Front. Agric. China 2009, 3(3): 311–318
DOI 10.1007/s11703-009-0022-1

RESEARCH ARTICLE

Yanxia GAO, Tao SUN, Jianguo LI

Effect of oilseeds rich in linoleic and linolenic acids on milk production and milk fatty acid composition in dairy cows

Abstract Nine multiparous cows averaging 93±13 days in milk production (DIM) were used in a triple 3×3 Latin square design to determine the effects of feeding them whole roasted flaxseed, cracked roasted soybean and fresh alfalfa in the diet on milk production, milk fatty acid profiles and the digestibility of nutrients. Each experimental period lasted 30 d and a sample collection was performed during the last 7 d. The cows were fed on the control basal diet (CON) or diets containing whole roasted flaxseed (FLA) or cracked roasted soybean (SOY). All diets were fed as a total mixed ration (TMR) and had similar concentrations of crude protein (CP), Net Energy Lactation (NEL), acid detergent fibre (ADF) and neutral detergent fibre (NDF). The dry matter intake (DMI) was not significantly different ($P > 0.05$), but tended to increase in FLA and SOY diets compared with the control ($P > 0.05$). Cows in all treatments had a similar milk yield, although 4% fat corrected milk (FCM) yield was higher on the FLA and SOY diets than on the CON diet. Milk fat percentage (3.45%) increased in the FLA diet compared with the control (3.31%) and SOY diets (3.39%). Milk protein percentages were similar among the diets ($P > 0.05$). There were similar digestibilities of DM, CP and ADF among the treatments and lower digestibilities of NDF and ether extract in the SOY diet compared with the CON diet. Feeding various oilseeds significantly increased the concentrations of $C_{18:1}$, $C_{18:3}$ and conjugated linoleic acid (CLA). The FLA diet decreased the ratio of omega-6 to omega-3 fatty-acids in the milk, which would improve the nutritive value of the milk.

Keywords oilseed, conjugated linoleic acid, milk fatty acid, dairy cow

Received September 8, 2008; accepted October 25, 2008

Yanxia GAO, Tao SUN, Jianguo LI (✉)
Department of Animal Science and Technology, Agricultural University of Hebei, Baoding 071001, China
E-mail: jgli@hebau.edu.cn

1 Introduction

Dietary polyunsaturated fatty acids (PUFA) are perceived to be healthier than saturated fatty acids (SFA). Research has shown that several benefits of omega-3 fatty acids to human health, including a decrease in the incidence of cancer, cardiovascular diseases, hypertension, arthritis, and an improvement in visual acuity (Wright et al., 1998; Simopoulos, 2001). As a result, there has been a great deal of interest in increasing the concentration of PUFA, especially conjugated linoleic acid (CLA) and omega-3 fatty acid in the milk fat to respond to the consumers' demands. Conjugated linoleic acid has been shown to protect against carcinogenesis *in vitro* in a wide range of human cancer cell lines, and *in vivo* in a number of animal models. Research findings suggest that CLA may have other potential health benefits including inhibition and regression of atherosclerosis, changes in body fat metabolism and partitioning, antidiabetic effects, and enhancement of bone growth (Huth et al., 2006).

The predominant CLA isomer in milk originates from endogenous synthesis from trans-11 $C_{18:1}$ via the enzyme delta 9-desaturase, and to a lesser extent from CLA produced in the rumen (Bauman et al., 2001). Several researchers have reported that the CLA concentration is higher in the milk from grazing animals than in the milk from animals fed on a TMR diet because of higher linolenic acid ($C_{18:3}$) in fresh grass than in corn silage (Kelly et al., 1998; Dhiman et al., 1999b; French et al., 2000). Feeding full-fat processed seeds allow the oil to become more gradually available in the rumen. Heat treatment is commonly used to protect the oilseeds from ruminal degradation (AbuGhazaleh et al., 2002; Mustafa et al., 2002; Mustafa et al., 2003). Kennelly (1996) suggested that the application of heat to high oil products such as flaxseed could denature the protein matrix surrounding the oil droplets and, therefore, protected oil from ruminal biohydrogenation. Feeding oilseeds to lactating dairy cows was often used to change the proportion of unsaturated FA in milk fat, which increased by as high as 40% (Casper et al., 1990; Stegeman et al., 1992; Kim et al., 1993), although extensive biohydrogenation occurred normally in the rumen (Palmquist and Jenkins, 1980). Flaxseed contains a high oil level (40% of total seed weight), and α-linolenic acid constituting approximately 55% of total fatty acids of the oil (Mustafa et al., 2002; Petit, 2002, 2003). Feeding flaxseed to dairy cows may reduce the concentrations of short-chain fatty acids (SCFA) and medium-chain fatty acids (MCFA) and increase those of long-chain fatty acids (LCFA) in the milk fat (Mustafa et al., 2003; Petit, 2003). Full fat soybeans are rich in polyunsaturated fatty acids (PUFA), especially linoleic acid ($C_{18:2}$), and feeding soybeans may increase the proportion of PUFA (Tice et al., 1994; Dhiman et al., 1999a).

The objective of this study was to evaluate the CLA content and fatty acid profile of milk fat from cows offered diets rich in linoleic or linolenic acid.

2 Methods

2.1 Experimental design and diets

Nine multiparous Holstein cows with 578±11 kg BW and 93±13 d DIM were used in a replicated ($n=3$) 3×3 Latin square design to determine the effects of feeding whole roasted flaxseed and cracked roasted soybean on milk production and milk composition, fatty acid profile of the milk fat and nutritive digestibility. Each experimental period lasted 30 d, which included a 10-d adjustment to experimental diets. The sample collection was performed during the last 7 d. The cows were fed on the control diet (CON) with corn silage (DM basis) as forage or diets containing 7.5% whole roasted flaxseed (FLA) or 7.5% cracked roasted soybean (SOY) with corn silage and fresh alfalfa (DM basis) as forage. All diets were formulated to contain 13% crude protein and were composed (dry basis) of 50% concentrate mixture and 50% forage to meet the requirements of a 600 kg cow at 90 DIM, producing 25 $kg \cdot d^{-1}$ milk with 3.5% fat (NRC, 2001)(Table 1). To prepare the cracked soybeans, the soybeans were passed through a roller mill to provide a particle size of 25% or less than that of the whole soybean seed. Soybean and flaxseed were roasted at 146ºC and steeped for 30 and 20 min using a Jet-Pro Roaster (Jet Pro Co., Springfield, OH). The diets were fed ad libitum to allow for 5%–10% feed refusal. The diets were offered in an equal portion three times daily at 06:00, 12:00 and 17:30. Cows were housed in the stalls, fed individually, had free access to water, and milked three times daily at 06:30, 12:30 and 18:00. Milk production was recorded at each milking. The cows were weighed on 3 consecutive days at the beginning and the end of each period.

2.2 Sample collection and analysis

Feed ingredients were sampled weekly. Composite samples were mixed thoroughly and subsampled for chemical analyses. The feed were offered and refusals from individual cows were recorded daily. Orts were mixed in each treatment and a representative sample was frozen daily. Samples of alfalfa and corn silage were frozen daily, and samples of individual TMR ingredients were taken once weekly. Weekly composite samples of forage and TMR were used for analysis of DM, ether extract, NDF, ADF, and CP.

Weekly feed samples were dried at 60°C in a forced air oven for 48 h (AOAC, 1990) and then grounded through a 1 mm screen of a standard Wiley mill (model 3; Arthur H. Thomas Co., Philadelphia, PA). Daily DMI for individual cows was calculated by subtracting the weekly mean of orts from the weekly mean of feed offered. The NEL content of the diet was calculated using the NEL values for the analysis of individual ingredients (NRC, 2001).

The DM contents of TMR, orts and fecal samples were determined by oven drying at 105°C for 48 h (AOAC 1990). The total N contents of TMR, orts and feces were determined by thermal conductivity (LECO model FP-428 Nitrogen Determinator, LECO, St. Joseph, MI). Crude proteins were calculated as N×6.25. The fatty acid content and compositions of dietary ingredients were analyzed using a Soxlec system HT6 apparatus (Tecator, Fisher Scientific) according to the procedure of Sukhija and Palmquist (1988). NDF and ADF were determined using the modified filter bag method of Van Soest et al. (1991) using an ANKOM200 Fibre Analyzer (ANKOM Technology Corp., Fairport, NY).

Ether extraction contents of feed ingredients and diets were conducted with a Soxlec system HT6 apparatus (Tecator, Fisher Scientific) according to method 920.39 (AOAC, 1990).

Milk samples were obtained from each cow by three consecutive milkings and mixed daily to analyze milk compositions during each experimental period. Daily milk yields were recorded. Milk samples were collected three times daily at 06:30, 12:30 and 18:00, then mixed according to the weights at each milking for 5 consecutive days at the end of each period. The samples were split into two portions (with bronopol-B2 preservative and without preservative) for analysis. The milk samples with preservative were analyzed for fat, protein, lactose and SNF by near-infrared spectroscopy (Foss MikoScan 4000; Foss Technology, Eden Prairie, MN). The remaining portion without preservative was stored at −20°C until analysis of fatty acids by GLC (HP 6890 series GC system) according to the method described by Chouinard et al. (1997).

The total feces were collected for five consecutive days at the last week of each period. On the 25th day of each experimental period, the cows were fitted with

Table 1 Ingredient and nutrient composition of experimental diets for lactating dairy cows

item		treatment			
		CON	FLA	SOY	SEM
ingredient/% DM basis	corn silage	50	33.3	33.3	—
	fresh alfalfa	0	16.7	16.7	—
	corn, ground shelled	25.5	24.5	26.5	—
	roasted whole flaxseed	0	7.5	0	—
	roasted cracked soybean	0	0	7.5	—
	wheat bran	2	2	2	—
	soybean meal	2.5	2.5	0	—
	peanut meal	2.5	0	1	—
	cottonseed meal	6	4.5	1.5	—
	flaxseed meal	2.5	0	2.5	—
	rapeseed meal	2	2	2	—
	malt root	2	2	2	—
	distillers dried grains with solubles	2.5	2.5	2.5	—
	sodium bicarbonate,	0.5	0.5	0.5	—
	dicalcium phosphate	0.7	0.7	0.7	—
	premix	0.5	0.5	0.5	—
	salt	0.8	0.8	0.8	—
nutrient contents of the diets	NE_L/(Mcal·kg^{-1} of DM)	1.24	1.35	1.30	0.04
	CP/% of DM	13.1	13.1	13.0	0.3
	NDF/% of DM	34.7	35.8	35.4	1.3
	ADF/% of DM	23.7	22.8	23.2	0.8
	calcium/% of DM	0.74	0.73	0.77	0.03
	phosphorus/% of DM	0.46	0.45	0.47	0.015
	ether extract/% of DM	3.7^a	5.0^b	5.2^b	—
fatty acids/% of total fatty acids	$C_{12:0}$	0.8^a	0^b	0^b	0.1
	$C_{14:0}$	1.2^a	0^b	0.5^b	0.1
	$C_{16:0}$	31.4^a	11.5^b	15.2^b	0.4
	$C_{16:1}$	0.42^a	0.12^b	0.31^a	0.015
	$C_{18:0}$	2.7	2.1	2.4	0.1
	$C_{18:1}$	24.6^a	17.8^b	20.2^{ab}	0.8
	$C_{18:2}$	29.8^a	31.4^a	47.2^b	1.3
	$C_{18:3}$	9.1^a	36.9^b	14.2^b	0.3

Note: FLA represents fat supplement based on roasted whole flaxseed, and SOY represents fat supplement based on roasted soybeans. Premix provided means 1 kg diet contains 0.5 mg I, 20 mg Fe, 10 mg Cu, 12.5 mg Mn, 40 mg Zn, 0.03 mg Se, 0.01 mg Co, 4750 IU vitamin A, 1000 IU vitamin D, 27.5 IU vitamin E. The Data were estimated using the NE_L values for feedstuffs from NRC (2001); a, b, c mean values in the same row with different superscripts are significantly different ($P < 0.05$).

harnesses and tubes allowing the collection of feces separately. The feces was collected from a rubber mat placed behind the animals and stored in plastic containers. The mass of the feces was recorded on an individual cow basis. The collected amount was thoroughly mixed, and 10% subsample was taken and stored at −20°C for subsequent analyses. The fecal samples were dried in a forced draft oven at 60°C for 72 h, ground through a 1-mm screen (Wiley mill), and were analyzed for DM, NDF, ADF, ether extract and CP as described earlier for feeds.

2.3 Statistical analyses

The Data were analyzed as a triple 3×3 Latin square design using the mixed procedures of SAS (SAS Institute 1999). Model sums of squares were separated into overall mean, cow (within square), square, period, treatment (effect of diet), and square × treatment interaction. All the variables were considered fixed, except cow (within square) and overall error, which were considered randomly. The interaction term square × treatment was removed from the model when $P > 0.25$. Statistical significance was declared at $P < 0.05$ and tendencies at $0.05 < P < 0.10$.

3 Results

3.1 BW, intake, milk production and composition, digestibility

The body weight, DMI, milk yield and milk compositions of the cows treated with diets are shown in Table 2. The body weight, DMI and milk yield of the cows were not affected significantly by the treatment diets. The initial and final BW averaged 570 and 586 kg, respectively, and the BW gain was numerically higher for the cows fed with FLA and SOY diet than for those fed with CON (178 and 178 vs. 155 $g \cdot d^{-1}$, respectively). The total DMI measured during the experiment was similar among treatments. The percentage of milk fat was higher for cows fed with FLA than cows fed with CON, and there was no difference between cows fed with SOY and FLA. The milk protein concentrations were similar among treatments, although numerically higher comparing the cows fed with FLA than with cows fed with SOY and CON diets. The digestibility of DM, CP and ADF was similar among the treatments (Table 3). But the apparent digestibility of NDF was lower in the SOY diet compared with the CON and FLA diets ($P < 0.05$). The apparent digestibility of ether extract was higher in the control group ($P < 0.05$).

3.2 Milk fatty acid profile

Dietary treatments had a significant impact on milk fatty acid compositions (Table 4). Concentrations of $C_{4:0}$, $C_{6:0}$, $C_{8:0}$, $C_{10:0}$, $C_{12:0}$, $C_{14:0}$, $C_{14:1}$, $C_{16:0}$, and $C_{16:1}$ in milk fat were similar among diets ($P > 0.05$). The concentration of $C_{18:0}$ was numerically higher comparing the cows fed with FLA and SOY diets than with those fed CON diet, but the difference was not significant ($P > 0.05$). Cows fed with FLA and SOY diets significantly increased

Table 2 BW, DMI, milk yield and milk composition from cows fed experimental diets

item	treatment			
	CON	FLA	SOY	SEM
initial BW/kg	565	559	587	11
finial BW/kg	579	575	603	14
BW change/($g \cdot d^{-1}$)	155	178	178	13
average BW/kg	572	567	595	14
DMI/($kg \cdot d^{-1}$)	15.70	17.00	16.46	0.61
milk yield				
actual/($kg \cdot d^{-1}$)	17.73	19.19	19.05	2.41
fat/%	3.31^{a}	3.45^{b}	3.39^{ab}	0.18
fat yield/($kg \cdot d^{-1}$)	0.59	0.66	0.65	0.04
protein/%	2.92	3.04	2.93	0.08
protein yield/($kg \cdot d^{-1}$)	0.52	0.58	0.56	0.04
4% FCM/($kg \cdot d^{-1}$)	15.89^{a}	17.61^{b}	17.31^{b}	1.61
ECM/($kg \cdot d^{-1}$)	16.95	18.85	18.41	1.21

Note: FLA represents fat supplement based on roasted whole flaxseed, and SOY represents fat supplement based on roasted soybeans. 4% FCM = 0.4×kg milk + 15.0×kg fat. ECM =(0.3246×kg milk) + (12.86×kg fat) + (7.04×kg protein). a–c indicate that the means in rows with different superscripts are significantly different at the level $P < 0.05$.

Table 3 Effect of dietary on apparent total tract digestibility of nutrients

apparent total tract digestibility,% of intake	treatment			
	CON	FLA	SOY	SEM
DM	67.3	67.9	67.1	2.1
CP	60.71	63.77	63.85	3.35
NDF	39.39^{a}	38.30^{a}	34.49^{b}	4.48
ADF	32.18	32.39	34.50	1.49
ether extract	60.67^{a}	54.11^{b}	55.84^{b}	5.45

Note: a–c indicate that the means in rows with different superscripts are significantly different at the level $P < 0.05$.

Table 4 Fatty acid composition of milk fat from feeding different fat sources (mg·g^{-1} total fatty acid)

item	treatment			
	CON	FLA	SOY	SEM
$C_{4:0}$	26.30	28.58	26.84	1.58
$C_{6:0}$	14.69	14.33	13.54	0.75
$C_{8:0}$	10.10	9.06	8.93	1.08
$C_{10:0}$	20.84	18.18	18.25	1.58
$C_{12:0}$	22.37	18.72	19.51	1.61
$C_{14:0}$	77.93	77.87	75.88	2.57
$C_{14:1}$	6.51	6.76	7.07	0.56
$C_{16:0}$	197.37	189.84	194.94	5.98
$C_{16:1}$	7.28	7.85	9.39	1.21
$C_{18:0}$	89.54	114.59	106.12	10.31
total $C_{18:1}$	206.78^{a}	276.03^{b}	268.86^{b}	21.82
t11$C_{18:1}$	14.07^{a}	19.06^{b}	20.85^{b}	1.61
t12$C_{18:1}$	1.56^{a}	2.43^{b}	2.54^{b}	0.16
c9$C_{18:1}$	189.38^{a}	251.61^{b}	242.45^{b}	11.28
c12$C_{18:1}$	1.76^{a}	2.93^{b}	3.02^{b}	0.22
$C_{18:2}$	13.35^{a}	16.41^{a}	24.62	1.01
$C_{18:3}$	1.55^{a}	4.62^{b}	4.52^{b}	0.18
total CLA	7.08^{a}	10.18^{b}	12.01^{b}	0.93
c9t11CLA	5.22^{a}	8.23^{b}	9.38^{b}	0.56
t10c12CLA	0.39	0.34	0.54	0.03
c9c11CLA	0.68	0.60	0.69	0.03
t9t11CLA	0.70^{a}	1.01^{b}	1.41^{c}	0.11
$C_{20:3}$	1.40^{ab}	1.12^{b}	1.53^{a}	0.18
$C_{20:5}$	0.41^{a}	0.43^{a}	0.54^{b}	0.04
$C_{22:4}$	0.59	0.46	0.68	0.06
Omega-6	13.34^{a}	16.41^{a}	24.62^{b}	1.71
Omega-3	3.94^{a}	6.63^{b}	7.27^{b}	0.36
Omega-6/ Omega-3	3.39^{a}	2.48^{b}	3.39^{a}	0.28

Note: Data with different letters in rows are different at the level $P<0.05$.

the content of $C_{18:1}$, $C_{18:2}$, $C_{18:3}$, c9, t11CLA, t9, t11CLA and total CLA compared with those fed with CON ($P<0.05$). The concentrations of long-chain fatty acid, monounsaturated fatty acid (MUFA) and PUFA were significantly increased ($P<0.05$), but the concentrations of short-chain and medium-chain in milk fat were numerically decreased on the diet of FLA and SOY compared with the CON diet ($P>0.05$) (Table 5). The desaturated enzyme index was different among treatments ($P<0.05$). The ratio of $C_{14:1}$ to $C_{14:0}$ and C_9, t_{11}CLA to $t_{11}C_{18:1}$ was significantly higher when the cows were fed with FLA and SOY diet ($P<0.05$). The SOY diet also significantly increased the ratio of $C_{16:1}$ to $C_{16:0}$ and $C_{18:1}$ to $C_{18:0}$ compared with the CON and FLA diet ($P<0.05$).

Table 5 The content of different kind of fatty acid in milk and desaturated index (mg·g^{-1} total fatty acid)

item	treatment			
	CON	FLA	SOY	SEM
short (< C_{14})	94.29	88.87	87.06	3.12
medium (C_{14}–C_{16})	289.08	282.32	287.28	8.21
long (> C_{18})	524.46^{a}	699.76^{b}	687.57^{b}	28.87
saturated FA (SFA)	459.13	471.17	463.99	9.39
SFA/% total FA	64.56	58.52	57.63	4.21
monounsaturated FA (MUFA)	220.56^{a}	290.64^{b}	285.32^{b}	13.83
MUFA/ % total FA	31.02^{a}	36.10^{b}	35.44^{b}	2.34
polyunsaturated FA (PUFA)	31.45^{a}	43.28^{b}	55.74^{c}	3.38
PUFA, % total FA	4.42^{a}	5.38^{b}	6.92^{c}	0.35
desaturated index				
$C_{14:1}$/$C_{14:0}$	0.081^{a}	0.089^{b}	0.091^{b}	0.004
$C_{16:1}$/$C_{16:0}$	0.037^{a}	0.042^{a}	0.048^{b}	0.003
$C_{18:1}$/$C_{18:0}$	2.29^{a}	2.44^{a}	2.55^{b}	0.09
C_9,t_{11}CLA/$t_{11}C_{18:1}$	0.37^{a}	0.43^{b}	0.45^{b}	0.04

Note: "Short" indicates short-chain fatty acid (< $C_{14:0}$). "Medium" indicates medium-chain fatty acid ($C_{14:0}$ to $C_{16:1}$). "Long" indicates long-chain fatty acid (> $C_{18:0}$). a–c indicate that the means in rows with different superscripts are different at the level $P<0.05$.

4 Discussion

4.1 BW, intake, milk production and composition, digestibility

In our study, the body weight, DMI and milk yield of cows were not affected significantly by the treatment diets. These results were consistent with a similar trial by Petit (2002). The total DMI measured during the experiment was similar among treatments. Decreases in DMI with fat-supplemented diets appear to be related to the ruminal effects of fats, whereas the ruminal effects of fat were not observed on any depression in the DMI (Petit, 2002). In the present study, DMI was numerically higher for cows fed with FLA and SOY than for those fed with CON, probably because of the better taste of alfalfa, roast flaxseed or soybean in the diets.

Milk production was similar among the treatments. This would corroborate the fact that the milk production of dairy cows was not affected by flaxseed (Petit, 2002) or soybean (Mohamed et al., 1988). The percentage of milk fat was higher for the cows fed on FLA than the cows fed on CON, and there was no difference between the cows fed on SOY and FLA. The fat intake through seeds may maintain or increase the milk fat content (Dhiman et al., 2000). The addition of polyunsaturated oils in the free form tended to depress the milk fat percentage (Selner and Schultz, 1980), whereas supplementation of oil through seeds may maintain or increase the milk fat content (Mohamed et al., 1988). The oil in the seeds may be released slowly during ruminal digestion, possibly reducing the accumulation and amount of $C_{18:1}$ trans-fatty acids leaving the rumen, thus, reducing the potential for milk fat depression with the raw cracked soybeans and roasted cracked soybeans treatments (Grummer, 1991). Scott et al. (1991) hypothesized that both an altered rumen environment and the presence of unsaturated FA in the diet were necessary conditions for a substantial decrease in the percentage and yield of milk fat. In the present experiment, feeding whole roasted flaxseed may have physically protected a high proportion of PUFA against ruminal hydrogenation, thus resulting in no milk fat depression. The cracking process of the soybean likely increased the surface area, but not enough to cause a rapid release of oil and not enough to result in milk fat depression. In the present study, milk protein concentrations were similar among treatments. Other work has shown that flaxseed may increase milk protein concentration when compared with other fat sources such as sunflower seed (Petit, 2003), calcium salts of palm oil, or micronized soybeans (Petit, 2002). Flaxseed, which is smaller in size than cracked soybean, might have increased its rate of passage from the rumen and increased its supply of AA absorbed from the small intestine for milk fat and protein synthesis. Methionine and lysine may play a role in milk fat synthesis through increasing de novo synthesis of short- and medium-chain fatty acids or through increasing synthesis of chylomicrons and very low-density lipoproteins (NRC, 2001). The differences in AA composition between soybean and flaxseed may also affect milk fat and protein synthesis. In the present study, the production of 4% FCM was higher for the cows fed on FLA and Soy diets than those fed on CON diet, with similar production of energy-corrected milk (ECM) among the treatments.

The digestibility of DM, CP and ADF was similar among the treatments. Cows fed on SOY had lower digestibility of NDF compared with cows fed on FLA and CON. Feeding whole flaxseed compared to cracked soybean could result in less oil release in the rumen, which would limit the negative effect of oil on fibre digestion. Cows fed on FLA and SOY had lower ether extract digestibility than those fed on CON. The higher concentration of ether extract in FLA and SOY diets, together with the slow release of oil in seeds might result in the lower ether extract digestibility.

4.2 Milk fatty acid profile

In the present study, the concentration of total $C_{18:1}$ in milk fat increased by 33.5% and 30.0% for cows fed on FLA and SOY than those fed on CON, which agrees with the findings by other researchers (Dhiman et al., 1995; Petit, 2002). Full fat soybean and flaxseed are rich in $C_{18:2}$ and $C_{18:3}$ and these fatty acids likely serve as precursors of c9, t12 CLA and $C_{18:1}$ following ruminal biohydrogenation. Diets rich in $C_{18:2}$ and $C_{18:3}$ have shown an increase in the $C_{18:1}$ content of milk fat through rumen biohydrogenation (Dhiman et al., 2000). Oleic acid is considered to be neutral in its effect upon human cholesterol levels while $C_{18:1}$ and PUFA may lower the blood cholesterol in humans (Kennelly, 1996). Therefore, the milk produced by cows fed on full fat soybean and flaxseed can benefit human health.

Concentrations of $C_{18:2}$, total CLA and $C_{18:3}$ in milk fat were higher for cows fed on SOY and FLA than those fed on CON. The supplement of flaxseed and full fat soybean increased the amount of $C_{18:2}$ and $C_{18:3}$ reaching the duodenum because of the increase of total intake of linoleic acid and linolenic acid, although extensive biohydrogenation occured normally in the rumen, which would explain the higher milk concentration of $C_{18:2}$ and $C_{18:3}$ for cows fed on SOY and FLA relative to those fed on CON. The long-chain polyunsaturated fatty acids ($C_{18:2}$ and $C_{18:3}$) were subject to biohydrogenation processes in the rumen, and the intermediate steps for converting $C_{18:2}$ to CLA were suggested by Kepler and Tove (1967). Other results (Dhiman et al., 1999b) suggested that $C_{18:3}$ might be a substrate for conversion to CLA in the rumen. In the present study, the concentration of CLA increased significantly in the milk fat for cows fed on FLA and SOY diets because of the increase of total intake of linoleic acid and linolenic acid, which was consistent with the findings (Kepler and Tove, 1967; Dhiman et al., 1999b).

Feeding flaxseed may result in the lowest omega-6 to omega-3 fatty acids ratio in milk fat, which would improve the nutritive value of milk from the human health point of view. This agreed with the result of Petit (2002). Feeding flaxseed to dairy cows could contribute to improving human health by a greater intake of omega-3 fatty acids in enriched dairy products.

Feeding FLA and SOY could significantly increase the concentration of long-chain fatty acid, monounsaturated fatty acid (MUFA) and PUFA, and numerically decrease the concentration of short-chain and medium-chain in milk fat compared with those fed on CON. An increased supply of dietary long-chain fatty acids has shown an increase in the secretion in milk fat and an inhabitation of de novo synthesis of medium-chain fatty acids in the mammary gland (Grummer, 1991), resulting, in low medium-chain fatty acids in milk.

The increase of PUFA concentration in the milk fat indicated that a portion of the unsaturated fatty acids contained in feeds escaped ruminal metabolism and hydrogen saturation. Therefore, modifying milk fatty acid profiles via diet may improve the profile of milk fat and may be beneficial to human health (Ney, 1991).

The desaturation index (substrate/product) was used to assess the extent of desaturation of specific fatty acids during milk fat synthesis (Sol Morales et al., 2000). The basal ratio of $C_{14:1}/C_{14:0}$ and c9, t11CLA/t11$C_{18:1}$ was higher when the cows were fed on SOY and FLA compared with CON (Table 5). The ratio of $C_{16:1}/C_{16:0}$

and $C_{18:1}/C_{18:0}$ was higher compared to the cows fed on SOY with those fed on FLA and CON, and there was no difference between FLA and CON. Higher ratios indicated more fatty acids as the substrates were desaturated when they were fed on SOY and FLA compared with CON. The increase in the ratios suggested exogenous oilseeds could improve the amount and (or) activities of desaturates in the mammary gland.

5 Conclusions

Feeding the whole roasted flaxseed and cracked roasted soybean at 7.5% of dietary DM to the dairy cows in mid-lactation had no detrimental effects on milk yield and concentrations of milk fat and protein. Overall, our study indicated that CLA content in the milk fat of dairy cows was increased by feeding full fat roasted flaxseed and soybean. The addition of full fat flaxseed and soybean enriched in unsaturated fatty acids markedly enhanced the CLA, MUFA and PUFA content in milk fat. The greatest increase was observed when the cracked roasted soybean was added to the diet. Feeding flaxseed resulted in the lowest ratio of omega-6 to omega-3 fatty acids in milk fat, which would improve the nutritive value of milk from the human health point of view. The dietary supplementation of full fat flaxseed and soybean may result in the increase of long-chain and unsaturated fatty acids.

Acknowledgements This work was supported by the National Key Technology R&D Program, China (No. 2006BAD04A10), Scientific and Technical Innovation Project of Hebei province, China (Nos. 07227146Z; 06547004D-6) and Special Scientific Research Program of Ministry of Agriculture, China (Nos. nyhyzx07-036-04; nycytx-10).

References

AbuGhazaleh A, Schingoethe D J, Hippen A R (2002). Feeding fish meal and extruded soybeans enhances the conjugated linoleic acid (CLA) content of milk. J Dairy Sci, 85: 624–631

AOAC (1990). Official Methods of Analysis. 15th ed. Arlington, VA: Association of Official Analytical Chemists International

Bauman D E, Corl B A, Baumgard L H, Griinari J M (2001). Conjugated linoleic acid (CLA) and the dairy cow. In: Garnsworthy P C, Wiseman J, eds. Recent Advances in Animal Nutrition-2001. Nottingham: Nottingham University Press, 221–250

Casper D P, Schingoethe D J, Eisenbeisz W A (1990). Response of early lactation cows to diets that vary in ruminal degradability of carbohydrates and amount of fat. J Dairy Sci, 73: 425–444

Chouinard P Y, Le'vesque J, Girard V, Brisson G J (1997). Dietary soybeans extruded at different temperatures: Milk composition and in situ fatty acid reactions. J Dairy Sci, 80: 2913–2924

Dhiman T R, Anand G R, Satter L D, Pariza M W (1999b). Conjugated linoleic acid content of milk from cows fed different diets. J Dairy Sci, 82: 2146–2156

Dhiman T R, Helmink E D, McMahon D J, Fife R L, Pariza M W (1999a). Conjugated linoleic acid content of milk and cheese from cows fed extruded oilseeds. J Dairy Sci, 82: 412–419

Dhiman T R, Satter L D, Pariza M W, Galli M P, Albridht K, Tolosa M X (2000). Conjugated linoleic acid (CLA) content of milk from cows offered diets rich in linoleic and linolenic acid. J Dairy Sci, 83: 1016–1027

Dhiman T R, Zanten K V, Satter L D (1995). Effect of dietary fat source on fatty acid composition of cow's milk. J Sci Food Agric, 69: 101–107

French P, Stanton C, Lawless F, O'Riordan E G, Monahan F J, Caffrey P J, Moloney A P (2000). Fatty acid composition, including conjugated linoleic acid, of intramuscular fat from steers offered grazed grass, grass silage, or concentrate-based diets. J Anim Sci, 78: 2849–2855

Grummer R R (1991). Effect of feed on the composition of milk fat. J Dairy Sci, 74: 3244–3257

Huth P J, DiRienzo D B, Miller G D (2006). Major scientific advances with dairy foods in nutrition and health. J Dairy Sci, 89: 1207–1221

Kelly M L, Kolver E S, Bauman D E, Van Amburgh M E, Muller L D (1998). Effect of intake of pasture on concentrations of conjugated linoleic acid in milk of lactating cows. J Dairy Sci, 81: 1630–1636

Kennelly J J (1996). The fatty acid composition of milk fat as influenced by feeding oilseeds. Anim Feed Sci Technol, 60: 137–152

Kepler C R, Tove S B (1967). Biohydrogenation of unsaturated fatty acids. J Biol Chem, 242: 5686–5692

Kim Y K, Schingoethe D J, Casper D P, Ludens F C (1993). Supplemental dietary fat from extruded soybeans and calcium soaps of fatty acids for lactating dairy cows. J Dairy Sci, 76: 197–204

Mohamed O E, Satter L D, Grummer R R, Ehle F R (1988). Influence of dietary cottonseed and soybean on milk production and composition. J Dairy Sci, 71: 2677–2688

Mustafa A F, Chouinard P Y, Christensen D A (2003). Effects of feeding micronised flaxseed on yield and composition of milk from Holstein cows. J Sci Food Agric, 83: 920–926

Mustafa A F, McKinnon J J, Christensen D A, He T (2002). Effects of micronization of flaxseed on nutrient disappearance in the gastrointestinal tract of steers. Anim Feed Sci Techno, 95: 123–132

National Research Council (NRC) (2001). Nutrient Requirements of Dairy Cattle. 7th ed. Washington D C: National Acacemy Press

Ney D M (1991). Potential for enhancing the nutritional properties of milk fat. J Dairy Sci, 74: 4002–4012

Palmquist D L, Jenkins T C (1980). Fat in lactation rations: A review. J Dairy Sci, 63: 1–14

Petit H V (2002). Digestion, milk production, milk composition, and blood composition of dairy cows fed whole flaxseed. J Dairy Sci, 85: 1482–1490

Petit H V (2003). Digestion, milk production, milk composition, and blood composition of dairy cows fed formaldehyde treated flaxseed or sunflower seed. J Dairy Sci, 86: 2637–2646

SAS User's Guide: Statistics, Version 8 Edition (1999). Cary (NC): SAS Inst, Inc

Scott T A, Combs D K, Grummer R R (1991). Effects of roasting, extrusion, and particle size on the feeding value of soybeans for dairy cows. J Dairy Sci, 74: 2555–2565

Selner D R, Schultz L H (1980). Effects of feeding oleic acid or hydrogenated vegetable oils to lactating cows. J Dairy Sci, 63:

1235–1241

Simopoulos A P (2001). n-3-fatty acids and human health, Defining strategies for public policy. Lipids, 36(Suppl 1): S83–89

Sol Morales M, Palmquist D L, Weiss W P (2000). Effects of fat source and copper on unsaturation of blood and milk triacylglycerol fatty acids in Holstein and Jersey cows. J Dairy Sci, 83: 2105–2111

Stegeman G A, Casper D P, Schingoethe D J, Baer R J (1992). Lactational responses of dairy cows fed unsaturated dietary fat and receiving bovine somatotropin. J Dairy Sci, 75: 1936–1945

Sukhija P S, Palmquist D L (1988). Rapid method for determination of total fatty acid content and composition of feedstuffs and feces. J Agric Food Chem, 36: 1202–1206

Tice E M, Eastridge M L, Firkins J L (1994). Raw soybeans and roasted soybeans of different particle sizes. 2. Fatty acid utilization by lactating cows. J Dairy Sci, 77: 166–180

Van Soest P J, Robertson J B, Lewis B A (1991). Methods for dietary fiber, neutral detergent fiber, and non-starch polysaccharides in relation to animal nutrition. J Dairy Sci, 74: 3583–3597

Wright T, McBride B, Holub B (1998). Docosahexaenoic acid enriched milk. World Rev Nutr Diet, 83: 160–165