Conjugated Linoleic Acid Content of Milk from Cows Fed Different Diets[1]

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ABSTRACT

Conjugated linoleic acid in milk was determined from cows fed different diets. In Experiment 1, cows were fed either normal or high oil corn and corn silage. Conjugated linoleic acid was 3.8 and 3.9 mg/g of milk fatty acids in normal and high oil treatments, respectively. In Experiment 2, cows consumed one-third, two-thirds, or their entire feed from a permanent pasture. Alfalfa hay and concentrates supplied the balance of feed for the one-third and two-third pasture treatments. Conjugated linoleic acid was 8.9, 14.3, and 22.1 mg/g of milk fatty acids in the one-third, two-third, and all pasture treatments, respectively. Cows grazing pasture and receiving no supplemental feed had 500% more conjugated linoleic acid in milk fat than cows fed typical dairy diets (Experiment 1). In Experiment 3, cows were fed either a control diet containing 55% alfalfa silage and 45% grain, or similar diets supplemented with 3% fish meal, or 250 g of monensin/cow/per day, or fish meal and monensin together. Conjugated linoleic acid was 5.3, 8.6, 6.8, and 8.9 mg/g of milk fatty acids in the control, fish meal, monensin, and fish meal plus monensin treatments, respectively. In Experiment 4, cows were fed either finely chopped alfalfa hay (Treatment 1), or coarsely chopped alfalfa hay (Treatment 2) in a 50% forage and 50% grain diet, or 66.6% grass hay and 33.4% grain (Treatment 3), or 98.2% grass hay (Treatment 4). Conjugated linoleic acid was 7.3, 8.3, 9.0, and 7.9 mg/g of milk fatty acids in treatments 1 through 4, respectively.
(**Key words:** conjugated linoleic acid, dairy cow, milk, rumen)

Abbreviation key: CLA = conjugated linoleic acid, **CS** = corn silage, **FM** = fish meal, **HOC** = high oil corn, **HMEC** = high moisture ear corn, **MN** = monensin, **NC** = normal corn.

INTRODUCTION

Conjugated dienoic derivatives of linoleic acid are a series of positional and geometric isomers of linoleic acid (*cis*-9, *cis*-12 octadecadienoic acid). Conjugated linoleic acid (**CLA**) occurs naturally in foods; however, the principal dietary sources are dairy products and other foods derived from ruminant animals (3). The primary isomer of CLA is *cis*-9, *trans*-11 octadecadienoic acid. It accounts for more than 82% of the total CLA isomers in dairy products (3).

Conjugated linoleic acid has been shown to have anticarcinogenic properties (13, 14, 16, 28), and to be antiatherogenic in hamsters (25) and rabbits (23). The average CLA content of milk will vary between 3 and 5.5 mg/g of fat. Typical consumption of CLA by humans is lower (on an equivalent BW basis) than the dose that has been shown to be effective in reducing tumors in animal models (17). The intake of CLA can be increased either by increasing the consumption of foods of ruminant origin, or by increasing the CLA content of milk and meat. The latter approach is more practical. Increasing the CLA content of milk has the potential of increasing the nutritive and therapeutic value of milk.

The *cis*-9, *trans*-11 CLA isomer is an intermediate product of biohydrogenation of linoleic acid by the rumen bacterium, *Butyrivibrio fibrisolvens* (20). Biohydrogenation in the rumen is affected by the type and amount of fatty acid substrate (27), forage to grain ratio (8), and nitrogen content of the diet (10). Changes in substrate supply and extent of biohydrogenation will affect the supply of intermediate and end products of biohydrogenation, thus influencing the CLA content of milk and meat from ruminants (4, 19).

Recently, Jiang et al. (18) reported a variation of 2.5 to 17.7 mg of CLA/g of milk fatty acids and suggested that the CLA content of milk could be increased through manipulation of the cow's diet. The nutritional and management factors that influence the CLA content of milk have not been studied extensively. The objective of the present research was to determine the CLA con-

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tent of milk from cows managed under different dietary regimens.

MATERIALS AND METHODS

Four experiments were conducted to determine the CLA content of milk from cows managed under different dietary regimens. The experimental details for each experiment are given below.

Experiment 1

The main objective of this experiment was to study the influence of feeding additional fat through high oil corn grain and corn silage (**CS**) compared with normal corn and CS on feed intake, milk yield, milk composition and milk fatty acid composition in dairy cows (5). The objective of this paper is to report on that part of the experiment relating fat content of corn to CLA content of milk fat.

Animals and treatments. Thirty-two multiparous and 22 primiparous cows were blocked according to milk yield in the previous lactation and expected calving date, respectively. Cows within each block were randomly assigned to either normal corn treatment (**NC**) or high oil corn treatment (**HOC**). Immediately after calving, all cows were fed a pretrial diet containing (DM basis): 15.5% alfalfa silage, 12.0% chopped alfalfa hay, 22.5% CS, 31.5% high moisture ear corn (**HMEC**), 10% soybean meal, 4.6% roasted soybeans, 2.0% blood meal, 0.2% sodium bicarbonate, 1.0% di-calcium phosphate, and 0.7% trace-mineralized salt. Vitamins were supplemented in the following amounts: vitamin A, 148,500; vitamin D, 49,500; and vitamin E, 495 IU/d and per cow. The CS and HMEC in the pretrial diet were a 50:50 mixture of normal and high oil types. Experimental diets were fed from wk 3 to 24 of lactation. The ingredient composition of the experimental diets was the same as the pretrial diet, except that alfalfa hay was replaced by alfalfa silage. All alfalfa silage used in this and subsequent experiments was inoculated with *Lactobacillus plantarum*, *Pediococcus acidilactici*, and *Streptococcus faecium* at the time of ensiling with 10^5 cfu/g of silage (Medipharm, USA, Des Moines, IA). Cows in the NC treatment were fed normal HMEC and normal CS, whereas cows in the HOC treatment received high oil HMEC and high oil CS. The corn was grown at the U.S. Dairy Forage Research Center farm at Prairie du Sac, Wisconsin. A mixture of corn hybrids LOL-5501 and LOL-4401 was used for high oil HMEC, and a hybrid LOL-522 of comparable maturity was used for normal HMEC. High oil corn hybrid LOL-671 and normal corn variety DK-512 of comparable maturity were used for making corn silage. The hybrids LOL-5501, LOL-4401, LOL-522, and LOL-671 were obtained from Cenex Land O'Lakes Company (Cenex Land O'Lakes, Webster City, IA). The corn cultivar DK-512 was obtained from Dekalb Genetics Corp. (Dekalb Genetics Corp., Dekalb, IL).

Sampling and analysis. Diets were fed as a TMR once daily. Feed orts were restricted to 5 to 10% of intake on an as-fed basis. Daily feed intake and milk yield were recorded. Weekly milk samples were collected from two consecutive a.m. and p.m. milkings. Milk samples were preserved with bronopol-B2 and stored at 4°C until further analysis. Preserved milk samples were analyzed for fat and protein content by the AgSource Cooperative Services (Milk Analysis Laboratory, Menomonie, WI) by infrared procedures with a Fossomatic-605, with a B filter. Final milk composition was expressed on weighted a.m. and p.m. milk yield.

Milk samples were collected for fatty acid analysis from a subset of 13 cows (6 and 7 cows in NC and HOC treatments, respectively) twice during the experiment at an interval of 5 wk from two consecutive a.m. and p.m. milkings. Cows were between 73 to 162 DIM at the time of first milk sampling for fatty acid analysis. Weighted composite milk samples from a.m. and p.m. milkings were analyzed for fatty acid composition, including CLA by the following procedure. Fat was extracted by boiling in a detergent solution as described by Hurley et al. (15). Net fat was weighed, capped under argon gas and stored, if necessary, at –20°C until further analysis. Extracted fat was derivatized to methyl esters by mixing 30 mg of fat with 5 ml of 4% HCl-methanol (3). Heptadecanoic acid was used as an internal standard. The methyl esters were extracted with 5 ml of hexane and 1 ml of distilled water. The hexane extract was washed twice with distilled water and dried over anhydrous sodium sulfate.

Fat samples were analyzed in a gas chromatograph (Model 5890 Series II, Hewlett-Packard Co., Wilmington, DE) fitted with a flame ionization detector and 3396A integrator. Samples containing methyl esters in hexane (1 to 3 μl) were directly injected through the splitless injection port onto Supelcowax 10, fused silica capillary column (60 m × 0.32 mm i.d, 0.25-μm film thickness; Supelco Inc., Bellefonte, PA). Gas chromatography conditions were: injector temperature, 250°C; oven temperature, initial 50°C, increased to 200°C at 20°C per min and held for 50 min; oven temperature was then increased to 225°C at 10°C per min and held for 20 min; detector temperature was 250°C. The CLA standards used were synthesized as described by Chin et al. (3). Fatty acids were identified by comparing the retention times with the methylated fatty acid standards including CLA. The CLA isomer reported is *cis*-9, *trans*-11 $C_{18:2}$. The percentage of each fatty acid was

TABLE 1. Fatty acid profile and amount of total fatty acids in diets and supplements used in Experiments 1 through 4.

Diets and treatments	Fatty acids reported (%)							Total fatty acids[1] (g/100 g of DM)
	$C_{14:0}$	$C_{16:0}$	$C_{16:1}$	$C_{18:0}$	$C_{18:1}$	$C_{18:2}$	$C_{18:3}$	
Experiment 1[2]								
NC	0.65	21.1	0.86	2.67	17.6	43.8	12.9	3.43
HOC	0.65	20.2	0.81	3.00	19.9	41.2	12.7	4.38
Experiment 2[3]								
1/3 PS + supplement	2.42	19.8	0.25	2.70	20.4	43.9	10.5	6.20
2/3 PS + supplement	4.82	22.9	0.47	3.34	14.6	35.5	18.4	5.73
All PS	1.48	20.0	1.24	2.58	4.2	18.9	51.6	1.4
Experiment 3[4]								
Control and MN	1.08	24.0	0.95	1.98	11.0	35.2	25.0	2.85
FM[5] and MN + FM	1.45	24.6	1.52	2.06	11.0	33.5	25.1	2.87
Experiment 4[6]								
Treatments 1 and 2	3.35	20.7	0.45	2.34	21.4	43.0	6.8	4.28
Treatment 3	1.03	22.2	0.53	2.05	10.8	29.7	32.0	2.55
Treatment 4	1.51	22.4	0.78	2.04	5.0	20.2	46.3	1.36

[1]Sum of $C_{14:0}$ through $C_{18:3}$.

[2]Treatment diets contained either normal (NC) or high oil (HOC) high moisture ear corn and corn silage.

[3]Cows on one-third pasture (1/3PS), two-third pasture (2/3PS), and all pasture (PS) treatments consumed one-third, two-third or all of their daily feed from pasture, respectively. The balance of feed for treatments 1/3PS and 2/3PS was supplied by a supplement.

[4]Cows were fed either a control diet, or diets containing 3% fish meal (FM), or 250 mg of monensin/cow per d (MN) or both (MN + FM).

[5]Fish meal supplied some longer chain fatty acids such as $C_{18:4}$, $C_{20:1}$, $C_{20:5}$, $C_{22:1}$, $C_{22:5}$, and $C_{22:6}$.

[6]Cows on treatments 1 and 2 were fed diets containing 50% forage and 50% grain (DM basis). Alfalfa hay used in treatment 1 was finely chopped, and high moisture ear corn was finely ground. For treatment 2, hay was coarsely chopped, and high moisture ear corn was coarsely ground. Cows on treatments 3 and 4 were fed diets containing 66.6% and 98.2% coarsely chopped grass hay, respectively.

calculated by dividing the area under the fatty acid peak by the sum of the areas under the total reported fatty acid peaks. Total fatty acid content and fatty acid profile of feed ingredients collected during the milk sampling period were determined by the procedure of Sukhija and Palmquist (32). Fatty acid composition of the experimental diets was calculated from fatty acid composition of individual dietary ingredients (Table 1). Diets contained (DM basis): 19.1% CP and 3.43% fatty acids (sum of $C_{14:0}$ to $C_{18:3}$ fatty acids) in the NC treatment and 19.3% CP and 4.38% fatty acids in the HOC treatment.

Experiment 2

The main objective of this experiment was to measure the impact of grain supplementation on milk yield, milk composition, milk fatty acid composition, and reproductive performance of Holstein dairy cows in a seasonal calving and grazing system. The objective of this paper is to report on the impact of grazing on CLA content of milk fat.

Animals and treatments. Fifty-four Holstein dairy cows were randomly assigned before calving to one of three treatment groups according to the expected calving date. The three treatment groups differed in the amount of feed consumed in the form of pasture and were: one-third pasture (1/3PS), two-third pasture (2/3PS), and all pasture (PS). Cows calved between March and May of 1995. From early May until the end of September cows grazed a permanent pasture containing primarily a mixture of: *Poa pratensis* (blue grass), *Elytrigia repens* (quackgrass), *Bromus inermis* (bromegrass) and *Trifolium repens* (white clover). During the grazing season, cows in 1/3PS, 2/3PS, and PS groups consumed approximately 1/3, 2/3 or all of their daily feed from the pasture, respectively. The balance of feed for the 1/3PS and 2/3PS treatments was supplied by a supplement. The supplement for the 1/3PS group contained (DM basis): 25% alfalfa hay, 48.3% coarsely ground HMEC, 6% soybean meal, 18% roasted cracked soybeans, and 2.7% of a mineral and vitamin mix. The supplement for the 2/3PS group contained (DM basis): 50% alfalfa hay, 28.4% coarsely ground HMEC, 18% roasted cracked soybeans, and 3.6% of a mineral and vitamin mix. Cows in the three treatment groups were grazed as a single group. Pasture was managed under an intensive rotational grazing system. Cows were moved daily to a new paddock after the morning milking, and were offered a constant amount of supplement after each milking throughout the grazing season. The CP contents of the 1/3PS and 2/3PS supplements were

19.5 and 19.6% (DM basis), respectively. Cows were fed supplement as a group. Supplement offered and orts were recorded daily. The amount of grass consumed was estimated from calculated net energy needs. The NE_L intake from grass was calculated using milk energy output (33) plus energy spent for maintenance and BW gain (24) minus energy intake from the supplement. The estimated NE_L value used for grass was 1.36 Mcal/kg of grass DM.

Daily milk yield was recorded. Weekly milk samples collected from consecutive a.m. and p.m. milkings were analyzed for fat and protein contents as described in Experiment 1. Milk samples were collected from consecutive a.m. and p.m. milkings for fatty acid analysis from a subset of randomly selected 35 cows (12, 11, and 12 cows in 1/3PS, 2/3PS, and PS treatments, respectively) once during the month of September. Weighted composite milk from a.m. and p.m. milkings and feed samples were analyzed for fatty acid composition, including CLA, as described in Experiment 1. Fatty acid composition of the diets was calculated from fatty acid composition of individual dietary ingredients (Table 1). During each grazing cycle, forage samples were collected from 4 paddocks before grazing to monitor the quality of the pasture. Pasture samples collected during the time of milk sampling for fatty acid analysis were dried at 60°C for 48 h and analyzed for total fatty acid content and fatty acid composition as described in Experiment 1. During the entire grazing season, forage from the pasture contained (DM basis) the following contents of CP, NDF, and ADF: 19.0% ± 3.9 SD; 50.6% ± 7.5 SD and 25.8% ± 4.6 SD, respectively.

Experiment 3

The main objective of this experiment was to determine the influence of feeding fish meal (SEA-LAC®, ruminant-grade menhaden fish meal; Zapata Hayne Corp., Hammond, Los Angeles, CA) and monensin (Rumensin®; Elanco Animal Health, Lilly Corporate Center, Indianapolis, IN) on milk yield, milk composition, and milk fatty acid composition of lactating dairy cows fed diets containing alfalfa silage (2). The objective of this paper is to report on that part of the study relating dietary influence on CLA content of milk fat.

Animals and treatments. Forty-eight multiparous Holstein dairy cows in two groups of 24 each were blocked according to milk yield. At the beginning of the experiment, cows were an average 53 to 80 DIM in groups 1 and 2, respectively. Cows within each block were randomly assigned to four treatment groups. The four treatment groups were control, fish meal (**FM**), monensin (**MN**), and monensin + fish meal (**MN + FM**). Cows in control treatment were fed diets containing (DM basis): 55% alfalfa silage, 40% coarsely ground HMEC, 2.9% soybean meal, 1.0% ground dry shelled corn, and 1.1% of a mineral and vitamin mix. Diets for FM, MN, and MN + FM treatments contained either 3% fish meal (DM basis) or 250 mg of monensin per cow/d or both, respectively. Fish meal was added by replacing HMEC and monensin was mixed with ground dry shelled corn. Diets were fed as a TMR once daily. Cows were fed experimental diets for 12 wk. Feed orts were restricted to 5 to 10% of intake on an as fed basis.

During the experiment, daily feed intake and milk yield were recorded. Milk samples for fatty acid composition were collected from consecutive a.m. and p.m. milkings during wk 5, 6, and 12 of the experiment. Weighted composite milk samples from a.m. and p.m. milkings were analyzed for fatty acid composition as described in Experiment 1. Feed samples collected during the sampling period for milk fatty acid analysis were dried at 60°C for 48 h and analyzed for total fatty acid content and fatty acid composition as described in Experiment 1. Fatty acid composition of the diets was calculated from fatty acid composition of the individual dietary ingredients (Table 1).

Experiment 4

The objective of this experiment was to study the influence of feeding diets containing either high proportions of grain or grass hay on CLA content of milk fat. The grass hay was harvested from paddocks that were adjacent to those grazed in Experiment 2.

Animals and treatments. Twenty mid-lactation Holstein dairy cows were blocked according to milk yield. Cows within each block were randomly assigned to four treatments. At the beginning of the experiment, cows in four treatments were between 182 to 216 DIM and were producing an average 34 kg of milk/d. The experiment lasted 8 wk. Cows in treatments 1 and 2 were given diets containing (DM basis): 22.5% alfalfa hay, 22.5% corn silage, 36% HMEC, 8% soybean meal, 9% roasted cracked soybeans, and 2.0% of a mineral and vitamin mix. In treatment 1, alfalfa hay was finely chopped and HMEC was finely ground, whereas in treatment 2, alfalfa hay was coarsely chopped and HMEC was coarsely ground. The coarsely ground HMEC was prepared by passing through a roller mill and finely ground HMEC was prepared by passing HMEC through a hammer mill (Meter/Mill; Clay Equipment Corp., Cedar Falls, IA) with a 0.95-cm screen. Cows in treatment 3 were fed a diet containing (DM basis): 66.6% coarsely chopped grass hay, 16% coarsely ground HMEC, 11.6% soybean meal, and 4% roasted cracked soybeans. Cows in treatment 4 were fed a diet containing 98.2% coarsely chopped grass hay (DM basis). The grass hay was made from pasture similar to that used for grazing cows in Experiment 2. The remainder of the diet for treatments 3 and 4 was a mineral and vitamin mix.

Daily feed intake and milk yield were recorded from wk 3 through 10 of the experiment. Milk samples were collected from two consecutive a.m. and p.m. milkings during wk 6, 9, and 10 of the experiment. Weighted composite milk samples from wk 6, 9, and 10 were analyzed for fat and protein content. Weighted composite milk samples from wk 6 and 10 were analyzed for fatty acid composition, including CLA. Samples of feed ingredients were collected during each week and composite samples for the entire experiment were analyzed for total fatty acid content and fatty acid composition. The procedures used for the milk and feed analyses were similar to the ones described in Experiment 1. The fatty acid composition of the diets was calculated from fatty acid composition of the individual dietary ingredients (Table 1).

Statistical Analysis

Data were analyzed as a mixed model by general linear models procedures of SAS (29). The model used for the analysis of feed intake, milk yield, and milk fatty acid data from Experiment 1 included: treatment, cow within treatment, week, and week by treatment interaction. The error term used for treatment was cow within treatment. The model used for Experiment 2 was the same as in Experiment 1, except there was no week or week by treatment interaction. The model used for fatty acid analysis in Experiment 3 included: group, treatment, treatment by group interaction, cow within treatment by group interaction, week, and week by treatment interaction. The error term used for the treatment and treatment by group interaction was cow within treatment by group interaction. The random error term used was cow within treatment. The model used for the analysis of Experiment 4 included: block, treatment, cow within block by treatment interaction, week, and week by treatment interaction. The error term used for the treatment in this model was cow within block by treatment interaction. Least square means were compared using protected least significant difference. Results were expressed as least squares means. Significance was declared at $P < 0.05$ unless otherwise noted.

RESULTS AND DISCUSSION

Experiment 1

Cows in Experiment 1 were fed diets containing forage and grain in a 50:50 ratio that were formulated to meet the nutrient demand of cows according to NRC (24). The NC and HOC diets had similar CP content. Additional oil from high oil HMEC and CS increased the total fatty acid content in the HOC diet by approximately one percentage unit compared with NC (Table 1). The HOC diet had a slightly higher proportion of $C_{18:0}$ and $C_{18:1}$ fatty acids, whereas the NC diet had a slightly higher proportion of $C_{18:2}$ fatty acids. Because of the presence of corn and soybeans, both diets were rich in $C_{18:2}$ fatty acid. No detectable CLA was found in any of the dietary ingredients.

Dry matter intake, milk yield, and milk composition data from the week during which milk was collected for fatty acid analysis are presented in Table 2. Cows in the NC and HOC treatments had similar daily DMI, milk, FCM, milk fat yield, milk fat, and milk protein content. In the studies of Atwell et al. (1) and LaCount et al. (21), daily DMI was not changed by high oil CS, however, DMI was higher for cows fed diets containing high oil corn grain. Others have reported that DMI was not affected by the inclusion of either high oil CS (30) or high oil corn grain (7) in the diets of lactating dairy cows. Milk yield and composition were not affected by feeding high oil CS (1, 21, 30) or high oil corn grain (1,

TABLE 2. Feed intake, milk yield, and fatty acid composition of milk from cows fed diets containing normal (NC) versus high oil corn (HOC) and corn silage (Experiment 1).[1]

	Treatment[2]			
Item	NC	HOC	SEM	P
Feed intake, kg/d	27.7	28.4	1.1	0.7
Milk yield, kg/d	40.4	43.5	2.4	0.5
3.5% FCM Yield, kg/d	40.7	40.7	2.3	0.4
Milk fat, %	3.60	3.10	0.26	0.7
Milk protein, %	3.07	2.90	0.08	0.8
Milk fat yield, kg/d	1.44	1.34	0.08	0.5
Fatty acid composition, mg/g of fatty acids				
$C_{10:0}$	30.7	27.1	1.3	0.4
$C_{12:0}$	41.0	35.2	1.6	0.4
$C_{14:0}$	130	120	3.0	0.6
$C_{16:0}$	325	301	9.0	0.7
$C_{16:1}$	16.8	14.3	0.6	0.2
$C_{18:0}$	93	119	4.0	0.2
$C_{18:1}$	206	228	6.0	0.5
$C_{18:2}$	35.6	36.4	1.3	0.5
CLA[2]	3.8	3.9	0.3	0.6
$C_{18:3}$	7.6	6.6	0.3	0.7
SFA[3]	620	602	11.0	0.6
UFA[4]	269	290	7.0	0.6
Others[5]	111	109	4.0	0.6

[1]Feed intake and milk yield data are from those cows whose milk was sampled for fatty acid analysis and represents the mean of two 1-wk periods during which milk was sampled for fatty acid analysis.

[2]Conjugated linoleic acid (*cis*-9, *trans*-11 $C_{18:2}$).

[3]Sum of $C_{10:0}$, $C_{12:0}$, $C_{14:0}$, $C_{16:0}$, and $C_{18:0}$.

[4]Sum of $C_{16:1}$, $C_{18:1}$, $C_{18:2}$, CLA, and $C_{18:3}$.

[5]100-sum of $C_{10:0}$ through $C_{18:3}$.

21). A one percentage unit increase in dietary fat content of the HOC treatment was probably not sufficient to support the changes in milk and milk components needed for detection. The data from all animals used in this experiment also showed no significant change in milk and milk composition (5). The proportions of medium and long chain fatty acids in milk were the same in the NC and HOC treatments (Table 2). Numerically, there was a decrease in the proportion of $C_{10:0}$ to $C_{16:1}$, and an increase in $C_{18:0}$ and $C_{18:1}$ fatty acids in the HOC treatment compared with the NC treatment, however, these changes were not statistically significant. The additional dietary fat from high oil HMEC and CS probably was not enough to produce significant changes in milk fatty acid composition in this experiment.

Feeding high oil HMEC and CS did not affect the concentration of CLA in milk compared with normal HMEC and CS. Average CLA concentration in the NC and HOC treatments was 3.9 mg/g of fatty acids in milk. Conjugated linoleic acid is considered as an intermediate product of biohydrogenation of $C_{18:2}$ fatty acid in the rumen (20). No change in CLA concentration was observed in the HOC treatment, probably because high oil HMEC and CS were slightly higher in $C_{18:0}$ and $C_{18:1}$ than $C_{18:2}$. Once again, the additional fat in the HOC treatment was probably not enough to produce significant change in milk fatty acid composition, including CLA. Total saturated and unsaturated fatty acid concentrations were not different between treatments.

Experiment 2

Supplements fed to cows in the 1/3PS and 2/3PS treatments were relatively high in $C_{18:2}$ content because HMEC and roasted soybeans were present. More than 50% of the total fatty acids in HMEC and roasted soybeans are $C_{18:2}$, whereas grasses are a rich source of $C_{18:3}$ (Table 1). Decreasing the supplement and increasing the proportion of pasture in the diet decreased total dietary fat, decreased the proportion of $C_{18:1}$ and $C_{18:2}$, and increased the proportion of $C_{18:3}$ in the diets (Table 1). The relative amounts of $C_{16:1}$ were greater with diets containing larger amounts of pasture.

Daily intake of supplement, calculated grass intake, and milk yield data from the week during which milk was collected for fatty acid analysis are presented in Table 3. As per the design of the experiment, cows in the 1/3PS treatment consumed a larger amount of supplement compared with cows in the 2/3PS treatment. Grass consumption from pasture increased as the amount of supplement was decreased in 2/3PS. Cows in PS treatment consumed only pasture grass, and were eating an estimated 14.1 kg of grass DM per day (Table 3). As expected, cows in the 1/3PS treatment, because of higher intake of nutrients through the supplement, produced more milk. The 2/3PS and PS treatments followed them. Milk fat and protein percentages were not different among treatments. Higher milk yield and no change in milk fat content resulted in higher milk fat yield in the 1/3PS followed by the 2/3PS and PS treatments.

Concentrations of $C_{10:0}$ to $C_{16:0}$ fatty acids were not different among treatments (Table 3). A reduction in the proportion of short and medium chain fatty acids is typically observed following supplementation of a diet with long-chain fatty acids (6, 12). This effect was not observed in the present experiment, probably because the oil was added through seeds. Feeding oilseeds will result in slow release of oil from the seed and may not curtail rumen fermentation and de novo synthesis of fatty acids (12). Moreover, the severity of depression in short and medium chain fatty acids is positively correlated with the amount of lipid available from the diet (12). An increased supply of $C_{16:1}$ from the pasture resulted in a higher concentration of $C_{16:1}$ in the milk fat of cows in the PS treatment compared with cows in the 1/3PS and 2/3PS treatments. The concentration of $C_{18:0}$ in milk fat was higher in cows given supplement (1/3PS and 2/3PS) compared with cows that grazed pasture only (PS). The proportion of $C_{18:1}$ remained unaffected by treatments.

The CLA in milk increased linearly as the amount of pasture was increased in the diet. Cows grazing pasture alone (PS) had 150 and 53% more CLA in milk fat than cows in the 1/3PS and 2/3PS treatments, respectively, and 500% more CLA in milk fat than cows fed diets containing forage and grain in a 50:50 ratio (Experiment 1, Table 2). Calculated forage and grain ratios in 1/3PS, 2/3PS, and all PS treatments were 46:54, 80:20 and 100:0, respectively. However, majority of forage in the Experiment 2 was supplied as green grass from the pasture compared with conserved forage in Experiment 1. In another study, milk from cows fed conserved alfalfa hay in diets containing forage to grain ratio of 50:50 or 20:80 had 3.5 and 3.3 mg of CLA/g of fatty acids, respectively (11).

The proportion of $C_{18:3}$ increased in milk fat as the amount of feed from pasture increased in the diet (Table 3). Long-chain polyunsaturated fatty acids ($C_{18:2}$ and $C_{18:3}$) are subjected to biohydrogenation processes in the rumen (20). The intermediate steps for converting $C_{18:2}$ to CLA have been suggested by Kepler and Tove (20). It is not known if $C_{18:3}$ can be converted to CLA. This study suggests that $C_{18:3}$ might be a substrate for conversion to CLA simply because $C_{18:3}$ is the predominant unsaturated fatty acid in pasture grass, and it

TABLE 3. Consumption of supplement, milk yield, and fatty acid composition of milk from cows grazing permanent pasture (Experiment 2).[1]

	Treatment[2]				
Item	1/3PS	2/3PS	PS	SEM	P^3
Supplement intake	11.6[a]	6.0[b]	. . .	0.2	0.01
Grass intake,[4] kg/d	4.5[c]	9.0[b]	14.1[a]	0.3	0.01
Milk yield, kg/d	24.5[a]	17.5[b]	14.5[c]	0.9	0.01
3.5% FCM Yield, kg/d	24.5[a]	18.0[b]	14.2[c]	0.9	0.01
Milk fat, %	3.51	3.64	3.37	0.1	0.2
Milk protein, %	2.90	2.73	2.86	0.06	0.09
Milk fat yield, kg/d	0.86[a]	0.64[b]	0.49[c]	0.03	0.01
Fatty acid composition, mg/g of fatty acids					
$C_{10:0}$	21.1	18.0	18.0	1.1	0.09
$C_{12:0}$	26.0	22.3	23.3	1.3	0.2
$C_{14:0}$	94	89	91	3	0.5
$C_{16:0}$	247	240	251	5	0.2
$C_{16:1}$	12.3[b]	13.1[b]	17.6[a]	0.6	0.001
$C_{18:0}$	152[a]	151[a]	121[b]	6	0.001
$C_{18:1}$	314	333	326	7	0.12
$C_{18:2}$	42.7[a]	27.1[b]	14.0[c]	1.5	0.001
CLA[5]	8.9[c]	14.3[b]	22.1[a]	0.9	0.001
$C_{18:3}$	8.1[c]	14.6[b]	20.2[a]	0.5	0.001
SFA[6]	540[a]	520[b]	505[b]	7	0.002
UFA[7]	386	402	400	7	0.2
Others[8]	74[b]	78[b]	95[a]	3	0.001

[a,b,c]Means with unlike superscripts within row differ according to *P* value indicated.

[1]Milk yield data are from only those cows whose milk was sampled for fatty acid analysis and represents the 1-wk period during which milk was sampled for fatty acid analysis.

[2]Cows on one-third pasture (1/3PS), two-third pasture (2/3PS), and all pasture (PS) treatments consumed one-third, two-third or all of their daily feed from pasture, respectively. The balance of feed for treatments 1/3PS and 2/3PS was supplied by a supplement.

[3]$P < 0.001$ is shown as $P = 0.001$.

[4]Estimated from net energy intake from grass. The NE_L intake from grass was calculated as milk energy output (33) + energy spent for maintenance and BW gain (24) – energy intake from the supplement. The NE_L value used for grass was 1.36 Mcal/kg of grass DM.

[5]Conjugated linoleic acid (*cis*-9, *trans*-11 $C_{18:2}$).

[6]Sum of $C_{10:0}$, $C_{12:0}$, $C_{14:0}$, $C_{16:0}$, and $C_{18:0}$.

[7]Sum of $C_{16:1}$, $C_{18:1}$, $C_{18:2}$, CLA, and $C_{18:3}$.

[8]100-sum of $C_{10:0}$ through $C_{18:3}$.

was the pasture dominated diets that supported the highest CLA content of milk fat.

Experiment 3

There was no group by treatment interaction for feed intake or milk yield. Therefore, combined results for both groups of cows from wk 5, 6, and 12 during which milk was collected for fatty acid analysis are presented in Table 4. Daily feed intake, milk yield, 3.5% FCM yield and milk protein content of cows were the same in control, FM, MN, or MN + FN treatments. Milk fat content was reduced in FM and MN + FM treatments compared with cows in control and MN treatments. Others (31) have observed similar decreases in milk fat content with diets containing fish meal. Decreased milk fat contents in FM and MN + FN treatments were balanced by a small increase in milk yield, resulting in similar milk fat yield among treatments.

Group by treatment interaction was nonsignificant for all fatty acids, except for CLA. Combined results for milk fatty acids of cows in both groups are presented in Table 4. The CLA concentrations were also analyzed separately for each group and are discussed later. Concentrations of $C_{10:0}$ to $C_{18:0}$ fatty acids were similar among treatments. Long-chain fatty acids ($C_{18:1}$, $C_{18:2}$, and $C_{18:3}$) remained unaffected in FM, MN, and MN + FM treatments compared with the control treatment.

Concentrations of CLA were increased in FM and MN + FM treatments compared with cows in the control treatment (Table 4). The average values for CLA in control, FM, MN, and MN + FN treatments were 5.5^c, 7.4^{bc}, 8.1^b, and 10.3^a mg/g of milk fatty acids (SEM = 0.4) in cows of group 1 and 5.1^c, 9.8^a, 5.5^{bc}, and 7.5^b mg/g of milk fatty acids (SEM = 0.5) in cows of group 2, respectively. The increases in CLA in FM and MN + FM treatments were small compared with increases observed in cows grazing pasture in Experiment 2. Cows in group 1 showed a trend for increased CLA in FM, MN, and MN + FN treatments, however, cows in group 2 had increased CLA concentrations in the FM and MN + FM treatments only. Further research is needed to determine the influence of fish oil on ruminal biohydrogenation of fatty acids, production of intermediate products during this process, and influence of biohydrogenation on milk CLA content. The concentrations of total saturated and unsaturated fatty acids in milk fat remained unaffected by treatments (Table 4).

Experiment 4

Treatment by week interaction was nonsignificant for feed intake and milk yield measurements. The combined results from wk 3 through 10 are presented in Table 5. As expected, cows fed high grain diets in treatments 1 and 2 had higher feed intake and produced more milk compared with cows fed high forage diets in treatments 3 and 4. Feeding alfalfa hay as finely chopped and finely ground HMEC (treatment 1) versus coarsely chopped hay and coarsely ground HMEC (treatment 2) had no influence on feed intake, milk yield, 3.5% FCM, milk protein content, or daily milk fat yield.

Decreased fat content in milk of cows in treatment 2 compared with cows in treatment 1 was opposite to what would be expected under normal circumstances. The observation of pretrial fat content of cows assigned to treatment 2 suggested that they may have inherently low milk fat content. As expected, cows fed 98.2% grass hay diet in treatment 4 ate less feed and produced less milk and milk fat than cows fed 66.6% grass hay in treatment 3 (Table 5). Milk fat and protein content were the same in treatments 3 and 4.

Treatment by week interaction was nonsignificant for milk fatty acids, except for $C_{18:2}$, CLA, and $C_{18:3}$ fatty acids. Average values for fatty acids from wk 6 and 10 are presented in Table 5. The data for $C_{18:2}$, CLA, and $C_{18:3}$ fatty acids were also analyzed separately for wk 6 and 10 and results are discussed later.

Cows fed high forage diets (treatments 3 and 4) had lower concentrations of $C_{10:0}$, $C_{12:0}$, and $C_{14:0}$ fatty acids

TABLE 4. Feed intake, milk yield, and fatty acid composition of the milk from cows fed diets containing fish meal and monensin (Experiment 3).[1]

	Treatment[2]					
Item	Control	FM	MN	MN + FM	SEM	P
Feed intake, kg/d	24.3	25.1	23.7	25.1	0.6	0.3
Milk yield, kg/d	35.1	37.9	35.1	36.8	1.6	0.5
3.5% FCM Yield, kg/d	33.1	32.0	32.0	32.7	1.3	0.9
Milk fat, %	3.19[a]	2.58[b]	3.00[a]	2.91[ab]	0.15	0.05
Milk protein, %	3.07	2.98	3.10	3.09	0.09	0.8
Milk fat yield, kg/d	1.11	0.96	1.04	1.04	0.05	0.3
Fatty acid composition, mg/g of fatty acids						
$C_{10:0}$	31.7	29.4	30.1	28.7	1.2	0.3
$C_{12:0}$	39.5	36.7	37.7	35.7	1.4	0.3
$C_{14:0}$	126	120	124	122	2	0.2
$C_{16:0}$	306	296	294	295	6	0.5
$C_{16:1}$	14.9	15.3	15.5	15.1	0.7	0.9
$C_{18:0}$	105	106	101	102	3	0.5
$C_{18:1}$	224	245	241	243	6	0.08
$C_{18:2}$	27.3	27.1	27.8	27.0	0.8	0.9
CLA[3]	5.3[b]	8.6[a]	6.8[b]	8.9[a]	0.5	0.001
$C_{18:3}$	9.0	8.7	9.4	8.9	0.3	0.3
SFA[4]	608	588	586	584	8	0.16
UFA[5]	290	314	311	312	8	0.1
Others[6]	112	108	113	114	3	0.4

[a,b]Means with unlike superscripts within row differ according to P value indicated.

[1]Feed intake and milk yield data represent the period during which milk was sampled for fatty acid analysis (mean of wk 5, 6, and 12).

[2]Cows were fed either the control diet or diets containing 3% fish meal (FM) or 250 mg of monensin/cow per d (MN) or both (MN + FM).

[3]Conjugated linoleic acid (*cis*-9, *trans*-11 $C_{18:2}$). The group by treatment interaction was significant (P = 0.2) for CLA. The CLA concentrations were also analyzed separately for each group and presented in the text.

[4]Sum of $C_{10:0}$, $C_{12:0}$, $C_{14:0}$, $C_{16:0}$, and $C_{18:0}$.

[5]Sum of $C_{16:1}$, $C_{18:1}$, $C_{18:2}$, CLA, and $C_{18:3}$.

[6]100-sum of $C_{10:0}$ through $C_{18:3}$.

TABLE 5. Feed intake, milk yield, and fatty acid composition of milk from cows fed diets containing high grain or conserved forage (Experiment 4).

	Treatment[1]					
Item	1	2	3	4	SEM	*P*
Feed intake, kg/d	19.4[a]	19.2[a]	14.2[b]	13.8[b]	1.2	0.01
Milk yield, kg/d	27.7[a]	28.2[a]	18.2[b]	11.7[c]	1.7	0.01
3.5% FCM Yield, kg/d	29.5[a]	27.3[a]	19.4[b]	12.8[c]	1.6	0.01
Milk fat, %	3.77[a]	3.24[b]	4.00[a]	4.01[a]	0.17	0.03
Milk protein, %	3.64	3.47	3.13	3.28	0.17	0.2
Milk fat yield, kg/d	1.06[a]	0.93[a]	0.72[b]	0.48[c]	0.06	0.01
Fatty acid composition, mg/g of fatty acid						
$C_{10:0}$	30.3[a]	27.7[a]	21.6[b]	20.3[b]	1.7	0.004
$C_{12:0}$	40.0[a]	35.7[a]	25.9[b]	25.9[b]	2.3	0.002
$C_{14:0}$	121[a]	114[ab]	102[c]	106[bc]	3	0.009
$C_{16:0}$	286[b]	271[b]	276[b]	322[a]	9	0.004
$C_{16:1}$	17.8	14.8	17.1	20.3	1.3	0.08
$C_{18:0}$	102[bc]	116[ab]	123[a]	92[c]	6	0.01
$C_{18:1}$	244[b]	265[ab]	286[a]	256[b]	7	0.01
$C_{18:2}$	41.7[a]	44.8[a]	39.5[a]	26.1[b]	2.2	0.003
CLA[2]	7.3	8.3	9.0	7.9	0.9	0.6
$C_{18:3}$	6.9[d]	8.5[c]	10.2[b]	13.0[a]	0.5	0.001
SFA[3]	579	564	548	566	12	0.3
UFA[4]	319[b]	344[ab]	363[a]	326[b]	10	0.03
Others[5]	103	94	90	110	4	0.02

[a,b,c]Means with unlike superscripts within row differ according to *P* value indicated.

[1]Cows on treatments 1 and 2 were fed diets containing forage and grain in 50:50 ratio. The alfalfa hay used in treatment 1 was finely chopped, and high moisture ear corn was finely ground. For treatment 2, hay was coarsely chopped, and high moisture ear corn was coarsely ground. Cows on treatments 3 and 4 were fed diets containing 66.6 and 98.2% coarse chopped grass hay, respectively.

[2]Conjugated linoleic acid (*cis*-9, *trans*-11 $C_{18:2}$).

[3]Sum of $C_{10:0}$, $C_{12:0}$, $C_{14:0}$, $C_{16:0}$, and $C_{18:0}$.

[4]Sum of $C_{16:1}$, $C_{18:1}$, $C_{18:2}$, CLA, and $C_{18:3}$.

[5]100-sum of $C_{10:0}$ through $C_{18:3}$.

compared with cows fed high grain diets (treatments 1 and 2), except the $C_{14:0}$ in treatments 2 and 4 were different at $P = 0.1$. Lower amounts of $C_{10:0}$, $C_{12:0}$, and $C_{14:0}$ in milk were probably due to an increased supply of long chain fatty acids from the grass. Five fatty acids ($C_{16:0}$, $C_{16:1}$, $C_{18:1}$, $C_{18:2}$, and $C_{18:3}$) generally account for over 90% of the total fatty acids in plant leaves. As discussed earlier, an increased supply of dietary fat as long chain fatty acids has been shown to inhibit de novo synthesis of short and medium chain fatty acids in the mammary gland (6, 12). The concentration of $C_{16:0}$ was highest in the milk of cows fed all grass hay (treatment 4). The concentration of $C_{16:0}$ did not differ in treatments 1, 2, and 3. Others have reported increased $C_{16:0}$ in milk when cows were switched from low roughage to high roughage diets (12). Small differences in the dietary supply of $C_{16:1}$ among treatments did not influence the $C_{16:1}$ content of milk.

Diets containing all grass hay (treatment 4) supplied large amounts of $C_{18:3}$ and small amounts of $C_{18:1}$ fatty acid. However, in the milk of cows fed all grass hay, the proportion of $C_{18:0}$ was slightly lower and the proportion of $C_{18:1}$ was comparable to the diets containing high grain (treatments 1 and 2). Higher levels of $C_{18:1}$ in cows fed all grass hay suggest that there was incomplete ruminal biohydrogenation of unsaturated fatty acids in cows fed grass hay compared with cows fed high grain.

Reduced $C_{18:2}$ and increased $C_{18:3}$ in the milk fat of cows in treatment 4 compared with other treatments are reflective of the dietary supply of these fatty acids (6, 12). Average values for $C_{18:2}$ in treatments 1 through 4 were 42.9[a], 44.7[a], 38.9[ab], and 31.3[b] mg/g of milk fatty acids during wk 6 and 40.6[a], 44.9[a], 40.0[a], and 20.9[b] mg/g of milk fatty acids during wk 10 of the experiment, respectively. The $C_{18:3}$ fatty acid concentrations in treatments 1 through 4 were 8.2[c], 9.9[b], 11.1[b], and 16.0[a] mg/g of milk fatty acids during wk 6 and 5.6[c], 7.1[b], 9.2[a], and 10.0[a] mg/g of milk fatty acids during wk 10 of the experiment. Increased amounts of forage in the diet resulted in more $C_{18:3}$ in the milk of cows in treatment 4 followed by treatments 3, 2, and 1.

Average values of CLA in treatments 1 through 4 were 6.4[b], 6.9[b], 9.8[a], and 9.4[a] mg/g of milk fatty acids during wk 6 and 8.2, 9.7, 8.3, and 6.4 mg/g of milk fatty acids during wk 10 of the experiment. Conjugated linoleic acid concentrations in milk were higher in treatments 3 and 4 compared with treatments 1 and 2 during wk 6; however, there were no treatment differences during wk 10 of the experiment. The reason for this discrepancy is not clear. However, cow to cow variation and microbial adaptation in the rumen could contribute to this difference (18). Fatty acid composition of milk, including CLA, was not influenced by feeding finely chopped alfalfa hay and finely ground HMEC compared with coarsely chopped alfalfa hay and coarsely ground HMEC, except that $C_{18:3}$ fatty acid concentration was higher in treatment 2 compared with treatment 1.

Ruminal pH measurements were not made in these cows. However, no change in milk fat percentage in treatments 1 and 2 suggests that the difference in ruminal pH of cows fed coarsely chopped alfalfa hay and coarsely ground HMEC (treatment 2) versus finely chopped hay and finely ground HMEC (treatment 1) were probably not significant. Decreased lipolysis in the rumen has been observed in cows with low ruminal pH (22). Interestingly, grazing increased CLA content in milk significantly (Experiment 2), however, the CLA content in milk remained unaffected when grass from neighboring paddocks was harvested and fed as hay at levels of 66.6 or 98.2% in the diets. During normal drying and storing, the $C_{18:3}$ content is decreased and $C_{16:0}$ is increased in forage (26). Overall lipid composition of the preserved forage remains relatively unchanged from that prior to preservation unless there is gross deterioration. The lipolysis of triacyl-glycerol and hydrogenation of linoleic acid was reduced in vitro with increasing maturity of ryegrass (9). The overall concentration of saturated fatty acid did not differ among treatments. Unsaturated fatty acid content of milk was high in cows fed 66.6% grass hay in treatment 3 compared with cows in treatments 1 and 4. Cows in treatments 2 and 3 had similar unsaturated fatty acid content in milk.

CONCLUSION

Results from the present research show that CLA content of cow's milk fat can be increased through different nutritional and management practices. Supplying additional 1% fat in the diet through high oil corn and high oil corn silage did not influence CLA content of milk. Increasing the proportion of grazed grass from pasture in the diet of dairy cows linearly increased the CLA content of milk. Cows grazing permanent natural pasture had 500% more CLA compared with cows fed TMR containing conserved forage and grain in a 50:50 ratio. Feeding pasture grass in dry form as hay did not influence milk CLA content. Feeding fish meal increased CLA content of milk by a small margin. Studies to determine the influences of a wider range of ruminal pH on lipid biohydrogenation in the rumen are recommended.

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REFERENCES

1 Atwell, D. G., E. H. Jaster, K. J. Moore, and R. L. Fernando. 1988. Evaluation of high oil corn and corn silage for lactating dairy cows. J. Dairy Sci. 71:2689–2698.

2 Broderick, G. A. 1997. Effect of supplementing low levels of monensin to lactating dairy cows fed alfalfa silage. Pages 66–70 *in* US Dairy Forage Research Center 1997 Research Summaries. US Dept. Agric., ARS, Madison, WI.

3 Chin, S. F., W. Liu, J. M. Storkson, Y. L. Ha, and M. W. Pariza. 1992. Dietary sources of conjugated dienoic isomers of linoleic acid, a newly recognized class of anticarcinogens. J. Food Comp. Anal. 5:185–197.

4 Dhiman, T. R., E. D. Helmink, D. J. McMahon, R. L. Fife, and M. W. Pariza. 1999. Conjugated linoleic acid content of milk and cheese from cows fed extruded oilseeds. J. Dairy Sci. 82:412–419.

5 Dhiman, T. R., B. Hoogendijk, R. P. Walgenbach, and L. D. Satter. 1996. Feeding high oil corn to lactating dairy cows. J. Dairy Sci. 79(Suppl. 1):136. (Abstr.)

6 Dhiman, T. R., K. van Zanten, and L. D. Satter. 1995. Effect of dietary fat source on fatty acid composition of cow's milk. J. Sci. Food Agric. 69:101–107.

7 Elliott, J. P., J. K. Drackley, D. J. Schauff, and E. H. Jaster. 1993. Diets containing high oil corn and tallow for dairy cows during early lactation. J. Dairy Sci. 76:775–789.

8 Gerson, T., A. John, and A.S.D. King. 1985. The effects of dietary starch and fiber on the *in vitro* rates of lipolysis and hydrogenation by sheep rumen digesta. J. Agric. Sci. (Camb.) 105:27–30.

9 Gerson, T., A. John, and A.S.D. King. 1986. Effects of feeding ryegrass of varying maturity on the metabolism and composition of lipids in the rumen of sheep. J. Agric. Sci. (Camb.) 106:445–448.

10 Gerson, T., A. John, and B. R. Sinclair. 1983. The effect of dietary N on *in vitro* lipolysis and fatty acid hydrogenation in rumen digesta from sheep fed diets high in starch. J. Agric. Sci. (Camb.) 101:97–101.

11 Griinari, J. M., D. A. Dwyer, M. A. McGuire, D. E. Bauman, D. L. Palmquist, and K.V.V. Nurmela. 1998. *Trans*-Octadecenoic acids and milk fat depression in lactating dairy cows. J. Dairy Sci. 81:1251–1261.

12 Grummer, R. R. 1991. Effect of feed on the composition of milk fat. J. Dairy Sci. 74:3244–3257.

13 Ha, Y. L., N. K. Grimm, and M. W. Pariza. 1987. Anticarcinogens from fried ground beef: heat-altered derivatives of linoleic acid. Carcinogenesis 8:1881–1887.

14 Ha, Y. L., J. Storkson, and M. W. Pariza. 1990. Inhibition of benzo(a)pyrene-induced mouse forestomach neoplasia by conjugated dienoic derivatives of linoleic acid. Cancer Res. 50:1097–1101.

15 Hurley, W. L., G. J. Warner, and R. R. Grummer. 1987. Changes in triglyceride fatty acid composition in mammary secretions during involution. J. Dairy Sci. 70:2406–2410.

16 Ip, C., S. F. Chin, J. A. Scimeca, and M. W. Pariza. 1991. Mammary cancer prevention by conjugated linoleic acid. Cancer Res. 51:6118–6124.
17 Ip, C., J.A.M. Singh, H. J. Thompson, and J. A. Scimeca. 1994. Conjugated linoleic acid suppresses mammary carcinogenesis and proliferative activity of the mammary gland in the rat. Cancer Res. 54:1212–1215.
18 Jiang, J., L. Bjoerck, R. Fonden, and M. Emanuelson. 1996. Occurrence of conjugated *cis*-9, *trans*-11-octadecadienoic acid in bovine milk: effects of feed and dietary regimen. J. Dairy Sci. 79:438–445.
19 Kelly, M. L., J. R. Berry, D. A. Dwyer, J. M. Griinari, P. Y. Chouinard, M. E. Van Amburg, and D. E. Bauman. 1998. Dietary fatty acid sources affect conjugated linoleic acid concentrations in milk from lactating dairy cows. J. Nutr. 128:881–885.
20 Kepler, C. R., and S. B. Tove. 1967. Biohydrogenation of unsaturated fatty acids. J. Biol. Chem. 242:5686–5692.
21 LaCount, D. W., J. K. Drackley, T. M. Cicela, and J. H. Clark. 1995. High oil corn as silage or grain for dairy cows during an entire lactation. J. Dairy Sci. 78:1745–1754.
22 Latham, M. J., J. E. Storry, and M. E. Sharpe. 1972. Effect of low-roughage diets on the microflora and lipid metabolism in the rumen. Appl. Microbiol. 24:871–877.
23 Lee, K. N., D. Kritchevsky, and M. W. Pariza. 1994. Conjugated linoleic acid and atherosclerosis in rabbits. Atherosclerosis 108:19–25.
24 National Research Council. 1989. Nutrient Requirements of Dairy Cattle. 6th rev. ed. Natl. Acad. Sci., Washington, DC.
25 Nicolosi, R. J., and L. Laitinen. 1996. Dietary conjugated linoleic acid reduces aortic fatty streak formation greater than linoleic acid in hypercholesterolemic hamsters. Fed. Am. Soc. Exp. Biol. 10(3):A477. (Abstr. 2751)
26 Noble, R. C. 1981. Digestion, absorption and transport of lipids in ruminant animals. Pages 57–93 *in* Lipid Metabolism in Ruminant Animals. W. W. Christie, ed. Pergamon Press, New York, NY.
27 Noble, R. C., J. H. Moore, and C. G. Harfoot. 1974. Observations on the pattern of biohydrogenation of esterified and unesterified linoleic acid in the rumen. Br. J. Nutr. 31:99–108.
28 Parodi, P. W. 1997. Cow's milk fat component as potential anticarcinogenic agents. J. Nutr. 127:1055–1060.
29 SAS® User's Guide: Statistics, Version 6.03 Edition. 1988. SAS Inst., Inc., Cary, NC.
30 Spahr, S. L., J. H. Byers, and J. H. Clark. 1975. No advantage for high oil corn silage when feeding dairy cows. Illinois Res. 17(1):16–17.
31 Spain, J. N., M. D. Alvarado, C. E. Polan, C. N. Miller, and M. L. McGilliard. 1990. Effect of protein source and energy on milk composition in midlactation dairy cows. J. Dairy Sci. 73:445–452.
32 Sukhija, P. S., and D. L. Palmquist. 1988. Rapid method for determination of total fatty acid content and composition of feedstuffs and feces. J. Agric. Food Chem. 36:1202–1206.
33 Tyrrell, H. F., and J. T. Reid. 1965. Prediction of the energy value of cow's milk. J. Dairy Sci. 48:1215–1223.