Response of Milk Fatty Acid Composition to Dietary Supplementation of Soy Oil, Conjugated Linoleic Acid, or Both¹

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ABSTRACT

Thirty-six Holstein cows were blocked by parity and allotted by stage of lactation to 6 treatments to evaluate the effects of dietary soy oil, conjugated linoleic acid (CLA; free acid or calcium salt), or both, on CLA content of milk. Diets were fed for 4 wk and are as follows: (1) control, (2) control + 5% soy oil, (3) control + 1% CLA, (4) control + 1% Ca(CLA)₂, (5) control + 1% CLA + 4% soy oil, and (6) control + 1% Ca(CLA)₂ + 4% soy oil. Rumen volatile fatty acid concentrations, blood fatty acid concentrations, milk yield, and milk composition were measured weekly or biweekly. Dry matter intake and milk yield were recorded daily. Dietary supplementation of soy oil or CLA had no effect on daily milk yield, milk protein concentration and production, or milk lactose concentration and production. Supplementation of unsaturated fatty acids as soy oil, CLA, or Ca(CLA)₂ increased total fatty acid concentration in plasma, decreased milk fat concentration and production, and had no effect on rumen volatile fatty acid concentrations. The weight percentage of CLA in milk was increased from 0.4 to 0.7% with supplementation of 1% CLA, to 1.2% with supplementation of soy oil, and to 1.3% with supplementation of 1% CLA plus soy oil. Supplementation with Ca(CLA)₂ or Ca(CLA)₂ + soy oil increased the CLA content of milk fat to 0.9 and 1.4%, respectively. In summary, adding 5% soy oil was as effective as supplementing CLA, Ca(CLA)₂, or a combination of 1% CLA (free acid or calcium salt) + 4% soy oil at increasing CLA concentrations in milk fat. Feeding CLA as the calcium salt resulted in greater concentrations of CLA in milk fat than did feeding CLA as the free acid. Dietary supplementation

of 5% soy oil or 4% soy oil + 1% CLA as the free acid or the calcium salt increased the yield of CLA in milk.

Key words: calcium, conjugated linoleic acid, rumen, volatile fatty acid

INTRODUCTION

Conjugated linoleic acid (CLA) has been reported to have a wide range of beneficial effects including anticarcinogenic (Parodi, 1994), antiatherogenic (Lee et al., 1994), and antiobesity activities (Pariza et al., 1996) as well as the ability to stimulate immune function (Miller et al., 1994). Ruminant meat, milk, and dairy products are the predominant sources of CLA in the human diet (Lawson et al., 2001). Total CLA content in milk or dairy products ranges from 0.34 to 1.07% of total fat, and it is currently estimated that the average adult consumes only one-third to one-half of the amount of CLA that has been shown to decrease cancer incidence in animal studies (Dhiman et al., 2005). For this reason, increasing the CLA content of milk has the potential to raise the nutritive and therapeutic values of dairy products.

Conjugated linoleic acid originates from either incomplete biohydrogenation of linoleic or linolenic acid to stearic acid in the rumen (Fellner et al., 1995) or from endogenous synthesis in the mammary gland or adipose tissue. Endogenously, *cis*-9, *trans*-11 CLA (the primary isomer found in milk) is synthesized from *trans* vaccenic acid, another intermediate of ruminal biohydrogenation, via Δ^9 -desaturase in tissues (Corl et al., 2001). The CLA content of milk and meat is affected by several factors including the animals breed, age, and diet. Providing plant (soybean, sunflower, corn, canola, flaxseed) and marine oils in the diet (Dhiman et al., 2000; Ramaswamy et al., 2001; Abu-Ghazaleh et al., 2003), pasture feeding (Dhiman et al., 1999), decreasing the forage-to-concentrate ratio (Kelly and Bauman, 1996), and supplementing diets with ionophores such as monensin (Fellner et al., 1997) all increase ruminal production of CLA and its secretion into milk fat. Protecting supplemental CLA from ruminal biohydrogenation may be an additional strategy to increase the CLA content of milk.

Received May 4, 2007.

Accepted September 20, 2007.

¹Publication of the Iowa Agriculture and Home Economics Experiment Station, Ames, Project Number 3801. Research funding granted by the USDA Center for Designing Foods to Improve Nutrition (Iowa State Univ., Ames).

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Calcium salts of long-chain fatty acids have been utilized extensively as an energy source in diets of lactating dairy cows. The salts remain relatively inert in the rumen under normal pH conditions and do not inhibit ruminal bacteria as do free long-chain fatty acids; the calcium salts then dissociate in the acidic conditions of the abomasum (Jenkins and Palmquist, 1984). Feeding calcium salts of CLA has been reported to increase CLA and decrease monounsaturated fatty acids in sheep tissues (Wynn et al., 2006), beef tissues (Gillis et al., 2004), and milk fat of early-lactation cows (Castaneda-Gutierrez et al., 2005) and midlactation cows (Giesy et al., 2002) but not cows in established lactation (Perfield et al., 2002). We hypothesize that supplementing dairy diets with the calcium salt of CLA, because they are less subject to ruminal biohydrogenation, will increase the CLA content in milk to a greater extent than supplementing the diet with the free acid of CLA or with soy oil. Thus, the objective of this study was to determine the effect of different dietary fat sources on milk fatty acid content.

MATERIALS AND METHODS

Experimental Design

Twelve primiparous and 24 multiparous Holstein cows in midlactation were used in a completely randomized block design experiment to evaluate the effects of dietary soy oil or CLA (free acid or calcium salt) on CLA content of milk. Cows were blocked by parity and were randomly assigned to 1 of 6 treatments. Diets were fed for 4 wk, and cows were managed in accordance with guidelines provided by the Iowa State University Committee on Animal Care. The 6 different diets were as follows: (1) control, (2) control + 5% soy oil, (3) control + 1% CLA, (4) control + 1% Ca(CLA)₂, (5) control + 1% CLA + 4% soy oil, and (6) control + 1% Ca(CLA)₂ + 4% soy oil. All diets were formulated to meet NRC (1989) requirements and were fed as TMR (Table 1). Supplemental CLA was included in the diet as an oil (provided courtesy of Conlinco Inc., Detroit Lakes, MN). The oil contained 67% CLA (Table 2) by weight and was added to the diet at 1.67% (DM basis) to provide 1% CLA in the diet. The Ca(CLA)₂ was prepared (courtesy of Eiler Frederiksen of Bioproducts Inc., Las Vegas, NV) by combining 18.09 g of CaO, 17.79 g of water, and 100.4 g of CLA-containing oil (FFA form, see above) and was added to the diet at 1.91% to provide 1% CLA in the diet. Dry matter content was approximately 60% for all 6 diets. Diets containing added soy oil or CLA had an increased concentration of total fatty acids, an increased percentage of C_{18:0}, and a decreased percent-

Table 1. Ingredients and chemical composition of diets¹

Composition	Control diet ²	Soy-oil diets ²
Ingredient, % of DM		
Alfalfa haylage	7.4	7.0
Corn silage	31.3	29.8
Alfalfa hay	9.6	9.1
High-moisture corn	24.2	23.0
Corn gluten feed	13.0	12.3
Soybean meal	11.0	10.5
Vitamin-mineral supplement ³	3.5	3.3
Soybean oil	0.0	5.0
Chemical composition		
CP	16.8	15.9
ADF	17.9	16.9
NDF	30.5	29.0
Lipid	3.0	8.0
Ash	6.9	6.6
NE _L ⁴ Mcal/kg	1.67	1.87
NFC ⁵	42.8	40.5

¹Dry matter on an as-fed basis = 62.4%.

²To provide 1% conjugated linoleic acid (CLA) in the CLA-containing diets, CLA-containing oil was added at 1.67% of the diet (DM basis) for the free acid diets and 1.91% (DM basis) for the Ca(CLA)₂ diets.

³Contained 13.83% Ca, 3.66% P, 3.14% Mg, 0.07% K, 7.70% Na, 5.42% Cl, 0.22% S, 8.03 mg/kg of Co, 451.41 mg/kg of Cu, 6,327.41 mg/kg of Fe, 21.58 mg/kg of I, 1,855.15 mg/kg of Mn, 16.20 mg/kg of Se, 2,535.57 mg/kg of Zn, 152.03 kIU/kg of vitamin A, 33.05 kIU/kg of vitamin D, and 1,316.54 kIU/kg of vitamin E. The mineral supplement was prepared by The Heart of Iowa Cooperative (Gilbert, IA).

⁴Calculated using NRC (2001) values.

⁵Calculated as 100 – (CP + NDF + lipid + ash).

age of C_{16:0}, C_{18:1}, C_{18:2}, and C_{18:3} compared with the control diet (Table 2).

Sample Collection and Measurements

The TMR samples were collected at wk 0, 2, and 4. Milk samples, from the a.m. and p.m. milkings, were collected, and equal volumes were combined for each cow during wk 0, 1, 2, 3, and 4. Rumen fluid was collected via an esophageal tube from each cow during wk 0 and 4. Blood was collected from coccygeal veins during wk 0, 2, and 4, and plasma was prepared from heparinized blood samples by centrifugation at 800 × g for 10 min at 4°C. All samples were stored at –20°C until analysis. Milk production was recorded daily. A duplicate set of milk samples was stored at 4°C until analysis of fat, protein, and lactose content by midinfrared spectrophotometry (MilkoScan 203, Foss Food Technology Corp., Eden Prairie, MN; AOAC, 1991). Milk samples were analyzed by Dairy Lab Services (Dubuque, IA). Dry matter in feed was quantified by drying feed at 65°C for 48 h (AOAC, 1991) in a forced-air oven. Nitrogen in feed samples was quantified by Kjeldahl analysis (AOAC, 1991). Lipid content of feed was determined gravimetrically by the procedure of

Table 2. Fatty acid composition of the experimental diets

Fatty acid	Diet ¹						
	Control	SO	CLA	SO + CLA	Ca(CLA) ₂	SO + Ca(CLA) ₂	CLA oil ²
	(g/100 g of fatty acids)						
C _{14:0}	0.17	0.20	0.22	0.19	0.26	0.12	0.36
C _{16:0}	17.00	13.78	11.18	12.04	11.02	12.34	5.18
C _{16:1}	0.21	0.15	0.32	0.16	0.24	0.21	0.43
C _{18:0}	2.32	3.16	4.02	3.32	3.89	3.22	5.92
C _{18:1}	20.29	19.74	15.06	17.01	14.87	16.67	12.80
C _{18:2}	51.30	50.67	32.80	35.85	33.26	35.74	5.30
C _{18:3}	5.92	7.42	4.03	5.66	4.55	5.57	1.66
CLA	ND ⁵	0.85	27.40	12.45	26.60	13.02	67.18
<i>cis</i> -9, <i>trans</i> -11 ³	ND ⁵	0.16	10.71	4.97	10.43	5.30	28.02
<i>trans</i> -10, <i>cis</i> -12 ⁴	ND ⁵	0.46	13.09	5.88	12.95	5.95	37.05
C _{20:0}	0.31	0.28	0.21	0.33	0.23	0.20	0.08
Total fatty acids, % of DM	1.80	6.80	3.50	7.40	3.40	7.20	

¹Control = control diet; SO = control diet + 5% soy oil; CLA = control diet + 1% conjugated linoleic acid (CLA) as free acid; SO + CLA = control diet + 4% soy oil + 1% CLA as free acid; Ca(CLA)₂ = control diet + 1% CLA as calcium salt; SO + Ca(CLA)₂ = control diet + 4% soy oil + 1% CLA as calcium salt.

²Supplemental CLA was included in the diet as an oil (provided courtesy of Conlinco Inc., Detroit Lakes, MN). The oil contained 67% CLA by weight and was added to the diet at 1.67% (DM basis) to provide 1% CLA in the diet.

³Conjugated linoleic acid isomers, mainly *cis*-9, *trans*-11 CLA, with a small proportion of other isomers like *trans*-9, *cis*-11 CLA.

⁴Conjugated linoleic acid isomers, mainly *trans*-10, *cis*-12 CLA, with a small proportion of other isomers like *cis*-9, *cis*-11 CLA.

⁵Not detected.

Bligh and Dyer (1959). Acid detergent fiber and NDF were determined by the procedures of Goering and Van Soest (1970). Net energy for lactation was calculated using NRC (2001) equations. Nonfiber carbohydrate was calculated according to the following equation: 100 – (CP, % + NDF, % + lipid, % + ash, %; NRC, 2001).

Volatile fatty acids in samples of rumen fluid were analyzed by a purge-and-trap apparatus connected to a gas chromatograph following the procedure of Erwin et al. (1961). A percept II and a Purge-and-Trap Concentrator 3000 (Tekmer-Dohrmann, Cincinnati, OH) were used to purge and collect volatiles. Two milliliters of sample was placed in a sample vial (40 mL) and purged with helium gas (40 mL/min) for 15 min. Volatiles were trapped at 20°C using a Tenax/Silica gel/Charcoal column (Tekmar-Dohrmann) and desorbed for 2 min at 220°C. The desorbed volatiles were concentrated at –100°C using a cryofocusing unit before being thermally desorbed (220°C) and injected (30 s) into a capillary gas chromatograph column. Ramped oven temperature was used. The initial oven temperature was 0°C and was held for 1.50 min. After that, the temperature was increased to 20°C at 4°C/min, increased to 80°C at 10°C/min, then increased to 180°C at 20°C/min and held there for 4.50 min. The column used was an HP-Wax (7.5 m) and HP-5 (30 m, Hewlett-Packard Co., Wilmington, DE) combined column with the flow pressure set at 0.84 kg/cm². A mass selective

detector (HP 5973, Hewlett-Packard Co.) was used to determine volatile components. The ionization potential of the mass selective detector was 70 eV, and the scan range was 40 to 450. Identification of volatiles was achieved by comparing mass spectral data of samples with those of the Wiley library (Hewlett-Packard Co.) and also with those of the standards. The area of each peak was integrated by using ChemStation software (Hewlett-Packard Co.), and total ion counts × 10³ were reported as an indicator of volatiles generated from rumen fluid samples.

Milk samples were centrifuged at 7,000 × g for 20 min to separate milk fat. Lipids of blood plasma were extracted by the procedure of Bligh and Dyer (1959). The extracted lipids from milk and blood were dissolved in methanol, and fatty acids were quantified by GLC (model 5890, Hewlett-Packard, Palo Alto, CA) by using a NaOCH₃-catalyzed methanolysis procedure (Kramer et al., 1997). Separation of the fatty acid methyl esters was performed on an SP-2560 fused silica capillary column (100 m × 0.25 mm i.d. × 0.2 μm film thickness, Supelco Inc., Bellefonte, PA) by using helium as carrier gas at a pressure of 2.11 kg/cm². Oven temperature was maintained at 70°C for 4 min, then increased at 13°C/min to 175°C, held there for 27 min, increased at 4°C/min to 215°C, and finally held there for 31 min. The C_{19:0} was added as an internal standard. Identification and quantification of CLA and

Table 3. Effect of dietary treatment on DMI, milk yield, and milk composition in dairy cows

Variable	Diet ¹						SE
	Control	SO	CLA	SO+CLA	Ca(CLA) ₂	SO+Ca(CLA) ₂	
DMI, kg/d	21.53	19.75	20.72	20.09	21.48	18.92	0.95
Milk yield, kg/d	30.26	28.79	29.69	29.09	29.87	28.74	0.39
Protein, g/100 g of milk	3.18	3.06	2.99	3.15	3.09	3.11	0.07
Protein yield, kg/d	1.05	0.97	0.96	1.00	1.02	0.97	0.02
Lactose, g/100 g of milk	4.80	4.74	4.79	4.87	4.76	4.61	0.09
Lactose yield, kg/d	1.53	1.47	1.56	1.51	1.57	1.49	0.03
Fat, g/100 g of milk	3.53 ^a	2.73 ^b	2.62 ^b	2.17 ^b	2.62 ^b	2.22 ^b	0.13
Fat yield, kg/d	1.12 ^a	0.85 ^b	0.87 ^b	0.64 ^c	0.87 ^b	0.66 ^c	0.04

^{a-c}Means in the same row with different superscripts differ ($P < 0.05$).

¹Control = control diet; SO = control diet + 5% soy oil; CLA = control diet + 1% conjugated linoleic acid (CLA) as free acid; SO + CLA = control diet + 4% soy oil + 1% CLA as free acid; Ca(CLA)₂ = control diet + 1% CLA as calcium salt; SO + Ca(CLA)₂ = control diet + 4% soy oil + 1% CLA as calcium salt.

other long-chain fatty acids were obtained by comparison with commercially available reference standards (Nu-Check Prep, Elysian, MN).

Statistical Analysis

Dry matter intake, milk production, blood plasma, and milk fatty acid composition data were adjusted by analysis of covariance using data taken during wk 0 as covariables. Covariable adjusted data were then analyzed as a completely randomized block design with repeated measures by using the MIXED procedures of SAS (Version 8.0, SAS Institute Inc., Cary, NC). The covariance structure used was compound symmetry. The model used was: $Y_{ijkl} = \mu + T_i + B_j + TB_{ij} + W_l + TW_{il} + E_{ijk}$, where Y_{ijkl} = dependent variable for cow k on treatment i and block j during time l ; μ = population mean; T_i = treatment effect; B_j = block effect (random); TB_{ij} = interaction of block and treatment; W_l = time effect; TW_{il} = interaction of treatment and time; and E_{ijk} = residual error. All pairwise comparisons were made using Tukey's multiple comparison when protected by a significant ($P < 0.05$) F -value.

RESULTS AND DISCUSSION

Feed Intake and Milk Yield

Dry matter intake averaged 20.4 kg/d across treatments and was not affected by addition of soy oil or CLA (Table 3). Milk production and protein and lactose content of milk were not affected by treatment, and treatment $\times$ week interactions were not observed. However, CLA and soy oil supplementation did result in a 30% decrease of milk fat concentration compared with the control diet ($P < 0.05$). The 2 CLA-supplemented diets [free acid – CLA or the calcium salt – Ca(CLA)₂] and the soy oil-alone diet resulted in a 22.9% decrease in milk fat yield compared with the

control diet ($P < 0.05$). The combination of soy oil with either CLA or Ca(CLA)₂ caused a 42% decrease in milk fat yield ($P < 0.05$).

Chouinard et al. (1999), when supplementing diets with 50 to 100 g/d of CLA; Lawless et al. (1998), when supplementing diets with full-fat soybeans at 3.1 kg/d; and Dhiman et al. (2000), when supplementing diets with 4% soy oil, also failed to observe treatment effects on milk protein and lactose yields. In contrast, other researchers have observed decreases in milk protein percentages when supplementing diets with fat (Coppock and Wilks, 1991); diets supplemented with fats rich in unsaturated fatty acids had a particularly negative effect (Firkins and Eastridge, 1994). Decreases in milk protein seen by others could be a result of an adverse effect on microbial fermentation and subsequent decline in microbial protein reaching the small intestine (Jenkins, 1993) or could be a result of increased milk yield diluting milk protein (Drackley and Elliott, 1993). Neither seems likely in the present trial, because dietary protein was adequate and milk production was not affected.

Milk fat depression has long been known to be caused by feeding diets containing significant concentrations of fats rich in polyunsaturated fatty acids (PUFA), like soybean oil (Hippen et al., 1996; Lawless et al., 1998). Dhiman et al. (2000) also observed decreased milk fat percentage in cows when supplemented with soy oil at 3 to 4% of diet DM basis but not when supplemented with soy oil at 1 to 2% of diet DM, suggesting that the hydrogenation ability of rumen microorganisms may have been exceeded at higher inclusion rates. Hippen et al. (1996) partly attributed milk fat depression seen when feeding fats rich in PUFA compared with fats rich in saturated fatty acids to decreased DMI and a subsequent low-fiber intake. However, cows given a daily abomasal infusion of 0.14 to 0.5% CLA of the diet on a DM basis

Table 4. Rumen VFA of cows fed different diets¹

Acid	Diet ²						SE
	Control	SO	CLA	SO + CLA	Ca(CLA) ₂	SO + Ca(CLA) ₂	
Total VFA (mmol/L)	85.0	64.3	64.8	71.2	64.5	71.0	4.9
	(mol/100 mol)						
Acetic	63.6	61.2	62.7	62.1	64.8	62.1	1.1
Propionic	18.1	20.2	19.4	20.7	17.9	21.5	1.1
Isobutyric	1.1	1.2	0.9	1.1	1.0	1.1	0.1
Butyric	14.0	13.9	13.7	13.1	13.3	12.0	0.6
Isovaleric	2.0	1.8	1.9	1.7	1.8	1.6	0.2
Valeric	1.3	1.7	1.4	1.3	1.3	1.7	0.2
Acetate:propionate	3.6	3.2	3.3	3.1	3.7	3.0	0.2

¹Samples were collected 4 h after morning feeding.

²Control = control diet; SO = control diet + 5% soy oil; CLA = control diet + 1% conjugated linoleic acid (CLA) as free acid; SO + CLA = control diet + 4% soy oil + 1% CLA as free acid; Ca(CLA)₂ = control diet + 1% CLA as calcium salt; SO + Ca(CLA)₂ = control diet + 4% soy oil + 1% CLA as calcium salt.

still exhibit a decrease in milk fat percentage without any significant effect on fiber intake (Chouinard et al., 1999). Alternatively, decreased milk fat yield in the present study may be due to an increase in the inhibitory isomers of CLA. Studies involving relatively pure CLA isomers have demonstrated that the *trans*-10, *cis*-12 (Baumgard et al., 2000); the *cis*-10, *trans*-12 (Sæbo et al., 2005); and the *trans*-9, *cis*-11 (Perfield et al., 2007) isomers of CLA decrease milk fat synthesis, but the *cis*-9, *trans*-11 (Baumgard et al., 2000) isomer does not. In agreement, Giesy et al. (2002) and Castaneda-Gutierrez et al. (2005) reported linear increases in CLA and linear decreases in milk fat production in mid and early lactation cows, respectively, when cows were supplemented with Ca(CLA)₂ compared with when cows were not supplemented with any lipid. Perfield et al. (2002) reported that supplementing Ca(CLA)₂ to dairy cows during established lactation caused a reduction in milk fat yield while yields of milk and other milk components were unaltered.

Rumen VFA

Concentrations of total VFA in the rumen were decreased slightly but not significantly ($P > 0.05$ for every pairwise comparison) by supplementing the diet with CLA, soy oil, or both (Table 4). A treatment $\times$ week interaction was not observed ($P > 0.05$). Feeding CLA or Ca(CLA)₂ had similar effects. Also, no significant differences ($P > 0.05$) for individual VFA percentages or acetate-to-propionate ratio were observed among the experimental diets, suggesting that the fat supplements had little or no effect on rumen fermentation. The lack of an effect at this low concentration of dietary fat on rumen fermentation is consistent with previous reports (Murphy et al., 1987).

Plasma

No treatment $\times$ week interactions were observed ($P > 0.05$) for plasma fatty acid concentration (Table 5). Dietary supplementation of soy oil and CLA resulted in an increase in total plasma fatty acid concentrations ($P < 0.05$). Specifically, fat-supplemented cows had greater concentrations of C_{16:0}, C_{18:1}, C_{18:2}, and C_{18:3}. The increase in C_{18:0} concentration was not significant ($P > 0.05$) because of large variation among cows. Hippen et al. (1996) reported similar results when cows were fed a diet supplemented with highly unsaturated fat. Increased concentrations of saturated fatty acids (C_{16:0} and C_{18:0}) in plasma in the present study when unsaturated fatty acids were supplemented suggests that absorption of unsaturated fatty acids facilitates the absorption of saturated fatty acids (Ockner et al., 1972). Additionally, dietary supplementation of fats rich in unsaturated fatty acids, followed by ruminal biohydrogenation, could have provided for the higher concentrations of saturated fatty acids in the plasma.

Increased concentrations of C_{18:1}, C_{18:2}, and C_{18:3} in plasma suggests that dietary supplementation of unsaturated fatty acids resulted in a greater load of unsaturated fatty acids than the rumen had capacity to biohydrogenate, resulting in more C_{18:1}, C_{18:2}, and C_{18:3} passing to the duodenum and being absorbed into the bloodstream. Alternatively, supplementing fat rich in PUFA can lead to a greater release of C_{18:2} from tissues to blood or a decreased uptake of fatty acids by tissues through lipoprotein lipase action (Grummer and Carroll, 1991). The effect of degree of saturation of dietary fat on its partitioning toward body reserves or milk production has been difficult to evaluate because of variations in milk production and stage of lactation and because of the short duration of feeding trials (Firkins and Eastridge, 1994). However, increased

Table 5. Fatty acid (FA) concentrations in blood plasma of cows fed different diets

Fatty acid	Diet ¹						SE
	Control	SO	CLA	SO + CLA	Ca(CLA) ₂	SO + Ca(CLA) ₂	
	(mg/100 mL)						
C _{14:0}	0.75	0.93	1.76	1.14	1.61	1.03	0.28
C _{14:1}	1.05	1.07	1.61	1.39	1.68	1.13	0.20
C _{15:0}	0.68	0.63	0.80	0.76	1.07	0.73	0.10
C _{16:0}	11.08 ^a	14.44 ^{ab}	16.36 ^b	16.98 ^b	14.06 ^{ab}	16.69 ^b	1.11
C _{16:1}	1.74	2.17	1.98	2.26	2.09	2.27	0.22
C _{17:0}	1.07	1.56	1.59	1.69	1.26	1.01	0.15
C _{17:1}	0.49	0.36	0.37	0.51	0.39	0.49	0.05
C _{18:0}	18.27	24.05	23.07	25.25	25.09	25.69	1.82
C _{18:1}	10.24 ^a	21.28 ^{bcd}	16.31 ^{abc}	25.25 ^d	12.37 ^{ab}	22.34 ^{cd}	1.67
C _{18:2}	2.89 ^a	3.89 ^{abc}	3.46 ^{ab}	4.47 ^c	3.55 ^{abc}	3.91 ^{bc}	0.23
Total FA	103.89 ^a	146.52 ^b	142.28 ^{ab}	170.11 ^b	138.48 ^{ab}	155.43 ^b	8.97

^{a-d}Means in the same row with different superscripts differ ($P < 0.05$).

¹Control = control diet; SO = control diet + 5% soy oil; CLA = control diet + 1% conjugated linoleic acid (CLA) as free acid; SO + CLA = control diet + 4% soy oil + 1% CLA as free acid; Ca(CLA)₂ = control diet + 1% CLA as calcium salt; SO + Ca(CLA)₂ = control diet + 4% soy oil + 1% CLA as calcium salt.

concentrations of C_{18:1}, C_{18:2}, and C_{18:3} in plasma of cows in the present study seem unlikely to derive from adipose tissue lipolysis.

Milk Fatty Acids

There was no significant interaction of treatment × week for milk fatty acids; therefore, overall means of milk fatty acids are presented in Table 6. Supplementing the diet with soy oil, CLA, or both, as the free acid or as Ca(CLA)₂ decreased the content of short- and medium-chain fatty acids (C_{4:0}–C_{14:0}) as well as C_{16:0} in the milk fat compared with the control diet ($P < 0.05$); the extent of the decrease was greatest for those diets containing the most fat (soy oil and soy oil + CLA). The decrease of C_{4:0}–C_{14:0} and C_{16:0} concentrations in milk caused by CLA or soy oil supplementation are consistent with the decrease reported by others (Chouinard et al., 1999; Dhiman et al., 2000; Perfield et al., 2002). Because almost all milk C_{4:0}–C_{14:0} and about half of C_{16:0} is synthesized de novo by the mammary epithelial cells, these changes suggest that the mechanism of milk fat depression seen by many when supplementing CLA involves the inhibition of de novo fatty acid synthesis. The lack of effect of treatment on ruminal VFA concentrations (Table 4) suggests that the inhibition of de novo synthesis of long-chain fatty acids by CLA was not primarily because of a shortage of substrate. However, total ruminal VFA concentration was numerically decreased, and production rates of VFA were not measured. Studies involving pure CLA isomers have demonstrated that *trans*-10, *cis*-12 CLA decreases milk fat synthesis, but *cis*-9, *trans*-11 CLA does not (Baumgard et al., 2000). The molecular mechanism mediating the inhibitory effect of CLA iso-

mers on milk fat depression is not well understood. Harvatine and Bauman (2006), however, demonstrated that the sterol response element binding protein transcription factor system, by binding to response elements located in lipogenic enzyme genes, may be a central signaling pathway by which CLA regulates fatty acid synthesis in the mammary gland. Thyroid hormone responsive spot 14, which is down-regulated during diet-induced milk fat depression, may also be involved in the molecular mechanism of milk fat depression, possibly as a secondary cellular signal for sterol response element binding protein 1 (Harvatine and Bauman, 2006).

In the present study, the concentrations of C_{18:0}, C_{18:1}, and C_{18:2} were increased ($P < 0.05$) because of fat supplementation, regardless of source; however, dietary fat source did affect the magnitude of increase. The concentration of C_{18:1} was increased 16.3 to 18.3% by supplementing CLA as the free acid or as Ca(CLA)₂ was increased by 60.7 to 62.5% by supplementing with soy oil or a combination of soy oil and CLA [free acid or Ca(CLA)₂]. Increases in C_{18:1} in milk fat when dairy cows are fed diets rich in unsaturated 18-carbon fatty acids are consistently observed (Bu et al., 2007). Feeding the free acid of CLA increased the C_{18:2} concentration in milk fat compared with the control diet, but feeding soy oil or Ca(CLA)₂ increased C_{18:2} concentration in milk fat to an even greater extent ($P < 0.05$). The combination of CLA and soy oil did not have an additive effect on milk C_{18:2} concentration, but the combination of Ca(CLA)₂ and soy oil did have an additive effect, indicating that a quantity of Ca(CLA)₂ was able to bypass ruminal biohydrogenation.

Feeding CLA as a free acid had no effect on C_{18:3} proportions in milk fat, whereas feeding Ca(CLA)₂ in-

Table 6. Effect of dietary treatments on the profile of milk fatty acids

Fatty acid	Diet ¹						SE
	Control	SO	CLA	SO + CLA	Ca(CLA) ₂	SO + Ca(CLA) ₂	
	(g/100 g of fatty acids)						
C _{4:0}	2.75 ^a	1.99 ^b	2.37 ^{ab}	2.19 ^b	2.33 ^{ab}	2.11 ^b	0.12
C _{6:0}	2.50 ^a	1.52 ^c	2.17 ^{ab}	1.62 ^{bc}	1.98 ^b	1.52 ^c	0.10
C _{8:0}	1.52 ^a	0.81 ^c	1.29 ^{ab}	0.80 ^c	1.13 ^b	0.76 ^c	0.07
C _{10:0}	3.09 ^a	1.63 ^c	2.52 ^b	1.55 ^c	2.48 ^b	1.57 ^c	0.10
C _{12:0}	3.08 ^a	1.90 ^c	2.79 ^b	1.72 ^c	2.72 ^b	1.72 ^c	0.07
C _{14:0}	10.58 ^a	7.70 ^b	9.89 ^a	7.67 ^b	9.88 ^a	7.96 ^b	0.17
C _{14:1}	1.27 ^a	0.94 ^b	1.18 ^a	0.99 ^b	1.17 ^a	0.91 ^b	0.04
C _{15:0}	1.13 ^a	0.74 ^c	0.96 ^b	0.74 ^c	0.93 ^b	0.78 ^c	0.04
C _{15:1}	0.26 ^{abc}	0.25 ^{abc}	0.28 ^{ab}	0.20 ^{ac}	0.33 ^b	0.18 ^c	0.03
C _{16:0}	32.43 ^a	23.71 ^c	29.18 ^b	24.28 ^c	28.27 ^b	24.79 ^c	0.47
C _{16:1}	2.01 ^{ab}	2.33 ^a	1.69 ^{bc}	2.25 ^a	1.62 ^c	2.18 ^a	0.09
C _{17:0}	0.53 ^a	0.41 ^{bc}	0.54 ^a	0.39 ^c	0.56 ^a	0.45 ^b	0.01
C _{17:1}	0.24 ^a	0.16 ^c	0.22 ^{ab}	0.19 ^{bc}	0.20 ^{bc}	0.17 ^c	0.01
C _{18:0}	9.62 ^a	11.51 ^b	11.06 ^b	11.47 ^b	10.84 ^b	11.33 ^b	0.41
C _{18:1}	20.98 ^a	33.79 ^c	24.41 ^b	34.10 ^c	24.82 ^b	33.71 ^c	0.66
C _{18:2}	2.90 ^a	3.85 ^c	3.42 ^b	3.83 ^c	4.12 ^c	4.74 ^d	0.10
C _{18:3}	0.30 ^a	0.24 ^b	0.32 ^a	0.25 ^b	0.40 ^c	0.31 ^a	0.02
C _{20:0}	0.12	0.11	0.13	0.11	0.13	0.14	0.02
CLA	0.40 ^a	1.23 ^c	0.72 ^b	1.27 ^c	0.90 ^b	1.41 ^c	0.05
Ratio of specific isomer/total CLA							
<i>cis</i> -9, <i>trans</i> -11 ²	0.66 ^a	0.80 ^b	0.67 ^a	0.77 ^b	0.49 ^c	0.56 ^c	0.02
<i>trans</i> -10, <i>cis</i> -12 ³	0.11 ^a	0.09 ^a	0.07 ^a	0.08 ^a	0.19 ^b	0.20 ^b	0.02
Ratio							
C _{14:0} /C _{14:1}	8.52 ^a	9.16 ^{ab}	8.65 ^a	8.38 ^a	8.73 ^a	10.57 ^b	0.42
C _{16:0} /C _{16:1}	17.21 ^a	11.28 ^b	18.05 ^a	11.68 ^b	18.27 ^a	12.62 ^b	0.56
C _{18:0} /C _{18:1}	0.49 ^a	0.36 ^b	0.47 ^a	0.34 ^b	0.46 ^a	0.34 ^b	0.01
Index ⁴							
P/S	0.06 ^a	0.11 ^d	0.07 ^b	0.10 ^d	0.09 ^c	0.12 ^e	0.003
M/S	0.37 ^a	0.74 ^c	0.46 ^b	0.74 ^c	0.47 ^b	0.72 ^c	0.02
IA	2.80 ^a	1.37 ^c	2.25 ^b	1.35 ^c	2.17 ^b	1.38 ^c	0.06
IT	3.59 ^a	2.02 ^c	3.03 ^b	2.00 ^c	2.86 ^b	1.99 ^c	0.07

^{a-c}Means in the same row with different superscripts differ ($P < 0.05$).

¹Control = control diet; SO = control diet + 5% soy oil; CLA = control diet + 1% conjugated linoleic acid (CLA) as free acid; SO + CLA = control diet + 4% soy oil + 1% CLA as free acid; Ca(CLA)₂ = control diet + 1% CLA as calcium salt; SO + Ca(CLA)₂ = control diet + 4% soy oil + 1% CLA as calcium salt.

²Conjugated linoleic acid isomers, mainly *cis*-9, *trans*-11 CLA with a small proportion of other isomers like *trans*-9, *cis*-11 CLA.

³Conjugated linoleic acid isomers, mainly *trans*-10, *cis*-12 CLA with a small proportion of other isomers like *cis*-9, *cis*-11 CLA.

⁴P/S = sum of polyunsaturated fatty acids/sum of saturated fatty acids; M/S = sum of monounsaturated fatty acids/sum of saturated fatty acids; IA = index of atherogenicity; IT = index of thrombogenicity.

creased C_{18:3} proportions in milk fat compared with the control diet ($P < 0.05$). Consistent with Dhiman et al. (2000), who fed soy oil at 4% of the diet DM, feeding soy oil in the present study decreased C_{18:3} proportions in milk fat ($P < 0.05$). Feeding CLA in combination with soy oil also decreased C_{18:3} proportions in milk fat, whereas feeding Ca(CLA)₂ in combination with soy oil did not decrease C_{18:3} proportions in milk fat ($P < 0.05$). The fact that C_{18:3} concentration in milk fat was increased to the greatest extent by feeding Ca(CLA)₂, and was maintained by including Ca(CLA)₂ with soy oil, indicates that ruminal biohydrogenation of C_{18:3} was lessened. Alternatively, the Ca(CLA)₂ that was not able to escape rumen fermentation may have been biohydrogenated in place of C_{18:3}. It may also be that supplementing Ca(CLA)₂ provided mammary

gland Δ^9 -desaturase with substrate for production of C_{18:3}.

Adding CLA as the free acid to the diet increased the CLA weight percentage in milk fat, and feeding Ca(CLA)₂ tended to increase the percentage even more ($P < 0.10$). The ratio of the *cis*-9, *trans*-11 isomer and the ratio of the *trans*-10, *cis*-12 isomer to total CLA in milk fat did not differ ($P > 0.05$) between the control diet and the diet supplemented with the free acid of CLA. The ratio of the *cis*-9, *trans*-11 isomer increased, and the ratio of the *trans*-10, *cis*-12 isomer to total CLA in milk fat decreased compared with control when diets were supplemented with Ca(CLA)₂ and when diets were supplemented with soy oil + Ca(CLA)₂ ($P < 0.05$). Adding 5% soy oil to the diet had a greater effect on increasing CLA content in milk than did add-

ing CLA in either form ($P < 0.05$). Soy oil alone or in combination with the free acid of CLA increased the ratio of the *cis*-9, *trans*-11 isomer and had no effect on the ratio of the *trans*-10, *cis*-12 isomer to total CLA in milk fat. There was no significant difference on milk CLA concentration between feeding 5% soy oil and feeding 4% soy oil + 1% CLA as the free acid. However, feeding 4% soy oil + 1% Ca(CLA)₂ tended to increase milk CLA concentration compared with feeding soy oil alone ($P < 0.10$). Soy oil has a large proportion of C_{18:2} (51% wt/wt), the precursor of CLA, and has been an effective dietary supplement for increasing the CLA content in milk (Dhiman et al., 2000). Kelly et al. (1998) showed that CLA concentration in milk fat could be increased by dietary PUFA supplementation, especially oils rich in linoleic acid. The *cis*-9, *trans*-11 CLA isomer is derived directly from C_{18:2} isomerization during ruminal biohydrogenation (Kepler et al., 1966) and is the major CLA isomer found in animal-derived foods.

Results from our study indicate that feeding soy oil at 5% of the diet to lactating cows is a more effective method to increase CLA concentrations in milk than feeding CLA as a free acid or as Ca(CLA)₂ at a concentration of 1% of the diet. Supplemental CLA is highly vulnerable to hydrogenation because of its conjugated double bonds. Huang (2000) reported that, in sheep, supplemental dietary CLA was 97% biohydrogenated when it was fed as the free acid and 90% biohydrogenated when it was fed as Ca(CLA)₂. Wynn et al. (2006) observed that, in sheep, 91.5% of CLA supplemented in the free acid form was biohydrogenated in the rumen, whereas only 35% of the supplemented Ca(CLA)₂ was biohydrogenated in the rumen. It may be possible that not enough CLA reached the small intestine in the present study because of extensive biohydrogenation in the rumen. Because feeding 3 to 4% of the diet DM as soy oil resulted in higher CLA concentrations in milk, and feeding 1 to 2% of the diet DM as soy oil did not, Dhiman et al. (2000) suggested that the biohydrogenation ability of rumen microorganisms could be exceeded when larger amounts of C_{18:2} are presented to the rumen, thus allowing increased escape of CLA to the intestine for absorption. Results from our study also indicate that feeding a combination of Ca(CLA)₂ and soy oil is the most effective strategy at increasing CLA content in milk while maintaining or increasing PUFA content of milk.

The ratios of C_{16:0} to C_{16:1} and C_{18:0} to C_{18:1} were decreased ($P < 0.05$) by soy oil supplementation, whereas feeding CLA had no significant effect on these ratios. The only dietary treatment that had an effect on the ratio of C_{14:0} to C_{14:1} compared with the control diet was the combination of soy oil and Ca(CLA)₂,

which increased it ($P < 0.05$). Supplemental dietary Ca(CLA)₂ has had no effect on the ratio of fatty acid pairs in early lactation cows (Castaneda-Gutierrez et al., 2005) and midlactation cows (Giesy et al., 2002). When fed to pregnant cows in established lactation, Perfield et al. (2002) observed a decrease in the ratio of fatty acid pairs (C_{14:0} to C_{14:1}, C_{16:0} to C_{16:1}, and C_{18:0} to C_{18:1}), indicating that Δ^9 -desaturase activity was elevated. In contrast, abomasal infusion of CLA increases the ratio of saturated to unsaturated fatty acids, indicating decreased Δ^9 -desaturase activity (Chouinard et al., 1999). When abomasally infused, the *trans*-10, *cis*-12 and *trans*-9, *cis*-11 isomers of CLA have been demonstrated to decrease the desaturase index, whereas the *cis*-9, *trans*-11 isomer does not (Baumgard et al., 2000; Perfield et al., 2007). Further, studies involving growing rodents have demonstrated that the *trans*-10, *cis*-12 isomer decreases both activity and gene expression for Δ^9 -desaturase (Park et al., 2000). Ruminal biohydrogenation of a portion of CLA, when it is included in the diet, would effectively lower the dose of *trans*-10, *cis*-12 CLA and *trans*-9, *cis*-11 CLA reaching the mammary gland compared with when the isomers are abomasally infused.

Dietary supplementation of CLA as the free acid increased the ratio of PUFA to saturated fatty acids (P/S) and the ratio of monounsaturated fatty acids to saturated fatty acids (M/S) compared with the control diet ($P < 0.05$). Feeding Ca(CLA)₂ increased the P/S but not the M/S to a greater extent than feeding CLA as the free acid ($P < 0.05$). Soy oil addition increased both the P/S and M/S of milk compared with the control diet and did so to a greater extent than either CLA treatment ($P < 0.05$). Addition of CLA to the soy oil diet as Ca(CLA)₂ but not as the free acid increased the P/S even further ($P < 0.05$). Addition of CLA in either form to the soy oil diet did not affect the M/S ratio compared with addition of soy oil alone.

Fatty acids can promote or prevent atherosclerosis and coronary thrombosis based on their effects on serum cholesterol and low-density lipoprotein-cholesterol concentrations (Ulbricht and Southgate, 1991). The equations proposed by Ulbricht and Southgate (1991) for the atherogenic and thrombogenic indices indicated that the C_{12:0}, C_{14:0}, and C_{16:0} FA are atherogenic and that C_{14:0}, C_{16:0}, and C_{18:0} are thrombogenic. The n-3, n-6, and monounsaturated FA are antiatherogenic and antithrombogenic. The ratio between the saturated and unsaturated fatty acids is used to calculate the atherogenic and thrombogenic indices. These data are in Table 6. The addition of soy oil, CLA, or both, to the diets decreased the atherogenic and thrombogenic index of milk ($P < 0.05$). The decrease caused by adding CLA in either form was similar but was not

Table 7. Effect of dietary treatment on the yields of milk fatty acids

Fatty acid	Diet ¹						SE
	Control	SO	CLA	SO + CLA	Ca(CLA) ₂	SO + Ca(CLA) ₂	
	(g/d)						
C _{4:0}	31.8 ^a	14.8 ^{bcd}	15.6 ^b	9.8 ^d	16.4 ^b	10.4 ^{cd}	1.2
C _{6:0}	27.9 ^a	17.6 ^b	19.0 ^b	11.6 ^c	19.7 ^b	13.0 ^c	0.9
C _{8:0}	17.8 ^a	8.7 ^b	10.7 ^b	4.8 ^c	11.3 ^b	5.8 ^c	0.7
C _{10:0}	33.7 ^a	15.5 ^c	22.0 ^{bc}	7.2 ^d	22.7 ^b	10.1 ^{cd}	1.6
C _{12:0}	37.8 ^a	17.7 ^c	21.7 ^{bc}	8.4 ^d	24.4 ^b	11.2 ^d	1.3
C _{14:0}	114.9 ^a	71.5 ^c	89.1 ^b	44.9 ^d	89.8 ^b	56.2 ^{cd}	4.2
C _{14:1}	15.1 ^a	8.2 ^{bc}	10.1 ^b	5.7 ^c	10.4 ^b	6.1 ^c	0.6
C _{15:0}	13.6 ^a	6.5 ^{bc}	8.3 ^b	3.7 ^d	8.2 ^b	5.0 ^{cd}	0.5
C _{15:1}	3.0 ^a	2.1 ^b	2.4 ^{ab}	1.2 ^c	2.7 ^{ab}	1.1 ^c	0.2
C _{16:0}	371.4 ^a	206.9 ^c	258.1 ^b	138.0 ^d	257.7 ^b	166.7 ^{cd}	11.5
C _{16:1}	24.2 ^a	18.5 ^b	14.3 ^{bc}	13.6 ^c	14.3 ^{bc}	14.1 ^c	1.0
C _{17:0}	5.9 ^a	3.6 ^c	4.8 ^b	2.1 ^d	4.5 ^b	3.0 ^c	0.2
C _{17:1}	2.6 ^a	1.4 ^{cd}	2.0 ^b	1.1 ^d	1.8 ^{bc}	1.1 ^d	0.1
C _{18:0}	103.7 ^a	93.1 ^{ab}	99.5 ^{ab}	85.0 ^{ab}	98.8 ^{ab}	79.7 ^b	5.3
C _{18:1}	222.3 ^{ab}	270.0 ^b	217.3 ^{ab}	250.2 ^b	186.3 ^a	229.0 ^{ab}	13.5
C _{18:2}	28.4	31.3	30.3	32.7	30.0	33.1	1.9
C _{18:3}	3.2 ^a	2.1 ^c	2.8 ^{ab}	2.2 ^{bc}	3.0 ^a	2.2 ^{bc}	0.2
C _{20:0}	1.3 ^a	1.1 ^{abc}	1.2 ^{ab}	0.7 ^c	1.2 ^{ab}	0.9 ^{bc}	0.1
CLA	5.1 ^a	10.4 ^{bc}	6.6 ^a	10.2 ^{bc}	7.8 ^{ab}	11.2 ^c	0.7

^{a-d}Means in the same row with different superscripts differ ($P < 0.05$).

¹Control = control diet; SO = control diet + 5% soy oil; CLA = control diet + 1% conjugated linoleic acid (CLA) as free acid; SO + CLA = control diet + 4% soy oil + 1% CLA as free acid; Ca(CLA)₂ = control diet + 1% CLA as calcium salt; SO + Ca(CLA)₂ = control diet + 4% soy oil + 1% CLA as calcium salt.

as great as for the diets containing soy oil. These data support the antiatherogenic effect of dietary CLA as proposed by Lee et al. (1994).

There was no significant interaction of treatment $\times$ week for yield of milk fatty acids; therefore, overall means of milk fatty acids are presented in Table 7. Despite our observation that VFA concentrations did not change, daily yields of all milk fatty acids containing fewer than 16 carbons were decreased ($P < 0.05$) by supplementing the control diets with soy oil, CLA, or both. The decreased de novo synthesis is consistent with the previous work (Chouinard et al., 1999; Dhiman et al., 2000; Perfield et al., 2002). Because milk C_{4:0} can be supplied by pathways not involving acetyl-coenzyme A carboxylase, the decrease in the incorporation of C_{4:0} into the milk fat suggests that the milk fat depression may be partly attributable to a decreased supply of substrates in addition to the depression of acetyl-coenzyme A carboxylase. Daily output of C_{18:0} in milk fat was decreased by supplementing 4% soy oil plus 1% Ca(CLA)₂, whereas the differences between the other 4 supplementations and the control were not significant ($P > 0.05$). Consistent with the observation by Hippen et al. (1996), daily incorporation of C_{18:1} and C_{18:2} was not affected by the dietary treatments ($P > 0.05$). Daily yield of milk CLA was increased by supplementing 5% soy oil and 4% soy oil + 1% CLA as the free acid or as Ca(CLA)₂ ($P < 0.05$).

CONCLUSIONS

Supplementing a control diet with 5% soy oil, 1% CLA as a free acid, 1% CLA as Ca(CLA)₂, 4% soy oil + 1% CLA as a free acid, or 4% soy oil + 1% CLA as Ca(CLA)₂ had no effect on DMI, rumen VFA concentrations, milk yield, or milk protein and lactose concentrations and yields but decreased milk fat concentration and fat yield principally by decreasing short- and medium-chain fatty acid synthesis. Dietary supplementation of 5% soy oil or 4% soy oil + 1% CLA as the free acid or as Ca(CLA)₂ increased the concentration of total fatty acids in plasma. Dietary supplementation of 1% CLA as free acid or as Ca(CLA)₂ increased CLA content in milk, whereas feeding 5% soy oil or 4% soy oil + 1% CLA as the free acid or as Ca(CLA)₂ caused an even greater CLA content in milk and increased daily yield of CLA in milk. To maximize the CLA content of milk and daily incorporation of CLA into milk, an ideal supplement would seem to be either C_{18:2}-rich oils or a combination of C_{18:2}-rich oils and CLA as the free acid or as Ca(CLA)₂. The combination of C_{18:2}-rich oils and Ca(CLA)₂ would also maintain PUFA concentration in milk. However, it may be more economical to feed soy oil to cows to increase the CLA content and yield in milk rather than to feed them a CLA supplement.

ACKNOWLEDGMENTS

This research was supported by the Iowa State University's USDA Center for Designing Foods to Improve

Nutrition. The CLA was donated by Daria Jerome of Conlinco Inc. (Detroit Lakes, MN). The calcium salt of CLA was prepared by Eiler Frederiksen (Bioproducts Inc., Las Vegas, NV).

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