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Effectiveness of Oils Rich in Linoleic and Linolenic Acids to Enhance Conjugated Linoleic Acid in Milk from Dairy Cows

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ABSTRACT

Forty Holstein dairy cows were used to determine the effectiveness of linoleic or linolenic-rich oils to enhance $C_{18:2}$ *cis*-9, *trans*-11 conjugated linoleic acid (CLA) and $C_{18:1}$ *trans*-11 (vaccenic acid; VA) in milk. The experimental design was a complete randomized design for 9 wk with measurements made during the last 6 wk. Cows were fed a basal diet containing 59% forage (control) or a basal diet supplemented with either 4% soybean oil (SO), 4% flaxseed oil (FO), or 2% soybean oil plus 2% flaxseed oil (SFO) on a dry matter basis. Total fatty acids in the diet were 3.27, 7.47, 7.61, and 7.50 g/100 g in control, SO, FO, and SFO diets, respectively. Feed intake, energy-corrected milk (ECM) yield, and ECM produced/kg of feed intake were similar among treatments. The proportions of VA were increased by 318, 105, and 206% in milk fat from cows in the SO, FO, and SFO groups compared with cows in the control group. Similar increases in $C_{18:2}$ *cis*-9, *trans*-11 CLA were 273, 150, and 183% in SO, FO, and SFO treatments, respectively. Under similar feeding conditions, oils rich in linoleic acid (soybean oil) were more effective in enhancing VA and $C_{18:2}$ *cis*-9, *trans*-11 CLA in milk fat than oils containing linolenic acid (flaxseed oil) in dairy cows fed high-forage diets (59% forage). The effects of mixing linoleic and linolenic acids (50:50) on enhancing VA and $C_{18:2}$ *cis*-9, *trans*-11 CLA were additive, but not greater than when fed separately. Increasing the proportion of healthy fatty acids (VA and CLA) by feeding soybean or flaxseed oil would result in milk with higher nutritive and therapeutic value.
Key words: milk, linoleic acid, linolenic acid, conjugated linoleic acid

INTRODUCTION

Manipulating the diet of dairy cow is one way to alter the fatty acid (**FA**) profile of milk fat. Health-conscious consumers are demanding milk with higher proportions of healthy FA. Conjugated linoleic acid (**CLA**) has been shown to have potential health benefits, including anticarcinogenic properties in experimental animals (Hughes and Dhiman, 2002). The primary isomers of CLA that have been associated with health benefits are the $C_{18:2}$ *cis*-9, *trans*-11 (***c*-9, *t*-11**) and $C_{18:2}$ *trans*-10, *cis*-12 (***t*-10, *c*-12**). The principal dietary sources of CLA for humans are dairy and meat products from ruminant animals. Vaccenic acid ($C_{18:1}$ *trans*-11; **VA**) also has potential health benefits because VA can be converted to $C_{18:2}$ *c*-9, *t*-11 in human (Turpeinen et al., 2002) and animal (Corl et al., 2001) body tissues through the enzyme Δ^9-desaturase. Milk that is high in $C_{18:2}$ *c*-9,*t*-11 CLA is generally higher in VA (Griinari et al., 2000).

Conjugated linoleic acid in milk originates from ruminal biohydrogenation of linoleic acid ($C_{18:2}$) as an intermediate product (Harfoot and Hazlewood, 1997) or from endogenous synthesis in mammary gland from VA, another intermediate of ruminal biohydrogenation, via Δ^9-desaturase (Griinari et al., 2000).

The CLA content of milk and meat products from ruminants can be increased through manipulation of the diet, such as grazing on pasture or feeding feed sources rich in linoleic and linolenic acids (Kelly et al., 1998; Dhiman et al., 1999, 2000; Chouinard et al., 2001), or feeding fish oil (Abu-Ghazaleh et al., 2001, 2003; Ramaswamy et al., 2001). In 2 separate experiments, feeding soybean oil at 4.0% or linseed oil at 4.4% of diet DM decreased milk fat content by 15 and 28%, respectively, and increased the proportion of $C_{18:2}$ *c*-9,*t*-11 CLA in milk fat by 4.2-fold in both experiments (Dhiman et al., 2000). The $C_{18:2}$ *c*-9, *t*-11 CLA yields were 18 and 13 g/d in soybean and linseed oil treatments, respectively, compared with control (4.5 g/d). These data suggest that oil supplements rich in linoleic or linolenic acid influence milk fat content and the amount of CLA in milk differently. The above studies feeding linoleic and linolenic acid were conducted in 2 separate experiments. A further study was conducted to quantify the effectiveness of linoleic- or linolenic-rich oil supplements alone or in combination under similar feeding conditions (same experiment) at 4% of diet DM in dairy cow fed high forage (59% forage) diets on feed

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intake, milk components, FA profile of milk, and blood parameters.

MATERIALS AND METHODS

Experimental Design and Treatments

Forty Holstein dairy cows were divided into 4 groups according to a random number table (Steel et al., 1997). Cows within groups were randomly assigned to 1 of 4 treatments. At the start of the experiment cows were 169.8 ± 8 DIM and produced 24.8 ± 5.9 kg of milk/d. Total duration of the experiment was 9 wk. The first 3 wk of the experiment was considered the standardization period; measurements were made during the last 6 wk. During the entire 9 wk experiment, cows in 3 treatments were fed a basal diet (control) or the basal diet supplemented with 4% soybean (*Glycine max*) oil (**SO**), 4% flaxseed (*Linum usitatissimum*) oil (**FO**), or 2% soybean oil plus 2% flaxseed oil (**SFO**) on a DM basis. Ingredient and chemical composition of diets is in Table 1. Oils were added by replacing mainly corn in the diet.

Diets were formulated to meet the nutrient requirements of cows producing 32 kg of 3.5% FCM/d according to the Feeding Standards of Dairy Cattle, China NongYe HangYe Boaozhun/Tuijian-34 (China, NY/t34, 2004). Cows were housed in a tie-stall barn and fed individually. Diets were mixed once a day and fed as a TMR 3 times a day. At the start of the experiment, cows were gradually adjusted to the experimental diets over a 7-d period. The oils were stored in a cold room at 4°C and were added fresh to the diet each morning to avoid oxidation. The oils were added as the final ingredient after mixing the other dietary ingredients. The TMR containing oil supplements was stored under shade in a cool place until fed at a later time. Orts were restricted to 5 to 10% of intake on an as-fed basis. Animal care and procedures were approved and conducted under established standards of the Institute of Animal Science, Chinese Academy of Agricultural Sciences, Beijing, China.

Sampling and Analysis

Amount of daily feed offered and refused was recorded for individual cows. Samples of TMR were collected daily and frozen at –20°C. Orts were sampled twice a week from each cow, composited for each treatment, and frozen at –20°C for further analysis. Weekly representative samples of TMR and orts from each treatment were analyzed for DM content. The DM content of the feed ingredients was determined by oven-drying at 60°C for 48 h. Dietary formulations were adjusted weekly during the experiment, if necessary, to account for small changes in ingredient DM content.

Dried feed samples from each week during the experimental period were ground through a Cyclotec 1093 Mill (Tecator 1093, Hoganas, Sweden) and analyzed for composition. The CP content of feed samples was determined using the macro-Kjeldahl nitrogen test (AOAC, 2000; method 954.01) with a Kjeltec digester 20 and Kjeltec System 1026 distilling unit (Tecator AB, Hoganas, Sweden). The NDF and ADF contents were determined using the basic procedure of Van Soest et al. (1991). Sodium sulfite was not used in the procedure for NDF determination, but pretreatment with heat-stable amylase (type XI-A from *Bacillus subtilis*; Sigma-Aldrich Corporation, St. Louis, MO) was included. Weekly samples of TMR were analyzed for total FA content and FA profile (Sukhija and Palmquist, 1988). During analysis, the samples were further dried at 105°C for 8 h to determine the absolute DM, and chemical analyses were exressed on the basis of this final absolute DM.

Daily DMI for individual cows was calculated by subtracting the weekly mean of orts from the weekly mean of feed offered. The NE_L content of the diet was calculated by using the NE_L table values (China NY/t34, 2004) for the individual dietary ingredients (Table 1). The NE_L values (Mcal/kg of DM) used for alfalfa hay, Chinese wildrye, corn silage, corn, wheat bran, soybean meal, cottonseed meal, rapeseed meal, corn meal, corn germ meal, soybean oil, and flaxseed oil were 1.19, 1.12, 1.45, 1.91, 1.61, 2.38, 1.71, 1.86, 1.80, 1.81, 5.65, and 5.65, respectively (China NY/t34, 2004).

Weekly mean NE_L intakes were calculated by multiplying the NE_L values of the diet by the mean DMI of the individual cows for the respective week. Cows were milked 3 times a day. Milk yield from individual cows was recorded on Tuesday and Friday of each week. Duplicate milk samples (100 mL) from individual cows were collected from the a.m. and p.m. milkings on Friday of each week. One set of milk samples from each milkings with bronopol-B2 preservative was analyzed for fat, protein, lactose, and SNF by near mid infrared procedures using a MilkoScan Minor machine (MilkoScan Type 78110, Foss Electric, Hillerød, Denmark). Final milk composition for each week was expressed on weighted milk yield of a.m. and p.m. samples. Average fat and protein yields were calculated by multiplying the milk yield by the fat and protein content for the respective week on an individual cow basis. A second set of milk samples from individual cows was composited from 2 milkings according to the milk yield and stored at –70°C for further analysis of FA profile. The ECM yield was calculated using the equation ECM = $0.327 \times$ milk (kg) + $12.95 \times$ fat (kg) + $7.20 \times$ protein (kg)

Table 1. Ingredient and chemical composition of experimental diets

Composition	Treatment[1]				SD
	Control	SO	FO	SFO	
Ingredient, g/100 g of DM					
Alfalfa hay	13.7	13.9	13.9	13.9	
Chinese wildrye	22.3	22.6	22.6	22.6	
Corn silage	22.9	22.6	22.6	22.6	
Corn coarse ground	19.7	14.8	14.8	14.8	
Wheat bran	0.2	0.2	0.2	0.2	
Soybean meal	7.5	8.7	8.7	8.7	
Cottonseed meal	3.5	3.3	3.3	3.3	
Rapeseed meal	1.9	1.6	1.6	1.6	
Corn meal	4.7	4.7	4.7	4.7	
Soybean oil	0.0	4.0	0.0	2.0	
Flaxseed oil	0.0	0.0	4.0	2.0	
Ca and P source[2]	2.6	2.6	2.6	2.6	
Sodium chloride	0.5	0.5	0.5	0.5	
Vitamin-mineral mix[3]	0.5	0.5	0.5	0.5	
Chemical, g/100 g of DM					
DM	50.2	50.6	50.6	50.6	0.20
NE_L, Mcal/kg of DM[4]	1.48	1.64	1.64	1.64	0.08
CP	16.05	16.03	16.03	16.03	0.01
NDF	41.82	41.33	41.33	41.33	0.24
ADF	26.07	25.99	25.99	25.99	0.04
Ca	1.04	1.04	1.04	1.04	0.01
P	0.58	0.56	0.56	0.56	0.01

[1]Cows were fed a basal diet (control) or basal diet supplemented with either 4.0% soybean oil (SO), 4.0% flaxseed oil (FO), or 2.0% soybean oil + 2.0% flaxseed oil (SFO).

[2]Contained 50% limestone (32.27% Ca) and 50% calcium phosphate monobasic (18.04% Ca and 18.13% P).

[3]Contained (per kilogram of DM) a minimum 250,000 IU of vitamin A; 65,000 IU of vitamin D; 2,100 IU of vitamin E; Fe 400 mg; Cu 540 mg; Zn 2,100 mg; Mn 560 mg; Se 15 mg; I 35 mg; and Co 68 mg.

[4]The NE_L values (Mcal/kg of DM) used for alfalfa hay, Chinese wildrye, corn silage, corn, wheat bran, soybean meal, cottonseed meal, rapeseed meal, corn meal, corn germ meal, soybean oil, and flaxseed oil were 1.19, 1.12, 1.45, 1.91, 1.61, 2.38, 1.71, 1.86, 1.80, 1.81, 5.65, and 5.65, respectively (China NY/t 34, 2004).

(Tyrrell and Reid, 1965) on an individual cow basis. Gross feed efficiency was calculated as daily ECM yield/kg of feed DMI on an individual cow basis.

For milk FA analysis, frozen milk samples from individual cows were thawed in a refrigerator at 4°C and centrifuged at 17,800 × *g* for 30 min at 8°C to separate fat. Fat cake (1 g) was transferred to a 5-mL tube and centrifuged again at 20,000 × *g* for 20 min at 20°C. Fat (20 mg) was esterified using a method described by Kramer et al. (1997). Separation of FA was achieved by gas chromatography (model 6890 Series II; Hewlett Packard Co., Avondale, PA) fitted with a flame-ionization detector. Samples containing methyl esters in hexane (1 μL) were injected through the split injection port (100:1) onto an SP-2560 fused silica 100 m × 0.25 mm column with a 0.20-μm film (Supelco Inc., Bellefonte, PA). The oven temperature was initially 170°C for 30 min, and then increased to 200°C at 1.5°C/min and held for 20 min. The temperature was then increased again at 5°C/min to 220°C and held for 20 min. Injector and detector temperatures were maintained at 240°C; total run time was 94 min. Heptadecadienoic acid was used as a qualitative internal standard. Each peak was identified using FA and FA methyl esters (Nu-Chek Prep, Elysian, MN; Matreya, Pleasant Gap, PA; and Supelco 37 Component FAME mix, Supelco, Bellefonte, PA). The percentage of each FA was calculated by dividing the area under the FA peak (minus the area under the peak for heptadecanoic acid) by the sum of the areas under the total reported FA peaks. Fatty acids were reported as grams per hundred grams of FA methyl esters. The CLA yield was calculated by multiplying CLA content (*c*-9, *t*-11 CLA) with total fat yield on an individual cow basis.

During wk 9 of the experiment, duplicate blood samples (15 mL) from individual cows were collected from the coccygeal vein or artery at 4 h postfeeding. One set of blood samples was collected in serum separator tubes (Serum Clot Activator, Greiner Bioone GmbH, A-4550 Kremsmunster, Austria). Blood samples were allowed to clot for a minimum of 30 min at room temperature and stored in the refrigerator overnight. The samples were centrifuged at 3,000 × *g* for 15 min at 4°C to separate the serum. A second set of blood samples was

collected in Vacutainer tubes containing anticoagulant (lithium heparin, Greiner bioone GmbH) for separating plasma. Blood samples were centrifuged at 3,000 × *g* for 15 min at 4°C to separate plasma. Blood plasma and serum samples were stored at –70°C until further analysis. Blood serum samples were analyzed for NEFA (Matsubara et al., 1983) and BHBA (McMurray et al., 1984) by an enzymatic method using kits (catalog nos. FA115 and RB1007, respectively; Randox Laboratories Ltd., Antrim, UK). Blood plasma samples were analyzed for glucose (Trinder, 1969), cholesterol (Fossati and Prencipe, 1982), and triglycerides (McNamara and Schaefer, 1987) using kits (Zhongsheng Beikong Bio-Technology and Science Inc., Beijing, China). The intra- and interassay CV for cholesterol assay were 1.2 and 2.7%, respectively. Blood plasma samples were also analyzed for phospholipids using an enzymatic choline oxidase and N-ethyl-N-(2-hydroxy-3-sulfopropyl)-3,5-dimethoxyaniline phospholipids kit (Sino-German Beijing Leadman BioTech Ltd., Beijing, China), low-density lipoprotein cholesterol (Lichtenstein et al., 1993; LDLP-C kit, Shanghai Fortune Long March Medical Science Co. Ltd., Shanghai, China), growth hormone (Reynaert and Franchimont, 1974; hGH kit, Beijing Furui BioTech Ltd., Beijing, China), and insulin (Starr et al., 1979; INS kit, Beijing Furui BioTech Ltd.). The intra- and interassay CV for growth hormone were 9 and 13%, and for the insulin assay were 10 and 15%, respectively.

Vaccenic acid is converted to CLA in the mammary gland (Corl et al., 2001) via the Δ^9-desaturase enzyme. The Δ^9-desaturase index was calculated for selected milk FA using product-to-substrate ratios of FA. The FA ratios used to determine the Δ^9-desaturase index were $C_{14:1}$:$C_{14:0}$, $C_{16:1}$:$C_{16:0}$, $C_{18:1}$ *cis*-9:$C_{18:0}$, and $C_{18:1}$ *trans*-11:$C_{18:2}$ *c*-9, *t*-11 CLA.

Chemical Composition of Diets

The chemical composition of experimental diets is in Table 1. The DM content of experimental diets ranged from 50.2 to 50.6% throughout the experiment. Replacing corn with an energy-dense oil supplement increased the energy content of diets in the SO, FO, and SFO diets compared with the control diet (1.64 vs. 1.48 Mcal/kg of feed DM). An attempt was made to keep diets isonitrogenous by adjusting the proportions of alfalfa hay, soybean, and cottonseed meals among treatments (average 16.4 ± 0.01% CP on DM basis). Fiber (NDF and ADF), Ca, and P contents of the diets met the minimum levels recommended for dairy cows with production levels used in the experiment (China NY/t 34-2004; NRC, 2001).

Table 2. Fatty acid (FA) content and composition of experimental diets

	Treatment[1]			
	Control	SO	FO	SFO
Total FA, % diet DM	3.27	7.47	7.61	7.50
FA,[2] % of FA reported				
$C_{14:0}$	3.13	1.25	1.10	1.18
$C_{16:0}$	23.25	20.72	11.43	16.33
$C_{18:0}$	1.99	3.12	3.34	3.22
$C_{18:1}$ *cis*-9	9.42	21.94	17.28	19.74
$C_{18:2}$	37.27	40.62	20.18	30.97
$C_{18:3}$	24.94	12.35	46.67	28.56

[1]Cows were fed a basal diet (control) or basal diet supplemented with either 4.0% soybean oil (SO), 4.0% flaxseed oil (FO), or 2.0% soybean oil + 2.0% flaxseed oil (SFO).

[2]Expressed as number of carbons: number of double bonds.

Total FA content and FA profile of experimental diets is given in Table 2. Adding oil doubled the FA content of diets in the SO, FO, and SFO treatments compared with the control (7.53 vs. 3.27% of diet DM). Dietary fat in oil treatments approached the maximum recommended level for dairy cows (7 to 8% of diet DM; NRC, 2001). However, as mentioned earlier, our objective was to determine the effectiveness of 2 different oils when included at 4% in the diet. Soybean oil is rich in $C_{18:2}$ and $C_{18:1}$ *cis*-9, whereas flaxseed oil is rich in $C_{18:3}$ FA. Increased proportions of (long-chain) $C_{18:1}$ *cis*-9, $C_{18:2}$, and $C_{18:3}$ in SO, FO, and SFO compared with the control diet reflect the supply of these FA from the soybean and flaxseed oils in the diet.

Statistical Analysis

The data for milk production variables and FA composition were analyzed as a completely randomized design with repeated measures using the MIXED models of SAS (SAS Institute, 1999–2000). Fixed effects included treatment, week, and treatment × week interaction. The variance for cow nested within treatment was used as random error term to test the main effect of treatment. The cow variance was considered random and data of DIM throughout the whole experiment were used as covariates. Covariance structure was AR(1). Data are presented as covariate adjusted least squares means. The significance level was declared at $P < 0.05$ unless otherwise noted. Trends for significance were declared at $P = 0.05$ to 0.10.

The model used for milk composition and FA analysis was:

$$Y_{ijk} = \mu + T_i + D_{j(i)} + W_t + (TW)_{it} \times (b + \Phi_j) \times X_{ij} + E_{ijk}$$

where Y_{ijk} = dependent variable measured at week t on the jth cow assigned to the ith treatment; μ = population

Table 3. Nutrient intake and milk production of cows fed oils rich in linoleic acid (soybean oil) and linolenic acid (flaxseed oil) alone or in combination

	Treatment[1]					
Item	Control	SO	FO	SFO	SEM[2]	*P*
Intake (kg/d)						
DM	16.2	16.2	15.9	16.2	0.22	0.10
NE_L (MCal/d)	24.0[b]	25.6[a]	26.0[a]	26.6[a]	0.35	**
Fat	0.50[b]	1.12[a]	1.08[a]	1.10[a]	0.06	**
Production (kg/d)						
Milk yield	21.7[b]	25.8[a]	25.0[a]	25.2[a]	1.54	*
ECM[3]	23.1	24.6	24.2	24.3	0.90	0.77
ECM/DM intake	1.43	1.52	1.56	1.51	0.08	0.64
Fat yield	0.77	0.83	0.81	0.82	0.05	0.19
Protein yield	0.72	0.81	0.78	0.77	0.04	0.40
Milk composition (%)						
Fat	3.49	3.21	3.26	3.30	0.12	0.36
Protein	3.15	3.20	3.17	3.15	0.08	0.95
Lactose	4.99	5.00	5.00	5.04	0.09	0.99
Total solids	12.52	12.26	12.31	12.34	0.21	0.82

[a,b]Means in the same row with different superscripts differ significantly for treatment effect with *P*-value as mentioned in column for significance.

[1]Cows were fed a basal diet (control) or basal diet supplemented with either 4.0% soybean oil (SO), 4.0% flaxseed oil (FO), or 2.0% soybean oil + 2.0% flaxseed oil (SFO).

[2]SEM = Standard error of least squares means.

[3]ECM = 0.327 × milk (kg) + 12.95 × fat (kg) + 7.20 × protein (kg); Tyrrell and Reid, 1965.

*$P < 0.05$; **$P < 0.01$.

mean; T_i = treatment effect; $D_{j(i)}$ = is the random effect of the jth cow within the ith treatment; W_t = week effect; $(TW)_{it}$ = fixed interaction effect between treatment and week; b = the common regression coefficient of DIM of X_{ij}; Φ_j = the slope deviation of the ith treatment from the common slope b; X_{ij} = the DIM of cow j on treatment i; and E_{ijk} = random error associated with the jth cow assigned to the ith treatment at week t.

RESULTS AND DISCUSSION

Treatment by week interaction was nonsignificant for intake and milk production parameters. The average values for production parameters of each treatment are in Table 3. Daily DMI was similar among treatments and averaged 16.2 ± 0.2 kg/d. Supplementing soybean or flaxseed oil at 4% of dietary DM alone or in combination increased daily fat and NE_L intake of cows in SO, FO, and SFO treatments but had no effect on DMI compared with the control treatment. Effect of supplemental fat on daily feed DMI has been variable. Unsaturated FA are more likely to depress feed intake than are saturated FA (Bremmer et al., 1998). Other factors that can affect DMI are palatability, FA chain length, and form (triglycerides, free FA). Feeding free oils up to 4% of diet DM had no negative effects on feed DMI in this study. These results are in agreement with recent reports pertaining to dairy cows (Dhiman et al., 2000) or goats (Chilliard et al., 2002) fed supplemental soybean oil or linseed oil at 2 to 6% of dietary DM. In another study, Kelly et al. (1998) fed supplemental peanut oil, sunflower oil, or linseed oil at a level of 5.3% of diet (total dietary fat 8.5%) and reported no significant change in DMI. However, Mohamed et al. (1988) observed negative effects of feeding 4% oil in the diet on feed intake, and attributed it to a reduction in digestibility of DM by free oil.

Supplementing fat through SO or FO alone or in combination (SFO) at 4% of diet DM resulted in a 16.7% increase in milk yield compared with the control treatment (Table 3). Previous studies have reported little or no effect of oil supplementation on milk yield (Chouinard et al., 1998; Kelly et al., 1998; Dhiman et al., 2000; Lock and Garnsworthy, 2002). In the present study we observed an increase in milk yield. However, as discussed later, the yield of ECM was not different among treatments. These results suggest that energy excreted in milk was the same across treatments.

Milk fat content, although statistically not different among treatments (Table 3), was numerically lower in milk from cows fed the SO, FO, and SFO treatments (average 3.26%) compared with the control treatment (3.49%). This difference is of significant economic importance to producers when milk price is based on kilograms of fat and protein. Milk fat depression commonly occurs when diets high in free oil are fed. The lower milk fat content could have been due to the increased dietary supply of $C_{18:2}$ and $C_{18:3}$ FA through soybean and flax oils, respectively. Increased dietary supply of $C_{18:2}$ and $C_{18:3}$ FA has been shown to increase *trans*-

$C_{18:1}$ FA in milk through ruminal biohydrogenation. There is a direct relationship between increased levels of *trans*-10 isomer of $C_{18:1}$ FA in milk and reduction in de novo FA synthesis (Piperova et al., 2000). Reduction in de novo FA synthesis is mediated through a reduction in mammary acetyl-CoA carboxylase and FA synthase activity, and in acetyl-CoA carboxylase mRNA abundance (Piperova et al., 2000).

Lower fat content in treatments with supplemental oil resulted in similar ECM yield among treatments. Gross feed efficiency calculated as ECM produced/kg of DMI did not differ among treatments (Table 3). Milk fat yield, milk protein content, protein yield, lactose contents, and total solids in milk were not different among treatments. Lock and Garnsworthy (2002) reported no change in milk protein content of cows fed 375 g of oil rich in linoleic and linolenic acid. Depression in milk protein content has been observed in other studies when dairy cows are fed supplemental oil or fat (Wu and Huber, 1994; Dhiman et al., 2000). Cows in early lactation appear more prone to a fat-induced milk protein depression than those in mid to late lactation due to increased vulnerability in early lactation to a negative nitrogen balance (DePeter and Cant, 1992). Cows in this study were in mid lactation and may not have experienced negative nitrogen balance or decrease in microbial protein synthesis. Overall results from this study suggest that supplementing oils through soybean or flaxseed oil alone or in combination at 4% of diet DM increased the energy content of diets but had no influence on feed DMI, ECM yield, or milk components compared with cows in the control group (without supplemental oil). Interestingly, the performance of cows in SO or FO treatments was the same, suggesting that oils rich in linoleic (soybean oil) or linolenic acid (flaxseed oil) affected the performance of animals in similar way.

Proportions of short- and medium-chain FA ($C_{10:0}$, $C_{12:0}$, $C_{14:0}$, and $C_{16:0}$) were decreased in milk from cows fed unsaturated oils (SO, FO, and SFO treatments) compared with controls (Table 4). The reduction in the proportions of these FA was of similar magnitude in cows fed SO and FO alone or in combination (SFO). This result supports the findings of others (Dhiman et al., 2000; Allred et al., 2006), who reported a decrease in short-chain FA as soybean or linseed oil was added to the diet of lactating dairy cows. The calculated yield of short- and medium-chain FA after correcting for the glycerol content of milk fat (Chouinard et al., 2001) was 412, 375, 349, and 366 g/d in control, SO, FO, and SFO treatments, respectively. The addition of long-chain unsaturated oils in free form has been shown to increase their secretion in milk fat and inhibit de novo synthesis of short- and medium-chain FA in the mammary gland (Grummer, 1991).

The proportions of $C_{18:0}$, $C_{18:1}$, and $C_{18:2}$ *c*-9, *t*-11 CLA increased in milk fat from cows fed unsaturated oils but the magnitude of change differed by type of oil used. Cows fed FO alone or in combination with SO had higher proportions of $C_{18:0}$ in milk fat compared with controls. The proportions of VA were increased by 318, 105, and 206% in milk fat from cows in SO, FO, and SFO compared with cows in the control group, respectively (Table 4). Similar increases in $C_{18:2}$ *c*-9, *t*-11 CLA were: 273, 150, and 183% in SO, FO, and SFO treatments, respectively. As expected, feeding unsaturated oils increased VA and $C_{18:2}$ *c*-9, *t*-11 CLA in milk (Kelly et al., 1998; Dhiman et al., 2000). However, a large increase in VA and $C_{18:2}$ *c*-9, *t*-11 CLA in SO compared with FO, higher levels of $C_{18:0}$ in FO compared with SO treatment, and higher proportions of $C_{18:1}$ *c*-9 in milk from FO compared with SO all suggest that ruminal biohydrogenation of unsaturated FA was comparatively more complete in cows fed FO than in cows fed SO. Comparing the independent effects of linoleic and linolenic acid on milk CLA content Lock and Garnsworthy (2002) concluded that diets high in linoleic acid increased $C_{18:2}$ *c*-9, *t*-11 CLA production in the rumen. Amounts of VA and CLA flowing through the duodenum were higher in cows fed diets supplemented with oils rich in linoleic acid compared with cows fed oils rich in linolenic acid. Our study supports the observations of Lock and Garnsworthy (2002) that oils rich in linoleic acid are more effective in enhancing VA and CLA in milk fat than oils containing linolenic acid.

The effects of mixing linoleic and linolenic acids (50:50) toward enhancing VA and $C_{18:2}$ *c*-9, *t*-11 CLA were approximately additive, but not greater than when fed separately (Table 4). In a study by Lock and Garnsworthy (2002), the effects of mixing oils rich in linoleic and linolenic acids had greater effects on enhancing $C_{18:2}$ *c*-9, *t*-11 CLA in milk than when fed separately. Differences in these studies could be due to different amounts of oils in the diet (373 vs. 650 g/d per cow). However, Doreau and Ferlay (1994) found no relationship between dietary concentrations of linoleic acid and the extent of rumen biohydrogenation.

As mentioned earlier, $C_{18:2}$ *c*-9, *t*-11 CLA in milk originates from either ruminal biohydrogenation of $C_{18:2}$ as an intermediate product (Harfoot and Hazlewood, 1997) or from endogenous synthesis in mammary gland from VA (Griinari et al., 2000). The endogenous synthesis of $C_{18:2}$ *c*-9, *t*-11 CLA from VA has been proposed as the major pathway of CLA synthesis in lactating cows, accounting for an estimated 78 to 85% of the CLA in milk fat (Corl et al., 2001; Lock and Garnsworthy,

Table 4. Fatty acid (FA) composition of milk from cows fed feed sources rich in linoleic acid (soybean oil) and linolenic acid (flaxseed oil) alone or in combination

	Treatment[2]					
FA,[1] g/100 g of FA reported	Control	SO	FO	SFO	SEM[3]	*P*
$C_{8:0}$	2.05	1.72	2.01	1.81	0.14	0.35
$C_{10:0}$	4.70^{a}	3.83^{b}	4.00^{b}	3.77^{b}	0.22	*
$C_{12:0}$	4.78^{a}	3.66^{b}	3.75^{b}	3.69^{b}	0.24	**
$C_{14:0}$	15.13^{a}	12.55^{b}	12.82^{b}	12.24^{b}	0.54	**
$C_{14:1}$	1.00	0.70	0.94	1.02	0.10	0.23
$C_{16:0}$	34.85^{a}	30.19^{b}	27.23^{b}	29.93^{b}	0.97	**
$C_{16:1}$	1.01	0.95	0.96	0.84	0.09	0.43
$C_{18:0}$	13.20^{c}	14.84^{bc}	17.36^{a}	16.03^{ab}	0.90	**
$C_{18:1}$ *t*-11, (VA)	1.48^{c}	6.19^{a}	3.04^{b}	4.53^{ab}	0.48	**
$C_{18:1}$ *c*-9	21.68^{c}	20.16^{bc}	26.89^{a}	24.38^{ab}	0.71	**
$C_{18:2}$	2.35^{b}	2.84^{a}	2.13^{b}	2.40^{ab}	0.15	*
$C_{18:3}$ *c*-9,12,15	0.37^{b}	0.37^{b}	1.00^{a}	0.44^{b}	0.07	**
$C_{18:2}$ *c*-9, *t*-11 CLA	0.64^{c}	2.39^{a}	1.60^{b}	1.81^{b}	0.12	***
$C_{18:2}$ *t*-10, *c*-12 CLA	0.04	0.08	0.09	0.07	0.02	0.24
$C_{18:2}$ *c*-9, *t*-11 CLA yield, (g/d)	6.12^{d}	20.52^{a}	13.44^{c}	16.30^{b}	2.03	***
$C_{20:3}$ *c*-8,11,14	0.11	0.12	0.10	0.11	0.02	0.77
$C_{20:5}$ (eicosapentaenoic acid)	0.10	0.12	0.12	0.08	0.02	0.73
$C_{22:4}$ c-5,8,11,14,17	0.08	0.09	0.10	0.12	0.02	0.64
$C_{22:6}$ (docosahexaenoic acid)	0.10	0.11	0.12	0.09	0.02	0.90
Total n-3 FA[4]	0.60^{b}	0.69^{b}	1.13^{a}	0.80^{b}	0.07	***
Total n-6 FA[5]	2.42	2.89	2.30	2.50	0.12	0.07
n-6:n-3	4.25^{a}	4.35^{a}	2.13^{c}	3.44^{b}	0.20	***
Monounsaturated FA[6]	24.24^{c}	27.45^{b}	31.37^{a}	28.73^{ab}	0.88	***
Polyunsaturated FA[7]	3.75^{c}	6.18^{a}	5.08^{b}	5.20^{b}	0.26	***
Saturated FA[8]	72.06^{a}	66.45^{b}	63.61^{b}	66.25^{b}	4.07	***
Unsaturated FA[9]	27.94^{b}	33.55^{a}	36.39^{a}	33.75^{a}	1.02	***

[a–d]Means in the same row with different superscripts differ significantly for treatment effect with *P* value as mentioned in column for significance.

[1]Expressed as number of carbons: number of double bonds; *c* = *cis*; *t* = *trans*.

[2]Cows were fed a basal diet (control) or basal diet supplemented with either 4.0% soybean oil (SO), 4.0% flaxseed oil (FO), or 2.0% soybean oil + 2.0% flaxseed oil (SFO).

[3]SEM = Standard error of least squares means.

[4]Sum of $C_{18:3}$ *c*-9,12,15; $C_{20:5}$ (EPA); $C_{22:5}$ and $C_{22:6}$ (DHA).

[5]Sum of $C_{18:2}$, $C_{20:3}$ *c*-8,11,14 and $C_{22:4}$.

[6]Sum of $C_{14:1}$, $C_{16:1}$, $C_{18:1}$ *t*-11, (VA), $C_{18:1}$ c-9).

[7]Sum of $C_{18:2}$, $C_{18:3}$, CLA,$C_{20:3}$, $C_{20:5}$, $C_{22:4}$ and $C_{22:6}$.

[8]Including $C_{8:0}$, $C_{10:0}$, $C_{12:0}$, $C_{14:0}$, $C_{16:0}$, and $C_{18:0}$.

[9]Total saturated FA.

*$P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

2002). Higher levels of VA in the SO treatment than the FO treatment in our study suggested that feeding cows oil rich in linoleic acid enhanced $C_{18:2}$ *c*-9, *t*-11 CLA in milk more through endogenous synthesis than did oils rich in linolenic acid. Further studies are recommended to quantify the contribution of different types of oils and combinations to endogenous synthesis of $C_{18:2}$ *c*-9, *t*-11 isomer of CLA.

As expected, feeding FO increased the proportions of $C_{18:3}$ in milk fat compared with control, SO, and SFO (Table 4). The proportions of $C_{18:2}$ *t*-10, *c*-12 and long-chain polyunsaturated FA with 20 and 22 carbons were not different among treatments. Higher milk yield and CLA content of milk in SO, FO, and SFO treatments resulted in higher CLA yields compared with the control treatment. The highest CLA yield was in SO followed by SFO, FO, and control treatments. Overall, feeding SO or FO alone or in combination to cows enhanced the proportions of monounsaturated FA, polyunsaturated FA, and total unsaturated FA in milk compared with cows on the control diet (with no supplemental oil).

Higher CLA levels are correlated with higher levels of VA, as shown in Table 4. It is important to estimate the index of the Δ^9-desaturase enzyme that is responsible for the conversion of VA to CLA. The mammary gland of cows has substantial Δ^9-desaturase activity. An increase in the CLA content of milk can be partially attributed to the increased index of Δ^9-desaturase. There are 4 major products of Δ^9-desaturase activity in the mammary gland: $C_{14:1}$, $C_{16:1}$, $C_{18:1}$ *c*-9, and $C_{18:2}$ *c*-9, *t*-11 CLA, which are produced from $C_{14:0}$, $C_{16:0}$, $C_{18:0}$, and VA, respectively. The Δ^9-desaturase indexes for

Table 5. Estimated Δ^9-desaturase activity of milk fatty acids in the mammary gland of cows fed oils rich in linoleic acid (soybean oil) and linolenic acid (flaxseed oil) alone or in combination

	Treatment[1]					
Fatty acid ratios[2]	Control	SO	FO	SFO	SEM[3]	P[4]
$C_{14:1}$:$C_{14:0}$	0.06	0.05	0.07	0.08	0.013	0.53
$C_{16:1}$:$C_{16:0}$	0.02	0.03	0.03	0.02	0.004	0.98
$C_{18:1}$ *c*-9:$C_{18:0}$	1.84	1.49	1.68	1.58	0.164	0.10
$C_{18:1}$ *t*-11:$C_{18:2}$ *c*-9, *t*-11	0.43	0.39	0.53	0.40	0.115	0.31

[1]Cows were fed a basal diet (control) or basal diet supplemented with either 4.0% soybean oil (SO), 4.0% flaxseed oil (FO), or 2.0% soybean oil + 2.0% flaxseed oil (SFO).

[2]Expressed as number of carbons: number of double bonds; *c* = *cis*; *t* = *trans*.

[3]SEM = Standard error of least squares means.

[4]Significance for treatment effect.

$C_{14:1}$:$C_{14:0}$, $C_{16:1}$:$C_{16:0}$, $C_{18:1}$ *cis*-9:$C_{18:0}$, and VA:$C_{18:2}$ *c*-9, *t*-11 CLA were not different among treatments (Table 5). Results indicate that there was no effect of adding oils rich in linoleic or linolenic acid alone or in combination to the diet on Δ^9-desaturase activity in the mammary gland. The $C_{14:0}$ is synthesized in the mammary gland, and therefore $C_{14:1}$ can only be produced by desaturation through Δ^9-desaturase enzyme. The average Δ^9-desaturase activity for $C_{14:1}$:$C_{14:0}$ was 0.065 in our study. Others have reported Δ^9-desaturase activity for $C_{14:1}$:$C_{14:0}$ ranging from 0.048 to 0.085 (Chouinard et al., 1999; Lock and Garnsworthy, 2002; Allred et al., 2006).

Advantages of adding oils to the cow's diet are increased energy density of the diet and energy intake by the animal. Adding supplemental fat can also alter the fat mobilization and deposition in animal tissues. Feeding SO or FO alone or in combination (SFO) increased the concentrations of cholesterol, phospholipids, low-density lipoprotein cholesterol, and NEFA in blood compared with feeding the control diet (Table 6). These results suggest that considerable amounts of triglyceride-rich lipoproteins were secreted into the lymphatic system and eventually reached the general circulation via the thoracic duct. Similar increases in cholesterol, lipoprotein cholesterol, and NEFA in blood plasma of cows fed fat have been reported by others (Grummer and Carroll, 1991; Petit et al., 2002; Petit, 2002). Blood insulin, growth hormone, and BHBA remained relatively unchanged during SO or FO supplementation compared with controls (Table 6). Inability of supplemental oil or fat to change insulin or growth hormone concentrations has been reported (Gagliostro and Chilliard, 1991; Grummer and Carroll, 1991). Also, growth hormone concentrations in blood are cyclic in nature and detecting treatment differences based on one sample is difficult. Despite increased levels of NEFA in blood from oil-supplemented cows, BHBA remained stable. No increase in ketone concentrations while NEFA were elevated in oil-supplemented cows indicated that FA were oxidized to CO_2 (Grummer and Carroll, 1991).

Table 6. Blood parameters of cows fed oils rich in linoleic acid (soybean oil) and linolenic acid (flaxseed oil) alone or in combination

	Treatment[1]					
Blood parameter, mmol/L	Control	SO	FO	SFO	SEM[2]	P
Cholesterol	5.74[c]	9.83[a]	8.39[b]	8.42[b]	0.05	**
Triglycerides	0.12	0.11	0.12	0.13	0.01	0.80
Phospholipids	2.99[b]	4.89[a]	4.07[a]	4.36[a]	0.07	**
Low-density lipoprotein cholesterol	1.13[c]	2.94[a]	2.27[b]	2.10[b]	0.02	**
Insulin, μIU/mL	13.35	14.15	15.36	16.38	0.12	0.87
Growth hormone, ng/mL	0.83	0.99	1.11	1.15	0.02	0.27
NEFA	0.08[c]	0.19[b]	0.26[a]	0.14[b]	0.09	0.03
BHBA	1.01	1.33	1.17	0.62	0.18	0.35

[a–c]Means in the same row with different superscripts differ significantly for treatment effect with P value as mentioned in column for significance.

[1]Cows were fed a basal diet (control) or basal diet supplemented with either 4.0% soybean oil (SO), 4.0% flaxseed oil (FO), or 2.0% soybean oil + 2.0% flaxseed oil (SFO).

[2]SEM = Standard error of least squares means.

**$P < 0.01$.

CONCLUSIONS

Under similar feeding conditions, oils rich in linoleic acid are more effective in enhancing contents and yield of VA and CLA in milk fat than oils containing linolenic acid when fed at 4% of dietary DM to dairy cows fed high forage (59% forage) diets. The effects of mixing linoleic and linolenic acids (50:50) toward enhancing VA and $C_{18:2}$ *c*-9, *t*-11 CLA were additive, but not greater than when fed separately. An increased proportion of healthy FA (CLA and VA) means milk with higher nutritive and therapeutic value.

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