Dose Response of Milk Fat to Intravenous Administration of the *trans*-10, *cis*-12 Isomer of Conjugated Linoleic Acid¹

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ABSTRACT

Intravenous infusion of conjugated linoleic acid (CLA) was evaluated as a simpler method than abomasal infusion and the feeding of calcium salts to examine milk fat depression. The objectives were to determine the dose-dependent response of milk fat and plasma metabolites to intravenous administration of the *trans*-10, *cis*-12 isomer of CLA, an isomer identified to possess an inhibitory effect on milk fat synthesis. Four multiparous Holstein cows averaging 123 ± 30 d in milk were randomly assigned to treatments in a 4×4 Latin square design. Catheters were inserted into the jugular vein for infusions and blood sampling. Treatments consisted of intravenous infusions of 0, 2, 4, and 6 g/d CLA (>95% *trans*-10, *cis*-12 CLA). Infusates contained 72 g/d of a parenteral solution, saline, and CLA to 90 ml. Periods were of 5 d duration with a 7 d wash out. Milk was sampled at each milking and analyzed for fat, protein, and fatty acids. Blood samples were obtained on the last day of each period. Dry matter intake (22.4 ± 2.4 kg/d), milk yield (28.5 ± 3.3 kg/d), and protein percent ($3.26 \pm 0.08\%$) of cows were not affected by treatment. However, milk fat percentage was reduced linearly with increasing doses of CLA. Milk fat percentage was 4.17, 3.53, 3.29, and 2.92% on d 5 for treatments 0, 2, 4, and 6 g/d CLA, respectively. Concentrations (4.2 mg/g of fat) of *cis*-9, *trans*-11 CLA in milk fat were not affected by treatment. However, an increase in the *trans*-10, *cis*-12 CLA content of milk fat was observed. Milk fat contained 0.00, 0.02, 0.06, and 0.10 mg of *trans*-10, *cis*-12 CLA per g of fat (SEM = 0.065) for treatments 0, 2, 4, and 6 g/d CLA, respectively. Plasma NEFA concentration increased linearly with the dose of the *trans*-10, *cis*-12 CLA. Intravenous infusion of the *trans*-10, *cis*-12 isomer of CLA depressed milk fat in a linear manner over the range of

infusion studied and, therefore, is an alternative to abomasal infusion.

(**Key words:** conjugated linoleic acid, milk fat, dairy cattle)

Abbreviation key: CLA = conjugated linoleic acid.

INTRODUCTION

Conjugated linoleic acid (CLA) refers to a mixture of positional and geometrical isomers of octadecadienoic acids with conjugated double bonds. Conjugated linoleic acid has been demonstrated to possess a wide range of health benefits, which include suppression of carcinogenesis (Pariza and Hargraves, 1985; Ha et al., 1987; Ip et al., 1994), reduction in atherogenesis (Nicolosi et al., 1997), improvement in glycemic status in diabetic rats (Houseknecht et al., 1998), and modulation of the immune system (Cook et al., 1993). Conjugated linoleic acid has been identified in various food sources. Milk and other dairy products are considered to be good sources of CLA, while vegetable oils and seafood contain minimal amounts of these fatty acids (Chin et al., 1992). Conjugated linoleic acid is formed in the rumen as an intermediate in the biohydrogenation pathway (Shortland et al., 1955). It can also be formed in tissues from *trans* vaccenic acid by the action of the enzyme Δ^9 -desaturase (Griinari et al., 2000). *trans*-Vaccenic acid is an intermediate in the biohydrogenation of several polyunsaturated fatty acids.

Apart from the potential health benefits mentioned, CLA has profound effects on lipid metabolism. Research has demonstrated that mixtures of CLA isomers either infused abomasally (Chouinard et al., 1999a) or when fed as calcium salts (Giesy et al., 2002) cause a dose dependent reduction in milk fat. Baumgard et al. (2000) demonstrated that the *trans*-10, *cis*-12 isomer of CLA but not the *cis*-9, *trans*-11 isomer of CLA was responsible for milk fat depression. Abomasal infusion requires surgically prepared animals, and feeding of calcium salts of CLA requires manufacturing and does not necessarily deliver the expected dose of CLA due to incomplete protection from rumen biohydrogenation. The objective of the study was to examine

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Table 1. Fatty acid profile of *trans*-10, *cis*-12 conjugated linoleic acid¹ (CLA) infused intravenously into lactating dairy cows.

Fatty acid	Percent
C _{16:0}	1.1
C _{18:0}	0.3
C _{18:1} <i>cis</i> -9	0.3
C _{18:2} <i>cis</i> -9, <i>trans</i> -11 (CLA)	1.6
C _{18:2} <i>trans</i> -10, <i>cis</i> -12 (CLA)	94.8
Unknown	1.9

¹Natural Lipids, Hovdebygda, Norway.

whether intravenous infusion could be utilized to simplify the experimental examination of the metabolic effects of CLA on milk fat synthesis. The study evaluated the dose-dependent response of milk parameters and plasma metabolites to intravenous administration of the *trans*-10, *cis*-12 isomer of CLA. Intravenous administration of the *trans*-10, *cis*-12 CLA was hypothesized to depress milk fat linearly with dose, and the extent of its incorporation into milk fat would vary positively with the dose of the *trans*-10, *cis*-12 CLA infused.

MATERIALS AND METHODS

All procedures involving animals were approved by the Animal Care and Use Committee of the University of Idaho. Four multiparous Holstein cows averaging 123 ± 30 DIM were used for the purpose of this study. Cows were randomly assigned to a treatment sequence in a 4 × 4 Latin square design. Treatments consisted of intravenous infusions of 0, 2, 4, and 6 g/d of *trans*-10, *cis*-12 CLA (Natural Lipids, Hovdebygda, Norway; Table 1) suspended in 72 g of 10% Intralipid (Baxter; Deerfield, IL) and brought to 90 ml with 0.9% saline. Sonication for 45 s ensured thorough mixing. The daily infusate was divided into six equal doses, loaded into syringes, and infused every 4 h. The dose range was based upon the amount of *trans*-10, *cis*-12 CLA infused abomasally by Chouinard et al. (1999a, 1999b). Periods were of 5-d duration with a 7-d interval to minimize any carryover effects of CLA. Cows were housed in free stalls but fed individually through the Calan (American Calan Inc., Northwood, NH) system. Cows were fed a diet formulated to meet their nutrient requirements (NRC, 1989; Table 2). Cows were fed to ad libitum intake with equal portions of feed offered at 0730 and 1730 h daily. In addition, water was available at all times. Daily feed refusals were weighed and removed before feeding at 0730 h. Cows were milked at 0500 and 1700 h daily. Milk yield was determined at each milking and samples were obtained. One aliquot was analyzed for fat, protein, and lactose content

Table 2. Ingredient and chemical composition of the diet.

Composition	Content
Ingredient, % DM	
Chopped alfalfa hay	27.6
Pea and triticale silage	7.7
Whole cottonseed	10.7
Steam-rolled corn	15.0
Energy Booster ¹	1.4
Barley	21.8
Dried distillers' grains	5.4
Soybean meal	4.9
Blood meal	0.8
Fish meal	0.8
Trace mineral premix ²	0.7
Vitamin premix ³	0.3
Limestone	1.4
Dicalcium phosphate	0.5
Magnesium oxide	0.2
Sodium bicarbonate	0.9
Chemical analysis	
CP, % DM	20.4
Crude fat, % DM	6.3
NDF, % DM	30.3
ADF, % DM	17.8
Ash, % DM	8.6
NE _L , Mcal/kg	1.675

¹Milk Specialties, Dundee, IL.

²Trace mineral mix (mg/kg mix) contained: Mn, 3500; Zn, 3000; Cu 750; Fe, 20; I, 85; Co, 15; and Se, 35.

³Vitamin premix provided 2,110,000 IU/kg of retinyl acetate, 390,000 IU/kg cholecalciferol, and 7,550 IU/kg dl- α -tocopheryl acetate.

by infrared analysis (Washington DHI, Burlington, WA).

Lipid extraction of milk was performed using the Folch procedure (1957) with modifications (Clark et al., 1982). Milk fatty acids were transesterified with sodium methoxide according to the method of Christie (1982). Analysis of methyl esters was performed on a GLC (Hewlett Packard 6890 Series with auto injector) fitted with a flame-ionization detector. Fatty acid profile was determined by split injection (20:1) onto a CP-Sil 88 fused silica capillary column (100 m × 0.25 mm, Chrompack, Raritan, NJ) using a programmed temperature gradient method. The hydrogen carrier gas pressure was constant, and the injector and detector temperatures were 255°C. Initial oven temperature was 70°C. Following injection of sample, oven temperature was increased at 1°C/min to 185°C and held for 20 min. Oven temperature was then increased at 3°C/min to 215°C followed by an increase at 10°C/min to 240°C and held for 5 min after which oven temperature was returned to 70°C. Individual fatty acids were identified by comparison of retention times to those of pure standards (Matreya Inc., Pleasant Gap, PA). A response correction factor for each fatty acid methyl ester was used to convert peak area percentage to weight percentage. Correction factors were determined by an

alyzing butter oil of a known fatty acid profile with certified values (CRM 164; European Community Bureau of Reference, Brussels, Belgium).

Catheters were inserted into the jugular vein for infusions and blood sampling using polyvinyl tubing. An intramuscular injection (1 g/d) of Naxcel (Pharmacia & Upjohn, Kalamazoo, MI) was administered to the animals immediately after catheterization as a prophylactic measure. Catheters were installed from 2 d before through the end of each period.

Blood samples were obtained once each period at 1400 h on d 5 of CLA infusion. Plasma was harvested after centrifugation at $2300 \times g$ for 15 min and stored at -20°C until analyzed. Plasma glucose concentration was determined by enzymatic colorimetric analysis using a commercial kit (510-DA; Sigma, St. Louis, MO). Triacylglycerol and glycerol concentrations were estimated using a commercial kit (336-10; Sigma) according to the manufacturer's instructions. Nonesterified fatty acids were determined by enzyme colorimetric analysis (Wako Pure Chemical Industries, Osaka, Japan) with modifications (Sechen et al., 1990).

Statistical Analysis

Data were statistically analyzed as a 4×4 Latin square design using the general linear model procedure of SAS version 8.0 (1999) according to the following model:

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

where Y_{ijkl} is the observation, μ is the overall mean, T_i is the treatment ($i = 0, 2, 4$, and 6 g/d of CLA), P_j is the period ($j = 1, 2, 3$, and 4), C_k is the cow ($k = 1, 2, 3$, and 4), and E_{ijkl} is the residual error. Preplanned comparisons were used to test the linear, quadratic or cubic effect of dose of CLA when effect of treatment was significant. Differences were declared at $P < 0.05$.

RESULTS

Infusion of the *trans*-10, *cis*-12 CLA had no effect on DMI, which averaged 22.4 kg/d (Table 3). Milk yield and milk protein were similar for all the treatment groups. In contrast, intravenous infusion of the *trans*-10, *cis*-12 CLA resulted in a dramatic reduction in milk fat percent over the 5-d infusion period. Milk fat percentage decreased gradually over the 5-d period (Figure 1). Milk fat percent and yield averaged 2.92% and 842 g/d for the 6 g/d dose of the *trans*-10, *cis*-12 CLA compared with 4.17% and 1055 g/d for the control group ($P < 0.05$; Table 3). Infusion of the *trans*-10, *cis*-12 CLA caused linear decreases ($P < 0.05$) in the milk

fat concentration of $C_{6:0}$, whereas the other short- and medium-chain fatty acids ($C_{4:0}$, $C_{8:0}$, $C_{10:0}$, $C_{12:0}$, and $C_{14:0}$) remained unchanged (Table 4). Most of the long-chain fatty acids were not affected except for linear increases ($P < 0.05$) in the concentration of $C_{18:2}$ and $C_{19:0}$ (Table 4). Combining milk fat yield and fatty acid composition, data demonstrated that infusion of *trans*-10, *cis*-12 CLA linearly decreased ($P < 0.05$) yields of $C_{6:0}$, $C_{8:0}$, $C_{10:0}$, $C_{12:0}$ and $C_{16:0}$, whereas yields of $C_{4:0}$, $C_{16:1}$, $C_{17:0}$, $C_{18:0}$, $C_{18:1}$, $C_{18:2}$, and $C_{18:3}$ remained unchanged (Table 5). Infusion of *trans*-10, *cis*-12 CLA linearly increased ($P < 0.05$) the *trans*-10, *cis*-12 CLA content of milk fat and yield. Milk fat concentration and yield of *trans*-10, *cis*-12 CLA increased from undetectable for the 0 g/d dose to 1.0 mg/g of fat and 0.9 g/d for the 6 g/d dose of the isomer. Milk fat content and yield of *cis*-9, *trans*-11 CLA, however, remained unchanged (Tables 4 and 5).

Plasma metabolites such as NEFA, glucose, triacylglycerol, and glycerol were also examined. Glucose concentrations were not affected by the *trans*-10, *cis*-12 CLA infusion and averaged 65 mg/dl (Table 6). However, a linear increase ($P < 0.05$) in the NEFA and glycerol concentration of plasma to the dose of the *trans*-10, *cis*-12 CLA infused was observed (Table 6). Concentration of plasma triacylglycerol was not affected by infusion of the *trans*-10, *cis*-12 CLA (Table 6).

DISCUSSION

Several theories have been proposed to explain the causes of milk fat depression in lactating cows. Dietary factors such as the amount of roughage (Jorgensen et al., 1965; Storry et al., 1974), forage to concentrate ratio (Sutton, 1989), and amount and type of dietary lipids (Sutton, 1989) bring about marked changes in the concentration of milk fat. *trans*-Fatty acids, derived from the diet or produced by rumen biohydrogenation or animal metabolism, have been suggested to cause milk fat depression (Davis and Brown, 1970; Astrup et al., 1975; Grinari et al., 1998). More recently, abomasal infusions of CLA mixtures have resulted in dramatic reductions in milk fat concentration (Chouinard et al., 1999a, 1999b; Looor and Herbein, 1998). Baumgard et al. (2000) identified the *trans*-10, *cis*-12 isomer of CLA as a potent inhibitor of milk fat synthesis and suggested that CLA containing a *trans*-double bond at carbon-10 appeared to be the key to reducing milk fat synthesis. In this study, the response of milk fat to intravenous administration of various doses of the *trans*-10, *cis*-12 isomer of CLA was examined.

Six grams of *trans*-10, *cis*-12 CLA infused intravenously resulted in a 30 and 20% reduction in milk

Table 3. Least squares means of DMI, milk yield, and composition of milk from lactating dairy cows during intravenous infusion of *trans*-10, *cis*-12 conjugated linoleic acid (CLA).^{1,2}

Variable	Dose of CLA (g/d)				SEM	Linear contrast ³
	0	2	4	6		
DMI, kg/d	21.4	24.4	21.1	22.0	2.4	—
Milk yield, kg/d	25.3	34.6	28.0	28.6	3.3	—
Milk protein, %	3.32	3.32	3.26	3.20	0.1	—
Milk protein yield, g/d	836	1177	905	918	92	—
Milk fat, %	4.17	3.53	3.29	2.92	0.2	**
Milk fat yield, g/d	1055	1215	893	842	83	*

¹ *Trans*-10, *cis*-12 CLA; Natural Lipids, Hovdebygd, Norway.

² Values represent d-5 of infusion.

³ Contrasts were evaluated only if main effect of dose was significant ($P < 0.05$). Lack of main effect of dose is designated by —. No significant quadratic or cubic effects of level of infusion were detected.

* $P < 0.05$.

** $P < 0.01$.

fat percentage and yield, respectively, over the 5-d infusion period and the depression in milk fat percentage varied linearly with the dose of CLA infused. Similar reductions in milk fat percentage were obtained when a mixture of CLA isomers (Chouinard et al., 1999a) or when the *trans*-10, *cis*-12 CLA was infused abomasally (Baumgard et al., 2000). Abomasal infusion of 150 g/d of CLA supplement containing 31 g/d of *trans*-10, *cis*-12 CLA caused a 56 and 63% reduction in milk fat percent and yield, respectively (Chouinard et al., 1999a), whereas abomasal infusion of 10 g/d of *trans*-10, *cis*-12 CLA reduced milk fat percentage and yield by 42 and 44%, respectively (Baumgard et al., 2000). When compared on a unit dose basis (decrease

in milk fat percentage per gram of *trans*-10, *cis*-12 CLA), intravenous administration of the *trans*-10, *cis*-12 isomer resulted in a greater reduction in milk fat percentage (5% per gram of *trans*-10, *cis*-12 CLA) than abomasal infusions of a CLA mixture (1.8% per gram of *trans*-10, *cis*-12 CLA; Chouinard et al., 1999a) but similar to abomasal infusions of *trans*-10, *cis*-12 CLA (4.2% per gram of *trans*-10, *cis*-12 CLA; Baumgard et al., 2000). However, the decrease in fat yield was less compared to previous studies (Chouinard et al., 1999b; Baumgard et al., 2000). This is mainly due to the variation in milk yield noted particularly during the 0 g/d *trans*-10, *cis*-12 CLA treatment. Baumgard et al. (2001) infused nearly identical *trans*-10, *cis*-12 CLA abomasally at doses of 3.5, 7.0, and 14.0 g/d and observed depression of milk fat yield similar to that in the current study. Clearly, intravenous infusion of *trans*-10, *cis*-12 CLA appears to affect milk fat production similar to abomasal infusion.

Reduction in milk fat output is sometimes associated with body fat accretion in dairy cattle (Davis and Brown, 1970). However, dietary CLA supplements have decreased body fat accretion in growing mice (Park et al., 1997) and pigs (Dugan et al., 1997; Ostrowska et al., 1999). Reduction in milk fat output with no change in body condition would therefore be expected to be associated with a decrease in energy intake of cows (Chouinard et al., 1999a). Abomasal infusions of CLA mixtures (Lor and Herbein, 1998; Chouinard et al., 1999a) or *trans*-10, *cis*-12 CLA (Baumgard et al., 2000) did not affect voluntary intake in lactating cows. Dry matter intake was not affected in the present study, but long-term studies would be necessary to evaluate the effect of the *trans*-10, *cis*-12 CLA on body fat and DMI. Infusion of the *trans*-10, *cis*-12 CLA, or CLA mixtures did not affect milk yield and milk protein similar to previous studies (Lor and Herbein,

Figure 1. Temporal pattern of milk fat content in dairy cows during intravenous infusion of 0, 2, 4, and 6 g/d of the *trans*-10, *cis*-12 conjugated linoleic acid (Natural Lipids, Norway). Values represent least squares means for four cows (SEM = 0.16).

Table 4. Least squares means of fatty acid composition of milk fat (g/100 g of fatty acids) during intravenous infusion of *trans*-10, *cis*-12 conjugated linoleic acid (CLA).^{1,2}

Fatty acid	Dose of CLA (g/d)				SEM	Linear contrast ³
	0	2	4	6		
C _{4:0}	4.12	3.79	3.64	3.71	0.47	—
C _{6:0}	1.97	1.95	1.81	1.67	0.09	*
C _{8:0}	1.11	1.00	0.93	0.88	0.06	—
C _{10:0}	2.31	2.20	1.96	1.75	0.15	—
C _{12:0}	2.44	2.41	2.23	1.90	0.18	—
C _{14:0}	7.16	7.54	7.39	8.13	0.70	—
C _{14:1 cis-9}	0.58	0.57	0.60	0.61	0.02	—
C _{15:0}	0.56	0.63	0.63	0.63	0.03	—
C _{16:0}	25.99	25.65	25.36	26.73	1.25	—
C _{16:1 cis-9}	1.39	1.33	1.15	1.03	0.14	—
C _{17:0}	0.54	0.51	0.47	0.50	0.02	—
C _{18:0}	15.80	15.99	14.34	15.13	0.58	—
C _{18:1 cis-9}	27.50	27.66	26.74	26.73	1.26	—
C _{18:2 cis-9, cis-12}	4.34	4.45	4.75	5.03	0.23	—
C _{18:3 cis-9, cis-12, cis-15}	0.39	0.40	0.38	0.37	0.02	—
C _{18:2 cis-9, trans-11} (CLA)	0.41	0.33	0.47	0.47	0.04	—
C _{18:2 trans-10, cis-12} (CLA)	0.00	0.02	0.06	0.10	0.01	***
C _{19:0}	0.30	0.31	0.36	0.45	0.02	—
C _{20:0}	0.11	0.13	0.14	0.14	0.02	—

¹*Trans*-10, *cis*-12 CLA; Natural Lipids, Hovdebygd, Norway.²Values represent d 5 of infusion.³Contrasts were evaluated only if main effect of dose was significant ($P < 0.05$). Lack of main effect of dose is designated by —. No significant quadratic or cubic effects of level of infusion were detected.* $P < 0.05$.*** $P < 0.001$.

1998; Chouinard et al., 1999a; Baumgard et al., 2000). The length of the infusion was likely too short to affect milk yield. Giesy (2000) demonstrated that long-term feeding of calcium salts of a mixture of CLA might increase milk yield without any effect on DMI in early lactation, whereas Perfield et al. (2002) did not detect any effect of calcium salts of CLA on milk yield or DMI from cows in late lactation.

The fatty acid profile of milk fat revealed a marked reduction in the yield of short-chain fatty acids that arise from de novo synthesis in the mammary gland during CLA infusion (Table 5). Thus, the *trans*-10, *cis*-12 isomer evidently inhibited de novo fatty acid synthesis. A possible mechanism could be via the suppression of acetyl-CoA carboxylase (Bauman et al., 2000). Acetyl-CoA carboxylase catalyzes the initial reaction in de novo synthesis of fatty acids in animal tissues and is considered the rate-limiting step. In the current study, infusion of the *trans*-10, *cis*-12 CLA had no effect on the yield of C_{14:0} and C_{14:1} unlike the observations by Chouinard et al. (1999b). Reasons for these inconsistencies may be related to the dose of the *trans*-10, *cis*-12 CLA infused (6 vs. 8.3 g/d), extent of milk fat depression (20 vs. 28%), the mode of administration (intravenous vs. abomasal), dietary lipid concentration, and dietary fatty acid profile. Yield of C_{16:0} decreased with infusion of the *trans*-10, *cis*-12 CLA.

Long-chain fatty acids secreted as milk fat are derived from the diet or body stores. A decrease in the output of long-chain fatty acids in milk could indicate a reduction in the mobilization from adipose tissue. However, an increase in concentration of NEFA in plasma was detected, suggesting that mobilization was increased, not decreased. Possibly a decrease in mammary uptake of the long-chain fatty acids caused an increase in the pool of NEFA in circulation as well as a decrease in their output in milk. Most of the long-chain fatty acids such as C_{18:1}, C_{18:2}, C_{18:3}, and C_{19:0} were unaffected. Milk fat content and yield of *trans*-10, *cis*-12 CLA increased ($P < 0.05$) linearly with the dose of the isomer infused, and the efficiency of incorporation into milk fat was calculated to be approximately 14% when compared to 10% observed by Chouinard et al. (1999a). No effect on *cis*-9, *trans*-11 CLA was observed. Griinari et al. (2000) suggested that the majority of *cis*-9, *trans*-11 CLA found in milk was produced by action of the Δ^9 -desaturase enzyme within mammary tissue. It appears that the formation of *cis*-9, *trans*-11 CLA from *trans*-11 vaccenic acid is unaffected by infusion of *trans*-10, *cis*-12 CLA. Thus, at the dosages infused, the *trans*-10, *cis*-12 CLA did not affect the activity of the Δ^9 -desaturase enzyme related formation of *cis*-9, *trans*-11 CLA from *trans*-11 vaccenic acid.

Table 5. Least squares means of fatty acids yields (g/d) during intravenous infusion of *trans*-10, *cis*-12 conjugated linoleic acid (CLA).^{1,2}

Fatty acid	Dose of CLA (g/d)				SEM	Linear contrast ³
	0	2	4	6		
C _{4:0}	45.7	46.5	32.7	33.1	7.7	—
C _{6:0}	21.2	24.0	16.2	14.5	2.2	*
C _{8:0}	11.7	12.3	8.4	7.7	1.1	*
C _{10:0}	24.3	26.6	17.4	14.6	2.0	**
C _{12:0}	25.6	29.6	19.7	16.3	2.2	**
C _{14:0}	76.5	95.5	64.7	68.6	7.7	—
C _{14:1cis-9}	6.4	7.6	5.3	6.4	0.7	—
C _{15:0}	5.9	7.7	5.7	5.3	0.5	—
C _{16:0}	275.0	316.5	222.1	224.1	19.1	*
C _{16:1cis-9}	15.2	16.3	10.8	8.6	2.3	—
C _{17:0}	5.7	6.1	4.1	4.2	0.5	—
C _{18:0}	163.0	192.3	127.6	129.9	14.1	—
C _{18:1cis-9}	290.4	333.0	235.8	224.7	33.2	—
C _{18:2cis-9, cis-12}	45.5	51.4	42.9	43.0	6.2	—
C _{18:3cis-9, cis-12, cis-15}	4.2	4.7	3.3	3.1	0.4	—
C _{18:2cis-9, trans-11} (CLA)	4.5	4.1	4.2	3.8	0.9	—
C _{18:2trans-10, cis-12} (CLA)	0.0	0.3	0.5	0.9	0.1	**
C _{19:0}	3.2	3.9	3.2	3.9	0.3	—
C _{20:0}	1.1	1.7	1.3	1.2	0.1	—

¹*Trans*-10, *cis*-12 CLA; Natural Lipids, Hovdebygd, Norway.²Values represent d 5 of infusion.³Contrasts were evaluated only if main effect of dose was significant ($P < 0.05$). Lack of main effect of dose is designated by —. A cubic effect ($P < 0.05$) of dose of CLA was detected for C_{16:0}.* $P < 0.05$.** $P < 0.01$.

Intravenous infusion of *trans*-10, *cis*-12 CLA brought about a drastic increase in the plasma NEFA concentration, and this increase was linear to the dose of the isomer infused. Baumgard et al. (2000) demonstrated a small increase (17%) in the plasma NEFA concentration upon abomasal infusion of the *trans*-10, *cis*-12 CLA; however, the increase observed upon intravenous infusion was much higher (217%). In lactating dairy cattle, elevation of plasma NEFA concentration is usually caused by a rapid decline in the net energy balance, either caused by a reduction in DMI or an increase in milk yield without an increase in DMI. This is usually followed by an increase in the

long-chain fatty acid content of milk fat that are characteristic of body fat reserves (Bauman et al., 1988). No increase in the long-chain fatty acid content of milk fat was detected, although there was a marked increase in the plasma NEFA concentration upon administration of the *trans*-10, *cis*-12 CLA. A plausible explanation for this observed increase in NEFA could be that fatty acids, during breakdown of triacylglycerides by lipoprotein lipase, were not completely taken up by extrahepatic tissues but were released into circulation (Grummer and Carroll, 1991). Gagliostro et al. (1991) suggested that some NEFA might be released during triacylglyceride hydrolysis by tissue lipopro-

Table 6. Least squares means of plasma concentrations of glucose, triacylglycerol, glycerol, and NEFA during intravenous infusions of *trans*-10, *cis*-12 conjugated linoleic acid (CLA).^{1,2}

Variable	Dose of CLA (g/d)				SEM	Linear contrast ³
	0	2	4	6		
Glucose, mg/dl	63.9	66.1	64.3	66.2	3.2	—
Triacylglycerol, μ M	222	267	243	276	30	—
Glycerol, μ M	894	1043	1098	1478	91	**
NEFA, μ M	365	486	652	1159	122	**

¹*Trans*-10, *cis*-12 CLA; Natural Lipids, Hovdebygd, Norway.²Values represent d 5 of infusion.³Contrasts were evaluated only if main effect of dose was significant ($P < 0.05$). Lack of main effect of dose is designated by —. No significant quadratic or cubic effects of dose of CLA were detected.** $P < 0.01$.

tein lipase. Infusions also resulted in a dose-dependent increase in the plasma glycerol concentration. Park et al. (1999) showed that *trans*-10, *cis*-12 isomer reduced lipoprotein lipase activity in cultured 3T3-L1 adipocytes and enhanced glycerol release into the medium. Increased plasma NEFA and glycerol might therefore result because of increased net fatty acid and glycerol release from adipose, a decrease in NEFA clearance by tissues or a combination of both. This might indicate a decrease in lipogenesis, probably due to inhibition of the enzyme lipoprotein lipase as shown by Park et al. (1999) with normal lipolytic activity in adipose tissue. Decreased lipogenesis with normal lipolytic activity in adipose tissue would affect fatty acid turnover, thus increasing the concentration of NEFA and glycerol in the blood stream.

CONCLUSION

Intravenous infusion of the *trans*-10, *cis*-12 CLA was evaluated as a simpler method compared with abomasal infusion and the feeding of calcium salts to examine milk fat depression. A dose-dependent decrease in milk fat percentage and yield was detected with intravenous infusion of *trans*-10, *cis*-12 CLA. The response of milk fat percentage to intravenous administration of *trans*-10, *cis*-12 CLA was similar to other studies using abomasal infusion of CLA or the feeding of calcium salts of CLA. Furthermore, the *trans*-10, *cis*-12 CLA isomer was incorporated into milk fat in a dose-dependent manner without affecting the content of *cis*-9, *trans*-11 CLA over the dose range studied. Intravenous administration of *trans*-10, *cis*-12 CLA can be used to perform studies evaluating the mechanism of milk fat depression.

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