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Effects of Protected Conjugated Linoleic Acid Supplementation on Milk Fatty Acid in Dairy Cows

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ABSTRACT : The objective was to determine the effects of supplementation of protected conjugated linoleic acid (CLA), CLA-20 comprising 10% each of *cis-9, trans-11* and *trans-10, cis-12*, on milk production and fatty acid profiles in plasma and milk in lactating dairy cows. Five mid-lactation, multiparous crossbred Holstein Friesian cows with average 402±20 kg BW were used in a 5×5 Latin square design for 21-d periods. Cows were given a total mixed ration (TMR) and supplemented with CLA-20 at 0, 20, 40, 80 and 160 g/d. The results showed that dry matter intake depression occurred in cows supplemented with CLA-20 at 160 g/d. Milk production slightly increased when CLA-20 supplementation was at 20, 40 and 80 g/d. However, 3.5% fat-corrected milk (FCM) was not affected by CLA-20 supplementation. Increased levels of CLA-20 supplementation resulted in a significantly decreased percentage of milk fat. Plasma concentrations of fatty acid were not altered by the amounts of CLA-20 supplementation except for the concentration of *trans-10, cis-12* CLA. For all dietary treatments, percentages of fatty acids (C4:0, C6:0, C8:0, C13:0, C14:0 C14:1 C15:0 C15:1 C16:0, C16:1, C18:1n9t, C18:2n6t, C18:2n6c, C20:0, C18:3n6, C18:3n3, C20:1 and C20:3n6) in milk fat were similar. Concentrations of C10:0, C11:0, C12:0 and C18:1n9c were decreased cubically and C18:0 was elevated linearly ($p<0.01$) according to the increased amounts of CLA-20 supplemented. The linear increase was observed for *cis-9, trans-11* CLA (0.62, 1.17, 1.94, 1.87 and 1.82% of total fatty acid), *trans-10, cis-12* CLA (0.01, 0.63, 0.67, 0.93 and 0.95% of total fatty acid) and total CLA (0.80, 2.25, 3.16, 3.97 and 3.94% of total fatty acid) in milk fat from 0 to 160 g/d of CLA-20 supplement. In conclusion, concentration of *cis-9, trans-11* CLA in milk fat was concomitantly elevated at an increasing rate with the increased amounts of CLA-20. Based on the results in this study, supplementation of CLA-20 at 80 g/d optimally enhanced total CLA in milk fat. (**Key Words :** Protected Conjugated Linoleic Acid, Plasma, Fatty Acid, Milk, Cows)

INTRODUCTION

Apparently, most biologically active isomer of conjugated linoleic acid (CLA) is *cis-9, trans-11*, which is accounted for more than 80% of the isomers of CLA in milk fat (Chin et al., 1992). The *cis-9, trans-11* CLA is an important component of milk fat and beneficial to human health due to its anti-carcinogenic properties. Research has therefore focused on methods of altering CLA content of milk fat. In corporation of commercial sources of CLA into dairy cattle rations is one potential means of increasing CLA content of milk (Hanson et al., 1998). Rumen microorganisms can biohydrogenate unsaturated fatty acids; therefore, dietary sources of CLA must be protected from biohydrogenation. Use of monensin has been reported to inhibit biohydrogenation (Wang et al., 2005). Supplementation of monensin and fish oil increased CLA content in milk fat (Dhiman et al., 1999) and in fat tissues of Korean Native steers (Wang et al., 2006). Abomasal infusion of 50 g/d of CLA increased the content of all CLA isomers in milk fat (Chouinard et al., 1999b). Supplementing CLA-60 as a calcium salt reduced the *de novo* synthesis of C8:0, C10:0 and C12:0. In contrast, concentration of *cis-9, trans-11* CLA and *trans-10, cis-12* CLA in milk fat increased at an increasing rate as doses of CLA increased (Giesy et al., 2002). Perfield et al. (2002) have also shown that supplementing rumen-protected CLA to pregnant dairy cows during established lactation causes an immediate and consistent reduction in milk fat yield while milk yield and other milk components were unaltered.

The objective of this study was therefore to determine

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Table 1. Ingredient and chemical composition of the experimental diet

Composition	
Ingredients, % DM	
Chopped rice straw	20.30
Cassava chip	35.87
Soybean meal	11.19
Whole cotton seed	9.97
Brewer's dried grain	10.04
Molasses	6.43
Urea	1.82
Salt	0.45
Oyster shell	0.64
Di-calcium phosphate	0.09
Mineral-vitamin[1]	0.27
Sulphur	0.18
Tallow	1.84
Sodium bicarbonate	0.91
Chemical composition (%)	
Dry matter (DM)	71.3
	---------- % DM ---------
Organic matter (OM)	91.2
Crude protein (CP)	13.8
Ether extract (EE)	8.7
Neutral detergent fiber (NDF)	53.6
Acid detergent fiber (ADF)	39.0
Ash	8.8

[1] Vitamin A = 44,000, D_3 = 60,000, E = 30,000 IU/kg; Fe = 11.6, Co = 0.03, Mn = 5.3, Cu = 5.6, Zn = 11.6, I = 0.07, P = 15.0, Mg = 10.0, Se = 0.06, g/kg.

Table 2. Fatty acid composition of experimental diets

Fatty acid	% of fatty acid
C4:0	0.03
C6:0	0.17
C8:0	0.04
C10:0	0.03
C11:0	0.03
C12:0	0.16
C14:0	1.30
C14:1	0.08
C15:0	0.19
C15:1	0.10
C16:0	26.40
C16:1	0.55
C17:0	0.39
C17:1	0.26
C18:0	9.65
C18:1n9*t*	0.44
C18:1n9*c*	21.18
C18:2n6*t*	0.11
C18:2n6*c*	36.75
C18:3n6	0.28
C18:3n3	0.19
C22:6n3	0.28

c = cis, t = trans

the effect of rumen protected CLA on fatty acid composition in milk fat in lactating cows.

MATERIALS AND METHODS

Animals and treatments

Five, multiparous Holstein Friesian crossbred cows with average 402±20 kg BW and 70.6±4 d DIM, were randomly assigned to receive dietary treatments in a 5×5 Latin square design for 21-day periods. Cows were housed individually and provided with water and mineral block throughout the experimental period. The dietary treatments were assigned to deliver 0, 20, 40, 80 and 160 g/d of CLA in the form coated by partly hydrogenated vegetable fats containing 10±1% of *cis-9, trans-11* and 10±1% of *trans-10, cis-12* isomers (CLA-20; BASF Co., Inc, Italy). Total mixed ration (TMR) (Table 1) was formulated according to their requirement (NRC, 1988) and was offered twice daily (0600 h and 1600 h) for *ad libitum* consumption to yield 5 to 10% feed refusal. CLA-20 was offered by top dressing daily.

Sampling and laboratory analysis

Dry matter intake (DMI, %BW) of the TMR was measured daily. TMR was sampled on the last 5 days of each period. Samples were dried in a forced-air oven at 60°C and stored in sealed containers at room temperature until analyzed. Equal amounts of samples from each period were combined to determine chemical composition and fatty acid profiles. In preparation for analyses, TMR was ground through a 2-mm screen (Thomas-Wiley Laboratory Mill, Arthur H. Thomas, Philadelphia, PA) and later ground through a 1-mm screen for crude protein (CP), ether extract (EE), ash (AOAC, 1990) and acid detergent fiber (ADF) and neutral detergent fiber (NDF) (Goering and Van Soest, 1970) and fatty acid content.

Cows were milked and recorded for milk weight twice daily at 0500 h and 1500 h. Milk samples were pooled on the last 5 days of each period by equal volume and analyzed for milk fat, protein, lactose, solids-not-fat (SNF) (Milkoscan 605; Foss Electric, Hillerod, Denmark), CLA isomers and fatty acid profiles.

Blood samples were collected at 3 h post-feeding (0800 h) from the jugular vein in vacutainer tubes and centrifuged at 3,000×g for 15 min. Serum was recovered and stored at -18°C until analyzed for fatty acid profiles.

Samples of TMR, milk fat and plasma lipid fractions were methylated by *in situ* transterification with 0.5 N methanolic NaOH followed by 14% boron trifluoride in methanol (Loor and Herbein, 2001). Fatty acid methyl esters were separated and quantified using gas chromatography. Samples were injected by auto-sampler into a Hewlett-Packard 5890A gas chromatograph equipped

with a flame ionization detector (Hewlett-Packard, Sunnyvale, CA, USA). Methyl esters from all samples were separated on a 100-mx 0.25-mm i.d.×0.20-μm film, Nonbonded Phase Fused Silica Column (SP-2560, Supelco, Bellefonte, USA). Supelco 37 component FAME Mix standards (C4:0-C24:1), Catalog No. 47885-U, Lot No. LB-26791 (Supelco, Bellefonte, PA, USA) and octadecadienoic acid, conjugated, methyl ester (Sigma Prod. No. O5632) were used to identify peaks and determine correction factors for individual fatty acids.

Statistical analysis

Data were subjected to analysis of variance according to a 5×5 Latin square design using the GLM procedure of SAS (1996). Significant differences were determined using Duncan's News Multiple Range Test and orthogonal polynomial contrast.

RESULTS AND DISCUSSION

Chemical and fatty acid compositions of TMR used in the experiment are presented in Table 1 and 2 whereas effect of CLA-20 supplementation on DMI, milk yield and milk composition are presented in Table 3.

DMI was linearly increased ($p<0.05$) by levels of CLA-20 supplementation from 0 to 80 g/d, but decreased ($p<0.05$) from 80 to 160 g/d. However, an increase DMI after CLA supplementation was not well understood. During short-term feeding of calcium salts of CLA, feed intake was unaffected (Hanson et al., 1998). Giesy et al. (2002) found that supplementation CLA-60 from 0 to 100 g/d did not alter DMI. Baumgard et al. (2000) demonstrated 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.

Milk yield was significantly increased (quadratic, $p<0.05$) as levels of CLA-20 supplementation increased. This occurrence was due mainly to the increased DMI. In contrast, other studies reported no effect on milk production when CLA was supplemented (Hanson et al., 1998; Giesy et al., 2002). However, increasing levels of CLA-20 supplementation did not alter 3.5% FCM.

Reduced milk fat was obviously a dose-dependent of CLA-20 supplement ($p<0.05$). Loor and Herbein (1998) demonstrated that CLA administration dramatically decreased milk fat yield and content. Short-term studies confirmed that abomasally infusing supplemental CLA caused milk fat depression (Chouinard et al., 1999b). Baumgard et al. (2002) suggested that, *trans-10, cis-12* CLA isomer is a very potent inhibitor of milk fat production. A daily dose of 13.6 g (0.08% of daily DMI) resulted in a 42% decrease in milk fat content and a 48% reduction in milk fat yield. Results demonstrated that the mechanisms by which *trans-10, cis-12* CLA decreased milk fat yield and content involves many aspects of milk fat synthesis. Specifically, this CLA isomer dramatically reduced the mammary gland's lipogenic capacity (rates of acetate incorporation into fatty acids) and decreased the expression of genes encoding enzymes, involving in the uptake and transport of circulating fatty acids, *de novo* fatty acid synthesis, desaturation of fatty acids, and formation of triglycerides. In this experiment, supplementation of CLA-20 also tended to decrease in milk protein, SNF and TS (cubic, $p<0.01$), but not on milk fat yield and milk specific gravity. In contrast, other works have shown that milk protein concentration was not altered by abomasal infusion of CLA sources or during feeding of calcium salts of CLA (Chouinard et al., 1999b; Loor and Herbein, 2001; Giesy et al., 2002; Perfield et al., 2004). However, long-term supplementation of calcium salts of CLA enhanced milk protein percentage in cows raised in a pasture-based management system (Medeiros et al., 2000).

Blood samples obtained at 3 h post-feeding was used to determine the distribution of 29 fatty acids and 2 isomers of

Table 3. Dry matter intake, milk yield and milk components in cows supplemented with different levels of CLA-20

Item	CLA-20 levels (g/d)					SEM	Contrast (P-value)			
	0	20	40	80	160		l	q	c	qr
DMI (kg/d)	12.7^{ab}	12.5^{ab}	13.3^{ab}	13.5^{a}	12.2^{b}	0.36	0.12	0.05	0.18	0.80
DMI (%BW)	3.07^{ab}	2.97^{ab}	3.16^{a}	3.22^{a}	2.89^{b}	0.09	0.03	0.08	0.27	0.85
MY (kg/d)	15.2^{c}	16.0^{ab}	16.4^{a}	16.0^{ab}	15.7^{bc}	0.22	0.04	<0.01	0.37	0.43
3.5% FCM (kg/d)	16.9	17.6	17.0	16.8	15.7	0.77	0.44	0.38	0.20	0.47
MFY (kg/d)	0.64	0.65	0.60	0.60	0.54	0.29	0.99	0.24	0.64	0.63
Protein (%)	3.91^{ab}	3.92^{a}	3.74^{c}	3.73^{c}	3.79^{bc}	0.04	0.72	0.09	<0.01	0.16
Fat (%)	4.26^{a}	4.12^{ab}	3.73^{bc}	3.82^{abc}	3.59^{c}	0.14	0.03	0.59	<0.01	0.19
SNF (%)	8.74^{ab}	8.84^{a}	8.74^{ab}	8.66^{b}	8.77^{ab}	0.05	0.20	<0.01	<0.01	0.82
TS (%)	13.0^{a}	13.0^{ab}	12.6^{abc}	12.5^{bc}	12.4^{c}	0.16	0.02	0.16	<0.01	0.22
SG	1.03	1.03	1.03	1.03	1.03	0.01	0.99	0.72	0.82	0.16

DMI = Dry matter intake, WC = Weight change, MY = Milk yield, 3.5% FCM = 3.5% of fat collected milk.

3.5% FCM = (0.432+(0.1625×% milk fat))×milk yield (kg/d), MFY = Milk fat yield, SNF = Solids-not-fat.

TS = Total solids, SG = Specific gravity, l = Linear, q = Quadratic, c = Cubic, qr = Quartic.

[a, b, c] Means on the same rows with different superscript letters differ ($p<0.05$).

Table 4. Concentration of plasma fatty acids from cows supplemented with CLA-20

Fatty acid (%)	CLA-20 levels (g/d)					SEM	Contrast (p-value)			
	0	20	40	80	160		l	q	c	qr
C4:0	1.32	1.26	0.48	0.75	1.10	0.29	0.31	0.12	0.45	0.37
C6:0	0.67	0.52	0.32	0.48	0.58	0.16	0.70	0.24	0.98	0.66
C8:0	0.28	0.25	0.15	0.33	0.34	0.08	0.49	0.34	0.76	0.36
C10:0	0.37	0.35	0.37	0.32	0.44	0.12	0.77	0.70	0.77	0.76
C11:0	0.08	0.17	0.01	0.04	0.03	0.04	0.15	0.99	0.19	0.14
C12:0	0.39	0.50	0.20	0.36	0.47	0.10	0.93	0.34	0.40	0.24
C14:0	1.57	2.21	1.43	1.42	1.47	0.34	0.76	0.38	0.90	0.88
C14:1	0.57	0.47	0.33	0.36	0.53	0.12	0.40	0.77	0.26	0.45
C16:0	15.17	18.62	14.33	16.22	15.57	1.32	0.72	0.72	0.28	0.11
C16:1	0.74	0.72	0.75	0.86	0.46	1.35	0.37	0.25	0.25	0.65
C18:0	18.30	18.95	19.21	16.84	17.87	0.91	0.34	0.64	0.26	0.40
C18:1n9*c*	9.66	10.64	10.63	10.79	9.23	0.93	0.82	0.22	0.83	0.76
C18:1n9*t*	0.31	0.27	0.30	0.35	0.21	0.10	0.77	0.69	0.50	0.86
C18:2n6*t*	0.20	0.08	0.11	0.20	0.11	0.59	0.71	0.62	0.15	0.81
C18:2n6*c*	31.94	28.51	36.03	36.40	32.37	2.71	0.46	0.60	0.30	0.07
C18:3n3	0.99	0.93	0.99	1.30	1.38	0.16	0.04	0.48	0.59	0.74
C18:3n6	1.03	0.84	1.06	0.64	0.80	0.15	0.18	0.91	0.74	0.14
C20:0	0.40	0.43	0.31	0.39	0.20	0.11	0.27	0.62	0.78	0.50
C20:1	0.50	0.41	0.42	0.94	0.26	0.23	0.92	0.36	0.04	0.26
C20:3n3	0.55	0.15	0.02	0.05	0.22	0.12	0.08	0.02	0.78	0.96
C20:3n6	2.09	1.73	2.00	2.33	2.57	0.27	0.09	0.29	0.48	0.87
C20:4n6	2.46	2.72	2.76	2.97	2.76	0.30	0.42	0.57	0.88	0.77
C20:5n3	0.08^{b}	0.19^{b}	0.32^{a}	0.11^{b}	0.11^{b}	0.08	0.90	0.02	0.39	0.14
C22:0	0.78	0.63	0.23	0.23	0.20	0.21	0.11	0.61	0.83	0.73
C22:1n9	0.57	0.08	0.07	0.10	0.19	0.17	0.16	0.07	0.46	0.78
C22:2	0.19	0.09	0.13	0.16	0.14	0.60	0.86	0.63	0.46	0.88
C22:6n3	0.29	0.23	0.49	0.23	0.17	0.11	0.33	0.13	0.66	0.05
C24:0	0.08^{ab}	0.31^{a}	0.12^{ab}	0.13^{ab}	0.06^{b}	0.07	0.23	0.10	0.09	0.13
C24:1	0.34	0.10	0.16	0.37	0.24	0.11	0.83	0.36	0.07	0.74

l = Linear, q = Quadratic, c = Cubic, qr = Quartic; *c* = *cis*, *t* = *trans*.

[a, b, c] Means on the same rows with different superscript letters differ ($p<0.05$).

CLA in plasma for transporting to the mammary gland. Concentrations of C4:0, C6:0, C8:0, C10:0, C11:0, C12:0, C14:0, C14:1, C16:0, C16:1, C18:0, C18:1n9c, C18:1n9t, C18:2n6t, C18:2n6c, C18:3n3, C18:3n6, C20:0, C20:1, C20:3n3, C22:0, C22:1n9, C22:2, C22:6n3 and C24:1 fatty acids in plasma were not altered by CLA-20 supplementation (Table 4). Both of CLA isomers supplementation did not affect the concentrations of dietary or ruminally derived fatty acids in the major plasma lipid fractions, which were directly proportional to the amounts of fatty acids absorbed from the small intestine into bloodstream. However, concentrations of C18:2n6 and C18:3n3 in plasma of ruminants were selectively incorporated into plasma cholesterol esters and phospholipids because they were the major substrates for ecithin:cholesterol acyl transferase (Noble et al., 1972). In this study, concentrations of C18:2n6t, C18:2n6c, C18:3n3 in plasma of all treatments were not changed. In contrast, concentration of C20:5n3, C24:0 in plasma of cows received CLA-20 supplementation were affected by treatments ($p<0.05$). Loor and Herbein (2003) showed that concentration of C14:0, C14:1, C16:0, C16:1 C18:0,

Table 5. Plasma CLA concentration from cows supplemented with CLA-20

Fatty acid (%)	CLA-20 levels (g/d)					SEM	Contrast (p-value)			
	0	20	40	80	160		l	q	c	qr
Conjugated 18:2										
cis-9,trans-11	0.19	0.24	0.51	0.80	0.65	0.26	0.51	0.70	0.89	0.52
Trans-10,cis-12	0.37^{b}	0.50^{ab}	0.55^{ab}	0.56^{ab}	0.66^{a}	0.04	0.03	0.99	0.07	0.10
trans-8, trans-10+trans-9, trans-11+trans-10,trans-12	0.25	0.40	0.39	0.22	0.50	0.10	0.54	0.79	0.09	0.11
Total CLA	0.81	1.14	1.45	1.58	1.81	0.36	0.02	0.89	0.06	0.03

l = Linear, q = Quadratic, c = Cubic, qr = Quartic; c = *cis*, t = *trans*.

[a, b] Means on the same rows with different superscript letters differ ($p<0.05$).

C18:3n3, C20:3n3, C20:4n6 and C20:5n3 were not different between *cis-9, trans-11* and *trans-10, cis-12* CLA infusion.

Plasma concentrations of *cis-9, trans-11* CLA and total CLA were not affected by treatments, but *trans-10, cis-12* CLA was increased when CLA-20-supplemented cows (Table 5). This result was in agreement with previous studies. Loor and Herbein (2003) showed that the concentration of *cis-9, trans-11* CLA in plasma was not affected by CLA isomers infusion, but *trans-10,cis-12* CLA in plasma was linearly increased ($p<0.01$). In contrast, the concentration of *cis-9, trans-11* and *trans-10, cis-12* CLA in plasma was increased after infusion of CLA isomers. These results could be explained that the concentration of *cis-9,trans-11* CLA may be originated from biohydrogenation by rumen microbe and from supplementation of CLA in feed. Both of CLA sources were mixed and subsequently absorbed into blood stream. For *trans-10, cis-12* CLA, that may be responsible from CLA-20 supplementation. CLA isomers were preferentially incorporated into plasma triglycerides, except for *trans-10, cis-12* CLA, which showed greater incorporation into plasma non-triglyceride fractions. This phenomenon as regards *trans-10,cis-12* CLA could be explained through its low transferring efficiency into milk fat (4.1-20.9%) after abomasal infusion of *trans-10,cis-12* CLA (Baumgard et al., 2001). In this experiment, the rumen microbes synthesized more *cis-9,trans-11* than *trans-10,cis-12* isomer. This result indicated that the *trans-10,cis-12* CLA involved the increased levels of CLA-20 supplementation.

Concentrations of C10:0, C11:0 and C12:0 fatty acid in milk fat showed a generally declining trend as dose of CLA-20 increased (Table 6). However, concentrations of other fatty acids such as C14:0, C14:1, C15:0, C15:1, C16:0, C16:1, C18:0, C18:1n9t, C18:1n9c, C18:2n6t, C18:2n6c, C18:3n3, C18:3n6, C20:0, C20:1 and C20:3n6 were not altered by levels of CLA. For C18:0 and C18:3n3 were increased (linear, $p<0.05$) and C18:1n9c (cubic, $p<0.05$) by levels of CLA-20 supplementation. The concentration of C16:0 in milk fat of all levels of CLA-20 supplementation were similar, which disagreed with the previous results (Giesy et al., 2002; Perfield et al., 2002; Mackle et al., 2003; Perfield et al., 2004). Baumgard et al. (2000) indicated that, *cis-9,trans-11* and *trans-10,cis-12* CLA infusion were decreased of ≤C16:0 in milk fat. Furthermore, in this study, the result illustrated similar concentration of C18:0 in milk fat when cows were supplemented by CLA-20. This result agreed with the work of Perfield et al. (2002), but, disagreed with Mackle et al. (2003). Perfield et al. (2004) and Giesy et al. (2002) showed that >C16:0 was increased, but, Baumgard et al. (2000) suggested that, *cis-9,trans-11* CLA and *trans-10,cis-12* CLA supplementation decrease C16:0 concentrations in milk fat. It was also observed in this experiment.

The concentrations of C18:0, C18:1n9c, C18:1n9t,

Table 6. Fatty acid composition in milk fat form cows supplemented with CLA-20

Fatty acid (%)	CLA-20 levels (g/d)					SEM	Contrast (p-value)			
	0	20	40	80	160		l	q	c	qr
C4:0	1.33	1.55	1.52	1.33	1.21	0.15	0.54	0.39	0.22	0.44
C6:0	0.97	0.42	0.52	0.37	0.29	0.26	0.26	0.31	0.23	0.78
C8:0	1.36	0.96	1.13	1.02	0.93	0.15	0.28	0.15	0.10	0.95
C10:0	2.89	2.07	2.56	2.26	2.10	0.28	0.12	0.03	0.01	0.53
C11:0	0.38[a]	0.26[b]	0.30[ab]	0.25[b]	0.25[b]	0.02	<0.01	<0.05	<0.01	0.80
C12:0	3.35[a]	2.55[b]	3.02[ab]	2.63[b]	2.67[b]	0.15	0.04	0.06	0.01	0.28
C13:0	0.72	0.14	0.14	0.12	0.14	0.23	0.13	0.23	0.42	0.79
C14:0	10.08	8.80	10.31	6.50	9.07	1.21	0.09	0.34	0.08	0.36
C14:1	0.97	0.86	0.93	0.78	0.91	0.10	0.29	0.90	0.49	0.63
C15:0	1.15	0.9	0.95	0.84	0.89	0.20	0.09	0.37	0.20	0.97
C15:1	0.01	0.01	0.01	0.01	0.01	<0.01	0.19	0.28	0.88	0.37
C16:0	32.98	31.38	33.55	32.18	30.71	1.49	0.79	0.56	0.19	0.77
C16:1	1.63	1.61	1.71	1.30	2.03	0.27	0.29	0.30	0.73	0.32
C18:0	13.12	16.50	16.25	17.00	17.19	1.38	0.02	0.33	0.62	0.72
C18:1n9*t*	0.35	0.22	0.17	0.31	0.35	0.37	0.26	0.25	0.16	0.75
C18:1n9*c*	20.51	23.24	24.16	25.01	24.24	2.26	0.50	0.98	0.02	0.46
C18:2n6*t*	0.13	0.16	0.17	0.16	0.17	0.03	0.23	0.16	0.52	0.42
C18:2n6*c*	0.42	0.64	1.02	1.46	0.04	0.51	0.08	0.47	0.58	0.73
C18:3n3	0.14	0.15	0.18	0.19	0.17	0.02	0.04	0.91	0.26	0.20
C18:3n6	0.03	0.04	0.03	0.03	0.03	<0.01	0.65	0.43	0.83	0.31
C20:0	0.13	0.15	0.19	0.20	0.19	0.03	0.21	0.87	0.60	0.38
C20:1	0.07	0.1	0.13	0.1	0.13	0.03	0.49	0.35	0.79	0.37
C20:3n6	0.09	0.1	0.11	0.11	0.09	0.07	0.12	0.84	0.28	0.77

l = Linear, q = Quadratic, c = Cubic, qr = Quartic; *c = cis, t = trans.*

[a, b] Means on the same rows with different superscript letters differ ($p<0.01$).

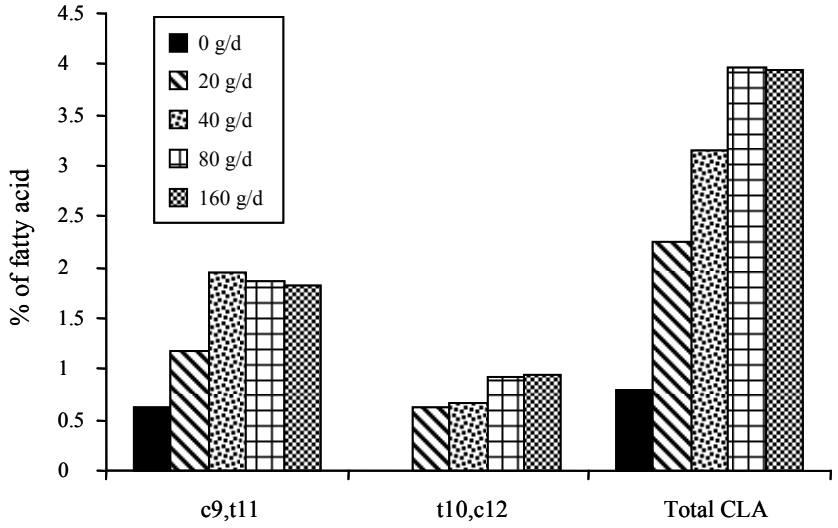

Figure 1. Dominant CLA in milk fat of cows supplemented with CLA-20 at 0, 20, 40, 80 and 160 g/d.

C18:2n6t, C18:2n6c, C18:3n3, C18:3n6, C20:0, C20:1 and C20:3n6 fatty acids in milk fat in this experiment were not affected by CLA-20 supplementation. These results disagreed previous reports (Baumgard et al., 2000; Giesy et al., 2002; Bernal-Santos et al., 2003; Mackle et al., 2003; Perfield et al., 2004). The possible reason of this result was that the alteration of concentration in these fatty acids required to meet different amount of each CLA isomers supplementation. Remarkably, the amount of *cis-9,trans-11* and *trans-10,cis-12* CLA supplementation might be too low, which was caused not to affect any fatty acids, in this study. In contrast with previous studies (Chouinard et al., 1999a, 1999b; Baumgard et al., 2000), the short-to medium chain fatty acids were reduced to a greater extent by CLA infusion than longer chain fatty acids. As in the study of Baumgard et al. (2000), between 74 and 78% of the reduction (mmol basis) in milk fat that occurred during CLA infusion was accounted for by fatty acids of chain length C4:0 to C16:0. Recently, Baumgard et al. (2001) demonstrated that at low concentrations of *trans-10,cis-12* CLA (3.5 g/d), the inhibition of milk fat synthesis involved more uniform reductions in short chain, medium chain and long chain fatty acids. Although the mechanisms by which CLA inhibits milk fat synthesis are still unknown.

Concentrations of *cis-9,trans-11* CLA in milk fat were linearly increased (0.62, 1.17, 1.94, 1.87 and 1.82% of fatty acid) of cows supplemented with CLA-20 at 0, 20, 40 , 80 and 160 g/d, respectively ($p<0.01$) (Figure 1, Table 7). An increase of *trans-10,cis-12* CLA was similar to *cis-9,trans-11* CLA by linearly increasing in milk fat (0.01, 0.63, 0.67,

Table 7. Concentration of CLA in milk fat from cows supplemented with CLA-20

CLA	CLA-20 levels (g/d)					SEM	Contrast (p-value)			
(% of fatty acid)	0	20	40	80	160		l	q	c	qr
Conjugated 18:2										
*c*9,*t*11	0.62^{c}	1.17^{b}	1.94^{a}	1.87^{a}	1.82^{a}	0.15	<0.01	<0.01	0.67	0.15
*t*8,*c*10	0.02^{b}	0.07^{a}	0.09^{a}	0.07^{a}	0.09^{a}	0.01	<0.01	<0.01	0.05	0.58
*c*10,*t*12	0.03^{bc}	0.06^{b}	0.01^{c}	0.06^{b}	0.10^{a}	0.01	<0.01	0.13	<0.01	<0.01
*t*9,*c*11	0.05^{b}	0.02^{b}	0.11^{a}	0.06^{b}	0.07^{a}	0.01	0.33	0.97	0.22	<0.01
*c*11,*t*13	0.00^{b}	0.00^{b}	0.00^{b}	0.05^{a}	0.00^{b}	0.01	<0.01	<0.01	0.05	0.48
*t*10,*c*12	0.01^{b}	0.63^{a}	0.67^{a}	0.93^{a}	0.95^{a}	0.15	<0.01	0.08	0.46	0.34
*c*8,*c*10	0.01^{bc}	0.06^{a}	0.03^{ab}	0.03^{ab}	0.00^{c}	0.01	0.08	0.42	0.27	<0.01
*c*9,*c*11	0.01^{b}	0.02^{ab}	0.02^{ab}	0.03^{ab}	0.05^{a}	0.01	0.55	0.33	<0.01	0.06
*c*10,*c*12	0.03^{d}	0.12^{c}	0.05^{cd}	0.44^{b}	0.54^{a}	0.03	0.01	<0.01	0.01	<0.01
*c*11,*c*13	0.03^{b}	0.02^{b}	0.01^{b}	0.15^{a}	0.01^{b}	0.01	<0.01	<0.01	0.20	0.07
*t*11,*t*13	0.03^{c}	0.05^{c}	0.17^{b}	0.19^{b}	0.24^{a}	0.02	<0.01	0.16	0.27	0.75
*t*8,*t*10+*t*9, *t*11+*t*10,*t*12	0.04^{c}	0.05^{bc}	0.07^{b}	0.09^{a}	0.07^{b}	0.01	<0.01	0.04	<0.05	0.07
Total CLA	0.80^{c}	2.25^{b}	3.16^{a}	3.97^{a}	3.94^{a}	0.30	<0.01	<0.01	0.87	0.74

l = Linear, q = Quadratic, c = Cubic, qr = Quartic; *c = cis, t = trans.*

[a, b, c] Means on the same rows with different superscript letters differ ($p<0.01$).

0.93 and 0.95% of fatty acid, in cows supplemented with all CLA-20 concentrations (0, 20, 40, 80 and 160 g/d, respectively, $p<0.01$). Most of others CLA isomers were increased, but lesser extend, by CLA-20 supplementation. However, variation of the others CLA isomers found in this experiment could be affected by CLA synthesized from mammary gland. Moreover, the concentration of total CLA in milk fat was also increased by dose of CLA-20. Apparently, the elevation of CLA in milk fat in this experiment was corresponded to previous studies (Loor et al., 2002; Loor and Herbein, 2003; Mackle et al., 2003; Perfield et al., 2004).

Daily production of *cis-9,trans-11* CLA were 4.01, 7.71, 11.87, 11.43 and 10.26 g/d when CLA-20 was supplemented at 0, 20, 40, 80 and 160 g/d equivalent to 0, 2, 4, 8 and 16 g *cis-9,trans-11* CLA/d, respectively. The presence of *cis-9,trans-11* CLA in milk fat in cows fed diet without CLA-20 supplement may be influenced by endogenous synthesis in the mammary gland from transvaccenic acid (TVA, *trans-11* c18:1) by the Δ^9-desaturase reaction (Griinari and Bauman, 1999). The control diet contained high concentration of linoleic acid (C18:2n6c) (Table 2), which was hydrogenated to *trans-11* C18:1, then led to increase precursors for Δ^9-desaturation in mammary gland, and resulted in an increase *cis-9,trans-11* CLA content in milk fat (AbuGhazaleh et al., 2003; Loor and Herbein, 2003). Apparent transferring efficiencies of *cis-9,trans-11* CLA from the supplement to milk fat was calculated according to Piperova et al. (2004) were 100, 100, 92 and 39% for 20, 40, 80 and 160 g/d of CLA-20 supplement, respectively. This observation was unexpected. Piperova et al. (2004) demonstrated that the apparent transferring efficiency of *cis-9, trans-11* CLA from the supplement to milk was 11%, which was similar to Giesy et al. (2002) who reported the 9-10% of transferring efficiency for *cis-9, trans-11* CLA in lactating cows by increasing doses of Ca-CLA-60. While Chouinard et al. (1999a) indicated transferring efficiency was about 33.5% during abomasal infusion of *cis-9, trans-11* CLA. The different transferring efficiency between this observation and previous reports was probably due to the fact that the fat percentage in our experiment was probably higher (3.59-4.26%) than in Piperova et al. (2004) (2.54-3.39%), Giesy et al. (2002) (2.29-3.45%), and Choinard et al. (1999a) (2.40-3.34%). Incomplete protection exogenous CLA source from ruminal biohydrogenation of CLA and amount of CLA supplementation could be the main reason for the difference of apparent transferring efficiencies between the current study and those reports.

CONCLUSIONS

Supplementation of CLA as CLA-20 on milk fat content and composition were examined. DMI and milk yield was slightly increased when the cows were supplemented with CLA-20 from 20 to 80 g/d. Increased supplementation of CLA up to 160 g of CLA-20/d resulted in milk fat depression. Content of *cis-9,trans-11* and *trans-10,cis-12* CLA in milk fat increased at an increasing rate of CLA-20 supplementation. Based on this experiment, supplementation with CLA-20 at 80 g/d was potentially increased the concentration of *cis-9,trans-11*, *trans-10,cis-12* CLA and total CLA in milk fat.

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