Milk Conjugated Linoleic Acid Response to Fish Oil and Sunflower Oil Supplementation to Dairy Cows Managed Under Two Feeding Systems

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ABSTRACT

Earlier research showed that conjugated linoleic acid (CLA) content in milk fat is highest when cows' diets are supplemented with a blend of fish oil (FO) and linoleic acid-rich oils. The objective of this study was to compare the effect of FO and sunflower oil (SFO) supplementation on milk *cis*-9, *trans*-11 CLA when dairy cows managed on pasture or in confinement. Fourteen Holstein cows were assigned into 2 treatment groups: cows grazed on alfalfa-grass pasture (PAS) or were fed corn silage-alfalfa hay mix ad libitum (LOT). Both groups were supplemented with a 8.2 kg/d grain supplement containing 640 g of FO and SFO (1:3 wt/wt). Grain supplement was fed in 2 equal portions after each milking, for a period of 3 wk. Milk samples were collected during the last 3 d of the experimental period. Milk yield was greater with the LOT diet (23.1 kg/d) compared with the PAS diet (19.4 kg/d). Milk fat percentages (2.51 and 2.95 for the LOT and PAS, respectively) and yields (0.57 and 0.51 kg/d) were similar for the 2 diets. Milk protein percentages were not affected by diets (3.34 and 3.35 for the LOT and PAS diets, respectively), but protein yields were lower for the PAS diet (0.61 kg/d) compared with the LOT diet (0.75 kg/d). Treatment diets had no effect on milk *trans* C18:1 concentrations [10.64 and 9.82 g/100 g of total fatty acids (FA) for the LOT and PAS, respectively] or yields (60.65 and 64.01 g/d), but did affect isomers distributions. Concentration (g/100 g of total FA) of vaccenic acid was lower with the LOT diet (2.15) compared with the PAS diet (4.52), whereas concentration of *trans*-10 C18:1 was greater with the LOT diet (4.99) compared with the PAS diet (1.69). Milk *cis*-9, *trans*-11 CLA concentration was greater with the PAS diet (1.52) compared with the LOT diet (0.84). In conclusion, the increase in milk *cis*-9, *trans*-11 CLA content was greater when pasture-based diets were supplemented with FO

and SFO. The lower *cis*-9, *trans*-11 CLA concentration in milk from the confinement-fed cows resulted from *trans*-10 C18:1 replacing vaccenic acid as the predominant *trans* C18:1 isomer.

Key words: forage source, fish oil, sunflower oil, milk conjugated linoleic acid

INTRODUCTION

Conjugated linoleic acid (CLA) is a collective term for positional and geometric isomers of linoleic acid (C18:2) in which the 2 double bonds are conjugated rather than separated by a methyl group. Due to the fact that the double bonds are present at various positions on the 18-carbon chain of the molecule and can have the *cis* and *trans* geometry, numerous different CLA are possible. Following reports (Sehat et al., 1998) that *cis*-9, *trans*-11 CLA, the major CLA isomer in milk fat, inhibits the growth of a number of human cancer cell lines and suppresses chemically induced tumors development in animal models (Parodi, 1999), there has been growing interest in developing dairy products enriched with *cis*-9, *trans*-11 CLA.

The *cis*-9, *trans*-11 CLA is formed in the rumen as an intermediate in the biohydrogenation of linoleic acid or in tissues by Δ^9 -desaturase from vaccenic acid (VA, *trans*-11 C18:1), another intermediate in the ruminal biohydrogenation of oleic, linoleic, and linolenic acids (Grinari and Bauman, 1999; AbuGhazaleh et al., 2005). Recent studies (Pipero et al., 2002; Kay et al., 2004) have estimated that more than 90% of milk *cis*-9, *trans*-11 CLA is made by the activity of the Δ^9 -desaturase enzyme.

Cows on pasture diets have been shown to have higher concentrations of CLA (Kelly et al., 1998; Lawless et al., 1998; White et al., 2001; Boken et al., 2005) in their milk than those consuming conserved forages. It also has been shown that milk CLA concentration can be enhanced in pasture grazing cows when their feed is supplemented with oils (Lawless et al., 1998; Boken et al., 2005; AbuGhazaleh and Holmes, 2007). Lawless et al. (1998) found that supplementation of grazing cows with full-fat soybeans or full-fat rapeseeds

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increased milk CLA concentration by 24.3 and 61%, respectively, relative to controls. Schroeder et al. (2003) reported that compared with grazing cows receiving corn-based concentrate, milk CLA increased by 173% when Ca-salts of fatty acids (FA; 30% linoleic acid) partially replaced corn grain. Cows grazing on annual rye-ryegrass pasture supplemented with a grain mix containing 500 g/d of soybean oil had the CLA content in milk increased by 46% compared with cows not supplemented with oil (Boken et al., 2005).

Inclusion of fish oil (FO) in TMR is an effective means of increasing milk *cis*-9, *trans*-11 CLA content (Donovan et al., 2000; Whitlock et al., 2002). This appears to occur because of the inhibitory effect of docosahexaenoic acid (C22:6n3) in FO on VA reduction to stearic acid (AbuGhazaleh and Jenkins, 2004). Milk *cis*-9, *trans*-11 CLA response to FO is further enhanced when given in combination with linoleic or linolenic acid-rich oil sources (AbuGhazaleh et al., 2002, 2003). AbuGhazaleh et al. (2003) concluded that supplementing dairy cows' diets with a combination of FO and linoleic acid-rich oils is the most effective dietary regimen to increase milk *cis*-9, *trans*-11 CLA content. Despite the volume of research on the impact of dietary FO and plant oils on milk's *cis*-9, *trans*-11 CLA content, there are surprisingly few data in the literature evaluating the effect that dietary oil supplements may have under different feeding systems (pasture vs. confinement) on *cis*-9, *trans*-11 CLA content. Therefore, the objective of this study was to compare milk *cis*-9, *trans*-11 CLA response in cows managed on pasture or in confinement when supplemented with a blend of FO and sunflower oil (SFO).

MATERIALS AND METHODS

Fourteen multiparous Holstein cows, selected from the herd of Southern Illinois University Dairy Cattle Research Center (Carbondale, IL), were used in a 5-wk study between August and September of 2006. All procedures for this study were conducted under approval of the Southern Illinois University Institutional Animal Care and Use Committee. During the first 2 wk (preexperiment), cows were housed in a free-stall barn and fed corn silage-alfalfa hay mix (65:35; DM basis) ad libitum plus 8.2 kg/d of basal grain supplement. The basal grain supplement contained (DM basis) 51% cracked corn, 29% soybean meal, 10% dried molasses, 5% meat and bone meal, 3.0% limestone, 1.5% dicalcium phosphate, and 1.5% vitamins and minerals (vitamin ADE premix, vitamin E premix, magnesium oxide, and high zinc trace mineralized salt). Milk production was recorded daily and milk samples were taken from each cow during the last 3 d (0600 and 1800 h) of wk 2 for composition and FA analysis. Milk production and

composition data generated during these 2 wk were used for covariance analysis. At the start of wk 3, cows were paired according to milk yield (24.3 ± 3.7 kg/d) and DIM (195 ± 18) and randomly assigned into 2 treatment groups using a complete randomized design with a 21-d experimental period. The first 18 d of the experimental period were used for adjustments to diets, and the last 3 d for data collections. Cows in group 1 remained on confinement and kept receiving the corn silage-alfalfa hay mix ad libitum (LOT), whereas cows in group 2 grazed on alfalfa-grass pasture (PAS). Both groups also received an 8.2-kg/d grain supplement containing 640 g of FO and SFO (1:3 wt/wt). The grain supplement contained (DM basis) 44.2% cracked corn, 27.1% soybean meal, 9.8% dried molasses, 6.4% SFO, 2.1% FO, 3.9% meat and bone meal, 3.3% limestone, 1.6% dicalcium phosphate, 0.7% high zinc trace mineralized salt, 0.3% magnesium oxide, 0.3% vitamin E premix, and 0.3% vitamin ADE premix. Menhaden FO (Omega Protein Inc., Hammond, LA) and SFO (purchased from a local dealer) were used in this study. Cows on pasture grazed together under intensive rotational grazing management. The pasture was diverse and consisted of alfalfa, orchardgrass, fescue, and weeds (35:35:15:15 wt/wt). The pasture area was divided into twenty-one 1-acre paddocks, and cows were moved to a new paddock daily. Polywire fencing prevented cows from grazing the next paddock or the previously grazed areas. Cows had free access to water. Grain supplement was offered in 2 equal portions after the morning and afternoon milking in the barn using Calan Broadbent feeder doors (American Calan Inc., Northwood, NH) for individual feed intakes. Amounts of grain supplement offered andorts were recorded daily for individual cows. Cows remained on the pasture daily for about 21 h.

Cows were milked twice daily at 0600 and 1800 h during the study, with individual milk weights recorded at each milking. Milk from individual cows was sampled during three 24-h periods on the last 3 d of the experiment and made into daily composites, amounts proportional to milk yield at each time. Composites were divided into 2 aliquots for analyses. One was refrigerated at 4°C and sent to Prairie Farms (Carlinville, IL) for analysis of fat, protein, lactose, and total solids (AOAC, 1997) by midinfrared spectrophotometry and SCC (AOAC, 1997) using a Fossomatic 90 (Prairie Farms). The second sample was stored at -20°C until analysis for FA composition. Representative pasture forage samples were collected on the last day of each week before grazing at 6 different locations per 1 acre using a 0.50-m² quadrat. The plants were clipped to a 2.5-cm stubble height using metal shears. Pasture samples were composited per week and stored at -20°C until analysis for chemical and FA composition. On the last day of each

week, samples of grain supplement, corn silage, and alfalfa hay were collected for analyses.

Pasture forage, corn silage, alfalfa hay, and grain supplement were freeze dried (Labconco Freeze Dry System, Labconco, Kansas City, MO) and then grounded through a standard model No. 3 Wiley mill (Arthur H. Thomas Co., Philadelphia, PA) with a 2-mm screen. Samples were analyzed for CP, ether extract, and ash according to AOAC methods (1997). Samples were reground (Brinkman ultracentrifuge mill) through a 1-mm screen prior to analyses for FA (Kramer et al., 1997) and fiber. Neutral detergent fiber (procedure B of Van Soest et al., 1991) and ADF (Robertson and Van Soest, 1981) were determined by Ankom fiber analyzer using the filter bag technique (Ankom Technology Corp., Fairport, NY). Body weights and BCS were recorded at the beginning and end of the study.

Milk and feed samples were methylated using the 2-step procedure outlined by Kramer et al. (1997). Milk samples were thawed in a 35°C water bath, and 1 mL was transferred into a glass test tube (16 × 200 mm) with Teflon-lined screw cap, stored at -80°C for 6 h, freeze dried, and then methylated. Methylated samples were then analyzed for FA using a Shimadzu GC-2010 gas chromatograph (Shimadzu Scientific Instruments Inc., Columbia, MD) equipped with a flame ionization detector and a Supelco 100-m SP-2560 fused-silica capillary column (0.25 mm i.d. × 0.2 µm film thickness). The helium carrier gas was maintained at a linear velocity of 23 cm/s. The oven temperature was programmed for 135°C for 5 min, then increased at 5°C/min to 165°C, held there for 80 min, then increased at 1.5°C/min to 180°C, then increased at 5°C/min to 245°C and held there for 9 min. Peaks were identified by comparing the retention times with FA in the corresponding standards (Nu-Chek Prep, Elysian, MN; Supelco, Bellefonte, PA; and Larodan Fine Chemicals, Malmö, Sweden).

The ANOVA was conducted using the MIXED procedure of SAS for a complete randomized design. The model contained the effects of diet, sample, and covariate. Cow within diet was used as an error term. Least squares means are reported throughout, and significance was declared at $P < 0.05$.

RESULTS AND DISCUSSION

The FA and nutrient compositions for the grain supplement, pasture, corn silage, and alfalfa hay are presented in Table 1. Oleic acid was the major FA in grain supplement, and it accounted for 45.6% of total FA, whereas linoleic acid accounted only for 24.9% of total FA. The unexpected high oleic acid concentration found in the grain supplement was due to high oleic levels

from the sunflower seed oil. Linoleic acid accounts for 70% of total FA in typical SFO (Casper et al., 1988) compared with 28% in our SFO supplement (Table 1). As expected, linolenic acid was the major FA in pasture (40.5% of total FA), whereas linoleic acid was the major FA in corn silage (48.5% of total FA). Linoleic and linolenic acids accounted for 24.4 and 23.2% of alfalfa hay total FA. The high NDF and ADF values for the pasture reflect its advanced phenological stage.

The effect of feeding systems on milk yield and composition is presented in Table 2. Compared with the LOT diet, the PAS diet resulted in a reduction ($P < 0.05$) in milk yield. By the end of the study, cows on the PAS diet produced 3.7 kg/d less milk than cows in the LOT diet. Reduction in milk yield is consistently seen in cows managed on pasture compared with cows managed in confinement (White et al., 2001; Bargo et al., 2002, 2006; Boken et al., 2005). Bargo et al. (2006) reported that cows kept on pasture for 19 wk and supplemented with grain mix (1 kg/4 kg of milk yield) produced 9.6 kg/d less milk than cows fed TMR. Additionally, when cows were managed under 2 different feeding systems (confinement vs. pasture) and fed oil supplement for 14-wk, Boken et al. (2005) reported that cows on pasture produced 5.8 kg/d less milk than cows in confinement.

Milk fat percentages (2.51 and 2.95 for the LOT and PAS, respectively) and yields (0.57 and 0.51 kg/d) were similar ($P > 0.05$) for the 2 diets. Milk protein percentages were not affected ($P > 0.05$) by diets averaging 3.34 and 3.35 for the LOT and the PAS diets, respectively. Boken et al. (2005) reported no difference in milk protein content between cows fed soybean oil and managed in confinement or on pasture. Recently, Holmes and AbuGhazaleh (2007) also observed no effect on milk protein content when equal amounts of linseed oil and FO were fed to cows managed under confinement or pasture feeding systems. Production of milk protein was lower ($P < 0.05$) with the PAS diet (0.61 kg/d) compared with the LOT diet (0.75 kg/d) as a result of lower milk yield with the PAS diet (Table 2). There were no differences in milk lactose, SCC, BW, BCS, and grain supplement intake between the 2 feeding groups (Table 2).

The effect of feeding systems on milk FA concentrations and yields is presented in Tables 3 and 4. Milk *trans* C18:1 concentrations (10.61 and 9.87 g/100 g of total FA for the LOT and PAS, respectively) and yields (60.65 and 64.01 g/d) were similar ($P > 0.05$) for both diets. Holmes and AbuGhazaleh (2007) also reported no differences in milk *trans* C18:1 when a blend of FO and linseed oil was fed to cows on pasture or in confinement. Even though milk *trans* C18:1 concentrations and yields were similar for the 2 diets, the distributions of individual isomers differed. The concentrations of

Table 1. Fatty acid and nutrient compositions for grain supplement, pasture, forages, and oil supplements

Fatty acid	Grain supplement ¹	Pasture ²	Alfalfa hay	Corn silage	Sunflower oil	Fish oil
C14:0	1.82	0.58	0.70	0.34	0.10	6.79
C16:0	9.72	13.95	29.98	16.59	4.76	16.67
C18:0	4.16	1.72	6.03	2.93	3.55	3.41
C18:1 <i>cis</i> -9	45.60	3.52	8.01	18.76	61.82	9.22
C18:2 <i>cis</i> -9, <i>cis</i> -12	24.86	15.65	24.43	48.50	27.89	1.13
C18:3n-3	0.73	40.53	23.15	11.09	0.10	1.49
C20:5n-3	1.90	ND ³	ND	ND	ND	10.72
C22:6n-3	2.03	0.27	ND	ND	ND	13.15
Composition, % of DM						
NE _L , ⁴ Mcal/kg	2.09					
CP	26.63	20.16	15.27	8.28		
NDF	16.03	61.39	56.74	51.36		
ADF	12.04	42.85	30.10	30.37		
Fatty acids	9.24	0.98	1.13	1.21		

¹Grain supplement = contained 640 g of fish oil and sunflower oil (1:3 wt/wt).

²Pasture = 35:35:20:10 alfalfa:orchard grass:fescue:weeds.

³ND = not detected or detected at < 0.01.

⁴NE_L = estimated NRC (2001).

milk VA and *trans*-16 were higher ($P < 0.05$) with the PAS diet than with the LOT diet, but the concentration of *trans*-10 was higher ($P < 0.05$) with the LOT diet than the PAS diet (Table 3). Vaccenic acid and *trans*-10 C18:1 were still the predominant *trans* C18:1 isomers in both diets accounting for 20 and 47% of *trans* C18:1 isomers in the LOT diet and 46 and 17% in the PAS diet, respectively. To the author's knowledge, there are no published data comparing the effect of dietary oil supplementations under pasture and confinement feed-

ing systems on the distributions of milk *trans* C18:1 isomers. Milk concentrations of VA increased and *trans*-10 C18:1 decreased in a linear manner as the proportion of fresh grass in the diet of lactating cows increased (Couvreur et al., 2006). Kay et al. (2005) reported that *trans*-10 C18:1 contributed <1% of the milk total FA in pasture-fed cows compared with about 25% in TMR fed cows. Furthermore, Looor et al. (2003) reported higher *trans*-10 C18:1 in milk of cows fed TMR compared with TMR plus pasture. Results from previous studies (Shingfield et al., 2005; AbuGhazaleh and Jacobson, 2007) suggest that diets that promote low ruminal pH (e.g., high starch intake) increase *trans*-10 C18:1 production in the rumen at the expenses of VA. *trans*-10 C18:1 in milk fat increased as the ratio of corn to alfalfa silage in the diet varied from 25 to 50% of diet DM and was further enhanced by lipid supplementation (Onetti et al., 2002). In this study, it appears that the intake of corn silage by the LOT cows may have provided ruminal conditions (low pH) favoring a shift in the biohydrogenation pathways toward more *trans*-10 C18:1 synthesis, possibly as a result of alteration in the microbial community profile, enzyme activities, or both. The concentrations of *trans*-6/8, *trans*-9, and *trans*-12 C18:1 were similar ($P > 0.05$) for the 2 diets, averaging 9.7, 8.3, and 12.5% of milk *trans* C18:1, respectively.

The main objective of this study was to compare milk *cis*-9, *trans*-11 CLA response to feeding a CLA-stimulating diet under pasture or confinement feeding systems. Even though the intake of supplemental oil was similar for both diets, the concentrations and yields of milk total CLA were higher ($P < 0.05$) for cows on the PAS diet (1.61 g/100 g of total FA and 7.93 g/d) than the LOT diet (0.87 g/100 g of total FA and 4.93 g/d). In both

Table 2. Effect of treatment diets on milk yield, milk composition, and grain intake

Item	Diet ¹		SEM
	LOT	PAS	
Milk yield, kg/d	23.10 ^a	19.60 ^b	1.351
Concentration (g/kg)			
Fat	2.51	2.95	0.254
Protein	3.34	3.35	0.220
Lactose	4.45	4.51	0.096
TS	11.29	11.65	0.450
Yield (kg/d)			
Fat	0.57	0.51	0.063
Protein	0.75 ^a	0.61 ^b	0.048
Lactose	1.02	0.88	0.115
TS	2.52	2.21	0.172
SCC, × 10 ³ /mL	424	329	66
BW, kg	580	613	34
BCS ²	2.82	2.77	0.140
Grain supplement intake, kg/d	8.19	8.18	0.015

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

¹LOT = corn silage-alfalfa hay mix plus 640 g of fish oil and sunflower oil (1:3 wt/wt); PAS = pasture plus 640 g of fish oil and sunflower oil (1:3 wt/wt).

²Scored on a 5-point scale, where 1 = emaciated to 5 = fat.

Table 3. Effect of treatment diets on milk fatty acid composition (g/100 g of fatty acids)

Fatty acid	Diet ¹		SEM
	LOT	PAS	
C4:0	1.34	1.23	0.112
C6:0	1.10	0.99	0.070
C8:0	0.62	0.53	0.038
C10:0	1.27 ^a	0.99 ^b	0.075
C12:0	1.68	1.26	0.080
C14:0	8.18 ^a	6.56 ^b	0.296
C14:1	0.91	0.76	0.106
C15:0	0.82	0.83	0.031
C16:0	23.81	22.17	0.915
C16:1	1.78	1.70	0.188
C17:0	0.48	0.52	0.021
C18:0	9.89	11.31	0.920
C18:1 <i>cis</i> -9	23.14 ^b	27.06 ^a	0.786
C18:1 <i>cis</i> -11	0.98 ^a	0.85 ^b	0.041
C18:1 <i>cis</i> -12	0.34 ^a	0.28 ^b	0.020
C18:1 <i>trans</i>	10.61	9.87	0.652
<i>trans</i> -6/8	0.99	1.00	0.073
<i>trans</i> -9	0.80	0.89	0.042
<i>trans</i> -10	4.99 ^a	1.69 ^b	0.409
<i>trans</i> -11	2.15 ^b	4.53 ^a	0.385
<i>trans</i> -12	1.33	1.22	0.052
<i>trans</i> -16	0.37 ^b	0.49 ^a	0.025
C18:2 <i>cis</i> -9, <i>cis</i> -12	2.32 ^a	1.80 ^b	0.105
C18:2 <i>cis</i> -9, <i>trans</i> -12	0.12 ^b	0.15 ^a	0.011
C18:2 <i>trans</i> -9, <i>cis</i> -12	0.02	0.03	0.007
C18:2 <i>trans</i> -9, <i>trans</i> -12	0.01	0.01	0.002
C18:2 <i>trans</i> -11, <i>cis</i> -15	0.14 ^b	0.23 ^a	0.018
CLA	0.87 ^b	1.61 ^a	0.178
<i>cis</i> -9, <i>trans</i> -11	0.84 ^b	1.53 ^a	0.133
<i>cis</i> -10, <i>cis</i> -12	0.01	0.01	0.003
<i>trans</i> -10, <i>cis</i> -12	ND ²	0.01	0.002
<i>trans</i> , <i>trans</i>	0.03 ^b	0.07 ^a	0.014
18:3n-3	0.16	0.16	0.015
C18:3n-6	0.01	0.01	0.003
C18:4n-3	0.01	0.02	0.004
C20:0	0.17 ^b	0.24 ^a	0.022
C20:1	0.03	0.01	0.008
C20:2	0.01	0.02	0.005
C20:4	0.05	0.04	0.005
C20:5n-3	0.04	0.03	0.006
C22:2	0.02	0.01	0.004
C22:3n-3	0.02	0.01	0.003
C22:4	0.12	0.12	0.016
C22:5n-3	0.07	0.06	0.004
C22:6n-3	0.05	0.04	0.004

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

¹LOT = corn silage-alfalfa hay mix plus 640 g of fish oil and sunflower oil (1:3 wt/wt); PAS = pasture plus 640 g of fish oil and sunflower oil (1:3 wt/wt).

²ND = not detected or detected at <0.01 .

diets, the predominant CLA isomer was *cis*-9, *trans*-11 CLA accounting for 95 and 94% of total CLA in the LOT and the PAS diets, respectively. Milk *cis*-9, *trans*-11 CLA concentration and yield were 81 and 60%, respectively, higher with the PAS diet than the LOT diet. Concentration of *cis*-9, *trans*-11 CLA is usually higher in milk fat from cows fed fresh forages compared with conserved forages (White et al., 2001; Boken et al., 2005;

Table 4. Effect of treatment diets on milk fatty acid yield (g/d)

Fatty acid	Diet ¹		SEM
	LOT	PAS	
C4:0	7.44	7.16	0.176
C6:0	6.14	5.61	0.936
C8:0	3.55	2.87	0.511
C10:0	7.26	5.27	0.935
C12:0	9.44	6.57	0.992
C14:0	45.70	33.94	4.388
C14:1	5.11	3.64	0.605
C15:0	4.57	4.28	0.471
C16:0	131.85	114.49	12.699
C16:1	9.50	8.26	0.765
C17:0	2.97	2.6	0.404
C18:0	58.36	60.35	11.084
C18:1 <i>cis</i> -9	132.84	139.00	13.116
C18:1 <i>cis</i> -11	5.18	4.71	0.384
C18:1 <i>cis</i> -12	1.86	1.47	0.185
C18:1 <i>trans</i>	60.65	64.01	7.530
<i>trans</i> -6/8	5.76	4.88	0.623
<i>trans</i> -9	4.49	4.44	0.350
<i>trans</i> -10	27.86 ^a	8.71 ^b	3.252
<i>trans</i> -11	13.35 ^b	23.10 ^a	2.910
<i>trans</i> -12	7.62	6.23	0.797
<i>trans</i> -16	2.13	2.62	0.360
C18:2 <i>cis</i> -9, <i>cis</i> -12	13.31 ^a	9.39 ^b	1.387
C18:2 <i>cis</i> -9, <i>trans</i> -12	0.63	0.76	0.053
C18:2 <i>trans</i> -9, <i>cis</i> -12	0.15	0.16	0.062
C18:2 <i>trans</i> -9, <i>trans</i> -12	0.04	0.03	0.009
C18:2 <i>trans</i> -11, <i>cis</i> -15	0.93	1.12	0.146
CLA	4.93 ^b	7.93 ^a	0.787
<i>cis</i> -9, <i>trans</i> -11	4.69 ^b	7.50 ^a	0.759
<i>cis</i> -10, <i>cis</i> -12	0.04	0.06	0.013
<i>trans</i> -10, <i>cis</i> -12	0.02	0.03	0.012
<i>trans</i> , <i>trans</i>	0.15 ^b	0.32 ^a	0.062
18:3n-3	0.93	0.84	0.16
C18:3n-6	0.03	0.05	0.015
C18:4n-3	0.04	0.07	0.018
C20:0	0.92	1.28	0.212
C20:1	0.15 ^a	0.05 ^b	0.034
C20:2	0.07	0.10	0.024
C20:4	0.30	0.23	0.046
C20:5n-3	0.23	0.17	0.39
C22:2	0.12	0.07	0.030
C22:3n-3	0.10 ^a	0.04 ^b	0.014
C22:4	0.71	0.54	0.072
C22:5n-3	0.39	0.35	0.049
C22:6n-3	0.27	0.22	0.035

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

¹LOT = corn silage-alfalfa hay mix plus 640 g of fish oil and sunflower oil (1:3 wt/wt). PAS = pasture plus 640 g of fish oil and sunflower oil (1:3 wt/wt).

Khanal et al., 2005; Bargo et al., 2006; Couvreur et al., 2006). It has been shown that when calcium-salts of FA (30% linoleic acid) partially replace corn in the grain mix supplemented to grazing dairy cows, milk *cis*-9, *trans*-11 CLA increases by 71% and by 366% when compared with cows fed TMR (Schroeder et al., 2003). Khanal et al. (2005) also showed that cows that were grazed on pasture and received 3.2 kg/d of grain mix containing full fat extruded soybeans had 211% more *cis*-9, *trans*-11 CLA in their milk when compared with cows fed

TMR. When fresh cut grass replaced corn silage in cows diet at 0, 30, 60, and 100%, milk *cis*-9, *trans*-11 CLA averaged 0.48, 0.54, 1.21, and 1.65 g/100 g of total FA, respectively (Couvreur et al., 2006). It has been suggested that the greater concentration of *cis*-9, *trans*-11 CLA in milk fat from grazing cows may result from the increase in linolenic acid intake (Ribeiro et al., 2005; Bargo et al., 2006), and subsequently, the increase in amounts of VA available postruminally for *cis*-9, *trans*-11 CLA synthesis by Δ^9 -desaturase. Couvreur et al. (2006) reported that milk *trans* C18:1 (VA in particular) linearly increased as the proportion of fresh cut grass in cows diet increased from 0 to 30, 60, and 100% of DM of forage. Looor et al. (2004) noted that compared with a control diet, adding linseed oil to dairy cows' diets (65:35 forage to concentrate) at 3% of DM increased the duodenal flow of C18:0 and *trans* C18:1 with VA accounting for 43% of *trans* C18:1. Additionally, when cows managed in confinement or on pasture were fed 500 g/d of soybean oil, milk C18:0, *trans* C18:1 and *cis*-9, *trans*-11 CLA contents were greater with pasture than the confinement cows (Boken et al., 2005). In this study, it is unlikely that the greater *cis*-9, *trans*-11 CLA concentration in milk fat with the PAS diet resulted from the increase in linolenic acid intake because the concentrations of *trans* C18:1 and C18:0 in milk fat were similar for both diets (Table 3). The underlying causal mechanism(s) by which grazing increases milk *cis*-9, *trans*-11 CLA remains unclear, but it is evident from this study that unknown pasture constituents create condition(s) in the rumen that favor higher ratio of VA to *trans*-10 C18:1, and therefore, increase VA availability for *cis*-9, *trans*-11 CLA synthesis by delta-9 desaturase. When dairy cows were fed TMR containing a blend of FO and SFO, Shingfield et al. (2006) showed that *cis*-9, *trans*-11 CLA in milk fat reached its highest concentration by d 5 of oil supplementation, but then declined thereafter as a result of *trans*-10 C18:1 replacing VA as the predominant FA. However, such shift was not observed when a blend of FO and SFO was added to grazing dairy cows' diets (AbuGhazaleh and Holmes, 2007).

Some of the variation in milk *cis*-9, *trans*-11 CLA response between the 2 diets tested in this study could also be attributed to differences in Δ^9 -desaturase activity. Product to substrate ratios of some FA represent a Δ^9 -desaturase index and can serve as a proxy for Δ^9 -desaturase activity (Baumgard et al., 2002). There are 4 major products of Δ^9 -desaturase activity in the mammary gland: C14:1, C16:1, *cis*-9 C18:1 and *cis*-9, *trans*-11 CLA, which are produced from C14:0, C16:0, C18:0, and VA, respectively. The Δ^9 -desaturase index was not different between the 2 diets (Table 5), suggesting simi-

Table 5. Effect of treatment diets on delta-9 desaturase index

Item	Diet ¹		SEM
	LOT	PAS	
C14:1/C14:0	0.13	0.10	0.013
C16:1/C16:0	0.08	0.07	0.006
C18:1 <i>cis</i> -9/C18:0	2.53	2.37	0.186
<i>cis</i> -9, <i>trans</i> -11 CLA/VA ²	0.37	0.38	0.035

¹LOT = corn silage-alfalfa hay mix plus 640 g of fish oil and sunflower oil (1:3 wt/wt); PAS = pasture plus 640 g of fish oil and sunflower oil (1:3 wt/wt).

²CLA = conjugated linoleic acid; VA = vaccenic acid.

lar Δ^9 -desaturase activity for the LOT and the PAS cows.

CONCLUSIONS

Milk yield was lower with pasture feeding system compared with the confinement feeding system. However, the feeding system had no effect on milk fat and protein percentages. Milk FA profile response to FO and SFO supplementation varied by feeding system. Fish oil and SFO supplementation resulted in higher milk VA, *cis*-9, *trans*-11 CLA and lower *trans*-10 C18:1 concentrations and yields in pasture-fed cows compared with confinement-fed cows. The lower *cis*-9, *trans*-11 CLA concentration in milk from the confinement-fed cows resulted from *trans*-10 C18:1 replacing VA as the predominant *trans* C18:1 isomer.

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