Effects of dietary supplementation of rumen-protected conjugated linoleic acid to grazing cows in early lactation

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ABSTRACT

Conjugated linoleic acids (CLA) are potent anti-carcinogens in animal and in vitro models as well as inhibitors of fatty acid synthesis in mammary gland, liver, and adipose tissue. Our objective was to evaluate long-term CLA supplementation of lactating dairy cows in tropical pasture on milk production and composition and residual effects posttreatment. Thirty crossbred cows grazing stargrass (*Cynodon nlemfuensis* Vanderyst var. *nlemfuensis*) were blocked by parity and received 150 g/d of a dietary fat supplement of either Ca-salts of palm oil fatty acids (control) or a mixture of Ca-salts of CLA (CLA treatment). Supplements of fatty acids were mixed with 4 kg/d of concentrate. Grazing plus supplements were estimated to provide 115% of the estimated metabolizable protein requirements from 28 to 84 d in milk (treatment period). The CLA supplement provided 15 g/d of *cis*-9,*trans*-11 and 22 g of *cis*-10,*trans*-12. Residual effects were evaluated from 85 to 112 d in milk (residual period) when cows were fed an 18% crude protein concentrate without added fat. The CLA treatment increased milk production but reduced milk fat concentration from 2.90 to 2.14% and fat production from 437 to 348 g/d. Milk protein concentration increased by 11.5% (2.79 to 3.11%) and production by 19% (422 to 504 g/d) in the cows fed CLA. The CLA treatment decreased milk energy concentration and increased milk volume, resulting in unchanged energy output. Milk production and protein concentration and production were also greater during the residual period for the CLA-treated cows. The CLA treatment reduced production of fatty acids (FA) of all chain lengths, but the larger effect was on short-chain FA, causing a shift toward a greater content of longer chain FA. The CLA treatment increased total milk CLA

content by 30% and content of the *trans*-10,*cis*-12 CLA isomer by 88%. The CLA treatment tended to decrease the number of days open, suggesting a possible effect on reproduction. Under tropical grazing conditions, in a nutritionally challenging environment, CLA-treated cows decreased milk fat content and secreted the same amount of milk energy by increasing milk volume and milk protein production.

Key words: composition, fatty acid, milk, tropical pasture

INTRODUCTION

The use of conjugated linoleic acids (CLA) as a supplement to dairy cows was initially conceived to increase its content in milk for the benefit of consumer health. The findings of Looor and Herbein (1998) and Chouinard et al. (1999) with abomasal infusion demonstrated that CLA supplements reduced milk fat yield. Subsequent work by Baumgard et al. (2000) demonstrated that the isomer involved in that effect was the *trans*-10,*cis*-12 isomer. These results suggested a great potential for the use of CLA as a tool to manipulate milk composition. The availability of mixtures of CLA isomers containing mainly *cis*-9,*trans*-11 and *trans*-10,*cis*-12 allowed the beginning of supplementation trials (Giesy et al., 2002; Bernal-Santos et al., 2003; Perfield et al., 2002). Those studies were done with high-producing cows and demonstrated decreased milk fat content but variable results regarding milk and protein yield. The high-producing cow is challenged to meet its nutritional requirements; thus, inhibition of fat secretion may have favored, in some studies (Mackle et al., 2003; De Veth et al., 2006; Kay et al., 2007), positive responses in milk and milk protein production. A similar situation may occur with dairy cows on tropical pastures, where several nutritional and climatic challenges may constrain milk production. Constraints such as availability and quality of forage, high temperatures and humidity, and greater maintenance requirement of

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Table 1. Composition of supplement¹ offered to grazing cows from 28 to 84 DIM

Ingredient	kg/d	%, DM
Corn, ground grain	2.35	58.5
Fishmeal	1.00	25
Wheat middlings	0.23	5.5
Soybean meal	0.22	5.5
Premix ²	0.20	5.5
Total	4.00	100.0

¹Fatty acid composition of the supplement (g/100 g of fat): palmitic acid: 22.74; stearic acid: 6.50; oleic acid: 27.45; linoleic acid: 33.92; linolenic acid: 1.35.

²Contained per kilogram: Ca, 187.5 g; P, 97.5 g; Mg, 22.5 g; S, 75 g; Na, 135 g; Cu, 1,200 mg; Fe, 1,500 mg; Mn, 1,500 mg; Zn, 2,250 mg; Co, 45 mg; I, 90 mg; Se, 38 mg; vitamin A, 100,000 IU; vitamin D, 30,000 IU; vitamin E, 500 IU.

cows because of physical activity can hinder the ability to achieve milk production potential. The ability to control the energetic density of milk could be used to reach greater milk volume production. The objective of this work was to determine the effects of CLA supplementation on milk production, milk composition, and fatty acid profile of cows grazing tropical forages.

MATERIALS AND METHODS

Animals, Management, and Treatments

The experiment was conducted with cows grazing stargrass (*Cynodon nlemfuensis* Vanderyst var. *nlemfuensis*) at Santa Monica Experimental Station (43°42'W; 22°21'S; altitude, 364 m above sea level) at Embrapa Dairy Cattle, Valença, Rio de Janeiro, Brazil. The cows were on a rotational grazing system consisting of 14 paddocks (0.5 ha/each). The grazing period was 2 d per paddock and the grazing cycle was 28 d. The pasture was sampled by simulated grazing and the mean nutritive value on a DM basis was CP, 13.3%; ether extract, 1.2%; NDF, 61.4%; sulfuric acid lignin, 3.2%; and ash, 9.1%.

Thirty crossbred Holstein × Zebu cows in early lactation received a dietary fat supplement of either Ca-salts of palm oil fatty acids (Megalac, Dwight & Church Co. Inc., Princeton, NJ; control treatment) or a mixture of Ca-salts of CLA (CLA treatment). The concentrate was formulated (Table 1) to supplement the estimated ingested pasture so that the diet would provide 115% of the estimated MP requirements (NRC, 2001). To formulate the supplements, the predicted DMI was 13.3 kg of DM/d with pasture intake at 9.7 kg of DM/d. Concentrate was supplemented to exceed the requirements for vitamins, macro-, and microelements (NRC, 2001). Concentrates were offered twice daily after each milking in individual recipients with the cows restrained

Table 2. Nutritional analysis from pasture and of the supplements during the treatment period (28 to 84 DIM) and residual period (85 to 112 DIM)¹

Chemical analysis	Pasture	Treatment period	Residual period
DM, %	30.9	89.6	88.2
CP, % of DM	13.3	24.1	17.9
Ether extract, % of DM	1.7	2.9	2.6
Ash, % of DM	9.1	14.4	14.1
NE _L , Mcal/kg of DM	1.29	1.95	1.60

¹Cows received 150 g/d of dietary fat supplement (top-dressed) consisting of Ca-salts of palm oil fatty acids (control treatment) or Ca-salts of CLA-60 (Natural Lipids Ltd., Hovdebygda, Norway).

in tie stalls until the supplements were completely consumed.

Cows were blocked by previous milk production and parity into 2 treatment groups. From 28 to 84 DIM they received supplements of either 150 g of Ca-salts of palm oil (control) or 150 g of Ca-salts of CLA.

In the CLA supplement, 65% of the lipid used in the Ca-salt formation (Church & Dwight Co. Inc.) 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% *trans*-8,*cis*-10, 17% *cis*-11,*trans*-13, and 9% other. Therefore, approximately 63 g of CLA-60 or 22 g of *trans*-10,*cis*-12 was fed daily considering that the calcium salts of CLA protected for this experiment had 70% as fatty acids, once 30% were measured as ash.

The residual effect of CLA was also evaluated once the supplementation of Ca-salts was discontinued at 84 DIM and the concentrate was replaced by a commercial concentrate based on wheat middlings from 85 to 112 DIM. The period from 28 to 84 DIM was considered the treatment period and that from 85 to 112 DIM the residual period. The chemical composition of concentrates from both periods is presented in Table 2.

Milk Production and Composition

Cows were milked twice daily (0600 and 1400 h), and yields were recorded at each milking from 28 to 114 DIM. Milk samples were collected 3 d a week (Monday, Thursday, and Saturday) from each milking and pooled by day of collection proportionally to yield. Samples were stored at 4°C with a preservative (bromopol tablet; D&F Control System, San Ramon, CA) until analyzed for fat, total protein, lactose, and total solids using infrared analysis (AOAC, 2000; method no. 972.160) with the analytical and calibration methods described by Bernal-Santos et al. (2003). Additional milk samples

were taken weekly (Friday) from each cow to evaluate MUN. Milk was also sampled for 5 consecutive days from 42 to 47 DIM (period 1) and from 70 to 74 DIM (period 2) within the treatment period, and another similar collection period in the middle of the residual period from 92 to 97 DIM and stored at -20°C without preservative until analyzed for fatty acids.

Blood was collected weekly via venipuncture from the coccygeal vessels following the afternoon milking and before providing the concentrate. Coagulation was prevented by EDTA; plasma was harvested following centrifugation ($2,800 \times g$, 15 min) and stored at -20°C until analysis for blood metabolites.

Body weights were determined weekly as the mean of 3 consecutive days, and BCS was determined weekly throughout the study as the average of scores given by 2 individuals. Cows were body scored on a 9-point system (NRC, 1996).

Fatty Acid Analysis

Milk fat (400 mg) was obtained after milk centrifugation ($17,800 \times g$, 30 min at 8°C). Lipid extraction was performed according to Hara and Radin (1978), and methylation was performed using a methanolic solution of sodium methoxide, as described by Christie (1982), with modifications by Chouinard et al. (1999). The fatty acid composition was determined by gas chromatography with a fused silica capillary column (SP-2560, $100 \text{ m} \times 0.25 \text{ mm} \times 0.2 \mu\text{m}$; Supelco, Bellefonte, PA) and a flame-ionization detector. A gradient temperature program was used, and total time for each run was 70 min. Injector temperature was 250°C and detector temperature was 300°C ; injection was via split mode (21:1). Carrier gas was helium (40 mL/min and 124 kPa on the column head). The CRM-164 (Commission of the European Communities, Community Bureau of Reference, Brussels, Belgium) was used as the standard to establish correction factors for each of the certified fatty acids, converting the percentage of area to weight (mg/g of total fatty acids).

MUN and Plasma Metabolite Analysis

Milk urea nitrogen analysis was done by an enzymatic method (Ureia Enz-Color, Laborclin, Curitiba, Parana, Brazil). A pooled sample was used as a control to inter-batch variability.

Plasma was analyzed for NEFA, triglyceride (TG), total cholesterol, and glucose. Concentrations of NEFA were determined using a colorimetric-enzymatic method (NEFA C, Wako Pure Chemical Industries, Osaka, Japan) and all samples were analyzed ($n = 360$). Concentrations of TG and glucose were analyzed

for plasma samples on DIM 28, 42, 56, and 70 in the treatment period and all 4 wk of the residual period. Cholesterol was assessed for plasma on samples similar to those used for TG and glucose in the treatment period, and at 91 and 105 DIM in the residual period using a colorimetric-enzymatic method (Cholesterol Enz-Color, Biodiagnostica, Curitiba, Parana, Brazil). Glucose determination was done by direct reading (glucose oxidase enzyme immobilized in membrane) on an YSI 2700 Select autoanalyzer (Biochemistry Analyzer, Yellow Springs, OH). Triglycerides were analyzed by an enzymatic method (TG Enz-Color, Biodiagnostica)

Statistical Analysis

For milk production, milk composition, BW, BCS, and metabolic parameters (MUN, NEFA, TG, cholesterol, and glucose), analyses were performed using the MIXED procedure of SAS (SAS Institute, 2000) for the treatment period and the residual period. The model included fixed effects of block, treatment, week, and treatment by week interaction, and a random effect of cow.

Fatty acid data were analyzed as a split-plot design through the GLM procedure of SAS (SAS Institute, 2000). The persistence of lactation was compared through the confidence interval test from the difference of the slope coefficient of the regressions as suggested by Neter et al. (1996). Standard error of the means are reported, and differences were considered significant when $P < 0.05$ unless otherwise stated.

RESULTS AND DISCUSSION

Milk Production and Composition

Cows supplemented with CLA had greater milk yield compared with control animals ($P = 0.06$) during the treatment period (Tables 3 and 4). There was no treatment by time interaction for milk yield. Milk fat concentration was depressed ($\sim 26\%$) in the treatment period (Tables 3 and 4) because of CLA supplementation ($P < 0.0001$). This effect was observed within 1 wk of treatment (Figure 1), when cows were about 30 DIM, and lasted until wk 1 of the residual period. The reduction in milk fat yield and concentration are in accordance with studies using pure CLA isomers, which demonstrated that the *trans*-10,*cis*-12 isomer reduced milk fat synthesis (Baumgard et al., 2000). Other CLA isomers, notably *trans*-9,*cis*-11 and *cis*-10,*trans*-12, have been associated with this effect (Harvatine et al., 2009).

The reduction in milk fat concentration was observed within 1 wk of treatment (Figure 2) when cows were

Table 3. Milk yield and composition, BW, and BCS of cows receiving rumen-protected fat supplements in the treatment period

Variable	Treatment ¹		SEM	Treatment	Time × treatment
	Control	CLA			
Milk yield, kg/d	15.2	16.3	0.16	0.056	0.861
Milk fat, %	2.90	2.14	0.037	<0.0001	<0.0001
Milk fat yield, g/d	437	348	6.1	0.002	0.010
Milk protein, %	2.79	3.11	0.018	0.001	0.005
Milk protein yield, g/d	422	504	5.1	0.001	0.353
Milk lactose, %	4.56	4.46	0.017	0.196	<0.0001
Milk lactose yield, g/d	692	726	7.8	0.277	0.849
Milk total solids, %	10.98	10.56	0.05	0.058	0.045
Milk total solids yield, g/d	1.662	1.716	17.4	0.494	0.799
Milk energy output, MJ/d	37.8	36.9	1.12	0.613	0.391
Milk energy concentration, MJ/kg	2.500	2.270	0.015	0.0004	<0.0001
BW, ² kg	439	451	8.6	0.498	0.548
BCS ³	3.7	3.9	0.12	0.417	0.204

¹Cows (n = 15) received a dietary fat supplement consisting of Ca-salts of palm oil fatty acids (control) or Ca-salts of a mixture of conjugated linoleic acid isomers from 28 to 84 DIM (CLA).

²Cows were weighed 3 d/wk throughout the study.

³Cows were scored (9-point scale) by 2 individuals 1 d/wk throughout the study, and average values were used.

about 30 DIM. Other studies show that the response to CLA inducing milk fat depression (MFD) occurred after several weeks of lactation, which was attributed to a possible attenuation of the cellular signaling to CLA *trans*-10, *cis*-12 at the onset of lactation (Bernal-Santos et al., 2003; Castañeda-Gutiérrez et al., 2005). In accordance with that, Moore et al. (2004) worked with periparturient cows (−10 to 21 d relative to calving) and observed that a higher CLA dose was required to reduce milk fat in early lactation. A greater relative

importance of de novo fat synthesis (i.e., from acetate) in the mammary gland for the low-producing cows of the present experiment, compared with high-producing cows in early lactation, may also explain the lower doses of CLA necessary to reduce milk fat content.

Milk fat yield was diminished by 20% in the treatment period for cows fed CLA. The intensity of MFD was similar to that obtained by Chouinard et al. (1999) when infusing 28 g/d of CLA-60 in the abomasum, but a simple comparison might be inadequate to estimate

Table 4. Milk yield and composition, BW, and BCS of cows from 85 to 112 DIM (residual period¹) after receiving rumen-protected fat supplements from 28 to 84 DIM (treatment period)

Variable	Treatment ²		SEM	Treatment	Time × treatment
	Control	CLA			
Milk yield, kg/d	11.8	13.1	0.19	0.017	0.720
Milk fat, %	3.29	3.17	0.06	0.268	0.007
Milk fat yield, g/d	387	411	8.0	0.311	0.001
Milk protein, %	2.84	3.05	0.02	0.017	0.001
Milk protein yield, g/d	335	398	6.3	0.006	0.266
Milk lactose, %	4.44	4.39	0.03	0.563	0.002
Milk lactose yield, g/d	526	574	9.4	0.082	0.395
Milk total solids, %	11.26	11.42	0.08	0.552	0.015
Milk total solids yield, g/d	1.605	1.759	33.6	0.070	0.378
Milk energy output, MJ/d	31.2	34.3	0.54	0.088	0.041
Milk energy concentration, MJ/kg	2.643	2.634	0.03	0.899	0.029
BW, ³ kg	440	457	9	0.372	0.972
BCS ⁴	3.7	3.9	0.1116	0.160	0.826

¹Cows (n = 15) received a dietary commercial diet.

²During the treatment period, cows (n = 15) received a dietary fat supplement consisting of Ca-salts of palm oil fatty acids (control) or Ca-salts of a mixture of conjugated linoleic acid isomers from 28 to 84 DIM (CLA).

³Cows were weighed 3 d/wk throughout the study.

⁴Cows were scored (9-point scale) by 2 individuals 1 d/wk throughout the study, and average values were used.

Figure 1. Dispersion of data and regressions of milk yield (kg/d) for cows fed a rumen-protected fat supplement with (CLA, ○) or without conjugated linoleic acid (control, ■) from 28 to 84 DIM (treatment period) and a low-fat supplement without CLA from 85 to 112 DIM (residual period).

the amount of CLA reaching the abomasum of the grazing cows in this work, as control cows already had milk fat content lower than usual for grazing cows of similar genetics; therefore, less CLA may be necessary to achieve the same effect. An explanation for the depressed milk fat observed in the control cows could be the inclusion of fishmeal in the concentrate. The 60 to 70 g of fishmeal oil present in the diet would be enough to inhibit milk fat synthesis (Chilliard et al., 2001; Gama et al., 2008).

Milk protein content for CLA-treated cows was about 11% greater ($P = 0.001$) in the treatment period. The greater protein content with the greater milk yield in the CLA-fed cows resulted in increased (19.4%) milk protein production compared with controls ($P = 0.002$). The increase in milk protein production could be the result of the relatively higher MP of the diet, which may have provided the amino acids demanded by the CLA-treated cows. It could also be hypothesized that protein synthesis was depressed by an energy deficit in the control cows and that reduction in milk fat energy secretion allowed spared energy to be used for protein synthesis in the mammary gland. As pointed out by Perfield et al. (2002), there are several studies in which CLA was supplemented to cows in established lactations without any change in milk yield or milk protein content. This supports the idea that for those changes to occur, animals may need to be in negative energy balance. Mackle et al. (2003) infused a CLA mixture containing almost 60% of CLA isomers in increasing

amounts and, although milk protein content was unchanged, protein production tended to increase at the 40 g/d dose. It can also be hypothesized that the better amino acid profile of the fishmeal could have helped in the achievement of the greater milk content and yield in our experiment.

There was no treatment effect for lactose. The greater osmotic effect because of the greater protein content of CLA milk and the high stability of milk osmolality (Jenness, 1985) could have had an effect on lactose, but no difference was observed, in accordance with the usually observed constancy of lactose content (Sutton, 1989; Kennelly, 1996). The large MFD resulted in greater total solids content for the control treatment ($P = 0.058$), but the difference was small because of the greater protein content of the CLA-treated animals that partly compensated for the lower fat production.

Milk energy density (Tables 3 and 4) was altered by treatment, and CLA-treated cows produced less energy-dense milk ($P < 0.001$). The total amount of energy secreted as milk was unchanged by CLA supplementation. There was also no difference in total DMI for the treatments, as determined by n-alkane technique in an accompanying experiment (Oliveira, 2003): 15.5 vs. 15.0 kg/d for control and CLA treatments, respectively ($P = 0.4588$). The average metabolizable energy of the forage was estimated at 2.0 Mcal/kg of DM (Oliveira, 2003). Therefore, in response to less energy-dense milk from the CLA-treated cows, there was clearly an appropriate increase in milk volume to reach the total

milk energy output potential of that pasture system of production, around 37 to 38 MJ/d (Table 3).

In the residual period, cows that were fed CLA during the treatment period produced 11% more milk than those that received Megalac (control treatment). The CLA-treated cows were able to maintain greater milk production even with poorer nutrient availability, because the residual period occurred in late summer, when pasture growth was decreasing and the capacity of selection by the cows was diminished. One explanation for the continued effect on milk production would be a higher peak of lactation for the CLA-treated cows and a resultant increase in persistency. It is interesting to note that the classic relation of 200 kg of additional milk to each additional 1 kg of milk in the peak of lactation might be independent from milk composition. The dispersion of the data of milk yield throughout the experimental period is presented in Figure 1. By comparing the slopes of the lines, it was determined that the persistency was not different between treatments (-0.0693 ± 0.0025 vs. -0.0736 ± 0.0021).

The mean milk fat content was not different between treatments in the residual period (Table 4), but there was a treatment by time interaction, as the values in the first week of the residual period were different between treatments, with values of 3.09 and 2.52%, respectively for control and CLA cows (Figure 2). These results were expected because CLA acts to inhibit lipogenic enzymes, and the reduced lipogenesis will last until the normal enzyme abundance is achieved again. Therefore, restoration of normal milk fat synthesis takes a few days to occur. There was no longer a difference between treatments in the second week of the residual period.

Protein content was still higher for CLA-treated cows during the residual period ($P = 0.017$) with a significant interaction of time by treatment: during the first week (wk 9), protein for the CLA treatment was higher (3.16 vs. 2.83). Nevertheless, the values for the final 3 wk of the residual period were not statistically different. This pattern was similar to that of fat content in the same period, which indicates that the increase in protein is an episode dependent of the fat reduction, as pointed out by Perfield et al. (2002). Interestingly, in the present work, there was no indication of change in energy balance due to CLA treatment.

Milk energy density (MJ/kg) was almost identical during the residual period once milk composition, except for protein in the first week, was the same. Net energy output (MJ/d), as in the treatment period, was not different, but tended to be greater for CLA cows ($P = 0.088$) as milk fat content was already similar, but production was still greater for CLA-treated cows.

There was a residual effect of CLA supplementation for protein production ($P = 0.006$) as a result of greater

Figure 2. Temporal pattern of milk fat content (A) and milk fat yield (B) for cows fed a rumen-protected fat supplement with (CLA, ♦) or without conjugated linoleic acid (control, ■) from 28 to 84 d (treatment period) and a commercial concentrate without CLA from 85 to 112 d (residual period).

protein content and milk volume. This result is mostly because of the greater protein content in the first week of the residual period, which would probably disappear if the investigation of the residual period continued further. In addition, as lactation proceeds, it is easier for the cow to be in a more positive energy balance, theoretically changing the response of the cow to CLA.

There was no effect of CLA supplementation on BW and BCS, and those variables were kept constant throughout the experiment (Tables 3 and 4). The absence of recovery of BCS is consistent with an energy-restricted environment. In fact, the restriction in nutritional resources was already clearly indicated by the increased milk production in response to CLA treatment. Some authors suggest that CLA could inhibit fat synthesis in adipose tissue; however, the CLA concentration required to affect mammary lipogenesis

Table 5. Fatty acid composition (g/100 g) of milk fat from cows receiving rumen-protected fat supplements from 28 to 84 DIM (treatment period) and without this supplement from 85 to 112 DIM (residual period)¹

Fatty acid ²	Treatment period 1 ³			Treatment period 2 ³			Residual period			SE
	Control	CLA	<i>P</i> -value	Control	CLA	<i>P</i> -value	Control	CLA	<i>P</i> -value	
C 4:0	3.59	2.65	**	3.28	2.19	**	3.69	3.52	NS	0.06
C 6:0	1.93	1.22	**	1.95	1.17	**	2.07	1.97	NS	0.05
C 8:0	0.98	0.59	**	1.03	0.60	**	1.07	1.01	NS	0.03
C 10:0	1.84	1.25	**	2.02	1.32	**	2.07	1.98	NS	0.56
C 12:0	2.10	1.77	*	2.39	1.96	*	2.42	2.37	NS	0.06
C 14:0	7.71	7.51	NS	8.67	8.47	NS	9.64	9.51	NS	0.17
C 14:1	0.86	0.85	NS	0.96	0.99	NS	1.29	1.35	NS	0.03
C 15:0	0.96	1.13	*	1.07	1.13	†	1.18	1.15	NS	0.02
C 16:0	26.34	25.44	**	26.90	27.00	NS	25.65	26.78	*	0.17
C 16:1 <i>c</i> 9	1.39	1.47	NS	1.26	1.58	*	1.37	1.59	*	0.03
C 18:0	10.73	11.07	NS	12.16	12.21	NS	11.94	11.21	NS	0.18
C 18:1 <i>c</i> 9	28.33	29.43	NS	24.68	26.13	NS	27.12	27.26	NS	0.34
C 18:1 <i>c</i> 11	1.77	1.85	†	1.56	1.52	NS	1.23	1.13	NS	0.03
C 18:1 <i>t</i> 9	1.29	1.35	†	1.34	1.27	NS	0.98	0.97	NS	0.08
C 18:1 <i>t</i> 10	0.77	0.85	NS	0.77	0.72	NS	0.45	0.52	NS	0.08
C 18:1 <i>t</i> 11	4.52	4.73	NS	4.32	4.16	NS	4.37	4.49	NS	0.08
C 18:1 <i>t</i> 12	0.88	0.96	NS	0.71	0.67	NS	0.45	0.80	NS	0.006
C 18:1 <i>t</i> 16	0.74	0.84	*	0.53	0.58	*	0.36	0.35	NS	0.02
C 18:2 <i>t</i> n-6	0.11	0.14	**	0.10	0.11	**	0.10	0.11	NS	0.02
C18:2 <i>c</i> n-6	1.73	1.79	NS	1.37	1.42	NS	1.51	1.52	NS	0.04
C 18:3 n-3	0.41	0.44	*	0.41	0.42	NS	0.50	0.51	NS	0.08
CLA ⁴	1.75	2.28	**	1.34	1.74	**	1.77	1.92	NS	0.05
CLA <i>c</i> 9, <i>t</i> 11	1.45	1.72	**	1.11	1.25	NS	1.58	1.64	NS	0.04
CLA <i>t</i> 10, <i>c</i> 12	0.04	0.08	**	0.03	0.05	**	0.03	0.03	NS	0.002
C 20:4 n-6	0.08	0.06	*	0.07	0.06	**	0.05	0.06	NS	0.002
C 20:4 n-3	0.04	0.04	NS	0.08	0.08	NS	0.03	0.03	NS	0.003
C 20:5 n-3	0.04	0.03	**	0.03	0.02	**	0.03	0.03	NS	0.001
C 22:5 n-3	0.11	0.11	NS	0.10	0.10	NS	0.12	0.13	NS	0.011
Summation										
<C12	10.44	7.48	**	10.67	7.24	**	11.32	10.85	NS	0.22
C14 to C16	35.87	27.42	*	37.60	37.59	NS	37.60	38.79	NS	0.30
>C18	44.82	42.01	NS	42.01	40.06	NS	44.68	42.82	NS	0.43

¹Cows received a supplement consisting of Ca-salts of palm oil fatty acids (control) or Ca-salts of a mixture of conjugated linoleic acid isomers (CLA). Both treatments provided about 105 g/d of fatty acids.

²*c* = *cis*; *t* = *trans*.

³Treatment period 1: milk collected from d 42 to 47; treatment period 2: milk collected from d 70 to 74.

⁴Sum of *cis*-9,*trans*-11, *trans*-10,*cis*-12, and other unrecognized fatty acids.

P* < 0.05; *P* < 0.01; †*P* < 0.10.

is much lower than that needed to inhibit lipogenesis in adipose tissue (Bauman et al., 2008).

Milk Fatty Acid Composition and Yield

Among all fatty acid chain lengths, there was a greater reduction in short-chain fatty acids in milk fat of the CLA-treated group (*P* < 0.001; Table 5). This reduction (Table 6) was consistent with the proposed CLA action in lipid metabolism (Bauman and Griinari, 2003; Bauman et al., 2008; Harvatine et al., 2009) because CLA acts on many lipogenic enzymes, like acetyl-CoA-carboxylase and fatty acid synthase (Piperova et al., 2000; Hayashi et al., 2007), although there are also effects on enzymes involved in long-chain fatty acid metabolism (Bauman et al., 2008).

Palmitic acid (C16:0) concentration for the CLA cows was lower (*P* = 0.0023) in the first period of sampling (42–47 DIM), but not (*P* = 0.8098) in the second one (70–74 DIM; Table 5). As C16:0 in milk can come from dietary fat and tissue mobilization or from de novo synthesis, this suggests a shift in the relative importance of those sources due to lactation stage; that is, de novo synthesis would proportionally decrease its participation as milk yield is less demanding. In the first period of sampling (42–47 DIM), cows were producing 15 to 18 kg of milk, whereas in the second period (70–74 DIM), cows were producing 13 to 16 kg. Nevertheless, the difference between fatty acids with <16 carbons in the first and second periods of sampling was small, with the recovery of C16:0 content occurring by the time of the second sampling.

Table 6. Yield of fatty acids (g/d) from cows receiving rumen-protected fat supplements from 28 to 84 DIM (treatment period) and without this supplement from 85 to 112 DIM (residual period)¹

Fatty acid ²	Treatment period 1 ³			Treatment period 2 ³			Residual period			SE
	Control	CLA	P-value	Control	CLA	P-value	Control	CLA	P-value	
C 4:0	15.7	11.5	**	14.0	7.1	**	14.5	15.5	NS	3.6
C 6:0	8.5	5.3	**	8.4	3.7	**	8.2	8.7	NS	2.3
C 8:0	4.4	2.6	**	4.5	1.9	**	4.3	4.5	NS	1.4
C 10:0	8.2	5.4	**	8.8	4.2	**	8.2	8.8	NS	2.7
C 12:0	9.3	7.5	*	10.4	6.4	**	9.6	10.5	NS	2.8
C 14:0	33.5	31.6	NS	37.3	27.9	*	38.0	41.9	NS	7.9
C 14:1	3.8	3.5	NS	4.1	3.3	NS	5.0	5.9	NS	1.3
C 15:0	4.1	4.8	*	4.6	3.8	†	4.6	5.0	NS	0.7
C 16:0	113.5	109.9	**	114.6	89.9	*	101.0	116.7	NS	15.0
C 16:1 <i>c</i> 9	6.0	6.2	NS	5.4	5.3	NS	5.4	6.9	†	1.3
C 18:0	46.3	48.1	NS	51.8	39.3	*	46.6	48.6	NS	8.4
C 18:1 <i>c</i> 9	122.5	127.6	NS	105.2	85.7	*	105.0	118.2	NS	16.9
C 18:1 <i>c</i> 11	7.7	8.0	†	6.5	5.0	**	4.8	4.9	NS	0.9
C 18:1 <i>t</i> 9	5.6	5.9	†	5.8	4.2	**	3.8	4.2	NS	2.9
C 18:1 <i>t</i> 10	3.3	3.7	NS	3.4	2.4	*	1.8	2.25	†	0.3
C 18:1 <i>t</i> 11	19.4	20.5	NS	18.6	13.55	**	17.4	19.6	NS	2.7
C 18:1 <i>t</i> 12	3.7	4.2	NS	3.1	2.3	*	1.8	3.4	NS	0.2
C 18:1 <i>t</i> 16	3.2	3.6	**	2.3	1.9	NS	1.4	1.5	NS	0.5
C 18:2 <i>t</i> n-6	0.5	0.6	**	0.4	0.4	NS	0.4	0.5	NS	0.1
C 18:2 <i>c</i> n-6	7.4	7.8	NS	5.8	4.7	*	6.1	6.6	NS	1.3
C 18:3 n-3	1.7	1.9	*	1.8	1.4	*	2.0	2.2	NS	0.4
CLA ⁴	7.5	9.8	**	5.7	5.8	NS	7.0	8.4	NS	1.6
CLA <i>c</i> 9, <i>t</i> 11	6.2	7.4	**	4.8	4.2	NS	6.3	7.2	NS	1.5
CLA <i>t</i> 10, <i>c</i> 12	0.2	0.3	**	0.1	0.1	*	0.1	0.1	NS	0.1
C 20:4 n-6	0.3	0.3	*	0.3	0.2	**	0.2	0.3	†	0.1
C 20:1	0.5	0.6	*	0.9	0.7	NS	0.8	1.0	NS	0.4
C 20:4 n-3	0.2	0.2	NS	0.4	0.2	*	0.1	0.1	NS	0.1
C 20:5 n-3	0.2	0.1	**	0.1	0.1	**	0.1	0.1	NS	0.1
C 22:6 n-3	0.5	0.5	**	0.3	0.2	*	0.1	0.2	NS	0.1

¹Cows received dietary supplement consisting of Ca-salts of palm oil fatty acids (control) or Ca-salts of a mixture of conjugated linoleic acid isomers (CLA). Both treatments provided about 105 g/d of fatty acids.

²*c* = *cis*; *t* = *trans*.

³Treatment period 1: milk collected from d 42 to 47; treatment period 2: milk collected from d 70 to 74.

⁴Sum of *cis*-9,*trans*-11, *trans*-10,*cis*-12, and other unrecognized fatty acids.

P* < 0.05; *P* < 0.01; †*P* < 0.10.

The CLA in milk fat originates mainly from endogenous synthesis involving the precursor 18:1 *trans*-11 and mammary Δ^9 -desaturase (Bauman and Lock, 2006). The reduction in CLA concentration (Table 5) observed from the first (42–47 DIM) to the second period (70–74 DIM) of milk sampling was possibly related to changes in the biohydrogenation pathway, with reductions in CLA precursors (18:1 *trans*-11; vaccenic acid). Consistent with that, there was a decrease in 18:1 *trans*-11 from the first to the second period (4.73 vs. 4.32%), and the same occurred for 18:1 *trans*-10 (0.85 vs. 0.72%). The opposite occurred with CLA *trans*-10,*cis*-12, which increased in the same comparison.

For total CLA yield, the greater concentration in the CLA-supplemented animals (Table 5) was not enough to compensate for the MFD, such that there was no difference in the total CLA yield in the second period (Table 6). However, in the first period, CLA yield was 32% greater for CLA-treated compared with control

cows. The yield of *trans*-10,*cis*-12 was higher for CLA-treated cows in both periods, but the difference was lower in the second period (*P* < 0.05) compared with the first (*P* < 0.001). The recovery of *trans*-10,*cis*-12 in milk of animals receiving CLA-60 of approximately 0.2 g/d represents less than 1% of estimated *trans*-10,*cis*-12 offered, suggesting that protection was not very effective. This was expected given that CLA-60 is rich in polyunsaturated fatty acids, which are less efficiently protected from biohydrogenation (Sukhija and Palmquist, 1990).

Secretion of *cis*-9,*trans*-11 (rumenic acid) was significantly decreased in the milk of CLA-treated animals in the second period of collection compared with the first period. In the second period, there was no difference between the CLA-treated cows and the control cows for rumenic acid yield (Table 6). This finding results from both a smaller difference in content of this isomer and a less pronounced difference in the total fat yield in the

Table 7. Desaturase index from cows during CLA treatment from 28 to 84 DIM (treatment period) and after CLA treatment from 85 to 112 DIM (residual period)¹

Fatty acid	Treatment period 1 ²			Treatment period 2 ²			Residual period			SE
	Control	CLA	<i>P</i> -value	Control	CLA	<i>P</i> -value	Control	CLA	<i>P</i> -value	
C14:1/C14:0	0.11	0.11	NS	0.11	0.12	NS	0.13	0.14	NS	0.02
C16:1/C16:0	0.05	0.06	NS	0.04	0.06	*	0.05	0.06	NS	0.01
C18:1/C18:0	3.37	3.41	NS	2.66	2.75	NS	2.93	3.16	NS	0.42

¹Cows received a dietary fat supplement consisting of Ca-salts of palm oil fatty acids (control) or Ca-salts of a mixture of conjugated linoleic acid isomers (CLA). Both treatments provided about 105 g/d of fatty acids.

²Treatment period 1: milk collected from d 42 to 47; treatment period 2: milk collected from d 70 to 74.

**P* < 0.05.

second period. This difference may be partly related to changes in the fatty acid profile of the pasture throughout the year, as reported by Auldist et al. (2002).

For fatty acid content in the residual period, there were only minor differences or tendencies (*P* < 0.10) between treatments for C16:1, C18:1 *trans*-10, and C20:4 n-6 (Table 5). For all other fatty acids, differences ceased after the end of treatment (*P* > 0.10).

Considering both sampling periods and all C18:1 isomers, only C18:1 *trans*-16 was higher for CLA-supplemented cows within the treatment period (Table 5). The C18:1 *cis*-9 isomer is the main unsaturated fatty acid in milk and was not affected by treatment during the whole experiment.

The reason for the concentration of several isomers >18 carbons being higher for CLA-supplemented cows (Table 5) could be the lower rate of de novo synthesis, which consequently diminished short-chain fatty acids with a proportional increase in long-chain fatty acids, as observed by many researchers (Baumgard et al., 2000; Perfield et al., 2006; Harvatine et al., 2009). It could also be because of fatty acid dissociation and biohydrogenation of the protected fat supplements in the rumen due to the less effective protection of polyunsaturated fatty acid calcium salts mentioned above.

Activity of Δ^9 -desaturase can be estimated through the relationship between unsaturated products and their saturated precursors (Table 7). The absence of the expected difference between monounsaturated fatty acids because of CLA inhibition of Δ^9 -desaturase could be explained by the presence of C20:5 in the fishmeal oil (Chilliard et al., 2001). This fatty acid, as well as other polyunsaturated fatty acids in fish oil, may inhibit de novo synthesis and Δ^9 -desaturase, but as indicated by the present results, to a lesser extent than *trans*-10,*cis*-12 (Xu et al., 2001). Differences were not significant, except for C16:1/16:0 in collection period 2; however, the effect was in the opposite direction from what would be expected for MFD. Nevertheless, the effect of reduced activity of Δ^9 -desaturase and MFD are probably overrated, as shown by Perfield et al. (2006)

who demonstrated a large reduction in this enzyme caused by *trans*-10,*trans*-12 CLA with no decrease in fat yield. Harvatine et al. (2009) noted that there are few biologically active isomers identified that are capable of reducing milk fat (doses of less than 10 g/d can decrease fat content by half), and it would be reasonable to accept that additional unidentified biohydrogenation intermediates could also be involved.

A regression between milk fat concentration and *trans*-10,*cis*-12 isomer using data from the treatment period shows that the *trans*-10,*cis*-12 concentration in milk increased while the milk fat percentage decreased (Figure 3), similarly to studies involving abomasal infusion of *trans*-10,*cis*-12 CLA as summarized by de Veth et al. (2004). The values agree with other trials conducted with higher producing cows.

Plasma Metabolites and MUN

Plasma concentrations of NEFA tended to be greater for the control treatment (*P* = 0.0991) during the treatment period. Concentrations of NEFA (Table 8) were lower than or equal to values normally observed in high-producing animals (Garcia-Bojalil et al., 1998; Knowlton et al., 1998; Putnam and Varga, 1998), but near those obtained by Sutton et al. (1998) with cows of similar production potential to the animals used in the present experiment. The tendency for greater NEFA in control cows (*P* = 0.099) compared with CLA-treated cows suggests greater mobilization of body reserves, consistent with greater long-chain fatty acid secretion (Castañeda-Gutiérrez et al., 2005). A review of studies involving CLA supplements or infusion of *trans*-10,*cis*-12 CLA indicates little or no effect on plasma variables (Bauman et al., 2008; Harvatine et al., 2009). The highest values for NEFA were obtained in the second week sampling: 323 versus 228 $\mu\text{mol/L}$ for control and CLA, respectively. Concentrations of NEFA suggest that negative energy balance was not pronounced in either treatment.

Table 8. Plasma metabolites and MUN of cows receiving rumen-protected fat supplements from 28 to 84 DIM (treatment period) and without this supplement from 85 to 112 DIM (residual period)¹

Variable	Treatment (Trt)		SEM	P-value	
	Control	CLA		Trt	Trt × time
Treatment period					
NEFA, μmol/L	224	176	11.0	0.0991	0.6611
Triglyceride, mg/dL	21.8	24.3	0.83	0.1923	<0.0001
MUN, mg/dL	19.3	19.7	0.40	0.5162	0.9225
Residual period					
NEFA, μmol/L	148	132	10.5	0.3782	0.1703
Triglyceride, mg/dL	19.2	27.5	0.75	<0.0001	<0.0001
MUN, mg/dL	17.6	18.3	0.50	0.4365	0.9790

¹Cows received a dietary fat supplement consisting of Ca-salts of palm oil fatty acids (control) or Ca-salts of a mixture of conjugated linoleic acid isomers (CLA). Both treatments provided about 105 g/d of fatty acids.

Differences were not observed during the treatment period for blood TG values although the treatment × time interaction was significant ($P < 0.0001$). During the residual period, means were different (19.2 and 27.5 mg/dL for control and CLA treatments, respectively; $P < 0.001$).

There were no treatment effects on MUN, plasma cholesterol, or glucose. The high MUN concentrations are in accordance with the level of protein in the diet. Milk protein content measured at the same time as MUN sampling was 3.10 and 2.82% for CLA and control cows, respectively. This result indicates that the

increase could be due to true protein synthesis and not merely by urea translocating from the blood to the milk. Perfield et al. (2002) did not observe any changes in the proportions of different nitrogen and protein fractions in milk from CLA-treated cows.

Reproductive Data

Reproductive data were collected throughout the trial to the end of lactation. Estrus was detected by visual observation. After the end of the residual period, cows continued to receive the same commercial concentrate,

Figure 3. Regression between milk fat percentage and conjugated linoleic acid (CLA) *trans*-10,*cis*-12 using data from cows receiving rumen-protected fat supplements from 28 to 84 DIM (treatment period).

Table 9. Reproductive data of cows receiving rumen-protected fat supplements from 28 to 84 DIM (treatment period) and without this supplement from 85 to 112 DIM (residual period) for over a year after the beginning of the experiment

Variable	Treatment ¹		SE	P-value
	Control	CLA		
Days to first estrus	111	89	10	0.22
Number of services	1.95	1.45	0.34	0.36

¹Cows received a dietary fat supplement consisting of Ca-salts of palm oil fatty acids (control) or Ca-salts of a mixture of conjugated linoleic acid isomers (CLA). Both treatments provided about 105 g/d of fatty acids.

but the amount given to each cow corresponded to 1 kg of ration for each 3 kg of milk above a baseline of 9 kg of milk. The reproductive data of the cows in the present study (Table 9) are consistent with the challenging environment of the tropical pasture. Although all reproductive variables were favorable to CLA-treated cows, only days open was significantly different ($P = 0.08$). Consistent with our results, a recent meta-analysis of 5 studies involving CLA supplementation in early lactation demonstrated an improvement in reproductive performance (de Veth et al., 2006). Reproductive variables examined included time to first ovulation, time to conception, and overall success of pregnancy. Interestingly, the improvements in reproduction variables were related to the dose of *trans*-10,*cis*-12 CLA but not associated with improvements in net energy balance (de Veth et al., 2006). This suggests that CLA effects may be independent of energy balance. In effect, the cows in the present trial did not improve their energy balance because total energy expenditure seemed to be unchanged (i.e., decreased milk energy content was compensated by greater milk volume). Results in vitro and in vivo with certain fat supplements, particularly fish oil and CLA, suggest that the effects on reproduction may be the result of CLA interfering with prostaglandin metabolism (Staples et al., 1998; Mattos et al., 2002).

CONCLUSIONS

Our results are consistent with the proposed hypothesis that CLA can be used to manipulate milk composition. Supplementation of CLA increased milk yield and protein production in an energy-limited environment. Energy output was unchanged but the relative protein to energy requirements were different in CLA-treated cows. Under these conditions there was an increase in protein content and a decrease in milk fat content. Treatment with CLA also tended to improve reproductive performance.

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