Consumer Acceptability of Conjugated Linoleic Acid-Enriched Milk and Cheddar Cheese from Cows Grazing on Pasture*

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ABSTRACT

Two experiments were conducted to study the consumer acceptability attributes of conjugated linoleic acid (CLA)-enriched milk and cheese from cows grazing on pasture. In experiment 1, 15 cows were fed either a diet containing 51% alfalfa hay plus corn silage and 49% concentrate [total mixed ration (TMR)], were grazed on pasture, or were grazed on pasture and received 3.2 kg/d of a grain mix. The grain mix contained 75% full-fat extruded soybeans (FFES), 10% corn, 10% beet pulp, and 5% molasses. During the final 3 wk of the 6-wk experiment, milk was evaluated for sensory attributes. In experiment 2, 18 cows were fed similar diets as in experiment 1, except replacing the group of cows grazed on pasture and receiving the grain mix was a group of cows grazed on pasture and receiving 2.5 kg/d per cow of the FFES; Cheddar cheese was manufactured from milk. Average CLA contents (g/100 g of fatty acid methyl esters) were 0.52, 1.63, and 1.69 in milk and 0.47, 1.47, and 1.46 in cheese from cows fed a TMR, grazed on pasture, and grazed on pasture and fed the grain mix, respectively. An open and trained panel evaluated CLA-enriched milk for mouth-feel, color, flavor, and quality and evaluated cheese for color, flavor, texture, and quality. Open and trained panel evaluations of milk and cheese showed no differences among treatments for any of the attributes, except that the trained panel detected a more barny flavor in milk from cows grazing pasture compared with milk from cows fed the TMR only. Results suggest that consumer acceptability attributes of CLA-enriched milk and cheese from cows grazing pasture is similar to those of milk and cheese with low levels of CLA.

(Key words: conjugated linoleic acid, milk, cheese, consumer)

Abbreviation key: CLA = conjugated linoleic acid, FA = fatty acid, FFES = full-fat extruded soybean.

INTRODUCTION

Conjugated linoleic acid (CLA) has been shown to have potential health benefits, including anticarcinogenic properties in experimental animals (Ip et al., 1991; Parodi, 1997; Pariza, 1999; Hughes and Dhiman, 2002). The primary isomers of CLA that have been associated with health benefits are the *cis*-9, *trans*-11 and *trans*-10, *cis*-12 octadecadienoic acids (Ip et al., 1991; Park et al., 1999). Throughout the remainder of this paper, the term CLA will refer to the *cis*-9, *trans*-11 isomer, unless otherwise specified. The principal dietary sources of CLA are dairy and meat products from ruminant animals. The average CLA content of whole milk varies from 0.30 to 0.55 g/100 g of fatty acids (Dhiman et al., 1999a). The CLA content of milk and meat products from ruminants can be increased through manipulation of the diet such as grazing on pasture or feeding feed sources rich in linoleic and linolenic acids (Jahries et al., 1997; Dhiman et al., 1999a; Lock and Garnsworthy, 2002).

Sensory attributes play a key role in determining consumer acceptability for dairy products. Consumer acceptability of synthetic CLA-enriched dairy products containing extremely high levels of CLA appears to be low and unrealistic because of cost constraints (Campbell et al., 2003). Ramaswamy et al. (2001a) studied the oxidized flavor scores of CLA-enriched milk and butter from cows fed fish oil, extruded soybeans, or their combination and found no difference between low- and high-CLA milk or butter. There is little information about the consumer acceptability and sensory attributes of CLA-enriched milk and cheese from cows grazing on pasture. Although one objective of this study was to produce CLA-enriched milk and cheese, the study was primarily conducted to test the hypothesis that CLA-enriched milk and cheese from pasture-fed cows would be similar in consumer acceptability attributes and specific flavor characteristics to that of milk and cheese with low levels of CLA.

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MATERIALS AND METHODS

Two experiments were conducted to study the consumer acceptability attributes of CLA-enriched milk (experiment 1) and cheese (experiment 2) from cows grazing on pasture.

Experiment 1

Fifteen multiparous Holstein cows with an initial milk yield of 41.4 ± 4.0 kg/d per cow and 228 ± 59 DIM were blocked according to average milk yield 1 wk before the start of the experiment. Cows within each block were randomly assigned to 1 of 3 treatments. Cows in positive energy balance (anabolic phase) of lactation were selected to avoid body fat mobilization factor, which could cause the release of aromatic compounds in fat tissue. Cows were fed either a TMR containing 51% forage and 49% concentrate, were grazed on pasture, or were grazed on pasture and supplemented with 3.2 kg/d per cow of a grain mix. The TMR contained (DM basis) 23.6% alfalfa hay, 27.8% corn silage, 10.7% steam-flaked corn, 19.3% commodity grain mix, 8.1% whole linted cottonseed, 2.1% soybean meal, 6.3% sugar beet pulp, 0.2% custom yeast mix, 1.2% molasses, and 0.8% fat. The grain mix contained (DM basis) 75% full-fat extruded soybeans (FFES), 10% dry ground corn, 10% dried sugar beet pulp, and 5% molasses on a DM basis.

Experimental duration was 6 wk (August to October), and measurements were made during the last 3 wk. Cows in the TMR group were housed in individual tie-stalls and were fed twice daily at 0800 and 1600 h to yield an average 5% orts on an as-fed basis. Cows grazed on pasture or those grazed on pasture and fed a grain mix were acclimated to pasture over a period of 1 wk (25, 50, and 75% pasture for 2 d each). All cows were grazed together under intensive rotational grazing management. The pasture area was 24 acres and was divided into 24 one-acre paddocks. All cows were moved to a new paddock every 24 h. Pasture area per cow was approximately $145 \text{ m}^2/\text{d}$. Cows had free access to water and minerals. To feed the grain mix, cows were brought to individual tie-stalls for 15 min after each milking before being turned out onto pasture. Cows remained on the pasture daily for about 22 h.

The FFES were prepared by extruding whole soybeans at 146 to 149°C using the Insta-Pro Extruder model 2500 (Insta-Pro Int., Division of Triple "F", Inc., Des Moines, IA). Animal care and procedures were approved and conducted under established standards of the Utah State University Institutional Animal Care and Use Committee.

Amounts of feed offered and orts were recorded daily for individual cows fed the TMR. Ort samples were

collected daily from individual cows fed the TMR during wk 4, 5, and 6, and weekly composite samples were used for analysis. Cows grazed on pasture and fed the grain mix consumed all of the grain mix offered. Samples of total mixed diet, individual dietary ingredients, and grain mix were collected weekly during wk 4, 5, and 6 of the experiment. Representative samples of pasture forage were collected from the paddock before grazing during wk 4 and 6 of the experiment using a 0.61-m^2 quadrant. Pasture forage clippings were obtained from 4 different locations per one-acre paddock. Samples from each paddock were composited, divided in half, and used for determining botanical and chemical composition.

Dry matter contents of the total mixed diet, individual feed ingredients, and orts were determined by oven drying at 60°C for 48 h. Pasture samples were freeze-dried (Labconco Freeze Dry System; Labconco, Kansas City, MO) to determine DM content. Forage and grain samples were ground through a Wiley mill (1-mm screen; Arthur H. Thomas, Philadelphia, PA) and analyzed for chemical composition. Pasture forage clipping samples were ground with dry ice to avoid causing damage to fatty acids. The CP content of feed samples was determined using the macro Kjeldahl nitrogen test using AOAC method 954.01 (AOAC, 2000) with a Kjeltex digester 20 and Kjeltex System 1026 distilling unit (Tecator AB, Hoganas, Sweden). The NDF and ADF contents were determined with the ANKOM²⁰⁰ Fiber Analyzer (ANKOM Technology Corporation, Fairport, NY), using the basic procedure of Van Soest et al. (1991). Sodium sulfite was not used in the procedure for NDF determination, but pretreatment with heat-stable amylase (Type XI-A from *Bacillus subtilis*; Sigma-Aldrich Corporation, St. Louis, MO) was included. Weekly samples of dietary ingredients were analyzed for total fatty acid (FA) content and FA profile (Sukhija and Palmquist, 1988). During analysis, the samples were further dried at 103°C overnight to determine the absolute DM, and chemical analyses were expressed on the basis of this final absolute DM.

Cows were weighed at the beginning, middle, and at the end of the experiment. Average weights were used for estimating DMI of cows in the groups in which cows were grazed on pasture or were grazed on pasture and fed the grain mix. Forage intakes for cows in these two groups were estimated from calculated net energy needs for maintenance, BW, and milk production using equations (Tyrrell and Reid, 1965; NRC, 2001). In the case of cows fed the TMR, the difference between the amount of feed DM offered and orts was used for calculating DMI.

Daily milk yield was recorded. Weekly milk samples were collected from 4 consecutive milkings in vials con-

taining preservative (Broad Spectrum Microtabs II; D & F Control Systems, Inc., San Ramon, CA) during wk 4 through 6. Milk samples from individual cows were analyzed at the Rocky Mountain DHIA Laboratory (Logan, UT) for fat and true protein contents with midinfrared procedures using a Bentley 2000 (Bentley Instruments, Chaska, MN). The infrared instrument was calibrated weekly using raw milk standards based on chemistry analysis (Eastern Laboratory Services Ltd., Fairlawn, OH). The fat measurement channel used was a combination of fat A and fat B. Final milk composition for each week was expressed on weighted milk yield of a.m. and p.m. samples. Milk pH was determined by a microprocessor (Orion Research, Inc., Cambridge, MA) at 21°C, and milk acidity was determined as described by Atherton and Newlander (1977) on weekly composite samples collected without preservative from wk 4 through 6 of the experiment.

Milk collected during wk 4, 5, and 6 was used for taste panel evaluation. Cows were milked into separate cans on d 1 of each week. Milk from a.m. and p.m. milkings of that day (10 kg per cow) was mixed for each treatment and pasteurized at 72°C for 15 s using APV model SR15-S (APV Equipment Inc., Tonawanda, NY). Milk was homogenized using a Gaulin model CGC 2-stage homogenizer (Gaulin, Everette, MA). Processed milk (10 kg) was vacuum-packaged, refrigerated at 4°C, and used for taste panel evaluation. Whole milk in a clear plastic container with a remaining shelf life of at least 21 d was purchased at a grocery store and used as a positive control. Sensory evaluation of milk was conducted within 72 h of procurement and 36 h of processing the milk.

Experiment 2

Eighteen Holstein cows (12 multiparous and 6 primiparous) with an initial milk yield of 31.0 ± 6.9 kg/d per cow that were 204 ± 37 DIM were blocked according to average milk yield from 1 wk before the start of the experiment. Within blocks, cows were randomly assigned to TMR, pasture, and pasture plus grain mix treatments similar to experiment 1, except that cows in the third group were fed only 2.5 kg/d of FFES per cow instead of the grain mix. This change in experiment 2 was made to feed more C_{18:2} FA through FFES than in experiment 1. Cow management, feeding practices, experimental duration, feed sampling, and milk sampling procedures were the same as described in experiment 1. The experiment was conducted during the months of May, June, and July.

Cheddar cheese was manufactured from milk collected in this experiment and used for FA analysis and consumer acceptability evaluation. To collect milk for

cheese manufacturing, cows were further divided randomly into 2 groups of 3 cows each per treatment. There was a total of 6 groups of cows: groups A and B were fed the TMR, groups C and D were grazed on pasture, and groups E and F were grazed on pasture and were fed FFES. Cheese was manufactured in 4 sessions because of the use of small-scale manufacturing facilities. In the first session, milk from cows in groups A, C, and E was used to make cheese. In the second session, milk from groups B, D, and F was used. The process was repeated using groups A, C, and E in the third session and B, D, and F in the fourth session. A total of 12 batches of cheeses (4 per treatment) were made over 16 d from wk 4 through 6 of the experiment. Cows were milked into separate cans. An equal amount of milk from each cow within a group was collected from 8 consecutive milkings and used for making one 14-kg batch of cheese.

Cheese was prepared using the basic small-scale manufacturing procedure of Kosikowski and Mistry (1997) with a few modifications as described. Raw whole milk was batch-pasteurized (153 kg per batch) in a water bath at 65.5°C for 30 min and subsequently cooled to 32.2°C. Calcium chloride (0.02% wt/wt) was added to aid coagulation. Annatto was added at a level of 19.8 mL/100 kg of milk to provide color and to avoid confounding effects of color differences among the cheeses caused by treatments. Lyophilized cultures of *Lactococcus lactis* ssp. *lactis* and *L. lactis* ssp. *cremoris* were added. Thirty minutes after inoculation, coagulant (Maxiran double strength; DSM Food Specialties, Menomonee Falls, WI) was added. After an additional 30 min, the coagulated milk was cut. The temperature was increased to 38.8°C, and the whey was drained off after 35 min. Curds were allowed to mat and cheddar until pH reached 5.4. Salt was added (0.28% wt/vol milk) in 3 applications (10 min apart) after which the curds were placed into stainless steel hoops. After processing overnight, cheese blocks were vacuum-packaged and refrigerated at 4°C until evaluation by an open panel of consumers after 40 d. After open panel evaluation, cheese was frozen at -20°C for 6 mo before conducting the trained panel evaluation. A small block of cheese was frozen separately for FA analysis.

FA Analysis of Milk and Cheese

Weekly composite milk samples from 6 consecutive milkings from individual cows during wk 4 to 6 of the experiment and individual batches of cheese were analyzed for FA composition including CLA using the procedures and gas chromatography conditions described by Dhiman et al. (1999b, 2002). Fatty acids were identified by comparing the retention times with methylated FA

standards including CLA (Nu-Chek Prep, Elysian, MN; Matreya, Pleasant Gap, PA; Supelco, Bellefonte, PA). Heptadecanoic acid was used as an internal standard. The CLA reported is C_{18:2} *cis*-9, *trans*-11. Percentage of each FA was calculated by dividing the area under the FA peak (minus the area under the peak for heptadecanoic acid) by the sum of the areas under the total reported FA peaks. Fatty acids were reported as grams per 100 g of fatty acid methyl esters. In the present study, an acidic catalyst was used for FA methyl ester preparation. Studies have shown that recovery of C_{18:2} *cis*-9, *trans*-11 is lower when using an acid catalyst as compared with a base catalyst (Kramer et al., 1997). Though the values reported in this study are lower than would be determined using a basic catalyst for methylation, relative comparisons among treatments should still be valid.

Consumer Evaluation of Milk and Cheese

Refrigerated fluid milk was tempered to $22.0 \pm 1.0^\circ\text{C}$ and was served (20 mL) in plastic cups to an open panel of consumers for evaluation. Panelists consisted of men and women ages 24 to 60 who were regular consumers of fluid milk and cheese. Random code numbers were assigned to each sample, but the order of sample presentation was not randomized among panelists. The evaluation of milk from wk 4, 5, and 6 was conducted in individual booths under fluorescent white light by 62, 86, and 92 judges, respectively. Water and spittoons were provided to rinse the mouth between samples. Judges were asked to rate each sample independently for color, mouth-feel, flavor, and overall quality on a continuous 9-point hedonic scale (where 9 = like extremely, 5 = neither like nor dislike, and 1 = dislike extremely).

An open panel of consumers evaluated each batch of cheese for color, flavor, texture, and overall quality on a continuous 9-point hedonic scale. Evaluation was conducted in 3 different sessions with 78, 83, and 73 consumers, respectively, in each session. Cheese (15 g) held at room temperature was served in plastic cups. Testing conditions were similar to those described previously for milk evaluation.

A trained panel of experts evaluated the fluid milk from each week for color, overall quality, and specific flavors on a scorecard patterned after the American Dairy Science Association scoring guide (Hammond et al., 1986) and as suggested by Bodyfelt et al. (1988a). Eight judges were selected from a group of people who had been regularly exposed to training and judging a variety of dairy products, including fluid milk. Judges were not specifically trained for the current study, but were familiar with the scorecard for fluid milk flavor.

The sensory testing scale was slightly modified by using continuous 10-point scale for flavor characteristics, where 10 = highly pronounced, 7 = moderate, 5 = slight, 3 = barely perceptible, and 1 = none.

For color and overall quality, a 9-point scale was used, where 9 = best in color and overall quality, 5 = average, and 1 = undesirable color and unfit for sale. Reference samples for specific attributes were provided for all flavor characteristics during sampling. Milk samples (20 mL) were served in plastic cups with random code numbers assigned to each sample. This was repeated after a 20-min break. Sample position was changed each time samples were offered to judges. The booth and judging conditions were the same as described for the open panel.

A trained panel evaluated each batch of cheese for overall quality, color, and flavor characteristics. Judges were selected from among people who had been regularly exposed to training on dairy products, including Cheddar cheese. Judges were trained for quantitative descriptive sensory analysis in 4 sessions over a period of 4 wk. The first 2 sessions were used for training on the Cheddar cheese lexicon, and the final 2 sessions were on the modified official American Dairy Science Association scorecard (Hammond et al., 1986) as suggested by Bodyfelt et al. (1988b) for cheese flavors. The scale used for specific flavors, uniformity of color, and overall quality of cheese by the trained panel was the same as was used for milk.

For evaluation, cheese blocks were thawed under refrigerated conditions for 4 d and then cut into cubes (1 cm³) and served to a panel of 7 trained judges. Cheese was served in plastic cups at room temperature with random code numbers assigned to each sample. All 12 batches of cheese were tested on the same day with breaks allowed for judges between trays of samples. This was repeated the following day. All judges tasted all samples. Flavor compounds were provided as references for specific flavor attributes to the judges during training and while performing the test. The testing conditions were the same as described previously in for milk. Judges were not provided information regarding the treatment of each sample in any of these evaluations.

Animal Diet Composition

Average botanical composition of pasture during experiments 1 and 2 on a DM basis was 2.55, 0.13, 0.27, and 0.28 tonne/ha of live grass (*Lolium perenne*), legumes (*Trifolium repens*), weeds, and dead material, respectively. The CP, NDF, ADF, and total FA contents in experiment 1 were 15.8, 33.7, 22.2, and 5.9% for the total mixed diet fed to cows in TMR; 19.9, 53.5, 28.7,

Table 1. Feed intake and milk production of cows in experiments 1 and 2.

Item	Treatment ¹			SEM	P = F
	TMR	PS	PES		
Experiment 1					
DMI, kg/d	25.7 ^a	21.8 ^b	22.0 ^b	0.49	<0.01
Milk yield, kg/d	33.9 ^a	25.7 ^b	26.3 ^b	0.98	<0.01
Milk fat, %	3.4	3.6	3.6	0.08	0.23
Milk protein, %	3.0	2.9	3.0	0.05	0.50
Experiment 2					
Milk yield, kg/d	24.3 ^a	18.6 ^b	22.2 ^a	1.07	0.05
Milk fat, %	4.4	4.1	4.3	0.27	0.36
Milk protein, %	3.1	3.2	3.2	0.06	0.71

^{a,b}Means with different superscripts within the same row differ with the *P* value shown in the last column.

¹Cows were fed a diet containing 51% conserved forage and 49% concentrate (TMR), were grazed on pasture (PS), or grazed on pasture and supplemented with a grain mix (3.2 kg/d per cow) containing 75% full-fat extruded soybeans, 10% beet pulp, 10% ground corn, and 5% molasses (DM basis) (PES). In experiment 2, cows on the PES treatment were supplemented with only 2.5 kg/d per cow of full-fat extruded soybeans only instead of the grain mix used in experiment 1.

and 4.7% for pasture forage available to cows grazed on pasture; and 33.4, 23.7, 17.8, and 19.9% of the DM for the grain mix offered to cows grazed on pasture and fed grain mix respectively. In experiment 2, the CP, NDF, ADF, and total FA contents for the TMR and pasture were similar to experiment 1 and were 40.9, 25.9, 17.5, and 19.8% for FFES offered to cows grazed on pasture and fed FFES respectively. Fatty acid composition was similar for diets in experiments 1 and 2. In the TMR, the major FA present were C_{16:0}, C_{18:1 cis-9}, C_{18:2}, and C_{18:3}. The C_{18:2} was the primary FA in FFES, comprising 44.5% of the total FA. In pasture forage, C_{18:3} comprised 56.4% of total FA. Neither C_{18:1 trans-11} nor CLA was detected in any of the feeds, except for small amounts of C_{18:1 trans-11} (0.11% of total FA) in TMR.

Statistical Analyses

All statistical analyses were performed using the statistical procedures of SAS (1999–2000). Analysis of DMI, milk yield, milk composition, and FA profile of milk in experiments 1 and 2 was carried out using PROC MIXED with repeated measures. Different covariance parameters were tested to find the one that best fit the model. Treatment (df = 2), block (df = 4), week (df = 2), and treatment × week (df = 4) were included in the model as the fixed effects with week as the repeated measure.

For cheese FA, group of cows within treatment was used as a random factor. Because the interest here is more on the FA composition of milk and cheese over time rather than differences between batches of cheese, cheese manufacturing sessions 1 and 2 were pooled to make “time 1,” and sessions 3 and 4 were pooled to make “time 2.” Therefore, time instead of batch was

used as the fixed factor in the model. This modification was necessary in view of the further grouping of cows within each treatment and cheese being made in 4 different sessions. The model for cheese FA included treatment (df = 2), time (df = 1), and treatment × time (df = 2) as fixed factors and group within treatment as a random factor.

Milk and cheese sensory evaluation scores were analyzed using PROC GLM of SAS (1999–2000). In the open panel scores, the model was built in a stepwise manner, assigning a critical α for inclusion in the final model and dropping those factors that did not meet the assigned critical value. The final model included the effects of treatment (df = 3 for milk and df = 2 for cheese), tasting session (for cheese; df = 2), testing week (for milk; df = 2), and their interactions. In the case of trained panel scores, judge (df = 7 for milk and df = 6 for cheese), treatment (df = 3 for milk and df = 2 for cheese), testing week (for milk; df = 2), tasting session within day (for cheese; df = 1), and their interactions were included in the model. Day of testing was used as a replicate. Means were separated by the Ryan-Elliot-Gabriel-Welsch multiple range test whenever there was a significant difference among the means caused by main effects. Statistical significance was declared at *P* < 0.05 unless otherwise noted. Trends were described at *P* ≤ 0.1.

RESULTS AND DISCUSSION

Feed Intake and Milk Production

There was no treatment × week effect for any of the intake and production variables in experiment 1. Week was also nonsignificant. The DMI was significantly higher for cows fed the TMR than for cows grazed on

pasture and fed or not fed the grain mix (Table 1). The DMI was not different for cows in these final 2 groups. As expected, cows fed the TMR had higher milk yields, and a reduction in DMI for cows grazed on pasture and for those grazed on pasture and fed the grain mix resulted in lower milk yields. Reduced milk yield in cows grazing pasture compared with cows consuming a mixed ration containing grain is common because of higher nutrient density of grain diet (Loor et al., 2002b). There was no difference in milk composition among treatments. No difference in milk fat and milk protein contents for cows grazing on pasture with or without supplementation of FFES compared with cows fed the TMR has been observed previously (Lawless et al., 1998; Dhiman et al., 1999a; Loor et al., 2002a). Milk pH did not differ across treatments and averaged 6.62, 6.62, and 6.61 for cows fed the TMR, cows grazed on pasture, and cows grazed on pasture and fed the grain mix, respectively. Milk acidity was 0.16 across all treatments ($P = 0.97$). Milk pH and acidity were within the normal range for Holstein cows (Walstra et al., 1999).

There was a significant treatment $\times$ week interaction for DMI in experiment 2. However, when analyzed separately within week, DMI was higher ($P < 0.01$) for cows fed the TMR compared with cows grazed on pasture and cows grazed on pasture and fed the grain mix. Milk yields were higher for cows fed the TMR and for cows grazed on pasture and fed the grain mix compared with cows grazed on pasture only. Milk fat and protein contents were similar across all treatments in experiment 2. Results from both experiments suggest that cows grazing on pasture alone had lower DMI and milk yield compared with cows consuming mixed grain and conserved forage diets.

FA Composition of Milk and Cheese

In experiment 1, the treatment $\times$ week interaction was nonsignificant ($P > 0.05$) for all FA in milk except for $C_{18:0}$ and CLA, which are shown in Figure 1. It should be mentioned that $C_{4:0}$ and $C_{6:0}$ FA were not measured; therefore, all FA are slightly overestimated. Provision of more long-chain and unsaturated FA in the diet through pasture forage and FFES increased the overall proportion of long-chain ($C_{18:0}$ or more) and unsaturated FA in milk from cows grazed on pasture and those grazed on pasture and fed a grain mix compared with milk of cows fed the TMR (Table 2). Similar changes in milk fat composition were observed by others when cows were grazed on pasture or fed diets supplemented with heated soybeans, FFES, or cottonseed (Dhiman et al., 1995, 1999a,b).

In experiment 1, the proportions of $C_{18:2}$ and $C_{18:3}$ in milk fat reflected the proportions of $C_{18:2}$ and $C_{18:3}$

Figure 1. The $C_{18:0}$ and $C_{18:2}$ *cis-9, trans-11* (CLA) contents (g/100 g of fatty acid methyl esters) in milk fat from cows fed a diet containing 51% conserved forage and 49% grain mix (TMR; ●), from cows grazed on pasture (▲), or from cows grazed on pasture and supplemented (3.2 kg/d per cow) with a grain mix (■) in experiment 1.

supplied by the diets. Cows grazed on pasture or grazed on pasture and fed the grain mix had 3 times the $C_{18:3}$ content in milk FA compared with milk FA from cows fed the TMR. Conversely, milk FA $C_{18:2}$ content of cows grazed on pasture was only 45% of that observed in the milk from cows fed TMR or from cows grazed on pasture and fed the grain mix.

The $C_{18:0}$ FA, when analyzed separately for wk 4, 5, and 6, showed no difference among treatments within week during experiment 1. The $C_{18:0}$ content in milk FA for cows grazed on pasture and those grazed on pasture and fed the grain mix ranged from 14.3 to 15.5 g/100 g of FA (Figure 1), which is slightly higher than has been reported for cows grazing on pasture with or without supplementation of oil seeds (Lawless et al., 1998; Dhiman et al., 1999a; Loor et al., 2002b). The reason for this may be that $C_{4:0}$ and $C_{6:0}$ were not measured in the current study, causing relative proportions of all other FA to be slightly higher.

The average CLA content in the milk of cows fed the TMR, those grazed on pasture, and those grazed on pasture and fed the grain mix was 0.52, 1.63, and 1.69 g/100 g of milk FA, respectively (Figure 1). One objective of this study was to produce milk with a high level of

Table 2. Fatty acid composition (g/100 g of total reported fatty acid methyl esters) of milk and cheese from experiments 1 and 2, respectively.

Fatty acid	Treatment ¹			SEM	P = F
	TMR	PS	PES		
Milk					
MCFA ²	12.9	12.5	11.2	0.95	0.33
C _{14:1}	2.38	2.33	2.03	0.12	0.10
C _{15:0}	1.26 ^b	1.64 ^a	1.35 ^{ab}	0.08	0.02
C _{16:0}	32.3 ^a	27.3 ^b	24.5 ^b	0.74	<0.01
C _{16:1}	1.26	1.65	1.42	0.11	0.10
C _{17:1}	0.23 ^b	0.35 ^a	0.31 ^a	0.01	<0.01
C _{18:1 trans-11}	3.10 ^b	5.47 ^a	5.98 ^a	0.33	<0.01
C _{18:1 other trans}	2.15 ^a	1.58 ^b	2.45 ^a	0.15	<0.01
C _{18:1 cis}	24.7	26.9	28.2	1.12	0.14
C _{18:2}	4.09 ^a	2.30 ^b	4.15 ^a	0.24	<0.01
C _{18:3}	0.41 ^b	1.22 ^a	1.17 ^a	0.07	<0.01
>C ₂₀ ³	0.33	0.33	0.32	0.02	0.99
SFA ⁴	60.8 ^a	56.2 ^b	52.3 ^b	0.99	<0.01
UFA ⁵	39.2 ^b	43.8 ^a	47.7 ^a	0.99	<0.01
Cheese					
MCFA	13.2	13.0	11.5	1.12	0.37
C _{14:1}	2.54	2.34	2.01	0.15	0.10
C _{15:0}	1.69 ^b	2.36 ^a	2.08 ^{ab}	0.07	0.02
C _{16:0}	32.9 ^a	27.8 ^b	25.1 ^c	0.62	<0.01
C _{16:1}	1.18 ^b	1.92 ^a	1.68 ^a	0.14	0.05
C _{17:1}	0.21 ^b	0.47 ^a	0.37 ^b	0.02	<0.01
C _{18:0}	14.5	12.6	14.4	0.74	0.27
C _{18:1 trans-11}	2.68 ^b	5.60 ^a	5.66 ^a	0.25	<0.01
C _{18:1 other trans}	1.86 ^a	1.17 ^b	1.97 ^a	0.08	<0.01
C _{18:1 cis}	24.5 ^b	28.0 ^a	29.3 ^a	0.32	0.14
C _{18:2}	3.55 ^a	2.10 ^b	3.15 ^a	0.15	0.01
C _{18:2 cis-9, trans-11} (CLA)	0.47 ^b	1.47 ^a	1.46 ^a	0.09	<0.01
C _{18:3}	0.49 ^b	0.97 ^a	0.97 ^a	0.08	0.03
>C ₂₀	0.27	0.25	0.31	0.02	0.60
SFA	62.3 ^a	55.7 ^b	53.1 ^b	0.58	<0.01
UFA	37.7 ^b	44.3 ^a	46.9 ^a	0.58	<0.01

^{a,b,c}Means with different superscripts within the same row differ with the *P* value shown in the last column.

¹Cows were fed a diet containing 51% conserved forage and 49% concentrate (TMR), were grazed on pasture (PS), or grazed on pasture and supplemented with a grain mix (3.2 kg/d per cow) containing 75% full-fat extruded soybeans, 10% beet pulp, 10% ground corn, and 5% molasses (DM basis) (PES). In experiment 2, cows on the PES treatment were supplemented with only 2.5 kg/d per cow of full-fat extruded soybeans instead of the grain mixed used in experiments.

²MCFA = Medium chain fatty acids (sum of C_{8:0}, C_{10:0}, C_{12:0}, and C_{14:0}).

³Sum of C_{20:2}, C_{20:3}, C_{20:4}, and C_{22:4}.

⁴Total saturated fatty acids (SFA). The average values used for C_{18:0} fatty acid in milk were 14.3, 14.8, and 15.2 for TMR, PS and PES treatments, respectively (Figure 1).

⁵Total unsaturated fatty acids (UFA). The average values used for C_{18:2 cis-9, trans-11} (CLA) in milk were 0.53, 1.63, and 1.69 for TMR, PS, and PES treatments, respectively (Figure 1).

CLA. The CLA content in the milk fat of cows grazed on pasture and those grazed on pasture and fed the grain mix was 3 times that in milk fat from cows fed the TMR. Similar increases in milk CLA content of cows grazing on pasture have been observed by other researchers (Dhiman et al., 1999a; Lawless et al., 1999; White et al., 2001). Interestingly, feeding supplemental FFES to cows grazing pasture did not enhance the CLA content of milk or daily CLA yield any further. The proportion of C_{18:1 trans-11} FA in milk fat was higher in cows grazing pasture with or without the additional

grain mix than in cows fed the TMR (Table 2). The C_{18:1 trans-11} FA is converted to CLA in the mammary gland via the Δ^9 -desaturase enzyme (Corl et al., 2001). No difference in the proportion of C₂₀ or higher carbon FA was observed among treatments. The C_{18:1 trans-11} and CLA contents in experiment 2 were 2.98, 5.74, and 5.93 and 0.50, 1.70, and 1.50 g/100 g of milk FA for cows fed the TMR, cows grazed on pasture, and cows grazed on pasture and fed the grain mix, respectively. The profile of the remaining FA in experiment 2 was very similar to that in experiment 1 (data not shown).

Table 3. Open panel scores¹ on consumer acceptability of milk and cheese from experiments 1 and 2, respectively.

Characteristics	Positive control ³	Treatment ²			SEM	P = F
		TMR	PS	PES		
Milk						
Color	6.7	6.6	6.6	6.6	0.29	0.88
Flavor	6.1	6.0	5.8	5.9	0.38	0.79
Mouth-feel	6.5	6.6	6.7	6.7	0.24	0.76
Overall	6.4	6.1	6.2	6.2	0.34	0.83
Cheese ⁴						
Color		6.4	6.8	6.4	0.51	0.67
Flavor		5.3	5.5	5.4	0.39	0.92
Texture		5.8	5.6	5.3	0.33	0.27
Overall		5.6	5.8	5.5	0.29	0.82

¹Nine-point scale, where 9 = like extremely, 5 = neither like nor dislike, and 1 = dislike extremely.

²Cows were fed a diet containing 51% conserved forage and 49% concentrate (TMR), were grazed on pasture (PS), or grazed on pasture and supplemented with a grain mix (3.2 kg/d per cow) containing 75% full-fat extruded soybeans, 10% beet pulp, 10% ground corn, and 5% molasses (DM basis) (PES). In experiment 2, cows on the PES treatment were supplemented with only 2.5 kg/d per cow of full-fat extruded soybeans instead of the grain mixed used in experiments.

³Whole milk purchased at a grocery store was used as a positive control.

⁴No positive control was used.

There were no significant interactions for any of the FA reported for cheese. The means for individual FA are given in Table 2. In general, the FA composition of cheese was similar to that of milk with some minor differences. The CLA content was 3 times greater in cheese manufactured from the milk of cows grazing pasture with or without the additional grain mix compared with that of cows fed the TMR, supporting a previous finding that cheese retains most of the CLA present in milk (Dhiman et al., 1999b).

Total unsaturated FA were higher in milk and cheese from cows grazing pasture with or without additional grain mix than in milk and cheese from cows fed the TMR. The proportions of unsaturated FA in the milk increase when feeds rich in C_{18:2} or C_{18:3}, such as FFES and green forages, are fed to animals (Kelly et al., 1998). Using both pasture treatments, we were able to produce milk and cheese enriched with CLA, meeting one of our objectives.

Consumer Acceptability and Flavor Characteristics of Milk and Cheese

The consumer panel scores of milk for mouth-feel, color, flavor, and overall quality did not differ across treatments (Table 3), indicating that CLA-enriched (0.11 to 0.12 g of CLA/227-mL serving) from cows grazing pasture was acceptable to consumers and was also comparable with milk purchased at a grocery store. Similarly, no significant differences were observed among treatments for color, flavor, texture, and overall quality of cheese when rated by the panel of consumers. On the hedonic scale, overall ratings (5.5 to 5.8) suggest

that milk and cheese from all treatments were liked slightly by consumers.

Trained panel evaluation showed no significant differences in overall quality and color of milk and cheese among treatments (Table 4). Milk and cheese from all treatments was liked moderately in terms of overall quality. Specific flavor attributes for milk were similar among treatments, except for barny flavor, which was scored higher in milk of cows grazing pasture (with or without the additional grain mix) compared with that of cows fed the TMR (Table 4). Interestingly, oxidized flavor was perceived to be at a higher level in milk purchased at the grocery store than in milk from experimental treatments. This may partially be because milk purchased at the store was in a non-light-shielding plastic container. All flavors in milk, including barny, were rated as barely perceptible or nonexistent. It is possible that vacuum-packaging procedures as used in this experiment might have removed some volatile off-flavors.

Specific flavors in cheese were rated as barely perceptible or nonexistent, except for acid and bitter flavors, which were rated as slightly perceived for all treatments. It should be noted that the Cheddar cheese produced in this study was aged to only 40 d. This is considered a positive finding by the researchers because differences detected in cheese would probably also have been present in milk.

Carpino et al. (2004) found that 4-mo-old cheese made from milk of pasture-fed cows contained 27 odor-active compounds, whereas only 13 were detected in cheese made from milk of cows fed a total mixed diet containing grain, suggesting that there are unique aroma compounds found in pasture plants that may be transferred

Table 4. Trained panel consumer acceptability scores¹ of milk and cheese from experiments 1 and 2, respectively.

Characteristic	Positive control ³	Treatment ²			SEM	P = F
		TMR	PS	PES		
Milk						
Overall quality	5.9	7.3	6.5	6.3	0.53	0.07
Color uniformity	6.1	7.0	6.9	6.3	0.48	0.47
Acid	1.3	1.3	1.3	1.2	0.11	0.94
Bitter	1.2	1.1	1.1	1.1	0.05	0.98
Feed	2.2	1.6	2.2	2.2	0.24	0.35
Fermented	1.1	1.2	1.3	1.2	0.13	0.72
Flat/low flavor	1.6	1.3	1.3	1.3	0.14	0.32
Rancid	1.3	1.1	1.3	1.3	0.11	0.80
Oxidized	2.3 ^a	1.2 ^b	1.1 ^b	1.5 ^b	0.09	<0.01
Barny	1.1 ^b	1.2 ^b	2.2 ^a	2.1 ^a	0.19	<0.01
Astringent	1.9 ^a	1.5 ^{ab}	1.3 ^b	1.8 ^{ab}	0.18	0.02
Cooked	2.3	1.8	2.1	1.9	0.20	0.19
Cowly	1.9 ^a	1.6 ^{ab}	1.6 ^{ab}	1.3 ^b	0.17	0.05
Foreign	1.2	1.1	1.2	1.7	0.21	0.09
Garlic/onion	1.1	1.0	1.0	1.0	0.08	0.40
Lacks freshness	2.3	1.8	2.0	2.4	0.23	0.36
Malty	1.2	1.3	1.0	1.2	0.13	0.47
Salty	1.3	1.4	1.3	1.2	0.10	0.75
Unclean	1.1	1.2	1.4	1.6	0.20	0.32
Others	1.3	1.4	1.7	1.5	0.16	0.79
Cheese ⁴						
Overall quality		6.6	6.5	6.3	0.32	0.52
Color uniformity		7.3	7.0	7.1	0.36	0.58
Acid		5.5	5.1	5.3	0.32	0.42
Bitter		3.9	3.5	4.0	0.23	0.37
Feed		2.3	2.3	2.2	0.16	0.88
Fermented		1.7	1.6	1.6	0.21	0.84
Flat/low flavor		2.2	2.0	2.3	0.22	0.34
Rancid		2.0	2.1	2.2	0.09	0.66
Oxidized		2.9	3.0	2.6	0.13	0.07
Fruity		1.7	1.2	1.7	0.21	0.09
Heated		1.7	1.2	1.7	0.22	0.08
Sulfide		2.3	2.0	2.3	0.19	0.92
Unclean		2.2	2.3	2.3	0.18	0.91
Whey taint		2.0	1.8	1.9	0.18	0.80
Yeasty		1.6	1.5	1.5	0.07	0.94
Others		1.5	1.4	1.6	0.08	0.11

^{a,b}Means with different superscripts within the same row differ with the *P* value shown in the last column.

¹Overall quality and uniformity of color rated as 9 = best in color and overall quality, 5 = average in color and overall quality, and 1 = not uniform color and quality unfit for sale. Other flavors rated as 9 = highly pronounced, 7 = moderate, 5 = slight, 3 = barely perceptible, and 1 = none.

²Cows were fed a diet containing 51% conserved forage and 49% concentrate (TMR), were grazed on pasture (PS), or grazed on pasture and supplemented with a grain mix (3.2 kg/d per cow) containing 75% full-fat extruded soybeans, 10% beet pulp, 10% ground corn, and 5% molasses (DM basis) (PES). In experiment 2, cows on the PES treatment were supplemented with only 2.5 kg/d per cow of full-fat extruded soybeans instead of the grain mixed used in experiments.

³Whole milk purchased from the store was used as positive control.

⁴No positive control was used.

to milk and cheese. High-grain diets that induce propionate metabolism in the rumen cause the formation of the sweet, raspberry-flavored γ -dodecanolactone from dietary oleic acid and sweet, raspberry-flavored γ -dodec-cis-6-enolactone from dietary linoleic acid (Urbach, 1990). Lush green pastures, on the other hand, produce a richly colored milk fat and introduce phytol, dihydrophytol, phytene, and probably their lower homologues into the milk fat, which, at high concentrations, may

give a grassy, bitter flavor (Urbach, 1990). Specific flavor compounds and their concentrations were not isolated in the current study. Although there may be unique compounds associated with milk from pasture-fed cows, it is also possible that differences in milk flavor may primarily be caused by concentration differences of a common set of flavor compounds rather than by the occurrence of compounds uniquely associated with a particular feed (Bendall, 2001). Phenolic com-

pounds, such as phytol, indole, and skatole, are present at higher levels in milk of cows grazing on pasture, and their effects on sensory attributes of dairy products are very subtle (O'Connell and Fox, 2001).

Results from the current study suggest that the consumer acceptability attributes of CLA-enriched milk and cheese from cows grazing on pasture were similar to milk and cheese with low levels of CLA. Sensory characteristics of CLA-enriched milk and butter from cows fed fish oil, extruded soybeans, or both have been reported as comparable with control milk and butter and did not show significant differences in oxidation or flavor (Ramaswamy et al., 2001a, b). Also, there was no significant difference in the flavor of butter with CLA at 0.7 or 2.58% of fat (Baer et al., 2001). Maynard and Franklin (2003) showed that high-CLA dairy products, including fluid milk, were acceptable to a substantial number of consumers. They found that survey respondents indicated a willingness to pay an average of \$0.41 per gallon more for CLA-enriched milk, and 80% of the respondents expressed a willingness to pay at least \$0.20 per gallon more for CLA-enriched milk.

CLA Intake from Dairy Products

Based on appropriate extrapolation of data from animals to humans, an adult human would require 0.72 to 0.80 g of CLA/d to inhibit tumor growth (Parrish et al., 2003; Watkins and Li, 2003). Correcting milk fat for glycerol content (Chouinard et al., 2001), based on fat and CLA contents of milk in this study, 500 mL of milk from cows fed the TMR, cows grazed on pasture, and cows grazed on pasture and fed the grain mix would provide 0.07, 0.25, and 0.26 g of CLA. Using CLA contents of cheese in the current study and a fat content of 32% (Shantha et al., 1995), 100 g of cheese from these 3 respective treatments would provide 0.13, 0.40, and 0.40 g of CLA. A person consuming 500 mL of milk and 100 g of cheese daily from cows fed the TMR would only be consuming about one-third of the estimated requirement for humans. However, consuming similar amounts of milk and cheese from cows grazed on pasture (with or without additional grain mix) will provide CLA close to the requirements.

In addition, there is a potential for higher levels of C_{18:1} *trans* FA in CLA-enriched products to be converted to CLA in human tissues (Palmquist and Santora, 1999), further increasing the supply of CLA that could be obtained from enriched products. Clearly, if the objective is to achieve health benefits from CLA, it is practical to consume CLA-enriched milk and cheese.

CONCLUSION

The CLA content of milk and cheese from cows grazing on pasture was 3 times higher than the CLA content

in milk and cheese from cows fed conserved forage plus grain. Supplementation of C_{18:2} to pasture in the form of FFES to cows grazing on pasture did not increase CLA content in milk compared with grazing on pasture alone. Results from the current study suggest that consumer acceptability attributes of CLA-enriched milk and cheese from cows grazing on pasture were similar to milk and cheese with normal levels of CLA from cows fed a conventional diet.

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