Effect of Dose of Calcium Salts of Conjugated Linoleic Acid (CLA) on Percentage and Fatty Acid Content of Milk Fat in Midlactation Holstein Cows¹

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ABSTRACT

Increasing conjugated linoleic acid (CLA) content of milk fat from lactating dairy cattle has become a research interest due to the possible health benefits afforded humans consuming CLA. Dietary supplementation of CLA to lactating dairy cows is one potential method by which CLA content of milk and dairy products may be enhanced. Feeding CLA in calcium salt form could potentially deliver CLA to the lower digestive tract through prevention of biohydrogenation by rumen microbes. Milk fat depression (MFD) occurs when cows receive CLA-60, a commercially available CLA source containing numerous CLA isomers, abomasally. Our objectives were to determine the quantity of CLA as calcium salts required to elicit maximal MFD and to evaluate the effects of CLA supplementation on fatty acid composition of milk fat. Five Holstein cows at approximately 93 DIM were utilized in a 5 × 5 balanced Latin square crossover design. Periods were 14-d in length with a 5-d treatment phase and 9-d rest phase. Treatments were 5-d supplementation of 0, 12.5, 25, 50, and 100 g of CLA-60 in calcium salt form. Milk samples were collected on d 5 of CLA supplementation and analyzed for composition and fatty acid profile. Regression analysis of milk fat data suggested that MFD was not maximized over the dose levels investigated, despite delivery of 34.5 g of *trans*-10, *cis*-12 CLA in the 100-g dose of CLA. Supplementation with 50 and 100 g of CLA per day resulted in a reduction of milk fat percent of 29 and 34%, respectively. Trend analysis indicated a linear decrease in the milk fat content of caprylic, capric, and lauric acids as the dose of CLA increased. Milk fat content of *cis*-9, *trans*-11, and *trans*-10, *cis*-12 CLA increased at an increasing rate as dose increased.

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

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

INTRODUCTION

Parodi (1997, 1999) reviewed the potential health benefits from human consumption of milk and dairy products. The *cis*-9, *trans*-11 isomer of conjugated linoleic acid (CLA) is one component of milk fat that is beneficial to human health due to anticarcinogenic properties. Research has therefore focused on methods of altering CLA content of milk fat. Incorporation of commercial sources of CLA into dairy cattle rations is one potential means of increasing CLA content of milk (Hanson et al., 1998). However, because ruminal microbial populations can biohydrogenate unsaturated fatty acids, dietary sources of CLA must be protected from biohydrogenation. Inclusion of dietary long-chain fatty acids as calcium salts reduces interaction of fats with microbial populations in vitro and in the rumen (Chalupa et al., 1984, 1986). Feeding calcium salts of CLA may therefore be an effective means of delivering CLA to the cow's lower digestive tract by reducing the extent of rumen biohydrogenation.

Abomasal infusion of 50 g/d of CLA (Chouinard et al., 1999b) increased the content of all CLA isomers in milk fat, but depressed milk fat percentage. Baumgard et al. (2000) determined that milk fat synthesis was inhibited by *trans*-10, *cis*-12 CLA, but not *cis*-9, *trans*-11 CLA. Both *cis*-9, *trans*-11, and *trans*-10, *cis*-12 CLA are contained in the commercial preparation (CLA-60) often utilized to supplement animal diets in research trials. Feeding calcium salts of CLA at 50 g/d increased CLA concentrations of milk by 61.5% (Hanson et al., 1998). The same study demonstrated that milk fat percentage was 34% lower in cows fed calcium salts of CLA than control cows.

Previous studies (Chouinard et al., 1999b; Hanson et al., 1998) have demonstrated the ability of milk fat to

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be depressed when 50 g of CLA per day was provided. Those studies had the goal of enhancing CLA, in particular *cis*-9, *trans*-11 CLA, content of milk fat. Due to the tremendous energy costs associated with milk fat secretion (approximately 50% of energy in milk; NRC, 1989), milk fat depression (**MFD**) may be a useful management tool to reduce energy requirements of lactating cows. However, the quantity of supplemental calcium salts of CLA necessary to elicit desired MFD has not been determined.

The objectives of this study were to determine the quantity of calcium salts of CLA required to achieve maximal depression of milk fat percentages, to determine the effect of CLA dose on fatty acid composition, and finally, to evaluate the relationship between milk fat percentage and the concentration of *cis*-9, *trans*-11, and *trans*-10, *cis*-12 isomers of CLA in milk fat.

MATERIALS AND METHODS

The University of Idaho Animal Care and Use Committee approved all of the procedures involving cows before the experiment began. Five multiparous Holstein cows were randomly assigned to a treatment sequence for a 5 × 5 balanced Latin square crossover design. Cows were 93 ± 8 DIM and producing an average of 46.5 ± 3.5 kg of milk per day at the initiation of the study. As part of herd management, cows received injections of recombinant bovine somatotropin (rbST; Posilac 1 Step, Monsanto Co., St. Louis, MO) on 14-d intervals during the study. For each 5-d period of the Latin square, cows began CLA supplementation 72 h following rbST injection. The timeline was established to have a 9-d rest phase separating each of the five periods. Doses (supplements) were designed to deliver 0, 12.5, 25, 50, and 100 g/d of CLA isomers in calcium salt form (Table 1). Sixty-five percent of the lipid used in calcium salt formation (Church & Dwight Co., Inc., Princeton, NJ) was a preparation containing CLA isomers (CLA-60; Natural Lipids LTD, Hovdebygda, Norway), and the balance of fatty acids were from palm oil. The profile of CLA isomers in CLA-60 was 24% *cis*-9, *trans*-11, 35% *trans*-10, *cis*-12, 15% *cis*-8, *trans*-10, 17% *cis*-11, *trans*-13 and 9% other. Quantity of individual CLA isomers supplemented for each dose was calculated based on this profile (Table 1). Levels of calcium salts and total lipid included in supplements for all groups were held constant by adjusting the amount of MegalacR in the topdress mix (Table 1). Doses were fed once daily following the a.m. milking and before TMR feeding (Table 2). Dry matter intakes of the TMR were measured daily, and the TMR was offered twice daily to yield 5 to 10% refusal.

Table 1. Ingredient composition of supplements containing conjugated linoleic acid (CLA) and quantity of CLA isomers provided as a calcium salt.

	Dose level of CLA				
	0	12.5	25	50	100
	g/d				
Supplement					
Calcium salts of CLA ¹	0.0	31.9	63.9	127.7	255.5
Megalac	255.5	223.5	191.6	127.7	0.0
Dried distillers grains	109.0	109.0	109.0	109.0	109.0
Dried molasses	104.5	104.5	104.5	104.5	104.5
	g supplemented per day				
Fatty acid					
C _{14:0}	4.7	4.4	4.0	3.2	1.7
C _{16:0}	104.4	96.5	88.2	72.7	45.1
C _{18:0}	8.2	7.8	7.4	6.7	7.5
C _{18:1 cis} -9	75.5	71.1	66.6	58.3	53.7
C _{18:2 cis} -9, <i>cis</i> -12	17.9	16.8	15.6	13.5	11.6
C _{18:2 cis} -8, <i>trans</i> -10 (CLA)	0	1.9	3.8	7.6	15.2
C _{18:2 cis} -9, <i>trans</i> -11 (CLA)	0	3.0	5.9	11.9	23.7
C _{18:2 trans} -10, <i>cis</i> -12 (CLA)	0	4.3	8.6	17.3	34.5
C _{18:2 cis} -11, <i>trans</i> -13 (CLA)	0	2.2	4.3	8.7	17.3
C _{18:2} (Other CLA)	0	1.2	2.3	4.7	9.3

¹Calcium salts of CLA contained 65% CLA-60 (Natural Lipids LTD, Hovdebygda, Norway) and 35% long-chain fatty acids of palm oil.

Cows were milked and milk yield was measured twice daily (0530 and 1700 h). Milk was sampled before treat-

Table 2. Ingredient and chemical composition of total mixed ration.

Composition	Concentration
Ingredient, % DM	
Alfalfa hay	35.5
Alfalfa silage	3.7
Whole cottonseed	3.7
Wheat millrun	3.7
Steam rolled corn	20.7
Barley	18.3
Dried distillers grains	4.0
Soybean meal	4.0
Fish meal, menhaden	1.9
Blood meal	0.8
Megalac	1.0
Sodium bicarbonate	0.98
Magnesium oxide	0.19
Trace mineral premix ¹	0.79
Vitamin premix ²	0.27
Dicalcium phosphate	0.50
Chemical analysis	
CP, % DM	19.2
ADF, % DM	20.3
NDF, % DM	29.8
Lipid, % DM	3.9
NE _L , Mcal/kg	1.67
Ca, % DM	0.95
P, % DM	0.53
Na, % DM	0.59
K, % DM	2.00

¹Trace mineral mix (mg/kg mix) contained: Mn, 3500; Zn, 3000; Cu 750; Fe, 20; I, 85; Co, 15; and Se, 35.

²Vitamin premix provided (per kg) 2,110,000 IU of retinyl acetate, 390,000 IU of cholecalciferol, and 7550 IU of *dl*- α -tocopheryl acetate.

ment was initiated and at each milking during CLA supplementation. Milk samples were pooled daily by equal volume and analyzed for milk fat, protein, lactose, SNF (near-infrared reflectance spectroscopy; Washington DHIA, Burlington, WA) and fatty acids. Milk fat was extracted following a modified Folch procedure (Clark et al., 1982). Methyl esters were formed using a methanolic sodium methoxide solution (Christie, 1982). Analysis of the methyl esters was performed on a gas-liquid chromatograph (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 $\times$ 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 4°C/min to 175°C and held for 3 min. Oven temperature was then raised 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 analyzing butter oil of a known fatty acid profile with certified values (CRM 164; European Community Bureau of Reference, Brussels).

Statistical Analyses

After completing period 1, one cow developed health problems during period 2 and was replaced with a cow of similar DIM, feed intake, and milk production. The replacement cow completed the final three periods of the study. Only data from the replacement cow were used in the statistical analyses. Data from samples collected on d 5 of each period were used in the statistical analyses. A previous study (Hanson et al., 1998) supplementing calcium salts of CLA reported that milk fat percentage reached nadir on the fifth day of supplementation, suggesting that milk fat content did not reach a low point at 2 to 3 d as reported with abomasal infusion of CLA (Chouinard et al., 1999b).

Hence, the response of milk fat percentage over CLA dose was analyzed using an exponential decay function of the form:

$$y = \beta_0 * e^{-\beta_1 x} + c + \varepsilon, \quad [1]$$

where y is the predicted milk fat percentage, x is the dose of CLA ($x = 0, 12.5, 25, 50$, or 100 g of CLA/d), and ε is the error term under standard regression assumptions. The regression coefficients β_0 and β_1 represent scale and rate of exponential decline, respectively, and c is a constant denoting the lower asymptote. Parameter estimation was accomplished using the Gauss-Newton nonlinear algorithm. Mean square error, residual structure, upper and lower 95% asymptotic confidence intervals, and asymptotic correlation coefficients were used as the statistical criteria to determine the adequacy of the model.

All other response variables were investigated using analysis of variance procedures. Data were analyzed assuming a Latin square design including the effects of cow, period, and dose in the model. Single degree of freedom contrasts were used to test for linear and quadratic trends within significant main effects ($P < 0.05$).

Regression procedures were also employed to explore the relationships between milk fat percentage and individual CLA isomers of interest. A linear regression model of the form:

$$y = \beta_0 + \beta_1 x + \varepsilon \quad [2]$$

was used, where y is the milk fat percentage, x is the content of the CLA isomer in milk fat, β_0 and β_1 are regression coefficients, and ε is the error term under standard linear regression assumptions. Model fit was evaluated using mean square error, significance of parameter estimates, examination of studentized residual plots and biological interpretation.

Statistical computations were performed using the procedures NLIN, GLM, and REG of SAS (1991).

RESULTS AND DISCUSSION

Least squares means from d 5 of CLA supplementation for DMI, milk yield, and milk components are presented in Table 3. During the 5-d supplementation period, DMI and milk yield were not affected ($P > 0.05$) by dose of CLA. Dose of CLA was not correlated with DMI, milk yield, or specific milk components other than milk fat percentage. Feed intake was unaffected during short-term feeding of calcium salts of CLA or abomasal infusion of CLA isomers (Hanson et al., 1998; Chouinard et al., 1999a, 1999b). Baumgard et al. (2000) reported no effect of abomasal infusion of *cis*-9, *trans*-11 CLA on DMI, but a tendency for reduced DMI when *trans*-10, *cis*-12 CLA was infused. An effect of CLA dose on lactose ($P < 0.05$) and SNF ($P < 0.05$) percentages of milk was detected (Table 3). Previous studies (Baumgard et al., 2000; Chouinard et al., 1999a, 1999b) evaluat-

Table 3. Means for DMI and production parameters after 5 d of supplementation with 0, 12.5, 25, 50, or 100-g dose of conjugated linoleic acid (CLA) per day as a calcium salt.^{1,2}

Variable	Dose of CLA g fed/d					SEM	Contrast ^{3,4}	
	0	12.5	25	50	100		L	Q
DM intake, kg/d	25.7	26.4	26.4	27.0	26.4	1.1	—	—
Milk yield, kg/d	42.3	43.5	47.9	44.0	43.1	1.0	—	—
Milk fat, %	3.45	2.97	2.96	2.46	2.29	0.13	***	NS
Milk protein, %	3.17	3.17	3.17	3.15	3.24	0.03	—	—
Milk lactose, %	4.76	4.83	4.73	4.66	4.65	0.04	**	NS
Milk SNF, %	8.75	8.82	8.69	8.59	8.69	0.04	**	NS

¹Least squares means from model including cow, period and dose effects.

²CLA-60; Natural Lipids, Hovdebygd, Norway.

³Contrasts were evaluated only if main effect of dose was significant. Lack of main effect of dose is designated by —.

⁴Probability of a linear (L) or quadratic (Q) effect of dose level.

NS = $P > 0.10$, ** $P < 0.01$, *** $P < 0.001$.

ing the effects of CLA supplementation have not reported changes in content of lactose and SNF of milk. Effects of short-term supplementation of CLA on content of lactose and SNF in milk were unexpected because milk yield and DMI were not altered. The decrease in SNF was mainly attributable to the decline in lactose concentration as milk protein concentration was unaffected by dose of CLA (Table 3). However, lactose concentrations were well within normal ranges (Newburg and Neubauer, 1995) suggesting little, if any, significant effect of dose of CLA on lactose synthesis. Milk protein concentration was not altered by abomasal infusion of CLA sources (Chouinard et al. 1999a, 1999b) or during feeding of calcium salts of CLA (Hanson et al., 1998). However, long-term supplementation of calcium salts of CLA enhanced milk protein percentage in cows in a pasture-based management system (Medeiros et al., 2000).

Milk fat percentage was reduced in a dose-dependent manner by feeding calcium salts of CLA (Table 3 and Figure 1). Parameter estimates for model [1] showed appropriate sign and magnitude to describe the reduction in milk fat percentage as dose increased (Table 4). Asymptotic standard errors were small relative to respective estimates and asymptotic confidence limits did not encompass zero, indicating that each of the parameters was significant and required in the model. The predicted equation (Figure 1) followed data well and showed the expected decrease in milk fat percentage as dose increased. Studentized residuals were randomly and uniformly distributed about zero with acceptable magnitudes (Giesy, 2000).

The estimated lower asymptote (2.25% milk fat) was less than the milk fat percentage for cows fed 50 and 100 g/d of CLA (2.46 and 2.29%, respectively) on d 5 of treatment, suggesting that MFD was not maximized within the range of CLA dose investigated in this study.

This phenomenon was not expected because Chouinard et al. (1999b) found near-maximal MFD with abomasal infusion of 50 g of CLA-60 (31.3 g of CLA isomers). In the current study, MFD was induced by supplementing cows with a source of CLA isomers in a form to reduce biohydrogenation by ruminal microorganisms. Abomasal infusion provides a means of delivery that is assured of being postruminal, whereas a potential exists for calcium salts of unsaturated fatty acids to be incompletely protected from microbial biohydrogenation in the rumen (Klusmeyer and Clark, 1991; Wu and Palmquist, 1991; Wu et al., 1991). Biohydrogenation of unsaturated C₁₈ fatty acids averaged 47% for in vitro (Wu and Palmquist, 1991) and 33 to 57% for in vivo (Klusmeyer and Clark, 1991; Wu et al., 1991) studies with calcium salts of palm oil. Thus, biohydrogenation of a portion of the CLA isomers might explain why maximal MFD was not established in the current study.

Figure 1. Relationship between dose of conjugated linoleic acid (CLA) as calcium salts and milk fat percentage in Holstein cows on the fifth day of CLA feeding. Graph illustrates predicted exponential regression model ($\hat{y} = 1.144 * e^{-0.033x} + 2.249$).

Table 4. Estimated coefficients for the exponential regression model used to describe milk fat percentage across five dose levels (0, 12.5, 25, 50, and 100 g/d) of calcium salts of conjugated linoleic acid (CLA).

Variable	Parameter estimate	SE ²	Confidence interval ¹	
			Lower	Upper
β_0	1.144	0.194	0.739	1.551
β_1	-0.033	0.015	-0.064	-0.002
C	2.249	0.173	1.887	2.612

¹Confidence interval is the asymptotic 95% confidence interval.²SE = Asymptotic standard error.

By inverting the exponential model, daily dose required to reduce milk fat percentage to 2.40% was 63 g of CLA. Interestingly, the nature of the exponential decay model is such that the quantity of CLA necessary to reduce milk fat percentage by a fixed amount, say 0.1% units, increases as milk fat percentage decreases. In other words, to reduce milk fat percentage from 3.35 to 3.25% required only 3.0 g of CLA, but reducing milk fat percentage from 2.40 to 2.30% required an additional 31.8 g of CLA. Thus, the depression in milk fat percentage for cows fed 100 g/d of CLA was only 18% greater than for those fed 50 g/d of CLA. Although the quantity of calcium salts of CLA required to reach maximal MFD was not determined, the inefficiency of additional CLA to decrease milk fat percentage at doses greater than 50 g/d of CLA suggested that a quantity between 50 and 100 g would yield near maximal MFD.

Concentrations of caprylic (C_{8:0}), capric (C_{10:0}), and lauric (C_{12:0}) acids showed a general trend of decline as dose of CLA increased (Table 5). However, concentrations of other fatty acids such as butyric (C_{4:0}), caproic (C_{6:0}), and myristic acid (C_{14:0}), synthesized within the mammary gland were not altered by dose of CLA. The linear trend of dose on palmitic acid (C_{16:0}) content ($P < 0.05$) was likely due to the reduction in supplemental C_{16:0} as dose increased, although a reduction in de novo synthesis can not be ruled out. Intake of palmitic acid was decreased approximately 59 g/d as dose of CLA increased from 0 to 100 g/d. This reduction in intake accounted for nearly 40% of the observed decline in palmitic acid output in milk. Chouinard et al. (1999b) reported that synthesis of de novo fatty acids decreased at a decreasing rate as abomasal infusion of CLA isomers increased. When comparing our results with Chouinard et al. (1999b), abomasal delivery of CLA isomers was more effective at lower doses in altering de novo fatty acid synthesis than supplementing diets with CLA as a calcium salts. Baumgard et al. (2000) observed a decrease in synthesis of de novo fatty acids (with the exception of C_{4:0}) when cows were supplemented with *trans*-10, *cis*-12 CLA but not during supplementation with *cis*-9, *trans*-11 CLA. The data by Baumgard et al. (2000) were the first to illustrate a direct effect of the *trans*-10, *cis*-12 CLA alone on MFD and milk fatty acid content. Results from Chouinard et al. (1999a) support the inhibitory role of *trans*-10, *cis*-

Table 5. Means of fatty acids in milk fat from cows after 5 d of supplementation with 0, 12.5, 25, 50, or 100 g of conjugated linoleic acid (CLA) per day as a calcium salt.^{1,2}

Fatty Acid	Dose of CLA g fed/d					SEM	Contrast ^{3,4}	
	0	12.5	25	50	100		L	Q
	mg/g fat							
C _{4:0}	34.3	35.9	35.3	35.0	36.8	1.3	—	—
C _{6:0}	22.3	22.1	21.7	20.5	19.5	0.7	—	—
C _{8:0}	12.7	12.1	11.8	11.2	10.2	0.5	**	NS
C _{10:0}	28.8	26.4	26.5	25.2	22.6	1.1	**	NS
C _{12:0}	31.9	29.1	29.7	28.9	27.1	0.9	**	NS
C _{14:0}	100.9	98.7	100.8	98.3	96.0	1.5	—	—
C _{16:0}	302.5	296.3	293.5	287.8	273.6	4.9	***	NS
C _{16:1 cis-9}	15.9	15.2	13.9	13.9	14.0	0.9	—	—
C _{18:0}	107.4	106.0	107.3	111.2	122.8	2.9	**	*
C _{18:1 cis-9}	238.0	245.5	244.2	256.0	256.9	2.7	***	NS
C _{18:2 cis-9, cis-12}	40.9	40.2	43.3	44.6	45.7	0.8	***	NS
C _{18:3 cis-9, cis-12, cis-15}	4.7	4.7	4.8	4.7	5.0	0.2	—	—
C _{18:2 cis-9, trans-11 (CLA)}	4.86	4.92	5.08	5.57	6.46	0.29	***	†
C _{18:2 trans-10, cis-12 (CLA)}	0.32	0.41	0.43	0.68	1.28	0.10	***	**

¹Least squares means from model including cow, period and dose effects.²CLA-60; Natural Lipids, Hovdebygda, Norway.³Contrasts were evaluated only if main effect of dose was significant ($P < 0.05$). Lack of main effect of dose is designated by —.⁴Probability of a linear (L) or quadratic (Q) effect of dose level.NS = $P > 0.10$, † $P < 0.10$, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

Figure 2. Linear regression of milk fat percentage by *cis*-9, *trans*-11 conjugated linoleic acid (CLA) content of milk fat and natural log of milk fat percentage by *trans*-10, *cis*-12 CLA content of milk fat.

12 CLA on de novo synthesis of milk fat, while illustrating a possible similar role for the *cis*-8, *trans*-10 CLA isomer.

Milk fat concentration of stearic ($C_{18:0}$), oleic ($C_{18:1}$), and linoleic ($C_{18:2}$) fatty acids increased ($P < 0.01$) as dose of calcium salts of CLA increased. Increases in the proportion of these long-chain fatty acids in milk fat are likely due to the dramatic reduction in de novo fatty acid synthesis. Yield of stearic, oleic, and linoleic acid were all decreased approximately 30% when comparing the 100 g of CLA/d versus the 0 g/d dose (data not shown). Approximately 30 and 56% of the decline in yield of oleic and linoleic acid, respectively, could be attributed to reduced intake from the supplements. Less than 3% of the decline in stearic acid output could be accounted for by a reduction in intake. Clearly, evaluation of milk fatty acid concentration fails to demonstrate that the use of preformed fatty acids is also reduced during feeding of calcium salts of CLA. Previous studies (Baumgard et al., 2000; Chouinard et al., 1999a, 1999b) demonstrated similar increases in proportions

of 18-carbon fatty acids in milk fat. In those studies, the ratio of $C_{18:0}$ to $C_{18:1}$ was increased, suggesting that activity of the Δ -9 desaturase enzyme was suppressed by abomasal supplementation of CLA isomers. In contrast, results of the current study show little change in the ratio of $C_{18:0}$ to $C_{18:1}$, 0.45 to 0.48 for 0 and 100 g/d of CLA treatments, respectively, suggesting that activity of the Δ -9 desaturase enzyme may not be a mechanism by which feeding of calcium salts of CLA modulates milk fat synthesis.

The concentration of CLA isomers in milk fat (Table 5) continually increased across all doses of CLA, suggesting the maximum incorporation of *cis*-9, *trans*-11, and *trans*-10, *cis*-12 CLA isomers into milk fat was not met by the levels of CLA supplemented in the current study. Supplementing cows with 100 g of CLA-60 required 255 g of the calcium salts. Use of more purified forms of CLA may be necessary to supplement cows with higher quantities of CLA due to potential restrictions in feeding levels of calcium salts. Apparent transfer efficiency of *cis*-9, *trans*-11, and *trans*-10, *cis*-12 CLA from supplement into milk fat were 0 and 3.5%, 9.0 and 2.4%, 11.4 and 3.7%, and 10.7 and 4.4% for doses 12.5, 25, 50, and 100, respectively. Chouinard et al. (1999b) reported apparent transfer efficiencies of 22.5 and 10.2% for *cis*-9, *trans*-11, and *trans*-10, *cis*-12, respectively, during abomasal infusion of CLA-60. Thus, site of supplementation may be an important determinant in the efficiency at which CLA isomers are transferred into milk fat. Further, ruminal biohydrogenation of CLA isomers during the current study may explain the lower transfer efficiencies of *cis*-9, *trans*-11, and *trans*-10, *cis*-12 CLA previously reported (Chouinard et al., 1999b). Incomplete protection from ruminal biohydrogenation of CLA when in the form of calcium salts is probably the main reason for lower apparent transfer efficiencies between the current study and Chouinard et al. (1999b).

A linear regression model [2] was chosen to relate milk fat percentage to CLA isomers. Best-fit regression of *trans*-10, *cis*-12 CLA content of milk fat to milk fat percentage was established by utilizing the natural log of milk fat, while no transformation was required for *cis*-9, *trans*-11 CLA. Estimated regression models (Figure 2) illustrate the relationship between milk fat percentage and content of *cis*-9, *trans*-11, and *trans*-10, *cis*-12 CLA in milk fat. All model coefficients were significantly different from zero ($P < 0.001$). Residual plots (Giesy, 2000) showed no unexpected patterns and residuals were randomly and uniformly distributed about zero. Generally, as content of CLA isomers in milk fat increased, percentage of fat in milk decreased. Negative correlation between milk fat and *cis*-9, *trans*-11 CLA ($r = -0.71$; $P < 0.001$), and *trans*-10, *cis*-12 CLA ($r = -0.81$;

$P < 0.001$) isomers further indicated the strong relationship among these response variables. The significant correlation between *trans*-10, *cis*-12 CLA in milk fat and milk fat percentage was consistent with the results of Baumgard et al. (2000) that demonstrated MFD when cows were infused with *trans*-10, *cis*-12 CLA, but not *cis*-9, *trans*-11 CLA. The significant correlation between *cis*-9, *trans*-11 CLA in milk fat and milk fat percentage was confounded by the simultaneous delivery of *cis*-9, *trans*-11 and *trans*-10, *cis*-12 CLA in the calcium salts.

CONCLUSIONS

The effects of supplementation of five levels of CLA as a calcium salt on milk fat content and composition were examined. Milk fat percentage declined in a dose-dependent manner, but maximal MFD was not achieved by feeding up to 100 g of CLA-60 per day as a calcium salt. However, increased dose levels were met by decreased efficiency to inhibit milk fat synthesis. Supplementing CLA-60 as a calcium salt reduced the *de novo* synthesis of C_{8:0}, C_{10:0}, and C_{12:0}. In contrast, content of *cis*-9, *trans*-11 and *trans*-10, *cis*-12 CLA in milk fat increased at an increasing rate as CLA supplementation increased. Supplementation with calcium salts of CLA reduced the yield of all other fatty acids, including the long-chain fatty acids from preformed sources. Calcium salts of CLA provide one with the opportunity to regulate milk fat synthesis with only a minor dietary addition.

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