Short Communication: Postruminal Infusion of Conjugated Linoleic Acids Negatively Impacts Milk Synthesis in Holstein Cows

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ABSTRACT

In view of the potential of rumen-protected conjugated linoleic acid (CLA) as a means to increase the CLA content of bovine milk, a study was undertaken to evaluate the effect of synthetic CLA on milk production and composition. Four Holstein cows received abomasal infusion of: 1) control, no lipid infusion, 2) 150 g/d of synthetic CLA, 31.7% cis-9, trans-11; 30.4% trans-10, cis-12, 3) 150 g/d of safflower oil, and 4) 150 g/d of tallow. Infusion was carried out for 20 to 22 h/d for 11-d periods in a 4 × 4 Latin square design. The milk fat concentration of cis-9, trans-11 and trans-10, cis-12 isomers of CLA was significantly increased with infusion of CLA. However, CLA infusion had other unexpected effects on milk production and composition. Milk yield dropped significantly during the period of CLA infusion. Furthermore, as well as the typical depression in milk fat reported with trans-10 isomers of CLA, other negative effects specific to CLA infusion were observed including a drop in lactose concentration and yield, a drop in protein yield, and an elevated somatic cell count. The important difference between synthetically produced CLA and CLA produced naturally in the cow is the much higher proportion of trans-10 isomers of CLA in the former. The results of this study suggest that the extent of enrichment possible for trans-10 isomers of CLA, and hence the usefulness of synthetic CLA for this purpose, may be limited because of unacceptable effects on milk yield and composition.

(Key words: abomasal infusion, conjugated linoleic acid, dairy cow, milk)

Abbreviation key: CLA = conjugated linoleic acid, CTL = control, SAFF = safflower, TALL = beef tallow, FA = fatty acid(s).

Conjugated linoleic acid (CLA) refers to a group of 18-carbon conjugated dienes of predominantly rumi-

nant origin. Ruminant animals produce CLA through the biohydrogenation of dietary fat in the rumen and also by desaturation of trans-11 18:1 in the mammary gland and adipose tissue (Griinari et al., 2000). Conjugated linoleic acid can also be synthesized in the laboratory from vegetable oils, such as sunflower oil. Mixtures of CLA isomers have been reported to have anticancer, anti-obesity, and anti-diabetic properties in various animal models (Belury, 2002).

Conjugated linoleic acid of ruminant origin contains predominantly one isomer (cis-9, trans-11 18:2), whereas synthetically produced CLA tends to contain a considerable proportion of trans-10, cis-12, as well as cis-9, trans-11 and smaller quantities of other isomers. This type of product has reduced subcutaneous fat and increased lean in pigs (Dugan et al., 1997) and will likely become commercially available in the near future. The trans-10, cis-12 isomer has been associated with these effects on body composition, whereas the anticarcinogenic properties have been attributed to the cis-9, trans-11 and trans-10, cis-12 isomers (Pariza et al., 2001). Isomers of CLA have also had an inhibitory effect on bovine milk fat synthesis (Lor and Herbein, 1999). The trans-10, cis-12 CLA appears to be the isomer responsible for this effect (Baumgard et al., 2000). Rumen-protected CLA may therefore be beneficial as a tool to increase the protein to fat ratio in milk (Bauman and Griinari, 2001), and potentially improve the energy balance of early lactation cows (Perfield II et al., 2002).

In view of the ability of the trans-10, cis-12 isomer to reduce body fat in animals, interest has been shown in whether this isomer could have a benefit for weight reduction in humans. Feeding rumen-protected CLA could be a means of elevating the concentration of CLA isomers in bovine milk fat, thereby increasing the supply of these specific fatty acids (FA) in the human diet. Gulati et al. (2000) fed goats 40g/d of a rumen-protected CLA mixture for 4 d and observed a significant increase in milk fat CLA concentration. Similarly, Chouinard et al. (1999a) showed that postruminal delivery of 150 g/d of a CLA mixture for 5 d was effective at increasing the concentration of CLA isomers in bovine milk fat. However, this level of CLA

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significantly depressed milk fat and reduced milk yield by more than 3 kg (Chouinard et al., 1999a). Our objective was to evaluate in greater detail the effect of post-ruminal delivery of CLA isomers on milk production and composition using abomasal infusion of 150 g/d for 11-d periods.

Four multiparous pregnant Holstein cows (168 ± 21 DIM; mean $\pm$ SD) were fed the same basal diet of 55% forage (alfalfa silage, barley silage, and alfalfa hay) and 45% concentrate (based on barley, corn, and canola meal). Each cow received abomasal infusion of: 1) control, no lipid infusion (CTL), 2) 150 g/d of synthetic CLA (Conlinco Inc, Detroit Lakes, MN), 31.7% cis-9, trans-11; 30.4% trans-10, cis-12 (CLA), 3) 150 g/d of safflower oil, 76% linoleic acid (SAFF), and 4) 150 g/d of beef tallow (TALL). Infusion was carried out for 20 to 22 h/d for 11-d periods in a 4×4 Latin square design. The lipids were infused daily in a 6-L carrier solution to give an adequate volume for infusion. The infusion solutions were made as follows. Briefly, 150 g of lipid was made into a paste with 56 g of spray-dried chicken protein (American Protein Corp., Ames, Iowa) in a steam-jacketed kettle. Six liters of water were added and the mixture was stirred and heated. When the temperature reached 70°C, 7 g of Tween-80 was added as an emulsifying agent. The emulsion was then homogenized in a one-stage homogenizer at 2500 psi and stored at 4°C until needed. The infusate was pumped using a Masterflex peristaltic pump with L/S-16 Tygon tubing (Labcor Inc., Anjou, Quebec). The tubing was passed through a rumen cannula and anchored in the abomasum with a plastisol flange. DMI and milk yield were recorded daily. Milk was sampled during the last 2 d of each period for composition analysis. Milk was analyzed for protein, fat, lactose, and SCC using near infrared spectroscopy at the Alberta

Agriculture, Food and Rural Development Central Milk testing laboratory (Edmonton, Alberta). Fatty acid composition of milk was analyzed following preparation of FA methyl esters with sodium methoxide (Chouinard et al., 1999b). The data were analyzed as a 4×4 Latin square design using the GLM procedure of SAS (SAS, 1989). Treatment differences were evaluated using the Duncan procedure with a significance level of $P < 0.05$.

The results of this study have been presented previously as an abstract (Bell and Kennelly, 2001). Infusion of CLA had dramatic effects on milk production and composition (Table 1). Milk yield declined steadily throughout the period of CLA infusion so that over the last 2 d of the period milk yield was 34.8 to 43.6% lower with CLA infusion compared to the other treatments. Concentration and yield of lactose and fat were also significantly lower with CLA infusion. Protein concentration was significantly higher with CLA although the yield of protein was lower compared to the other treatments. Analysis of FA composition showed that the concentration of 18:2n-6 and CLA increased significantly as a result of SAFF and CLA infusion, respectively (Table 2). Since the yield of milk fat was reduced with CLA infusion, the yield of all the FA (except CLA isomers) was significantly reduced with the CLA treatment (data not shown).

Most interesting was the effect of treatment on milk SCC (Table 1). The SCC was approximately five to seven times greater as a result of CLA infusion compared to the other treatments, which had values at levels considered normal for healthy cows. SCC is a count of white blood cells and sloughed off epithelial cells in milk. High counts are generally indicative of an infection in the udder. However, we believe that infection was not the cause of the high SCC observed

Table 1. Yield and composition of milk from cows receiving abomasal infusion of control (CTL), beef tallow (TALL), safflower oil (SAFF), or conjugated linoleic acid (CLA).

	CTL ²	TALL	SAFF	CLA	sem
DMI (kg/d) ¹	18.59	18.92	19.27	18.59	0.563
Milk yield (kg/d)	24.2 ^a	23.0 ^a	26.6 ^a	15.0 ^b	1.928
Lactose (%)	3.86 ^a	3.88 ^a	4.06 ^a	3.36 ^b	0.078
Lactose yield (kg/d)	0.95 ^a	0.92 ^a	1.08 ^a	0.50 ^b	0.071
Fat (%)	2.38 ^a	2.62 ^a	2.37 ^a	1.78 ^b	0.164
Fat yield (kg/d)	0.56 ^a	0.59 ^a	0.63 ^a	0.24 ^b	0.031
Protein (%)	3.06 ^a	2.99 ^a	3.16 ^a	4.42 ^b	0.198
Protein yield (kg/d)	0.70 ^{ab}	0.66 ^a	0.83 ^b	0.61 ^a	0.044
SCC, $\times 1000/\text{ml}$	187 ^a	193 ^a	133 ^a	991 ^b	144.3
Sodium, mg/kg	715 ^a	748 ^a	658 ^a	978 ^b	64.38
Chloride, (%)	0.192 ^a	0.192 ^a	0.188 ^a	0.252 ^b	0.006

^{a,b}Within a row, values with different superscripts are significantly different ($P < 0.05$).

¹Values for dry matter intake represent average for entire period since dry matter intake remained stable during each period regardless of treatment; milk parameters represent values for the last 2 d/period.

²CTL = control (no lipid infusion); TALL = infusion of 150 g/d beef tallow; SAFF = infusion of 150 g/d of safflower oil; CLA = infusion of 150 g/d of synthetic CLA.

Table 2. Fatty acid composition of milk fat from cows receiving abomasal infusion of control (CTL), beef tallow (TALL), safflower oil (SAFF), or conjugated linoleic acid (CLA).

Fatty acid ¹	CTL ²	TALL	SAFF	CLA	sem
4:0-15:0	23.6 ^a	22.3 ^a	21.9 ^a	18.2 ^b	0.910
16:0	32.3 ^a	31.9 ^a	29.9 ^a	39.5 ^b	1.120
16:1 n-7	1.41 ^{ab}	1.58 ^a	1.12 ^b	1.39 ^{ab}	0.120
17:0	0.62	0.58	0.57	0.62	0.024
18:0	11.0 ^a	11.0 ^a	11.2 ^a	13.5 ^a	0.801
18:1 trans	2.48	2.70	2.58	2.21	0.157
18:1 n-9	24.4 ^{ab}	25.6 ^a	22.8 ^b	18.2 ^c	0.696
18:1 n-12	1.13	1.08	1.09	0.96	0.077
18:2 n-6	1.8 ^a	2.1 ^a	7.6 ^b	2.3 ^a	0.203
18:3 n-3	0.15	0.15	0.17	0.19	0.022
Cis-9, trans-11 CLA	0.59 ^a	0.61 ^a	0.58 ^a	1.77 ^b	0.108
Trans-10, cis-12 CLA	ND ^a	ND ^a	ND ^a	0.85 ^b	0.060

^{a,b,c}Within a row, values with different superscripts are significantly different ($P < 0.05$).

¹Fatty acids expressed as percentage of fatty acid methyl esters.

²CTL = control (no fat infusion); TALL = infusion of 150 g/d beef tallow; SAFF = infusion of 150 g/d of safflower oil; CLA = infusion of 150 g/d of synthetic CLA.

ND = Not detected

with CLA infusion. The high SCC was observed in each cow but only during the period when that cow received CLA infusion. Milk from the period preceding or following the CLA period always had much lower counts. The cows also showed no physical signs that may have indicated an infection. For instance, there was no effect of treatment on DMI (Table 1). Furthermore, there were no visible signs of mastitis during milking at any time in the course of the experiment. Bacterial analysis of the milk showed counts of *Streptococcus* spp./*Enterococcus* spp. well within the normal range for raw milk and no signs of *Staphylococcus aureus* (data not shown). The surprisingly low concentration of lactose with CLA was counterbalanced by a higher concentration of sodium. The concentration of chloride was also higher with CLA infusion. During the early stages of involution, similar changes are seen in the mammary secretion as were observed with infusion of CLA. Although purely speculative, it is possible that infusion of relatively large amounts of these synthetic CLA isomers was initiating dry-off mechanisms in the udder. Further work will be necessary to explore this by examining the changes in expression of genes involved in the process of bovine mammary involution.

This study demonstrated that postruminal delivery of a mixture of CLA isomers could significantly increase the concentration of these various FA in bovine milk. This is in contrast to methods that use modifications to the cow's diet to increase the natural production of CLA where the increase is predominantly in cis-9, trans-11 CLA. This study showed that the extent of enrichment possible for trans-10 isomers of CLA is limited because of unacceptable effects on milk yield and composition. This places a constraint on the degree to which synthetic CLA preparations could be used to

enrich milk CLA and the extent to which bovine milk could be used as a vehicle to increase the supply of trans-10, cis-12 CLA in the human diet.

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