J. Dairy Sci. 88:2813–2819

Effects of Corn Silage Particle Length and Forage:Concentrate Ratio on Milk Fatty Acid Composition in Dairy Cows Fed Supplemental Flaxseed

H. W. Soita, M. Fehr, D. A Christensen, and T. Mutsvangwa
Department of Animal and Poultry Science, University of Saskatchewan, Saskatoon, Canada S7N 5A8

ABSTRACT

This study aimed to evaluate the effects of length of chop of corn silage and forage:concentrate ratio (F:C) on performance and milk fatty acid profiles in dairy cows supplemented with flaxseed. Our hypothesis was that decreasing forage particle length and F:C ratio would increase unsaturated fatty acid flow to the small intestine and subsequent transfer of these unsaturated fatty acids into milk. Eight Holstein cows (648.1 ± 71.5 kg body weight; 109.6 ± 43.6 days in milk) were used in a replicated 4 × 4 Latin square design with 21-d periods and a 2 × 2 factorial arrangement of dietary treatments. Dietary factors were: 1) F:C ratios (dry matter basis) of 55:45 and 45:55; and 2) corn silage particle lengths of 9.52 and 19.05 mm. All experimental cows received 1 kg of flaxseed to substitute for 1 kg of a rolled barley grain-based concentrate daily. Diets were fed twice daily as a total mixed ration. Corn silage particle length and F:C ratio had no effect on dry matter intake, milk yield, and milk composition; however, feeding short cut corn silage depressed milk protein yield. Significant particle size × F:C ratio interactions were observed for milk fat proportions of $C_{16:0}$, $C_{18:1}$ *cis*-9, and $C_{18:2}$ *cis*-9, *trans*-11 (a conjugated linoleic acid isomer). At short corn silage particle size, decreasing F:C ratio depressed milk fat proportion of $C_{16:0}$. Conversely, feeding short corn silage at high F:C ratio increased the proportion of $C_{18:1}$ *cis*-9 and $C_{18:2}$ *cis*-9, *trans*-11 in milk fat. The milk fat proportion of $C_{18:2}$ *trans*-10, *cis*-12, a conjugated linoleic acid isomer that is associated with milk fat depression, was not affected by dietary treatment. Our results show that corn silage particle length and F:C ratio influence milk fatty acid profiles in dairy cows fed supplemental flaxseed as a source of polyunsaturated fatty acids.
(**Key words:** corn silage, particle length, forage:concentrate ratio, milk fatty acids)

Abbreviation key: CLA = conjugated linoleic acid, **FA** = fatty acid, **F:C** = forage:concentrate ratio, **PSPS** = Penn State Particle Separator, **PUFA** = polyunsaturated fatty acid, **TLC** = theoretical length of cut.

INTRODUCTION

Currently, there is significant research interest aimed at manipulating bovine milk fat to improve its healthfulness to consumers (see Mansbridge and Blake, 1997; Chilliard et al., 2001; Jensen, 2002). This involves reducing the saturated fatty acid (**FA**) content of milk, especially myristic ($C_{14:0}$) and palmitic ($C_{16:0}$) acids, which have been demonstrated to have undesirable hypercholesterolemic effects and to increase the risk of coronary heart disease (Berner, 1993). In addition, this research has been aimed at enhancing the milk content of conjugated linoleic acids (CLA) and the long-chain polyunsaturated fatty acids (PUFA), especially oleic ($C_{18:1}$), linoleic ($C_{18:2n6}$), and linolenic ($C_{18:3n3}$), because of their anticarcinogenic (Parodi, 1997) and potential cardioprotective roles in humans (Massaro et al., 1999).

In practice, nutrition is the predominant environmental factor affecting bovine milk fat, and it represents a practical tool to alter its FA composition (Kennelly, 1996). The FA content of diets consumed by lactating cows affects both the type and the relative proportions of milk FA (Grummer, 1991). Various studies have reported that feeding ruminally unprotected or protected vegetable oils or oilseeds (Murphy et al., 1990; Chouinard et al., 1992; Ward et al., 2002), marine oils (Offer et al., 1999; Keady et al., 2000), and animal fats (Chilliard et al., 2001) can effectively alter milk FA profiles in dairy cows. However, ruminal biohydrogenation of dietary unsaturated FA is extensive, thus limiting intestinal flows and subsequent incorporation of diet-derived unsaturated FA into milk fat (see review by Jenkins, 1993). For this reason, numerous studies have focused on intraruminal factors that affect the extent of ruminal biohydrogenation of dietary unsaturated FA, with the objective of increasing ruminal escape of dietary unsaturated FA. For example, there is limited evidence indicating that dietary factors that alter ruminal pH and result in

Received March 15, 2005.
Accepted April 19, 2005.
Corresponding author: T. Mutsvangwa; e-mail: tim.mutsvan@usask.ca.

shifts in fermentation products may alter biohydrogenation pathways of PUFA in the rumen, leading to ruminal accumulation and increased postruminal flow of PUFA, especially CLA and $C_{18:1}$ *trans*-10 (Loor et al., 2003). Previously, Van Nevel and Demeyer (1996) showed that high concentrate diets depress lipolysis and biohydrogenation of dietary unsaturated FA by ruminal contents in vitro. Such diets with a high level of readily fermentable carbohydrates usually depress ruminal pH. More recently, Qiu et al. (2004) demonstrated that CLA, an intermediate arising from incomplete ruminal biohydrogenation of linoleic acid ($C_{18:2}$; Kepler et al., 1966), flows from a continuous culture system were increased by depressed pH. Feeding finely chopped forages or increasing dietary levels of readily fermentable carbohydrates reduced chewing and rumination, leading to depressed ruminal pH, impaired fiber digestion, and a depressed milk fat test (Beauchemin et al., 1994; Soita et al., 2000). Because of their influence on ruminal pH, dietary forage particle size and forage:concentrate ratio (**F:C**) may influence ruminal biohydrogenation of supplemental fats, and subsequent postruminal flow of unsaturated fatty acids and their incorporation into milk fat. There is very limited information available on how corn silage particle length and F:C may interact to alter milk FA profiles in dairy cows fed supplemental flaxseed. Therefore, the objective of the present study was to evaluate the effects of corn silage particle length and dietary F:C ratio on performance and milk FA profiles in dairy cows supplemented with flaxseed. Our hypothesis was that decreasing corn silage particle length and dietary F:C ratio would depress ruminal pH which, in turn, would impair ruminal FA biohydrogenation, thus potentially increasing unsaturated FA flow to the small intestine and their incorporation into milk fat.

MATERIALS AND METHODS

Cows and Experimental Design

Eight Holstein cows (648.1 ± 71.5 kg BW; 109.6 ± 43.6 DIM) were used in this study. The experiment was run as a replicated 4 × 4 Latin square design trial with 21-d periods and a 2 × 2 factorial arrangement of dietary treatments. Animals were cared for and handled in accordance with the Canadian Council on Animal Care regulations; and the University of Saskatchewan Animal Care Committee approved their use for this experiment.

Experimental Treatments, Feeding Management, and Sample Collection

The 4 dietary treatments were formulated by combining 2 factors, each with 2 levels. The dietary factors studied were: 1) F:C ratios (DM basis) of 55:45 and 45:55; and 2) corn silage theoretical lengths of cut (**TLC**) of 9.52 and 19.05 mm. The F:C ratio of the TMR regularly fed in the Dairy Unit at the University of Saskatchewan was 50:50, and the experimental F:C ratios were chosen to test the effects of a concentrate-rich (F:C = 45:55) or a forage-rich (F:C = 55:45) diet. The range in F:C ratios chosen was not wide to encompass typical F:C ratios that are commonly used by dairy producers in the field. The 9.52-mm TLC of corn silage was chosen because it is what we commonly use in the Dairy Unit at the University of Saskatchewan; the 19.05-mm TLC was used to test the effects of increasing TLC of corn silage. The composition and chemical analysis of experimental diets are shown in Table 1. The 19.05-mm TLC of corn silage was achieved with a forage harvester (model 900; New Holland, Burlington, IA) set with 26-tooth sprocket and 12 knives, and the 9.52-mm TLC was achieved with 12-tooth sprocket and 12 knives. The chemical compositions of the 9.52- and 19.05-mm TLC corn silages are presented in Table 2. Each 21-d experimental period consisted of 14 d for dietary adaptation and 7 d for data collection. Experimental diets were fed twice daily at 0800 and 1530 h as TMR for ad libitum intake. At both the morning and afternoon feeding, all cows received 0.5 kg of flaxseed to substitute for 0.5 kg of a rolled barley grain-based concentrate. The inclusion rate of flaxseed was based on a previous study in our laboratory (see Soita et al., 2003) that indicated that this level was efficacious in inducing changes in milk FA profiles. The flaxseed was top-dressed onto the TMR and mixed thoroughly by hand before feeding. Fatty acid composition (g/100 g of FA) of flaxseed was $C_{12:0}$, 0.04; $C_{14:0}$, 0.16; $C_{14:1}$, 0.03; $C_{16:0}$, 6.28; $C_{16:1}$, 0.15; $C_{18:0}$, 3.32; $C_{18:1}$, 16.7; $C_{18:2}$, 15.2; and $C_{18:3}$, 55.3.

During the 7-d data collection period, individual cow feed intake was recorded daily. Total mixed ration samples and orts were collected daily, stored at –20°C, and composited per cow for each experimental period. Pooled TMR and orts samples were analyzed for DM by drying in an oven at 60°C for 48 h (AOAC, 1990), CP using the macro-Kjeldahl procedure (AOAC, 1990), ADF (AOAC, 1990), and NDF (Van Soest et al., 1991). Amylase was used for NDF determination. During each data collection period, particle size distributions were determined for corn silage and all TMR using the Penn State Particle Separator (**PSPS**) as described by Heinrichs (1996) and Lammers et al. (1996). The PSPS used was equipped with 3 screens (19.0, 8.0, and 1.18 mm) and a bottom pan. Briefly, approximately 150 to 200 g of as-fed corn silage or TMR was placed on the top screen of the PSPS. The PSPS was shaken as described by Heinrichs (1996). Corn silage and TMR fractions retained on each screen and the bottom pan were then weighed to determine particle size distribution. Experimental cows were

Table 1. Ingredient and chemical composition of experimental diets varying in forage particle length[1] and forage:concentrate ratio.

	55% concentrate		45% concentrate	
Diet ingredients, % DM	Short cut	Long cut	Short cut	Long cut
Corn silage	45.4	45.4	55.3	55.3
Barley grain, rolled	31.2	31.2	20.58	20.58
Canola meal	7.01	7.01	7.59	7.59
Soybean meal	7.01	7.01	7.59	7.59
Corn gluten meal	2.76	2.76	2.78	2.78
Canola oil	1.08	1.08	0.89	0.89
WDDG[2]	1.38	1.38	1.39	1.39
Molasses	1.09	1.09	0.89	0.89
Mineral-vitamin mix[3]	1.52	1.52	1.57	1.57
Salt	0.31	0.31	0.29	0.29
Sodium bicarbonate	0.31	0.31	0.29	0.29
Dynamate	0.11	0.11	0.11	0.11
Limestone	0.78	0.78	0.71	0.71
Chemical composition				
DM, %	55.4	56.1	51.6	52.1
CP, % of DM	17.4	17.5	17.5	17.9
ADF, % of DM	15.8	15.4	17.2	16.5
NDF, % of DM	28.5	28.7	30.2	29.0
Crude fat, % of DM	4.3	4.5	5.9	5.6

[1]Corn silage particle length: short cut = 9.5-mm theoretical length of cut (TLC); long cut = 19-mm TLC.

[2]Wheat distillers dried grains.

[3]Contained (per kilogram of premix; DM basis): 330,000 IU of vitamin A, 60,000 IU of vitamin D, 1000 IU of vitamin E, 16% Ca, 8.5% P, 6.3% Na, 4.5% Mg, 2100 mg Zn, 1500 mg Mn, 535 mg Cu, 12 mg Se, 45 mg I.

milked twice daily at 0500 and 1500 h, and milk weights were recorded during the 7-d data collection period. Milk samples were collected daily from morning and afternoon milkings with or without preservative (2-bromo-2-nitropropane-1-2-diol). Milk samples were then pooled daily based on milk yield. Pooled samples with preservative were immediately submitted to the Provincial Milk Testing Laboratory (Saskatchewan Agriculture, Food and Rural Revitalization, Regina, SK, Canada) for compositional analysis. Milk samples were analyzed for CP and fat using a near infrared analyzer (Foss System 4000, Foss Electric, Hillerød, Denmark) according to AOAC (1990). Milk samples without preservative were stored at –20°C until further analysis for FA as described below.

Fatty Acid Analysis

Frozen milk samples for FA analysis were thawed in a 38°C water bath and mixed according to AOAC (1990) method 925.21, before milk fat extraction. Milk fat extraction was based on the method described by Mir et al. (1999). Briefly, an 8-mL milk sample was transferred into a screw-capped centrifuge tube, and 14 mL of hexane/isopropanol (3:2; vol/vol) was added. Contents were flushed with N_2 and then shaken for 3 min. The mixture was centrifuged at 1750 × *g* for 5 min at 5°C and the upper layer was transferred into another tube. Ten milliliters of hexane/isopropanol was added to the lower layer and re-extracted twice. The 3 extracts were pooled, 7 mL of 0.47 *M* aqueous sodium sulfate was added, and then the upper (hexane) layer was collected into preweighed tubes, and evaporated under N_2 in a 40°C water bath. Similarly, flaxseed fat was extracted from 5 g of a ground sample as described by Folch et al. (1957). Plasma fatty acids were determined on samples collected in tubes containing 15% (wt/vol) potassium EDTA. Briefly, 100 μL of plasma was placed in polytetraethylene screw-capped tubes and 4 mL of ethyl ether was added. Contents were shaken for 10 min. The extract was washed with 4 mL of saturated sodium chloride and centrifuged at 1750 × *g* for 5 min, and the organic phase transferred to a new tube. Extraction was repeated twice and the solvent was evaporated to dryness under a stream of N_2. The lipid extract was determined gravimetrically.

A known amount of extracted lipid was derivatized using tetramethylguanidine and methanol with heneicosanoic acid ($C_{21:0}$) as the internal standard. Briefly, to about 40 mg of extracted lipid in a test tube, 25 μL of 20 mg/mL $C_{21:0}$ was added to each sample, followed by 400 μL of methanol, and 100 μL of tetramethylguanidine. The tubes were flushed with N_2 and heated (while capped) in boiling water for 10 min. After cooling, 5 mL of saturated NaCl solution was added followed by 2 mL of petroleum ether. The tubes were flushed with N_2 and placed on a tube rocker for 5 min or until there was clear phase separation. The organic phase was transferred to a new test tube and evaporated under N_2. The contents were resuspended in 5 mL of hexane, ready to determine fatty acid profile by gas chromatography.

Fatty acid analysis was carried out in duplicate on an SP-2650, 100 m × 0.25 mm ID, 0.2 μm film column (Sigma Aldrich, Ontario, Canada) installed in a high-performance gas chromatograph using a flame ionization detector with capillary injection system at a split ratio of 1:100. The oven temperature was set at 140°C for 5 min, raised to 240°C at 4.0°C/min, and then held for 60 min. Helium was used as a carrier gas at a flow rate of 1.1 mL/min. Identification of the fatty acids was by comparison with retention times of known standards (Sigma Aldrich) and amounts present were determined by calculation based on the internal standard.

Statistical Analyses

All data on DMI, milk yield and composition, milk fatty acid composition, and blood fatty acid profiles were analyzed as a 4 × 4 replicated Latin square with a factorial arrangement of treatments using the Proc Mixed

Table 2. Chemical composition of short cut and long cut corn silage.[1]

	Corn silage	
Item	Short cut	Long cut
DM, %	30.9 (1.1)	31.0 (0.8)
CP, % of DM	9.7 (0.6)	9.7 (0.7)
ADF, % of DM	23.8 (0.6)	24.4 (0.3)
NDF, % of DM	45.8 (3.1)	45.1 (1.8)

[1]Means (SD) for short cut (9.5-mm theoretical length of cut; TLC) and long cut (19-mm TLC) corn silage.

procedure of SAS (SAS Institute, 1998). The statistical model included the effects of square, period, corn silage particle length, F:C ratio, and corn silage particle length × F:C ratio. Significant effects were declared at $P < 0.05$, unless otherwise indicated.

RESULTS AND DISCUSSION

The nutrient composition of experimental diets is presented in Table 1. Mean diet ADF concentrations for the concentrate-rich diet (15.6%) and the forage-rich diet (16.9%) were below NRC (2001) recommendations. This was a function of a lower than anticipated ADF concentration of the corn silage (see Table 2). It is possible that this deficiency in dietary ADF might have reduced chewing and rumination activity, thus potentially inducing a more severe ruminal acidosis than we intended with the reduced forage particle size and a higher dietary concentrate level. However, even though we did not measure ruminal pH in the present study, experimental cows did not show any clinical signs of ruminal acidosis such as loose feces or diarrhea. Furthermore, experimental periods were relatively short (21 d) and any adverse effects of a dietary ADF deficiency would likely be short-term. Table 3 shows particle size distribution of the short- and long-cut corn silage, and of the experimental diets. As expected, the proportion of as-fed long-cut silage retained on the PSPS 19-mm screen was 19 percentage units higher than that of short-cut corn silage. The percentage of long-cut corn silage retained on the PSPS 8-mm screen was 14.5 percentage units lower compared with short-cut corn silage. As a result, experimental diets containing long-cut corn silage had a higher proportion of as-fed particles being retained on the PSPS 19-mm screen, and a lower proportion of as-fed particles retained on the PSPS 8-mm screen compared with experimental diets containing short-cut corn silage (Table 3). Differences in the percentage of as-fed particles retained on both the PSPS 1.18-mm screen and bottom pan were small for short- and long-cut corn silage, and for experimental diets (Table 3).

Dry matter intake was unaffected ($P > 0.05$) by corn silage particle length (Table 4). These findings are in agreement with several other studies (Clark and Armentano, 1998; Bal et al. 2000; Onetti et al., 2003) that reported a lack of effect of corn silage particle length on DMI. However, other studies have reported a decrease in DMI of dairy cows when corn silage TLC was increased from 19 to 32 mm (Schwab et al., 2002) and from 30 to 40 mm (Timmermans et al., 2000), an observation likely related to higher rumen retention times with long-cut corn silage (Onetti et al., 2003). Corn silage TLC in our study were 9.52 and 19.05 mm, and were below the TLC range used by Timmermans et al. (2000) and Schwab et al. (2002); therefore, any direct comparisons between our study and these studies might be misleading. Feeding a higher level of concentrate tended ($P = 0.07$) to increase DMI by 1.6 kg/d (Table 4). Yang et al. (2001) observed that dietary F:C ratios of 35:65 or 55:45 had no effects on DMI in cows fed barley silage-based diets; however, Kalscheur et al. (1997) reported a higher DMI in cows fed 25% forage compared with those fed 60% with corn-silage-based diets. Milk yield, 3.5% FCM yield, and milk composition were unaffected ($P > 0.05$) by corn silage particle length and level of concentrate; however, cows fed diets containing long-cut corn silage had a higher ($P = 0.04$) milk protein yield compared with cows fed short-cut corn silage (Table 4). The reason for this higher milk protein yield is unclear, but we surmise that it was because of numerically lower milk yield for cows fed short-cut corn silage at 45% concentrate (Table 4). The lack of effect of corn silage TLC on milk yield and milk composition is in agreement with findings reported by others (Bal et al., 2000; Schwab et al., 2002; Onetti et al., 2003). Beauchemin et al. (1994) concluded that effects of

Table 3. Penn State Particle Separator (PSPS) determinations (% retained, as-is basis) of corn silage and experimental diets fed to dairy cows.[1]

	Corn silage		55% concentrate		45% concentrate	
PSPS screen size	Short cut	Long cut	Short cut	Long cut	Short cut	Long cut
>19 mm	4.24 (0.18)	23.2 (2.4)	2.35 (0.52)	17.7 (2.7)	2.87 (0.50)	17.3 (3.1)
>8 mm	73.0 (2.4)	58.5 (4.8)	61.2 (3.6)	45.2 (1.9)	65.3 (2.6)	42.6 (1.9)
>1.18 mm	21.7 (2.0)	17.5 (1.9)	33.9 (3.5)	35.6 (2.9)	29.6 (2.0)	31.6 (1.4)
Bottom pan	0.92 (0.50)	1.20 (0.41)	2.42 (0.21)	2.63 (0.22)	2.15 (0.77)	2.48 (0.50)

[1]Means (SD) for short cut (9.5-mm theoretical length of cut; TLC) and long cut (19-mm TLC) corn silage.

Table 4. Dry matter intake, milk yield, and milk composition of Holstein cows supplemented with flaxseed as affected by feeding diets varying in corn silage particle length[1] and forage:concentrate ratio.

	55% concentrate		45% concentrate			Contrasts: P value[2]		
Item	Short cut	Long cut	Short cut	Long cut	SE	L	C	L × C
DMI, kg/d	26.4	26.1	24.9	24.5	1.02	0.24	0.07	0.19
Milk yield, kg/d	41.4	42.3	38.9	40.8	1.42	0.37	0.39	0.64
3.5% FCM, kg/d	41.3	41.3	39.0	40.7	1.32	0.38	0.16	0.42
Fat, %	3.44	3.30	3.54	3.45	0.13	0.21	0.32	0.61
Fat yield, kg/d	1.42	1.39	1.32	1.39	0.06	0.69	0.38	0.71
Protein, %	3.04	3.07	3.02	3.09	0.14	0.39	0.61	0.64
Protein yield, kg/d	1.25	1.29	1.17	1.25	0.04	0.04	0.42	0.81

[1]Corn silage particle length: short cut = 9.5-mm theoretical length of cut (TLC); long cut = 19-mm TLC.
[2]L = Main effect of corn silage length of cut; C = main effect of forage:concentrate ratio; L × C = interaction.

particle size on milk fat content were likely to be observed when NDF levels were below minimum requirements recommended by NRC (2001). In our study, dietary NDF contents were higher than 28.5%.

The primary objective of the present study was to examine the effects of corn silage particle length and F:C ratio on FA composition of milk fat. We hypothesized that decreasing corn silage particle length and F:C ratio would depress ruminal pH, primarily as a result of reduced chewing and rumination activity due to smaller forage particle size, and higher ruminal VFA production due to feeding more grain. In turn, depressed ruminal pH would impair ruminal FA biohydrogenation as reported previously (Van Nevel and Demeyer, 1996; Loor et al., 2003), thus potentially increasing unsaturated FA flow to the small intestine and their subsequent incorporation into milk fat. Corn silage particle size and F:C ratio had no effect ($P > 0.05$) on milk fat proportions of C_{10} to $C_{14:1}$, and $C_{16:1}$ (Table 5). In dairy cows, short-chain fatty acids, i.e., C_{10} to $C_{14:1}$, are mainly derived from de novo synthesis in the mammary gland (Kennelly and Glimm, 1998); therefore, the lack of dietary treatment effects on milk fat levels of these short-chain fatty acids is indicative of a similar extent of mammary de novo synthesis.

Corn silage particle length had no effect ($P > 0.05$) on milk fat proportion of $C_{16:0}$; however, there was an interaction ($P < 0.01$) between corn silage particle length and F:C ratio (Table 5). In dairy cows, $C_{16:0}$ in milk fat can be derived from both dietary sources and de novo synthesis in the mammary gland (Grummer, 1991). There was significant interaction ($P < 0.05$) between corn silage particle length and concentrate level on milk fat proportions of $C_{18:1}$ *cis*-9, $C_{18:1}$ *trans*-10, and $C_{18:2}$ *cis*-9, *trans*-11 (Table 5). Feeding long-cut corn silage at high F:C ratio increased the proportions of $C_{18:1}$ *cis*-9 and $C_{18:2}$ *cis*-9, *trans*-11 in milk fat. Corn silage particle length and dietary F:C ratio had no effect ($P > 0.05$) on milk fat content of $C_{18:2}$ *trans*-10, *cis*-12 (Table 5), also a key

Table 5. Milk fatty acid composition (expressed as g/100 g of milk fat) of Holstein cows supplemented with flaxseed as affected by feeding diets varying in corn silage particle length[1] and forage:concentrate ratio.

	55% concentrate		45% concentrate			Contrasts: P value[2]		
Fatty acids	Short cut	Long cut	Short cut	Long cut	SE	L	C	L × C
$C_{10:0}$	3.36	3.62	3.92	3.49	0.23	0.72	0.95	0.14
$C_{12:0}$	3.99	4.23	4.02	4.20	0.19	0.29	0.94	0.87
$C_{14:0}$	13.1	13.4	13.2	13.1	0.35	0.68	0.94	0.65
$C_{14:1}$	1.56	1.53	1.53	1.58	0.10	0.92	0.93	0.66
$C_{16:0}$	30.9	27.2	30.8	32.4	0.86	0.05	0.01	0.01
$C_{16:1}$	1.98	1.86	1.91	1.92	0.07	0.55	0.81	0.35
$C_{18:0}$	12.5	12.3	12.1	12.6	0.41	0.75	0.97	0.45
$C_{18:1}$, *c*9	27.9	25.6	27.8	29.3	0.77	0.44	0.03	0.02
$C_{18:1}$, *c*6	0.39	0.36	0.44	0.35	0.06	0.46	0.98	0.85
$C_{18:1}$, *t*10	1.02	1.19	1.24	1.56	0.04	0.04	0.05	0.04
$C_{18:1}$, *t*11	0.46	0.41	0.56	0.61	0.05	0.41	0.04	0.10
$C_{18:2}$, *c*9, *c*12	2.67	2.77	2.69	2.56	0.15	0.91	0.54	0.47
$C_{18:2}$, *t*10, *c*12	0.047	0.058	0.056	0.047	0.006	0.94	0.94	0.61
$C_{18:2}$, *c*9, *t*11	0.60	0.57	0.61	0.69	0.02	0.37	0.02	0.02
$C_{18:3}$	0.93	1.01	0.99	0.88	0.02	0.89	0.65	0.17

[1]Corn silage particle length: short cut = 9.5-mm theoretical length of cut (TLC); long cut = 19-mm TLC.
[2]L = Main effect of corn silage length of cut; C = main effect of forage:concentrate ratio; L × C = interaction.

Table 6. Plasma fatty acid composition (expressed as g/100 g of fat) of Holstein cows supplemented with flaxseed as affected by feeding diets varying in corn silage particle length[1] and forage:concentrate ratio.

	55% concentrate		45% concentrate			Contrasts: P value[2]		
Fatty acids	Short cut	Long cut	Short cut	Long cut	SE	L	C	L × C
$C_{14:0}$	1.23	1.44	1.22	1.11	0.06	0.39	0.23	0.21
$C_{14:1}$	0.45	0.51	0.42	0.46	0.20	0.61	0.39	0.71
$C_{16:0}$	11.2	12.9	9.89	10.4	0.60	0.49	0.01	0.01
$C_{16:1}$	0.44	0.52	0.49	0.56	0.03	0.26	0.61	0.37
$C_{18:0}$	19.3	19.8	18.6	17.1	0.36	0.42	0.28	0.31
$C_{18:1}$, *c*9	9.31	11.7	9.56	10.1	0.28	0.21	0.03	0.02
$C_{18:1}$, *t*11	0.31	0.23	0.48	0.33	0.11	0.33	0.04	0.64
$C_{18:2}$, *c*9, *c*12	44.6	47.8	49.6	47.9	1.05	0.05	0.04	0.04
$C_{18:3}$	6.12	5.39	6.67	6.32	0.07	0.42	0.61	0.67

[1]Corn silage particle length: short cut = 9.5-mm theoretical length of cut (TLC); long cut = 19-mm TLC.
[2]L = Main effect of corn silage length of cut; C = main effect of forage:concentrate ratio; L × C = interaction.

CLA isomer. The changes in milk FA profiles observed in this study, especially for $C_{16:0}$, are in accordance with changes that occur during dietary-induced milk fat depression (Bauman and Griinari, 2001). The presence in milk fat of $C_{18:1}$ *trans* FA, which are usually intermediates of ruminal biohydrogenation, has been associated with milk fat depression. Baumgard et al. (2002) demonstrated that $C_{18:1}$ *trans*-10 may be a potent inhibitor of milk fat synthesis. In the current study, we did not measure dietary treatment effects on patterns of ruminal fermentation, but we can surmise that our dietary treatments might have caused only subtle changes in ruminal environment and shifts in biohydrogenation pathways; hence, milk fat was not significantly depressed. Flaxseed is rich in C18:3 and ruminal biohydrogenation of this FA leads to $C_{18:1}$ *trans*-11 that can be absorbed postruminally and be taken up by the mammary gland for use as a substrate for $C_{18:2}$ *cis*-9, *trans*-11 synthesis via γD^9-desaturase activity. We observed that the effects of corn silage particle length on $C_{18:1}$ *trans*-11, a precursor for CLA, showed significant trends when low concentrate, short-cut corn silage diets were fed. Flaxseed also contains a moderate proportion of $C_{18:2}$ (15.2 g/100 g of FA). Ruminal biohydrogenation of $C_{18:2}$ leads to $C_{18:2}$ *cis*-9, *trans*-11 CLA accumulation in the rumen, which can be absorbed postruminally and be taken up by the mammary gland and incorporated into milk fat. A further biohydrogenation of $C_{18:2}$ results in $C_{18:1}$ *trans*-11, which is also a precursor for CLA synthesis via Δ^9-desaturase activity in the mammary gland (Griinari et al., 2000). Our observations that both $C_{18:1}$ *trans*-10 and $C_{18:1}$ *trans*-11 in milk fat were increased when low concentrate, short-cut corn silage diets were fed was unexpected, and the reasons for this observation are unclear. In the absence of ruminal measurements, we speculate that the severity of ruminal pH depression due to feeding short-cut corn silage or more concentrate was somewhat mild, such that any changes in ruminal FA biohydrogenation due to altered ruminal pH were small. This is supported by our observation that, across dietary treatments, $C_{18:2}$ *cis*-9, *trans*-11 was the predominant CLA isomer in milk fat as compared with $C_{18:2}$ *trans*-10, *cis*-12 (Table 5). At higher ruminal pH (>5.8 to 6.0), the predominant CLA isomer produced in the rumen is $C_{18:2}$ *cis*-9, *trans*-11 (Bauman and Griiniri, 2001). This study demonstrated that when feeding high concentrate diets, short-cut corn silage would depress milk fat content of CLA; however, this is reversed when low concentrate diets are fed.

Blood concentrations of $C_{14:0}$, $C_{14:1}$, $C_{16:1}$, $C_{18:0}$, and $C_{18:3}$ were unaffected ($P > 0.05$) by corn silage particle length and level of dietary concentrate (Table 6). There was significant interaction ($P < 0.05$) between corn silage particle length and level of dietary concentrate on blood concentrations of $C_{16:0}$, $C_{18:1}$ *cis*-9, and $C_{18:2}$ *cis*-9, *cis*-12 (Table 6). Feeding long-cut corn silage at high F:C ratio increased the proportions of $C_{16:0}$ and $C_{18:1}$ *cis*-9 in blood, whereas $C_{18:2}$ *cis*-9, *cis*-12 was increased. Not surprisingly, the trends in blood FA profiles as affected by dietary treatment reflect trends observed in milk FA profiles.

CONCLUSIONS

This study demonstrates that the effects of supplementing flaxseed in diets of lactating dairy cows as a source of PUFA in milk is influenced by corn silage particle length and F:C ratio. The changes in milk FA profiles observed here are likely due to shifts in ruminal environmental kinetics that, in turn, affect bacterial FA biohydrogenation pathways. An interaction of corn silage particle length and F:C ratio was observed for milk fat contents of $C_{18:1}$ *cis*-9 and $C_{18:2}$ *cis*-9, *trans*-11, as milk fat of cows fed short-cut corn silage at low F:C ratio had higher proportions of these FA. Our results show that corn silage particle length and F:C ratio influence milk

FA profiles in dairy cows fed supplemental flaxseed as a source of polyunsaturated fatty acids.

ACKNOWLEDGMENTS

Partial financial support was provided by the Saskatchewan Department of Agriculture, Food and Rural Revitalization.

REFERENCES

AOAC. 1990. Official Methods of Analysis. 15th ed. Association of Official Analytical Chemists. Arlington, VA.

Bal, M. A., R. D. Shaver, A. G. Jirovec, K. J. Shinners, and J. G. Coors. 2000. Crop processing and chop length of corn silage: Effects on intake, digestion, and milk production by dairy cows. J. Dairy Sci. 83:1264–1273.

Bauman, D. E., and J. M. Graniiri. 2001. Regulation and nutritional manipulation of milk fat: Low-fat syndrome. Livest. Prod. Sci. 70:15–29.

Baumgard, L. H., E. Matitashvili, B. A. Corl, D. A. Dwyer, and D. E. Bauman. 2002. *trans*-10, *cis*-12 CLA decreases lipogenic rates and expression of genes involved in milk lipid synthesis in dairy cows. J. Dairy Sci. 85:2155–2163.

Beauchemin, K. A., B. I. Farr, L. M. Rode, and G. B. Schaalje. 1994. Effects of alfalfa silage chop length and supplementary long hay on chewing and milk production of dairy cows. J. Dairy Sci. 77:1326–1339.

Berner, L. A. 1993. Defining the role of milkfat in balanced diets. Adv. Food Nutr. Res. 37:131–257.

Chilliard, Y., A. Ferlay, and M. Doreau. 2001. Effect of different types of forages, animal fat or marine oils in cow's diet on milk fat secretion and composition, especially conjugated linoleic acid (CLA) and polyunsaturated fatty acids. Livest. Prod. Sci. 70:31–48.

Chouinard, P. Y., V. Girard, and G. J. Brisson. 1992. Fatty acid composition of cow's milk as influenced by feeding soybeans extruded at different temperatures. J. Dairy Sci. 75(Suppl. 1):277.

Clark, P. W., and L. E. Armentano. 1999. Influence of particle size on the effectiveness of the fiber in corn silage. J. Dairy Sci. 82:581–588.

Folch, J., M. Lees, and G. H. Sloane-Stanley. 1957. A simple method for the isolation and purification of total lipids from animal tissues. J. Biol. Chem. 226:497–509.

Griinari, J. M., B. A. Corl, S. H. Lacy, P. Y. Chouinard, K. V. Nurmela, and D. E. Bauman. 2000. Conjugated linoleic acid is synthesized endogenously in lactating dairy cows by Δ^9-desaturase. J. Nutr. 130:2285–2294.

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

Heinrichs, J. 1996. Pages 1–9 in Evaluating Particle Size of Forages and TMRs using the Penn State Particle Size Separator. Pennsylvania State University, State College.

Jenkins, T. C. 1993. Lipid metabolism in the rumen. J. Dairy Sci. 76:3851–3863.

Jensen, R. G. 2002. Invited review: The composition of bovine milk lipids: January 1995 to December 2000. J. Dairy Sci. 85:295–350.

Kalscheur, K. F., B. B. Teter, L. S. Piperova, and R. A. Erdman. 1997. Effect of dietary forage concentration and buffer addition on duodenal flow of *trans*-C18:1 fatty acids and milk fat production in dairy cows. J. Dairy Sci. 80:2104–2114.

Keady, T. W. J., C. S. Mayne, and D. A. Fitzpatrick. 2000. Effects of supplementation of dairy cattle with fish oil on silage intake, milk yield and milk composition. J. Dairy Res. 67:137–153.

Kennelly, J. J. 1996. The fatty acid composition of milk fat as influenced by feeding oilseeds. Anim. Feed Sci. Technol. 60:137–152.

Kennelly, J. J., and D. R. Glimm. 1998. The biological potential to alter the composition of milk. Can. J. Anim. Sci. 78(Suppl.):23–56.

Kepler, C. R., K. P. Hirons, J. J. McNeill, and S. B. Tove. 1966. Intermediates and products of the biohydrogenation of linoleic acid by *Butyrivibrio fibrisolvens.* J. Biol. Chem. 241:1350–1354.

Lammers, B. P., D. R. Buckmaster, and A. J. Heinrichs. 1996. A simple method for the analysis of particle sizes of forage and total mixed rations. J. Dairy Sci. 79:922–928.

Loor, J. J., J. H. Herbein, and C. E. Polan. 2002. *Trans*-18:1 and 18:2 isomers in blood plasma and milk fat of grazing cows fed a grain supplement containing solvent-extracted or mechanically extracted soybean meal. J. Dairy Sci. 85:1197–1207.

Mansbridge, R. J., and J. S. Blake. 1997. Nutritional factors affecting the fatty acid composition of bovine milk. Br. J. Nutr. 78(Suppl. 1):S37–S47.

Massaro, M., M. A. Carluccio, and R. de Caterina. 1999. Direct vascular antiatherogenic effects of oleic acid: A clue to the cardioprotective effects of the Mediterranean diet. Cardiologia 44:507–513.

Mir, Z., L. A. Goonewardene, O. Okine, S. Jeager, and H. D. Scheer. 1999. Effect of feeding canola oil on constituents, CLA, and long chain fatty acids in goat milk. Small Rumin. Res. 33:137–143.

Murphy, J. J., G. P. McNeill, J. F. Connolly, and P. A. Gleeson. 1990. Effect on cow performance and milk fat composition of including full fat soyabeans and rapeseeds in the concentrate mixture for lactating dairy cows. J. Dairy Res. 57:295–306.

NRC. 2001. Nutrient Requirements of Dairy Cattle. 7th rev. ed. National Academy Press. Washington, DC.

Offer, N. W., M. Marsden, J. Dixon, B. K. Speake, and F. E. Thacker. 1999. Effect of dietary fat supplements on levels of n-3 polyunsaturated fatty acids, trans acids and conjugated linoleic acid in bovine milk. Anim. Sci. 69:613–625.

Onetti, S. G., R. D. Shaver, S. J. Bertics, and R. R. Grummer. 2003. Influence of corn silage particle length on performance of lactating dairy cows fed supplemental tallow. J. Dairy Sci. 86:2949–2957.

Parodi, P. W. 1997. Cow's milk fat components as potential anti-carcinogenic agents. J. Nutr. 127:1055–1060.

Qui, X., M. L. Eastridge, and J. L. Firkins. 2004. Effects of dry matter intake, addition of buffer, and source of fat on duodenal flow and concentration of conjugated linoleic acid and *trans*-11 C18:1 in milk. J. Dairy Sci. 87:4278–4286.

SAS Institute. 1998. SAS/STAT User's Guide (Release 6.03). SAS Inst. Inc., Cary, NC.

Schwab, E. C., R. D. Shaver, K. J. Shinners, J. L. Lauer, and J. G. Coors. 2002. Processing and chop length effects in brown-midrib corn silage on intake, digestion and milk production by dairy cows. J. Dairy Sci. 85:613–623.

Soita, H. W., D. A. Christensen, and J. J. McKinnon. 2000. Influence of particle size on the effectiveness of the fiber in barley silage. J. Dairy Sci. 83:2295–2300.

Soita, H. W., J. A. Meier, M. Fehr, P. Yu, D. A. Christensen, J. J. McKinnon, and A. F. Mustafa. 2003. Effects of flaxseed supplementation on milk production, milk fatty acid composition and nutrient utilization by lactating dairy cows. Arch. Anim. Nutr. 57:107-116.

Timmermans, S. J., L. M. Johnson, J. H. Harrison, and D. Davidson. 2000. Estimation of the flow of microbial nitrogen to the duodenum using uric acid or allantoin. J. Dairy Sci. 83:1286–1299.

Van Nevel, C. J., and D. I. Demeyer. 1996. Influence of pH on lipolysis and biohydrogenation of soybean oil by rumen contents in vitro. Rep. Nutr. Dev. 36:53–63.

Van Soest, P. J., J. B. Robertson, and B. A. Lewis. 1991. Methods for dietary fiber, neutral detergent fiber, and non-starch polysaccharides in relation to animal nutrition. J. Dairy Sci. 74:3593–3597.

Ward, A. T., K. M. Wittenberg, and R. Przybylski. 2002. Bovine milk fatty acid profiles produced by feeding diets containing solin, flax and canola. J. Dairy Sci. 85:1191–1196.

Yang, W. Z., K. A. Beauchemin, and L. M. Rode. 2001. Effects of grain processing, forage to concentrate ratio, and forage particle size on rumen pH and digestion by dairy cows. J. Dairy Sci. 84:2203–2216.