Use of Conjugated Linoleic Acid (CLA) Enrichments to Examine the Effects of *trans*-8, *cis*-10 CLA, and *cis*-11, *trans*-13 CLA on Milk-Fat Synthesis*

J. W. Perfield, II,¹ A. Sæbø,² and D. E. Bauman¹

¹Department of Animal Science,
Cornell University, Ithaca, NY 14853

²Natural ASA, N-6160, Hovdebygda, Norway

Abstract

Conjugated linoleic acid (CLA) supplements have typically been comprised of 4 isomers (*trans*-8, *cis*-10; *cis*-9, *trans*-11; *trans*-10, *cis*-12; and *cis*-11, *trans*-13 CLA). Abomasal infusion of pure isomers has shown that *trans*-10, *cis*-12 CLA is a potent inhibitor of milk-fat synthesis, whereas *cis*-9, *trans*-11 CLA has no effect. However, there appear to be additional fatty acids that inhibit milk-fat synthesis, and the objective of this study was to investigate the effects of additional CLA isomers present in CLA supplements. Four rumen fistulated Holstein cows (141 ± 8 DIM, mean ± SE) were randomly assigned in a 4 × 4 Latin square experiment. Treatments were abomasal infusion of (1) skim milk (negative control), (2) *trans*-10, *cis*-12 CLA supplement (positive control), (3) *trans*-8, *cis*-10 CLA supplement, and (4) *cis*-11, *trans*-13 CLA supplement. Treatments 2 through 4 were targeted to provide 4 g/d of the CLA isomer of interest. The *trans*-8, *cis*-10 CLA supplement had no effect on milk-fat yield, whereas the *trans*-10, *cis*-12 CLA supplement reduced milk-fat yield by 35%. The *cis*-11, *trans*-13 CLA supplement contained some *trans*-10, *cis*-12 CLA, and when data were compared to the positive control treatment group, it was obvious that *cis*-11, *trans*-13 CLA also had no effect on milk-fat synthesis. Milk-fat content of specific CLA isomers was significantly elevated within respective treatment groups. Milk yield, DMI, and milk protein yield were unaffected by treatment. Overall, *trans*-10, *cis*-12 CLA reduced milk-fat synthesis, whereas the other major isomers present in CLA supplements (*trans*-8, *cis*-10 CLA and *cis*-11, *trans*-13 CLA) had no effect.

(Key words: conjugated linoleic acids, CLA, milk fat, lactation, cows)

Abbreviation key: CLA = conjugated linoleic acid, MFD = milk-fat depression.

INTRODUCTION

Conjugated linoleic acid (CLA) is a collective term for different positional and geometric isomers of octadecadienoic acid that contain a pair of double bonds in a conjugated configuration. Mixed isomers of CLA have been shown to reduce milk-fat content when abomasally infused into lactating cows (Lor and Herbein, 1998; Chouinard et al., 1999a, 1999b; Mackle et al., 2003). Commercial preparations of calcium salts of mixed CLA isomers provide a method for protecting the CLA from biohydrogenation in the rumen. Dietary supplements of these rumen-protected calcium salts of CLA cause a reduction in milk-fat synthesis of cows that are at different stages of lactation and fed a variety of diets (Medeiros et al., 2000; Giesy et al., 2002; Perfield et al., 2002; Bernal-Santos et al., 2003).

Commercial preparations of mixed CLA isomers are predominantly composed of 4 CLA isomers (*trans*-8, *cis*-10 CLA; *cis*-9, *trans*-11 CLA; *trans*-10, *cis*-12 CLA; and *cis*-11, *trans*-13 CLA). Abomasal infusion of relatively pure CLA isomers identified *trans*-10, *cis*-12 CLA as a potent inhibitor of milk-fat synthesis (Baumgard et al., 2000, 2002; Lor and Herbein, 2003) and the curvilinear relationship between percent reduction in milk fat and milk fat content of *trans*-10, *cis*-12 CLA has been established (Baumgard et al., 2001; Peterson et al., 2002). However, scenarios exist where the milk-fat content of *trans*-10, *cis*-12 CLA does not appear to account completely for the percent reduction in milk-fat synthesis. Peterson et al. (2003) induced milk fat depression (MFD) with a high concentrate, low roughage diet and noted that in their investigation, as well as in other studies where MFD was induced with polyunsaturated oil supplements (Pipero et al., 2000; Whitlock et al., 2002), the MFD was much greater than would be anticipated by the milk-fat content of *trans*-10, *cis*-12 CLA. A similar discrepancy between milk-fat content of *trans*-10, *cis*-12 CLA and reduction in milk fat was ob-

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Corresponding author: D. E. Bauman; e-mail: deb6@cornell.edu.

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Table 1. Ingredient and chemical composition of diet.

Composition	Concentration
Ingredient, % of DM	
Alfalfa hay	50.0
Corn meal	26.9
Soybean meal (51% CP)	3.4
Expeller soybean meal	1.8
Cottonseed	6.8
Urea	0.3
Dicalcium phosphate	0.3
Salt	0.5
Vitamin and mineral mix ¹	1.2
Chemical analysis, % of DM	
CP	17.1
Crude fat	4.1
NDF	43.7
ADF	30.7
NEL, Mcal/kg DM	1.54

¹Contained 20.0% Cl, 18.0% Na, 12.0% Ca, 8.0% S, 7.5% Mg, 1.0% K, 0.48% Zn, 0.40% Mn, 0.29% Fe, 0.07% Cu, 0.01% P, 0.006% Co, 550 IU/g of vitamin A, 132 IU/g of vitamin D, 2970 IU/g of vitamin E.

served by Perfield et al. (2002) and Bernal-Santos et al. (2003) in cows fed rumen-protected CLA. Likewise, Chouinard et al. (1999b) demonstrated that abomasal infusion of a CLA supplement containing a mixture of CLA isomers devoid of the *trans*-10, *cis*-12 CLA isomer caused MFD.

The substantial divergences from the relationship between milk-fat content of *trans*-10, *cis*-12 CLA and reduction in milk-fat yield suggest that other CLA isomers or unique fatty acids formed in the rumen might also affect lipid metabolism in the mammary gland. The objective of the present study was to examine milk-fat synthesis and compare the effects of CLA supplements enriched with other predominant CLA isomers found in CLA mixtures (*trans*-8, *cis*-10 CLA and *cis*-11, *trans*-13 CLA) to the effects of *trans*-10, *cis*-12 CLA, a known inhibitor of milk-fat synthesis. Similar to *trans*-10, *cis*-12 CLA, *trans*-8, *cis*-10 CLA, and *cis*-11, *trans*-13 CLA are CLA isomers that are naturally present in the rumen and milk fat but at very low concentrations

(Sehat et al., 1998; Bauman et al., 2000; Piperova et al., 2000, 2002).

MATERIALS AND METHODS

The Cornell University Institutional Animal Care and Use Committee approved all procedures involving animals. Four rumen fistulated lactating Holstein cows 141 ± 8 DIM (mean ± SE) were randomly assigned in a 4 × 4 Latin square experiment and housed in tie stalls at the Cornell University Large Animal Teaching and Research Unit. Cows were fed a TMR formulated to meet or exceed nutrient requirements for energy, protein, minerals, and vitamins (NRC, 2001) using the Cornell Net Carbohydrate and Protein System (Fox et al., 1992). Chopped alfalfa was the major forage component, with cornmeal as the major concentrate (Table 1). Cows were fed *ad libitum* with equal portions of feed offered at 0700 and 1900 h daily. Water was available at all times.

Cows were milked at 0700 and 1900 h daily. Yield was determined, and samples were taken from each milking. One aliquot was stored with preservative (bromopol tablet; D&F Control System, 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 follows: fat by modified Mojonnier ether extraction (AOAC 2000: method 989.05) and Kjeldahl true protein (AOAC 2000: method 991.22). A second aliquot was taken for analysis of fatty-acid composition and stored without preservative at -20°C.

Treatments were abomasal infusion of (1) skim milk (control), (2) *trans*-8, *cis*-10 CLA supplement (8,10 CLA), (3) *cis*-11, *trans*-13 CLA supplement (11,13 CLA), and (4) *trans*-10, *cis*-12 CLA supplement (10,12 CLA). The conditions by which the 8,10 CLA and 11,13 CLA supplements were manufactured resulted in a significant proportion of one other CLA isomer in each supple-

Table 2. Fatty acid profile of conjugated linoleic acid (CLA) supplements.

Fatty acid, %	8,10 CLA supplement	11,13 CLA supplement	10,12 CLA supplement
C16:0	<0.1	<0.1	<0.1
C18:0	<0.1	<0.1	<0.1
C18:1 <i>cis</i> -9	1.1	1.1	0.9
C18:2 <i>cis</i> -9, <i>cis</i> -12	0.3	<0.5	<0.1
Conjugated linoleic acid isomers			
C18:2 <i>trans</i> -8, <i>cis</i> -10	32.0	3.7	...
C18:2 <i>cis</i> -9, <i>trans</i> -11	52.0	4.3	7.3
C18:2 <i>trans</i> -10, <i>cis</i> -12	2.2	44.0	88.6
C18:2 <i>cis</i> -11, <i>trans</i> -13	1.4	39.0	...
Other CLA	7.3	7.5	2.8
Other	3.5	<0.1	<0.1

Table 3. Quantities of conjugated linoleic acid (CLA) isomers provided during the infusion period by emulsions of each supplement.¹

Conjugated linoleic acid, g/d	8,10 CLA supplement	11,13 CLA supplement	10,12 CLA supplement
C18:2 <i>trans</i> -8, <i>cis</i> -10	3.5	0.4	nd ²
C18:2 <i>cis</i> -9, <i>trans</i> -11	5.7	0.4	0.3
C18:2 <i>trans</i> -10, <i>cis</i> -12	0.2	4.2	4.0
C18:2 <i>cis</i> -11, <i>trans</i> -13	0.2	3.8	nd
Other CLA isomers	0.8	0.7	0.1

¹Values represent the average amount supplied over the 5 d infusion period (n = 4 cows) for each treatment; SE represented <10% of the mean.

²nd = Not detected.

ment. The fatty acid composition of the supplements (Natural ASA, Hovdebygda, Norway) is provided in Table 2. The presence of *trans*-10, *cis*-12 CLA in the 11,13 CLA supplement was accounted for by the positive control treatment group, which provided a comparable quantity of *trans*-10, *cis*-12 CLA. The CLA supplements were emulsified with skim milk using a microfluidizer (model 110T; Microfluidics, Newton, MA) at a pressure of 8000 lb/in² (563.6 kg/cm²) as previously described (Chouinard et al., 1999a). Emulsions were prepared fresh each experimental period, and target concentrations were formulated to provide 4.0 g/d of the isomer of interest. Actual CLA concentrations in the emulsions were determined by the difference in total solids content (AOAC, 2000: method 990.20) between the skim milk (control) and each of the emulsions, and average amounts of CLA isomers infused over the 4 treatment periods are presented in Table 3. Skim milk (control) and CLA emulsions were stored at 4°C until infused.

Treatment periods were 5 d in duration, and supplements were abomasally infused 4 times a day at 0100, 0700, 1300, and 1900 h. Equal volumes of supplement were infused at each time point via a 0.5-cm (i.d.) polyvinyl chloride tubing through the rumen fistula and sulcus omasi into the abomasum (Spires et al., 1975). Each treatment period was followed by a 7-d washout period to prevent carryover effects in the next treatment period.

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 transmethylation according to Christie (1982) with modifications by Chouinard et al. (1999a). Fatty acid methyl esters were quantified using a gas chromatograph (GCD system HP 6890+; 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). Gas chromatograph conditions were as de-

scribed by Perfield et al. (2002). Fatty acid peaks were identified using pure methyl ester standards (Nu-Chek Prep, Elysian, MN). Additional standards for CLA isomers were obtained from Natural ASA. A butter oil reference standard (CRM 164; Commission of the European Community Bureau of References, Brussels, Belgium) was used to determine recoveries and correction factors for individual fatty acids.

Statistical Analysis

Data were statistically analyzed as a 4 × 4 Latin square design using the PROC MIXED procedure of SAS (1998), where the model is:

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

where

Y_{ijk} = observation,
 μ = overall mean,
 T_i = treatment (i = 1, 2, 3, and 4),
 P_j = period (j = 1, 2, 3, and 4),
 C_k = cow (k = 1, 2, 3, and 4), and
 E_{ijk} = residual error.

RESULTS AND DISCUSSION

Abomasal infusion was used as a convenient experimental method to provide the CLA isomer supplements in a manner that would avoid biohydrogenation and metabolism of the fatty acids by rumen bacteria. In comparison with the control treatment, the 8,10 CLA supplement had no effect on milk-fat synthesis. Reductions in milk-fat percentage and milk-fat yield were observed for both the 10,12 CLA and 11,13 CLA treatment groups (Table 4). However, a similar quantity of *trans*-10, *cis*-12 CLA, a potent inhibitor of milk-fat synthesis, was provided by both supplements. The temporal pattern depicts a progressive reduction in milk-fat yield for the 10,12 CLA and 11,13 CLA treatments

Table 4. Performance of lactating dairy cows during abomasal infusion of conjugated linoleic acid (CLA) supplements.¹

Variable	Treatment				SEM	P
	Control	8,10 CLA supplement	11,13 CLA supplement	10,12 CLA supplement		
DMI, kg/d	22.1	23.4	20.7	21.8	1.9	0.44
Milk, kg/d	33.1	33.5	32.4	35.8	2.3	0.37
Milk fat %	3.19 ^a	3.12 ^a	2.07 ^b	1.95 ^b	0.19	<0.001
kg/d	1.057 ^a	1.027 ^a	0.676 ^b	0.687 ^b	0.070	0.004
Milk protein ² %	2.65	2.66	2.64	2.64	0.13	0.99
kg/d	0.875	0.879	0.853	0.935	0.047	0.22

¹Values represent an average of d 5 of infusion for 4 cows. Means within a row with different superscripts differ.

²True protein.

with a return to previous yields after the termination of infusions (Figure 1). In contrast to milk fat, yields of milk and milk protein were not affected by any of the treatments, and DMI did not differ significantly among treatment groups (Table 4). The lack of change in DMI, milk yield, and milk-protein yield is consistent with other studies that have abomasally infused CLA isomers and observed a reduction in milk fat (Chouinard et al., 1999a, 1999b; Baumgard et al., 2000, 2001; Peterson et al., 2002; Loor and Herbein, 2003).

The fatty-acid composition of milk fat from the 4 experimental groups is presented in Table 5. The fatty-acid composition of the 8,10 CLA treatment group was similar to that of the control group. In contrast, the 10,12 CLA and 11,13 CLA supplements caused a reduction in the secretion of most milk fatty acids. Milk fatty-acid composition was shifted due to a greater reduction in the secretion of fatty acids containing <16 carbons, resulting in a corresponding increase in the percentage

of most longer-chain fatty acids (>16 carbons). Milk-fat composition changes are also comparable to results previously observed in studies in which CLA infusions caused a reduction in milk fat (Chouinard et al., 1999a, 1999b; Baumgard et al., 2000, 2001; Peterson et al., 2002; Loor and Herbein 2003; Mackle et al., 2003). These previous studies also indicated that secretion of all milk fatty acids was reduced, with a greater reduction of the fatty acids containing ≤16 carbons resulting in a proportional increase of the fatty acids consisting of >16 carbons.

As expected, CLA supplements caused significant increases in milk fat of CLA isomers corresponding to those provided by the supplements. The 8,10 and 11,13 CLA supplements each contained a significant proportion of one other CLA isomer due to the manufacturing process (Table 2). The 8,10 CLA supplement contained a large proportion of the *cis*-9, *trans*-11 CLA isomer, and infusion of the 8,10 CLA supplement increased both the *trans*-8, *cis*-10 CLA and *cis*-9, *trans*-11 CLA content of milk fat. The 11,13 CLA supplement contained a significant amount of the *trans*-10, *cis*-12 CLA isomer, and it increased the milk-fat content of both *cis*-11, *trans*-13 CLA and *trans*-10, *cis*-12 CLA, whereas the relatively pure 10,12 CLA supplement only increased the concentration of *trans*-10, *cis*-12 CLA. The *trans*-8, *cis*-10 CLA isomer had the greatest transfer efficiency (36 ± 7%; mean ± SD), whereas the transfer efficiency of the *cis*-11, *trans*-13 CLA isomer was 23 ± 5%, and that of the *trans*-10, *cis*-12 CLA isomer was 17 ± 6% and 23 ± 8% when provided by the 11,13 and 10,12 CLA supplements, respectively. These transfer efficiencies are similar to those observed in other studies involving abomasal infusion of mixtures of CLA isomers, as well as infusions of pure *trans*-10, *cis*-12 CLA (Chouinard et al., 1999a, 1999b; Baumgard et al., 2001; Peterson et al., 2002).

Figure 1. Temporal pattern of milk fat yield during abomasal infusion of conjugated linoleic acid (CLA) supplements. Infusion period (5 d) indicated by the dotted lines. Values represent means from 4 cows; SEM = 0.07 kg/d.

Table 5. Composition of milk fat during abomasal infusion of conjugated linoleic acid (CLA) supplements.¹

Fatty acid, %	Treatment				SEM	P
	Control	8,10 CLA supplement	11,13 CLA supplement	10,12 CLA supplement		
4:0	4.07 ^a	4.23 ^a	3.93 ^a	3.34 ^b	0.22	0.003
6:0	1.88 ^a	1.96 ^a	1.45 ^b	1.21 ^c	0.11	<0.001
8:0	0.92 ^a	0.98 ^a	0.67 ^b	0.56 ^c	0.07	<0.001
10:0	1.85 ^a	2.02 ^a	1.41 ^b	1.18 ^c	0.14	<0.001
12:0	2.04 ^b	2.24 ^a	1.79 ^c	1.66 ^c	0.18	<0.001
14:0	8.67 ^b	9.62 ^a	8.35 ^{bc}	7.75 ^c	0.58	<0.001
14:1	0.72 ^a	0.74 ^a	0.57 ^b	0.75 ^a	0.17	<0.01
15:0	0.85 ^b	0.89 ^a	0.90 ^a	0.88 ^a	0.01	0.03
16:0	26.59 ^a	26.41 ^a	24.70 ^b	23.95 ^b	0.66	<0.01
16:1	1.24 ^a	1.04 ^b	1.04 ^b	1.28 ^a	0.12	0.01
17:0	0.57 ^b	0.53 ^b	0.64 ^a	0.63 ^a	0.04	<0.001
18:0	14.46 ^b	14.59 ^b	16.12 ^a	14.67 ^{ab}	0.78	0.09
18:1 <i>trans</i> -4	0.02	0.03	0.02	0.02	<0.01	0.41
18:1 <i>trans</i> -5	0.03	0.03	0.03	0.02	<0.01	0.71
18:1 <i>trans</i> 6-8	0.40 ^b	0.42 ^b	0.53 ^a	0.54 ^a	0.02	<0.001
18:1 <i>trans</i> -9	0.30 ^b	0.29 ^b	0.31 ^{ab}	0.33 ^a	0.02	0.03
18:1 <i>trans</i> -10	0.66	0.60	0.65	0.64	0.10	0.54
18:1 <i>trans</i> -11	1.33 ^d	1.40 ^c	1.71 ^a	1.50 ^b	0.13	<0.001
18:1 <i>trans</i> -12	0.60	0.62	0.61	0.65	0.03	0.30
18:1 <i>cis</i> -9	26.21 ^{bc}	24.37 ^c	27.43 ^b	29.99 ^a	1.19	<0.001
18:2 <i>cis</i> -9, <i>cis</i> -12	2.31 ^b	2.31 ^b	2.60 ^b	3.18 ^a	0.20	<0.001
18:2 <i>trans</i> -8, <i>cis</i> -10	<0.01 ^b	0.27 ^a	<0.01 ^b	<0.01 ^b	<0.01	<0.001
18:2 <i>cis</i> -9, <i>trans</i> -11	0.46 ^c	0.83 ^a	0.55 ^b	0.60 ^b	0.03	<0.001
18:2 <i>trans</i> -10, <i>cis</i> -12	<0.01 ^b	<0.01 ^b	0.19 ^a	0.18 ^a	<0.01	<0.001
18:2 <i>cis</i> -11, <i>trans</i> -13	<0.01 ^b	<0.01 ^b	0.23 ^a	<0.01 ^b	<0.01	<0.001
18:3	0.77	0.81	0.63	1.32	0.25	0.19
20:0	0.11	0.11	0.11	0.11	<0.01	0.71
Others	2.92	2.90	2.77	3.04	0.12	0.50
Summation						
<C16	21.02 ^b	22.70 ^a	19.06 ^c	17.31 ^d	1.36	<0.001
C16 & C16:1	27.83 ^a	27.44 ^a	25.74 ^b	25.22 ^b	0.68	0.003
>C16	51.15 ^b	49.83 ^b	55.17 ^a	57.49 ^a	1.97	<0.001

¹Values represent an average of d 5 of infusion. Means within a row with different superscripts differ.

The lack of an effect of the 8,10 CLA supplement on milk-fat yield and milk fatty-acid composition is a clear indication that *trans*-8, *cis*-10 CLA at a dose of 3.5 g/d does not cause MFD. The 8,10 CLA supplement did provide 5.7 g/d of *cis*-9, *trans*-11 CLA, but there have been a number of previous studies that focused on this specific isomer, in part because it is the major CLA isomer in milk fat. Whereas Loor and Herbein (1998) concluded that *cis*-9, *trans*-11 CLA and its metabolite *trans*-11 18:1 did cause MFD in an early study involving infusion of mixed isomers of CLA, this has not been supported by the present study (Figure 1) or more recent work. Abomasal infusion studies using relatively pure *cis*-9, *trans*-11 CLA have shown that this isomer has no effect on milk-fat synthesis even at high concentrations (15 g/d) (Baumgard et al., 2000, 2002; Loor and Herbein, 2003). Grinari et al. (2000) abomasally infused 25 g/d of a mixture containing equal quantities of *trans*-11 18:1 and *trans*-12 18:1 and observed no effect on milk-fat synthesis. Furthermore, it has been shown that milk-fat content of either *cis*-9, *trans*-11 CLA or *trans*-11 18:1 has little or no relationship to milk-fat

percent or milk-fat yield (Kelsey et al., 2003; Lock et al., 2003). Therefore, we conclude that, although *trans*-8, *cis*-10 CLA is taken up by the mammary gland and transferred to milk fat, it has no effect on milk-fat synthesis.

The 11,13 CLA supplement contained both *cis*-11, *trans*-13 and *trans*-10, *cis*-12 CLA, and it caused a reduction in milk-fat synthesis. To address this, a positive control treatment consisting of pure *trans*-10, *cis*-12 CLA was used. The *trans*-10, *cis*-12 CLA isomer has been shown to be a potent inhibitor of milk-fat synthesis (Baumgard et al., 2000, 2001; Loor and Herbein, 2003). Peterson et al. (2002) established that a curvilinear relationship exists between dose of *trans*-10, *cis*-12 CLA abomasally infused and the extent of reduction in milk-fat synthesis. In the present study, similar amounts of *trans*-10, *cis*-12 CLA were provided by both the 11,13 CLA and 10,12 CLA treatments, and they resulted in an equivalent decline in milk-fat synthesis. The reductions in milk-fat yield observed for these 2 treatment groups were also consistent with the dose response curve developed by Peterson et al. (2002). Likewise,

milk composition changes observed for the 11,13 CLA and 10,12 CLA supplements were similar to Peterson et al. (2002), and the percentage of *trans*-10, *cis*-12 CLA present in the milk fat of the 11,13 CLA and 10,12 CLA treatment groups was not different (Table 5). Therefore, we conclude that *cis*-11, *trans*-13 CLA has no effect on rates of milk-fat synthesis, and the decrease in milk-fat yield and changes in milk fatty-acid composition observed with the 11,13 CLA supplement were entirely due to the presence of *trans*-10, *cis*-12 CLA.

The impetus for this study was based in part on results from an earlier experiment where abomasal infusion of a supplement enriched with *cis/trans* 8,10 CLA and devoid of *cis/trans* 10, 12 CLA caused a reduction in milk-fat synthesis (Chouinard et al., 1999b). At the time, we were unable to identify which double bond was *cis* and which was *trans*, but in subsequent analysis, we established that this isomer was *trans*-8, *cis*-10 CLA. Results from the present experiment rule out *trans*-8, *cis*-10 CLA along with *cis*-11, *trans*-13 CLA as causes for that reduction in milk-fat synthesis. Castor oil was the starting material for the manufacture of the *trans*-8, *cis*-10 CLA enriched supplement used by Chouinard et al. (1999b), whereas the starting material used to make the *trans*-8, *cis*-10 CLA preparation in the present study was vegetable oil. Both preparations contained a high concentration of *trans*-8, *cis*-10 CLA. However, the difference in starting materials may have resulted in a slightly different overall fatty acid profile. Obviously, the preparation derived from castor oil contained a small quantity of a potent inhibitor of milk-fat synthesis other than the *trans*-10, *cis*-12 CLA isomer, and this is why a reduction in milk-fat synthesis was observed. In particular, castor oil has a high content of ricinoleic acid (12-hydroxy-9-octadecenoic acid), and perhaps this or some metabolite of it brought about the reduction in milk-fat synthesis.

There was no effect on milk-fat synthesis by either *trans*-8, *cis*-10 CLA or *cis*-11, *trans*-13 CLA, so there must be other explanations for the discrepancy between the milk-fat content of *trans*-10, *cis*-12 CLA and reduction in milk-fat synthesis observed when calcium salts of CLA were fed (Perfield et al., 2002; Bernal-Santos et al., 2003). One possibility consistent with the biohydrogenation theory of MFD (Bauman and Grinarii, 2001) is that a unique fatty acid intermediate(s) is formed in the rumen from one of the fatty acids present in the supplement, and this compound(s) is a potent inhibitor of milk-fat synthesis. Calcium salts of polyunsaturated fatty acids are only partially protected against rumen biohydrogenation and metabolism, and this varies according to the specific polyunsaturated fatty acid being protected and the rumen environment (Gulati et al., 1997; Wu and Papas, 1997). Studies that

have fed a mixture of CLA isomers in the form of rumen-protected calcium salts showed the specific transfer efficiency for *trans*-10, *cis*-12 CLA into milk fat was 2 to 4% (Perfield et al., 2002; Bernal-Santos et al., 2003), whereas the transfer from abomasal infusion was about 20% (Baumgard et al., 2000, 2001; Peterson et al., 2002). The rumen-protected supplement of CLA caused a greater reduction in milk-fat content than would be anticipated based on the milk-fat content of *trans*-10, *cis*-12 CLA. This suggests that some of the fatty acids in the supplement were metabolized in the rumen to unique fatty acids that may also be potent inhibitors of milk-fat synthesis. Alternatively, unidentified biohydrogenation intermediates that affect milk-fat synthesis have also been suggested to be involved in situations of diet-induced MFD based on the fact that *trans*-10, *cis*-12 CLA content of milk fat is inadequate to explain completely the degree of reduction in milk-fat synthesis (Bauman and Grinarii, 2003; Peterson et al., 2003).

Overall, abomasal infusion of *trans*-10, *cis*-12 CLA caused a reduction in the rates of milk-fat synthesis consistent with results from previous studies. Comparisons of results from the two CLA enrichments (8,10 CLA and 11,13 CLA) to those of a negative control (no CLA) and a positive control (*trans*-10, *cis*-12 CLA) demonstrated abomasal infusion of *trans*-8, *cis*-10 CLA, *cis*-11, *trans*-13 CLA, and *cis*-9, *trans*-11 CLA had no effect on milk-fat synthesis.

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