Effects of Intravenous Infusion of Conjugated Diene 18:3 Isomers on Milk Fat Synthesis in Lactating Dairy Cows

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ABSTRACT

It has been previously established that *trans*-10, *cis*-12 conjugated linoleic acid plays an important role in milk fat depression (MFD). However, in many situations of dietary induced MFD, the reduction in milk fat synthesis is much greater than what would be predicted based on the milk fat concentration of *trans*-10, *cis*-12 18:2. These observations suggest that other biohydrogenation intermediates could be implicated in MFD. The objective of this study was to evaluate the effects on milk fat synthesis of an intravenous administration of 2 conjugated diene 18:3 isomers (*cis*-9, *trans*-11, *cis*-15 and *cis*-9, *trans*-13, *cis*-15 18:3), which are intermediates in ruminal biohydrogenation of α -linolenic acid. Three multiparous Holstein dairy cows (days in milk = 189 ± 37 d; body weight = 640 ± 69 kg; mean $\pm$ standard deviation), fitted with indwelling jugular catheters, were randomly assigned to a 3×3 Latin square design. For the first 5 d of each period, cows were infused intravenously with a 15% lipid emulsion providing 1) *cis*-9, *trans*-11, *cis*-15 18:3 + *cis*-9, *trans*-13, *cis*-15 18:3 + *trans*-10, *cis*-12 18:2 (CD18:3 + CLA); 2) *cis*-9, *cis*-12, *cis*-15 18:3 + *cis*-9, *cis*-12 18:2 as a control (ALA + LA); or 3) *cis*-9, *cis*-12, *cis*-15 18:3 + *trans*-10, *cis*-12 18:2, as a positive control (ALA + CLA). Milk production was recorded, and milk was sampled daily at each milking for analyses of fat, protein, lactose, milk urea nitrogen, and somatic cell count. Dry matter intake, milk yield, and milk protein were not affected by treatment. Over the experimental period, milk fat content was decreased by 7% for cows that received either ALA + CLA or CD18:3 + CLA compared with ALA + LA. The temporal pattern of milk fat content showed a linear decrease during the infusion period for ALA + CLA and CD18:3 + CLA treatment groups. The transfer efficiencies of conjugated diene 18:3 isomers into milk fat averaged 39 and 32% for *cis*-9, *trans*-11, *cis*-15 18:3 and *cis*-9, *trans*-13, *cis*-15 18:3, respectively. The CD18:3 + CLA treatment had no effect on milk fat

concentration beyond that attributable to its *trans*-10, *cis*-12 18:2 content. In conclusion, results from the current study offered no support for a role of either *cis*-9, *trans*-11, *cis*-15 18:3 or *cis*-9, *trans*-13, *cis*-15 in MFD.

Key words: conjugated diene 18:3, conjugated linolenic acid, milk fat depression, biohydrogenation

INTRODUCTION

Diet has a significant effect on milk fat synthesis in dairy cows. Supplementing highly fermentable carbohydrates or lipid supplements rich in polyunsaturated fatty acids such as plant or fish oils are known to decrease milk fat when fed to dairy cows (Davis and Brown, 1970; Sutton, 1989; Griinari et al., 1998). This phenomenon is referred to as milk fat depression (MFD). Several studies suggest that dietary induced MFD is due to changes in the rumen biohydrogenation process (NRC, 2001). Indeed, the biohydrogenation theory proposed by Bauman and Griinari (2001) stipulates that when a milk fat depressing diet is fed, the pathways of rumen biohydrogenation are altered, resulting in the production of unique fatty acid intermediates that are potent inhibitors of milk fat synthesis in the mammary gland. *Trans*-10, *cis*-12 18:2, one of many isomers of conjugated linoleic acid (CLA), has been identified as one such intermediate, and relation between the isomer and decreased milk fat output is well established (Baumgard et al., 2000). de Veth et al. (2004) combined results from 7 studies and established a curvilinear relationship between the increasing dose of *trans*-10, *cis*-12 18:2 infused into the abomasum and the reduction in milk fat production. However, in many situations of dietary induced MFD, the reduction in milk fat synthesis is much greater than what would be predicted based on the milk fat concentration of *trans*-10, *cis*-12 18:2 observed (Peterson et al., 2003; Piperova et al., 2004; Loores et al., 2005). These previous results suggested that there must be biohydrogenation intermediates other than *trans*-10, *cis*-12 18:2 implicated in MFD. Subsequent studies have provided evidence of a role for *trans*-9, *cis*-11 18:2 (Perfield et al., 2007) and *cis*-10, *trans*-12 18:2 (Sæbø et al., 2005b) in MFD. Milk fat content of many other bio-

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hydrogenation intermediates increases under dietary conditions of MFD (Shingfield and Griinari, 2007). *Cis*-9, *trans*-11, *cis*-15 18:3 has been tentatively identified as the most common intermediate produced in the first step of the biohydrogenation process of α -linolenic acid (*cis*-9, *cis*-12, *cis*-15 18:3) by the action of the enzyme Δ^{12} -*cis*, Δ^{11} -*trans* isomerase in the rumen (Kepler and Tove, 1967). Subsequent studies also confirmed the presence, in small amounts, of *cis*-9, *trans*-13, *cis*-15 18:3 in milk (Destailats et al., 2005b) and muscle (Plourde et al., 2007) lipid extracts from cattle, which can be considered as a second intermediate produced in the biohydrogenation process of α -linolenic acid. According to Banni (2002) and Sæbø et al. (2005a), these isomerised 18:3 (*cis*-9, *trans*-11, *cis*-15 18:3 and *cis*-9, *trans*-13, *cis*-15 18:3) can be referred to as conjugated diene 18:3 (CD18:3) isomers.

Different CD18:3 isomers have been previously studied for their effects on lipid metabolism (Koba et al., 2002; Destailats et al., 2005a; Sæbø et al., 2005a; Plourde et al., 2006). Sæbø et al. (2005a) found no effect of an enrichment containing *cis*-6, *trans*-10, *cis*-12 18:3, a CD18:3 isomer produced by the Δ^6 -desaturation of dietary *trans*-10, *cis*-12 18:2. Koba et al. (2002) reported that compared with linoleic acid (*cis*-9, *cis*-12 18:2), CLA and, more potently, a mixture of conjugated diene and triene 18:3 isomers were found to decrease perirenal adipose tissue weight in rats. The same experiment demonstrated that conjugated 18:3, but not CLA, was found to increase serum and liver triacylglycerol, indicating that in rats, conjugated 18:3 modulates the body fat and triacylglycerol metabolism differently from CLA. The primary objective of the present study was to investigate the effect of intravenous infusion of an equal mixture of *cis*-9, *trans*-11, *cis*-15 18:3 and *cis*-9, *trans*-13, *cis*-15 18:3, two intermediates in the biohydrogenation of α -linolenic acid in the rumen, on milk fat synthesis in dairy cows.

MATERIALS AND METHODS

Animals and Diets

All procedures involving dairy cows were conducted according to the regulations of the Canadian Council on Animal Care and were approved by the Université Laval Animal Care Committee. Three multiparous Holstein cows (mean BW: 640 $\pm$ 69 kg) in midlactation (mean DIM: 189 $\pm$ 37 d) fitted with indwelling jugular catheters were intravenously infused with lipid emulsion enriched in 3 different mixtures of octadecatrienoic acid according to a 3 $\times$ 3 Latin square design. Experimental periods were 14 d in length. Animals were housed in a tie-stall facility. Throughout

the experiment, cows were fed a TMR once daily (1000 h), which was formulated to meet or exceed the NRC requirements (NRC, 2001; Table 1). Orts were weighed daily just before feeding, and the amount of feed offered was adjusted to ensure 10% feed refusal. Feed samples were taken weekly, and DM content of silages was measured by oven-drying at 65°C for 48 h. Proportions of TMR ingredients were adjusted weekly based on DM content of forages. Free access to water was provided at all time. Cows were weighed for 2 consecutive days at the beginning of each period.

Infusion Procedures

Three days before the infusion, 2 indwelling catheters were placed in each of the external jugular veins; one side was for infusion of lipid emulsion, and the contralateral side was for blood sampling. Catheters were kept patent with physiological saline plus either 200 IU/mL of heparin between experimental days or 20 IU/mL during the sampling period. Because pure CD18:3 supplements were not available, effects of these fatty acids were investigated using a supplement that contained 40% of an equal mixture of *cis*-9, *trans*-11, *cis*-15 18:3 and *cis*-9, *trans*-13, *cis*-15 18:3 as well as 5.2% of *trans*-10, *cis*-12 18:2 (Naturia Inc., Sherbrooke, Québec, Canada; Table 2). The amount infused for the first treatment (CD18:3 + CLA) was calculated to provide 10 g/d of CD18:3 and, as a consequence, supplied 1.3 g/d of *trans*-10, *cis*-12 18:2, a known potent inhibitor of milk fat synthesis. Therefore, as a positive control, another experimental fat mixture (ALA + CLA) was

Table 1. Composition of the TMR

Item	% (DM basis)
Ingredient	
Timothy silage	32.1
Corn silage	26.1
Soybean meal	11.3
Rolled barley	21.2
Cracked corn	7.5
Mineral and vitamin premix ¹	0.8
Iodized salt	0.3
Calcium carbonate	0.7
Chemical ²	
DM, % as fed	47.9
CP	16.2
NDF	38.6
ADF	20.0
Ca	0.7
P	0.4
NE _L , Mcal/kg	1.56

¹Contained 15% Ca, 5% P, 5% Mg, 25% NaCl, 40 mg/kg of Co, 3,500 mg/kg of Fe, 100 mg/kg of I, 850 mg/kg of Cu, 3,100 mg/kg of Mn, 30 mg/kg of Se, 3,400 mg/kg of Zn, 350 kIU/kg of vitamin A, 150 kIU/kg of vitamin D, and 2,000 IU/kg of vitamin E.

²Calculated from ingredient components using the average for intake and cow characteristics (NRC, 2001).

Table 2. Fatty acid composition of lipid supplements and amounts infused daily in each treatment group

Variable	Oleic acid ¹	Linoleic acid ²	Linolenic acid ³	Conjugated linoleic acid ⁴	Conjugated diene 18:3 ⁵
Fatty acid, % by weight					
14:0	2.93	0.10	<0.01	0.05	<0.01
16:0	3.55	7.11	<0.01	4.90	0.37
<i>cis</i> -9 16:1	4.20	0.13	<0.01	0.07	0.07
18:0	0.87	4.08	<0.01	2.31	0.14
<i>cis</i> -9 18:1	61.08	15.40	0.64	11.08	17.49
<i>cis</i> -11 18:1	4.78	1.31	<0.01	0.96	1.13
<i>cis</i> -9, <i>cis</i> -12 18:2	6.96	68.00	18.20	0.78	14.76
<i>cis</i> -9, <i>cis</i> -12, <i>cis</i> -15 18:3	0.35	0.14	80.89	0.17	9.13
<i>cis</i> -9, <i>trans</i> -11 18:2	0.07	0.08	<0.01	37.85	4.25
<i>trans</i> -10, <i>cis</i> -12 18:2	0.07	0.02	<0.01	38.43	5.24
<i>cis</i> -9, <i>trans</i> -11, <i>cis</i> -15 18:3	<0.01	<0.01	<0.01	<0.01	20.34
<i>cis</i> -9, <i>trans</i> -13, <i>cis</i> -15 18:3	<0.01	<0.01	<0.01	<0.01	20.34
Others	15.15 ⁶	3.64 ⁷	0.27 ⁸	3.41 ⁹	6.73 ¹⁰
Treatment					
	Amount infused (g/d)				
ALA + LA	5.8	3.8	15.1	0	0
ALA + CLA	5.5	0.1	15.1	3.3	0
CD18:3 + CLA	0	0	0	0	24.6

¹VWR International, Ville Mont-Royal, Québec, Canada.²Sigma-Aldrich Canada Ltd., Oakville, Ontario, Canada.³Larodan Fine Chemicals, Malmö, Sweden.⁴Clarinol A-80; Loders Croklaan, Channahon, IL.⁵Conjugated diene 18:3; Naturia, Sherbrooke, Québec, Canada.⁶Mainly other 18:1 isomers.⁷Mainly other 18:2 isomers (nonconjugated) and other 18:1 isomers.⁸Mainly γ -linolenic acid.⁹Mainly other CLA isomers and 20:0.¹⁰Mainly unknown and other isomers of 18:1, 18:2, and 18:3.

used, containing the same amount of *trans*-10, *cis*-12 18:2 (Clarinol A-80; Loders Croklaan, Channahon, IL), and in which CD18:3 was replaced with α -linolenic acid. Treatments also included a control (ALA + LA), in which CD18:3 and *trans*-10, *cis*-12 18:2 were replaced with α -linolenic acid and linoleic acid, respectively. Free fatty acid supplements (oleic acid, *cis*-9 18:1: VWR International, Ville Mont-Royal, Québec, Canada; linoleic acid: Sigma-Aldrich Canada Ltd., Oakville, Ontario, Canada; α -linolenic acid; Larodan Fine Chemicals, Malmö, Sweden) were included in experimental infusates to equilibrate the amounts of total 18:1, 18:2, and 18:3 among treatments (Table 3).

The experimental free fatty acid mixtures were diluted in 215 g of 20% Intralipid (Baxter Corporation, Toronto, Ontario, Canada), to which was added 2 g of phosphatidylcholine (Sigma-Aldrich Canada Ltd.) and 13 g of glycerine (Fisher Scientific Company, Ottawa, Ontario, Canada) as emulsifiers and brought to 450 mL with sterile water. Emulsions were obtained using a high-pressure homogenizer (Emulsi-flex-C50; Avestin Inc., Ottawa, Ontario, Canada), sterilized at 121°C for 20 min (MLS-3020U, Sanyo Electric Co. Ltd., Bensenville, IL) and subsequently cooled to 4°C until used.

All treatments were intravenously infused for 5 d with a 9-d interval between periods. Lipid emulsions were continuously infused by pumps (Plum Lifecare Pumps, series 1.6; Abbott Laboratories, Chicago, IL) so that daily infusion volume (450 mL) was delivered over a 24-h period.

Sampling, Measurements, and Analyses

Cows were milked twice daily at 0700 and 1700 h. Milk was sampled, and milk yield was recorded using calibrated milk meters (Flomaster Pro, DeLaval, Tumba, Sweden) at each milking. Milk samples were kept at 4°C using bronopol as a preservative, before analysis for fat, protein, lactose, MUN, and SCC using a Foss Milkoscan 4000 (Foss Electric, Hillerød, Denmark) combined with a Bentley 2000 (Bentley Instruments, Chaska, MN). All milk composition analysis was conducted at the Québec Dairy Production Centre of Expertise (Ste-Anne-de-Bellevue, Québec, Canada). Additional milk samples were collected and stored at -20°C without preservative for subsequent analysis.

Lipid extraction of milk samples was performed, and the extracted lipids were methylated according to

Table 3. Fatty acids provided on a daily basis during intravenous infusion

Fatty acid, g/d	Treatment		
	ALA + LA	ALA + CLA	CD18:3 + CLA
Free fatty acid ¹			
18:1			
<i>cis</i> -9	4.0	4.0	4.0
18:2			
<i>cis</i> -9, <i>cis</i> -12	5.8	3.3	3.5
<i>cis</i> -9, <i>trans</i> -11	0.0	1.2	1.0
<i>trans</i> -10, <i>cis</i> -12	0.0	1.3	1.3
Total	5.8	5.8	5.8
18:3			
<i>cis</i> -9, <i>cis</i> -12, <i>cis</i> -15	12.2	12.2	2.2
<i>cis</i> -9, <i>trans</i> -11, <i>cis</i> -15	0.0	0.0	5.0
<i>cis</i> -9, <i>trans</i> -13, <i>cis</i> -15	0.0	0.0	5.0
Total	12.2	12.2	12.2
Others	2.4	2.2	2.6
Esterified fatty acids ²			
16:0	23.0	23.1	22.9
<i>cis</i> -9 18:1	42.4	42.5	42.2
<i>cis</i> -9, <i>cis</i> -12 18:2	102.8	103.1	102.4
<i>cis</i> -9, <i>cis</i> -12, <i>cis</i> -15 18:3	11.0	10.2	10.9
Others	28.5	28.6	28.4

¹Fatty acids from the commercial supplements that were provided to balance total 18:1, 18:2, and 18:3 concentrations among treatments.

²Fatty acids in triacylglycerol and phospholipid from the commercial lipid emulsion (20% Intralipid; Baxter Corporation, Toronto, Ontario, Canada) in which the free fatty acids were emulsified.

Chouinard et al. (1997). Composition analyses of the fatty acids were carried out with a gas chromatograph (HP 5890A Series II, Hewlett-Packard, Palo Alto, CA) equipped with a 100-m CP-Sil 88 capillary column (i.d., 0.25 µm; film thickness, 0.20 µm; Chrompack, Middleburg, the Netherlands) and a flame ionization detector. Two oven temperature programs were used in this study. To screen milk fatty acid composition, a first temperature program was as follows: at the time of sample injection, the column temperature was 80°C for 1 min, then ramped at 2°C/min to 215°C and maintained for 30 min. A second temperature program was used to separate the 2 CD18:3 isomers: at the time of sample injection, the column temperature was 120°C for 180 min, then increased at 10°C/min to 220°C and maintained isothermal for 20 min (Plourde et al., 2007). For both programs, inlet and detector temperatures were 220 and 230°C, respectively. The split ratio was 100:1. The flow rate for hydrogen carrier gas was 1 mL/min. Most fatty acid peaks were identified and quantified using either a quantitative mixture or pure methyl ester standards (Larodan Fine Chemicals; Sigma-Aldrich Canada Ltd.; Matreya LLC, Pleasant Gap, PA; Nu-Chek Prep, Elysian, MN). The CD18:3 isomers were identified using a qualitative standard obtained from Naturia Inc. Because no quantitative standard was available, the peak response factor for

cis-9, *cis*-12, *cis*-15 18:3 was used to adjust the peak areas of the CD18:3 isomers. Standards for *trans*-5 18:1, *trans*-10 18:1, *trans*-15 18:1, *trans*-16 18:1, and *cis*-15 18:1 were not available commercially and were identified by order of elution according to Precht et al. (2001). The response factor for *cis*-9 18:1 was used to quantify these other 18:1 isomers.

Blood was collected on d 4 and 5 of infusion twice daily (0800 and 1700 h). Blood samples were withdrawn via the jugular catheter and transferred into Vacutainer tubes that contained no preservative (Vacutainer 366430, Becton Dickinson, Franklin Lakes, NJ) to determine serum glucose, triacylglycerol, and NEFA concentrations. Blood samples were allowed to clot at room temperature (20°C) for approximately 3 h and were centrifuged at 956 × *g* for 15 min at 4°C. The supernatants were transferred to microtubes and frozen at -20°C for later analysis. Frozen serum was submitted to the Faculté de Médecine Vétérinaire, Université de Montréal (St-Hyacinthe, Québec, Canada) for the measurement of serum NEFA (FA 115 kit; Randox Laboratories Ltd., Crumlin, UK) and glucose (reagent 442640; Beckman Coulter Inc., Mississauga, Ontario, Canada) using an automated autoanalyzer (Synchron CX5 Clinical System; Beckman Coulter Inc., Fullerton, CA). Blood triacylglycerol was measured using a commercial kit (Sigma-Aldrich Canada Ltd.).

Statistical Analysis

Data were analyzed as a 3×3 Latin square design using the PROC MIXED procedure of SAS (SAS Institute, Cary, NC) according to the model:

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

where Y_{ijk} = individual observation; μ = overall mean; T_i = effect of treatment ($i = 1, 2$, and 3); P_j = effect of period ($j = 1, 2$, and 3); C_k = effect of cow ($k = 1, 2$, and 3 ; treated as a random effect); and ε_{ijk} = residual error term.

Period was repeated with the subject being cow. In the event that treatment effects were established, multiple comparisons were performed using the Tukey-Kramer adjustment (SAS Institute).

To study the temporal pattern of milk fat content and selected milk fatty acids during intravenous infusion of fatty acid supplements, data were analyzed as repeated measures within the Latin square using a mixed model:

$$Y_{ijkl} = \mu + T_i + P_j + C_k + D_l + (T \times D)_{il} + \varepsilon_{ijkl},$$

where D_l = effect of day of infusion ($l = 0$ to 5) and $(T \times D)_{il}$ = effect of the interaction between treatment and day of infusion.

The subject of the repeated statement was the cow $\times$ period interaction. When an effect of day of infusion or a treatment $\times$ day of infusion interaction were detected, linear and quadratic contrasts for time effect were performed.

The latter statistical model with effect of day of infusion replaced by effect of sampling time ($l = 0800$ or 1700 h) was used to analyze serum glucose, NEFA, and triacylglycerol. Because there was no sampling time $\times$ treatment interaction, mean concentrations of serum glucose, NEFA, and triacylglycerol sampled at 0800 and 1700 h on d 4 and 5 were combined for statistical analysis according to the first model described previously.

Significance was declared at $P < 0.05$, and trends were declared at $P < 0.10$. The values reported are least squares means and standard errors.

RESULTS AND DISCUSSION

Many studies have demonstrated that *trans*-10, *cis*-12 18:2 plays a major role in MFD (Baumgard et al., 2000, 2001). Results from these previous trials led to the establishment of a curvilinear relationship between milk fat percentage and milk fat concentration of *trans*-10, *cis*-12 18:2 (Peterson et al., 2002; de Veth et al., 2004). However, in many situations of MFD, the magnitude of decrease in milk fat synthesis is much greater than the expected reduction based on the milk fat concentration of *trans*-10, *cis*-12 18:2 (Perfield et al., 2002; Peterson et al., 2003; Gervais et al., 2005). This suggests that other intermediates in the biohydrogenation of polyunsaturated fatty acids in the rumen could act as potent inhibitors of milk fat synthesis.

In the current study, a fatty acid supplement was provided to lactating dairy cows in an effort to study the effect on milk fat synthesis of isomers of CD18:3, intermediates produced during metabolism of α -linolenic

Table 4. Dry matter intake, milk yield, and milk composition from lactating cows during intravenous infusion of fatty acid supplements¹

Variable	Treatment ²			SED ³	P-value
	ALA + LA	ALA + CLA	CD18:3 + CLA		
DMI, kg/d	29.7	19.0	21.7	0.7	0.11
Milk yield, kg/d	26.7	27.1	28.4	0.9	0.35
FCM, kg/d	28.4	27.5	28.6	1.1	0.63
Milk protein					
Content, %	3.66	3.72	3.69	0.03	0.43
Yield, g/d	964	983	1,029	43	0.45
Milk fat					
Content, %	4.49 ^a	4.22 ^b	4.20 ^b	0.04	0.02
Yield, g/d	1,181	1,112	1,151	46	0.47
Milk lactose					
Content, %	4.51 ^b	4.61 ^a	4.50 ^b	0.02	0.04
Yield, g/d	1,209	1,253	1,282	47	0.45
MUN, mg/dL	14.7	15.7	14.0	0.8	0.31
SCC, $\times 10^3$ /mL	43	41	31	17	0.80

^{a,b}Superscript letters within a row denote differences ($P < 0.05$).

¹Values represent means from d 4 and 5 of infusion of fatty acid supplements for 3 cows.

²See Table 3 for composition.

³SED = standard error of the least squares means difference.

acid in the rumen. Intravenous infusion was used as a simple experimental procedure to ensure complete protection of fatty acid supplements from rumen biohydrogenation (Viswanadha et al., 2003). The CD18:3 + CLA treatment group received an intravenous infusion of 5 g of each isomer of CD18:3 daily to ensure that the effect of these isomers on milk fat synthesis could be compared with those from other biohydrogenation intermediates studied in published experiments (Peterson et al., 2002; Perfield et al., 2004, 2007; Sæbø et al., 2005a,b).

Dry matter intake and BW were not affected by intravenous infusion of the fatty acid supplements (Table 4). Milk yield and protein were not altered by treatment. In contrast, over the experimental period, milk fat content was decreased by 7% for cows that received ALA + CLA and CD18:3 + CLA compared with ALA + LA. The temporal pattern of milk fat content showed a linear decrease during the infusion period for ALA + CLA and CD18:3 + CLA treatment groups (Figure 1). At the end of the period, return to preinfusion milk fat content was observed for all treatments (Figure 1).

The decrease in milk fat content was similar for ALA + CLA and CD18:3 + CLA treatment groups (Table 4). Results from previous studies established a strong negative relationship between milk fat synthesis and concentration of *trans*-10, *cis*-12 18:2 in milk fat (Peterson et al., 2002; de Veth et al., 2004). The extent of decline in milk fat content observed in the current study was consistent with what would be predicted based on the increase in milk fat concentration of *trans*-10, *cis*-12 18:2 for ALA + CLA and CD18:3 + CLA treatment groups (Table 5). However, the reduction in milk fat content observed for these 2 treatment groups was not associated to a decrease in milk fat yield, as opposed to results from many other studies investigating the effects of *trans*-10, *cis*-12 18:2 on milk fat synthesis in dairy cows (Kay et al., 2007; Baumgard et al., 2000, 2001). In the current study, ALA + CLA and CD18:3 + CLA treatments both provided 1.3 g/d of *trans*-10, *cis*-12 18:2. This low dose of *trans*-10, *cis*-12 18:2 might explain the lack of effect on milk fat yield.

Viswanadha et al. (2003) observed a linear decrease with increasing doses of intravenously infused *trans*-10, *cis*-12 18:2 up to 6 g/d. Therefore, the daily amount of *trans*-10, *cis*-12 18:2 used in the present work was below the dose that would induce a maximum level of MFD, allowing the expression of any additive effect of other fatty acids on milk fat synthesis.

The CD18:3 + CLA treatment provided 5 g of each CD18:3 isomer daily. Bauman et al. (2008) established that, when abomasally infused, the median effective dose of *trans*-10, *cis*-12 18:2 was 2.5 g/d and corresponded to a 25% reduction in milk fat yield. Intra-

venous administration of *trans*-10, *cis*-12 18:2 affects milk fat synthesis as efficiently as abomasal infusion (Viswanadha et al., 2003). Consequently, the daily supply of CD18:3 isomers was calculated to allow an effect on milk fat yield if these isomers had to be considered as inhibitors of milk fat synthesis at a dose comparable to *trans*-10, *cis*-12 18:2.

Variations in fatty acid content of treatments resulted in differences in fatty acid composition of milk for the respective isomers infused, except for *cis*-9, *trans*-11 18:2, which showed a similar content in milk fat for all treatment groups (Table 5; Figure 2). Under the conditions used in this experiment, *cis*-9, *trans*-13, *cis*-15 18:3 was not detectable in milk fat, unless CD18:3 + CLA supplement was provided (Figure 3). Therefore, no statistical analysis was performed on this specific isomer. The transfer efficiency of *trans*-10, *cis*-12 18:2 into milk fat when cows received either ALA + CLA or CD18:3 + CLA treatments was 16% (Figure 4). This value is comparable to the average of 22% estimated by de Veth et al. (2004) from a summary of published studies in which cows received abomasal infusion of *trans*-10, *cis*-12 18:2. In contrast, the efficiency of transfer of *trans*-10, *cis*-12 18:2 observed in the present trial was much greater than the one calculated from data published by Viswanadha et al. (2003; <2%) when lactating dairy cows were intravenously infused 2 to 6 g/d of *trans*-10, *cis*-12 18:2. The greater transfer efficiency of *trans*-10, *cis*-12 18:2 might be explained by the fact that, in contrast to Viswanadha et al. (2003), the supplements were continuously infused in the jugular

Figure 1. Temporal pattern of milk fat content in lactating cows during intravenous infusion of ALA + LA, ALA + CLA, or CD18:3 + CLA; see Table 3 for composition; $n = 3$. P -values represent the interaction between treatment and linear (lin) and quadratic (quad) effects of time during the infusion (from d 0 to d 5).

Table 5. Fatty acid composition of milk fat from lactating cows during intravenous infusion of fatty acid supplements¹

Fatty acid, mg/g	Treatment ²			SED ³	P-value
	ALA + LA	ALA + CLA	CD18:3 + CLA		
4:0	30.9	29.2	30.8	1.3	0.49
6:0	20.8	19.2	20.0	0.9	0.41
8:0	13.8	13.1	13.4	0.7	0.80
10:0	32.2	32.0	32.7	1.8	0.92
12:0	38.7	38.9	39.5	2.0	0.93
14:0	111.6	116.0	118.9	2.0	0.12
<i>cis</i> -9 14:1	11.2 ^b	12.0 ^a	12.2 ^a	0.1	0.01
15:0	12.2	12.7	12.2	0.2	0.13
16:0	297.1 ^a	290.2 ^b	296.2 ^{ab}	1.2	0.05
<i>cis</i> -9 16:1	18.3	18.1	18.4	0.4	0.98
17:0	17.7	17.2	17.1	1.2	0.86
18:0	70.6	71.0	65.4	2.9	0.30
<i>trans</i> -5 18:1	0.18	0.24	0.20	0.07	0.44
<i>trans</i> -6-8 18:1	1.5	1.6	1.6	0.1	0.37
<i>trans</i> -9 18:1	1.3	1.2	1.2	0.1	0.94
<i>trans</i> -10 18:1	3.4	3.7	2.7	0.8	0.58
<i>trans</i> -11 18:1	4.0	3.3	5.5	0.4	0.06
<i>trans</i> -12 18:1	2.3	2.4	2.3	0.1	0.46
<i>cis</i> -9 18:1	127.6	133.1	126.3	3.2	0.28
<i>trans</i> -15 18:1	2.3	2.4	2.6	0.3	0.53
<i>cis</i> -11 18:1	3.5	4.2	3.7	0.3	0.22
<i>cis</i> -12 18:1	1.4	1.4	1.4	0.1	0.79
<i>cis</i> -13 18:1	0.30	0.30	0.32	0.01	0.27
<i>trans</i> -16 18:1	2.0	2.1	2.0	0.1	0.39
<i>cis</i> -15 18:1	0.96	0.97	0.98	0.04	0.88
<i>cis</i> -9, <i>cis</i> -12 18:2	25.9	25.4	25.9	0.7	0.49
20:0	1.14	1.13	1.03	0.05	0.24
<i>cis</i> -9, <i>cis</i> -12, <i>cis</i> -15 18:3	8.5 ^a	8.4 ^a	4.9 ^b	0.5	0.03
<i>cis</i> -9, <i>trans</i> -11 18:2	2.9	3.1	3.5	0.2	0.21
<i>trans</i> -10, <i>cis</i> -12 18:2	0.093 ^c	0.322 ^a	0.248 ^b	0.004	<0.001
<i>cis</i> -8 20:1	0.93	1.04	0.97	0.04	0.20
<i>cis</i> -9, <i>trans</i> -11, <i>cis</i> -15 18:3	0.19 ^b	0.17 ^b	2.05 ^a	0.13	0.006
<i>cis</i> -9, <i>trans</i> -13, <i>cis</i> -15 18:3	—	—	1.4	—	—
<i>cis</i> -5, <i>cis</i> -8, <i>cis</i> -11, <i>cis</i> -14, <i>cis</i> -17 20:5	0.85	0.93	0.57	0.10	0.13
<i>cis</i> -7, <i>cis</i> -10, <i>cis</i> -13, <i>cis</i> -16, <i>cis</i> -19 22:5	0.85	0.85	0.82	0.09	0.96
Glycerol	125.1	124.3	125.0	0.8	0.62
Summation					
De novo ⁴	264.2	268.2	274.9	8.7	0.56
16:0 + <i>cis</i> -9 16:1	356.3 ^a	349.0 ^b	356.2 ^{ab}	1.1	0.04
Preformed ⁵	336.6	344.4	331.1	7.8	0.41
Δ^9 -Desaturase index					
Specific ratio ⁶					
<i>cis</i> -9 14:1	0.092	0.095	0.094	0.001	0.09
<i>cis</i> -9 16:1	0.057	0.059	0.058	0.001	0.77
<i>cis</i> -9 18:1	0.648	0.650	0.657	0.006	0.43
<i>cis</i> -9, <i>trans</i> -11 18:2	0.44	0.50	0.42	0.07	0.55
Overall index ⁷	0.249	0.255	0.246	0.004	0.29

^{a-c}Superscript letters within a row denote differences ($P < 0.05$).

¹Values represent means from d 4 and 5 of infusion of fatty acid supplements for 3 cows.

²See Table 2 for composition.

³SED = standard error of the least squares means difference.

⁴Fatty acids with chain lengths from 6 to 14.

⁵Fatty acids with chain lengths of 15, 17, or longer.

⁶Specific ratios for Δ^9 -desaturase activity are ratio of the Δ^9 -desaturase product divided by the sum of the Δ^9 -desaturase product and substrate as described by Kelsey et al. (2003): *cis*-9 14:1 = (*cis*-9 14:1)/(*cis*-9 14:1 + 14:0); *cis*-9 16:1 = (*cis*-9 16:1)/(*cis*-9 16:1 + 16:0); *cis*-9 18:1 = (*cis*-9 18:1)/(*cis*-9 18:1 + 18:0); *cis*-9, *trans*-11 18:2 = (*cis*-9, *trans*-11 18:2)/(*cis*-9, *trans*-11 18:2 + *trans*-11 18:1).

⁷Overall Δ^9 -desaturase index was calculated as follows: [(*cis*-9 14:1) + (*cis*-9 16:1) + (*cis*-9 18:1) + (*cis*-9, *trans*-11 18:2)]/[(*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)].

vein of the cows. Another explanation may be related to the differences between the emulsion processes used in the 2 studies. In the work from Viswanadha et al.

(2003), the fatty acid supplements were obtained via sonication of the mixtures, as opposed to the present trial, in which the emulsion was achieved using a high-

Figure 2. Temporal pattern of selected milk fatty acids from lactating cows during intravenous infusion of ALA + LA, ALA + CLA, or CD18:3 + CLA; see Table 3 for composition; $n = 3$. P -values represent the interaction between treatment and linear (lin) and quadratic (quad) effects of time during the infusion (from d 0 to d 5).

pressure homogenizer. Transfer efficiencies of CD18:3 isomers averaged 39 and 32% for *cis*-9, *trans*-11, *cis*-15 18:3 and *cis*-9, *trans*-13, *cis*-15 18:3, respectively. These observations are consistent with those of Sæbø et al. (2005a), who reported a greater numerical value for transfer efficiency of different CD18:3 isomers when compared with *trans*-10, *cis*-12 18:2.

Isomers of CD18:3 were taken up from blood by the mammary gland and incorporated into milk fat. However, the uptake of these isomers did not result in any

additional effect on milk fat synthesis when compared with positive control (ALA + CLA). Therefore, the decrease in milk fat content observed in the CD18:3 + CLA treatment group is due solely to the presence of *trans*-10, *cis*-12 18:2 in the supplement.

Chouinard et al. (1999) reported that dietary supplements of CLA altered de novo fatty acid synthesis in lactating dairy cows. However, Baumgard et al. (2002) demonstrated that decrease in expression of lipoprotein lipase and fatty acid binding protein, 2 key enzymes in

Figure 3. Partial gas chromatograms of milk fat from cows after 5 d of intravenous infusion of ALA + LA (A), ALA + CLA (B), CD18:3 + CLA (C); see Table 3 for composition.

the use of preformed fatty acids for milk fat synthesis, occurred when cows received *trans*-10, *cis*-12 18:2. Consistent with these observations, many other studies reported reduction in the yields of most fatty acids, with effects primarily on de novo fatty acids when cows received high doses of abomasally or intravenously infused *trans*-10, *cis*-12 18:2 (Baumgard et al., 2000; Perfield et al., 2002). Nevertheless, results from trials in which lower doses of *trans*-10, *cis*-12 18:2 were provided to dairy cows, as in the current study, showed a reduction in milk fat yield more equally distributed among fatty acids (Baumgard et al., 2001; Peterson et al., 2002). This could explain the lack of effect of treatment on milk fat composition of short-, medium-, and long-chain fatty acids observed in the present experiment (Table 5).

Another effect of *trans*-10, *cis*-12 18:2 that has been observed in lactating dairy cows is its ability to alter the fatty acid composition of milk by reducing the product:substrate ratios related to Δ^9 -desaturase (Chouinard et al., 1999). Baumgard et al. (2002) demonstrated that abomasal infusion of *trans*-10, *cis*-12 18:2 decreased the mRNA abundance for Δ^9 -desaturase in the mammary gland of dairy cows. However, many studies showed no effect on Δ^9 -desaturase when cows received low doses of *trans*-10, *cis*-12 18:2 (Peterson et al., 2002; Sæbø et al., 2005a). As expected, based on the low dose of *trans*-10, *cis*-12 18:2 used in this study, no effect of treatment was observed on any of

the product:substrate ratios related to the desaturase index (Table 5).

Serum concentrations of NEFA and triacylglycerol were not affected by treatments (Table 6). Previously, *trans*-10, *cis*-12 18:2 has been shown to increase plasma NEFA from 30 to 200% when intravenously infused to dairy cows at doses ranging from 2 to 6 g/d (Viswanadha et al., 2003). Again, the smaller dose of *trans*-10, *cis*-12 18:2 (1.3 g/d) used in the current study could explain the discrepancy. However, many other trials in which *trans*-10, *cis*-12 18:2 was provided into the abomasum at greater doses did not report changes in blood concentrations of NEFA (Perfield et al., 2002; Castañeda-Gutiérrez et al., 2007). In contrast with the study by Viswanadha et al. (2003), the supplements used in this experiment were isolipidic and continuously infused in the jugular vein of the cows. This might explain the variation between the 2 studies. Serum concentration of glucose tended to be greater for cows receiving the CD18:3 + CLA supplement (Table 2). Giguère et al. (2006) investigated the effects of CLA and a mixture of CD18:3 similar to the one used in the current study on glucose metabolism in the piglet. Their work provided evidence that both dietary CLA and CD18:3 influence glucose homeostasis but through different metabolism in the piglet. Previous studies demonstrated that *trans*-10, *cis*-12 18:2 did not alter blood glucose concentrations in dairy cows (Moore et al., 2004; Selberg et al., 2004). In contrast, Odens et al. (2007) observed a

Table 6. Serum concentrations of glucose, triacylglycerol, and NEFA during intravenous infusion of fatty acid supplements¹

Variable	Treatment ²			SED ³	P-value
	ALA + LA	ALA + CLA	CD18:3 + CLA		
Glucose, mmol/L	3.19	3.28	3.46	0.06	0.09
Triacylglycerol, μ mol/L	221	226	230	11	0.71
NEFA, μ mol/L	283	193	290	135	0.79

¹Values represent an average for serum collected at 0800 and 1700 h on d 4 and 5 of infusion for 3 cows.

²See Table 3 for composition.

³SED = standard error of the least squares means difference.

significant increase in blood glucose (11%) when dairy cows were fed CLA supplements from 10 d precalving to 40 d postcalving at a dose of 174 or 58 g/d (depending upon DIM). In the current study, the effects of treatment on milk lactose were not consistent with the effects on blood glucose, suggesting that the latter might be of questionable biological importance. Additional studies would be necessary for a better understanding of possible interactions between CLA or CD18:3 and glucose metabolism in lactating dairy cows.

In conclusion, the current study investigated the effect of intravenous infusion of 2 CD18:3 isomers, intermediates in the biohydrogenation of α -linolenic acid in the rumen, on milk fat synthesis in dairy cows. Isomers of CD18:3 were taken up from blood by the mammary gland and incorporated into milk fat with

transfer efficiencies of 39 and 32% for *cis*-9, *trans*-11, *cis*-15 18:3 and *cis*-9, *trans*-13, *cis*-15 18:3, respectively. However, the uptake of these isomers did not influence milk fat synthesis of the CD18:3 + CLA treatment group. The decreases in milk fat content observed in the CD18:3 + CLA and ALA + CLA treatment groups were similar and, therefore, attributable solely to the presence of *trans*-10, *cis*-12 18:2 in both supplements. These results offered no support for a role of either *cis*-9, *trans*-11, *cis*-15 18:3 or *cis*-9, *trans*-13, *cis*-15 18:3 in MFD at a dose in which *trans*-10, *cis*-12 CLA is effective in reducing milk fat secretion.

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Figure 4. Apparent transfer efficiency of intravenously infused fatty acids into milk fat of dairy cows. Each column represents the mean ($\pm$ SD) for transfer, calculated on d 4 and 5 of infusion of fatty acid supplements for 3 cows. $n = 9$ for *cis*-9, *cis*-12 18:2 and *cis*-9, *cis*-12, *cis*-15 18:3, $n = 6$ for *trans*-10, *cis*-12 18:2, and $n = 3$ for *cis*-9, *trans*-11, *cis*-15 18:3 and *cis*-9, *trans*-13, *cis*-15 18:3.

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