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Conjugated Linoleic Acid in Milk Fat of Grazing Dairy Cows Fed Fish Oil and Linseed Oil

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Abstract: The objective of this study was to determine the effect of supplementing grazing dairy cows' diet with Fish Oil (FO) and Linseed Oil (LSO) on milk Conjugated Linoleic Acid (CLA). Sixteen Holstein cows (170±19 DIM) were divided into 2 groups (n = 8 per treatment) and fed a basal diet (7.2 kg d^{-1}; DM basis) consisting of corn, soybean meal, molasses, vitamin/mineral premix plus 800 g saturated animal fat (CONT) or a basal diet plus 200 g FO and 600 g LSO (FOLSO). All cows grazed together on Sudan grass pasture for ad libitum and fed the treatment diets for 3 weeks. Cows were milked twice a day and milk samples were collected during the last 3 days of the trial. Milk production (24.89 and 22.45 kg d^{-1}), milk protein percentages (2.76 and 2.82) and milk protein yield (0.68 and 0.64 kg d^{-1}) for the CONT and FOLSO diets, respectively, were not affected ($p>0.05$) by treatment diets. Milk fat percentages (3.90 and 2.86) and milk fat yields (0.97 and 0.64 kg d^{-1}) were lower ($p<0.05$) with the FOLSO diet compared with the CONT diet. The concentration and yield of milk *cis*-9 *trans*-11 CLA were higher ($p<0.05$) with the FOLSO diet (2.56% of total FA and 16.44 g d^{-1}, respectively) than the CONT diet (0.66% of total FA and 6.44 g d^{-1}, respectively). The concentrations of milk *trans* C18:1 and Vaacenic Acid (VA) were higher ($p<0.05$) with the FOLSO diet (36.99 and 7.48% of total FA, respectively) than the CONT diet (28.8 and 2.27% of total FA, respectively). In conclusion, supplementing grazing cows' diet with FO and LSO increased milk *cis*-9 *trans*-11 CLA content but reduced milk fat content and yield.

Key words: Pasture, milk, CLA, fish oil, linseed oil, vaacenic acid, FA

INTRODUCTION

Conjugated Linoleic Acid (CLA) refers to a mixture of positional and geometric isomers of octadecadienoic acid with conjugated double bonds. CLA are natural occurring Fatty Acids (FA) in foods derived from ruminants. In milk fat, the *cis*-9 *trans*-11 CLA account for at least 82% of total CLA isomers (Sehat *et al*., 1998). The *cis*-9 *trans*-11 CLA is synthesized either in the rumen as an intermediate during the biohydrogenation of linoleic acid or in tissues by Δ-9 desaturase from Vaccenic Acid (VA), another important intermediate in ruminal biohydrogenation of C18 unsaturated FA (Griinari and Bauman, 1999; AbuGhazaleh *et al*., 2005). Dietary *cis*-9 *trans*-11 CLA is now receiving great attention because of its recognized health benefits as a cancer chemopreventive and antitherogenic (Berlury *et al*., 2002).

Concentration of CLA is higher in milk fat from cows offered fresh compared with conserved forages (Kelly *et al*., 1998; Dhiman *et al*., 1999; Couvreur *et al*., 2006). Dhiman *et al*. (1999) conducted a study in which cows consumed either 1/3, 2/3, or all of their daily feed from pasture. They showed that CLA concentration in the milk fat was highest for cows on 100% pasture (22.1 mg g^{-1} of FA), intermediate for cows on 2/3 pasture (14.3 mg g^{-1} of FA) and least for cows receiving 1/3 pasture (8.9 mg g^{-1} of FA). Recently, Couvreur *et al*. (2006) reported a linear relationship between the proportion of fresh grass in dairy cows' diets and milk *cis*-9 *trans*-11 CLA content. Milk *cis*-9 *trans*-11 CLA content can be also enhanced by supplementing dairy cows' diets with lipid sources (Schroeder *et al*., 2003; Boken *et al*., 2005; Loor *et al*., 2005a; Flowers *et al*., 2007). Schroeder *et al*. (2003) reported 173% increase in milk CLA when corn grain in grazing dairy cows' diet was partially replaced with Ca-salts of unsaturated FA. When cows on annual rye-ryegrass pasture were supplemented with a grain mix containing 0 or 500 g d^{-1} of soybean oil, milk CLA content increased by 46% with the soybean oil supplementation (Boken *et al*., 2005). Loor *et al*. (2005a) reported a significant increase in milk *cis*-9 *trans*-11 CLA content when dairy cows' diet was supplemented with Linseed Oil (LSO) at 3% of diet DM. Recently, Flowers *et al*. (2007) reported a linear increase in milk *cis*-9 *trans*-11 CLA content with increasing the level of LSO in grazing dairy cows' diet.

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Inclusion of Fish Oil (FO) in dairy cows' diets has been also shown to increase milk *cis*-9 *trans*-11 CLA content (Donovan *et al*., 2000; Whitlock *et al*., 2002). Milk fat *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). Despite the volume of research on the impact of dietary FO and plants oils on milk *cis*-9 *trans*-11 CLA content under confinement feeding system, there is surprisingly little data in the literature evaluating the effect that dietary FO and plants oils may have under grazing feeding system on milk *cis*-9 *trans*-11 CLA content. Therefore, the objective of this trial was to evaluate milk *cis*-9 *trans*-11 CLA response to FO and LSO supplementation when cows fed a pasture-based diet.

MATERIALS AND METHODS

All procedures for this study involving the use of animals were conducted at The Southern Illinois University Carbondale Dairy Center and were approved by the Southern Illinois University Intuitional Animal Care and Use Committee. Sixteen multiparous Holstein cows of mean live weight 610±23.3 kg were divided into 2 groups (8 cows per group) based on milk yield (27±3.2 kg d^{-1}) and DIM (170±19), placed on Sudan-grass pasture and offered 8 kg of grain supplement containing either 800 g saturated animal fat (rumo-fat; CONT) or 800 g of FO-LSO (1:3 w/w; FOLSO) for a total of 21 days. The first 18 days were used for adjustment to diets and the last 3 days for milk samples collection. Grain supplements were formulated to be isocaloric and isonitrogenous (Table 1). Menhaden FO (Omega Protein Inc., Hammond, LA) and Rumo-fat (Robt Morgan Inc. Paris, IL), a source of saturated animal fat, were used in this study. Linseed oil was purchased from a local store. Cows were offered the grain supplement into two equal meals at 06:30 and 16:30 using Calan Broadbent feeder doors (American Calan, Inc., Northwood, NH) to monitor individual intakes. The Sudan-grass pasture was divided into 12 paddocks (1.5 acres) for rotational grazing and cows moved to a new paddock every day. Cows were on the experimental pasture for 21 h a day and had continuous access to water. Cows were milked twice daily at 06:00 and 16:00.

Milk yields were recorded daily, but measurements collected during the last 5 days were used for statistical analysis. Milk samples were collected from each cow during the last 3 days (d 19, 20 and 21) from the morning and afternoon milkings, made into daily composites based on milk yield at each milking and then split into two portions for analyses. One aliquot was stored at 4°C and sent to Prairie Farms (Carlinville, IL) for analyses of fat, protein, total solids and lactose (AOAC, 1997) by mid-infrared spectrophotometry (Multispec; Foss Food Technology Corp., Eden Prairie, MN) and Somatic Cell Counts (SCC; AOAC, 1997) using a Fossomatic 90 (Foss Food Technology Corp.). The second aliquot was stored at -20°C for FA analysis as described by AbuGhazaleh and Holmes (2007).

Grain supplements offered were recorded daily, but only measurements collected during the last 5 days were used for statistical analysis. Orts were removed and weighed after the morning feeding. Representative samples of Sudan grass (collected from 4 different locations per paddock then composited into one) and grain supplements were collected at d 7, 14 and 21 and stored at -20°C until analysis for chemical and FA composition. Grain supplements and pasture samples were freeze dried (Labconco Freeze Dry System, Labconco, Kansas City, MO) and then ground through a standard model No. 3 Wiley mill (Arthur H. Thomas Co., Philadelphia, PA) with a 2-mm screen. Samples were analyzed for CP, EE and ash according to AOAC methods (1997). A portion of each sample was reground through an ultracentrifuge mill (Brinkman Instruments Co., Westbury, NY) with a 1-mm screen and analyzed for NDF (Procedure B, Van Soest *et al*., 1991), ADF (Robertson and Van Soest, 1981) using ANKOM fiber analyzer using the filter bag technique (ANKOM Technology Corp., Fairport, NY) and FA as described for milk. The C17:0, dissolved in benzene, was used as an internal standard. Cows body weights and Body Condition Score (BCS; Wildman *et al*., 1982) were recorded at the beginning and end of the trial.

Table 1: Ingredient and nutrient content of grain supplement and pasture

	Treatment diets[1]		
Ingredient (% of DM)	CONT	FOLSO	Pasture
Corn ground cracked	42.20	42.20	
Linseed oil	0.00	7.40	
Fish oil	0.00	2.40	
Animal fat (Rumo-fat)	9.80	0.00	
Molasses cane deh	8.30	8.30	
Soybean meal, 48% CP	34.20	34.20	
Limestone	2.80	2.80	
Dical phosphate	1.40	1.40	
Magnesium oxide	0.30	0.30	
Hi Zn TM salt	0.50	0.50	
Vit ADE premix[2]	0.30	0.30	
Vit E premix[3]	0.30	0.30	
Chemical composition (% of DM)			
NDF	22.67±2.03	21.28±2.55	63.92±5.60
ADF	18.98±2.02	13.95±1.67	34.62±4.44
EE	10.91±0.58	10.07±1.47	0.74±0.17
CP	19.82±0.97	20.51±1.45	13.28±5.50

[1]CONT= grain supplement with 800 g of Rumo fat; FOLSO = grain supplement with 200 g FO and 600 g LSO, [2]Contained 4,400,000 IU of Vitamin A, 880,000 IU of vitamin D and 400 IU of vitamin E g^{-1}, [3]Contained 44,000 IU of vitamin E g^{-1}.

Experimental data was subjected to the PROC MIXED procedure of SAS (SAS, INC. Cary, NC) for a completely randomized design using following statistical model:

$$Y_{ijk} = \mu+D_i+C_j+S_k+e_{ijk}$$

Where Y_{ijk} = The dependent variable; μ = Overall mean; D_i = Diet effect; C_j = Cow effect;
S_k = Sample effect and e_{ijk} = residual error associated with Y_{ijk}. The values for least squares means are reported followed by Standard Error of Mean (SEM). Treatment effects were declared significance at $p<0.05$.

RESULTS AND DISCUSSION

Linolenic acid was the main FA in pasture, it accounted for 37.7% of total FA. Both palmetic and linoleic acids were the second main FA in pasture; they accounted for 13.5 and 14.0% of total FA, respectively (Table 2). As expected, the FA profile in the CONT diet was characterized by its high stearic acid content (46.4%). In the FOLSO diet, linolenic acid was the main FA accounting for 34.4% of total FA followed both linoleic and oleic acids at 17.2 and 16.1%, respectively. Stearic and Palmetic acids were the main FA in the rumo-fat at 52.1 and 20.6% of total FA, respectively. Linolenic acid was the main FA in LSO (52.1% of total FA) whereas; Eicosapentaenoic Acid (EPA; 13.0%) and Docosahexaenoic Acid (DHA; 16.0%) were the two main omega-3 FA in FO.

Milk yield, milk composition and grain supplement intake are presented in Table 3. Inclusion of FO and LSO in the diet had no effect ($p>0.05$) on milk yield (24.89 and 22.45 kg d^{-1} for the CONT and the FOLSO diets, respectively). Similar results were reported under confinement (Boken *et al*., 2005; Loor *et al*., 2005a) and grazing (Rego *et al*., 2005b; AbuGhazaleh and Holms, 2007; Flowers *et al*., 2007) feeding conditions when diets were supplemented with oils. Supplementing diets with oils rich in polyunsaturated FA often results in a reduction in feed intake and therefore milk yield (Donovan *et al*., 2000; Lock and Shingfield, 2004). The equal intake of grain supplements in this experiment may explain the similar milk yield between treatment diets.

Milk fat percentages (3.90 and 2.86) and yields (0.97 and 0.64 kg d^{-1}) were higher ($p<0.01$) in cows fed the CONT diet compared with the FOLSO diet (Table 3). Milk fat depression is frequently seen in dairy cows fed oil supplements (AbuGhazaleh *et al*., 2002; Rego *et al*., 2005a,b; Shingfield *et al*., 2006; Holmes and AbuGhazaleh, 2007). After reviewing eighteen experiments involving 25 comparisons to describe the effects of lipid supplementation on milk production and composition with grazing dairy cows, Schroeder *et al*. (2004) concluded that the decrease in milk fat was a common result when unsaturated lipid supplements were added to grazing dairy cows' diets. Different explanations have been suggested for the milk fat depression associated with lipids feeding. Jenkins (1993) and Schroeder *et al*. (2004) suggested that the reduction in milk fat content with dietary lipids may results from their negative effects in rumen fiber digestion with a consequent reduction in acetate production, the precursor for short and medium-chain FA synthesis in mammary glands (Harfoot and Hazelwood, 1997). Others (Grummer, 1991; Chillard and Doreau, 1997) suggested that the increase uptake and incorporation of dietary long-chain FA into triglycerides inhibits the *de novo* FA synthesis in the mammary gland and therefore, reduce milk fat content. Recently, Bauman and Griinari (2001) suggested that milk fat depression was related to the direct action on the mammary gland of specific FA isomers derived from the ruminal metabolism of dietary unsaturated FA. Piperova *et al*. (2004) and Shingfield *et al*. (2006) reported a close association between milk fat depression and levels of milk trans-10 C18:1. In the current study, the concentration of trans-10 C18:1 in milk fat was 808% higher with the FOLSO diet than the CONT diet.

Table 2: Fatty acid composition for grain supplement, pasture, rumo fat, linseed oil and fish oil

	Treatment diets[1]			Lipid supplements		
Fatty acid	CONT	FOLSO	Pasture[2]	Rumo-Fat	LSO[3]	FO[4]
	(g 100 g^{-1} FA)					
C14:0	1.41	1.34	0.33	2.35	0.05	8.25
C16:0	23.68	9.30	13.46	20.63	4.58	20.27
C18:0	46.38	3.49	3.31	52.05	2.85	4.14
C18:1	6.88	16.09	2.28	0.28	17.31	11.22
C18:2	13.70	17.18	13.95	0.04	13.63	1.37
C18:3n3	0.93	34.38	37.66	nd[5]	52.11	1.81
C20:5n3, EPA	0.01	1.42	0.02	nd	nd	13.03
C22:6n3, DHA	0.02	1.25	0.07	nd	nd	15.99

[1]CONT = grain supplement with 800 g of Rumo fat; FOLSO = grain supplement with 200 g FO and 600 g LSO, [2]Pasture = Sudan grass, [3]LSO = Linseed Oil, [4]FO = Fish Oil, [5]nd = Not detected or detected at <0.01

Table 3: Effect of treatment diets on milk yield, composition and grain supplement intake

Item	Treatment diets[1] CONT	FOLSO	SEM[2]	p-value
Milk, kg d^{-1}	24.89	22.45	0.88	0.07
Fat				
%	3.90	2.86	0.13	0.01
kg d^{-1}	0.97	0.64	0.04	0.01
Protein				
%	2.76	2.82	0.11	0.74
kg d^{-1}	0.68	0.64	0.03	0.30
Lactose				
%	4.60	4.63	0.07	0.77
kg d^{-1}	1.14	1.04	0.05	0.20
Total solids				
%	12.16	11.46	0.14	0.01
kg d^{-1}	3.02	2.57	0.10	0.01
SCC×10^3 mL^{-1}	133.93	938.76	290.80	0.07
Grain supplement intake,				
kg d^{-1}	7.58	7.83	0.21	0.42
Body weight, kg	3.85	5.77	5.50	0.43
BCS[3]	3.10	2.90	0.25	0.33

[1]CONT = grain supplement with 800 g of Rumo fat; FOLSO = grain supplement with 200 g FO and 600 g LSO, [2]SEM = Standard Error Mean, [3]Scored on a five-point scale where 1 = emaciated to 5 = overly fat

Table 4: Effect of treatment diets on milk fatty acid composition

Fatty acids	Treatment diets[1] CONT	FOLSO	SEM[2]	p-value
	-----g 100 g^{-1} FA-----			
C4:0	1.43	0.91	0.081	0.01
C6:0	1.24	0.68	0.067	0.01
C8:0	0.77	0.38	0.053	0.01
C10:0	1.57	0.76	0.106	0.01
C12:0	1.85	1.08	0.093	0.01
C14:0	7.78	5.76	0.172	0.01
C16:0	27.40	19.85	0.591	0.01
C17:0	0.87	0.48	0.030	0.01
C18:0	15.60	9.45	0.936	0.01
C18:1 trans	28.80	36.99	0.887	0.01
T6	0.28	0.71	0.050	0.01
T9	0.25	0.73	0.056	0.01
T10	0.23	2.09	0.465	0.02
T11, (VA)	2.27	7.48	0.758	0.04
T12	0.40	1.46	0.131	0.01
T16	0.23	1.03	0.056	0.01
C18:1 c9	23.96	22.41	1.194	0.37
C18:2 t11c15	0.22	2.09	0.120	0.01
C18:2 c9c12	1.99	1.66	0.128	0.09
C18:3n3	0.56	1.26	0.075	0.01
CLA c9t11	0.66	2.56	0.283	0.05
CLA tt	0.10	0.38	0.055	0.03
C20:5n3, EPA	0.03	0.07	0.004	0.01
C22:4n6	0.03	0.04	0.004	0.04
C22:5n3	0.05	0.07	0.002	0.01
C22:6n3, DHA	0.01	0.06	0.003	0.01
SMCFA[3]	43.73	30.64	1.042	0.01
SAT[4]	59.37	40.77	0.994	0.01
UNSAT[5]	34.75	47.93	0.986	0.01

[1]CONT = grain supplement with 800 g of Rumo fat; FOLSO = grain supplement with 200 g FO and 600 g LSO, [2]SEM = Standard Error Mean, [3]SMCFA = short and medium chain FA (C4:0-C16:0), [4]SAT = Sum of saturated FA, [5]UNSAT = Sum of Unsaturated FA

Table 5: Effect of treatment diets on milk fatty acid yield

Fatty acids	Treatment diets[1] CONT	FOLSO	SEM[2]	p-value
	---------(g d^{-1})---------			
C4:0	13.60	6.02	0.078	0.01
C6:0	11.90	4.49	0.077	0.01
C8:0	7.35	2.46	0.547	0.01
C10:0	15.06	4.85	1.121	0.01
C12:0	17.88	6.82	1.120	0.01
C14:0	75.40	36.00	3.392	0.01
C16:0	262.80	127.00	10.522	0.01
C17:0	8.37	3.05	0.313	0.01
C18:0	143.50	65.10	7.399	0.01
C18:1 trans	37.81	83.53	6.328	0.01
T6	2.73	4.48	0.340	0.01
T9	2.40	4.60	0.364	0.01
t10	2.27	12.39	1.916	0.01
t11, (VA)	22.88	47.79	5.529	0.01
t12	3.94	9.12	0.990	0.01
t16	2.29	6.45	0.282	0.01
C18:1 c9	236.17	144.67	14.784	0.01
C18:2 t11c15	2.03	13.49	1.018	0.01
C18:2 c9c12	19.30	10.82	1.317	0.01
C18:3n3	5.43	8.34	0.776	0.02
CLA c9t11	6.44	16.44	2.013	0.01
CLA tt	0.90	2.52	0.036	0.01
C20:5n3, EPA	0.32	0.46	0.055	0.05
C22:4n6	0.28	0.20	0.032	0.12
C22:5n3	0.51	0.48	0.004	0.54
C22:6n3, DHA	0.05	0.38	0.030	0.01
SMCFA[3]	404.84	186.85	15.646	0.01
SAT[4]	419.47	195.70	16.458	0.01
UNSAT[5]	1617.16	1622.96	17.888	0.82

1CONT = grain supplement with 800 g of Rumo fat; FOLSO = grain supplement with 200 g FO and 600 g LSO, [2]SEM = Standard Error Mean, [3]SMCFA = Short and medium chain FA (C4:0-C16:0), [4]SAT = Sum of saturated FA, [5]UNSAT = Sum of Unsaturated FA

Treatment diets had no effect (p>0.05) on milk protein percentages (2.76 and 2.82) and yields (0.68 and 0.64 kg d^{-1}). Supplementing grazing cow's diet with 500 g of sunflower oil or soybean oil did not affect milk protein content or yield (Rego *et al*., 2005b). Similarly, AbuGhazaleh and Holmes (2007) and Flowers *et al.* (2007) reported no difference in milk protein content in pasture cows fed a diet with or without plant oils. However, others (Dhiman *et al*., 2000; Whitlock *et al*., 2006; Shingfield *et al*., 2006) reported a significant reduction in milk protein content with oil supplementation, changes that have often been attributed to increases in milk yield (dilution effect) rather than decreases in milk protein synthesis (DePeters and Cant, 1992). Similar milk yield in the current study may explain the similar milk protein content between treatment diets. Grain supplement intake was similar (p>0.05) between treatment diets (7.58 and 7.83 kg d^{-1} for the CONT and the FOLSO diets, respectively). Treatment diets had no effect (p>0.05) on SCC, body weight and BCS.

The FOLSO diet resulted in marked alterations in milk FA concentrations and yields relative to the CONT diet (Table 4 and 5). The concentrations of milk short and medium chain FA (C4:0-C16:0) were significantly reduced (p<0.05) with the FOLSO diet (18.7% of total FA) compared with the CONT diet (40.5%). Even short and medium chain FA (C4:0-C14:0) are derived from the *de novo* synthesis by the mammary gland and from the mammary uptake of preformed FA (Jenkins, 1993). In the current study, the lower concentrations of even-number saturated FA with the FOLSO diet compared with the CONT diet suggest less *de novo* FA synthesis occurred with cows fed the FOLSO diet. As stated earlier, feeding oils are typically associated with a decrease in the *de novo* synthesis of short and medium chain FA (Rego *et al*., 2005b; Loor *et al*., 2005a,c).

The objective of this study, was to evaluate the effects of feeding a CLA-stimulating diet to grazing dairy cows on milk *cis*-9 *trans*-11 CLA content. Milk *cis*-9 *trans*-11 CLA concentration and yield increased by 288 and 155% with the FOLSO diet relative to the CONT diet, respectively (Table 4 and 5). The concentration of *cis*-9 *trans*-11 CLA averaged 0.66 and 2.56% of total FA with the CONT and the FOLSO diets, respectively. Diets containing FO, plant oils or their combinations consistently increased milk *cis*-9 *trans*-11 CLA content under confinement (Whitlock *et al*., 2002; Loor *et al*., 2005a; Bu *et al*., 2007) and grazing (Rego *et al*., 2005b; Boken *et al*., 2005; AbuGhazaleh and Holmes, 2007) feeding conditions. Supplementing grazing dairy cows with 500 g d^{-1} of sunflower oil or soybean oil resulted in 68 and 53% increase in milk CLA compared with control cows (Rego *et al*., 2005b). Compared with pasture cows on control diet, supplementing grazing dairy cows diet with 160 and 320 g of FO/d resulted in 44 and 62% increase in milk CLA concentration, respectively (Rego *et al*., 2005a). The higher milk *cis*-9 *trans*-11 CLA concentration seen in this study with the FOLSO diet compared with that reported by AbuGhazaleh *et al*. (2003); 1.06% of milk total FA, Loor *et al*. (2005a); 1.34% of milk total FA and Bu *et al*. (2007); 1.60% of milk total FA when dairy cows fed LSO at 524, 588 and 636 g d^{-1}, respectively, may have resulted from grazing and/or FO supplementation. Cows on pasture diets have been shown to have higher levels of *cis*-9 *trans*-11 CLA (Boken *et al*., 2005; Couvreur *et al*., 2006) in their milk than those on conserved forages. Recently, Couvreur *et al*. (2006) reported a linear relationship between the proportion of fresh grass in dairy cows' diets and milk *cis*-9 *trans*-11 CLA. AbuGhazaleh and Jenkins (2004) showed that FO promotes VA accumulations in rumen by inhibiting the reduction of VA to C18:0. As mentioned earlier, *cis*-9 *trans*-11 CLA in milk originates from either the ruminal biohydrogenation of linoleic acid (Harfoot and Hazlewood, 1997) or from the endogenous synthesis in mammary gland from VA (Griinari and Bauman, 1999). The endogenous synthesis of *cis*-9 *trans*-11 CLA from VA has been proposed as the major pathway of *cis*-9 *trans*-11 CLA synthesis in lactating cows, accounting for an estimated 78-90% of the *cis*-9 *trans*-11 CLA in milk fat (Corl *et al*., 2001; Piperova *et al*., 2002).

Concentrations and yields of milk *trans* C18:1 were also affected by treatment diets (Table 4 and 5). Dietary oil supplementation resulted in 232% increase in milk *trans* C18:1 concentrations relative to the CONT diet. The concentrations of *trans* C18:1 averaged 3.99 and 13.23% of total FA with the CONT and the FOLSO diets, respectively (Table 4). Vaccenic acid was the major *trans* C18:1 isomer in milk with both treatment diets accounting for 56.5% of total *trans* C18:1. Milk VA concentration was higher ($p<0.05$) with the FOLSO diet (7.48 g 100 g^{-1} FA) than the CONT diet (2.27 g 100 g^{-1} FA). The increase in concentrations of milk *trans* C18:1 isomers, VA in particular, with the FOLSO diet was expected. Similar increases in milk *trans* C18:1 and VA concentrations were observed under confinement (Loor *et al*., 2005a,b; Bu *et al*., 2007) and grazing (Flowers *et al*., 2007; Holmes and AbuGhazaleh, 2007) feeding conditions when cows were fed FO and/or LSO. AbuGhazaleh and Holmes (2007) observed a significant increase in milk VA content when partially grazed dairy cows were supplemented with a blend of sunflower oil (300 g d^{-1}) and FO (100 g d^{-1}) when compared to partially grazed dairy cows received control diet (5.11 vs. 2.20 g 100 g^{-1} of milk total FA). Milk VA accounted for 5.43, 3.23 and 3.04 % of milk FA when dairy cows fed LSO at 860, 588 and 636 g d^{-1}, respectively (Loor *et al*., 2005a,c; Bu *et al*., 2007). The higher milk VA concentration seen in this study (7.48%) may have resulted from grazing, FO or their combinations. Recently, Felton and AbuGhazaleh (2007) and Holmes and AbuGhazaleh (2007) showed that the increase in milk VA content was higher when oil supplements were fed to dairy cows managed under grazing than confinement feeding systems. Previously, AbuGhazaleh *et al*. (2002) and Whitlock *et al*. (2002) showed that combining FO with plant oils results in more increase in milk VA than when fed separately. Human body can convert some of the VA into *cis*-9 *trans*-11 CLA (Salminen *et al*., 1998); therefore, producing milk with high VA concentration could be also beneficial.

Concentrations of milk *trans*-6/8 C18:1 (0.28 and 0.71 for the CONT and the FOLSO diets, respectively), *trans*-9 C18:1 (0.25 and 0.73), *trans*-10 C18:1 (0.23 and 2.09), *trans*-12 C18:1 (0.40 and 1.46) and *trans*-16 C18:1 (0.23 and 1.03) were all higher ($p<0.05$) with the FOLSO diet than the CONT diet. The increase in concentrations of *trans*-6/8, *trans*-9, *trans*-10, *trans*-12 *and trans*-16 C18:1 in milk fat with the FOLSO diet is consistent with that reported by Holmes and AbuGhazaleh (2007), Loor *et al*. (2005a,c) and Flowers *et al*. (2007) when FO and/or LSO were added to dairy cows' diets. The presence of these different *trans* C18:1 isomers in milk fat provides comparative evidence for the existence of alternative pathways for the biohydrogenation of C18 polyunsaturated FA other than those established by Harfoot and Hazlewood (1997).

CONCLUSION

Results showed that supplementing grazing dairy cows with LSO and FO altered milk composition and FA profile. The FOLSO had no effect on milk yield, grain supplement intake and milk protein content compared with the CONT diet. Milk fat content and yield decreased with oil supplementation. The FOLSO diet increased milk *cis-9 trans-11* CLA and VA compared with the CONT diet. Supplementing grazing cows' diets with LSO and FO enhances milk *cis*-9 *trans*-11 CLA content and could be sued as a practical approach to broaden dairy market by increasing their competitiveness in the healthy food market.

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