Effect of Intake of Pasture on Concentrations of Conjugated Linoleic Acid in Milk of Lactating Cows[1]

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ABSTRACT

We examined the effect of intake of fresh pasture on concentrations of conjugated linoleic acid in milk fat. Sixteen Holstein cows were paired and divided into either the control group or the grazing group. The study involved initial, transition, and final periods. During the initial period, all cows consumed a total mixed diet. Cows in the control group were fed the total mixed diet throughout the study, and cows in the grazing group were gradually adjusted to a diet consisting of intensively managed pasture. Performance of cows in the grazing group was significantly reduced from that of cows in the control group during the final period (dry matter intake, 19% less; milk yield, 29.6 vs. 44.1 kg/d; and live weight, 40 kg less). During the initial period, when both groups were consuming a total mixed diet, concentrations of conjugated linoleic acid in milk fat were similar ($\overline{X}$ = 5.1 mg/g of milk fat). As the grazing group was gradually adjusted to pasture, concentrations of conjugated linoleic acid in milk gradually increased. During the final period, when cows in the grazing group were consuming a diet consisting of pasture only, conjugated linoleic acid concentrations in the milk fat were doubled (10.9 vs. 4.6 mg/g of milk fat). Furthermore, results showed the individual consistency of the milk fat content of conjugated linoleic acid over time but also demonstrated substantial variation among individual cows within treatment groups. Overall, this study indicated that the concentration of conjugated linoleic acid in milk fat is enhanced by dietary intake of fresh pasture.

(**Key words**: conjugated linoleic acid, pasture, anticarcinogen)

Abbreviation key: **CLA** = conjugated linoleic acid.

INTRODUCTION

Conjugated linoleic acid (**CLA**) is a mixture of geometric and positional isomers of linoleic acid with conjugated double bonds. Conjugated linoleic acid is a potent anticarcinogen, and the National Academy of Sciences (21) has pointed out that CLA is the only fatty acid that has been shown unequivocally to inhibit carcinogenesis in experimental animals. Conjugated linoleic acid is found predominately in food products from ruminant animals; milk and other dairy products are the major sources of CLA in the human diet (13, 23). Furthermore, the *cis*-9, *trans*-11 isomer is the major CLA isomer found in milk fat and is thought to be the biologically active form that possesses anticarcinogenic capabilities (2, 13, 24).

The content of CLA in milk fat varies widely among herds (15, 26). This variation may be related to factors that are associated with rumen fermentation because CLA originates from the incomplete biohydrogenation of unsaturated fatty acids in the rumen. The content of CLA in milk fat is affected by a number of factors, including forage to concentrate ratio (9), level of intake (14, 31), and intake of unsaturated fatty acids, especially plant oils that are high in linoleic acid (9, 16, 19). Seasonal variations in milk fat concentrations of CLA have also been reported; the highest concentrations are observed in summer (22, 26). Previous research (22, 26) has also suggested that this increase during summer is related to increased consumption of lush pastures. Consistent with this result, Timmen and Patton (31) showed higher concentrations of CLA in milk fat of cows grazing pasture, and Dhiman et al. (5) recently

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demonstrated that concentrations of CLA in milk increased as consumption of pasture increased. Banni et al. (1) also showed that concentrations of CLA in the milk fat of sheep were greater when lush pasture was consumed.

The objective of the present study was to investigate the effects of pasture in more detail by following the pattern of change in the milk fat concentration of CLA as the cow makes the transition from a total mixed diet to a diet consisting of pasture only. The design also allowed the evaluation of individual variation during the transition between diets. Although previous work (16) has demonstrated a substantial variation in the milk fat concentrations of CLA, the previous work with pasture (5, 31) involved dietary comparisons among different groups of animals.

MATERIALS AND METHODS

All research directly involving the use of animals was conducted at The Pennsylvania State University Dairy Cattle Research and Education Center (University Park) and was approved by The Pennsylvania State University Institutional Animal Care and Use Committee.

The study used 16 Holstein cows that had been exposed to intensive grazing during previous seasons. Cows were paired based on daily milk yield (46.3 ± 2.1 kg/d), live weight (603 ± 18 kg), and DIM (59 ± 7 d) ($\overline{X}$ ± SE for all cows) and divided into two groups. The study interval was divided into three periods: initial period, transition period, and final period. During the initial period, all cows were fed a total mixed diet and were confined to a free-stall unit. One group remained on the total mixed diet throughout the study (control group), and the other group was gradually adjusted to a diet consisting of intensively managed pasture (grazing group).

Pastures were grazed from May 12 to June 9, 1995, and trace mineral salt blocks and water were available on the pasture. The transition period was 2 wk in duration as cows in the grazing group were adapted to the pasture. During wk 1, cows in the grazing group were fed the total mixed diet at 50% of their previous DMI, and this amount was reduced to 25% of the previous DMI during wk 2. The total mixed diet offered to cows in the grazing group during the 2-wk transition period was identical to that offered to the control cows; no correction was made for the nutrient content of consumed pasture. During the transition period, the total mixed diet was fed to cows in the grazing group in a feed bunk located on the pasture. Supplementation of the total mixed diet was terminated after the transition period, and the diet for the final 2-wk period consisted of pasture only. Grazing management practices were as described by Kolver and Muller (18).

Details on the nutrient and chemical composition of the diets have been presented (18). The major feed components of the total mixed diet were (DM basis) 24.0% corn silage, 18.8% legume silage, 4.2% legume hay, 25.0% high moisture shelled corn, and 12.5% whole cottonseed. Overall, the total mixed diet was balanced to meet requirements (20) and, on a DM basis, had 19.1% CP and a NE_L value of 1.65 Mcal/kg. Pastures had been established and intensively grazed for 4 yr. Pastures contained approximately 53% ryegrass, 19% white clover, 21% other grasses (including orchardgrass, Kentucky bluegrass, smooth bromegrass, and tall fescue), 3% weeds, and 4% dead material, as determined by hand-plucked samples at randomly selected transects (18). To determine chemical composition, pasture was sampled by cutting five quadrants (0.268 m^2 per quadrant); the pasture was then processed and analyzed as described by Kolver and Muller (18). The chemical composition (DM basis) of the pasture averaged 25.1% CP and had a NE_L value of 1.65 Mcal/kg.

All cows were milked twice daily (0730 and 1730 h), and milk yield was recorded. Milk samples were collected at each milking for 2 consecutive d during the initial period, for 2 consecutive d at the end of the 2-wk transition period, and for the last 2 d of the final period. A preservative, 2-bromo-2-nitropropane-1,3-diol, was added to the milk samples. The milk samples were stored at 4°C until analyzed for fat and protein content by infrared spectrophotometry by the Pennsylvania DHIA Milk Testing Laboratory (State College). A duplicate set was stored at –20°C until analysis for fatty acid composition.

For fatty acid composition, milk samples were thawed and then centrifuged. Fat cakes were recovered, placed in sample vials, flushed with N_2, capped, and then placed in a –20°C freezer. Subsequently, milk fat samples were combined for each period to create a pool for each cow. Lipid extraction was according to the procedures of Hara and Radin (10) using a volume of 18 ml of hexane and isopropanol (3:2, vol/vol)/g of fat cake. After vortexing, a sodium sulfate solution (6.7% $NaSO_4$ in distilled H_2O) was added at a volume of 12 ml/g of fat cake. The hexane layer was transferred to a tube containing 1 g of $NaSO_4$, and, after 30 min, the hexane layer was removed and stored under N_2 gas at –20°C until methylation.

Fatty acid methyl esters were prepared by the transmethylation procedure described by Christie

(4). This procedure involved the addition of 2 ml of hexane (HPLC grade) to 40 mg of lipid, vortexing, and then the addition of 40 μl of methyl acetate. Then, 40 μl of methylation reagent (1.75 ml of methanol/0.4 ml of 5.4 *M* sodium methylate) were added. The mixture was vortexed and allowed to react for 10 min, and 60 μl of termination reagent (1 g of oxalic acid/30 ml of diethyl ether) were added. The sample was then centrifuged for 5 min at 2400 × *g* at 5°C, and the liquid portion was transferred to labeled vials (Wheaton, Millville, NJ) and stored at –20°C.

Fatty acid methyl esters were separated and quantified using gas chromatography (Hewlett Packard GCD system G1800 A; Hewlett Packard, Avondale, PA) equipped with HP G107A GCD software for peak integration. In general, specifications were based on those of Shantha and Decker (29) with optimization for the separation of CLA. Conditions were as follows: a fused silica capillary column (2-4082, SUPELCOWAX 10, 60-m × 0.32-mm i.d. × 0.25-μm film thickness; Supelco Inc., Bellefonte, PA), ultra-high purity He carrier gas, an electron ionization detector, and a split injection inlet (87.5:1, vol/vol). The injector and detector temperatures were maintained at 250°C. The column temperature was set at 50°C for 1 min and then was increased 20°C/min to 200°C and held for 38 min.

A butter oil standard (CRM 164; Commission of the European Communities, Community Bureau of Reference, Brussels, Belgium) was routinely chromatographed to determine rates of recoveries and correction factors for fatty acids. Additionally, a standard mixture of fatty acids (GLC-60; Nu Check Prep, Inc., Elysian, MN) was chromatographed to verify retention times and rates of recoveries. Furthermore, because CLA was of special interest, a CLA standard provided by M. Pariza (University of Wisconsin, Madison) was used to determine correct retention time for the *cis*-9, *trans*-11 isomer of CLA.

All cows completed the study. Least square means were calculated by period (initial, transition, and final) and treatment (control and grazing groups) for milk yield and milk composition data using the general linear models procedure of SAS (28). Least square means of milk yield and composition data were compared by the predicted difference method in the general linear models procedure of SAS (28). No differences ($P > 0.2$) existed among the three periods (same total mixed diet) for the control group or between the control group and the grazing group during the initial period when the same total mixed diet was fed. Differences were apparent for the grazing group when comparisons were made involving the initial period (total mixed diet) versus the transition period (total mixed diet and pasture) and the initial period versus the final period (pasture only). Interactions among cow, treatment, and period were tested and removed from the final model because they were not significant ($P > 0.2$).

RESULTS AND DISCUSSION

One focus of the present study was to compare nutrient intake of high yielding cows grazing pasture

TABLE 1. Milk yield and composition of cows fed a total mixed diet in confinement and that of cows grazing pasture.

	Dietary treatment[1]						
	Control group			Grazing group[2]			
Variable	Initial	Transition	Final	Initial	Transition	Final	SEM
Milk yield, kg/d	45.6	45.4	44.1	47.4	35.4**	29.6**	1.2
Fat							
%	3.55	3.54	3.48	3.52	3.77	3.72	0.10
kg/d	1.62	1.61	1.52	1.67	1.34†	1.10**	0.05
Protein							
%	2.69	2.75	2.80	2.75	2.84*	2.61**	0.02
kg/d	1.23	1.23	1.22	1.30	1.01**	0.77**	0.04

[1] During the initial period, both groups of cows were fed the total mixed diet. Cows in the control group remained on the total mixed diet throughout all periods. Cows in the grazing group shifted to the total mixed diet plus pasture during the transition period and then to a diet consisting of pasture only during the final period.

[2] Significant differences existed only for the grazing group; comparisons were between the initial and transition periods and the initial and final periods.

†$P < 0.1$.

*$P < 0.05$.

**$P < 0.01$.

or fed a total mixed diet and to identify nutrients that limit milk yield for cows grazing pasture. These results have been previously presented (18). Dry matter intake differed between groups. In the final period, cows grazing pasture only had reduced intake and consumed 19% less DM than did cows fed the total mixed diet. Intake of CP and NDF did not differ between treatments. However, cows in the grazing group had a reduced intake of net energy compared with cows in the control group (18).

Milk yield and composition data are presented in Table 1. Milk yield and composition remained consistent for cows in the control group throughout all periods. Cows in the grazing group had comparable milk yield and composition during the initial period when both groups were fed the total mixed diet. However, the transition from the total mixed diet to a diet consisting of pasture only resulted in decreased yields of milk, fat, and protein ($P < 0.001$). The decreased intake of net energy by cows grazing pasture was reflected not only in reduced milk yield, but also in live weight because the BW of grazing cows decreased by 40 kg from the initial period to the final period (18). Based on their reduced DMI, milk yield, and BW, cows in the grazing group had inadequate net energy intake and mobilized body reserves during the transition and final periods. During the final period, the milk yield that could be supported by the metabolizable energy intake, as determined by the Cornell Net Carbohydrate and Protein System (7, 27, 30), was 43.0 kg/d for the control group and 27.6 kg/d for the grazing group as compared with actual yields of 44.1 and 29.6 kg/d, respectively (18).

Total fatty acid composition of milk fat is presented in Table 2. The concentration of short- and medium-chain fatty acids in milk fat decreased for cows consuming pasture. During the final period, short- and medium-chain fatty acids ($C_{6:0}$ to $C_{14:0}$) made up 17.6% of the total milk fatty acids for cows in the control group, but these represented only 12.8% of the milk fat for cows in the grazing group. Thus, de novo synthesis of fatty acids in the mammary gland was decreased for cows in the grazing group. The reduction of short- and medium-chain fatty acids was offset in large part by changes in $C_{18:1}$. The content of $C_{18:1}$ in milk fat for cows in the grazing group was in-

TABLE 2. Fatty acid composition of the milk from lactating cows during initial, transition, and final periods.

	Dietary treatment[1]						
	Control group			Grazing group[2]			
Fatty acid[3]	Initial	Transition	Final	Initial	Transition	Final	SEM
	(%, wt/wt)						
6:0	2.55	2.10	2.13	2.55	2.08	1.79*	0.11
8:0	1.32	1.13	1.17	1.26	1.01*	0.91**	0.05
10:0	2.55	2.40	2.34	2.32	1.77**	1.66**	0.09
12:0	2.66	2.57	2.59	2.38	1.81**	1.70**	0.10
14:0	9.31	9.34	9.37	8.42	6.64**	6.70**	0.31
14:1	0.66	0.67	0.67	0.65	0.58	0.62	0.02
15:0	0.79	0.83	0.83	0.79	0.69**	0.88*	0.02
16:0	28.80	30.99	30.68	29.54	24.10**	24.17**	0.75
16:1	1.50	1.61	1.40	1.53	1.84**	1.85**	0.05
18:0	14.15	14.17	14.99	13.60	14.51	13.20*	0.27
18:1	28.37	26.70	26.57	29.54	35.04**	34.72**	0.91
18:2	2.58	2.51	2.62	2.60	2.53	2.25*	0.08
CLA[4]	0.47	0.43	0.46	0.54	0.71*	1.09**	0.06
18:3	0.30	0.27	0.25	0.31	0.59**	0.95**	0.05
Unidentified	3.98	4.29	3.96	4.25	6.10**	7.51**	0.29

[1] During the initial period, both groups of cows were fed the total mixed diet. Cows in the control group remained on the total mixed diet throughout all periods. Cows in the grazing group shifted to the total mixed diet plus pasture during the transition period and then to a diet consisting of pasture only during the final period.

[2] Significant differences existed only for the grazing group; comparisons were between the initial and transition periods and the initial and final periods.

[3] Method of analysis did not permit quantification of $C_{4:0}$, which is present in milk fat.

[4] Conjugated linoleic acid.

*$P < 0.05$.

**$P < 0.01$.

creased 30% compared with that for cows in the control group. Although the content of linolenic acid ($C_{18:3}$) in milk fat was low, the consumption of pasture tripled the concentration as well as increased the content of unidentified fatty acids in milk fat. The results of the present study are representative of previous work that describes changes in milk fatty acid composition and reflect both the change in the diet and the nutritional status. Increases in the content of $C_{18:0}$ and $C_{18:1}$ in milk fat and a decrease in $C_{16:0}$ are typically observed in cows grazing pasture as opposed to cows fed a total mixed diet (3, 25, 31). Additionally, previous work has demonstrated that the milk fat of cows fed less energy than required exhibited significant increases in $C_{18:1}$ and substantial reductions in short- and medium-chain fatty acids compared with the milk fat content of adequately fed cows (6, 31).

The major objective of the present study was to examine the effect of dietary intake of pasture on the CLA content of milk fat. Concentrations of CLA were affected by dietary treatment as cows consuming diets consisting of pasture had CLA concentrations that were twice as high as those of cows consuming the total mixed diet (Table 2; $P < 0.01$). Furthermore, the increase in milk fat concentrations of CLA was intermediate during the transition period when cows were consuming the total mixed diet plus pasture. In contrast, cows in the control group had relatively constant concentrations of CLA across all three periods.

Conjugated linoleic acid is formed in the rumen as an intermediate in the biohydrogenation pathway of linoleic acid. This pathway involves an initial isomerization step, resulting in the formation of a conjugated *cis*-9, *trans*-11-octadecadionic acid, which undergoes hydrogenation to *trans*-11-octadecenoic acid and further hydrogenation to stearic acid. A number of studies [see review by Harfoot and Hazlewood (11)] have investigated the specific rumen microorganisms involved in the completion of the various steps of the biohydrogenation pathway of linoleic acid. Although the linoleate isomerase and CLA reductase have been purified from the microorganism *Butyrivibrio fibrisolvens* (12, 17), apparently no one species of microorganisms carries out the full sequence of biohydrogenation.

Conjugated linoleic acid is just one of the fatty acids absorbed from the gastrointestinal tract and transported via circulation for use by the mammary gland. As for the anticarcinogenic effects of CLA, the interest is to increase the CLA content of milk fat rather than the absolute amount of CLA secreted in milk. Nevertheless, total CLA yield can be calculated in the present study and differs less between treatments than does CLA concentration because of treatment effects on milk yield (Table 1). The total yield of CLA in milk fat for the final period averaged 11.2 ± 3.4 g/d ($\overline{X} \pm SE$) for the grazing group versus 6.8 ± 1.9 g/d for the control group.

The milk fat content of CLA is affected by the intake of unsaturated fatty acids (9, 16, 19). In cows grazing pasture, the major dietary unsaturated fatty acid is linolenic acid. In a review, Viviani (33) presented a schematic diagram that suggested that CLA was formed as an intermediate in the biohydrogenation pathway of linolenic acid. However, studies (11) of the biohydrogenation of linolenic acid, *cis*-9, *cis*-12, *cis*-15-octadecatrienoic acid, have shown that it was converted to *cis*-9, *trans*-11, *cis*-15-conjugated triene, then to *trans*-11, *cis*-15-$C_{18:2}$, and finally to *trans*-11 $C_{18:1}$, *trans*-15-$C_{18:1}$, or *cis*-15-$C_{18:1}$. Therefore, linolenic pathways did not involve CLA as an intermediate, and published research does not support the suggestion of Viviani (33). However, *cis*-9, *trans*-11 CLA may be produced endogenously from *trans*-11-octadenoic acid by Δ-9-desaturase in the body tissues (8).

Other factors may affect CLA production in the rumen when cows are switched to pasture. The type and source of dietary carbohydrate may influence rates of microbial fermentation in a way that alters the rate of CLA production or utilization by rumen microbes and ultimately the concentration of CLA in milk fat. Such an effect could help explain the reported differences in the CLA content of milk fat observed between cows fed fresh forage (pasture) and cows fed preserved forages (5). Sugars, such as fructosans, starch, pectins, and soluble fiber content, greatly decline during the fermentation process used to preserve forage (32). Furthermore, during the drying process necessary to obtain hay, the forage is subject to respiration, which can lead to a 10 to 20% decrease in certain constituents, mainly, the carbohydrate fraction of the plant cell contents. Thus, the high concentrations of rapidly fermentable starch, sugars, and soluble fiber that are found in immature spring pastures may create a rumen environment and conditions that favor a greater production or a reduced utilization of CLA by the rumen bacteria. Furthermore, other factors that may affect the rumen environment and microbial population could differ in the grazing animal. Passage rate and fluid dilution rate increase because of the high water intake associated with grazing pasture. Meal size, feeding frequency, bite size, and time spent ruminating may

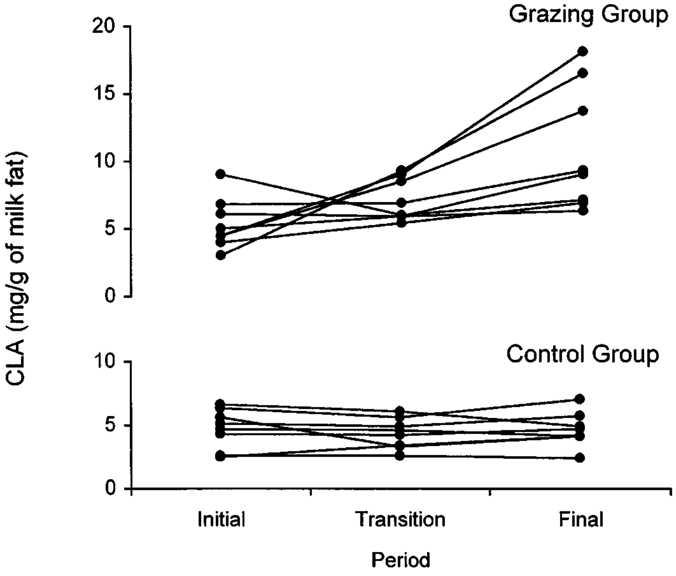

Figure 1. Individual variation among cows within the control and grazing groups across the study. Cows in the control group (n = 8) received a total mixed diet during all periods. Cows in the grazing group (n = 8) received the total mixed diet during the initial period, the total mixed diet plus pasture during the transition period, and a diet consisting of pasture only during the final period. Standard error of the mean for conjugated linoleic acid (CLA) averaged 0.6 and 0.8 mg/g of milk fat for cows in the control and grazing groups, respectively.

differ in cows grazing pasture, and these factors could be important in the alteration of rumen fermentation and the influence on rumen production and utilization of CLA.

Reil (26) and Kelly and Bauman (15) showed a wide variation in CLA concentrations among individual herds. However, variation among individual cows has been less extensively examined. This study is one of the first to examine individual differences in the effect of dietary change on CLA concentration in milk fat and the differences over time among individual cows at similar stages of lactation, consuming the same diet, and subjected to the same management regimen (Figure 1). The concentration of CLA in milk fat from the control cows was relatively constant, averaging 4.5 mg/g of lipid over the three periods of the study. Milk fat content of CLA for individual cows fed the total mixed diet was relatively consistent across study periods although there was a three-fold difference among individual cows with CLA concentrations ranging from 2.4 to 7.0 mg/g of milk fat (Figure 1). The CLA content in milk fat from cows in the grazing group showed more variation as the proportion of pasture increased. Cows in the grazing group averaged 5.4 mg of CLA/g of milk fat while consuming the total mixed diet, and the CLA content increased ($P < 0.01$) to 10.9 mg/g of milk fat when these cows consumed only pasture (Figure 1). However, not all cows responded to the same extent. The individual range in CLA concentrations for cows fed the total mixed diet was 3.0 to 9.0 mg/g of fat for the grazing group, but, when the cows were consuming only pasture, the range of CLA concentrations was 6.3 to 18.1 mg/g of milk fat. Three of the 8 cows grazing pasture only reached a CLA concentration that was >13.5 mg/g of milk fat. In contrast, 2 of the 8 cows grazing pasture had minimal response; content of CLA in the final period was increased <1 mg/g of milk fat compared with the concentration of CLA in the initial period when the total mixed diet was fed (Figure 1).

CONCLUSIONS

Overall, the present study indicated that CLA content in milk fat of dairy cows can be increased via dietary manipulation. The consumption of a diet that consisted of pasture enhanced the content of CLA in milk fat. The present study demonstrated that CLA concentrations in milk fat were doubled when cows consumed only pasture compared with those when cows were fed a total mixed diet. Additionally, this study showed the consistency of CLA content in milk fat for individual cows over time but also demonstrated a substantial individual variation in the CLA response to the change from a total mixed diet to a diet consisting of pasture only. This substantial individual variation indicated that additional factors must affect the rumen environment and conditions in a manner that alters microbial rates of production and utilization of CLA and subsequent milk fat concentrations of CLA.

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