Effects of Abomasal Infusion of Conjugated Linoleic Acid on Milk Fat Concentration and Yield from Pasture-Fed Dairy Cows

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ABSTRACT

The aim of this study was to investigate the effects of conjugated linoleic acid supplementation on the synthesis of milk fat in pasture-fed Friesian cows. In four cows, a commercial mixture containing 62.3% (wt/vol) conjugated linoleic acid was infused intraabomasally to avoid rumen fermentation and biohydrogenation. The design was a 4 × 4 Latin square in which each cow received infusions of 0, 20, 40, and 80 g/d of conjugated linoleic acid mixture for 4 d. Cows were fed freshly cut ryegrass/white clover pasture ad libitum. Milk fat concentration was decreased by 36, 43, and 62% and milk fat yield was decreased by 32, 36, and 60% by the 20, 40, and 80 g of conjugated linoleic acid/d treatments. Dry matter intake, milk protein concentration, and protein yield were unaffected by treatments; however, milk yield was increased by 11% during the 40-g conjugated linoleic acid/d treatment. The effects of conjugated linoleic acid infusion were most pronounced in reducing de novo fatty acid synthesis and desaturation. Results show that the inhibitory effect of this conjugated linoleic acid mixture on milk fat synthesis occurs in pasture-fed cows, and demonstrate the potential to dramatically alter gross milk composition. This technology could offer a management tool to manipulate milk composition and energy demands of pasture-fed cows.

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

Abbreviation key: BOH = beta-hydroxybutyrate, CLA = conjugated linoleic acid, FAME = fatty acid

methyl esters, NCN = noncasein nitrogen, TN = total nitrogen, TP = true protein, UN = urea nitrogen.

INTRODUCTION

The demand for milk fat relative to milk protein has declined, and many modern dairy products have little requirement for milk fat. Several world dairy markets already discourage the production of milk fat relative to milk protein through weighted payment schemes and quotas. Milk fat synthesis is also energetically expensive for the dairy cow, particularly in early lactation, when body lipid mobilization occurs to support lactation (Bauman and Davis, 1975). High-producing cows and those grazing pasture under a controlled allowance are subjected to even greater pressure to mobilize body fat reserves in support of lactation (Macdonald and Macmillan, 1993). Thus, reducing the concentration and yield of fat may provide benefits by diverting energy into more valuable milk components (e.g., protein) and enhancing energy balance in the high-producing dairy cow.

Milk fat depression, or a decrease in the rate of fat synthesis, has been characterized under several dietary situations including high concentrate diets, low fiber diets, unprotected dietary fats, small particle size forages and ionophores (Bauman and Griinari, 2001). The mechanism responsible is thought to involve specific fatty acid isomers arising from rumen biohydrogenation of dietary lipid inhibiting some aspect of milk fat synthesis; this has been referred to as the “biohydrogenation theory” of milk fat depression (see review by Bauman and Griinari, 2001). Recently, several studies have shown that administration of commercially available mixtures of conjugated linoleic acid (CLA) also inhibit lipid synthesis in lactating cows, causing substantial reductions in milk fat secretion (Lor and Herbein, 1998; Chouinard et al., 1999a, 1999b; Kraft et al., 1999; Medeiros et al., 2000). Baumgard et al. (2000) recently demonstrated that the *trans*-10 *cis*-12 CLA isomer in-

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Table 1. Nutrient composition of pasture diet fed to cows.¹

Diet	Quantity
Nutrient composition, % of DM ²	
CP	15.3
Crude fat	4.3
Ash	8.8
NDF	54.2
ADF	27.3
Lignin	2.7
Fatty acid composition, % of total fatty acids	
C _{12:0}	1.1
C _{14:0}	0.9
C _{16:0}	19.6
C _{16:1}	1.9
C _{18:0}	8.2
C _{18:1}	3.9
C _{18:2}	13.0
C _{18:3}	46.5
C _{20:0}	1.9
C _{22:0}	0.9
C _{24:0}	2.1

¹Mean of four weekly composites.

²Dietary DM % averaged 18.1% and in vitro digestibility was 68%.

hibited milk fat synthesis whereas the *cis*-9, *trans*-11 CLA did not. When these results are considered with earlier studies that used various CLA isomer enrichments (Chouinard et al., 1999a, 1999b), the *trans*-8 *cis*-10 CLA isomer may also inhibit milk fat synthesis.

The main objective of the current study was to evaluate the extent of milk fat depression in cows fed a diet comprising 100% pasture when a commercially available CLA mixture was infused intra-abomasally. At the same time, the dose response and time course effects of CLA infusion on milk fat concentration and yield and DMI were assessed. Blood metabolites and hormones were also examined during milk fat reduction to identify potential mechanisms.

MATERIALS AND METHODS

Animals, Design, and Treatments

The Ruakura Animal Committee approved all procedures involving dairy cows. Four lactating, rumen-fistulated, multiparous, pregnant Friesian cows were used. At the start of the study, cows were 80 ± 2 DIM and averaged 523 ± 45 kg BW (X ± SD).

Cows were fed a 100% pasture diet comprising mainly ryegrass (*Lolium perenne* L.) but with some white clover (*Trifolium repens* L.) present. Pasture was cut twice daily at approximately 0600 and 1500 h and offered ad libitum, in individual in-door stalls. Daily pasture samples were composited by treatment period for chemical analyses, which were conducted as described by Mackle et al. (1999); nutrient composition of the diet is presented in Table 1. Water was constantly available.

The experiment consisted of four 11-d periods; the initial 2-d allowed for acclimation to metabolism stalls, the following 4-d in metabolism stalls represented the treatment interval, and cows grazed pasture during the 5-d interval posttreatment. During treatment intervals, cows received abomasal infusions of one of four treatments according to a 4 × 4 Latin Square design. Treatments were abomasal infusions of 0, 20, 40 and 80 g/d of a commercial CLA source (CLA-60; Natural Lipids, Hovdebygda, Norway). Table 2 presents the fatty acid composition of the CLA supplement.

Preparation of Abomasal Infusates

The CLA mixture was infused as an emulsion with skim milk to provide a constant release of CLA into the abomasum; skim milk was reconstituted from skim milk powder (New Zealand Milk Products, Wellington, New Zealand) to provide consistency between periods. Three days before each infusion interval, a 2.0% CLA (wt/wt) emulsion in skim milk (40 L) was prepared essentially as described by Chouinard et al. (1999a).

Briefly, the skim milk powder (lot 140 D105 G2591; New Zealand Milk Products, Wellington, NZ) was rehydrated in water at 45°C for 20 min; a 50-ml sample was taken for total solids testing. The CLA mixture was briefly heated to 50°C before use to ensure liquid form, and was then incorporated into the skim milk using a high shear mixer (Ultra Turrax T50; Janke and Kunkel, IKA Labortechnik, Germany). The mixture was then heated to 74°C for 15 s to pasteurize and passed through a homogenizer (APV Rannie, Copenhagen, Denmark) at 74°C with a first stage pressure of 13.0 MPa (2000 psi) and a second stage pressure of 3.45 MPa (500 psi). The emulsion was cooled to <7°C in an ice bath, a sample was taken for particle size testing (average size = 0.9 µm), and a further 50-ml sample was taken for total solids testing. The day before start of infusions, two

Table 2. Fatty acid profile of the CLA mixture¹ infused.

Fatty acid	% of total fatty acids	% of total CLA
C _{16:0}	4.0	
C _{18:0}	2.1	
<i>cis</i> -9 C _{18:1}	21.4	
<i>cis</i> -9, <i>cis</i> -12 C _{18:2}	6.6	
Conjugated linoleic acid ²	(62.3)	(100.0)
<i>cis/trans</i> 8, 10 + 9, 11 C _{18:2}	22.0	35.3
<i>cis/trans</i> 10, 12 C _{18:2}	18.5	29.7
<i>cis/trans</i> 11, 13 C _{18:2}	10.1	16.2
Other CLA	11.7	18.8
Unknown	3.6	

¹CLA-60 (Natural Lipids Ltd., Hovdebygda, Norway, lot no. 10685).

²*cis/trans* indicates molecule has one *cis* and one *trans* double bond.

more skim milk/CLA emulsions were prepared containing 0.5 and 1.0% CLA concentration (wt/wt) by dilution of the 2.0% batch with skim milk. The remaining skim milk was used as the 0% CLA (control) infusate.

Abomasal infusates were delivered via polyvinyl chloride tubing (Nalgene 180 PVC; 0.47 cm i.d. $\times$ 0.78 cm OD; Nalge Co., Rochester, NY), which extended through the rumen fistula and sulcus omasi into the abomasum (Spires et al., 1975). Peristaltic pumps (STA-131900; Desaga, Heidelberg, Germany) delivered the 4 L of infusate continuously over 24 h. Infusion equipment was checked daily during treatment periods to ensure correct placement in the abomasum.

Milk Yield and Composition

Cows were milked at approximately 0700 and 1600 h. At each milking, milk yield was determined by weight and samples collected. Milk samples from PM and AM milkings were composited proportionately by weight according to milk yields. Fresh samples were analyzed for CP, fat, lactose, and total solids using an infrared milk analyzer (FT120; Foss Electric, Hillerød, Denmark), for SCC using an automated cell counter (Fosomatic 215; Foss Electric) and milk urea using a commercially available test kit (Boehringer Mannheim, Mannheim, Germany). The infrared analysis of milk protein was calibrated to measure milk protein according to total N (TN) $\times$ 6.38.

Because milkfat content was unusually low with CLA infusion, the infrared method was evaluated to ensure its accuracy. Composite milk samples from the initial 2-d acclimation period, the 4-d infusion interval, and the first 2-d of the 'washout' interval were tested for N fractions using macro-kjeldahl techniques (AOAC, 1995). Noncasein N (NCN) was determined according to the International Dairy Federation (1964); CN was precipitated at pH 4.6 and removed by filtration, leaving the filtrate for N analysis. From these N fractions, CP (TN $\times$ 6.38) and CN ((TN – NCN) $\times$ 6.38) were calculated. Milk NCN was also converted to protein equivalents by multiplying by 6.38 to allow comparison with other protein fractions. From the same composite samples, milk fat was extracted using a modification of the Rose Gottlieb technique (IDF, 1987) to determine reference fat concentration. The reference CP (Kjeldahl) and fat (Rose Gottlieb) measurements allowed direct comparison with infrared results. Reference and infrared measurements were highly correlated at all times, with r^2 values of 0.99 for both fat and protein.

On the last day of infusion interval (d 4), subsamples of milk fat obtained following the Rose Gottlieb fat extraction procedure were collected for further analyses. Citrate concentrations were determined using spectro-

photometric detection (Shimadzu UV-160A, Shimadzu Corporation, Kyoto, Japan) based on the method of (Dagley, 1974). Fatty acid methyl esters (FAME) were quantified by gas liquid chromatography after methylation using sodium methoxide as described by MacGibbon (1988). Gas chromatographic analyses of standard fatty acid compositions were performed on a Shimadzu GC17A (Shimadzu Corporation) equipped with a 15-m EC-1000 column (15 m $\times$ 0.53 mm i.d. and 1.2 μ m film thickness; Alltech Associates, Inc., Deerfield, IL) with hydrogen as the carrier gas (10 ml/min). The flame-ionization detector was set at 250°C. The column was operated at an initial temperature of 50°C for 1.5 min, then temperature programmed at 15°C/min to 150°C followed by 6°C/min to 220°C, where it was held for 3 min. Cold on-column injection (1.0 μ l) was used, with an initial temperature of 35°C before ramping to 150°C at 250°C/min and then 220°C at 150°C/min.

Concentrations of C_{18:1} *trans* positional isomers were determined by argentation thin-layer chromatography as described in Fong (1997). Briefly, an aliquot (100 μ l) of FAME solution (5 mg/ml) was loaded onto the argentation TLC plate and fractionated using toluene as mobile phase. *Trans* monoene and saturated FAME bands were extracted and reconstituted in 200 ml of hexane for analysis by capillary gas chromatography. Separation of the CLA and C_{18:1} *trans* positional isomers were carried out on a Shimadzu GC 17A (Shimadzu Corporation) equipped with a 50 m $\times$ 0.22 i.d. BPX70 capillary column (50 m $\times$ 0.22 mm i.d. and 0.25 μ m film thickness; SGE Incorporated, Austin, TX) with hydrogen as the carrier gas (50 ml/min). The column was operated at an initial temperature of 50°C for 1.5 min, then temperature programmed at 3°C/min to 220°C and held for 10 min. Cold on-column injection (0.2 μ l) was used, with an initial temperature of 50°C before ramping to 250°C at 150°C/min.

Blood Metabolites and Hormones

Blood samples were obtained on d 4 of infusion at 0800, 1000, 1200 and 1400 h by venipuncture of the coccygeal vein and were collected into both heparinized (~100 U of heparin/ml of blood) and plain Vacutainers. Plasma was harvested from heparinized Vacutainers, stored at –20°C, and later analyzed for insulin by a double-antibody radioimmunoassay as described by McMahon et al. (1998). A commercial kit was used (Coat-A-Count; Diagnostic Products Corporation, Los Angeles, CA), all samples were completed within one assay and the intra-assay CV was 12.9%.

Serum from plain vacutainers was analyzed for concentrations of urea N (UN; urease method), glucose (hexokinase method), beta-hydroxybutyrate (BOH)

Table 3. Least squares means for DMI, milk yield, and milk composition.

Variable	CLA-60 infused (g/d) ¹				SED	Contrast ²		
	0	20	40	80		Lin	Quad	Dev
DMI, kg/d	16.5	16.6	16.1	15.9	0.7	0.37	0.99	0.65
Milk yield, kg/d	19.7	21.2	21.9	20.3	0.8	0.68	0.02	0.88
Fat ³								
%	3.97	2.50	2.28	1.53	0.22	<0.01	0.01	0.05
g/d	784	534	501	313	51	<0.01	0.09	0.09
CP ³								
%	3.05	2.96	2.97	2.99	0.04	0.38	0.14	0.32
g/d	598	624	650	602	30	0.97	0.11	0.68
Lactose ⁴								
%	4.90	4.95	4.91	4.92	0.03	0.86	0.70	0.21
g/d	967	1,048	1,077	1,001	43	0.66	0.03	0.94
Total solids ⁴								
%	12.42	11.02	10.94	10.10	0.27	<0.01	0.05	0.06
g/d	2,449	2,335	2,393	2,048	91	<0.01	0.22	0.20
Casein ³								
%	2.39	2.34	2.33	2.29	0.04	0.06	0.59	0.70
g/d	468	494	508	462	24	0.66	0.08	0.84
NCN × 6.38 ³								
%	0.66	0.64	0.65	0.69	0.01	0.03	0.03	0.75
g/d	131	134	144	142	6	0.07	0.28	0.38
CP:Fat ³	0.78	1.23	1.32	2.01	0.19	<0.01	0.98	0.31
Milk urea, mmol/l	3.78	3.33	3.15	2.94	0.20	<0.01	0.18	0.67
Milk citrate, mg/ml	1.58	2.20	2.22	2.23	0.07	<0.01	<0.01	0.02

¹Treatments involved 4-d abomasal infusion of 0, 20, 40, or 80 g CLA-60/d. Values presented represent averages from PM and AM milking for the last day of the infusion periods (d-4).

²Contrast = probability of a linear (Lin) or quadratic (Quad) effect of the level of CLA supplement or deviations (Dev).

³Data derived from reference analyses (Rose Gottlieb for fat and Kjeldahl for CP).

⁴Data derived from infrared analysis.

and NEFA (colorimetric method) using commercially available enzymatic colorimetric kits (Boehringer Mannheim, Germany) and a spectrophotometric auto-analyzer (Hitachi 717; Hitachi Ltd., Tokyo, Japan). All samples were completed within one assay and the intra-assay CVs were 1.3, 2.2, 1.0, and 5.0% for UN, glucose, BOH, and NEFA, respectively.

Statistical Analyses

Mean values for each treatment were determined for milk yield, milk composition, and DMI on the last day of the infusion intervals. Reported concentrations of glucose, NEFA, UN, BOH, and insulin are mean values for the four samples taken on d 4 of infusion. Data were analyzed using Genstat (Payne et al., 1993) as a Latin Square design with linear, quadratic, and deviation (from linear and quadratic) contrasts of treatments.

RESULTS

All cows completed the study in good health. The three levels of CLA infusions represented ~0.08 to 0.3% of the total DMI. The DMI averaged 16.3 ± 0.7 kg/d

($\bar{x} \pm \text{SED}$) and was not affected ($P > 0.1$) by CLA infusion (Table 3). The dose response for milk yield displayed a quadratic response ($P = 0.02$) being highest for the 40 g/d dose. By d 4 of infusion, cows receiving the 40 g/d treatment produced 11% (+2.2 kg/d; $P < 0.02$) more milk, than those on control treatment. The temporal pattern of milk yield across the 2-d baseline, 4-d infusion, and 7-d postinfusion intervals is shown in Figure 1. There was evidence of residual affects of CLA on milk yield, with a linear effect ($P < 0.01$) of CLA dose apparent on d 3 to 5 of the post-infusion interval (Figure 1).

Milk fat concentration was reduced ($P < 0.01$) by 37, 43, and 62% to levels of 2.50, 2.28, and 1.53% fat (c.f. 3.97% fat for control) when CLA-60 was infused at 20, 40, and 80 g/d, respectively. Corresponding reductions in milk fat yield were 32, 36, and 60% (−250, −283, and −471 g/d; $P < 0.01$) for the 20, 40, and 80 g CLA/d treatments, respectively (Table 3). Neither milk fat concentration nor yield had appeared to reach a nadir by d 4 of infusion period (Figure 2). The residual effect of depressed milk fat synthesis was evident ($P < 0.01$) for 5-d after CLA infusions had ceased.

Neither concentration nor yield of milk CP were altered ($P > 0.05$) by CLA dose (Table 3); however, there

was a nonsignificant quadratic trend ($P = 0.11$) for milk protein yield to reach a maximum value at the 40 g/d dose. Lactose concentrations were unaltered by treatment, but the yield of lactose was highest for the 40 g/d dose levels, thereby fitting a quadratic dose response trend ($P = 0.03$). Mean SCC were $43,000 \pm 19,000$ cells/ml ($X \pm SD$) and did not differ among treatments ($P > 0.1$; data not presented).

Yield and concentration of total solids decreased with CLA dose ($P < 0.01$). The CP:fat ratio was increased by 58, 69, and 158% for the 20, 40, and 80 g/d dose rates, respectively. These changes resulted from the reduction in fat content and yield (Table 3).

Milk from cows treated with 80 g of CLA/d had 4% lower CN concentration compared with controls and there was a linear trend ($P = 0.06$) for CN concentration to decrease with increasing CLA dose rate (Table 3). There was a quadratic trend ($P = 0.08$) for an increase in casein yield with CLA treatment. Concentrations of NCN were highest for the 80 g/d dose and there was a non-significant ($P = 0.07$) linear trend for the yield of NCN to increase with increasing dose rate (Table 3). In contrast, concentrations of milk urea, a subcomponent of NCN, decreased linearly ($P < 0.01$) with increasing CLA dose; the 80 g/d treatment reduced urea concentrations by 22%.

Infusion of CLA linearly decreased ($P < 0.05$) the concentration of all short- and medium-chain FAME ($C_{4:0} - C_{14:0}$), $C_{16:0}$, $C_{16:1}$, and $C_{17:0}$ with the exception ($P > 0.05$) of $C_{15:0}$, and $C_{17:1}$ (Table 4). For $C_{16:0}$, the

Figure 1. Temporal pattern of milk yield across 11-d period. Abomasal infusion of CLA-60 was performed at either 0 ($\circ$), 20 ($\blacktriangle$), 40 ($\blacksquare$), or 80 ($\bullet$) g/d. Infusions began following the d-2 PM milking and continued through the d-6 PM milking (shown by dashed lines). Values are means of four cows and the standard error of the difference was 0.75 kg/d.

Figure 2. Temporal pattern of milk fat concentration (lower panel) and yield (upper panel) across 11-d period. Abomasal infusion of CLA-60 was performed at either 0 ($\circ$), 20 ($\blacktriangle$), 40 ($\blacksquare$), or 80 ($\bullet$) g/d. Infusions began following the d-2 PM milking and continued through the d-6 PM milking (shown by dashed lines). Values are means of four cows and the standard error of the differences were 0.22% and 51 g/d for milk fat concentration and yield, respectively.

decreasing trend was also quadratic ($P < 0.01$) with the response to CLA dose already maximized at 20 g/d. In contrast, concentrations of $C_{18:0}$, $C_{18:2}$, and $C_{18:3}$ increased ($P < 0.01$) linearly with CLA dose; levels of $C_{18:0}$ were 74% higher for cows receiving 80 g of CLA/d compared with controls (Table 4). Total concentrations of $C_{18:1}$ were altered by CLA infusion according to a quadratic trend, with highest levels reached at 20 g CLA-60/d (Table 4).

Concentrations of *trans*-11, *trans*-13+14, *trans*-15, and *trans*-16 $C_{18:1}$ increased ($P < 0.01$) with increasing dose of CLA-60. Total CLA concentrations also increased ($P < 0.01$) with increasing dose of CLA-60 infusion, with the 80 g/d treatment displaying a 230% increase compared to the control. All classes of CLA isomers presented were increased in a linear fashion by

Table 4. Least squares means for milk fatty acid methyl ester (FAME) concentrations and ratios.

Variable	CLA-60 infused (g/d) ¹				SED	Contrast ²		
	0	20	40	80		Lin	Quad	Dev
	% of total FAME							
C _{4:0}	3.53	3.60	3.49	2.91	0.27	0.04	0.24	0.94
C _{6:0}	2.36	1.99	1.75	1.35	0.18	<0.01	0.42	0.84
C _{8:0}	1.30	0.94	0.87	0.61	0.12	<0.01	0.21	0.31
C _{10:0}	2.83	2.01	1.92	1.37	0.26	<0.01	0.20	0.23
C _{10:1}	0.34	0.18	0.15	0.06	0.03	<0.01	0.02	0.09
C _{12:0}	3.20	2.48	2.51	2.07	0.24	<0.01	0.24	0.18
C _{14:0}	10.90	9.49	9.67	8.75	0.63	0.02	0.42	0.24
C _{14:1}	1.14	0.83	0.75	0.43	0.09	<0.01	0.32	0.22
C _{15:0}	1.52	1.62	1.61	1.64	0.08	0.24	0.44	0.56
C _{16:0}	26.42	22.39	22.51	21.86	0.82	<0.01	0.01	0.07
C _{16:1}	1.98	1.81	1.77	1.65	0.14	0.06	0.60	0.70
C _{17:0}	0.73	0.88	0.93	1.04	0.04	<0.01	0.10	0.24
C _{17:1}	0.47	0.51	0.49	0.48	0.03	0.95	0.42	0.37
C _{18:0}	11.08	14.10	14.57	19.27	0.42	<0.01	0.80	0.01
Total C _{18:1}	23.92	27.49	27.03	25.33	1.46	0.69	0.05	0.34
Total <i>cis</i>	19.03	21.36	20.71	18.44	1.27	0.36	0.06	0.34
Total <i>trans</i>	4.89	6.12	6.33	6.88	0.29	0.36	0.06	0.34
<i>trans</i> -6 and 9	0.18	0.20	0.22	0.23	0.03	0.13	0.68	0.81
<i>trans</i> -10	0.13	0.15	0.17	0.14	0.03	0.80	0.17	0.62
<i>trans</i> -11	2.97	3.71	3.79	4.22	0.19	<0.01	0.10	0.16
<i>trans</i> -12	0.27	0.32	0.33	0.33	0.05	0.35	0.43	0.74
<i>trans</i> -13 & 14	0.66	0.87	0.93	0.95	0.06	<0.01	0.03	0.47
<i>trans</i> -15	0.32	0.39	0.40	0.43	0.03	0.01	0.13	0.29
<i>trans</i> -16	0.36	0.49	0.49	0.59	0.06	<0.01	0.48	0.34
C _{18:2}	1.18	1.45	1.53	1.90	0.09	<0.01	0.70	0.29
Total CLA	1.64	2.56	2.98	5.44	0.19	<0.01	0.04	0.07
<i>cis</i> -9, <i>trans</i> -11 + <i>cis</i> -8, <i>trans</i> -10	1.34	1.68	1.79	2.56	0.12	<0.01	0.33	0.22
<i>cis/trans</i> and <i>trans/cis</i>	0.05	0.42	0.63	1.97	0.07	<0.01	<0.01	0.03
<i>cis/cis</i>	0.07	0.14	0.17	0.32	0.01	<0.01	0.17	0.07
<i>trans/trans</i>	0.18	0.31	0.39	0.58	0.02	<0.01	0.30	0.14
C _{18:3}	0.66	0.77	0.80	0.95	0.05	<0.01	0.74	0.43
	Ratios							
C _{10:0} /C _{10:1}	8.53	11.35	13.52	24.00	1.35	<0.01	0.07	0.45
C _{14:0} /C _{14:1}	9.97	11.70	13.37	20.85	0.91	<0.01	0.04	0.54
C _{16:0} /C _{16:1}	13.79	12.75	12.82	13.21	1.36	0.80	0.48	0.77
C _{18:0} / <i>cis</i> -C _{18:1}	0.58	0.67	0.72	1.05	0.05	<0.01	0.12	0.42

¹Treatments involved 4-d abomasal infusion of 0, 20, 40, or 80 g CLA-60/d. Values presented represent averages from PM and AM milking for the last day of the infusion periods (d 4).

²Contrast = probability of a linear (Lin) or quadratic (Quad) effect of the level of CLA supplement or deviations (Dev).

CLA dose rate; when 80 g/d of CLA-60 was infused, the *cis*-8, *trans*-10, and *cis*-9, *trans*-11 C_{18:2} isomers (which co-eluted) increased by 91% over the controls. Ratios of C_{10:0} to C_{10:1}, C_{14:0} to C_{14:1}, and C_{18:0} to *cis*-C_{18:1}, but not C_{16:0} to C_{16:1}, increased ($P < 0.01$) linearly with increasing dose rate of CLA-60.

Changes in milk fat synthesis were primarily due to a reduction in the de novo synthesized fatty acids (Figure 3). The fatty acids with chain length between C₄ and C₁₅ are synthesized de novo in the mammary gland—these fatty acids accounted for 45% of the reduction in milk fatty acid yield, on a molar basis. In contrast, fatty acids with chain length of 18 carbons and greater accounted for only 25% of the reduction in milk

fatty acid yield, with the balance accounted for by C₁₆ fatty acids.

Serum NEFA levels were reduced ($P < 0.01$) in a quadratic response by CLA-60 infusion; concentrations of NEFA in serum were 13, 26, and 0% lower for the 20, 40, and 80 g/d treatments, respectively (Table 5). Serum concentrations of BOH, urea, and glucose, and plasma concentrations of insulin were all unaffected by CLA infusion (Table 5).

DISCUSSION

The synthesis of milk fat by the dairy cow is energetically expensive, requiring reducing equivalents, glyc-

Table 5. Least squares means for blood serum metabolite and plasma insulin concentrations.

Variable	CLA-60 infused (g/d) ¹				SED	Contrast ²		
	0	20	40	80		Lin	Quad	Dev
NEFA, mmol/l	0.098	0.085	0.073	0.098	0.007	0.77	<0.01	0.38
BOH, mmol/l	0.58	0.59	0.55	0.56	0.04	0.63	0.82	0.46
Urea, mmol/l	3.82	3.78	3.56	3.39	0.30	0.16	0.99	0.73
Glucose, mmol/l	3.3	3.3	3.4	3.3	0.06	0.87	0.69	0.16
Insulin, ng/ml	0.45	0.57	0.52	0.56	0.06	0.17	0.37	0.14

¹Treatments involved 4-d abomasal infusion of 0, 20, 40, or 80 g CLA-60/d. Values presented represent averages from four samples taken on the last day of the infusion periods (d-4).

²Contrast = probability of a linear (Lin) or quadratic (Quad) effect of the level of CLA supplement or deviations (Dev).

erol, acetate, BOH, and preformed fatty acids (Bauman and Davis, 1974). The burden of producing milk fat is greater when energy supply is limiting, such as in early lactation under pasture grazing systems, where pasture allowance is often restricted as a pasture management practice or where pasture availability is limited due to environmental factors (Bryant, 1990). Several dairy markets already discourage the production of milk fat relative to protein through weighted payment schemes and quotas. Collectively, these two factors provide impetus to find ways of reducing milk fat production while maintaining output of other milk components.

An abomasal infusion technique was utilized to investigate controlled milk fat depression in cows fed 100% pasture, using a commercially available CLA mixture. Although recent studies have demonstrated that it is

the *trans*-10, *cis*-12 CLA isomer, and possibly the *trans*-8, *cis*-10 CLA isomer, that reduces milk fat synthesis (Baumgard et al., 2000, 2001; Chouinard et al., 1999a, 1999b), we opted to use a mixture of CLA isomers (CLA-60) as a supplement with potential for commercial use. The dose response and time course effects of CLA infusion on milk fat concentration and yield, and DMI were also examined over the 96-h infusion period. Animal well being was maintained at all times, and no milk or blood samples were missed. Thus, we were able to complete our objectives.

The reductions in milk fat concentration and yield observed in the current study are comparable to results from studies with TMR-fed cows which received either a mixture of CLA isomers (Lor and Herbein, 1998; Chouinard et al., 1999a, 1999b; Kraft et al., 1999; Medeiros et al., 2000) or pure *trans*-10, *cis*-12 CLA supplement (Baumgard et al., 2000, 2001). Increasing doses of CLA-60 reduced milk fat concentration and yield in a linear fashion, although there was no difference between the 20 and 40 g/d treatments (Table 3; Figure 2). The 60% reduction in concentration and yield of milk fat following the 4-d abomasal infusion of 80 g of CLA-60 per day is a reduction previously matched only in the study of Chouinard et al. (1999a). Concentration and yield of milk fat did not return to pre-infusion levels until d 5 of the postinfusion period (Figure 2).

In agreement 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 to C₁₆. 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

Figure 3. Yields of fatty acids in milk produced on d 4 of a 4-d period of abomasal infusion of CLA-60. Amounts of CLA-60 infused were 0, 20, 40, and 80 g/d. Yields were derived by summing for each cow, the individual yields of each fatty acid into the following categories: sum of C₄ to C₁₅ (black bar), sum of C_{16:0} plus C_{16:1} (gray bar), sum of C₁₈ isomers (open bar). Vertical brackets represent standard errors of the means.

fat synthesis are still unknown, our results support the suggestion by Baumgard et al. (2000) that inhibition of key enzymes involved in de novo fatty acid synthesis may be involved. Infusion of CLA also increased the ratios of C_{10:0} to C_{10:1}, C_{14:0} to C_{14:1}, and C_{18:0} to *cis* C_{18:1} in milk fat (Table 4). Similar effects have been noted previously (Chouinard et al. 1999a, 1999b; Baumgard et al., 2000), and these findings suggest that the delta-9 desaturase which catalyzes the desaturation of these medium to long-chain fatty acids, was inhibited by CLA. Studies with rats have showed that a dietary CLA supplement decreased expression of mRNA and enzyme activity for hepatic delta-9 desaturase (Bretillon et al., 1999; Lee et al., 1998).

In the current experiment DMI was not affected by any CLA dose rate, suggesting at least during these short-term infusion periods, a shift in energy partitioning. This is consistent with studies where mixtures of CLA (Lor and Herbein 1998; Chouinard et al., 1999a, 1999b) or *trans*-10, *cis*-12 CLA (Baumgard et al., 2000, 2001) were infused. In contrast to these previous studies, however, milk yield in the current experiment increased during the 40 g CLA/d treatment (+11% or 2.2 kg/d). This difference may relate to the difference in diets as cows in the previous studies were fed TMR (Lor and Herbein, 1998; Chouinard et al., 1999a, 1999b; Baumgard et al., 2000, 2001) as opposed to 100% pasture as fed in the current study. Sparing energy from fat synthesis is likely to have a greater positive impact on milk production in pasture fed cows than in cows fed a more energy dense TMR which would match cow energy requirements more adequately than pasture. An increase in energy availability resulting from a constant DMI and reduced milk fat secretion (Table 3) together with a reduction in glucose oxidation for NADPH production and a sparing of glucogenic precursors such as glycerol (Bauman and Davis, 1974), may have allowed for the increased milk yield with the moderate CLA treatments (Table 3). Interestingly, while milk yield reached a maximum at a rate of 40 g/d but decreased thereafter (Table 3), serum NEFA concentrations displayed the opposite trend (Table 5). The fact that no response in milk yield was observed during the 80 g of CLA/d treatment is difficult to explain and could indicate that the optimal dose rate has been surpassed by this treatment. Given that delta-9 desaturase was impaired and milk fatty acid synthesis was reduced by 60% at this dose, it could be that the supply and pattern of fatty acids required for the normal turnover of cell membranes would be compromised.

With the exception of a study by Medeiros et al. (2000), effects of CLA on milk synthesis in cows have been specific to milk fat with no effects on milk yield or milk protein concentration and yield (Lor and Herbein,

1998; Chouinard et al., 1999a, 1999b; Baumgard et al., 2000, 2001). The consistent factor between Medeiros et al. (2000) and the current study was the pasture-based diet. Key differences in response to CLA supplementation, however, were that increases in milk protein yield in the Brazilian study arose from an increase in milk protein concentration and a constant milk yield. In the current study milk yield increased for the moderate CLA treatments but protein concentration remained constant. Thus, at the 40 g of CLA/d dose milk yield was 11% greater than the control treatment while daily yields of crude protein and casein were increased by 9% (quadratic trend where $P < 0.11$ and $P < 0.08$, respectively). This discrepancy may relate to differences between our study and that of Medeiros et al. (2000) in breed of cow, pasture type, presence or absence of a protein supplement, and the length of the treatment periods over which responses were measured.

The reduction in milk energy output during CLA supplementation might also provide substantial benefits to the cow. After accounting for the combined effects of reduced milk fat production and increased lactose production (the latter effect occurred only for the 20 and 40 g treatments), the energy required for lactation was reduced by 13, 13, and 30% for the 20, 40, and 80 g/d treatments. Given the constant DMI observed over this 4-d treatment period, this apparent net gain in energy would be expected to result in partitioning of energy to body tissue reserves. Such a scenario would be beneficial during certain times of lactation, especially for the seasonally calving, pasture-fed cow. For example, enhanced energy balance could improve reproductive performance (Senatore et al., 1996), allow pasture grazing cows to maintain production of protein during periods of feed shortage (e.g., summer dry conditions), or allow pregnant seasonally calving cows to undergo longer lactations while tissue reserves are replenished for the next season. Longer-term studies with accurate feed intake and LW measurements are required to confirm a sustained improvement in energy balance would occur over extended treatment periods, and to develop a practical means of administering CLA to grazing dairy cows on farms. Although Medeiros et al. (2000) found cows receiving rumen-protected CLA supplement and offered a diet comprising summer pasture plus a concentrate mix had reduced milk fat content and yields for several weeks, neither DMI nor liveweight were apparently measured in this study.

Future studies should also examine the efficacy of practical delivery approaches such as once or twice daily feeding of rumen-protected CLA compared to continuous infusion as used in the current study. Examination of the interactions between energy intake in the cow and milk fat reduction should also be undertaken.

CONCLUSIONS

These data clearly demonstrate that the concentration and yield of milk fat in pasture-grazing dairy cows can be markedly reduced by abomasal infusion of CLA. Inhibition of both *de novo* fatty acid synthesis and the desaturation of fatty acids was clearly evident. Although treatments were imposed for only 4 d, the reductions in milk fat and energy output combined with a constant DMI suggests an increase in energy availability to the cow for other purposes. This apparent increase in energy supply probably contributed to increased milk yield when cows received moderate CLA dose rates.

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