Conjugated Linoleic Acid and Vaccenic Acid in Rumen, Plasma, and Milk of Cows Fed Fish Oil and Fats Differing in Saturation of 18 Carbon Fatty Acids¹

A. A. AbuGhazaleh,² D. J. Schingoethe, A. R. Hippen, and K. F. Kalscheur

Dairy Science Department, South Dakota State University, Brookings 57007-0647

ABSTRACT

The objective of this study was to examine the effect of feeding fish oil (FO) along with fat sources that varied in saturation of 18 carbon fatty acids (high stearic, high oleic, high linoleic, or high linolenic acids) on rumen, plasma, and milk fatty acid profiles. Four primiparous Holstein cows at 85 d in milk (± 40) were assigned to 4 $\times$ 4 Latin squares with 4-wk periods. Treatment diets were 1) 1% FO plus 2% commercial fat high in stearic acid (HS); 2) 1% FO plus 2% fat from high oleic acid sunflower seeds (HO); 3) 1% FO plus 2% fat from high linoleic acid sunflower seeds (HLO); and 4) 1% FO plus 2% fat from flax seeds (high linolenic; HLN). Diets were formulated to contain 18% crude protein and were composed of 50% (dry basis) concentrate mix, 25% corn silage, 12.5% alfalfa silage, and 12.5% alfalfa hay. Milk production, milk protein percentages and yields, and dry matter intake were similar across diets. Milk fat concentrations and yields were least for HO and HLO diets. The proportion of milk *cis*-9, *trans*-11 conjugated linoleic acid (CLA; 0.71, 0.99, 1.71, and 1.12 g/100 g fatty acids, respectively), and vaccenic acid (TVA; 1.85, 2.60, 4.14, and 2.16 g/100 g fatty acids, respectively) were greatest with the HLO diet. The proportions of ruminal *cis*-9, *trans*-11 CLA (0.09, 0.16, 0.18, and 0.16 g/100g fatty acids, respectively) were similar for the HO, HLO, and HLN diets and all were higher than for the HS diet. The proportions of TVA (2.85, 4.36, 8.69, and 4.64 g/100 g fatty acids, respectively) increased with the HO, HLO, and HLN diets compared with the HS diets, and the increase was greatest with the HLO diet. The effects of fat supplements on ruminal TVA concentra-

tions were also reflected in plasma triglycerides, (2.75, 4.64, 8.77, and 5.42 g/100 g fatty acids, respectively); however, there were no differences in the proportion of *cis*-9, *trans*-11 CLA (0.06, 0.07, 0.06, and 0.07 g/100 g fatty acids, respectively). This study further supports the significant role for mammary delta-9 desaturase in milk *cis*-9, *trans*-11 CLA production.

(**Key words:** conjugated linoleic acid, vaccenic acid, rumen digesta, plasma triglycerides)

Abbreviation key: CLA = conjugated linoleic acid, DHA = docosahexaenoic acid, ECM = energy-corrected milk, FO = fish oil, EPA = eicosapentaenoic acid, HLO = high linoleic acid, HLN = high linolenic acid, HO = high oleic acid, HS = high stearic acid, TVA = vaccenic acid.

INTRODUCTION

Milk fat is a rich natural dietary source of conjugated linoleic acid (CLA), which has been the focus of considerable research efforts in recent years that have revealed potential health-promoting properties associated with particular isomers of CLA, primarily, *cis*-9, *trans*-11 CLA and *trans*-10, *cis*-12 CLA. Conjugated linoleic acids have been shown to affect atherosclerosis, diabetes, the immune system, bone mineralization, body fat accretion, and nutrient partitioning (McGuire and McGuire, 2000). The reported health benefits of CLA make it desirable to increase the concentrations and yields of CLA in dairy products.

In the rumen, *cis*-9, *trans*-11 CLA is formed primarily from isomerization of dietary linoleic acid (C18:2n6) during the first step of the biohydrogenation process (Harfoot and Hazlewood, 1988). Once the *cis*-9, *trans*-11 CLA is formed, biohydrogenation of the *cis*-9 bond occurs by microbial reductase (group A microorganisms) to form vaccenic acid (TVA; *trans*-11C18:1). The extent to which TVA is hydrogenated to stearic acid (C18:0; group B microorganisms) depends on conditions in the rumen (Jenkins, 1993). Dietary linolenic acid (C18:3n3) also undergoes biohydrogenation by being first isomerized to a conjugated triene (*cis*-9, *trans*-11, *cis*-15 C18:3), followed by re-

Received November 8, 2002.

Accepted April 19, 2003.

¹Published with the approval of director of the South Dakota Agricultural Experiment Station as Publication Number # 3340 of the Journal Series.

²Present address: Department of Animal and Veterinary Science, Clemson University, Clemson, SC 29634.

Corresponding author: D. J. Schingoethe; e-mail: david_schingoethe@sdstate.edu.

ductions of double bonds at carbons 9, 15, and 11 to yield *trans*-11, *cis*-15 C18:2, TVA, and C18:0, respectively (Wilde and Dawson, 1966). The high correlation reported by Jiang et al. (1996) between *cis*-9, *trans*-11 CLA and TVA in milk fat led Griinari et al. (1997) to hypothesize that a portion of *cis*-9, *trans*-11 CLA appearing in milk fat was of endogenous origin. Subsequent work (Griinari et al., 2000; Corl et al., 2001) showed that approximately 64 to 78% of *cis*-9, *trans*-11 CLA appearing in milk fat is synthesized in the mammary gland from TVA via delta-9 desaturase.

Our previous research (Whitlock et al., 2002; AbuGhazaleh et al., 2002a; AbuGhazaleh et al., 2003) demonstrated that feeding fish oil (FO) along with mono- and polyunsaturated fatty acid oils, especially oils high in C18:2n6, increased the concentrations and yields of milk *cis*-9, *trans*-11 CLA and TVA. The purpose of the current study was to determine the effects of FO supplementation of diets differing in fatty acid profiles on rumen, plasma, and milk fatty acid profiles with emphasis on *cis*-9, *trans*-11 CLA and TVA.

MATERIALS AND METHODS

Four ruminally fistulated primiparous Holstein cows averaging 85 DIM (± 40) were used in a 4×4 Latin square design. Period length was 28 d of which the first 21 d were used as adjustment period to the experimental diets. Cows were housed in a free-stall barn and fed using Calan Broadbent feeding doors (American Calan, Inc., Northwood, NH) for monitoring individual feed intakes. Cows were individually fed a TMR once daily (0900 h) for ad libitum consumption. Amounts fed and refused were recorded daily. Treatment diets were 1) 1% FO plus 2% commercial fat high in stearic acid (HS); 2) 1% FO plus 2% fat from high oleic acid (HO) sunflower seeds; 3) 1% FO plus 2% fat from high linoleic acid (HLO) sunflower seeds; and 4) 1% FO plus 2% fat from flax seeds (high linolenic; HLN). Diets were formulated to be isonitrogenous at 18% CP and to contain a surplus of major nutrients (NRC, 2001). Diets (Table 1) were composed of 50% (dry basis) concentrate mix, 25% corn silage, 12.5% alfalfa silage, and 12.5% alfalfa hay. Menhaden FO (Omega Protein Inc., Hammond, LA) was used in this experiment. Rumo-fat (Robt Morgan Inc. Paris, IL) was used as a source of C18:0. Regular sunflower seeds and flax seeds were obtained from local dealers. A local farmer donated the high oleic sunflower seeds. Sunflower and flax seed shells were cracked by rollers; however, because flax seeds are quite small, most of the seed shells remained intact. Fatty acid profiles of diets and fat supplements are given in Table 2.

Cows were milked daily at 0600, 1400, and 2100 h, and milk yield was recorded at each milking during the final 7 d of each period. Milk samples from the three daily milkings were collected on 3 consecutive days (d 26, 27, and 28) of each period and composited by day. Composites were split into two portions for analysis. One portion was refrigerated at 4°C and sent to a laboratory (Valley Queen Cheese Factory, Milbank, SD) to be analyzed for fat, protein, lactose, and SNF (AOAC, 1997) by midinfrared spectrophotometry (Multispec; Foss Food Technology Corp., Eden Prairie, MN); SCC (AOAC, 1997) were determined using a Fossomatic 90 (Foss Food Technology Corp.). The other portion of each sample was stored at -20°C for analysis of fatty acids by GLC. Milk samples were analyzed for individual fatty acids by GLC of butyl esters. Briefly, 0.5 ml of milk was placed into 13 $\times$ 100 mm test tubes with Teflon-lined screw caps, followed by the addition of 750 μ l of n-butanol. Samples were vortexed at low speed while slowly adding 75 μ l of acetyl chloride, gassed with N₂, capped tightly, and heated at 100°C for 30 min. After samples cooled to room temperature, 5 ml of 6% K₂CO₃ and 1 ml of hexane were added and the samples vortexed for 30 s. Samples were centrifuged (20 min at 2500 $\times$ g), and the bottom layer was aspirated and discarded. The remaining layer was washed four times (20 min at 2500 $\times$ g) with distilled, deionized water. The upper layer was removed and placed in injection vials for analysis. Esters of fatty acids were separated using a fused silica capillary column (100 m $\times$ 0.25 mm, i.d. $\times$ 0.20 μ m thickness, SPTM-2560). The split ratio in the injector port (180°C) was 75:1 with a column flow of 1.5 ml/min of He. Oven temperature was programmed for 60°C for 5 min, then increased from 60 to 165°C at 3°C/min, held at 165°C for 10 min, raised to 220°C at 5°C/min, and finally held at 220°C for 28 min. The injection and detector temperatures were 230°C. Peaks for individual fatty acids were identified by order of elution and comparison to known commercially prepared standards.

Concentrate mixes, alfalfa hay, haylage, and corn silage were sampled weekly and stored frozen at -20°C until processed for further analysis. For analysis, samples were dried in an oven at 55°C for 48 h, ground through a model no. 3 Wiley mill (Arthur H. Thomas Co., Philadelphia, PA) with a 2-mm screen, and composited by period. Feed samples were dried for 24 h at 105°C for determination of DM content. Contents of CP, ether extract, ash, Ca, P, and Mg were determined by AOAC (1997) methods. For analysis of NDF, ADF, and acid detergent lignin, the sample was reground through an ultracentrifuge mill (Brinkman Instruments Co., Westbury, NY) with a 1-mm screen.

Table 1. Ingredient and nutrient content of experimental diets.

Item	Diet ¹			
	HS	HO	HLO	HLN
	(% of DM)			
Ingredient Composition				
Alfalfa hay	12.50	12.50	12.50	12.50
Alfalfa silage	12.50	12.50	12.50	12.50
Corn silage	25.00	25.00	25.00	25.00
Cracked corn	26.70	27.00	27.42	27.56
Soybean meal	14.50	13.50	13.34	12.54
Fish oil	1.00	1.00	1.00	1.00
Saturated-fat ²	2.00	...	...	...
Sunflower seeds, high oleic	...	4.60	...	...
Sunflower seeds, high linoleic	...	...	4.34	...
Flax seeds	...	...	...	5.00
Soy hulls	3.50	1.60	1.60	1.60
High Zn trace mineralized salt ³	0.20	0.20	0.20	0.20
Magnesium oxide	0.14	0.14	0.14	0.14
Dicalcium phosphate	0.60	0.60	0.60	0.60
Limestone	1.00	1.00	1.00	1.00
Vitamin A, D, & E premix ⁴	0.10	0.10	0.10	0.10
Vitamin E premix ⁵	0.06	0.06	0.06	0.06
Chemical composition				
DM, %	58.10	58.90	58.40	58.80
CP	16.40	16.75	16.68	16.91
Total fatty acids	4.35	4.15	4.20	4.06
ADF	19.14	18.64	18.29	19.03
NDF	29.87	29.33	29.19	30.04
Lignin	2.94	3.03	3.02	3.02
Ash	6.94	7.02	6.81	6.85
Ca	1.05	1.09	1.05	0.98
P	0.43	0.45	0.43	0.41
Mg	0.32	0.30	0.34	0.34
NE _L ⁶ , Mcal/kg	1.80	1.76	1.76	1.74

¹HS = 1% fish oil + 2% fat source high in stearic acid; HO = 1% fish oil + 2% fat from high oleic acid sunflower seeds; HLO = 1% fish oil + 2% fat from high linoleic acid sunflower seeds; and HLN = 1% fish oil + 2% fat from flax seeds (high linolenic).

²Rumo-fat (Robt Morgan Inc. Paris, IL).

³Contained (%): NaCl (95.5-98.8), Zn (1.00), Mn (0.75), Fe (0.60), Mg (0.05) Cu (0.032), I (0.028), and Co (0.011).

⁴Contained 4,400,000 IU of vitamin A; 880,000 IU of vitamin D, and 440 IU of vitamin E/kg.

⁵Contained 44,000 IU of vitamin E/kg.

⁶Estimated from NRC (2001).

NDF (procedure B, Van Soest et al., 1991), ADF (Robertson and Van Soest, 1981), and acid detergent lignin (Lowry et al., 1994) were determined by ANKOM fiber analyzer using Filter Bag Technique (ANKOM Technology Corp., Fairport, NY). The BCS (Wildman et al., 1982) and BW were recorded at the beginning of the experiment and the end of each period.

Samples of ruminal contents were collected at 2 and 6 h after the morning feeding on d 27 and at 4 h on d 28 of each period. Contents (approximately 450 g) were removed by hand from four different locations in the rumen and mixed. Additional ruminal contents were taken and squeezed through four layers of cheesecloth, and 100 ml of the fluid was added to each sample. Ruminal samples were then placed into resealable plastic bags and stored on ice until processing

in the laboratory. Every sample was mixed one more time by hand, subsampled (approximately 200 g), and oven dried at 38°C for 48 h. Dried samples were then ground to pass a 0.5-mm screen and composited into one sample per cow per period. Methods used to extract and determine ruminal fatty acids composition were described previously (AbuGhazaleh et al., 2002b).

Blood samples (50 ml) were obtained from the jugular vein at 4 h after the morning feeding on d 28 of each period. Blood was collected into a 50-ml tube containing 0.5-ml heparin and placed on ice immediately until processing in the laboratory. Blood was centrifuged at 3000 × g for 15 min for harvesting plasma. Plasma was stored at -20°C until analysis. Lipids were extracted from plasma (15 ml) with chlo-

Table 2. Fatty acid composition of dietary treatments and fat supplements.

Fatty acids ²	Diet ¹				Fat supplements				
	HS	HO	HLO	HLN	Rumo-fat	Oleic sunflower	Regular sunflower	Flax seeds	Fish oil
					(g/100 g of fatty acids)				
C14:0	2.50	1.72	1.58	1.58	2.81	0.04	0.07	0.05	7.70
C15:0	0.30	0.21	0.19	0.19	0.38	0.01	0.01	0.02	0.73
C16:0	16.39	11.17	11.19	11.10	22.11	3.79	5.87	5.66	17.10
C16:1	2.03	2.14	1.98	2.00	0.05	nd ³	nd	nd	10.26
C17:0	0.78	0.31	0.26	0.23	1.67	nd	nd	nd	0.69
C18:0	22.92	4.17	3.88	3.57	64.20	3.42	4.18	3.76	3.21
C18:1c9	10.72	36.52	19.84	18.16	1.10	83.90	24.45	20.92	6.97
C18:1c11	0.03	0.04	0.04	0.03	1.10	0.75	0.50	0.02	2.99
C18:2c9c12	19.46	21.80	42.10	25.88	0.88	4.29	59.95	16.60	1.31
C18:3n3	4.44	4.86	4.36	23.24	0.05	0.22	0.14	50.12	1.10
C18:3n6	0.33	0.38	0.33	0.31	0.04	0.03	0.01	0.02	0.37
C20:1	0.07	0.06	0.06	0.05	nd	nd	nd	nd	0.76
C20:0	0.89	0.43	0.41	0.34	0.45	nd	nd	nd	1.40
C21:0	0.64	0.63	0.58	0.62	0.02	0.1	0.12	nd	0.85
C20:2	0.08	0.08	0.04	0.08	nd	nd	nd	nd	0.21
C20:3	0.06	0.06	0.06	0.06	nd	nd	nd	nd	0.20
C22:0	0.41	0.58	0.57	0.34	0.05	0.10	0.10	nd	0.27
C22:1	0.13	0.13	0.11	0.12	nd	nd	nd	nd	1.61
C20:5 (EPA) ⁴	1.97	1.98	1.87	1.93	nd	nd	nd	nd	10.93
C22:6 (DHA) ⁵	2.10	1.97	1.95	2.04	nd	nd	nd	nd	11.92
Others ⁶	13.94	10.83	8.46	8.21	5.11	3.35	4.60	2.83	19.25

¹HS = 1% fish oil + 2% fat source high in stearic acid; HO = 1% fish oil + 2% fat from high oleic acid sunflower seeds; HLO = 1% fish oil + 2% fat from high linoleic acid sunflower seeds; and HLN = 1% fish oil + 2% fat from flax seeds (high linolenic).

²Expressed as number of carbons:double bonds.

³nd = not detectable or detected at < 0.01g/100g of fatty acids.

⁴EPA = eicosapentaenoic acid.

⁵DHA = docosahexaenoic acid.

⁶Unidentified peaks.

roform/methanol (2:1, vol/vol). The plasma triglyceride fraction was separated on a 500-mg silicic acid column (309250, Alltech Assoc., Deerfield, IL) according to Christie (1982). The residues (phospholipids, cholesterol esters, and free fatty acids) were dissolved in hexane, dried under N₂, and analyzed for fatty acid profiles at 65:1 split ratio as described for milk samples (AbuGhazaleh et al., 2002a). Analysis of total fatty acids of plasma triglyceride fraction required injection of 3 μ l of butyl esters at a 15:1 split ratio. Fatty acids were then determined as described for milk samples (AbuGhazaleh et al., 2002a).

Samples of alfalfa hay, alfalfa haylage, corn silage, and concentrate mix were composited into TMR within each treatment period and fat was extracted in a mixture of ether and acetone (1:1, vol/vol). Approximately 15 mg of the extracted fat was transferred into 13- $\times$ 100-mm test tubes with Teflon-lined screw caps and then analyzed for fatty acids as described by AbuGhazaleh et al. (2002a). Nonadecanoic acid (C19:0), dissolved in butanol, was used as an internal standard for feed fatty acid analysis.

Data were analyzed as a Latin square using the mixed model procedures of SAS (1996) with fixed effects of periods and treatments; the random effect was cow. Least significant difference at $P < 0.10$ was used to determine significant differences among means.

RESULTS AND DISCUSSION

The ingredient and chemical composition of the four diets are shown in Table 1. Diets contained similar DM, CP, ADF, and NDF. Acid detergent fiber and NDF concentrations for the HS were balanced by adding soy hulls (Table 1). The CP content of all diets were lower than anticipated because of a lower CP for alfalfa hay than anticipated. The four fat supplements were chosen because of the differences in their fatty acids composition (Table 2) and the measured profiles were typical of the fat supplements. Rumo-fat showed high contents (64.2%) of C18:0. Oleic acid (*cis*-9 C18:1, 83.9%) was the major fatty acid in high oleic sunflower seeds, while C18:2n6 (60%) was the major fatty acid in regular sunflower seeds. The flax seeds and FO were characterized by their contents of omega-3 fatty

Table 3. Milk yield, milk composition, BW, BCS, and DMI from cows fed fish oil with different fat sources.

Item	Diet ¹				SE	P
	HS	HO	HLO	HLN		
Milk, kg/d	31.69	31.52	28.91	29.61	1.58	0.30
3.5 % FCM, kg/d	29.79 ^a	27.61 ^{ab}	24.97 ^b	27.59 ^{ab}	1.64	0.10
ECM ² , kg/d	30.01 ^a	28.24 ^{ab}	25.79 ^b	27.91 ^{ab}	1.42	0.10
Fat						
%	3.10 ^a	2.74 ^b	2.64 ^b	3.09 ^a	0.15	0.08
kg/d	0.99 ^a	0.86 ^{ab}	0.77 ^b	0.91 ^a	0.07	0.09
Protein						
%	3.00	3.01	3.12	3.04	0.09	0.54
kg/d	0.95	0.94	0.89	0.89	0.04	0.18
Lactose						
%	4.96	5.00	4.94	4.99	0.05	0.59
kg/d	1.57	1.58	1.43	1.48	0.08	0.32
Total solids						
%	12.10	12.00	11.80	12.20	0.85	0.39
kg/d	3.84	3.78	3.40	3.59	0.30	0.59
SCC, X 10 ³ /ml	123	78	110	110	45.82	0.81
BW, kg	580	575	592	586	10	0.54
BCS ³	3.35	3.40	3.30	3.35	0.07	0.18
DMI, kg/d	22.30	21.80	22.30	22.00	1.09	0.95

^{a,b,c}Means in the same rows with unlike superscripts differ ($P < 0.10$).

¹HS = 1% fish oil + 2% fat source high in stearic acid; HO = 1% fish oil + 2% fat from high oleic acid sunflower seeds; HLO = 1% fish oil + 2% fat from high linoleic acid sunflower seeds; and HLN = 1% fish oil + 2% fat from flax seeds (high linolenic).

²Energy-corrected milk (Orth, 1992).

³Scored on a five-point scale where 1 = emaciated to 5 = overly fat (Wildman et al., 1982).

acids. C18:3n3 (50.1%) is the major fatty acid in flax seeds, while C20:5n3 (eicosapentaenoic acid, **EPA**, 10.9%) and C22:6n3 (docosahexaenoic acid, **DHA**, 11.9%) were the main polyunsaturated fatty acids in FO.

Milk Yield, Milk Composition, and DMI

Table 3 gives mean values for concentrations and yields of major milk constituents. Treatment had no significant effect on milk production ($P = 0.30$), although there was a trend towards higher milk production for cows fed the HS and the HO diets ($P < 0.15$). The HO and the HLO diets depressed milk fat concentration compared with values of the other two diets. Cows fed the HS had similar milk fat percentages to cows fed the HLN. The HLO caused the greatest depression in milk fat percentages and accordingly had the lowest fat, 3.5% FCM, and energy-corrected milk (ECM) yields, while the HS had the highest fat, 3.5% FCM, and ECM yields. Milk true protein percentages and yields, total milk solids percentages and yields, SCC, BW, BCS, and DMI were not different among treatments ($P > 0.10$). Further discussions about the effect of these treatments on milk yield, milk composition, and DMI by cows in a companion study are reported elsewhere (AbuGhazaleh et al., 2003).

Fatty Acid Composition of Ruminal Digesta

Our previous studies demonstrated an increase in milk fat *cis*-9, *trans*-11 CLA concentration when diets were supplemented with FO and lipids rich in *cis*-9 C18:1, C18:2n6, and C18:3n3 acids (Whitlock et al., 2002; AbuGhazaleh et al., 2002a; AbuGhazaleh et al., 2003). Feeding lactating dairy cows a blend of FO and a high C18:2n6 source resulted in the greatest increases in the concentrations and yields of milk *cis*-9, *trans*-11 CLA and TVA (AbuGhazaleh et al., 2003). The overall objective of our work here was to determine the effect of treatments on ruminal and plasma fatty acid profiles, especially TVA and *cis*-9, *trans*-11 CLA. The rumen is the site of intense microbial lipid metabolism. Lipolysis of dietary glycolipids, phospholipids, and triglycerides leads to free fatty acids that are hydrogenated by microbes to more saturated end products. Until recently, it was believed that C18:0 was the only end product of *cis*-9 C18:1 hydrogenation in the rumen. However, a recent study by Mosley et al. (2002) demonstrated that *cis*-9 C18:1 could also be a precursor for several *trans*-fatty acid isomers including TVA. Biohydrogenation of C18:2n6 acid involves an initial isomerization of the *cis*-12 double bond to a *trans*-11 bond, forming *cis*-9, *trans*-11 CLA. Microbial reductase hydrogenates the *cis*-9 bond, resulting in formation of TVA, which in turn is reduced to C18:0

(Harfoot and Hazlewood, 1988). The pathway for reduction of C18:3n3 acid to C18:0 involves TVA but not *cis*-9, *trans*-11 CLA as an intermediate (Wilde and Dawson, 1966).

The high proportion of *cis*-9 C18:1, C18:2n6, and C18:3n3 acids in ruminal content of cows fed the HLN diet indicates that flax seeds were partially protected from microbial biohydrogenation. Additionally, the high proportion of ruminal *cis*-9 C18:1 and C18:2n6 acids with the HO and the HLO diets, respectively, indicates that biohydrogenation of these fatty acids did not proceed to completion. Greater intake of dietary C16:0 (132, 91, 94, and 89 g/d for HS, HO, HLO, and HLN, respectively) and C18:0 (185, 33, 32, and 29 g/d for HS, HO, HLO, and HLN, respectively) explain the higher proportion of these fatty acids in ruminal digesta of cows fed the HS diet.

The degree of biohydrogenation of individual fatty acids could not be estimated in this study; however, others (Wu et al., 1991; Kalscheur et al., 1997; Wachira et al., 2000; Lock and Garnsworthy, 2002) have reported different rates of biohydrogenation of *cis*-9 C18:1, C18:2n6, and C18:3n3 fatty acids. Wachira et al. (2000) reported that biohydrogenation of C18:2n6 and C18:3n3 ranged between 80 and 93% when lambs were fed FO and/or whole linseed. When Kalscheur et al. (1997) fed high *cis*-9 C18:1 and C18:2n6 sunflower oil to dairy cows at 3.7% of dietary DM, biohydrogenation of *cis*-9 C18:1c9, C18:2n6, and C18:3n3 acids ranged from 72 to 85%. Wu et al. (1991), however, observed a lesser biohydrogenation of *cis*-9 C18:1 where the level of biohydrogenation varied from 71% when cows were offered a linseed diet to 60% when cows were offered a FO diet. Previous researchers (Kalscheur et al., 1997; Beam et al., 2000; Jenkins and Adams, 2002) reported that the factors with the greatest influence on rates of biohydrogenation of unsaturated fatty acids were rumen pH, amount of added fat, number of double bonds in fatty acid, and ruminal turnover. Variation in rates was relatively small according to diet, time of day that the inoculum was collected, or degree of esterification of the added fat (Beam et al., 2000).

The proportion of C18:1 *trans* isomers in ruminal digesta was increased ($P < 0.10$) when cows were fed the HO, HLO, and the HLN compared with the HS diet, and was greatest with the HLO diet (Table 4). As has been noted herein and by others (AbuGhazaleh et al., 2002b; Piperova et al., 2002), TVA is the major *trans*-C18:1 isomer in the rumen and duodenum fatty acids. The significant increase in *trans*-C18:1 fatty acids in general, and TVA in particular, with the HO compared with the HS diet support the finding of Mosley et al. (2002) that *cis*-9 C18:1 could serve as a pre-

cursor for several *trans*-fatty acid isomers including TVA. The *cis*-9 C18:1 might have also interfered with biohydrogenation of other polyunsaturated fatty acids in the diet, resulting in the accumulation of *trans*-C18:1 (Mosley et al., 2002). In addition, cows fed the HO diet had a slightly greater intake of C18:2n6 (177 vs. 156 g/d.) compared with cows fed the HS diet. This also could have contributed to greater ruminal content of TVA for cows fed the HO diet compared with cows fed the HS diet. The fact that the HLN diet had lesser ruminal TVA concentration compared with HLO diet further support our speculation that flax seeds were partially protected from ruminal biohydrogenation.

Under normal ruminal conditions, biohydrogenation of C18:2n6 and C18:3n3 fatty acids leads to TVA formation as an intermediate (Wilde and Dawson, 1966; Harfoot and Hazlewood, 1988). The C18:2n6 interferes with its own biohydrogenation when present in ruminal contents at higher concentrations. Harfoot et al. (1973) reported that a concentration of C18:2n6 exceeding 1.0 mg/ml of culture contents interfered with biohydrogenation of TVA, leading to the accumulation of TVA at the expense of C18:0. Beam et al. (2000) reported the overall rate of biohydrogenation of C18:2n6 was $14.3\% \text{ h}^{-1}$, but declined by $1.2\% \text{ h}^{-1}$ for each percentage unit increase in C18:2n6 added to the substrate. The inhibitory effects of C18:2n6 on biohydrogenation also appear to be more severe when it is supplied as the free acid rather than supplied as a triglyceride (Beam et al., 2000). AbuGhazaleh et al. (2002b) reported that FO can also cause incomplete biohydrogenation of C18:2n6; however, the mechanism is not clear but may involve an inhibition of the enzyme that catalyzes the final biohydrogenation step in the rumen, possibly by altering group B microorganism populations which hydrogenate TVA into C18:0 (Harfoot and Hazlewood, 1988).

The concentration of *cis*-9, *trans*-11 CLA in ruminal digesta was also increased ($P < 0.04$) with the HO, HLO, and the HLN compared with the HS diet (Table 4), and there were no significant differences between cows fed the HO, HLO, and the HLN diets. This lack of differences might indicate that hydrogenation of C18:2n6 into *cis*-9, *trans*-11 CLA in the HLO diet was not a rate-limiting step. It is not clear why *cis*-9, *trans*-11 CLA concentration was high with the HO and the HLN diets. The *cis*-9 C18:1 and C18:3n3 or their derivatives might have caused a shift in rumen microbial populations and/or interfered with the biohydrogenation of C18:2n6 in the diet, resulting in the formation of *cis*-9, *trans*-11 CLA. The effect of mono- and polyunsaturated fatty acids in rumen microbial populations and biohydrogenation is an area that needs further investigation. The greater TVA to *cis*-9, *trans*-11 CLA

Table 4. Fatty acid composition of rumen digesta.

Fatty acid ²	Diet ¹				SE	P
	HS	HO	HLO	HLN		
	(g/100 g of fatty acids)					
C12:0	0.17	0.17	0.17	0.16	0.01	0.80
C13:0	0.05 ^b	0.04 ^b	0.05 ^b	0.08 ^a	0.01	0.07
C14:0	1.53 ^a	1.04 ^b	1.02 ^b	0.91 ^c	0.04	<0.01
C15:0	0.60 ^a	0.54 ^{cb}	0.57 ^{ab}	0.50 ^c	0.03	0.06
C16:0	16.48 ^a	12.81 ^b	13.06 ^b	12.57 ^b	0.27	<0.01
C16:1	0.53 ^a	0.45 ^{ab}	0.40 ^b	0.44 ^{ab}	0.06	0.10
C17:0	0.43 ^b	0.35 ^b	0.54 ^a	0.34 ^c	0.07	<0.01
C18:0	32.72 ^a	27.03 ^b	25.62 ^b	23.10 ^b	2.85	0.07
C18:1 Δ 6-8	0.66 ^b	1.20 ^a	1.22 ^a	1.07 ^a	0.20	0.09
C18:1 Δ 9	0.33 ^b	0.54 ^{ab}	0.82 ^a	0.30 ^b	0.19	0.10
C18:1 Δ 10	0.75 ^b	1.27 ^b	2.18 ^a	0.97 ^b	0.40	0.05
C18:1 Δ 11(TVA)	2.85 ^c	4.36 ^b	8.69 ^a	4.64 ^b	0.72	0.09
C18:1 Δ 9	6.76 ^b	12.63 ^a	8.16 ^b	11.34 ^a	1.28	0.01
C18:1 Δ 11	0.47 ^b	0.43 ^b	0.60 ^a	0.45 ^b	0.05	0.06
C18:2 Δ 9 Δ 12	0.17 ^a	0.11 ^c	0.14 ^b	0.11 ^c	0.01	<0.01
C18:2 Δ 9 Δ 12	5.72 ^b	6.48 ^b	8.45 ^a	8.57 ^a	0.60	<0.01
C20:0	1.18 ^a	0.79 ^b	0.81 ^b	0.80 ^b	0.08	<0.01
C18:3 Δ 3	1.27 ^b	1.30 ^b	1.32 ^b	10.13 ^a	0.68	<0.01
C18:2 Δ 9 Δ 11	0.09 ^b	0.16 ^a	0.18 ^a	0.16 ^a	0.02	0.04
C18:2 Δ 10 Δ 12	0.09 ^b	0.12 ^a	0.13 ^a	0.10 ^b	<0.01	0.04
C18:2 Δ 9 Δ 11	0.07	0.09	0.10	0.08	0.03	0.83
C21:0	0.16	0.17	0.35	0.15	0.10	0.28
C20:3	0.08	0.09	0.09	0.15	0.04	0.41
C22:0	0.56 ^b	0.65 ^a	0.65 ^a	0.50 ^c	0.03	<0.01
C24:0	0.50 ^b	0.65 ^a	0.67 ^a	0.65 ^a	0.04	0.02
C23:0	0.14 ^b	0.17 ^a	0.16 ^{ab}	0.15 ^{ab}	0.01	0.10
C20:5 (EPA) ³	0.32 ^a	0.29 ^{ab}	0.27 ^{ab}	0.24 ^b	0.03	0.11
C22:5	0.02	0.01	0.01	0.03	0.01	0.37
C22:6 (DHA) ⁴	0.18 ^a	0.16 ^{ab}	0.11 ^{bc}	0.08 ^c	0.03	0.08
Others ⁵	24.46	24.64	24.01	21.10	2.52	0.51
Short ⁶	0.21	0.21	0.22	0.25	0.02	0.46
Medium ⁷	19.57 ^a	15.19 ^b	15.58 ^b	15.76 ^c	0.31	<0.01
Long ⁸	55.77 ^b	59.96 ^{ab}	60.11 ^{ab}	64.14 ^a	2.57	0.09
Saturated	54.51 ^a	44.41 ^b	43.65 ^b	39.92 ^b	3.06	0.01
Unsaturated	21.04 ^c	30.96 ^b	32.25 ^b	39.22 ^a	2.92	<0.01
TVA:c9 Δ 11CLA	32.30 ^b	29.43 ^b	44.05 ^a	31.65 ^b	5.45	0.09

^{a,b,c}Means in the same rows with unlike superscripts differ ($P < 0.10$).

¹HS = 1% fish oil + 2% fat source high in stearic acid; HO = 1% fish oil + 2% fat from high oleic acid sunflower seeds; HLO = 1% fish oil + 2% fat from high linoleic acid sunflower seeds; and HLN = 1% fish oil + 2% fat from flax seeds (high linolenic).

²Expressed as number of carbons:double bonds.

³EPA = eicosapentaenoic acid.

⁴DHA = docosahexaenoic acid.

⁵Unidentifiable peaks.

⁶Short-chain FA: (C12:0 to C13:0).

⁷Medium-chain FA: (C14:0 to C17:0).

⁸Long-chain FA: ($\geq$ C18:0).

ratio in the rumen digesta for cows fed all diets compared with milk indicated that fat supplements increased milk *cis*-9, *trans*-11 CLA concentration mainly by increasing ruminal production of TVA, which also illustrates the significant role the mammary delta-9 desaturase plays in milk CLA production (Piperova et al., 2002).

Another CLA isomer of interest is *trans*-10, *cis*-12. Recent studies (Baumgard et al., 2001; Peterson et al., 2002) have established a relation between milk

fat depression and the increase *trans*-10, *cis*-12 CLA content in milk fat. Milk fat concentration was reduced by 24, 37, and 46% when cows received 3.5, 7.0, and 14.0 g/d of *trans*-10, *cis*-12 CLA (Baumgard et al., 2001). Baumgard et al. (2002) reported that *trans*-10, *cis*-12 CLA inhibits milk fat synthesis by decreasing the mRNA expression of acetyl CoA carboxylase, fatty acid synthetase, lipoprotein lipase, and other enzymes involved in uptake and transport, de novo synthesis, and triglyceride formation of circulating fatty acids.

Ruminal concentration of *trans*-10, *cis*-12 CLA tended to be greater ($P < 0.11$) with the HO and the HLO diets compared with the HS and HLN diets (Table 4) and corresponds with the decrease in milk fat with the HO and the HLO diets (Table 3). It is not clear why *trans*-10, *cis*-12 CLA is high in the HO diet, but as indicated before, *cis*-9 C18:1 or its derivatives might have interfered with the biohydrogenation of C18:2n6 in the diet, resulting in the formation of *trans*-10, *cis*-12 CLA. Evidence indicates that under some dietary conditions, such as a high grain diet, an increased activity of bacterial *cis*-9, *trans*-10 isomerase becomes the dominant first step in biohydrogenation, resulting in formation of *trans*-10, *cis*-12 CLA from C18:2n6 (Bauman et al., 2000).

There was a substantial decrease in the concentrations of EPA and DHA in the ruminal digesta fatty acids compared with dietary fatty acids (Tables 2 and 4). This was probably caused by biohydrogenation of EPA and DHA. Wachira et al. (2000) reported biohydrogenation values between 72 and 79% for EPA and DHA in diets that contained FO or linseed and FO. The lower proportion of DHA in rumen digesta across diets compared with EPA might indicate that DHA disappeared at a greater rate than EPA.

Fatty Acid Composition of Plasma Triglycerides and Nontriglyceride Fraction

Phospholipids and cholesteryl esters are the principal components of blood lipid and together account for about 95% of the total lipids in the plasma of ruminant animals. However, triglycerides and free fatty acids represent <5% and 1% of total plasma lipid, respectively (Christie, 1981). Polyunsaturated fatty acids that escape ruminal biohydrogenation are preferentially esterified to the plasma cholesteryl esters and phospholipid (Christie, 1981). Plasma cholesteryl esters and phospholipids have comparatively slow turnover, while triglyceride and free fatty acids fractions have a rapid turnover and supply fatty acids to other tissues such as the mammary gland and adipose tissue (Christie, 1981). Therefore, the profile of fatty acids of plasma triglycerides represents the profile of fatty acids available to the mammary gland.

The profile of fatty acids in plasma triglycerides and nontriglyceride fractions are shown in Tables 5 and 6. There is a clear difference in the distributions of fatty acids in the plasma triglycerides and nontriglyceride fractions. The proportion of polyunsaturated fatty acids, C18:2n6, C18:3n3, and EPA, were higher in the nontriglyceride fraction. Another important difference between the fatty acid profiles of plasma triglycerides and nontriglyceride fractions is the distri-

bution of *trans* C18:1 and CLA isomers (Tables 5 and 6). Both *trans* C18:1 and CLA isomers were preferentially incorporated into plasma triglycerides, except for *trans*-10, *cis*-12 CLA, which showed greater incorporation into plasma nontriglyceride fractions. This greater incorporation of *trans*-10, *cis*-12 CLA into plasma nontriglyceride fractions may explain the low transfer efficiency (20.9 4.1%) of this isomer into milk fat when Baumgard et al. (2001) infused *trans*-10, *cis*-12 CLA into the abomasum. Loor et al. (2002) studied the effect of feeding extruded soybeans to lactating cows on plasma fatty acid profiles. They observed that TVA was exclusively found in plasma triglycerides. In our study, the free fatty acids were part of the nontriglyceride fraction, which might explain the presence of small amounts of TVA in this fraction. Kiteessa et al. (2001); however, fed tuna oil to sheep and observed that *trans*-C18:1 were increased in plasma triglyceride, free fatty acid, and phospholipid fractions.

The concentrations of *trans* C18:1 in general, and TVA in particular, in plasma triglycerides were increased ($P < 0.10$) when cows were fed the HO, HLO, and the HLN compared with the HS diet, and were greatest with the HLO diet (Table 5). However, there were no differences among treatments in the concentration of *cis*-9, *trans*-11 CLA in plasma triglycerides. This lack of increase in plasma *cis*-9, *trans*-11 CLA was unexpected but might be related to changes in the delta-9 desaturase enzyme activity in the intestinal mucosa. Bickerstaffe and Annison (1969) demonstrated the presence of delta-9 desaturase in the intestinal tissues of ruminants, but enzyme activity was low. Baumgard et al. (2002) reported that the activity of delta-9 desaturase was reduced when *trans*-10, *cis*-12 CLA was infused into the abomasum at a higher level (7 and 14 g/d) but not at a low level (3.5 g/d). Cows fed the HS diet had the least proportion of *trans*-10, *cis*-12 CLA in their ruminal digesta (Table 4).

The addition of high *cis*-9 C18:1 sunflower, C18:2n6 sunflower, and flax seed increased ($P < 0.10$) the proportion of *cis*-9 C18:1, C18:2n6, and C18:3n3 FA in plasma triglycerides, respectively (Table 5). The high proportion of *cis*-9 C18:1, C18:2n6, and C18:3n3 acids in plasma triglycerides indicates that biohydrogenation of these fatty acids did not proceed to completion in the rumen. The proportion of DHA in plasma triglycerides was greater than that of EPA, which explains why DHA concentration is greater than EPA in milk fat (Table 7). When Kiteessa et al. (2001) fed tuna oil to sheep, they observed a greater concentration of DHA in plasma triglycerides compared with EPA; however, EPA was incorporated to a greater de-

Table 5. Fatty acid composition of plasma triglycerides fraction.

Fatty acids ²	Diet ¹				SE	P
	HS	HO	HLO	HLN		
	(g/100 g of fatty acids)					
C12:0	0.21 ^b	0.24 ^{ab}	0.25 ^{ab}	0.28 ^a	0.03	0.12
C13:0	0.07 ^b	0.09 ^{ab}	0.10 ^{ab}	0.11 ^a	0.02	0.11
C14:0	2.63	2.28	2.19	2.38	0.23	0.36
C15:0	1.28	1.17	1.28	1.37	0.10	0.35
C16:0	18.82 ^a	16.45 ^b	17.89 ^{ab}	19.04 ^a	0.97	0.09
C16:1	0.82	0.64	0.63	0.70	0.12	0.38
C17:0	1.27 ^a	1.01 ^b	0.97 ^b	1.09 ^b	0.09	0.06
C18:0	23.45 ^b	27.08 ^{ab}	27.56 ^{ab}	29.59 ^a	2.88	0.11
C18:1 α 6-8	0.53 ^c	1.32 ^a	1.41 ^a	0.80 ^b	0.06	<0.01
C18:1 α 9	0.36 ^d	0.75 ^b	0.84 ^a	0.51 ^c	0.04	<0.01
C18:1 α 10	0.61 ^b	1.45 ^{ab}	2.17 ^a	1.02 ^b	0.56	0.07
C18:1 α 11 (TVA)	2.75 ^c	4.64 ^b	8.87 ^a	5.42 ^b	1.01	0.01
C18:1 α 9	4.65 ^c	7.63 ^a	6.40 ^b	6.55 ^b	0.38	<0.01
C18:1 α 11	0.63 ^b	0.55 ^b	1.16 ^a	0.72 ^b	0.11	<0.01
C18:2 α 9 α 12	0.24	0.22	0.23	0.25	0.04	0.95
C18:2 α 9 α 12	2.08 ^b	2.13 ^b	2.63 ^a	2.14 ^b	0.22	0.10
C20:0	0.70	0.60	0.58	0.72	0.08	0.34
C18:3 α 3	0.58 ^b	0.60 ^b	0.56 ^b	1.06 ^a	0.13	0.02
C18:2 α 9 α 11	0.06	0.07	0.06	0.07	0.01	0.21
C18:2 α 10 α 12	0.02 ^b	0.05 ^a	0.06 ^a	0.03 ^b	<0.01	0.10
C18:2 α 9 α 11	0.05 ^b	0.12 ^a	0.11 ^a	0.09 ^{ab}	0.02	0.11
C21:0	0.09	0.11	0.08	0.09	0.02	0.63
C20:3	0.06 ^b	0.11 ^a	0.08 ^{ab}	0.09 ^{ab}	0.01	0.10
C22:0	0.30	0.36	0.34	0.32	0.03	0.48
C22:1	0.18 ^a	0.15 ^{ab}	0.12 ^b	0.16 ^{ab}	0.03	0.12
C24:0	0.35	0.43	0.35	0.25	0.09	0.41
C23:0	0.08	0.11	0.07	0.12	0.03	0.68
C20:5 (EPA) ³	0.30 ^a	0.21 ^b	0.19 ^b	0.23 ^{ab}	0.04	0.09
C22:5	0.34	0.35	0.26	0.28	0.05	0.24
C22:6 (DHA) ⁴	0.65	0.62	0.47	0.47	0.10	0.31
Others ⁵	34.82 ^a	27.84 ^b	22.96 ^b	24.17 ^b	3.37	0.05
Short ⁶	0.28 ^b	0.33 ^{ab}	0.35 ^{ab}	0.38 ^a	0.04	0.09
Medium ⁷	24.82 ^a	21.54 ^b	22.93 ^{ab}	24.58 ^a	1.33	0.10
Long ⁸	40.37 ^b	50.62 ^a	54.11 ^a	51.24 ^a	2.58	<0.01
Saturated	49.23	49.91	51.63	55.36	3.57	0.39
Unsaturated	16.23 ^c	22.57 ^b	25.76 ^a	20.85 ^b	1.61	<0.01
TVA: <i>c</i> 9 α 11CLA	48.68 ^c	68.58 ^{bc}	137.74 ^a	81.54 ^b	13.65	0.02

^{a,b}Means in the same rows with unlike superscripts differ ($P < 0.10$).

¹HS = 1% fish oil + 2% fat source high in stearic acid; HO = 1% fish oil + 2% fat from high oleic acid sunflower seeds; HLO = 1% fish oil + 2% fat from high linoleic acid sunflower seeds; and HLN = 1% fish oil + 2% fat from flax seeds (high linolenic).

²Expressed as number of carbons:double bonds.

³EPA = eicosapentaenoic acid.

⁴DHA = docosahexaenoic acid.

⁵Unidentifiable peaks.

⁶Short-chain FA: (C12 to C13:0).

⁷Medium-chain FA: (C14:0 to C17:0).

⁸Long-chain FA: ($\geq$ C18:0).

gree into plasma cholesteryl esters compared with DHA.

High percentages of unidentified peaks in rumen and plasma samples (Tables 4, 5, and 6) were seen in this study. The temp and run program used for fatty acid analysis on our gas chromatograph lead to an extensive amount of fatty acid isomers separation (100 to 130 fatty acid isomers), where most of them were

unknown to us since we did not have standards for them.

Fatty Acid Composition of Milk

Another objective of this study was to determine the effects of *cis*-9 C18:1, C18:2n6, and C18:3n3 acids on *cis*-9, *trans*-11 CLA and TVA concentration in milk

Table 6. Fatty acid composition of plasma non-triglycerides fractions.

Fatty acid ²	Diet ¹				SE	P
	HS	HO	HLO	HLN		
	(g/100 g of fatty acids)					
C14:0	0.65 ^b	0.73 ^a	0.59 ^b	0.64 ^b	0.04	0.09
C15:0	0.41 ^{ab}	0.43 ^a	0.38 ^b	0.39 ^b	0.02	0.11
C16:0	2.48 ^{ab}	2.64 ^a	2.32 ^b	2.36 ^b	0.14	0.12
C16:1	1.21	1.09	1.01	1.38	0.25	0.75
C17:0	0.06 ^a	0.05 ^a	0.05 ^a	0.02 ^b	0.01	0.12
C18:0	0.26 ^b	0.49 ^a	0.26 ^b	0.27 ^b	0.09	0.02
C18:1 τ 9	0.04 ^b	0.06 ^a	0.03 ^b	0.03 ^b	<0.01	0.03
C18:1 τ 11 (TVA)	0.02 ^c	0.06 ^a	0.05 ^{ab}	0.04 ^b	<0.01	0.03
C18:1 c 9	1.24 ^b	1.92 ^a	1.12 ^b	1.18 ^b	0.06	<0.01
C18:1 c 11	0.31 ^c	0.33 ^{bc}	0.41 ^a	0.35 ^b	0.02	<0.01
C18:2 c 9 c 12	46.91 ^a	48.12 ^a	49.39 ^a	41.60 ^b	2.06	0.04
C20:0	0.30 ^a	0.26 ^b	0.21 ^c	0.22 ^c	0.02	0.01
C18:3 n 3	6.34 ^b	6.69 ^b	4.70 ^c	10.97 ^a	0.33	<0.01
C18:2 τ 10 c 12 (CLA)	0.11 ^c	0.53 ^b	1.04 ^a	0.36 ^b	0.12	0.09
C22:0	0.12	0.14	0.10	0.10	0.02	0.36
C22:1	0.48	0.76	0.67	0.64	0.15	0.38
C20:5 (EPA) ³	2.21	2.30	1.99	2.16	0.21	0.53
C22:6 (DHA) ⁴	0.13	0.15	0.12	0.12	0.02	0.24
Others ⁵	36.71	33.27	35.57	37.15	2.31	0.41
Medium ⁶	4.80	4.95	4.34	4.79	0.30	0.31
Long ⁷	58.48	61.78	60.09	58.05	2.36	0.13
Saturated	4.27 ^{ab}	4.74 ^a	3.91 ^b	3.99 ^b	0.28	0.08
Unsaturated	59.02	61.99	60.52	58.85	2.09	0.46

^{a,b,c}Means in the same rows with unlike superscripts differ ($P < 0.10$).

¹HS = 1% fish oil + 2% fat source high in stearic acid; HO = 1% fish oil + 2% fat from high oleic acid sunflower seeds; HLO = 1% fish oil + 2% fat from high linoleic acid sunflower seeds; and HLN = 1% fish oil + 2% fat from flax seeds (high linolenic).

²Expressed as number of carbons:double bonds.

³EPA = eicosapentaenoic acid.

⁴DHA = docosahexaenoic acid.

⁵Unidentifiable peaks.

⁶Medium-chain FA: (C14:0 to C17:0).

⁷Long-chain FA: ($\geq$ C18:0).

fat. We are not aware of previous investigations of milk fat CLA with diets containing FO and high *cis*-9 C18:1 or C18:3 n 3 sources. However, others (Dhiman et al., 2000; Chouinard et al., 2001; Lock and Garnsworthy, 2002) have added plant oil alone or Ca salts of plant oil high in *cis*-9 C18:1 and C18:3 n 3 and observed an increase in milk *cis*-9, *trans*-11 CLA concentrations. In this study, the concentrations of *cis*-9, *trans*-11 CLA were increased ($P < 0.10$) when cows were fed the HO, HLO, and the HLN compared with the HS, and were greatest with the HLO (Table 7). Because of the drop in milk fat content, *cis*-9, *trans*-11 CLA yields were increased ($P < 0.10$) only with the HLO diet. Milk fat concentrations of *cis*-9, *trans*-11 CLA did not differ ($P > 0.10$) between cows fed the HO and the HLN. The fact that our HLN did not increase *cis*-9, *trans*-11 CLA concentrations over the HO might have been due to the low availability of flax seed oil for ruminal biohydrogenation (Table 4). High milk *trans*-10, *cis*-12 CLA concentrations for the HO and HLO compared with the HLN and HS explain the

lower milk fat content with these diets. Recent studies (Baumgard et al., 2001; Peterson et al., 2002) have established a relation between milk fat depression and the increase *trans*-10, *cis*-12 CLA content in milk fat.

Unfortunately, the presence of *trans*-9, *trans*-11 CLA at this level in milk fat indicates that some isomerization of *cis*-9, *trans*-11 CLA occurred during butylation. Consequently, the reported isomer distributions should be interpreted with caution. Acid catalyzed methylation has been shown to cause isomerization of *cis*-, *trans*-CLA to their corresponding *trans*-, *trans*-CLA isomers and/or convert them to allyl methoxy derivatives (Kramer et al., 1997).

As with *cis*-9, *trans*-11 CLA, the concentration of TVA in milk fat increased ($P < 0.10$) when cows were fed the HO, HLO, and the HLN compared with the HS and were greatest with the HLO (Table 7). However, TVA yields were similar for the HS and the HO diets (Table 8). Additionally, milk fat concentrations and yields of TVA did not differ ($P > 0.10$) between cows fed the HO and the HLN diets. Feeding FO (Whit-

Table 7. Fatty acid composition of milk fat.

Fatty acid ²	Diet ¹				SE	P
	HS	HO	HLO	HLN		
	(g/100 g of fatty acids)					
C4:0	3.56 ^a	3.27 ^{ab}	2.98 ^b	3.36 ^a	0.18	0.08
C6:0	2.03 ^a	1.72 ^{bc}	1.58 ^c	1.89 ^{ab}	0.11	0.03
C8:0	1.19 ^a	0.96 ^b	0.88 ^b	1.10 ^a	0.07	0.02
C10:0	2.69 ^a	2.14 ^{bc}	1.92 ^c	2.40 ^{ab}	0.15	0.01
C11:0	0.05 ^a	0.04 ^b	0.04 ^b	0.04 ^b	<0.01	0.09
C12:0	3.11 ^a	2.55 ^c	2.34 ^c	2.80 ^b	0.11	<0.01
C13:0	0.09 ^a	0.07 ^b	0.08 ^{ab}	0.08 ^{ab}	<0.01	0.10
C14:0	11.26 ^a	10.31 ^b	9.42 ^c	10.19 ^b	0.20	<0.01
C14:1	1.05	1.01	1.15	1.07	0.20	0.91
C15:0	1.03 ^a	0.96 ^b	0.94 ^b	0.94 ^b	0.02	<0.01
C16:0	27.33 ^a	23.78 ^b	23.16 ^b	24.00 ^b	0.47	<0.01
C16:1	1.48	1.42	1.84	1.64	0.37	0.69
C17:0	0.67 ^a	0.54 ^b	0.50 ^c	0.53 ^b	0.01	<0.01
C18:0	9.05	10.07	8.54	9.13	1.09	0.59
C18:1 $\neq$ 6-8	0.38 ^c	0.83 ^a	0.80 ^a	0.50 ^b	0.06	<0.01
C18:1 $\neq$ 9	0.40 ^b	0.70 ^a	0.75 ^a	0.50 ^b	0.06	<0.01
C18:1 $\neq$ 10	0.59 ^b	1.44 ^{ab}	2.17 ^a	0.84 ^b	0.57	0.09
C18:1 $\neq$ 11 (TVA)	1.85 ^b	2.60 ^b	4.14 ^a	2.60 ^b	0.33	0.08
C18:1 $\neq$ 9	17.71 ^c	21.45 ^a	20.02 ^b	19.32 ^b	0.65	<0.01
C18:1 $\neq$ 11	0.54 ^{bc}	0.46 ^c	0.90 ^a	0.62 ^b	0.07	<0.01
C18:2 $\neq$ 9 $\neq$ 12	0.05 ^b	0.07 ^b	0.07 ^b	0.10 ^a	0.01	0.03
C18:2 $\neq$ 9 $\neq$ 12	2.24 ^b	2.08 ^b	2.95 ^a	2.24 ^b	0.10	<0.01
C20:0	0.30 ^a	0.24 ^b	0.20 ^b	0.24 ^b	0.03	0.06
C18:3 $\neq$ 3	0.77 ^b	0.74 ^b	0.67 ^b	1.21 ^a	0.06	<0.01
C18:2 $\neq$ 9 $\neq$ 11	0.71 ^c	0.99 ^b	1.71 ^a	1.12 ^b	0.13	0.06
C18:2 $\neq$ 10 $\neq$ 12	0.03 ^c	0.06 ^b	0.11 ^a	0.04 ^c	0.01	0.07
C18:2 $\neq$ 9 $\neq$ 11	0.08	0.09	0.12	0.12	0.03	0.41
C21:0	0.09	0.08	0.07	0.07	0.02	0.38
C20:2	0.09 ^a	0.06 ^b	0.08 ^{ab}	0.07 ^b	0.01	0.11
C20:3	0.06 ^a	0.05 ^b	0.06 ^{ab}	0.06 ^{ab}	<0.01	0.12
C22:0	0.12 ^b	0.13 ^a	0.13 ^a	0.11 ^b	<0.01	0.01
C22:1	0.10 ^a	0.07 ^b	0.09 ^{ab}	0.09 ^{ab}	0.01	0.13
C20:4	0.25	0.22	0.21	0.22	0.02	0.34
C23:0	0.12	0.11	0.11	0.12	0.01	0.82
C20:5 (EPA) ³	0.13 ^a	0.09 ^b	0.10 ^b	0.12 ^a	0.01	<0.01
C22:5	0.14	0.14	0.14	0.12	0.01	0.70
C22:6 (DHA) ⁴	0.17	0.14	0.13	0.14	0.02	0.29
Others ⁵	8.25 ^b	8.06 ^b	8.71 ^b	10.01 ^a	0.54	0.04
Short ⁶	12.72 ^a	10.75 ^{bc}	9.81 ^c	1.68 ^{ab}	0.62	0.02
Medium ⁷	42.81 ^a	38.02 ^b	37.01 ^b	38.35 ^b	0.85	<0.01
Long ⁸	36.22 ^c	43.18 ^a	44.47 ^a	39.95 ^b	0.69	<0.01
Saturated	62.67 ^a	56.96 ^b	52.88 ^c	56.99 ^b	1.46	<0.01
Unsaturated	29.08 ^c	34.98 ^b	38.41 ^a	32.99 ^b	1.09	<0.01
TVA: $\neq$ 9 $\neq$ 11CLA	2.55	2.69	2.35	2.40	0.29	0.66

^{a,b,c}Means in the same rows with unlike superscripts differ ($P < 0.10$).

¹HS = 1% fish oil + 2% fat source high in stearic acid; HO = 1% fish oil + 2% fat from high oleic acid sunflower seeds; HLO = 1% fish oil + 2% fat from high linoleic acid sunflower seeds; and HLN = 1% fish oil + 2% fat from flax seeds (high linolenic).

²Expressed as number of carbons:double bonds.

³EPA = eicosapentaenoic acid.

⁴DHA = docosahexaenoic acid.

⁵Unidentifiable peaks.

⁶Short-chain FA: (C4:0 to C13:0).

⁷Medium-chain FA: (C14:0 to C17:1).

⁸Long-chain FA: ($\geq$ C18:0).

lock et al., 2002) or FO as fish meal with extruded soybeans (AbuGhazaleh et al., 2002a) increased milk TVA concentrations by three- to five-fold. When Chouinard et al. (2001) fed dietary supplements of Ca

salts of fatty acids from canola oil, soybean oil, and linseed oil, milk TVA concentrations increased and were greatest for cows fed Ca salts of soybean oil, intermediate for cows fed Ca salts of linseed oil, and

least for cows fed Ca salts of canola oil. The lower availability of flax seed oil for ruminal biohydrogenation may have been the reason for lack of differences in TVA concentration in milk from cows fed the HO and the HLN. Further discussion about the effect of these treatments on milk fatty acid profiles by cows in a companion study are reported elsewhere (AbuGhazaleh et al., 2003).

Milk concentration of *trans*-10 C18:1 was highest when cows were fed the HLO (Table 7). Previously, this isomer was misidentified as *cis*-6 C18:1 by our laboratory (AbuGhazaleh et al. 2002a, AbuGhazaleh et al., 2002b; Whitlock et al., 2002). A few milk samples from this experiment were sent to Bauman's laboratory, where it was confirmed that the peak previously identified by our laboratory as *cis*-6 C18:1 was *trans*-10 C18:1. As a result, values previously reported as *cis*-6 C18:1 by our group should be amended and considered values for *trans*-10 C18:1.

CONCLUSIONS

Conjugated linoleic acid concentration in milk fat can be enhanced by the addition of FO along with mono- and polyunsaturated fatty acids to the diet, especially oils high in C18:2n6. Feeding lactating dairy cows a blend of FO and a high C18:2n6 source (e.g., regular sunflower seeds) resulted in the greatest increases in the concentrations and yields of milk *cis*-9, *trans*-11 CLA, and TVA. The increase in milk *cis*-9, *trans*-11, CLA resulted mainly from high ruminal production of TVA that was exclusively incorporated into plasma triglycerides. This study further supports the significant role for mammary delta-9 desaturase in milk *cis*-9, *trans*-11 CLA production.

ACKNOWLEDGMENTS

We thank the employees of the South Dakota State University Dairy Research Facility for care of the cows and assistance in obtaining research data and Valley Queen Cheese Factory, Milbank, SD, for milk analysis. We also thank Tom Young, Onida, SD, for donating the high oleic sunflower seeds. This research was partially supported by a grant from the South Dakota Oilseeds Council.

REFERENCES

- AbuGhazaleh, A. A., D. J. Schingoethe, A. R. Hippen, and L. A. Whitlock. 2002a. Feeding fish meal and extruded soybeans enhances the conjugated linoleic acid (CLA) content of milk. *J. Dairy Sci.* 85:624–631.
- AbuGhazaleh, A. A., D. J. Schingoethe, A. R. Hippen, K. F. Kalscheur, and L. A. Whitlock. 2002b. Fatty acid profiles of milk and rumen digesta from cows fed fish oil, extruded soybeans or their blend. *J. Dairy Sci.* 85:2266–2276.
- AbuGhazaleh, A. A., D. J. Schingoethe, A. R. Hippen, and K. F. Kalscheur. 2003. Milk conjugated linoleic acid response to fish oil supplementation of diets differing in fatty acid profiles. *J. Dairy Sci.* 86:944–953.
- Association of Official Analytical Chemists. 1997. Official Methods of Analysis. 16th ed. AOAC Intl., Gaithersburg, MD.
- Bauman, D. E., L. H. Baumgard, B. A. Corl, and J. M. Griinari. 2000. Biosynthesis of conjugated linoleic acid in ruminants. *Proc. Am. Soc. Anim. Soc.*, 1999. Available at: <http://www.asas.org/jas/symposia/proceedings/0937.pdf>. Accessed July 2001.
- Baumgard, L. H., E. Matitashvili, B. A. Corl, D. A. Dwyer, and D. E. Bauman. 2002. *trans*-10, *cis*-12 conjugated linoleic acid decrease lipogenic rates and expression of genes involved in milk lipid synthesis in dairy cows. *J. Dairy Sci.* 85:2155–2163.
- Baumgard, L. H., J. K. Sangster, and D. E. Bauman. 2001. Milk fat synthesis in dairy cows is progressively reduced by increasing supplemental amounts of *trans*-10, *cis*-12 conjugated linoleic acid (CLA). *J. Nutr.* 131:1764–1769.
- Beam, T. M., T. C. Jenkins, P. J. Moate, R. A. Kohn, and D. L. Palmquist. 2000. Effects of amount and source of fat on the rates of lipolysis and biohydrogenation of fatty acids in ruminal contents. *J. Dairy Sci.* 83:2564–2573.
- Bickerstaffe, R., and E. F. Annison. 1969. Glycerokinase and desaturase activity in pig, chicken, and sheep intestinal epithelium. *Comp. Biochem. Physiol.* 31:47–54.
- Chouinard, P. Y., L. Corneau, W. R. Butler, Y. Chilliard, J. K. Drackley, and D. E. Bauman. 2001. Effect of dietary lipid source on conjugated linoleic acid concentrations in milk fat. *J. Dairy Sci.* 84:680–690.
- Christie, W. W. 1981. Lipid Metabolism in Ruminant Animals. Pergamon Press, New York, NY.
- Christie, W. W. 1982. Pages 30–38 in Lipid Analysis. 2nd ed. Pergamon Press, New York, NY.
- Corl, B. A., L. H. Baumgard, D. A. Dwyer, J. M. Griinari, B. S. Phillips, and D. E. Bauman. 2001. The role of Δ^9 -desaturase in the production of *cis*-9, *trans*-11 CLA. *J. Nutr. Biochem.* 12:622–630.
- Dhiman, T. R., L. D. Satter, M. W. Pariza, M. P. Galli, K. Albright, and M. X. Tolosa. 2000. Conjugated linoleic acid (CLA) content of milk from cows offered diets rich in linoleic and linolenic acid. *J. Dairy Sci.* 83:1016–1027.
- Griinari, J. M., P. Y. Chouinard, and D. E. Bauman. 1997. Pages 208–216 in Trans Fatty Acid Hypothesis of Milk Fat Depression Revised. Proceedings Cornell Nutrition Conference for Feed Manufacturers. Cornell University, Ithaca, NY.
- Griinari, J. M., B. A. Corl, S. H. Lacy, P. Y. Chouinard, K. V. V. Nurmela, and D. E. Bauman. 2000. Conjugated linoleic acid is synthesized endogenously in lactating dairy cows by Δ^9 desaturase. *J. Nutr.* 130:2285–2291.
- Harfoot, C. G., and G. P. Hazlewood. 1988. Lipid metabolism in the rumen. Pages 285–322 in The Rumen Microbial Ecosystem. P. N. Hobson, ed. Elsevier Applied Science Publishers, London, UK.
- Harfoot, C. G., R. C. Noble, and J. H. Moore. 1973. Factors influencing the extent of biohydrogenation of linoleic acid by rumen micro-organisms in vitro. *J. Sci. Food Agric.* 24:961–970.
- Ip, C., S. Banni, E. Angioni, G. Carta, J. McGruley, H. J. Thompson, D. Barbano, and D. Bauman. 1999. Conjugated linoleic acid enriched butter fat alters morphogenesis and reduces cancer risk in rats. *J. Nutr.* 129:2135–2142.
- Jenkins, T. C. 1993. Lipid metabolism in the rumen. *J. Dairy Sci.* 76:3851–3863.
- Jenkins, T. C., and C. S. Adams. 2002. The biohydrogenation of linoleamide in vitro and its effects on linoleic acid concentration in duodenal contents and sheep. *J. Anim. Sci.* 80:533–540.
- Jiang, J., L. Bjoerck, R. Fonden, and M. Emanuelson. 1996. Occurrence of conjugated *cis*-9, *trans*-11-octadecadienoic acid in bovine milk: effects on feed and dietary regimen. *J. Dairy Sci.* 79:438–445.
- Kalscheur, K. F., B. B. Teter, L. S. Piperova, and R. A. Erdman. 1997. Effect of fat source on duodenal flow of *trans*-C18:1 fatty

- acids and milk fat production in dairy cows. *J. Dairy Sci.* 80:2115–2126.
- Kitessa, S. M., S. K. Gulati, J. R. Ashes, E. Fleck, T. W. Scott, and P. D. Nichols. 2001. Utilization of fish oil in ruminants I. Fish oil metabolism in sheep. *Anim. Feed Sci. Technol.* 89:189–199.
- Kramer, J. K. C., V. Fellner, M. E. R. Dugan, F. D. Sauer, M. M. Mossoba, and M. P. Yurawecz. 1997. Evaluating acid and base catalysts in the methylation of milk and rumen fatty acids with special emphasis on conjugated dienes and total trans fatty acids. *Lipids* 32:1219–1228.
- Lock, A. L., and P. C. Garnsworthy. 2002. Independent effects of dietary linoleic and linolenic fatty acids on the conjugated linoleic acid content of cows' milk. *Anim. Sci.* 74:163–176.
- Loor, J. J., J. H. Herbein, and C. E. Polan. 2002. Trans18:1 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.
- Lowry, J. B., L. L. Conlan, A. C. Schlink, and C. S. McSweeney. 1994. Acid detergent dispersible lignin in tropical grasses. *J. Sci. Food Agric.* 65:41–50.
- McGuire, M. A., and M. K. McGuire. 2000. Conjugated linoleic acid (CLA): A ruminant fatty acid with beneficial effects on human health. *Proceedings of the American Society of Animal Sci.* 1999. Available at <http://www.asas.org/jas/symposia/proceedings>.
- Mosley, E. E., G. L. Powell, M. B. Riley, and T. C. Jenkins. 2002. Microbial biohydrogenation of oleic acid to trans isomers in vitro. *J. Lipid Res.* 43:290–296.
- National Research Council. 2001. *Nutrient Requirements of Dairy Cattle*. 7th rev. ed. Natl. Acad. Sci., Washington, DC.
- Orth, R. 1992. Sample day and lactation report, DHIA 200. Fact Sheet A-2, Mid-States DRPC, Ames, IA.
- Peterson, D. G., L. H. Baumgard, and D. E. Bauman. 2002. Dose-dependent reduction in milk fat secretion with abomasal infusion of *trans*-10, *cis*-12 conjugated linoleic acid (CLA) and comparison to diet-induced milk fat depression. *J. Dairy Sci.* 85 (Suppl. 1): 703. (Abstr.)
- Piperova, L. S., J. Sampugna, B. B. Teter, K. F. Kalscheur, M. P. Yurawecz, Y. Ku, K. M. Morehouse, and R. A. Erdman. 2002. Duodenal and milk trans octadecenoic acid and conjugated linoleic acid (CLA) isomers indicate that postabsorptive synthesis is the predominant source of *cis*-9-containing CLA in lactating dairy cows. *J. Nutr.* 132:1235–1241.
- Robertson, J. B., and P. J. Van Soest. 1981. The detergent system of analysis and its application to human foods. Pages 123–129 in *The Analysis of Dietary Fiber*. W. P. T. James and O. Theander, ed. Marcel Dekker, New York, NY.
- SAS/STAT. 1996. *Software: Changes and Enhancements Through Release 6.11*. SAS Inst., Inc., Cary, NC.
- 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.* 74:3583–3597.
- Wachira, A. M., L. A. Sinclair, R. G. Wilkinson, K. Hallett, M. Enser, and J. D. Wood. 2000. Rumen biohydrogenation of n-3 polyunsaturated fatty acids and their effects on microbial efficiency and nutrient digestibility in sheep. *J. Agric. Sci.* 135:419–428.
- Whitlock, L., D. J. Schingoethe, A. R. Hippen, K. F. Kalscheur, R. J. Baer, N. Ramaswamy, and K. M. Kasperon. 2002. Fish oil and extruded soybeans fed in combination increase CLA in milk of dairy cows more than when fed separately. *J. Dairy Sci.* 85:234–243.
- Wilde, P. F., and R. M. C. Dawson. 1966. The biohydrogenation of alpha-linolenic acid and oleic acid by rumen microorganisms. *Biochem. J.* 98:469–475.
- Wildman, E. E., G. M. Jones, P. E. Wagner, R. L. Bowman, H. F. Troutt, and T. N. Lesch. 1982. Dairy cow body condition scoring and its relationship to selected production characteristics. *J. Dairy Sci.* 65:495–501.
- Wu, Z., O. A. Ohajuruka, and D. L. Palmquist. 1991. Ruminal synthesis, biohydrogenation and digestibility of fatty acids by dairy cows. *J. Dairy Sci.* 74:3025–3034.