Production Responses of Dairy Cows to Dietary Supplementation with Conjugated Linoleic Acid (CLA) During the Transition Period and Early Lactation^{1,2}

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ABSTRACT

Holstein cows (n = 30) entering second or greater lactation were fed fat supplements (90 g/d of fatty acids) consisting of Ca salts of either palm fatty acid distillate (control) or a mixture of palm fatty acid distillate and mixed isomers of conjugated linoleic acid (CLA, 30.4 g/d) from 2 wk prepartum through 20 wk postpartum to determine whether CLA would inhibit milk fat synthesis during early lactation and, in turn, affect energy metabolism of dairy cows during the transition period and early lactation. Feeding CLA did not affect DMI or plasma concentrations of glucose, nonesterified fatty acids, or β -hydroxybutyrate during the prepartum period and did not affect postpartum DMI. Feeding CLA reduced milk fat content by 12.5% during early lactation; however, cows fed CLA tended to produce approximately 3 kg/d more milk during the first 20 wk of lactation. Feeding CLA tended to decrease the contribution of short- and medium-chain (C $\leq$ 16) fatty acids to milk fat. Changes in milk yield, milk fat content, and milk fatty acid composition were not apparent until after the second week of lactation. Yield of 3.5% fat-corrected milk, milk protein content, milk protein composition, and calculated energy balance were not affected by

treatment. Postpartum concentrations of glucose, nonesterified fatty acids, and β -hydroxybutyrate in plasma and hepatic content of glycogen and triglycerides were similar between treatments. These data imply that with CLA treatment in early lactation, dairy cows decreased milk fat synthesis and appeared to respond by partitioning more nutrients toward milk synthesis rather than improving net energy balance.

(**Key words:** conjugated linoleic acid, milk fat, lactation, transition cow)

Abbreviation key: CLA = conjugated linoleic acid, NCN = noncasein nitrogen, NEB = net energy balance, TN = true nitrogen, TP = true protein.

INTRODUCTION

Conjugated linoleic acid (CLA) is a collective term for several positional and geometric configurations of octadecadienoic acid with a conjugated double-bond system. During established lactation studies that ranged in duration from 5 d (Lor and Herbein, 1998; Chouinard et al., 1999a, 1999b; Giesy et al., 2002) to 20 wk (Perfield et al., 2002), feeding supplements containing mixed isomers of CLA caused a reduction in the content and yield of milk fat by dairy cows. *trans*-10, *cis*-12 Conjugated linoleic acid has been shown to be a CLA isomer that affects milk fat and is a very potent inhibitor of milk fat synthesis (Baumgard et al., 2000, 2001; Peterson et al., 2002). Conjugated linoleic acid and the *trans*-10, *cis*-12 isomer also have been implicated in the biohydrogenation theory of diet-induced milk fat depression (Bauman and Griinari, 2001), and there is an inverse relationship between milk fat content of *trans*-10, *cis*-12 CLA and milk fat yield (Bauman and Griinari, 2001; Giesy et al., 2002; Peterson et al., 2002).

Economics generally favor prevention of milk fat depression on commercial dairy farms, but there are some scenarios in which reduced output of milk fat could be advantageous (Bauman et al., 2001; Perfield et al., 2002). These include markets where producers are regulated by a quota system based on milk fat and situa-

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tions in which animals cannot consume sufficient energy to meet requirements such as occurs during heat stress or when poor weather limits forage growth in pasture-based systems. Parturition and early lactation is an additional period when intake is inadequate to meet nutrient requirements, so cows are dependent on the use of body reserves. If an imbalance occurs during this period, stress or metabolic disorders can occur, and prolonged negative energy balance can also compromise reproductive performance (Bell, 1995; Butler, 2001). Current management recommendations and most experimental approaches to decrease the severity and duration of negative energy balance involve strategies to maximize DMI during this transition period. However, the energy deficit also could be less pronounced if the fat content of milk were reduced. Our objective was to determine the effect of feeding a supplement containing rumen-protected CLA on milk fat synthesis at parturition and during early lactation in dairy cows. To obtain a more complete evaluation, we monitored lactational performance, metabolic status, and variables related to reproductive performance.

MATERIALS AND METHODS

Animals, Treatments, and Sampling

The Cornell University Institutional Animal Care and Use Committee approved all procedures involving animals. Thirty pregnant Holstein cows entering their second or greater lactation were assigned to one of two treatment groups based on parity, and housed in tie stalls at the Cornell University Dairy Teaching and Research facility. Treatment administration was initiated 14 d before anticipated calving date and continued until 140 DIM. Each treatment group received a fat supplement that was top-dressed once daily on their TMR. Both fat supplements were provided as calcium salts of long-chain fatty acids to partially protect them from ruminal biohydrogenation. Rumen-protected fat supplements were 116 g/d of Ca-salts of palm fatty acid distillate (EnerGII; Bioproducts Inc. Fairlawn, OH) for the control treatment and 126 g/d of a mixture of Ca-salts of palm fatty acid distillate plus Ca-salts of CLA (Agribrands Purina Canada Inc., Woodstock, Ontario, Canada) for the CLA treatment group. Fatty acid composition of the supplements has been previously reported (Perfield et al., 2002), and each supplement provided about 90 g/d of fatty acids. The CLA supplement provided 30.4 g/d of CLA and the four predominant CLA isomers were: *trans*-8, *cis*-10 (9.2%), *cis*-9, *trans*-11 (25.1%), *trans*-10, *cis*-12 (28.9%), and *cis*-11, *trans*-13 (16.1%) (Perfield et al., 2002).

Cows were fed diets as TMR that were formulated using the Cornell Net Carbohydrate and Protein Sys-

Table 1. Ingredient and chemical composition of the prepartum and postpartum diets.¹

Variable	Prepartum	Postpartum
Ingredient, % of DM		
Corn silage	38.1	30.9
Mixed grass-legume silage	12.0	15.5
Ground shelled corn	21.1	24.6
Grass hay	12.6	...
Alfalfa hay	...	8.6
Whole cottonseed	3.6	5.6
Soybean meal (47.5% CP)	4.0	3.7
Expeller soybean meal	5.6	7.7
Sodium bicarbonate	...	0.5
Mineral and vitamin mix ^{2,3}	2.9	2.9
Chemical analysis, % of DM		
CP	15.7	16.4
NDF	35.6	34.3
ADF	23.3	22.0
NFC ⁴	43.6	43.4
Ether Extract	4.3	4.8
Ca	0.92	1.07
P	0.45	0.49
Mg	0.45	0.32
K	1.48	1.40
NE _L , Mcal/kg DM	1.58	1.63

¹Values represent averages of samples composited every 4 wk.

²The mix fed prepartum contained 11.9% Ca, 6.2% Mg, 4.3% Na, 6.6% Cl, 1.55% S, 32 mg/kg of Co, 302 mg/kg of Cu, 760 mg/kg of Fe, 32 mg/kg of I, 1126 mg/kg of Mn, 16 mg/kg of Se, 1489 mg/kg of Zn, 146 IU/g of vitamin A, 59 IU/g of vitamin D, and 2594 IU/g of vitamin E.

³The mix fed postpartum contained 20.0% Cl, 18.0% Na, 12.0% Ca, 8.0% S, 7.5% mg, 1.0% K, 0.48% Zn, 0.40% Mn, 0.29% Fe, 0.07% Cu, 0.01% P, 0.006% Co, 550 IU/g of vitamin A, 132 IU/g of vitamin D, 2970 IU/g of vitamin E.

⁴100 - ([NDF - NDICP] + CP + ash + ether extract).

tem (Fox et al., 1992) to meet or exceed predicted requirements for energy, protein, minerals, and vitamins (NRC, 2001). All cows were fed the same basal diet during the prepartum period beginning between 28 and 21 d before expected parturition and then switched to a common diet during the postpartum period. Ingredient and nutrient composition of the diets fed during the prepartum and postpartum phases of the experiment are provided in Table 1. Feeds were sampled weekly throughout the experiment, and DM content was determined by drying at 54°C until static weight. Amounts of individual feed components in the TMR were adjusted weekly based on changes in the DM content of feed components. Feed samples were composited at 4-wk intervals and analyzed by wet chemistry methods for CP, ADF, NDF, ether extract, and minerals (Dairy One Cooperative Inc., Ithaca, NY). Cows were fed ad libitum, with fresh feed provided each morning after milking. Orts were weighed and recorded daily. Water was available at all times.

Body weights and BCS of each animal were recorded on 1 d each week throughout the study. Body condition

scores were assigned using a five-point system (Wildman et al., 1982) by two individuals, and the average of these two scores was the assigned value. Daily observations and general health records were maintained throughout the study.

After parturition, cows were milked three times per day and yields were recorded at each milking. Through the 20-wk treatment period, milk samples were collected from each milking on one day per week and composited. The composite samples were stored at 4°C with a preservative (bronopol tablet; D&F Control System, San Ramon, CA) until analyzed for fat, protein, and lactose using infrared analysis (AOAC 2000: method 972.160), and SCC by an optical fluorescent method (AOAC 2000: method 978.26) (Dairy One Cooperative Inc.). The calibration reference methods for the infrared milk analysis were as follows: fat by modified Mojonnier ether extraction (AOAC 2000: method 989.05), Kjeldahl true protein (AOAC 2000: method 991.22), and lactose by difference of oven drying total solids (AOAC 2000: method 990.20) minus the sum of modified Mojonnier ether extraction, Kjeldahl true protein (AOAC 2000: method 991.22), estimated nonprotein nitrogen (0.19% on a protein basis), and estimated ash [(true protein $\times$ 0.596) + 0.5379]. Additional aliquots of the composite milk samples collected on wk 1, 2, 4, 6, 8, 12, 16, and 20 were stored at -20°C (without preservative) until analyzed for fatty acid and protein composition.

Blood samples were collected every other day beginning 1 wk before treatment (21 d prepartum) and continuing through 56 DIM, and thereafter once weekly until the study terminated (140 DIM). Blood was collected via venipuncture from the coccygeal vessels during the 30-min interval following the morning milking and before providing fresh feed. Sodium heparin (100 U/ml of blood) was used to prevent coagulation. Plasma was harvested following centrifugation (2800 $\times$ g, 15 min at 4°C) and stored at -20°C until analyses for metabolites and progesterone.

Liver samples were obtained from each cow via percutaneous trochar biopsy (Veenhuizen et al., 1991) on 1 d during the week preceding assignment to treatment and on d 10 and 21 postpartum. Liver was blotted to remove excess blood and connective tissue, snap frozen in liquid nitrogen, and stored at -80°C until analyzed for triglyceride and glycogen content.

Milk Fatty Acid Analysis

Milk fat was extracted using the method of Hara and Radin (1978) and fatty acid methyl esters were prepared by base-catalyzed transmethylation according to Christie (1982) with modifications by Chouinard et al. (1999a). Fatty acids in the rumen-protected supple-

ments were methylated using 1% sulfuric acid in methanol as described by Christie (1989). Fatty acid methyl esters were quantified using a gas chromatograph (GCD system HP 6890+; Hewlett Packard, Avondale, PA) equipped with a SP-2560 fused silica capillary column (100 m $\times$ 0.25 mm [i.d.] with 0.2- μ m film thickness; Supelco, Bellefonte, PA) under conditions previously described (Perfield et al., 2002). Fatty acid peaks were identified using pure methyl ester standards (Nu-Chek Prep, Elysian, MN). Additional standards for CLA isomers were obtained from Natural Lipids Ltd AS (Hovdebygd, Norway). A butter oil reference standard (CRM 164; Commission of the European Community Bureau of References, Brussels, Belgium) was used to determine recoveries and correction factors for individual fatty acids as well as acting as a reference sample for routine quality control.

Milk Protein Analysis

Total N (**TN**) (AOAC, 2000; method number 991.20) and N fractions of milk were determined using macro-Kjeldahl techniques. Milk NPN consisted of N that was soluble in 12% trichloroacetic acid (AOAC, 2000; method number 991.21). Noncasein N (**NCN**) was determined by precipitating casein at pH 4.6 and filtering, leaving the filtrate for N analysis (AOAC, 2000; method number 998.05). These milk N fractions were used in calculations as follows: CP equaled (TN $\times$ 6.38); true protein (**TP**) equaled (TN - NPN) $\times$ 6.38; casein protein equaled (TN - NCN) $\times$ 6.38; and whey protein equaled (NCN - NPN) $\times$ 6.38. Milk NCN and NPN were multiplied by 6.38 to convert to protein equivalents so they could be compared with other protein fractions.

Plasma and Tissue Analysis

Plasma glucose was determined by enzymatic analysis (glucose oxidase) using a commercial kit (kit 510-A; Sigma Chemical, St. Louis, MO). Plasma concentrations of NEFA were analyzed by enzymatic analysis (NEFA-C; WAKO Pure Chemical Industries, Osaka, Japan) using modifications described by McCutcheon and Bauman (1986) and Sechen et al. (1990). Plasma concentrations of BHBA were quantified (BHBA dehydrogenase) using a commercial kit (kit 310-UV; Sigma Chemical). All spectrophotometric measurements were conducted using a Versa_{max} tunable microplate reader (Molecular Devices, Sunnyvale, CA). Interassay variation was maintained at <5%.

Plasma collected during the postpartum period was assayed for progesterone by radioimmunoassay (Staigmiller et al., 1979; Nara and First, 1981). Intra- and interassay CV were 5 and 13%, respectively. Ovulation

Table 2. Least squares means for measurements of performance and plasma metabolites during the prepartum period.

Variable	Treatment ¹		SEM	P-value
	Control	CLA		
Performance				
DMI, kg/d	12.1	13.1	0.6	0.25
BW, ² kg	709	720	6	0.24
BCS ³	3.47	3.48	0.09	0.91
Net energy balance, ⁴ Mcal/d	5.3	6.8	0.9	0.28
Plasma metabolites ⁵				
Glucose, mg/dl	48.3	50.0	1.0	0.27
NEFA, uEq/L	258	247	33	0.82
BHBA, mg/dl	6.5	6.1	0.3	0.25

¹Cows received a dietary fat supplement consisting of Ca-salts of palm fatty acid distillate (control treatment; n = 15 cows) or Ca-salts of a mixture of conjugated linoleic acid isomers plus palm fatty acid distillate (CLA treatment; n = 15 cows).

²Cows were weighed on 1 d each week throughout the prepartum period.

³Cows were scored (five-point scale) by two individuals on 1 d each week throughout the study and values averaged.

⁴Calculated based on NRC (2001). $(DMI \times NE_L \text{ diet}) - ([0.08 \times BW^{0.75}] + ([0.00318 \times 0 - 0.352] \times (\text{calf BW}/45)))/0.218$.

⁵Blood samples were obtained every other day throughout the prepartum period.

was identified by three consecutive samples with progesterone levels greater than 1 ng/ml.

Glycogen content of liver was determined according to the procedures described in Hawk and Bergeim (1926) with modifications. Frozen liver tissue was pulverized in liquid nitrogen and approximately 0.1 g of powdered liver tissue was placed in a 15-ml polycarbonate centrifuge tube with 1.2 ml of 30% KOH. The tube was capped, vortexed, and placed in a boiling water bath for 20 min. The mixture was brought to room temperature, 1.4 ml of 95% ethanol added, and centrifuged at 4°C for 20 min at $1730 \times g$ in a Beckman GS-6KR centrifuge. The supernatant was removed and the pellet was washed twice with 60% ethanol, using 10 ml the first time and 5 ml the second. Following each wash, the mixture was centrifuged under the same conditions as described above. After the second wash the pellet was resuspended in 8 ml of 0.2 N acetate buffer. Two hundred microliters of hydrolyzed liver sample was combined with 30 μ l of amyloglucosidase (Sigma A-3514; Sigma Chemical) and incubated at 55°C for 1 h to convert liver glycogen to glucose. Glucose was then analyzed as described for plasma.

Liver triglyceride content was determined using the Folch extraction method (Folch et al., 1957) followed by a colorimetric method for estimating serum triglycerides (Fletcher, 1968) with modifications described by Foster and Dunn (1973).

Statistical Analysis

Daily intake and milk yield values were reduced to weekly means before data analysis. Pretreatment val-

ues for DMI, BW, BCS, blood metabolites, and liver composition were used as covariates during analysis of covariance applied to their corresponding measurements during the treatment period. Analysis of variance was conducted using the MIXED procedure of SAS (2001) for a completely randomized design with repeated measures. The model included the effects of treatment, time, and the interaction of treatment and time, with the random variable being the cow $\times$ treatment interaction. Parity was included as a term in the original model, but was not significant and was eliminated from the final model. Days to first ovulation for the two treatments were compared using a two-tailed *t*-test (Number Cruncher Statistical System; NCSS, 2000), median days to first ovulation was evaluated using nonparametric survival analysis (NCSS, 2000), and the number of cows with days to first ovulation less than or equal to 30 d was evaluated by chi-square analysis (NCSS, 2000). Least squares means are reported throughout, and significance was declared at $P < 0.05$.

RESULTS

Cows were fed rumen-protected CLA beginning 2 wk before expected calving date. Performance parameters and plasma metabolites were measured during this timeframe, and no differences were observed between treatment groups for DMI, BW, BCS, or net energy balance (NEB; Table 2). Likewise, plasma concentrations of glucose, NEFA, and BHBA were similar between treatment groups during the prepartum period (Table 2).

Table 3. Least squares means for performance measurements over the first 20 wk of lactation.

Variable	Treatment ¹		SEM	P-value
	Control	CLA		
Cows, n	15	15		
DMI, kg/d	23.5	23.9	0.5	0.65
Milk yield, kg/d	44.3	47.1	1.3	0.13
Milk fat %	3.60	3.15	0.08	<0.001
Yield, g/d	1570	1452	50	0.11
Milk protein %	2.77	2.74	0.04	0.60
Yield, g/d	1214	1264	32	0.28
Milk lactose %	4.74	4.73	0.05	0.98
Yield, g/d	2099	2227	62	0.16
SCC, × 1000	382	426	157	0.84
3.5% FCM, kg/d	44.5	43.9	1.3	0.75
Milk net energy, ² Mcal/d	29.8	29.4	0.8	0.80
Body weight, ³ kg	599	616	7	0.09
Body condition score ⁴	2.92	3.06	0.06	0.15
Net energy balance, ⁵ Mcal/d	-1.3	-0.6	0.7	0.49

¹Cows received a dietary fat supplement consisting of Ca-salts of palm fatty acid distillate (control treatment) or Ca-salts of a mixture of conjugated linoleic acid isomers plus palm fatty acid distillate (CLA treatment).

²Calculated based on NRC (2001). Milk yield (kg/d) × (0.0929 × milk fat % + 0.0563 × milk protein % + 0.0395 × milk lactose %).

³Cows were weighed on 1 d each week throughout the study.

⁴Cows were scored (five-point scale) by two individuals on 1 d each week throughout the study, and values were averaged.

⁵Calculated based on NRC (2001). (DMI × NE_L diet) – [(0.08 × BW^{0.75}) + [0.0929 × milk fat % + 0.0563 × milk protein % + 0.0395 × milk lactose %] × milk yield).

Performance data for the first 20 wk postpartum are presented in Table 3. During this period, CLA-supplemented animals tended ($P < 0.13$) to yield approximately 3 kg/d more milk. Milk fat percentage was decreased by 12.5% for the CLA-supplemented group over the 20-wk period ($P < 0.001$), but milk fat yield was reduced by only 7.5% ($P < 0.11$) because of the increased milk yield (Table 3). The numerical differences in milk yield and milk fat percentage were offsetting, such that yield of 3.5% FCM and the net energy required for milk synthesis were essentially identical between the two treatment groups (Table 3). Temporal patterns for milk yield, milk fat percentage, and 3.5% FCM are shown in Figure 1. The yield and fat content of milk were nearly identical for the two treatment groups during the first 2 wk postpartum, and then differences occurred that persisted through the remainder of the period. In contrast, there were no significant differences in content of protein and lactose in milk between treatment groups throughout the treatment period (Table 3).

Similar to the prepartum period, DMI and NEB were not significantly affected by supplementation with rumen-protected CLA (Table 3). The temporal pattern of NEB also was similar between groups during the entire period of supplementation (Figure 2). Postpartum BW (Table 3; Figure 3) of cows fed CLA tended ($P < 0.09$)

to be increased compared with controls; postpartum BCS (Table 3; Figure 3) tended ($P < 0.14$) to follow a similar pattern.

The CLA supplementation resulted in a shift in the fatty acid composition of milk fat (Table 4). When averaged over the entire postpartum treatment period, the reduction in the yield of the short- and medium-chain fatty acids (<16 carbons) was proportionally greater, causing a shift such that the milk fat of cows receiving CLA tended ($P < 0.09$) to have a greater content of long-chain fatty acids (>16 carbons) (Table 4). Statistically significant ($P < 0.05$) increases in content of *trans*-C18:1 fatty acids in milk fat also were measured; however, the total changes represented less than 0.3% of the total fatty acids. Similar to the temporal pattern of response observed for milk yield and milk fat content, the shift in milk fatty acid composition occurred after the second week of lactation and persisted through the remainder of the treatment period (Figure 4). In contrast, milk fat content of *trans*-10, *cis*-12 CLA was elevated during the first week of lactation and remained constant over the entire 20-wk period (Figure 5). Pairs of fatty acids comprising the desaturase index (Perfield et al., 2002) were not different between treatment groups (Table 4), indicating that Δ^9 -desaturase was not affected by CLA supplementation.

Figure 1. Temporal pattern of milk yield, milk fat, and 3.5% FCM for cows fed a rumen-protected fat supplement without (control) or with conjugated linoleic acid (CLA). Values are least squares means, $n = 15$ for each treatment group; SEM averaged 1.3 kg/d for milk yield, 0.08% for milk fat and 1.3 kg/d for 3.5% FCM; P -values for treatment $\times$ week of treatment interaction are 0.48 for milk yield, 0.06 for milk fat, and 0.97 for 3.5% FCM.

Figure 2. Temporal pattern of calculated net energy balance by week relative to calving for cows fed a rumen-protected fat supplement without (control) or with conjugated linoleic acid (CLA). Values are least squares means, $n = 15$ for each treatment group; SEM averaged 0.7 Mcal/d.

Figure 3. Temporal pattern of BW and BCS for cows fed a rumen-protected fat supplement without (control) or with conjugated linoleic acid (CLA). Values are least squares means, $n = 15$ for each treatment group; SEM averaged 6.5 kg for BW and 0.1 for BCS; P -values for treatment $\times$ week of treatment interaction are 0.92 for BW and 0.96 for BCS.

Milk protein was analyzed for specific protein fractions as well as TP (Table 5). Consistent with the lack of change in yield and content of protein in milk during the entire 20-wk treatment period, there were no differences between treatment groups for any of the specific protein or N fractions in milk.

Data for postpartum plasma metabolites and liver composition are presented in Table 6. Plasma concentrations of glucose and NEFA were not affected during either d 2 through 56 of lactation or d 63 through 140 of lactation. Consistent with the lack of effect of treatment on the temporal pattern of calculated NEB described above (Figure 2), the similar temporal pattern for plasma NEFA concentrations between treatments confirms the lack of effect on mobilization of body fat reserves (Figure 6). Concentrations of BHBA in plasma were increased ($P < 0.01$) during d 63 through 140; however, given the lack of effect ($P > 0.15$) of treatment on plasma BHBA concentrations from d 2 through 56 of lactation and the small magnitude of the effect during d 63 through 140, this effect is not likely to be of biological significance. Concentrations of glycogen and triglycerides in liver were not affected significantly by treatment (Table 6).

Table 4. Least squares means for fatty acid composition of milk fat from cows receiving rumen-protected fat supplements.

	Treatment ^{1,2}		SEM	P-value
	Control	CLA		
Fatty acid, %				
4:0	4.76	4.79	0.07	0.80
6:0	2.21	2.10	0.05	0.15
8:0	1.15	1.05	0.04	0.08
10:0	2.34	2.15	0.10	0.17
12:0	2.50	2.33	0.10	0.22
14:0	8.73	8.60	0.20	0.66
14:1	0.52	0.49	0.03	0.58
15:0	0.72	0.69	0.02	0.29
16:0	27.17	25.40	0.41	0.005
16:1	1.29	1.19	0.06	0.23
17:0	0.51	0.53	0.01	0.21
18:0	13.43	14.54	0.27	0.007
18:1 <i>trans</i> -4	0.03	0.03	0.001	0.48
18:1 <i>trans</i> -5	0.03	0.03	0.004	0.68
18:1 <i>trans</i> -6-8	0.40	0.45	0.01	0.005
18:1 <i>trans</i> -9	0.30	0.33	0.007	0.003
18:1 <i>trans</i> -10	0.55	0.64	0.02	0.03
18:1 <i>trans</i> -11	1.15	1.12	0.04	0.47
18:1 <i>trans</i> -12	0.64	0.72	0.02	0.01
18:1 <i>cis</i> -9	24.12	24.64	0.63	0.63
18:2 <i>cis</i> -9, <i>cis</i> -12	3.38	3.75	0.08	0.002
18:2 <i>cis</i> -9, <i>trans</i> -11	0.36	0.36	0.01	0.66
18:2 <i>trans</i> -10, <i>cis</i> -12	<0.01	0.03	0.001	0.001
18:3	0.42	0.47	0.01	0.001
20:0	0.09	0.09	0.002	0.07
Other	2.72	2.99	0.05	0.001
Summation, %				
<16 carbons	22.92	22.15	0.51	0.29
16 carbons	28.46	26.37	0.45	0.002
>16 carbons	47.60	49.75	0.87	0.09
Desaturase index				
14:1/14:0	0.06	0.06	0.004	0.62
16:1/16:0	0.05	0.05	0.002	0.77
18:1/18:0	1.82	1.72	0.06	0.20
c9,t11 CLA/18:1 t11	0.32	0.33	0.01	0.19

¹Cows received a dietary fat supplement consisting of Ca-salts of palm fatty acid distillate (control treatment) or Ca-salts of a mixture of conjugated linoleic acid isomers plus palm fatty acid distillate (CLA treatment).

²There was no treatment $\times$ time interaction and values represent average of milk samples obtained at treatment wk 1, 2, 4, 6, 8, 12, 16, and 20 postpartum.

Reflective of the lack of effect of treatment on plasma and liver measurements, incidence of displaced abomasum, retained placenta, ketosis, and milk fever was low in this experiment (data not shown). Although the number of cows per treatment was too small to enable us to statistically assess metabolic disorder incidence as affected by treatment, occurrence of each of these disorders was numerically similar between the two treatments.

Several variables of reproductive performance are presented in Table 7. Concentrations of progesterone in plasma from cows during early lactation were used to determine days to first ovulation. Cows fed rumen-protected CLA during the transition period and early lactation tended to have lower mean days ($P < 0.13$)

and median days ($P < 0.07$) to ovulation, whereas the number of cows whose first ovulation occurred on or before 30 DIM was similar (Table 7). Although the number of cows assigned to each treatment was too small to assess other reproductive parameters statistically, it is interesting to note that the CLA treatment group had a numerical advantage in number of cows that became pregnant and conception rate. Overall, there was no evidence that supplementation with CLA had any negative effects on reproduction variables.

DISCUSSION

The period of transition between late pregnancy and early lactation is characterized by a shift in nutrient

Figure 4. Temporal pattern of milk fatty acid compositional change over the first 20 wk of lactation for cows fed a rumen-protected fat supplement without (control) or with conjugated linoleic acid (CLA). Values are least squares means, $n = 15$ for each treatment group; SEM averaged 0.51, 0.45, and 0.87 g/100 g fatty acids and P -values for treatment $\times$ week of treatment interactions are 0.29, 0.08, and 0.16 for < 16, 16, and >16 carbons, respectively.

partitioning that requires extensive coordination of metabolism to ensure an adequate supply of nutrients to support milk synthesis (Bell, 1995; Drackley et al., 2001). We included these metabolic adaptations in the treatment period by beginning dietary supplementation with the two fat sources at 2 wk before the predicted calving date. This represents the first study to feed rumen-protected CLA during the prepartum period, and supplementation had no effect on measured performance variables or plasma metabolites (Table 2).

In the present study, CLA supplementation did not affect milk fat content until several weeks after the initiation of lactation (Figure 1). Likewise, there was no shift in milk fatty acid composition until several weeks postpartum (Figure 4). This differs from studies conducted with cows during established lactation where abomasal infusion of CLA (Baumgard et al., 2000, 2001) or feeding rumen-protected CLA (Giesy et al., 2002;

Figure 5. Temporal pattern of concentration of *trans*-10, *cis*-12 CLA in milk fat over the first 20 wk of lactation for cows fed a rumen-protected fat supplement without (control) or with conjugated linoleic acid (CLA). Values are least squares means, $n = 15$ for each treatment group; SEM averaged 0.001 g/100 g; P -value for treatment $\times$ week of treatment interaction is 0.06.

Perfield et al., 2002) resulted in an immediate reduction in milk fat content and shift in milk fatty acid composition. A delayed effect of treatment also was observed by Selberg et al. (2002) when CLA supplementation was initiated 28 d prepartum.

An explanation for the lack of a CLA response in milk fat during the first few weeks postpartum is unknown. A CLA isomer that causes a reduction in milk fat is *trans*-10, *cis*-12 CLA (Baumgard et al., 2000), and our analysis indicates it was consistently transferred to milk fat throughout the treatment period (Figure 5). Thus, the lack of an effect immediately postpartum is unlikely to be related to any difference in uptake of *trans*-10, *cis*-12 CLA by the mammary gland. The source of fatty acids for milk fat synthesis is also a consideration, and this differs during the early postpartum period. The NEB characteristic of the onset of lactation is associated with an increased mobilization of body fat reserves, and this results in an increased mammary uptake of circulating NEFA and their use to synthesize milk fat triglycerides (Bell, 1995). However, it is not obvious why an increased contribution of NEFA for milk fat synthesis would completely abolish the CLA-induced reduction in milk fat. Another consideration is the mechanism and studies investigating the effect of *trans*-10, *cis*-12 CLA on milk fat synthesis have shown the mechanism involves coordinated decreases in mRNA abundance for key enzymes involved in the production of milk fat (Baumgard et al., 2002). Perhaps at the onset of lactation the essential cellular signaling systems are attenuated such that *trans*-10, *cis*-12 CLA is unable to elicit the coordinated reduction in the expression of genes for key lipogenic enzymes. Additional studies will be required to understand the basis for the delayed response to CLA, but the overall effect is that CLA supplementation did not reduce milk fat synthesis in the immediate postpartum period.

Table 5. Least squares means for milk nitrogen fractions from cows receiving rumen-protected fat supplements.

Milk variable	Treatment ^{1,2}		SEM	P-value
	Control	CLA		
TP, ³ %	2.79	2.71	0.04	0.16
Casein, ⁴ %	2.31	2.25	0.04	0.27
Whey protein, ⁵ %	0.48	0.46	0.01	0.28
NPN $\times$ 6.38, ⁶ %	0.19	0.18	0.003	0.22
NCN $\times$ 6.38, ⁶ %	0.66	0.64	0.02	0.20
Casein, % of TP	82.86	83.18	0.49	0.64
NPN, % of TP	6.75	6.75	0.16	0.99

¹Cows received a dietary fat supplement consisting of Ca-salts of palm fatty acid distillate (control treatment) or Ca-salts of a mixture of conjugated linoleic acid isomers plus palm fatty acid distillate (CLA treatment).

²There was no treatment $\times$ time interaction and values represent least square means of milk samples obtained at wk 2, 4, 8, 12, 16, and 20 postpartum.

³True protein (TP) calculated as $(\text{TN} - \text{NPN}) \times 6.38$.

⁴Casein protein calculated as $(\text{TN} - \text{NCN}) \times 6.38$.

⁵Whey protein calculated as $(\text{NCN} - \text{NPN}) \times 6.38$.

⁶Multiplied by 6.38 to allow comparison with other protein fractions.

Results from the present study with transition cows also differ in other ways from those observed with administration of CLA to cows in established lactation. In established lactation, CLA administration or feeding affected the yield and content of milk fat, but milk yield and the yield of other milk components were relatively unaffected (Baumgard et al., 2000, 2001; Chouinard et al., 1999a; 1999b; Giesy et al., 2002; Perfield et al., 2002). In the present study the CLA-supplemented

group exhibited an apparent increase in milk yield, beginning several weeks postpartum and continuing throughout the remainder of the treatment period (Figure 1). While statistically the milk yield increase (~ 3 kg/d) was only a trend ($P < 0.13$), it is underpinned by the simultaneous temporal pattern changes involving an increase in milk lactose yield (pattern not shown), a decrease in milk fat percentage (Figure 1), and shift in the fatty acid composition of milk fat (Figure 4).

Table 6. Least squares means for concentrations of metabolites in plasma and liver during the postpartum treatment period.

	Treatment ¹			
Variable	Control	CLA	SEM	P-value
Plasma ²				
Glucose, mg/dl				
d 2 through 56	42.7	44.2	1.0	0.29
d 63 through 140	48.7	48.7	1.0	0.98
NEFA, uEq/L				
d 2 through 56	603	563	51	0.58
d 63 through 140	268	274	32	0.89
BHBA, mg/dl				
d 2 through 56	8.4	10.2	0.9	0.18
d 63 through 140	6.0	7.1	0.3	0.01
Liver, ³ % wet weight				
Glycogen ⁴	1.21	1.16	0.18	0.85
Triglycerides ⁵	10.1	11.9	1.8	0.51

¹Cows received a dietary fat supplement consisting of Ca-salts of palm fatty acid distillate (control treatment) or Ca-salts of a mixture of conjugated linoleic acid isomers plus palm fatty acid distillate (CLA treatment).

²Values represent plasma samples obtained every other day from 2 to 56 DIM, and weekly thereafter.

³Values represent liver samples obtained at 10 and 21 DIM.

⁴Values for d 10 were 1.17 and 1.01% and for d 21 were 1.25 and 1.32% for cows fed the control and CLA treatments, respectively. The interaction of treatment and time was not significant ($P = 0.44$).

⁵Values for d 10 were 10.8 and 12.3% and for d 21 were 9.4 and 11.4% for cows fed the control and CLA treatments, respectively. The interaction of treatment and time was not significant ($P = 0.66$).

Figure 6. Temporal pattern of concentration of NEFA in plasma during the first 140 d of lactation for cows fed a rumen-protected fat supplement without (control) or with conjugated linoleic acid (CLA). Values are least squares means, $n = 15$ for each treatment group; SEM averaged 43 $\mu\text{Eq/L}$; P -value for treatment $\times$ time interaction is 0.74.

Thus, feeding rumen-protected CLA to early-lactation cows had a net effect of shifting nutrient partitioning, where energy spared from the reduction in milk fat was used to increase milk synthesis so that NEB was unchanged (Figure 2). This shift in nutrient partitioning when dietary CLA supplements were fed also has been reported by others. An increase in milk yield coinciding with decreased milk fat content was reported in studies in which cows were fed CLA supplements in early lactation (Giesy et al., 1999; Selberg et al., 2002) or on pasture-based systems (Medeiros et al., 2000). These studies have been reported only in abstract form, but are all situations in which energy intake would likely be less than optimal. In the case of pasture-fed cows where amino acid supply would likely be in excess,

supplementation with rumen-protected CLA also resulted in a simultaneous increase in milk protein yield (Medeiros et al., 2000; Gulati et al., 2001).

The present study is the first to supplement CLA during the first 20 wk of lactation, an interval in which cows would typically be returned to reproductive service. Effects on reproduction were of special interest because studies with avian species indicated that CLA supplements resulted in high embryonic mortality and reduced hatchability of eggs (Aydin et al., 1999a, 1999b). However, no adverse effects were observed for conception, maintenance of pregnancy throughout the treatment period, or for the remainder of the pregnancy (Table 7). Perfield et al. (2002) reported that CLA supplementation over the last 20 wk of the lactation cycle caused no adverse effects on the maintenance of pregnancy or the development of the fetus. The CLA isomers are metabolized and a portion of their beneficial effects—for example, effects on enhancing immune function—are postulated to involve the formation of eicosanoids (Pariza et al., 2000). Polyunsaturated fatty acids, such as linoleic acid, may affect prostaglandin synthesis through a variety of mechanisms, which may contribute to a reduction in embryonic mortality (Mattos et al., 2000). Due to the limited number of animals per treatment group, it is difficult to make any definitive conclusions about the reproductive benefits of CLA supplementation, but all of the numerical changes in reproductive variables were all positive. Larger scale experiments will be required to elucidate the mechanisms underpinning these potential effects of CLA.

CONCLUSIONS

Feeding rumen-protected CLA to dairy cows during the transition period and early lactation resulted in a modest decrease in milk fat content and tended to increase milk yield. These changes, together with a shift in the fatty acid composition of milk fat, were not apparent until several weeks postpartum, indicating that milk fat synthesis was refractory to inhibition by CLA during the immediate postpartum period. The offsetting effects of feeding CLA on milk yield and milk fat content resulted in comparable NEB and plasma concentrations of NEFA and BHBA between treatments; however, days to first ovulation tended to decrease in cows fed CLA, and other indices of health and reproductive performance were not adversely affected by feeding rumen-protected CLA during the transition period and early lactation. Future research should investigate the carryover effects of feeding CLA on milk yield and extend these results on reproductive performance.

Table 7. Reproductive performance during the transition period and early lactation.

Variable	Treatment ¹		P -value
	Control	CLA	
First ovulation, d			
Mean	39.3	29.3	0.13
Median	32	20	0.07
First ovulation ≤ 30 d, ² n	8	9	0.91
Cows pregnant, ³ n	8	12	
Conception rate (all eligible cows), ³ %	27	42	

¹Cows received a dietary fat supplement consisting of Ca-salts of palm fatty acid distillate (control treatment) or Ca-salts of a mixture of conjugated linoleic acid isomers plus palm fatty acid distillate (CLA treatment).

²15 Cows/treatment.

³14 Cows/treatment eligible for breeding. Data for cows pregnant reflect cows confirmed pregnant to a breeding occurring before 140 DIM. Data for conception rate reflect cows confirmed pregnant expressed as a percentage of the total number of breedings occurring before 140 DIM.

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