.02	0.56
16:0	15.5	16.7	17.4	14.5	16.9	18.8	0.7	0.34	0.14	0.43
*cis*9-16:1	1.8	1.9	2.8	2.2	2.5	2.8	0.4	0.47	0.18	0.51
18:0	40.1	43.4	41.4	39.4	42.0	41.7	1.7	0.76	0.96	0.34
Cis 18:1										
9	8.1	8.3	8.9	6.4	4.6	5.1	0.5	0.01	0.32	0.86
11	0.5	0.5	0.6	0.4	0.5	0.6	0.02	0.26	0.01	0.49
12	1.1	0.8	0.9	1.0	1.3	1.6	0.1	0.01	0.03	0.30
13	1.2	0.9	1.2	0.9	0.9	1.3	0.1	0.34	0.01	0.27
15	0.4	0.4	0.5	0.4	0.6	0.6	0.02	0.01	0.22	0.71
Trans 18:1										
6 + 7 + 8	0.4	0.6	0.7	0.4	0.4	0.5	0.04	0.01	0.03	0.70
9	0.3	0.4	0.5	0.3	0.3	0.4	0.03	0.15	0.03	0.93
10	0.7	0.7	0.9	0.6	0.8	1.2	0.1	0.05	0.01	0.46
11	1.6	1.4	1.5	2.3	2.4	3.0	0.3	0.01	0.31	0.52
12	0.6	0.6	0.7	0.6	0.7	0.9	0.1	0.01	0.05	0.44
13 + 14	1.2	1.2	1.2	1.1	1.5	1.6	0.1	0.01	0.20	0.74
16	0.5	0.5	0.6	0.6	0.7	0.8	0.1	0.02	0.09	0.77
Nonconjugated 18:2										
*cis*9,*cis*12	5.7	5.2	5.4	12.0	13.3	12.8	1.0	0.01	0.89	0.76
Conjugated 18:2										
*cis*9,*trans*11	0.3	0.7	0.3	0.3	0.7	0.4	0.1	0.18	0.01	0.66
*trans*10,*cis*12	0	0	0.8	0	0	0.7	0.04	0.36	0.01	0.19
18:3n-3	0.7	1.0	1.2	0.7	0.7	0.9	0.1	0.01	0.01	0.75
Total	93.2	94.7	97.5	95.1	97.9	106.7	3.6	0.16	0.19	0.46

[1]Values are the average of means ± pooled SEM for four cows, except for OLE-9/11CLA with three cows, obtained at the end of CLA infusion (48 h).

[2]Probability of difference between treatment means due to oil (O), CLA isomer (I), and their interaction (O × I).

[3]Basal = the means of observations at -12 and 0 h before infusion are shown for comparison only.

they were readily available to the mammary gland. The low transfer efficiency (8% at 48 h) of infused 10/12CLA into milk fat found in the present and previous (Baumgard et al., 2000, 2001) studies may be explained by its greater incorporation (total micrograms per milliliter plasma) into plasma phospholipids, which are not readily taken up by the mammary gland.

Availability of *cis*9-18:1, *trans*9-18:1, and 18:3n-3 for extraction from TG+FFA was greater when high-oleic oil was fed compared with high-linoleic oil. In contrast, feeding high-linoleic oil increased the availability of *cis*12-, *cis*15-, *trans*10- through *trans*16-18:1, and 18:2n-6. There were minor but significant increases in the concentrations of 14:0 and some *cis*- or *trans*-isomers of 18:1 and 18:3n-3 in response to 10/12CLA infusion compared with 9/11CLA (Table 6). This response to exogenous 10/12CLA was associated with an overall 30% increase in total fatty acids in the FFA fraction (data not shown). Higher concentrations of total plasma FFA were previously found during abomasal infusions of CLA mixtures (Loor and Herbein, 1998) or various doses (2 to 10 g/d) of *trans*10,*cis*12-18:2 (Baumgard et al., 2000).

Despite the increase in concentrations of the various *cis*- and *trans*-18:1 isomers in plasma TG+FFA due to oils or 10/12CLA, extraction ratios for these fatty acids did not change. Assuming that mammary blood flow remained constant, the extent of the increases in concentration apparently was not large enough to influence extraction. Only the extraction of 18:2n-6 was greater when high-linoleic oil was fed, regardless of isomer, because its concentration in TG+FFA was 147% higher compared with feeding high-oleic oil, and it accounted for 13% of total TG+FFA. Although concentrations of 18:0 in TG+FFA were constant across treatments, infusion of 10/12CLA decreased the extraction ratio for 18:0 by 6 percentage units regardless of diet. In contrast, the extraction ratio for *cis*9,*trans*11-18:2 increased due to 10/12CLA infusion. Our values are the first estimates of extraction of the major CLA isomers from blood plasma TG+FFA by the mammary gland of lactating cows.

Compared with 9/11CLA, infusion of 10/12CLA decreased milk fat concentration from 3.5% before infusion to 2.1% at 72 h (Figure 1). This represented a 40% reduction in concentration and led to an overall 25%

Table 7. Mammary gland extraction ratios of fatty acids in blood plasma triglycerides plus FFA in cows fed diets containing (2.5% of DM) high-oleic sunflower oil (OLE) or high-linoleic safflower oil (LIN) and infused into the abomasum for 2 d with 15 g/d *cis*9,*trans*11-CLA (9/11) or *trans*10,*cis*12-CLA (10/12).[1]

		OLE			LIN			P^2		
	Basal[3]	9/11	10/12	Basal	9/11	10/12	SEM	O	I	O × I
Fatty acid	%									
14:0	31	32	28	30	27	31	4	0.95	0.95	0.33
16:0	31	32	29	30	31	32	2	0.72	0.60	0.34
*cis*9-16:1	42	39	43	43	48	47	4	0.45	0.21	0.18
18:0	40	41	35	38	39	34	1	0.27	0.01	0.09
Cis 18:1										
9	42	44	43	41	44	40	3	0.70	0.42	0.55
11	39	36	39	41	43	40	4	0.43	0.96	0.49
12	34	35	47	33	37	43	5	0.87	0.17	0.63
13	30	48	55	48	51	67	5	0.62	0.06	0.37
15	23	22	23	31	32	30	4	0.08	0.92	0.60
Trans 18:1										
6 + 7 + 8	47	49	47	52	54	55	3	0.06	0.97	0.59
9	68	67	57	62	66	65	2	0.14	0.04	0.07
10	64	63	58	69	70	66	2	0.07	0.25	0.83
11	61	59	52	56	58	59	4	0.48	0.60	0.35
12	52	50	53	59	61	62	6	0.16	0.77	0.89
13 + 14	60	63	60	62	64	62	2	0.62	0.28	0.82
16	59	61	61	54	55	55	5	0.30	0.97	0.95
Nonconjugated 18:2										
*cis*9,*cis*12	21	20	15	30	30	38	4	0.02	0.78	0.21
Conjugated 18:2										
*cis*9,*trans*11	56	62	84	62	67	71	3	0.23	0.01	0.02
*trans*10,*cis*12	—	—	81	—	—	80	6	0.72	0.01	0.79
18:3n-3	45	43	40	41	44	43	4	0.54	0.11	0.07
Total	40	41	38	39	39	39	1	0.99	0.34	0.17

[1]Values are the average of means ± pooled SEM for four cows, except for OLE-9/11CLA with three cows, obtained at the end of CLA infusion (48 h).

[2]Probability of difference between treatment means due to oil (O), CLA isomer (I), and their interaction (O × I).

[3]Basal = the means of observations at -12 and 0 h before infusion are shown for comparison only.

reduction in yield (Table 3). Basal concentrations of saturated fatty acids with 6 to 16 carbons averaged 370 mg/g of total fatty acids, and were 34% lower than typically seen in milk fat from cows fed diets without supplemental oil (Palmquist et al., 1993). A large portion of the reduction in de novo synthesis due to feeding unsaturated oils occurs as a result of greater uptake and secretion of dietary and ruminally derived fatty acids (Palmquist et al., 1993). Exogenous fatty acids compete for esterification with newly synthesized short-chain fatty acids in mammary cells and could lead to feedback inhibition of lipogenic enzymes (Palmquist et al., 1993). Results from a recent study indicated that supplemental *cis*9-18:1 was preferentially incorporated into the sn-2 position of the milk fat triglyceride at the expense of 16:0 (DePeters et al., 2001). The net effect was lower concentration of 16:0 but higher *cis*9-18:1 concentration in milk fat.

Because of the small amount of *trans*10,*cis*12-18:2 required to reduce milk fat percentage (Figure 1A) and fatty acid yield (Table 8; Figure 4A), this isomer appears to depress de novo fatty acid synthesis in a manner distinct from that caused by dietary unsaturated fatty acids. Despite lower concentrations of 16:0 in milk fat when 18:2n-6 was infused into the abomasum, only the infusion of a CLA mixture reduced 16:0 and milk fat concentrations (Loor and Herbein, 1998). It seems that a *trans*-10 double bond in the CLA is required for inhibition of lipogenesis. For example, infusions of *trans*10,*cis*12-18:2 or *cis*8,*trans*10-18:2 (in combination with *cis*9,*trans*11-18:2) reduced milk fat synthesis to a similar extent (Loor and Herbein, 1998; Chouinard et al., 1999b). A common response to CLA mixtures or 10/12CLA not observed when unsaturated oils are fed is a marked accumulation of 18:0 in milk fat.

In the present study, the extent of the decrease in milk fat yield observed with 10/12CLA infusion was unexpected because the transfer efficiency for supplemental dietary lipid to milk fat is high (Palmquist et al., 1993). Oil supplementation had the potential to overcome reductions in de novo synthesis caused by either CLA. However, lower concentrations (Figure 1B) and yields of saturated 6:0 to 16:0 (Table 8) corresponded with the gradual increase in concentration of

Table 8. Milk fatty acid yields by cows fed diets containing (2.5% of DM) high-oleic sunflower oil (OLE) or high-linoleic safflower oil (LIN) and infused into the abomasum for 2 d with 15 g/d *cis*9,*trans*11CLA (9/11) or *trans*10,*cis*12-CLA (10/12).[1]

		OLE			LIN			P[2]		
	Basal[3]	9/11	10/12	Basal	9/11	10/12	SEM	O	I	O × I
Fatty acid	g/d									
4:0	36.4	34.8	31.4	39.0	36.4	31.8	0.8	0.28	0.01	0.54
6:0	19.4	19.8	14.4	19.8	20.2	14.2	0.5	0.81	0.01	0.60
8:0	8.6	9.1	6.2	9.0	9.4	6.2	0.2	0.28	0.01	0.42
10:0	16.8	17.4	11.8	17.0	18.4	11.4	0.5	0.58	0.01	0.18
12:0	16.0	16.8	11.8	14.6	15.6	11.8	0.4	0.47	0.01	0.53
14:0	71.8	74.8	56.8	70.4	73.2	53.2	2.8	0.12	0.01	0.51
*cis*9-14:1	7.0	7.2	4.6	6.2	6.8	3.8	0.2	0.05	0.01	0.57
16:0	162.2	164.8	124.8	160.2	162.6	121.2	6.5	0.30	0.01	0.81
6:0-16:0	258.1	268.2	194.7	251.6	263.2	186.4	6.4	0.42	0.01	0.61
*cis*9-16:1	7.4	7.2	5.4	7.0	6.8	4.6	0.1	0.04	0.01	0.36
18:0	115.4	116.8	113.2	120.8	120.2	116.0	4.1	0.36	0.28	0.92
Cis 18:1										
9	277.4	277.8	231.1	244.8	248.4	194.8	7.5	0.01	0.01	0.44
11	3.0	2.8	2.8	2.8	2.8	2.4	0.1	0.21	0.32	0.27
12	0.6	0.6	0.4	1.2	1.2	1.0	0.02	0.01	0.02	0.68
13	1.0	1.0	0.8	0.8	0.8	0.6	0.02	0.08	0.02	0.33
15	1.6	1.6	1.4	1.6	1.8	1.4	0.1	0.37	0.04	0.38
Trans 18:1										
6 + 7 + 8	3.4	3.6	3.2	2.0	2.0	1.8	0.1	0.01	0.01	0.29
9	3.2	3.2	3.0	2.6	2.6	2.2	0.1	0.01	0.01	0.62
10	5.0	5.0	4.0	5.0	5.0	4.4	0.1	0.21	0.01	0.14
11	8.4	8.8	7.2	13.0	13.2	12.0	0.3	0.01	0.02	0.79
12	1.8	1.8	1.4	2.2	2.2	1.8	0.1	0.01	0.01	0.83
13 + 14	8.6	9.0	7.8	10.8	10.6	9.6	0.3	0.01	0.01	0.92
16	3.2	3.6	2.8	3.4	3.2	3.0	0.1	0.75	0.12	0.99
Nonconjugated 18:2										
*cis*9,*cis*12	21.4	21.8	18.6	36.6	36.0	31.6	0.8	0.01	0.01	0.44
*trans*9,*trans*12	0.4	0.2	0.2	0.4	0.4	0.2	0.03	0.28	0.06	0.18
*cis*9,*trans*12	1.2	1.2	1.0	1.6	1.6	1.4	0.1	0.01	0.03	0.52
*trans*9,*cis*12	0.4	0.4	0.2	0.6	0.4	0.4	0.03	0.02	0.03	0.75
*trans*11,*cis*15	1.2	2.0	1.8	1.1	1.8	1.6	0.1	0.16	0.27	0.67
Conjugated 18:2										
*cis*9,*trans*11	5.2	7.4	3.8	7.0	10.2	5.4	0.2	0.01	0.01	0.09
*trans*10,*cis*12	0.0	0.0	1.8	0.0	0.0	2.0	0.1	0.20	0.01	0.34
18:3n-3	2.2	2.0	2.0	2.4	2.4	2.0	0.2	0.08	0.04	0.36
20:3n-6	0.8	0.8	0.6	1.0	1.0	0.6	0.01	0.02	0.01	0.23
20:4n-6	1.2	1.2	0.8	1.4	1.4	1.0	0.04	0.01	0.01	0.46
20:5n-3	0.6	0.6	0.6	0.8	0.6	0.6	0.03	0.11	0.10	0.19
Total	808.0	822.8	676.4	807.2	819.0	656.2	17.0	0.36	0.01	0.53

[1]Values are the average of means ± pooled SEM for four cows, except for OLE-9/11CLA with three cows, obtained from 12 through 96 h of infusion.

[2]Probability of difference between treatment means due to oil (O), CLA isomer (I), and their interaction (O × I).

[3]Basal = the means of observations at -12 and 0 h before infusion are shown for comparison only.

*trans*10,*cis*12-18:2 in milk fat from 0 to 48 h during 10/12CLA infusion (data not shown), and accounted for the overall reduction in milk fat yield. The temporal nature of the decrease in lipogenesis we observed with very small concentrations (6 mg/g) of *trans*10,*cis*12-18:2 were consistent with a sequence of events, which may have begun with reductions in synthesis of mRNA for acetyl-CoA carboxylase and fatty acid synthase. Hence, between 0 and 48 h of 10/12CLA infusion, reductions in yields of saturated 4:0-16:0 accounted for more than 50% of the decrease in total fatty acid yield (Figure 5). The present study confirmed initial evidence (Baumgard et al., 2000, 2001; Peterson et al., 2002) indicating *trans*10,*cis*12-18:2 at very small concentrations is extremely effective in reducing milk fat percentage and de novo fatty acid synthesis in dairy cows.

Stearic acid in plasma TG+FFA across treatments accounted for 42% of total fatty acids available for extraction (Table 6). After uptake by the mammary gland, 18:0 becomes the primary substrate for Δ^9 desaturase (Enjalbert et al., 1998). *Trans*11-18:1 derived from the rumen also is a substrate for Δ^9 desaturase and leads to endogenous synthesis of *cis*9,*trans*11-18:2 in the mammary gland (Bauman et al., 2001). As indicated

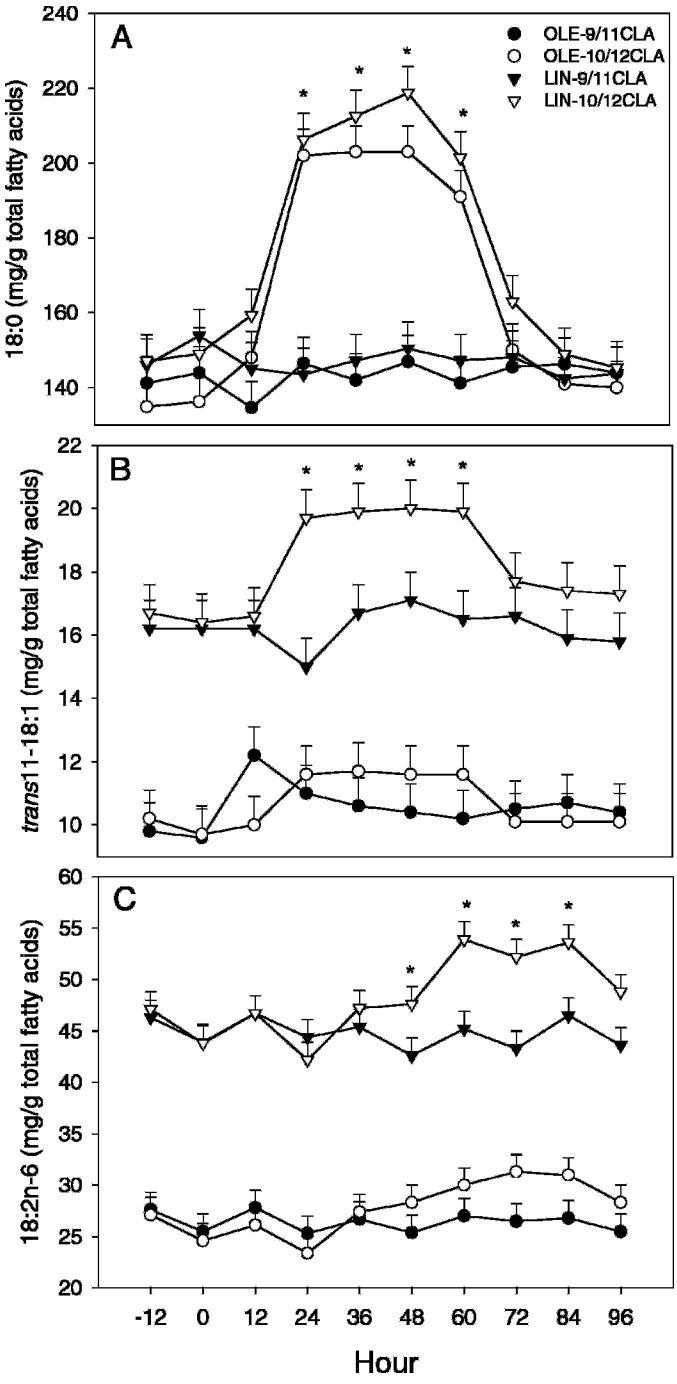

Figure 3. Concentrations of 18:0 (panel A), *trans*11-18:1 (panel B), and 18:2n-6 (panel C) in milk fat from cows fed high-oleic (OLE) or high-linoleic (LIN) oil, and infused into the abomasum with *cis*9,-*trans*11-18:2 (9/11CLA) or *trans*10,*cis*12-18:2 (10/12CLA) for 48 h. Values are means plus pooled SEM for four cows, except for OLE-9/11CLA with three cows, at each 12-h interval. Asterisks denote significant ($P < 0.05$) time by CLA isomer interaction.

by normalized ratios (Table 9), the extent of desaturation of 16:0, 18:0, and *trans*11-18:1 before infusion of any CLA was higher when high-oleic oil was fed compared with high-linoleic oil. However, the marked increase in milk fat 18:0 and *trans*11-18:1 concentrations from 24 through 48 h when 10/12CLA was infused (Figure 3A,B), regardless of diet, suggested desaturation of fatty acids derived from plasma was severely decreased by exogenous *trans*10,*cis*12-18:2. Desaturation of *trans*11-18:1 to *cis*9,*trans*11-18:2 apparently was very sensitive to exogenous 10/12CLA (Figure 4B), because yield of *cis*9,*trans*11-18:2 in milk fat was reduced by 50% regardless of diet (Figure 5). The depressed ratios of *cis*9-14:1/14:0 and *cis*9-16:1/16:0, despite their lower concentrations in milk fat, provide evidence that desaturation of endogenously synthesized fatty acids also is sensitive to 10/12CLA.

Reduced desaturation, resulting in accumulation of 18:0 in the mammary gland during infusion of 10/12CLA, might have lowered 18:0 extraction from plasma TG+FFA. In contrast, the reduction in desaturation of *trans*11-18:1 to *cis*9,*trans*11-18:2 may have decreased endogenously synthesized *cis*9,*trans*11-18:2 concentration in the mammary gland. The lower amount of *cis*9,*trans*11-18:2 in the gland could have enhanced its extraction from TG+FFA when 10/12CLA was infused (Table 7). The reduction in yield of *cis*9,-*trans*11-18:2, despite greater extraction from blood, in combination with the lower normalized ratio for *cis*9,-*trans*11-18:2/*trans*11-18:1 during 10/12CLA infusion provides additional evidence (Bauman et al., 2001) that endogenous synthesis (via desaturation) may be the primary source of *cis*9,*trans*11-18:2 in milk fat.

Expression of Δ^9 desaturase activity is markedly reduced by *trans*10,*cis*12-18:2, but not *cis*9,*trans*11-18:2, in rodent adipose, liver, and mammary gland tissue (Pariza et al., 2001; Lin, 2000). The negative effect of *trans*10,*cis*12-18:2 on desaturation activity in the bovine mammary gland might be mediated by reductions in the transcription of the Δ^9 desaturase gene (Lin, 2000). We postulate that a large portion of the reduction in milk fat concentration and yield due to *trans*10,*cis*12-18:2, and not observed when unsaturated oils are fed, is a consequence of its acute effects on Δ^9 desaturase. Even when availability of *trans*11-18:1 and 18:0 were elevated due to supplemental oils, both *cis*9,*trans*11-18:2 and *cis*9-18:1 concentrations in milk fat were negatively correlated with *trans*10,*cis*12-18:2 (Figure 4B and C). Nearly 40% of the reduction in total fatty acid yield between 0 and 48 h of infusion was due solely to oleic acid (Figure 5). With mice with a null mutation in the Δ^9 desaturase gene, it was conclusively shown that triglyceride synthesis in the liver relies heavily on endogenous synthesis of oleic acid (Miyazaki et al.,

Table 9. Normalized ratios[1] of fatty acids in milk fat from cows fed diets containing (2.5% of DM) high-oleic sunflower oil (OLE) or high-linoleic safflower oil (LIN) and infused into the abomasum for 2 d with 15 g/d *cis*9,*trans*11-CLA (9/11) or *trans*10,*cis*12-CLA (10/12).[2]

		OLE			LIN			P^3		
Ratio	Basal[4]	9/11	10/12	Basal	9/11	10/12	SEM	O	I	O × I
*c*9-14:1/14:0	0.089	0.087	0.074	0.081	0.084	0.066	0.01	0.05	0.01	0.36
*c*9-16:1/16:0	0.044	0.043	0.041	0.042	0.041	0.037	0.002	0.04	0.04	0.36
*c*9-18:1/18:0	0.71	0.70	0.67	0.67	0.67	0.63	0.01	0.01	0.01	0.13
*c*9*t*11-18:2/*t*11-18:1	0.39	0.46	0.34	0.35	0.43	0.31	0.01	0.01	0.01	0.86
20:4n-6/18:2n-6	0.052	0.048	0.040	0.034	0.035	0.028	0.003	0.01	0.01	0.60
20:5n-3/18:3n-3	0.13	0.14	0.15	0.17	0.16	0.14	0.01	0.40	0.79	0.16

[1]Normalized ratio = mg/g product/[mg/g substrate + mg/g product] (Sol Morales et al., 2000).

[2]Values are the average of means ± pooled SEM for four cows, except for OLE-9/11CLA with three cows, obtained from 12 through 96 h of infusion.

[3]Probability of difference between treatment means due to oil (O), CLA isomer (I), and their interaction (O × I).

[4]Basal = the means of observations at -12 and 0 h before infusion are shown for comparison only.

2001). Results from the present study provide evidence of a similar mechanism present in bovine mammary tissue.

Plasma-derived 18:2n-6, through elongation and desaturation via Δ^5 and Δ^6 desaturases (Hermansen et al., 1995), is the major source of 20:3n-6 and 20:4n-6 in milk fat. Before infusion, feeding high-linoleic oil compared with high-oleic oil resulted in greater extraction of 18:2n-6 from TG+FFA (Table 7) and led to greater concentration (data not shown) and yield of 20:4n-6 in milk fat (Table 8). However, from 60 through 84 h after infusion of 10/12CLA, the concentration of 18:2n-6 in milk fat (Figure 3) increased but 20:4n-6 decreased (data not shown) regardless of diet. Similar responses were not found when 18:2n-6 was infused into the abomasum of lactating cows (Loor and Herbein, 1998), suggesting that the presence of a *trans*10-double bond in the CLA is associated with the reduction in 20:4n-6 concentration and yield.

As mentioned earlier, the presence of the *trans*10-double bond (either in CLA or as *trans*10-18:1) may be required to induce lower milk fat synthesis in the mammary gland of dairy cows. It has been speculated that *trans*10,*cis*12-18:2 is an intermediate in the hydrogenation of 18:2n-6, which accumulates when high-grain low-forage diets are fed (Bauman et al., 2001). In response to a high-grain diet plus 5% soybean oil, *trans*10-18:1 accounted for 60% of all *trans*-18:1 isomers (16% of total milk fatty acids) but *trans*10,*cis*12-18:2 represented only 10% of total CLA isomers (1% of total milk fatty acids) (Piperova et al., 2000). Others did not detect (Griinari et al., 1998) or found no correlation (Loor et al., 2002b) between *trans*10,*cis*12-18:2 and reduced milk fat percentage in response to high-concentrate diets with or without unsaturated oil. Thus, the involvement of *trans*10,*cis*12-18:2 in diet-induced milk fat depression is still unclear. Greater production of *trans*10-18:1 (and to a lesser extent *trans*10,*cis*12-18:2) in the rumen depressed milk fat percentage and yield by inhibiting the activity and mRNA abundance for acetyl-CoA carboxylase and fatty acid synthase (Piperova et al., 2000). Desaturation of 18:0 did not seem to be affected, as the concentrations of 18:0 and *cis*9-18:1 in milk fat were similar compared with controls. At least in mouse liver, *trans*10-18:1 did not inhibit Δ^9 desaturase activity compared with *trans*10,*cis*12-18:2 (Park et al., 2000). Hence, *trans*10,*cis*12-18:2 and *trans*10-18:1 might affect overall lipogenesis in the bovine mammary gland by different mechanisms. In this regard, profiles of stearic plus oleic acid (along with *trans*10-18:1 and *trans*10,*cis*12-18:2) in milk fat from cows with depressed milk fat content could be used to assess the relative involvement of 18:1 and CLA isomers with a *trans* 10-double bond in diet-induced milk fat depression.

CONCLUSIONS

High-oil feed ingredients increase availability of unsaturated fatty acids and rumen-derived *trans*18:1 isomers in blood for uptake and incorporation into milk fat. *Trans*11-18:1 and *cis*9,*trans*11-18:2 are the major intermediates of dietary 18:2n-6 hydrogenation. In contrast, *trans*6/7/8- and *trans*9-18:1 are the major intermediates produced during isomerization of *cis*9-18:1. Under normal rumen conditions *trans*10-18:1 may arise from isomerization of oleic acid or via a *cis*9-18:1 intermediate from linoleic acid. Despite its ruminal origin, the majority of *cis*9,*trans*11-18:2 in milk fat is synthesized within the mammary gland from *trans*11-18:1 via Δ^9 desaturase. Transfer of dietary *trans*10,*cis*12-18:2 into milk fat may be low due to its preferential incorpo-

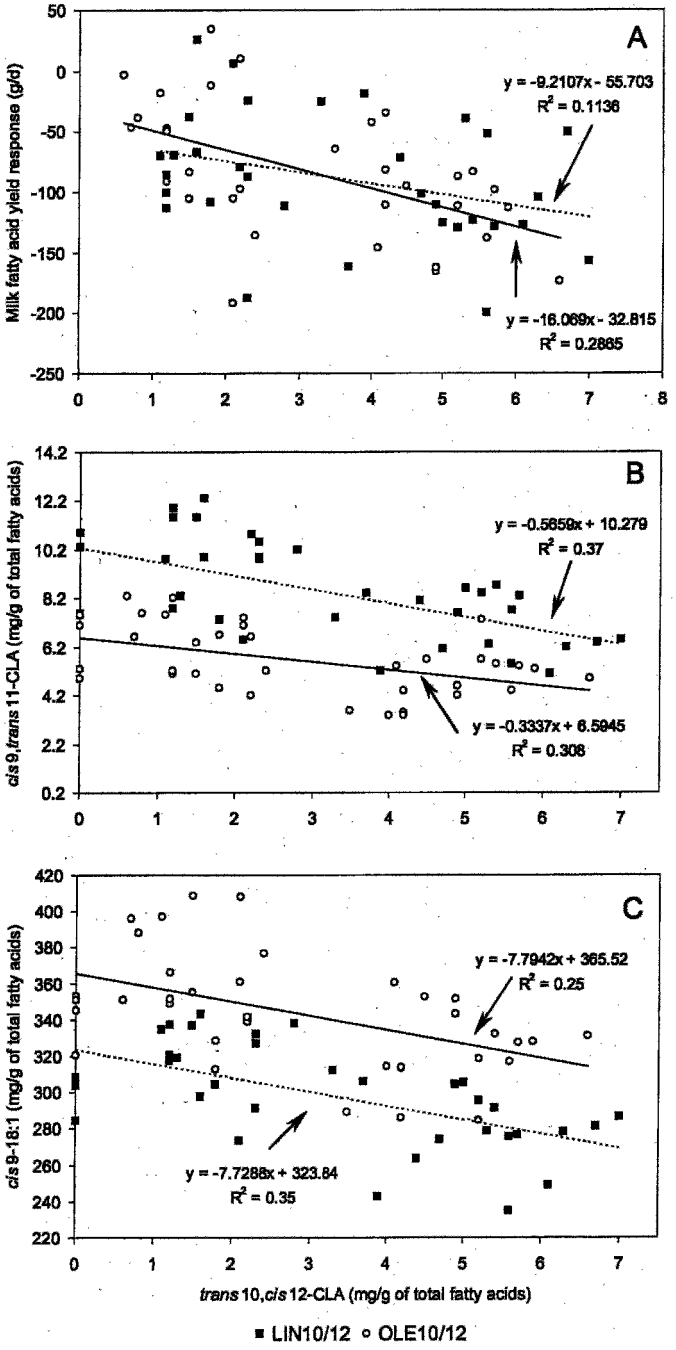

Figure 4. Relationships between milk fat concentration of *trans*10,*cis*12-CLA and milk fatty acid yield response (Panel A), concentration of *cis*9,*trans*11-CLA in milk fat (Panel B), or concentration of *cis*9-18:1 in milk fat (Panel C) from cows fed high-oleic (OLE) or high-linoleic (LIN) oil, and infused into the abomasum with *trans*10,*cis*12-18:2 (10/12CLA).

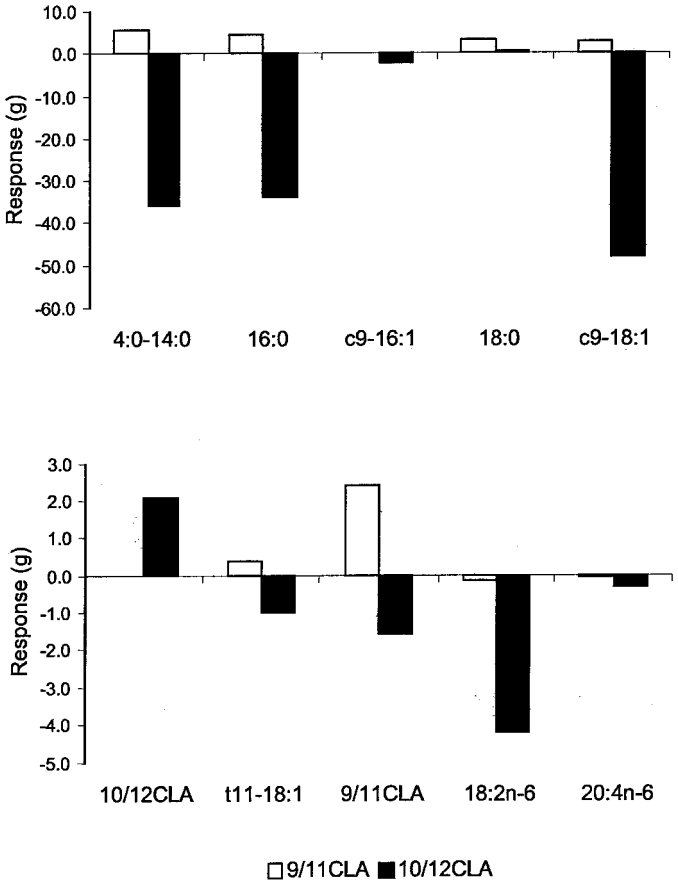

Figure 5. Effect of a 48 h infusion of *cis*9,*trans*11-CLA (9/11CLA) or *trans*10,*cis*12-CLA (10/12CLA) into the abomasum of lactating cows on the secretion response (yield at 48 h - yield at 0 h) of selected milk fatty acids.

ration into plasma phospholipids. However, if diet were capable of enhancing rumen-outflow of *trans*10,*cis*12-18:2, its uptake by the mammary gland could decrease de novo fatty acid synthesis and desaturation of long-chain fatty acids. Reduced milk fat production due to *trans*10,*cis*12-18:2 is closely associated with a lack of endogenously synthesized oleic acid for triglyceride formation.

REFERENCES

Ågren, J. J, A. Julkunen, and I. Penttila. 1992. Rapid separation of serum lipids for fatty acid analysis by a single aminopropyl column. J. Lipid Res. 33:1871–1876.

Association of Official Analytical Chemists. 1990. Official Methods of Analysis. 15th ed. AOAC, Arlington, VA.

Bauman, D. E., B. A. Corl, L. H. Baumgard, and J. M. Griinari. 2001. Conjugated linoleic acid (CLA) and the dairy cow. Pages 221–250 in Recent Advances in animal Nutrition. P. C. Garnsworthy and J. Wiseman, ed. Nottingham University Press, Nottingham, United Kingdom.

Baumgard, L., B. A. Corl, D. A. Dwyer, A. Saebo, and D. E. Bauman. 2000. Identification of the conjugated linoleic acid isomer that inhibits milk fat synthesis. Am. J. Physiol. 278:R179–R184.

Baumgard, L., J. K. Sangster, and D. E. Bauman. 2001. Milk fat synthesis in dairy cows is progressively reduced by increasing amounts of *trans*-10,*cis*-12 conjugated linoleic acid. J. Nutr. 131:1764–1769.

Bickerstaffe, R., D. E. Noakes, and E. F. Annison. 1972. Quantitative aspects of fatty acid biohydrogenation, absorption and transfer into milk fat in the lactating goat, with special reference to the *cis*- and *trans*-isomers of octadecenoate and linoleate. Biochem. J. 130:607–617.

Chilliard, Y., A. Ferlay, R. Mansbridge, and M. Doreau. 2000. Ruminant milk fat plasticity: nutritional control of saturated, polyunsaturated, *trans* and conjugated fatty acids. Ann. Zootech. 49:181–205.

Chouinard, P., L. Corneau, D. M. Barbano, L. E. Metzger, and D. E. Bauman. 1999a. Conjugated linoleic acids alter milk fatty acid composition and inhibit milk fat secretion in dairy cows. J. Nutr. 129:1579–1584.

Chouinard, P., L. Corneau, A. Saebo, and D. E. Bauman. 1999b. Milk yield and composition during abomasal infusions of conjugated linoleic acids in dairy cows. J. Dairy Sci. 82:2737–2745.

DePeters, E. J., J. B. German, S. J. Taylor, S. T. Essex, and H. Perez-Monti. 2001. Fatty acid and triglyceride composition of milk fat from lactating Holstein cows in response to supplemental canola oil. J. Dairy Sci. 84:929–936.

Enjalbert, F., M. Nicot, C. Bayourthe, and R. Moncoulon. 1998. Duodenal infusions of palmitic, stearic, or oleic acids differently affect mammary gland metabolism of fatty acids in lactating dairy cows. J. Nutr. 128:1525–1532.

Giesy, J. G., M. A. McGuire, B. Shafii, and T. W. Hanson. 2002. Effect of dose of calcium salts of conjugated linoleic acid (CLA) on percentage and fatty acid content of milk fat in midlactation Holstein cows. J. Dairy Sci. 85:2023–2029.

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.

Hermansen, J. E., F. Jonsbo, J. O. Andersen, K. F. Michaelsen, and M. R. Weisbjerg. 1995. On the transfer of gamma-linolenic acid into milk-fat and its possible elongation to arachidonic acid by cows. Milchwissenschaft 50:3–6.

Kemp, P., R. W. White, and D. J. Lander. 1975. The hydrogenation of unsaturated fatty acids by five bacterial isolates from the sheep rumen, including a new species. J. Gen. Microbiol. 90:100–114.

Kepler, C. R., and S. B. Tove. 1967. Biohydrogenation of unsaturated fatty acids. III. Purification and properties of a linoleate delta-12-*cis*,delta11-*trans*-isomerase from *Butyrivibrio fibrisolvens*. J. Biol. Chem. 242:5686–5692.

Lin, X. 2000. Stearoyl-CoA desaturase gene transcription, mRNA, and activity in response to *trans*-vaccenic acid and conjugated linoleic acid isomers. Ph.D. Diss., Virginia Tech, Blacksburg.

Loor, J. J., and J. H. Herbein. 1998. Exogenous conjugated linoleic acid isomers reduce bovine milk fat concentration and yield by inhibiting de novo fatty acid synthesis. J. Nutr. 128:2411–2419.

Loor, J. J., and J. H. Herbein. 2001. Alterations in blood plasma and milk fatty acid profiles of lactating Holstein cows in response to ruminal infusion of a conjugated linoleic acid mixture. Anim. Res. 50:463–476.

Loor, J. J., A. B. P. A. Bandara, and J. H. Herbein. 2002a. Characterization of 18:1 and 18:2 isomers produced during microbial biohydrogenation of unsaturated fatty acids from canola or soybean oil in the rumen of lactating cows. J. Anim. Phys. Anim. Nutr. 86:422–432.

Loor, J. J., A. Ferlay, A. Ollier, M. Doreau, and Y. Chilliard. 2002b. Conjugated linoleic acids (CLA), *trans* fatty acids, and lipid content in milk from Holstein cows fed a high- or low-fiber diet with two levels of linseed oil. J. Dairy Sci. 85(Suppl. 1):1188.(Abstr.)

Loor, J. J., L. E. Quinlan, A. B. P. A. Bandara, and J. H. Herbein. 2002c. Distribution of trans-vaccenic acid and *cis*9,*trans*11- conjugated linoleic acid (rumenic acid) in blood plasma lipid fractions and secretion into milk fat of Jersey cows fed canola or soybean oil. Anim. Res. 51:119–134.

Miyazaki, M., Y. C. Kim, and J. M. Ntambi. 2001. A lipogenic diet in mice with a disruption of the stearoyl-CoA desaturase gene 1 reveals a stringent requirement of endogenous monounsaturated fatty acids for triglyceride synthesis. J. Lipid Res. 42:1018–1024.

Mosley, E. E., G. L. Powell, M. B. Riley, and T. C. Jenkins. 2002. Microbial biohydrogenation of oleic acid to *trans* isomers in vitro. J. Lipid Res. 43:290–296.

National Research Council. 1989. Pages 90–110 in Nutrient Requirements of Dairy Cattle. 6th rev. ed. Natl. Acad. Sci., Washington, DC.

O'Shea, M., R. Devery, F. Lawless, J. Murphy, and C. Stanton. 2000. Milk fat conjugated linoleic acid inhibits growth of human mammary MCF-7 cancer cells. Antican. Res. 20:3591–3602.

Palmquist, D. L., A. D. Beaulieu, and D. M. Barbano. 1993. Feed and animal factors influencing milk fat composition. J. Dairy Sci. 76:1753–1771.

Pariza M., Y. Park, and M. Cook. 2001. The biologically active isomers of conjugated linoleic acid. Prog. Lipid Res. 40:283–298.

Park, Y., J. M. Storkson, J. M. Ntambi, M. E. Cook, C. J. Sih, and M. W. Pariza. 2000. Inhibition of hepatic stearoyl-CoA desaturase activity by *trans*-10, *cis*-12 conjugated linoleic acid and its derivatives. Biochim. Biophys. Acta 1486:285–292.

Peterson, D. G., L. H. Baumgard, and D. E. Bauman. 2002. Short Communication: Milk fat response to low doses of *trans*-10, *cis*-12 conjugated linoleic acid (CLA). J. Dairy Sci. 85:1764–1766.

Piperova, L. S., B. B. Teter, I. Bruckental, J. Sampugna, S. E. Mills, M. P. Yurawecz, J. Fritsche, K. Ku, and R. A. Erdman. 2000. Mammary lipogenic enzyme activity, *trans* fatty acids and conjugated linoleic acids are related in lactating dairy cows fed a milk fat-depressing diet. J. Nutr. 130:2568–2574.

Piperova, 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 *cis*9-containing CLA in lactating dairy cows. J. Nutr. 132:1235–1241.

SAS User's Guide: Statistics, Version 8 Edition. 2000. SAS Inst., Inc., Cary, NC.

Sol Morales, M., D. L. Palmquist, and W. P. Weiss. 2000. Effects of fat source and copper on unsaturation of blood and milk triacylglycerol fatty acids in Holstein and Jersey cows. J. Dairy Sci. 83:2105–2111.

Stallings, C. C., G. Kroll, J. C. Kelley, and M. L. McGilliard. 1985. A computer ration evaluation program for heifers, dry cows, and lactating cows. J. Dairy Sci. 68:1015–1019.

Ulberth, F., and M. Henninger. 1994. Quantitation of *trans* fatty acids in milk fat using spectroscopic and chromatographic methods. J. Dairy Res. 61:517–527.

Van Soest, P. J., J. B. Robertson, and B. A. Lewis. 1991. Methods of dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. J. Dairy Sci. 74:3583–3597.