Milk Fatty Acid Composition of Grazing Dairy Cows When Supplemented with Linseed Oil

G. Flowers,* S. A. Ibrahim,† and A. A. AbuGhazaleh*¹

*Department of Animal Science, Food and Nutrition, Southern Illinois University, Carbondale 62901

†Department of Family and Consumer Sciences, North Carolina A&T State University, Greensboro 27411

ABSTRACT

The effects of varying amounts of linseed oil (LSO) in grazing dairy cows' diet on milk conjugated linoleic acid (*cis*-9, *trans*-11 CLA) were investigated in this study. Twelve Holstein cows in midlactation (150 ± 19 DIM) were placed on alfalfa-based pasture and assigned to 4 treatments using a 4×4 Latin square design with 3-wk periods. Treatments were: 1) control grain supplement; 2) control grain supplement containing 170 g of LSO (LSO1); 3) control grain supplement containing 340 g of LSO (LSO2); and 4) control grain supplement containing 510 g of LSO (LSO3). Grain supplements were offered at 7 kg/d. Additional 100 g/d of algae, divided evenly between the 2 feeding times, were added to every treatment diet. Milk samples were collected during the last 3 d of each period and analyzed for chemical and fatty acid composition. Treatments had no effect on milk production (18.9, 18.5, 19.6, and 19.1 kg/d for treatments 1 to 4, respectively). Linseed oil supplementation caused a quadratic increase in milk fat (3.23, 3.44, 3.35, and 3.27% for treatments 1 to 4, respectively) and protein (3.03, 3.19, 3.12, and 3.08%) contents. Concentrations (g/100 g of fatty acids) of milk *cis*-9, *trans*-11 CLA (1.12, 1.18, 1.39, and 1.65 for treatments 1 to 4, respectively) and VA (3.39, 3.62, 4.25, and 4.89) linearly increased with LSO supplementations. Results from this trial suggest that the increase in milk *cis*-9, *trans*-11 CLA was proportional to the amounts of LSO fed. In conclusion, adding LSO to grazing dairy cow diets can improve the nutritional value of milk without compromising milk composition or cow performance.

Key words: conjugated linoleic acid, dairy cow, linseed oil, milk fatty acid composition

INTRODUCTION

Conjugated linoleic acid (CLA) isomers are potentially valuable fatty acids chiefly found in the milk of

ruminants. The most common of these isomers, *cis*-9, *trans*-11, has been found to have anticarcinogenic properties and possibly other positive effects for human health (Bhattacharya et al., 2006).

The *cis*-9, *trans*-11 CLA is synthesized as an intermediate product in the rumen during the biohydrogenation of dietary linoleic acid or in animal tissues by Δ^9 -desaturase from vaccenic acid (VA, *trans*-11 C18:1), another intermediate in ruminal biohydrogenation (Griinari and Bauman, 1999). Unlike linoleic acid, biohydrogenation of linolenic acid (C18:3n-3) in the rumen leads to the formation of VA, not *cis*-9, *trans*-11 CLA (Harfoot and Hazlewood, 1997). Approximately 80% of *cis*-9, *trans*-11 CLA appearing in milk fat is synthesized in the mammary gland via Δ^9 -desaturase (Mosley et al., 2006).

Because of their potential health benefits, there is considerable interest in increasing CLA concentration levels in milk fat. Efforts to do this have involved modifying, supplementing, or both, the diets of dairy cows. Studies reporting supplementing dairy cows' diet with plant oils (Dhiman et al., 2000; Rego et al., 2005b; Bu et al., 2007), marine oil (Donovan et al., 2000; Rego et al., 2005a), or algae (Franklin et al., 1999) have all resulted in substantial increases in milk *cis*-9, *trans*-11 CLA content. AbuGhazaleh et al. (2002, 2003) and Whitlock et al. (2002) demonstrated that the greatest concentrations of *cis*-9, *trans*-11 CLA in milk fat can be obtained by adding fish oil along with plant oils high in linoleic acid (sunflower oil) or linolenic acid (linseed oil) to dairy cow diets. AbuGhazaleh and Jenkins (2004b) showed that the stimulatory effect of dietary fish oil and algae on milk *cis*-9, *trans*-11 CLA production is due to the inhibitory effect of docosahexaenoic acid (C22:6; DHA) on the ruminal reduction of VA to stearic acid. However, including fish oil in dairy cow diets can cause a reduction in feed intake and milk fat content (Donovan et al., 2000; Whitlock et al., 2002; Rego et al., 2005a).

Moreover, despite evidence that it can lead to higher CLA content, the effect of dietary oil level on milk CLA content has not been consistent. Milk CLA levels significantly increased when linseed oil (LSO) was fed to

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¹Corresponding author: aabugha@siu.edu

dairy cows at 460 g/d but did not additionally increase when LSO intake was increased to 880 g/d (Dhiman et al., 2000). Additionally, Rego et al. (2005a) reported similar increases in milk CLA when fish oil was added to grazing dairy cow diets at 160 and 320 g/d. When soybean oil was included in dairy cow diets at 0 (control), 0.5, 1, 2, and 4% of diet DM, the CLA content of milk significantly increased only when soybean oil was added at 2 and 4% of diet DM when compared with the control (Dhiman et al., 2000).

Another factor of interest here is the combination of feeding regimen and diet oil supplementation. Although the effect of plant oils on milk *cis*-9, *trans*-11 CLA is well documented under confinement feeding system, there is less information in the literature concerning the effect of oil supplementation levels on milk *cis*-9, *trans*-11 CLA content of grazing dairy cows. Studies have shown that milk CLA levels are typically higher in cows fed pasture-based diets than TMR (Lor et al., 2003; Schroeder et al., 2003; Bargo et al., 2006). To improve our understanding of the effects of plant oil supplementation on CLA content on pasture grazing dairy cows, this trial was conducted to determine the effects of varying amounts of LSO in grazing dairy cow diets on milk *cis*-9, *trans*-11 CLA.

MATERIALS AND METHODS

All procedures for this trial were approved by the Southern Illinois University Intuitional Animal Care and Use Committee. Cows were selected from the Southern Illinois University Carbondale Dairy Research Center. The experiment was conducted between April and July 2006. Twelve multiparous Holstein cows at 150 ± 19 DIM and averaging 31.5 ± 3.7 kg/d of milk yield were used in a 4×4 Latin square design with 3-wk periods. The first 18 d were for diet acclimatization, and the last 3 d were for sampling. Cows within a square were randomly assigned to dietary treatments. All cows were grazed together on alfalfa-fescue-clover-weeds mixed pasture (50:20:20:10 wt/wt) during the entire experiment and fed grain supplements. An 8-ha pasture subdivided into ten 0.8-ha paddocks, yielding approximately 270 m² of pasture area per cow per day, was used. Cows were rotated throughout the 10 paddocks to promote continuous grazing. Water was available to cows at all times.

Treatments were 1) control grain supplement, 2) control grain supplement with 170 g/d of LSO (**LSO1**), 3) control grain supplement with 340 g/d of LSO (**LSO2**), and 4) control grain supplement with 510 g/d of LSO (**LSO3**). The grain supplement contained corn, dried molasses, soybean meal, meat and bone meal, minerals,

and vitamins (Table 1). Grain supplements (7 kg/d) were 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. Fifty grams of S-type gold algae (source of DHA; Martek Inc., Baltimore, MD) was added as a top dress to each grain feeding. The algae were stored at 5°C until use. Amounts of grain supplement offered, and orts were recorded daily for each cow.

The cows were milked daily at 0630 and 1730 h and milk yield was recorded at each milking. Milk samples from both the morning and evening milkings were collected during the last 3 d of each period. Twenty-four hour composites of each cow's milk, amounts proportional to milk yield at each time, were divided into 2 portions for analysis. One portion was stored at 5°C and sent to a laboratory (Prairie Farms, Carterville, IL) to be analyzed for fat, true protein, and lactose (AOAC, 2000; method 972.16) by midinfrared spectrophotometry (Foss 303 Milk-O-Scan; Foss Foods Inc.; Prairie Farms, Carlinville, IL) and SCC (Fossomatic 6000; Foss Incorporated, Eden Prairie, MN; AOAC, 2000, method 975.16). The other portion of each sample was stored at -20°C for fatty acid analysis.

Pasture, grain supplement, and algae samples were taken during wk 2 and 3 of the each period and stored at -20°C until analysis for chemical and fatty acid composition. Pasture samples were taken randomly from 5 different locations per paddocks using a 0.50-m² quadrant. The plants were clipped to a 2.5-cm stubble height using metal shears. Pasture samples were composited per period and stored at -20°C until analysis for chemical and fatty acid composition.

Pasture and grain supplement 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 (method 976.05), and ash (method 942.05) according to AOAC methods (2000). Samples were reground (Brinkman ultracentrifuge mill) through a 1-mm screen prior to analyses for fatty acids (Kramer et al., 1997) and fiber. The NDF was determined using the Van Soest et al. (1991) procedure. The heat stable amylase and sodium sulphite were used to determine NDF. Acid detergent fiber was determined using cetyl trimethyl ammonium bromide and 1 N H₂SO₄ as described by Robertson and Van Soest (1981). The NDF and ADF are expressed including the residual ash. Body weights and BCS were recorded at the end of each period.

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 caps, stored at -80°C for 6 h, freeze-dried, and then methylated using the NaOCH₃

Table 1. Ingredient and chemical composition of treatments and pasture

Item (% of DM)	Treatment ¹				Pasture
	Control	LSO1	LSO2	LSO3	
Cracked corn	49.00	48.10	45.00	42.00	
Linseed oil	0.00	2.57	5.12	7.67	
Dried molasses	10.40	10.40	10.40	10.40	
Soybean meal, 48% CP	29.40	27.80	28.20	28.80	
Meat and bone meal	4.18	4.16	4.16	4.14	
Limestone	3.48	3.47	3.46	3.45	
Dicalcium phosphate	1.74	1.74	1.73	1.73	
Magnesium oxide	0.35	0.35	0.35	0.35	
Zn trace mineralized salt	0.69	0.69	0.69	0.69	
Vitamin E premix ²	0.35	0.35	0.35	0.35	
Vitamin ADE premix ³	0.35	0.35	0.35	0.35	
Composition, % of DM					
Ash	10.2 ± 1.07	9.45 ± 0.73	8.85 ± 0.86	9.11 ± 0.67	8.04 ± 2.03
CP	23.2 ± 1.81	21.0 ± 1.25	20.8 ± 1.33	21.4 ± 1.27	11.3 ± 1.26
NDF	23.7 ± 0.49	23.7 ± 0.47	24.7 ± 0.62	25.9 ± 0.46	64.1 ± 7.96
ADF	13.2 ± 0.69	11.9 ± 0.88	11.9 ± 0.38	11.7 ± 0.80	37.3 ± 5.76
NFC ⁴	51.90	50.76	48.68	46.52	
NE _L , ⁵ Mcal/kg	1.76	1.85	1.95	2.05	

¹Control = control with no linseed oil; LSO1 = control with 170 g/d of linseed oil; LSO2 = control with 340 g/d of linseed oil; LSO3 = control with 510 g/d of linseed oil.

²Contained 44,000 IU of vitamin E/kg.

³Contained 4,400,000 IU of vitamin A, 880,000 IU of vitamin D, and 400 IU of vitamin E/kg.

⁴Estimated NRC (2001).

⁵Estimated NRC (2001).

and HCl 2-step procedure as outlined by Kramer et al. (1997). Methylated fatty acids were then analyzed for fatty acids 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. The injector and detector temperatures were set at 255°C. Peaks were identified by comparing the retention times with those of corresponding standards (Nu-Chek Prep., Elysian, MN; Supelco, Bellefonte, PA; and Larodan Fine Chemicals, Malmo, Sweden). The *trans* C18:1 isomers that were not available commercially (*trans*-6/8, *trans*-10, *trans*-12, *trans*-16) were identified according to the elution sequence reported by Loo et al. (2005). Heptadecanoic acid (C17:0) was added to all feed samples as an internal standard.

Data for fatty acid composition, grain supplement intake, milk production and composition, body weight, and body condition scores are reported as least squares means. Data were analyzed as a Latin square using the PROC MIXED of SAS (SAS Institute Inc., Cary, NC). The statistical model includes cow, period, sample, diet, and residual error. Fixed effects were period and

diet, whereas cow was the random effect. Preplanned comparisons were linear, quadratic, and LSO vs. control. Significance was declared at $P < 0.05$.

RESULTS

The nutrient content and fatty acid composition of grain supplements and pasture are presented in Tables 1 and 2. The average CP, ADF, NDF, and ash content (DM basis) for pasture were 11.3, 37.3, 64.3, and 8.04%, respectively. Pasture fatty acids content averaged 15.0 mg/g of DM. As expected, fatty acid content in grain supplements increased as amounts of LSO increased averaging 18.4, 34.7, 53.2, and 72.1 mg/g of DM for treatments 1 to 4, respectively. Linolenic acid was the major fatty acid in the pasture grasses accounting for approximately 40.8% of total fatty acids (Table 2). The second major fatty acid in the pasture was linoleic acid accounting for 13.7% of total fatty acids. Addition of LSO increased the concentrations (mg/g of DM) of oleic, linoleic, and linolenic acids in grain supplements (Table 2). Concentrations (mg/g of grain DM) of linolenic acid (0.69, 8.2, 17.6, and 28.2 with treatments 1 to 4, respectively), linoleic acid (5.8, 11.7, 15.0, and 18.1) and oleic acid (4.7, 7.7, 11.1, and 14.8) increased as the amounts of LSO in grain supplements increased. The major fatty acid in algae was DHA accounting for 38.3% of the total fatty acids.

Table 2. Fatty acid composition for treatments, pasture, algae, and linseed oil

Fatty acids	Treatment ¹				Supplement		
	Control	LSO1	LSO2	LSO3	Pasture ²	Algae ³	LSO ⁴
	(g/100 g of FA)						
C14:0	0.29	0.18	0.15	0.11	0.47	8.97	0.05
Unknown ⁵	1.78	1.06	0.55	0.38	6.60	ND ⁶	ND
C16:0	14.86	11.02	9.31	8.27	12.38	23.80	4.58
C18:0	4.74	4.44	4.40	4.27	1.39	0.58	6.43
C18:1c9	25.53	22.47	20.99	20.58	4.88	0.20	19.15
C18:2c9c12	33.52	34.02	28.24	25.07	13.69	0.18	14.57
C18:3n-3	3.72	23.91	32.92	37.43	40.76	0.28	55.70
C20:5n-3	ND	ND	ND	ND	ND	2.14	ND
C22:6n-3, DHA ⁷	0.04	0.01	0.02	ND	0.14	38.29	ND
	(mg/g of DM)						
C14:0	0.05	0.06	0.07	0.08	0.07		
Unknown ⁵	0.33	0.35	0.29	0.28	0.85		
C16:0	2.69	3.74	4.91	5.95	1.84		
C18:0	0.86	1.49	2.29	3.06	0.20		
C18:1c9	4.70	7.68	11.12	14.81	0.68		
C18:2c9c12	5.84	11.67	14.96	18.09	2.03		
C18:3n-3	0.69	8.21	17.58	28.24	6.05		
C20:5n-3	ND	ND	ND	ND	ND		
C22:6n-3, DHA ⁷	0.01	0.00	0.01	ND	0.02		
Total FA	18.39	34.74	53.17	72.08	15.02		

¹Control = control with no linseed oil; LSO1 = control with 170 g/d of linseed oil; LSO2 = control with 340 g/d of linseed oil; LSO3 = control with 510 g/d of linseed oil.

²Pasture = alfalfa-fescue-clover-weeds (50:20:20:10 wt/wt).

³S type Gold algae (Martek Inc., Baltimore, MD).

⁴LSO = linseed oil.

⁵Unknown = unknown peak between C14:0 and C16:0.

⁶ND = not detected or detected at <0.01.

⁷DHA = docosahexaenoic acid.

The effect of treatments on milk yield and composition is presented in Table 3. There was no effect ($P = 0.87$) of treatments on milk yield (18.9, 18.5, 19.6, and 19.1 kg/d for treatments 1 to 4, respectively). Linseed oil supplementation resulted in a quadratic increase ($P < 0.02$) in milk fat and protein contents. However, when LSO diets were contrasted against the control treatment diet, milk fat percentages and yield were similar ($P > 0.10$). Milk fat percentages averaged 3.23, 3.44, 3.35, and 3.27 for treatments 1 to 4, respectively. Milk protein percentages (3.03, 3.19, 3.12, and 3.08 for treatments 1 to 4, respectively) and total solids (11.70, 12.07, 11.90, and 11.90) were higher ($P < 0.05$) with LSO diets compared with control treatment. Treatments had no effect ($P > 0.10$) on grain supplement intake, SCC, BW, and BCS (Table 3).

The effects of treatments on milk fatty acid composition are presented in Table 4. Compared with the control treatment, the concentrations of the even chained C6:0 to C16:0 were lower ($P < 0.05$) with the LSO treatments (Table 4). Similarly, yields of even-chained C6:0 to C16:0 isomers were lower ($P < 0.05$) with the LSO treatments compared with the control treatment (data not shown). As the amounts of LSO in grain supple-

ments increased, the concentrations of even-chained C4:0 to C16:0 decreased in a linear manner ($P < 0.001$). The concentrations of even-chained C4:0 to C16:0 averaged 39.3, 38.8, 36.8, and 33.7 g/100 g of total fatty acids for treatments 1 to 4, respectively. Addition of LSO to grain supplements linearly decreased ($P < 0.001$) the concentrations of milk C11:0, C15:0, and C17:0 compared with the control treatment. Treatments had no effect on milk C18:0 concentrations or yields (data not shown).

Milk *trans* C18:1 concentrations were higher ($P < 0.001$) with the LSO treatments compared with the control treatment diet. Concentrations of milk *trans* C18:1 linearly increased ($P < 0.001$) by 9, 32, and 52% with the LSO1, LSO2, and LSO3 treatments, respectively, compared with the control treatment diet. Vaccenic acid was the major *trans* C18:1 isomer in all treatments, accounting for approximately 53% (ranged from 52 to 54%) of total *trans* C18:1 isomers. The addition of LSO to the grain supplements increased ($P < 0.001$) milk VA concentrations when compared with the control treatment. Increasing the amount of LSO in grain supplements caused in a linear increase ($P < 0.001$) in milk VA (3.39, 3.62, 4.25, and 4.89 g/100 g of fatty acids

Table 3. Effect of treatments on milk yield, milk composition, BW, and BCS

Item	Treatment ¹				SEM	Contrast ²		
	Control	LSO1	LSO2	LSO3		Lin	Quad	Control vs. LSO
						<i>P</i> -value		
Milk, kg/d	18.93	18.50	19.60	19.10	1.26	0.63	0.94	0.87
Fat								
%	3.23	3.44	3.35	3.27	0.08	0.71	0.02	0.10
kg/d	0.60	0.64	0.66	0.63	0.04	0.42	0.12	0.18
Protein								
%	3.03	3.19	3.12	3.08	0.05	0.60	0.02	0.05
kg/d	0.59	0.59	0.58	0.56	0.07	0.32	0.82	0.50
Lactose								
%	4.56	4.40	4.59	4.66	0.09	0.14	0.11	0.89
kg/d	1.90	1.88	1.98	2.02	0.12	0.10	0.53	0.47
Total solids								
%	11.70	12.07	11.90	11.90	0.12	0.08	0.04	0.05
kg/d	2.99	2.94	3.12	3.61	0.45	<0.01	0.10	0.23
MUN, %	16.70	17.0	15.40	16.20	0.54	0.12	0.54	0.35
SCC × 10 ³ /mL	272	275	367	410	80.30	0.15	0.79	0.39
BW, kg	631	599	600	604	29.23	0.16	0.82	0.23
BCS ³	2.83	2.63	2.71	2.74	0.12	0.49	0.12	0.09
Grain supplement intake, kg/d	6.92	6.94	6.90	6.96	0.10	0.90	0.89	0.93

¹Control = control with no linseed oil; LSO1 = control with 170 g/d of linseed oil; LSO2 = control with 340 g/d of linseed oil; LSO3 = control with 510 g/d of linseed oil.

²Lin = linear effect, Quad = quadratic effect, and control vs. LSO = control versus linseed oil supplementation.

³Scored on a 5-point scale where 1 = emaciated to 5 = overly fat.

for treatments 1 to 4, respectively) and *trans*-10 C18:1 (0.56, 0.59, 0.72, and 0.82 g/100 g of fatty acids for treatments 1 to 4, respectively) concentrations. The addition of LSO to grain supplements also linearly increased ($P < 0.001$) the concentrations of milk *trans*-6/8, 9, 12, and 16 when compared with the control treatment.

Linseed oil supplementation increased concentrations ($P < 0.001$) of milk *cis*-9, *trans*-11 CLA when compared with the control treatment (Table 4). As the amount of LSO in the grain supplements increased, milk *cis*-9, *trans*-11 CLA concentrations (1.12, 1.18, 1.39, and 1.65 g/100 g of fatty acids for treatments 1 to 4, respectively) and yields (5.45, 5.73, 6.79, and 7.99 g/d) increased in a linear manner ($P < 0.001$). Milk *trans*, *trans* CLA concentrations increased by 36.4, 54.5, and 72.7% with the LSO1, LSO2, and LSO3 treatments, respectively, relative to the control treatment (Table 4).

Linseed oil supplementation also affected milk n-3 fatty acids concentrations (Table 4). Milk n-3 fatty acids concentrations (0.86, 1.09, 1.26, and 1.25 g/100 g of fatty acids for treatments 1 to 4, respectively) and yields (4.16, 5.29, 6.11, and 6.05 g/d) linearly increased ($P < 0.001$) as the level of LSO in grain supplements increased. Milk linolenic acid content increased by 32.2, 71.2, and 74.5% with the LSO1, LSO2, and LSO3 treatments, respectively, compared with the control treatment. Treatments had no effect ($P > 0.008$) on milk

DHA content (0.14, 0.19, 0.12, and 0.11 g/100 g of fatty acids for treatments 1 to 4, respectively).

DISCUSSION

Supplementing grazing dairy cow diets with different amounts of LSO had no effect on milk yield and grain intake. Others (Schroeder et al., 2003; Rego et al., 2005b; Boken et al., 2005; Shingfield et al., 2005) have also reported similar effects when cow diets were supplemented with plant oils. Supplementing dairy cow diets with high amounts of plant oils often cause a drop in feed intake and therefore milk yield (Chilliard et al., 2001; Rego et al., 2005b) possibly as a result of their negative affects on feed digestibility and rumen fermentation (Jenkins, 1994). In the present study, all cows consumed equal amounts of grain supplements, which may explain the similar milk yield among treatments.

Effect of dietary plant oil supplementation on milk fat content has not been consistent. Bu et al. (2007) and AbuGhazaleh and Holmes (2007) reported no effect of dietary oil supplementations on milk fat content. In contrast, others (AbuGhazaleh et al., 2002; Rego et al., 2005b; Shingfield et al., 2006) reported marked reduction in milk fat content with dietary oil supplementations. Bauman and Griinari (2003) have suggested that milk fat depression (MFD) was related to the direct action on the mammary gland of specific fatty acid iso-

Table 4. Effect of treatments on milk fatty acid composition

Item	Treatment ¹				SEM	Contrast ²		
	Control	LSO1	LSO2	LSO3		Lin	Quad	Control vs. LSO
	g/100 g of fatty acids					P-value		
C4:0	1.72	1.72	1.57	1.40	0.12	0.01	0.35	0.15
C6:0	1.59	1.53	1.42	1.22	0.11	0.01	0.42	0.05
C8:0	0.94	0.92	0.83	0.71	0.06	0.01	0.20	0.01
C10:0	1.69	1.64	1.51	1.31	0.09	<0.01	0.19	<0.01
C11:0	0.25	0.24	0.22	0.20	0.02	0.01	0.58	0.02
C12:0	1.87	1.75	1.69	1.51	0.08	<0.01	0.41	<0.01
C13:0	0.07	0.06	0.06	0.06	0.01	0.51	0.45	0.26
C14:0	7.91	7.48	7.38	6.71	0.27	<0.01	0.26	<0.01
C14:1n-5 <i>t</i>	0.01	0.01	0.01	0.02	0.01	0.01	0.08	0.20
C14:1n-5 <i>c</i>	0.72	0.68	0.67	0.66	0.06	0.04	0.36	0.03
C15:0	0.99	0.88	0.92	0.84	0.02	<0.01	0.23	<0.01
C16:0	23.27	22.67	21.20	19.76	0.62	0.01	0.16	<0.01
C16:1n-5 <i>t</i>	0.17	0.18	0.21	0.25	0.01	<0.01	0.16	<0.01
C16:1n-5 <i>c</i>	1.28	1.32	1.12	1.14	0.07	<0.01	0.77	0.03
C17:0	0.64	0.58	0.55	0.49	0.01	<0.01	0.95	<0.01
C18:0	12.08	12.35	12.29	12.24	0.41	0.64	0.39	0.34
<i>trans</i> C18:1	6.19	6.76	8.17	9.43	0.34	<0.01	0.12	<0.01
<i>t</i> -6/8	0.46	0.51	0.64	0.75	0.02	<0.01	0.10	<0.01
<i>t</i> -9	0.43	0.47	0.58	0.67	0.02	<0.01	0.26	<0.01
<i>t</i> -10	0.56	0.59	0.72	0.82	0.03	<0.01	0.19	<0.01
<i>t</i> -11(VA)	3.39	3.62	4.25	4.89	0.22	<0.01	0.10	<0.01
<i>t</i> -12	0.63	0.71	0.96	1.14	0.04	<0.01	0.10	<0.01
<i>t</i> -16	0.62	0.69	0.85	0.97	0.03	<0.01	0.30	<0.01
C18:1 <i>c</i> 9	25.44	25.67	25.14	25.83	0.72	0.54	0.33	0.69
C18:1 <i>c</i> 11	0.79	0.79	0.72	0.74	0.04	0.02	0.66	0.92
C18:1 <i>c</i> 12	0.27	0.25	0.34	0.42	0.02	<0.01	0.01	<0.01
C18:2 <i>c</i> 9 <i>t</i> 12	0.11	0.11	0.14	0.19	0.02	0.01	0.19	0.10
C18:2 <i>t</i> 9 <i>c</i> 12	0.05	0.08	0.1	0.12	0.01	<0.01	0.28	<0.01
C18:2 <i>t</i> 11 <i>c</i> 15	0.37	0.47	0.74	1.07	0.05	<0.01	<0.01	<0.01
C18:2 <i>c</i> 9 <i>c</i> 12	1.93	1.96	1.97	1.95	0.07	0.69	0.55	0.52
CLA <i>c</i> 9 <i>t</i> 11	1.12	1.18	1.39	1.65	0.05	<0.01	0.02	<0.01
CLA <i>c</i> 9 <i>c</i> 11	0.02	0.03	0.04	0.04	0.01	0.01	0.40	0.01
CLA <i>tt</i>	0.11	0.15	0.17	0.19	0.01	<0.01	0.33	<0.01
C18:3n-3	0.59	0.78	1.01	1.03	0.04	<0.01	<0.01	<0.01
C18:3n-6	0.01	0.01	0.01	0.01	0.01	0.01	0.21	0.01
C19:0	0.05	0.05	0.06	0.05	0.01	0.78	0.27	0.48
C20:0	0.20	0.18	0.17	0.14	0.01	<0.01	0.60	<0.01
C23:0	0.02	0.03	0.03	0.02	0.01	0.44	0.34	0.86
C24:0	0.05	0.05	0.05	0.05	0.01	0.05	0.46	0.22
C20:1n-9	0.05	0.03	0.02	0.02	0.01	0.01	0.40	0.02
C20:3n-6	0.02	0.01	0.02	0.01	0.01	0.42	0.54	0.45
C20:4n-6	0.18	0.16	0.14	0.11	0.01	<0.01	0.30	<0.01
C20:5n-3	0.05	0.04	0.04	0.04	0.01	0.01	0.54	0.01
C22:2n-6	0.02	0.02	0.02	0.02	0.01	0.73	0.94	0.95
C22:4n-6	0.03	0.04	0.03	0.03	0.01	0.59	0.41	0.70
C22:5n-3	0.08	0.08	0.08	0.07	0.01	0.02	0.10	0.26
C22:6n-3, DHA ³	0.14	0.19	0.12	0.11	0.02	0.08	0.15	0.92
Short and medium ⁴	39.30	38.80	36.80	33.70	0.96	<0.01	0.06	<0.01
Long ⁵	50.78	52.11	53.22	55.99	0.89	<0.01	0.25	<0.01
Saturated ⁶	52.30	52.10	49.90	46.70	0.89	<0.01	0.03	<0.01
Unsaturated ⁷	38.90	40.20	41.60	44.30	0.83	<0.01	0.07	<0.01
n3 ⁸	0.86	1.09	1.26	1.25	0.05	<0.01	<0.01	<0.01

¹Control = control with no linseed oil; LSO1 = control with 170 g/d of linseed oil; LSO2 = control with 340 g/d of linseed oil; LSO3 = control with 510 g/d of linseed oil.

²Lin = linear effect, Quad = quadratic, and control vs. LSO = control versus linseed oil supplementation.

³DHA = docosahexaenoic acid.

⁴Short- and medium-chain fatty acids C4:0–16:0.

⁵Long = sum of long-chain fatty acids from C17:0–C24:0.

⁶Saturated = sum of saturated fatty acids from C4:0–C24:0.

⁷Unsaturated = sum of unsaturated fatty acids from C14:1–C22:6n-3.

⁸Sum of all n3 fatty acids C18:3n-3–C22:6n-3.

mers derived from the ruminal metabolism of dietary unsaturated fatty acids. It is well established that post-ruminal infusions of *cis*-9, *trans*-12 CLA inhibit de novo milk fatty acids synthesis in dairy cows (Baumgard et al., 2002), and dietary induced MFD has been related to increased formations of this isomer in the rumen (Bauman and Griinari, 2003). In the current study, *trans*-10, *cis*-12 CLA was not detected in milk fat. *Trans*-10 C18:1 has been also reported to be associated with MFD (Piperova et al., 2004; Shingfield et al., 2006). Although milk *trans*-10 C18:1 concentration linearly increased with LSO supplementations, the concentration was still lower than those reported by Homes and AbuGhazaleh (2007; 4.1 g/100 g of total fatty acids), Loor et al. (2004, 2.84 g/100 g of total fatty acids), and Shingfield et al. (2006; 7.72 g/100 g of total fatty acids) where MFD was reported.

As expected, milk *trans* C18:1 concentrations, VA in particular, increased with LSO supplementation. Supplementing dairy cow diets with plant oils increased milk *trans* C18:1 content under confinement (Whitlock et al., 2002; Shingfield et al., 2006; Bu et al., 2007) and grazing (Boken et al., 2005; Rego et al., 2005b; AbuGhazaleh and Holmes, 2007) feeding systems. Milk VA content averaged 3.23% (Loor et al., 2005) and 3.04% (Bu et al., 2007) of milk total fatty acids when dairy cows were fed LSO at 588 and 636 g/d, respectively. The higher milk VA concentration seen in this study with LSO diets compared with the previous 2 studies may have resulted from grazing, algae supplementation, or both. Cows on pasture-based diets have been shown to have higher levels of VA in their milk than those on conserved forages (Boken et al., 2005; Couvreur et al., 2006). Recently, Couvreur et al. (2006) reported a linear relationship between the proportion of fresh grass in dairy cow diets and milk VA. AbuGhazaleh and Jenkins (2004b) showed that DHA promotes VA accumulations in rumen by inhibiting the reduction of VA to stearic acid. In the current study, milk stearic acid concentration did not increase with LSO supplementation as seen with Bu et al. (2007) and Loor et al. (2005) when LSO was fed to dairy cows supporting the DHA effect on VA accumulation.

The concentration of *trans*-10 C18:1 in milk fat in this study was relatively low compared with that seen by Rego et al. (2005b) and Shingfield et al. (2006). Fresh forage intake may explain, in part, the lower concentration of milk *trans*-10 C18:1 in this study. 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 dairy cows increased (Couvreur et al., 2006). Kay et al. (2005) reported that *trans*-10 C18:1 contributed <1% of the milk total *trans* C18:1 isomers in pasture fed cows compared with about 23% in TMR fed

cows. Results from previous studies suggest that the interaction of high-dietary starch and oils leads to the largest increases in *trans*-10 C18:1 concentration as a result of *trans*-10 C18:1 replacing VA as the predominant *trans* C18:1 isomer (Shingfield et al., 2005; AbuGhazaleh and Jacobson, 2007a). Oil source may also explain the low milk *trans*-10 C18:1 concentration in this study. AbuGhazaleh and Jacobson (2007b) showed that linolenic acid was less efficient than linoleic acid (the primary fatty acid in soybean oil) in promoting *trans*-10 C18:1 formation.

Supplementing dairy cow diets with linolenic acid oil source increased milk *cis*-9, *trans*-11 CLA content under confinement (Dhiman et al., 2000; Loor et al., 2005; Bu et al., 2007) and grazing (Brown et al., 2006; Holmes and AbuGhazaleh, 2007) feeding systems. The linear increase in milk VA concentration with LSO and the subsequent conversion of VA to *cis*-9, *trans*-11 CLA by Δ^9 -desaturase in mammary gland may explain the linear increase in milk *cis*-9, *trans*-11 CLA content. As mentioned earlier, *cis*-9, *trans*-11 CLA in milk originates from ruminal biohydrogenation of linoleic acid as an intermediate product or from 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 80% of the *cis*-9, *trans*-11 CLA in milk fat (Mosley et al., 2006).

The increases in milk linolenic acid and DHA contents with the LSO treatments were relatively small compared with their intake (Table 2). Similar low increases in n-3 fatty acids were reported by others (Loor et al., 2005; Rego et al., 2005a; Bu et al., 2007) when n-3 lipid supplements were used. This low transfer of n-3 fatty acids from feed to milk fat may be explained by extensive ruminal biohydrogenation of linolenic acid (Harfoot and Hazlewood, 1997) and DHA (AbuGhazaleh and Jenkins, 2004a) or by their partitioning toward other tissues within the body. Kitessa et al. (2001) reported that n-3 fatty acids are almost totally confined to plasma cholesterol ester and phospholipids, which are poorly taken up by the mammary gland (Offer et al., 1999).

CONCLUSIONS

Supplementing grazing dairy cow diets with algae and LSO increased milk *cis*-9, *trans*-11 CLA content without affecting cow's milk yield. Linseed oil supplementation caused a quadratic increase in milk fat and protein contents. The increase in milk *cis*-9, *trans*-11 CLA content was proportional to the amounts of LSO in grazing dairy cow diets. Supplementing grazing

dairy cow diets with algae and LSO at up to 510 g/d can improve the nutritional value of milk without compromising milk composition or cow performance.

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