Trans-10, Trans-12 Conjugated Linoleic Acid Does Not Affect Milk Fat Yield but Reduces Δ^9 -Desaturase Index in Dairy Cows¹

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Abstract

Trans-10, *cis*-12 conjugated linoleic acid (CLA) is a potent inhibitor of milk fat synthesis, and the magnitude of milk fat depression is often correlated with the fat content of this isomer. However, the *trans*-10, *cis*-12 CLA content does not always correspond to the extent of milk fat depression, and in some instances, an increase in the milk fat content of *trans*-10, *trans*-12 CLA has been observed. We synthesized *trans*-10, *trans*-12 CLA (>90% purity) and investigated its effect on milk fat synthesis and incorporation into plasma lipids. Three rumen-fistulated Holstein cows were randomly assigned in a 3 × 3 Latin square experiment. Treatments were a 4-d abomasal infusion of 1) ethanol (control), 2) a *trans*-10, *cis*-12 CLA supplement (positive control), and 3) a *trans*-10, *trans*-12 CLA supplement; 5 g/d of the CLA isomer of interest was provided. Milk yield, dry matter intake, and milk protein were unaffected by treatment. Treatment with *trans*-10, *trans*-12 CLA had no effect on milk fat yield, whereas treatment with *trans*-10, *cis*-12 CLA reduced milk fat yield by 28%. Incorporation of CLA was greatest for the plasma triglyceride fraction, and the milk fat content was subsequently elevated within the respective treatment groups. The milk fatty acid composition indicated that Δ^9 -desaturase was reduced significantly for both CLA treatments, but the reduction was greater for the treatment with *trans*-10, *trans*-12 CLA. Overall, abomasal infusion of *trans*-10, *trans*-12 CLA and *trans*-10, *cis*-12 CLA altered the desaturase ratios, but only *trans*-10, *cis*-12 CLA reduced milk fat synthesis. **Key words:** conjugated linoleic acid, milk fat, lactation, desaturase

INTRODUCTION

For over 100 yr, producers and scientists have recognized that certain diets reduce milk fat production in

dairy cows, and this phenomenon is commonly referred to as milk fat depression (MFD). Recent investigations have resulted in the biohydrogenation theory to explain MFD and the concept that, under certain dietary conditions, pathways of rumen biohydrogenation are altered to produce unique fatty acid intermediates, some of which are potent inhibitors of milk fat synthesis (Bauman and Griinari, 2001, 2003). *Trans*-10, *cis*-12 conjugated linoleic acid (CLA) is one such intermediate (Baumgard et al., 2000), and the relationship between this isomer and a reduced milk fat output is well established (de Veth et al., 2004). The mechanism for MFD involves a coordinated reduction in the pathways of milk fat synthesis (Baumgard et al., 2002; Peterson et al., 2003). This often includes effects on Δ^9 -desaturase, resulting in a shift in the desaturase index for milk fatty acids (Baumgard et al., 2000, 2001), and it has been postulated that a large portion of the reduction in milk fat during MFD is due to a limitation in the oleic acid supply as a consequence of effects on Δ^9 -desaturase (Loor and Herbein, 2003; Loor et al., 2005b).

In many situations of diet-induced MFD, changes in *trans*-10, *cis*-12 CLA are minimal or inadequate to completely account for the reduction in milk fat. This has led investigators to propose that there must be additional unique biohydrogenation intermediates involved in the regulation of milk fat synthesis (Bauman and Griinari, 2001; Peterson et al., 2003; Piperova et al., 2004; Griinari and Bauman, 2006; Loor et al., 2005b). Dietary situations in which MFD occurs are associated with alterations in the pathways of biohydrogenation, and as a consequence, changes in many *trans*-18:1 and CLA isomers correlate with the reduction in milk fat yield (e.g., Bauman and Griinari, 2003; Loor et al., 2005b). Of these changes, Bauman and Griinari (2003) noted that an increase in the milk fat content of *trans*-10 18:1 was consistently observed in studies of diet-induced MFD. Loor et al. (2005b) summarized data from 13 studies and demonstrated convincingly that MFD was strongly correlated with changes in the milk fat content of *trans*-10 18:1. Furthermore, their own data indicated a significant correlation between MFD and the occurrence of CLA isomers containing 2 *trans* double bonds. McConnell (2004) supplemented the diets of lactating dairy cows with fish

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oil and observed that the temporal pattern of milk fat content of both *trans*-12 and *trans*-10 18:1 increased in a manner reciprocal to the decrease in milk fat yield, but that the milk fat content of *trans*-10, *cis*-12 CLA was negligible and unaffected. Recently, Loor et al. (2005a) also reported a reduction in milk fat yield after 4 wk of fish oil supplementation and similarly observed no change in the milk fat content of *trans*-10, *cis*-12 CLA, whereas both *trans*-10 18:1 and *trans*-12 18:1 were increased.

Based on the aforementioned results, we speculated that *trans*-10, *trans*-12 CLA, a potential precursor for both *trans*-10 18:1 and *trans*-12 18:1, could be involved in the regulation of milk fat during diet-induced MFD. Oil from *Chilopsis linearis* seeds is unusual because it contains *trans*-10, *trans*-12 CLA as a major fatty acid (Aitzetmüller et al., 2003). A lipid extract from these seeds was used as a reference standard, and preliminary gas chromatographic analysis of samples from an earlier study (Perfield et al., 2002) indicated that the milk fat content of *trans*-10, *trans*-12 CLA was increased 3-fold in situations of MFD (data not shown). This was more thoroughly examined by Shingfield et al. (2003) in dairy cows fed fish oil supplements; they observed an increase in the milk fat content of *trans*-10, *trans*-12 CLA as well as *trans*-10 and *trans*-12 18:1; however, the milk fat content was unaltered and the observed reduction in milk fat yield was confounded by a parallel reduction in milk yield. The primary objective of the present study was to investigate the effects of *trans*-10, *trans*-12 CLA on milk fat synthesis and the desaturase index in dairy cows. For comparison, a treatment with *trans*-10, *cis*-12 CLA was included as a positive control. A secondary objective was to compare the incorporation of these CLA isomers into the plasma fractions involved in lipid transport and their subsequent transfer into milk fat.

MATERIALS AND METHODS

Animals and Experimental Design

The Cornell University Institutional Animal Care and Use Committee approved all procedures involving animals. Rumen-fistulated lactating Holstein cows (168 ± 80 DIM; mean ± SE) were housed in tie stalls at the Cornell University Large Animal Teaching and Research Unit. Throughout the study, cows (n = 3) were fed a TMR formulated to meet or exceed nutrient requirements for energy, protein, minerals, and vitamins (National Research Council, 2001) using the Cornell Net Carbohydrate and Protein System (Fox et al., 2004). Chopped alfalfa was the major forage component, with cracked corn as the major concentrate (Table 1). Cows were fed ad libitum with equal portions of feed offered

Table 1. Ingredient composition and chemical analysis of diet

Composition	Concentration
Ingredient, % of DM	
Alfalfa hay	55.5
Cracked corn	27.9
Cottonseed	6.8
Soybean meal	3.8
Roasted soybean meal	3.7
Limestone	0.7
Salt	0.3
Magnesium oxide	0.1
Urea	0.1
Vitamin and mineral mix ¹	0.2
Chemical analysis, % of DM	
CP	16.0
Crude fat	4.7
NDF	39.9
ADF	28.8
NE _L , Mcal/kg of DM	1.59

¹Contained 34.9% Fe, 30.2% Zn, 24.5% Mn, 9.2% Cu, 0.5% I, 0.2% Co, 0.2% Se, 0.1% biotin, 14 KIU/kg of vitamin A, 3.5 KIU/kg of vitamin D, and 87.4 IU/kg of vitamin E.

at 0700 and 1900 h daily, and water was available at all times.

The experimental design was a 3 × 3 Latin square, and treatments were abomasal infusion of 1) ethanol (control), 2) *trans*-10, *trans*-12 CLA (**t-10, t-12**), and 3) *trans*-10, *cis*-12 CLA (**t-10, c-12**). The CLA supplements were solubilized in 95% ethanol at a ratio of 5:1 (ethanol:CLA), flushed with oxygen-free nitrogen, and stored at 4°C until used (maximum of 4 d). The infusate provided 5.0 g/d of the isomer of interest and small amounts of other CLA isomers present in the supplement (Table 2). Treatment periods were 4 d in duration, and supplements were abomasally infused 4 times/d at equal time intervals. At each infusion, an equal volume of supplement was infused into the abomasum via 0.5-cm (i.d.) polyvinyl chloride tubing that passed through the rumen fistula (Spires et al., 1975). Each treatment period was followed by a 7-d washout period to minimize carryover effects in the next treatment period.

Table 2. Quantity of fatty acids provided by the conjugated linoleic acid (CLA) supplements¹

CLA isomer, g/d	Treatment ²	
	<i>t</i> -10, <i>t</i> -12	<i>t</i> -10, <i>c</i> -12
<i>Trans</i> -10, <i>trans</i> -12	5.0	<0.1
<i>Trans</i> -10, <i>cis</i> -12	0.2	5.0
<i>Cis</i> -10, <i>trans</i> -12	0.2	<0.1
<i>Cis</i> -10, <i>cis</i> -12	0.1	<0.1
<i>Cis</i> -9, <i>trans</i> -11	<0.1	0.3
<i>Trans</i> -9, <i>trans</i> -11	<0.1	<0.1

¹Supplements were abomasally infused every 6 h during 4-d treatment periods.

²Abomasal infusion of *trans*-10, *trans*-12 CLA (*t*-10, *t*-12 treatment) or *trans*-10, *cis*-12 CLA (*t*-10, *c*-12 treatment).

Figure 1. Gas chromatograms of the starting material (A), intermediate mixture (B), and purified final product (C) in the synthesis of the *trans*-10, *trans*-12 conjugated linoleic acid (CLA) for the *t*-10, *t*-12 CLA treatment.

Cows were milked at 0700 and 1900 h daily. At each milking, milk yield was determined and duplicate milk samples were obtained. One aliquot was stored with preservative (bronopol tablet; D&F Control Systems, San Ramon, CA) at 4°C until analyzed for fat and protein using infrared analysis (AOAC, 2000, method 972.160; Dairy One Cooperative Inc., Ithaca, NY). The calibration reference methods for the infrared milk analysis were as described by Bernal-Santos et al. (2003). A second aliquot was stored without preservative at -20°C until analyzed for fatty acid composition. On the last day of each treatment period, blood samples were also taken from the coccygeal vein, with sodium heparin (100 U/mL of blood) used to prevent coagulation. Plasma was harvested (2,300 × *g* for 15 min at 4°C) and stored at -20°C until analyzed for fatty acid composition.

Source of CLA Supplements

The *trans*-10, *cis*-12 CLA isomer was purchased from Natural ASA (Hovdebygda, Norway) and its purity was >95%. Infusion of the *t*-10, *c*-12 treatment served as a positive control for the study.

The isomer of interest, *trans*-10, *trans*-12 CLA, was not commercially available, so we synthesized it in our laboratory. The FFA form of *trans*-10, *cis*-12 CLA (Figure 1A; >95% purity) was used as the starting material. The synthesis was initially optimized on a small scale and then applied on a larger scale. Briefly, the production involved addition of a concentrated iodine solution (1 g/L of petroleum ether) to a suspension containing 20 g of *trans*-10, *cis*-12 CLA in 200 mL of petroleum ether until a light pink color was achieved. This solution was placed in a UV hood with constant stirring and with further addition of iodine as necessary to maintain color. After 2 h, the suspension was washed several times with 200

mL of 0.01 *M* sodium thiosulfate until the pink color disappeared, indicating that the reaction was neutralized. The lower aqueous layer was discarded and the fatty acids were recovered by evaporating the solvent layer under a gentle stream of nitrogen. The resulting fatty acid mixture, analyzed by gas chromatography, contained around 70% *trans*-10, *trans*-12 CLA, 12% each of the *trans*-10, *cis*-12 and *cis*-10, *trans*-12 CLA isomers, and 1% of the *cis*-10, *cis*-12 CLA isomer (Figure 1B). The fatty acid mixture was solubilized in acetone and stored at -80°C to achieve selective crystallization of the *trans*-10, *trans*-12 CLA isomer. After 3 d, the crystallized fraction was isolated by vacuum filtration of the solution, followed by rinses with cold acetone. The resulting purity of the crystals was >90% *trans*-10, *trans*-12 CLA (Figure 1C, Table 2). Typically, 60% of the starting material was recovered as *trans*-10, *trans*-12 CLA, and the procedure was repeated until the quantity of enriched *trans*-10, *trans*-12 CLA product was sufficient for abomasal infusions.

Fractionation of Plasma Lipids

Lipids were extracted from a 3-mL plasma sample using the method of Hara and Radin (1978) with the modifications described by Corl et al. (2001). The plasma lipid classes of interest were FFA, triglycerides (TG), phospholipids (PL), and cholesterol esters (CE), and these were separated using solid-phase column chromatography (500-mg Amino SPE column; Orochem Technologies, Lombard, IL). The fractionation was performed according to Agren et al. (1992) and adapted for bovine plasma, with the modification of an increased solvent volume (12 mL) used to extract each fraction.

Internal standards were used to determine the adequacy of the fractionation method and determine recoveries for each plasma lipid fraction. Prior to fractionation, internal standards containing unique fatty acids for each of the 4 lipid classes were added to the plasma lipid extract. The standards utilized were nonadecanoic acid (19:0 FFA; Nu-Chek Prep, Inc., Elysian, MN); Δ 11-14 *cis* trieicosadienoin (TG containing 20:2; Nu-Chek Prep, Inc.); cholesteryl pentadecanoate (CE containing 15:0; Nu-Chek Prep, Inc.); and 1,2-diheptadecanoyl-*sn*-glycerol-3-phosphorylcholine (PL containing 17:0; Matreya, LLC, Pleasant Gap, PA). Fractionation of the plasma lipids for each sample was performed with and without the addition of internal standards to allow correction for endogenous quantities of the fatty acids used as internal standards. Each sample was also spiked with a known amount of tridecanoic acid methyl ester (13:0; Nu-Chek Prep, Inc.) prior to gas chromatographic analysis to allow for quantification of fatty acids within a sample. Following gas chromatographic analysis of these

Table 3. Recovery of internal standards added to the lipid extract and separated by solid-phase column chromatography

Internal standard, %	Plasma fraction ¹			
	FFA	TG	CE	PL
15:0 _{CE}	<0.1	3.7	96.3	<0.1
17:0 _{PL}	<0.1	<0.1	1.2	98.8
19:0 _{FFA}	99.9	<0.1	<0.1	<0.1
20:2 _{TG}	<0.1	99.9	<0.1	<0.1

¹CE = Cholesteryl esters; PL = phospholipids; TG = triglycerides.

samples (described in the following section), the fractionation procedure was evaluated by determining the distribution of the 4 internal standards in each fraction after accounting for endogenous levels of the fatty acids. Results indicated that proper separation of the plasma lipid classes occurred with 95 to nearly 100% of the internal standard being associated with the appropriate plasma lipid fraction (Table 3).

Fatty Acid Analysis

Milk fat was extracted using the method of Hara and Radin (1978), and fatty acid methyl esters were prepared by base-catalyzed transesterification according to Christie (1982), with modifications by Chouinard et al. (1999a). Fatty acid methyl esters were quantified using an HP 6890+ gas chromatograph (Hewlett-Packard, Avondale, PA) equipped with a CP-Sil 88 fused-silica capillary column [100 m × 0.25 mm (i.d.) with 0.2-μm film thickness; Varian, Inc., Walnut Creek, CA], and a flame ionization detector with hydrogen as the carrier gas. Gas chromatographic conditions for milk fat separation were as described by Perfield et al. (2002).

Fatty acid methyl esters were prepared from the plasma lipid fractions obtained following the procedure for solid-phase column chromatography. This involved using 1% methanolic sulfuric acid as described by Christie (1989). Using the same gas chromatographic system just described, conditions for plasma fatty acids had an initial oven temperature of 80°C that was increased at 2°C/min to 190°C and held for 20 min, followed by an increase of 10°C/min to 225°C, where the temperature was maintained for 32 min. The inlet port and detector were maintained at 250°C, and the split ratio was 50:1. The flow rate of the hydrogen carrier gas was 1 mL/min, the hydrogen flow to the detector was 25 mL/min, the airflow was 300 mL/min, and the flow of nitrogen makeup gas was 40 mL/min.

Fatty acid peaks were identified in the gas chromatographic analysis using pure methyl ester standards (Nu-Chek Prep). Additional standards for CLA isomers were obtained from Natural ASA. A butter oil reference standard (CRM 164; Commission of the European Commu-

Table 4. Performance of lactating dairy cows during abomasal infusion of conjugated linoleic acid (CLA) supplements¹

Variable	Treatment ²			SEM	P
	Control	<i>t</i> -10, <i>t</i> -12	<i>t</i> -10, <i>c</i> -12		
DMI, kg/d	25.7	26.2	24.5	0.9	0.50
Milk, kg/d	31.5	31.4	32.3	2.3	0.69
Milk fat					
%	3.41 ^a	3.32 ^a	2.41 ^b	0.11	0.02
kg/d	1.072 ^a	1.043 ^a	0.776 ^b	0.077	0.01
Milk protein ³					
%	2.76	2.68	2.72	0.09	0.06
kg/d	0.866	0.839	0.871	0.036	0.50
SCC × 1,000	46	29	41	9	0.53

^{a,b}Means (n = 3) within a row with different superscripts differ.

¹Values represent an average of d 4 of infusion for 3 cows.

²Abomasal infusion of ethanol (control), *trans*-10, *trans*-12 CLA (*t*-10, *t*-12 treatment), or *trans*-10, *cis*-12 CLA (*t*-10, *c*-12 treatment).

³True protein.

nity Bureau of References, Brussels, Belgium) was also analyzed periodically to control for column performance and the calculation of correction factors for individual fatty acids.

Statistical Analysis

Data were statistically analyzed as a 3 × 3 Latin square design using the PROC MIXED procedure of SAS (SAS Institute, 1998) in which the model is

$$Y_{ijk} = \mu + T_i + P_j + C_k + E_{ijk}$$

where Y_{ijk} is the observation, μ is the overall mean, T_i is the treatment ($i = 1, 2$, and 3), P_j is the period ($j = 1, 2$, and 3), C_k is the cow ($k = 1, 2$, and 3), and E_{ijk} is residual error. Significance was set at $P < 0.05$.

RESULTS AND DISCUSSION

Abomasal infusion of treatments was used as a convenient means of avoiding metabolism of the CLA supplements by rumen bacteria and of accurately providing the desired quantity of supplement (Table 2). Treatments provided 5.0 g/d of either *trans*-10, *trans*-12 CLA, the fatty acid of interest, or *trans*-10, *cis*-12 CLA, a known inhibitor of milk fat synthesis whose potency is well established (de Veth et al., 2004). In comparison with the control treatment, the *t*-10, *t*-12 treatment had no effect on milk fat synthesis, whereas the *t*-10, *c*-12 treatment resulted in the expected reductions in milk fat percentage and milk fat yield (Table 4). The temporal pattern (Figure 2) illustrates a progressive reduction in milk fat yield for the *t*-10, *c*-12 treatment period, with a return to the previous yield when the infusion was terminated. Recently, Sæbo et al. (2005) also reported

Figure 2. Temporal pattern of milk fat yield during abomasal infusion of conjugated linoleic acid (CLA) supplements (control = ethanol; *t*-10, *t*-12 treatment = *trans*-10, *trans*-12 CLA; *t*-10, *c*-12 treatment = *trans*-10, *cis*-12 CLA). Animals received supplements for 4 d (dotted lines). Values represent means from 3 cows; SEM = 0.08 kg/d.

that abomasal infusions of *trans*-10, *trans*-12 CLA had no effect on milk fat synthesis. Likewise, the magnitude of the observed reduction and the temporal pattern were consistent with previous studies in which *trans*-10, *cis*-12 CLA was abomasally infused (Baumgard et al., 2001; Peterson et al., 2002). In contrast to the effects on milk fat, the milk and milk protein yields were not affected by either of the treatments, and DMI was similar among the treatment periods (Table 4). The lack of change in milk yield, milk protein yield, and DMI is consistent with other studies in which a reduction in milk fat synthesis was observed when CLA isomers were abomasally infused (e.g., Baumgard et al., 2000, 2001; Peterson et al., 2002; Loor and Herbein, 2003; Sæbo et al., 2005).

Blood samples obtained on the last day of each treatment period were used to determine the distribution of the *trans*-10, *trans*-12 and *trans*-10, *cis*-12 CLA isomers in plasma lipid fractions (Table 5). Regardless of treatment, *trans*-10, *trans*-12 CLA was present in all plasma fractions. When cows received the *t*-10, *t*-12 treatment, the concentration of this CLA isomer significantly increased in the TG fraction of plasma. The *trans*-10, *cis*-12 CLA isomer differed in that it was present in the plasma lipid fractions only when cows received the *t*-10, *c*-12 treatment, and during this period the greatest increase was also observed in the TG fraction (Table 5). These results agree with a previous study in which a marked increase in the *trans*-10, *cis*-12 CLA concentration was observed for the plasma TG fraction when a larger dose of this isomer (15 g/d) was infused for a shorter period (2 d; Loor and Herbein, 2003). Therefore, short-term supplementation of CLA isomers caused their concentration to be increased predominantly in the TG fraction of plasma lipids. This result is consistent with the increased presence of these CLA isomers in milk fat, as the TG fraction is the primary pool of plasma fatty acids taken up by the mammary gland and incorporated into milk fat (Bauman and Grinari, 2001).

The content of CLA isomers in milk fat was consistent with the plasma TG pattern and was increased by the respective CLA supplements, as expected (Table 6). The *trans*-10, *trans*-12 CLA isomer was present in milk fat at low concentrations (0.03% of total fatty acids) in the control and *t*-10, *c*-12 treatment periods and increased to 0.11% of total fatty acids when cows received the *t*-10, *t*-12 treatment. After correcting for endogenous levels in milk fat, the transfer efficiency of abomasally infused *trans*-10, *trans*-12 CLA into milk fat averaged $17 \pm 3\%$ (mean $\pm$ SD). This is comparable to the $23 \pm 5\%$ (mean $\pm$ SD) transfer efficiency for *trans*-10, *trans*-12 CLA re-

Table 5. Fractional concentration of infused fatty acids in plasma lipids

Fraction ²	Treatment ¹			SEM	P
	Control	<i>t</i> -10, <i>t</i> -12	<i>t</i> -10, <i>c</i> -12		
	———— (<i>trans</i> -10, <i>trans</i> -12 CLA, % total fatty acids) ————				
FFA	0.04	0.10	0.06	0.026	0.12
TG	0.20 ^b	0.33 ^a	0.16 ^b	0.041	<0.01
CE	0.25	0.25	0.26	0.025	0.78
PL	0.11	0.07	0.11	0.025	0.26
	———— (<i>trans</i> -10, <i>cis</i> -12 CLA, % total fatty acids) ————				
FFA	<0.01 ^b	<0.01 ^b	0.02 ^a	0.008	0.02
TG	<0.01 ^b	<0.01 ^b	0.13 ^a	0.041	<0.001
CE	<0.01 ^b	<0.01 ^b	0.03 ^a	0.004	<0.001
PL	<0.01	<0.01	0.02	0.014	0.28

^{a,b}Means within a row with different superscripts differ.

¹Abomasal infusion of ethanol (control), *trans*-10, *trans*-12 CLA (*t*-10, *t*-12 treatment), or *trans*-10, *cis*-12 CLA (*t*-10, *c*-12 treatment).

²TG = triglycerides; CE = cholesteryl esters; PL = phospholipids.

Table 6. Composition of milk fat during abomasal infusion of conjugated linoleic acid (CLA) supplements¹

Milk fat composition	Treatment ²			SEM	P
	Control	<i>t</i> -10, <i>t</i> -12	<i>t</i> -10, <i>c</i> -12		
Fatty acid, %					
4:0	5.00	5.14	5.33	0.36	0.56
6:0	2.38 ^a	2.43 ^a	2.01 ^b	0.11	<0.001
8:0	1.12 ^a	1.14 ^a	0.89 ^b	0.04	<0.001
10:0	2.21 ^b	2.30 ^a	1.78 ^c	0.14	<0.001
12:0	2.42 ^a	2.48 ^a	2.19 ^b	0.19	<0.001
14:0	9.86 ^b	10.17 ^a	9.54 ^c	0.40	<0.001
14:1	0.73 ^a	0.61 ^b	0.63 ^b	0.17	<0.01
15:0	0.92	0.93	0.93	0.02	0.72
16:0	29.23 ^a	29.78 ^a	26.95 ^b	0.85	<0.001
16:1	1.20 ^a	1.00 ^b	1.03 ^b	0.07	<0.001
17:0	0.53 ^b	0.56 ^b	0.61 ^a	0.02	<0.001
18:0	11.65 ^b	13.95 ^a	13.71 ^a	1.40	<0.001
18:1 <i>trans</i> -4	0.03	0.04	0.03	<0.01	0.25
18:1 <i>trans</i> -5	0.03	0.03	0.02	<0.01	0.22
18:1 <i>trans</i> -6-8	0.42 ^b	0.44 ^b	0.50 ^a	0.01	<0.001
18:1 <i>trans</i> -9	0.32	0.32	0.33	0.02	0.71
18:1 <i>trans</i> -10	0.59	0.62	0.62	0.05	0.26
18:1 <i>trans</i> -11	1.64	1.63	1.74	0.05	0.24
18:1 <i>trans</i> -12	0.71	0.71	0.71	0.06	0.99
18:1 <i>cis</i> -9	22.22 ^b	19.97 ^c	23.35 ^a	1.39	<0.001
18:2 <i>cis</i> -9, <i>cis</i> -12	2.88 ^b	2.87 ^b	3.06 ^a	0.11	<0.04
18:2 <i>cis</i> -9, <i>trans</i> -11	0.62 ^a	0.49 ^c	0.56 ^b	0.08	<0.001
18:2 <i>trans</i> -10, <i>cis</i> -12	<0.01 ^b	<0.01 ^b	0.18 ^a	0.01	<0.001
18:2 <i>trans</i> -10, <i>trans</i> -12	0.03 ^b	0.11 ^a	0.03 ^b	<0.01	<0.001
18:3	0.39	0.39	0.38	0.01	0.57
20:0	0.12	0.12	0.12	0.01	0.15
Others	2.76 ^a	2.65 ^b	2.77 ^a	0.13	0.03
Summation by source ³					
<16:0	24.65 ^b	25.22 ^a	23.30 ^c	0.68	<0.01
16:0 and 16:1	30.43 ^a	30.77 ^a	27.98 ^b	0.89	<0.001
>16:0	44.93 ^b	44.90 ^b	48.72 ^a	0.62	<0.001
Desaturase index ⁴					
14:1/(14:0 + 14:1)	0.068 ^a	0.055 ^c	0.061 ^b	0.014	<0.001
16:1/(16:0 + 16:1)	0.039 ^a	0.032 ^b	0.037 ^a	0.002	<0.001
18:1/(18:0 + 18:1)	0.656 ^a	0.590 ^b	0.630 ^a	0.040	<0.001
CLA/(18:1 <i>t</i> 11 + CLA)	0.272 ^a	0.230 ^b	0.244 ^b	0.028	<0.001

^{a-c}Means within a row with different superscripts differ.

¹Values represent an average of 4 of infusion.

²Abomasal infusion of ethanol (control), *trans*-10, *trans*-12 CLA (*t*-10, *t*-12 treatment), or *trans*-10, *cis*-12 CLA (*t*-10, *c*-12 treatment).

³Fatty acids <16:0 originated from de novo synthesis, fatty acids >16:0 were preformed fatty acids taken up by the mammary gland, and 16:0 and 16:1 fatty acids came from both de novo and preformed sources.

⁴Values represent the ratio of product/(substrate + product) for Δ^9 -desaturase. Double bonds are in the *cis* orientation unless otherwise indicated; CLA = *cis*-9, *trans*-11 CLA.

ported by Sæbo et al. (2005). The milk fat content of *trans*-10, *cis*-12 CLA was at the limit of detection in the control and *t*-10, *t*-12 treatment periods. However, in the *t*-10, *c*-12 treatment group, the milk fat content of this isomer increased to 0.18% of total fatty acids, representing an average transfer efficiency of $28 \pm 7\%$ (mean $\pm$ SD). This value is within the range of previous observations but was slightly greater than the average of 22% reported by de Veth et al. (2004) from a summary of published studies ($n = 6$) in which *trans*-10, *cis*-12 CLA was abomasally infused across a range of doses. In general, similar transfer efficiencies have been reported for

the limited number of other CLA isomers that have been investigated in lactating cows (Chouinard et al., 1999a,b; Perfield et al., 2004; Sæbo et al., 2005).

The fatty acid composition of milk fat from the 3 experimental periods is presented in Table 6. The *t*-10, *c*-12 treatment caused a reduction in the secretion of most milk fatty acids, but a greater reduction occurred in the secretion of fatty acids containing <16 carbons. Consequently, the milk fat composition was shifted to reflect a corresponding increase in the percentage of most longer-chain fatty acids (>16 carbons). These changes in milk fat composition are comparable to results previously ob-

served in which CLA infusions caused a reduction in milk fat (Chouinard et al., 1999a,b; Baumgard et al., 2000, 2001; Peterson et al., 2002; Looor and Herbein 2003; Mackle et al., 2003). Although the *t*-10, *t*-12 treatment caused no difference in the overall yield of milk fatty acids, we observed that secretion of fatty acids representing the substrate and products of Δ^9 -desaturase shifted in a manner indicative of an inhibition of this enzyme (Table 6). This was also observed by Sæbo et al. (2005).

The desaturase index comprises 4 ratios of fatty acids that represent a proxy for the Δ^9 -desaturase enzyme in the mammary gland (Bauman et al., 2003), and studies in lactating mice and goats have demonstrated a strong correlation between these fatty acid pairs and the activity and abundance of the enzyme (Singh et al., 2004; Bernard et al., 2005). When compared with the control, ratios for the *t*-10, *c*-12 treatment were significantly decreased for some of the desaturase pairs, whereas the *t*-10, *t*-12 treatment caused a greater magnitude of reduction and significant effects for all ratios (Table 6).

The fatty acids comprising TG dictate their fluidity characteristics, and this is an important consideration in the packaging and secretion of milk fat. Triglycerides can become more fluid by increasing the content of unsaturated fatty acids. Thus, Δ^9 -desaturase and its conversion of stearic acid to oleic acid in the mammary tissue play an important role in the supply of unsaturated fatty acids (Chilliard et al., 2000). Recently, it has been proposed that a reduced supply of oleic acid resulting from an inhibition of Δ^9 -desaturase is the basis for the reduced milk fat yield during treatment with *trans*-10, *cis*-12 CLA and for the diet-induced MFD (Looor and Herbein, 2003; Looor et al., 2005a,b). The present study offers no support for this concept, as the *t*-10, *t*-12 treatment reduced the oleic acid content of milk fat and markedly altered the desaturase index but had no effect on milk fat yield. Furthermore, sterculic acid, a potent inhibitor of Δ^9 -desaturase (Jeffcoat and Pollard, 1977; Gomez et al., 2003), causes a marked reduction in the oleic acid content of milk fat and a shift in the desaturase index but has no effect on milk fat yield when administered to dairy cows (Griinari et al., 2000; Corl et al., 2001; Kay et al., 2004). Thus, a reduction in Δ^9 -desaturase is not a prerequisite for MFD. Likewise, low doses of *trans*-10, *cis*-12 CLA reduce milk fat yield without affecting the desaturase index; the desaturase index is altered only at higher doses in which the reduction in milk fat secretion is greater than approximately 25% (Baumgard et al., 2000, 2001; Peterson et al., 2002). The regulation of Δ^9 -desaturase by *trans*-10, *cis*-12 CLA was also initially speculated to be the mechanism by which this CLA isomer regulates the lipid metabolism associated with body fat accretion in mice (Choi et al., 2000); however, recent

evidence indicates that the mechanism is independent of effects on Δ^9 -desaturase (Kang et al., 2004).

Overall, providing supplements containing *trans*-10, *trans*-12 CLA and *trans*-10, *cis*-12 CLA increased the concentrations of these specific CLA isomers in the TG fraction of plasma lipids and milk fat. Supplementation of 5 g/d of the *trans*-10, *cis*-12 CLA isomer for 4 d caused a reduction in milk fat synthesis accompanied by a decrease in the desaturase index. A similar supply of *trans*-10, *trans*-12 CLA had no effect on the total quantity of milk fatty acids secreted. However, the *t*-10, *t*-12 treatment did decrease Δ^9 -desaturase, as indicated by the desaturase index, thereby altering the milk fatty acid composition. Thus, results from the current study suggest that effects on Δ^9 -desaturase are not an essential component in the mechanism by which *trans*-10, *cis*-12 CLA regulates fatty acid synthesis.

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