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Fatty Acid Profile of Ruminal Fluid, Plasma and Milk Fat of Dairy Cows Fed Soybean and Sunflower Oil-Rich Diets, Without Effects on Milk Production

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ABSTRACT

The objective of the study was to evaluate the effect of feeding soybean oil (SB) and sunflower oil (SF) on ruminal fluid, plasma and milk fatty acid profiles. Eighteen cows were obtained for the experiment and received 20 kg corn silage and 7.5 kg concentrate as a base diet, containing 6.2% palm oil on a dry matter basis, for four weeks. Subsequently, after the four-week experimental period, six cows were assigned to each of the dietary treatments. The treatments consisted of three groups: 1) the same base diet, containing 6.2% palm oil (control); 2) 6.2% SB; and 3) 6.2% SF. All treatments did not alter milk production. The milk fat percentages were significantly ($P < 0.05$) lower in the cows fed the SB- and SF-rich diets at day 7 and 14, whereas milk fat yields were significantly ($P < 0.05$) lower only in the cows fed the SF-rich diet at day 7. The proportion of ruminal C16:0 was significantly ($P < 0.001$) lower and C18:0 was significantly ($P < 0.05$) higher for the SB and SF diets, while in addition, C18:1n-9 tended to be lower on the SF diet ($P = 0.062$). The proportions of plasma C16:0 and C18:1n-9 were significantly ($P < 0.05$) lower for the SB and SF diets, whereas C18:0 was significantly ($P = 0.027$) higher with the SB diet. Even though no differences in polyunsaturated fatty acids, in either the ruminal fluid or plasma, were found among the treatments, the proportion of milk linoleic acids (C18:2n-6) was similar in the SB and SF diets and both diets had proportions that were significantly ($P < 0.001$) higher than in the control. In addition, milk linolenic acids (C18:3n-3) were significantly ($P < 0.05$) greater in the SB diet than for the SF diet and for the control. An increase in the ruminal pH and a reduction in blood non-esterified fatty acid (NEFA) were detected in cows fed either the SB or SF diet. The results suggested (with the balance between dietary fatty acids hydrogenated in the rumen and taken up by the mammary gland) that SB is an acceptable fat source for high linoleic acid and linolenic acid (omega-6 and 3) and that SF is suitable for high linoleic acid (omega-6)-produced milk, without adverse effects on milk yield and composition. This practical feeding trial would reflect the use of these oils to produce health-enhancing dairy products.

Keywords: soybean oil, sunflower oil, polyunsaturated fatty acid, milk, cow

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INTRODUCTION

Unsaturated lipids have been added to the diet of dairy cows in order to modify the fatty acid profile of the milk fat to meet customer demands for health-enhancing products. Even though there are many sources of lipid feed (including forage, oils and oilseeds, fish oil and other fat supplements), oils and oilseeds are one particular means of feeding unsaturated fatty acids in supplementary rations to lactating cows to increase the amount of unsaturated fatty acids in the milk fat. In addition, oils and oilseeds have been suggested as: 1) appropriate energy sources in order to improve the energy value of maintenance and production diets in dairy cows, especially during early lactation; 2) as a means of overcoming cases of severe negative energy balance (NEB); and 3) as an aid in assisting dairy cows to express their productive potential, through positive effects on reproduction (Robinson *et al*., 2002; Zheng *et al*., 2005) and immune responses (Lessard *et al*., 2004; Thanasak *et al*., 2004; Thanasak *et al*., 2005).

Adding a sunflower oilseed supplement to the diet altered the milk fatty acid profile by increasing the concentration of unsaturated fatty acids and decreasing saturated fatty acids (Bett *et al*., 2004). In addition, supplements of sunflower and soybean oils decreased the concentration of short and medium chain fatty acids and increased the concentration of long chain fatty acids (Rego *et al*., 2005). Feeding fish oil and/or extruded soybeans reduced the amounts of short (C6:0-C13:0) and medium (C14:0-C17:0) chain fatty acids, whereas the proportion of long chain fatty acids (> C17:0) in milk fat was increased (AbuGhazaleh *et al*., 2002). Moreover, the hypercholesterolemic fatty acids in milk fat (for example C12:0, C14:0 and C16:0) decreased, while the concentrations of oleic and linoleic acids increased following supplementation with vegetable oils (Rego *et al*., 2005). Another report suggested that both extruded soybeans and sunflower seeds can be used as dietary fat supplements to increase milk yield and the proportion of unsaturated fatty acids in milk fat (Schingoethe *et al*., 1996). Feeding high (20%) soybean oil concentrate has been reported to increase the concentration of linoleic acid in blood plasma and the mammary fat pad (Thibault *et al*., 2003). Instead of soybean and sunflower oilseeds, feeding diets rich in other vegetable oils and with an oilseed origin, such as corn oil, flaxseed, linseed, solin, canola, rapeseed and evening primrose oil have been reported to affect the proportion of polyunsaturated fatty acids in milk (Ward *et al*., 2002; Leonardi *et al*., 2005).

To regulate the fatty acid profile in milk fat, the previous studies indicated that the polyunsaturated fatty acids (PUFA) present in milk fat are derived either directly or indirectly from the diet. However, in measuring the effects of a high oil diet, fatty acid composition has been evaluated mostly in the milk fat and sometimes in plasma, but rarely in the ruminal fluid and its metabolism has not been discussed (AbuGhazaleh *et al*., 2002). Moreover, most of the previous experiments have been carried out under particular research conditions, which might not reflect field practice or be able to be applied in some countries. Cows under different conditions react differently to supplemented fat in their diet. For example, a special feeding regimen, such as using protected fat or abomasal infusion, which allows the unsaturated fatty acid to escape from ruminal biohydrogination, has promoted a higher proportion of these fatty acids in milk fat (Ashes *et al*., 1997; Perfield *et al*., 2002) and often depressed milk fat synthesis (Abu-Ghazaleh *et al*., 2001; Rego *et al*., 2005; Zheng *et al*., 2005). In addition, other potential factors include: the quantity of supplemented fat; different sources of dietary energy (crops and local feedstuffs); environmental status; and management. Thus, to promote these unsaturated fatty acids in the milk

of dairy cows in different countries, more research trials need to be conducted.

The aim of the current study was to examine the effect of supplemented diets containing polyunsaturated oils in modifying the fatty acids composition in ruminal fluid, blood plasma and milk fat, with particular reference to the effects on milk n-3 and n-6 PUFA. To simulate a practical feeding trial, soybean oil and sunflower oil were chosen, as they are locally available, generally used and represent good sources of linolenic acid (C18:3n-3) and linoleic acid (C18:2n-6), respectively. Polyunsaturated oils were used at a level (<7% in ration) that was intended to not interfere with either dry matter intake (DMI) or milk production. The output of this experiment would reflect the ability of feeding PUFA-rich diets to improve the quality of dairy production in Thailand and other tropical countries.

MATERIALS AND METHODS

Animals, diets and experimental design

The experiment used 18 multiparous 75% Holstein Friesian cows, with an average body weight (mean ± SE) of 373 ± 12.50 kg, aged 3.60 ± 0.45 years, average parity of 2.06 ± 0.37, having 119.77 ± 21.84 days in milk and an average milk yield 14.65 ± 0.86 kg per day. The study was performed with the approval of the Faculty of Veterinary Science - Animal Care and Use Committee (FVS-ACUC), Mahidol University, Thailand.

To evaluate responses to supplementary diets containing vegetable oils, a completely randomized design (CRD) was used. During the pre-experimental period (4 weeks), all cows (n=18) were fed a base diet rich in palm oil, in order to adjust the nutritional status and prepare the cows for the oil-supplemented diets. The base diet consisted of 20 kg of corn silage and 7.5 kg of concentrate containing palm oil (PO), which was fed daily. Subsequently, during the experimental period (4 weeks), six cows (n=6) were assigned to each of the three dietary treatments, which were fed the diets containing palm oil (PO) for the control group or soybean oil (SB) or sunflower oil (SF) for the test groups. The ingredients and fatty acid compositions of the concentrates are presented in Table 1. The analyzed composition of the diets is shown in Table 2. The rations were fed four times a day at 8:00, 10:00, 16:00 and 20:00 h. Feed was restricted and refusals were checked.

Sampling procedure

Cows were milked twice daily at 06:00 and 15:00 h and the milk yields were recorded. Milk sampling involved approximately 60 mL per cow per day (30 mL at each milking time) on the last day of the pre-experimental period (day 0 of the experimental period) and subsequently at days 7, 14, 21 and 28 of the experimental period. Milk samples were kept at 4°C for milk composition analysis. Extra samples were collected for fatty acid profile determination on days 0 and 28 and kept at -20°C for further analysis.

Blood samples were collected (on day 0 and 28) by venipuncture from the jugular vein of each cow, with the first 10 mL being drawn into an evacuated blood collecting tube (BD Vacutainer® BD Franklin Lakes NJ, USA) containing sodium heparin. The blood was centrifuged at 3,000 × g for 15 min and the plasma was harvested and stored at -20°C until fatty acid analysis. Another 10 mL sample was drawn into a plain evacuated tube. The blood was centrifuged at 3,000 × g for 5 min and the serum was harvested and stored at -20°C until NEFA analysis.

Ruminal fluid samples were collected (approximately at 12:00-14:00 h on days 0 and 28) using a rumenocentesis technique on the left site of the ventral sac of the rumen, which was identified 15-20 cm caudoventral to the costochondral junction of the last rib. These

Table 1 Composition of experimental concentrates and their analyzed fatty acid profiles.

	Pre-experimental concentrate	Experimental concentrate		
	PO	Control	SB	SF
Ingredients (kg dry matter)				
Constant component[1]	938	938	938	938
Palm oil	62	62	-	-
Soybean oil	-	-	62	-
Sunflower oil	-	-	-	62
Total	1,000	1,000	1,000	1,000
Fatty acids[2] (%)				
C8:0	1.26	1.26	1.39	1.23
C10:0	1.55	1.55	1.63	1.46
C11:0	0.01	0.01	0.01	0.01
C12:0	18.93	18.93	19.34	17.18
C13:0	0.02	0.02	0.02	0.02
C14:0	7.92	7.92	7.57	7.14
C14:1	0.13	0.13	0.04	0.02
C15:1	0.00	0.00	0.01	0.00
C16:0	25.92	25.92	12.14	12.42
C16:1	0.17	0.17	0.13	0.18
C17:0	0.07	0.07	0.00	0.04
C18:0	3.65	3.65	3.54	3.23
C18:1	28.16	28.16	18.68	24.33
C18:2n-6	11.67	11.67	32.11	32.43
C18:3n-6	0.18	0.18	0.40	0.08
C18:3n-3	0.31	0.31	2.91	0.18

[1] The constant component (in kg dry matter) consisted of: cassava, 231; soybean meal,179; palm meal,192; copra meal,192; wheat bran,51; ground bone,15; cotton seed,38; sodium chloride,17; premix,9; urea, 17.

[2] Fatty acid (%) values are expressed as g/100g of total fatty acid methyl esters

Table 2 Analyzed macronutrient composition of experimental diets.

	Corn silage [a]	Concentrate [b]		
		Control	SB	SF
Daily intake per cow [c] (kg dry matter)	4.02	7.48	7.48	7.48
Macronutrients (% dry matter)				
Crude fat	1.45	10.74	10.24	10.44
Crude fiber	28.52	8.08	8.20	8.08
Crude protein	6.07	23.06	21.27	19.36

[a] The corn silage contained 201g dry matter/kg.

[b] All vegetable oil supplement concentrates (PO, SB and SF) contained 997 g dry matter/kg.

[c] R:C ratio = 35:65.

samples were drawn into separate 30-mL tubes and the ruminal pH was determined immediately using a glass electrode pH meter, before the samples were kept at -20°C for further fatty acid composition analysis.

Milk composition analysis

Milk samples were homogenized at 40° C and then the milk composition (fat, protein, lactose and solid not fat) was determined using automated infrared analysis (MilkoScan™ Minor4, IDF and AOAC approved IR-technology).

Determination of non-esterified fatty acid (NEFA) in serum

Concentrations of serum NEFA (NEFA C, Wako Pure Chemical Industries Ltd., Osaka, Japan) were determined by an enzymatic method using spectrophotometry and a commercially available test kit.

Analysis of fatty acid composition in ruminal fluid, plasma and milk

Total lipids were extracted from the ruminal fluid (2mL) or plasma (2 mL) or milk (4 mL) with chloroform/methanol (2:1, v/v), as described by Folch *et al*. (1957). The extracted lipids were saponified with 0.5 N methanolic sodium hydroxide and methylated with 14% boronitrifluoride-methanol complex (Batch no. 045k5302, SIGMA-ALDRICH, Louis,USA) as described by Metacalfe *et al*. (1966).

The fatty acid methyl esters were separated and quantified by gas chromatography (GC) using a Konik HRGC 4000 series gas chromatography apparatus equipped with a flame ionization detector (FID) and a high polar fused-silica capillary column (SP-2560, 100m × 0.25mm × 0.2μm film). Helium was used as the carrier gas at a flow rate of 1 mL/min. Pure methyl ester standards were used to identify peaks and to determine correction factors for individual fatty acids.

Statistical analysis

Prior to statistical analysis, all data, assumed to have a normal distribution, were checked by the Kolmogorov-Smirnov test. All data were subjected to a one way ANCOVA according to a completely randomized design, with dietary treatments as a fixed factor, parameters observed as dependent variables and pre-experimental parameters observed (day 0) as covariates, in order to correct for variability of different beginning variables. Where the influence of dietary treatment was significant, multiple comparisons using least significant differences were used to compare diets with different effects on the variable involved. Whole data statistical calculations were performed as a general linear model using the univariate procedure of the software package SPSS version 17. Data for fatty acid composition in the ruminal fluid, plasma and milk were reported as means of percentages. Overall differences between treatment means were considered significant when $P < 0.05$. All P values were presented in tables.

RESULTS AND DISCUSSION

Intake, milk yield and milk composition

Two cows in the control group suffered from mastitis, so the sixteen cows that remained healthy were used throughout the experiment. There were no refusals of the experimental diets during the experimental period.

All treatments had no effect on milk yield throughout the experiment, but the SF diet significantly ($P < 0.05$) decreased the 3.5% fat corrected milk (FCM) when compared to the control diet after the first week of the experimental period (Figure 1). The lack of any effect on milk yield in this experiment was similar to previous reports of cows fed vegetable oil supplements, such as corn oil (CO), cottonseed oil (CS), linseed oil (LO) and soybean oil (SB) (Thibault *et al*., 2003; Whitlock *et al*., 2003; Zheng *et al*., 2005). In addition, no change in milk production or 4%

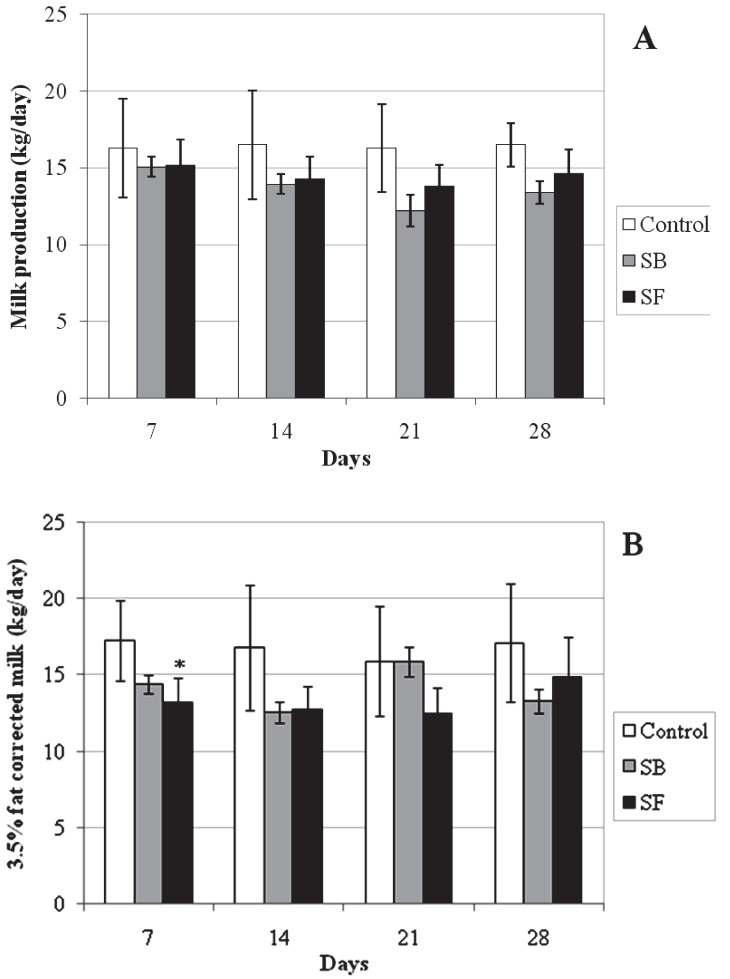

Figure 1 Milk production (mean kg per day ± SE) of cows fed diets supplemented with soybean oil (SB) and sunflower oil (SF): (A) milk production; (B) 3.5% fat corrected milk.
* = $P < 0.05$, when compared with control values.

FCM was reported in cows infused with LO in the duodenum (Petit *et al*., 2002). However, another report showed higher 4% FCM production in cows fed extruded soybean and sunflower seed diets (Schingoethe *et al*., 1996).

At days 7 and 14, the SB and SF supplements significantly ($P < 0.05$) depressed milk fat percentages compared to the control, based on the mean percentage of milk fat at day 7 being significantly ($P < 0.05$) lower in the SF compared to the SB supplement, whereas at day 7, milk fat yield was significantly ($P < 0.05$) lower only in cows fed SF. However, milk fat percentages and milk fat yields did not alter throughout the rest of the experiment (Figure 2). A search of the literature on the effect of oil supplements on milk fat concentration indicated that some reports revealed that milk fat production can be suppressed by feeding SB , SF- and CO-supplemented diets (Abu-Ghazaleh *et al*., 2001; Rego *et al*., 2005; Zheng *et al*., 2005). In addition, no effect on milk fat percentages and yields of cows fed SF, LO and FO has been reported over a four-week experimental period (Loor *et al*., 2005). However,

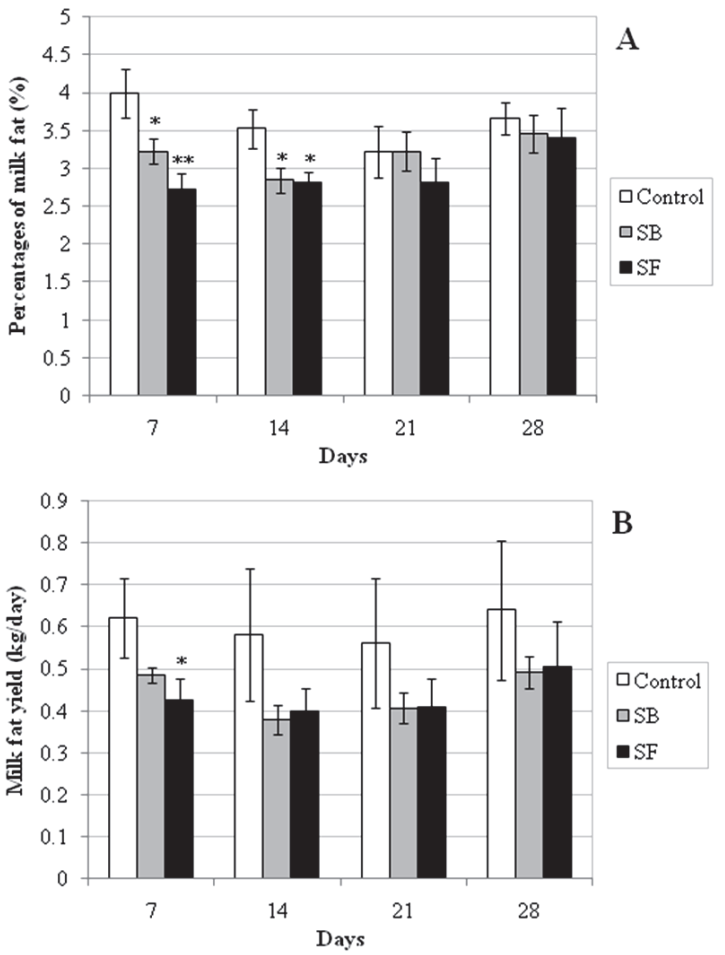

Figure 2 Milk fat of cows fed diets supplemented with soybean oil (SB) and sunflower oil (SF): (A) percentage of milk fat (mean percentage ± SE); (B) milk fat yield (mean kg per day ± SE). * = $P < 0.05$; ** = $P < 0.001$, when compared with control values.

an increase in milk fat production has been reported by feeding treated oil seeds (Petit, 2003).

In addition, the percentage and yield of milk lactose, protein and SNF were within the normal range and were not different among treatments (data not shown). These results, where there was no effect, were similar to previous reports (Schingoethe *et al.*, 1996; Petit, 2003; Thibault *et al.*, 2003). However, a greater protein concentration in milk has been reported in cows fed flaxseed (Petit, 2003). While the literature on vegetable oil supplementation indicated that changes in milk production and milk composition were inconsistent, the current study suggested that the effects of SB and SF oil-rich diets on these variables were rather small and could not be discriminated within a couple weeks after the replacement of these oil-rich diets.

Ruminal fluid composition and characteristics

There was a significant ($P<0.001$) reduction in palmitic acid (C16:0) and a significant ($P < 0.05$) increase in stearic acid (C18:0) in the ruminal fluid of cows fed SB- and SF-

supplemented diets when compared to the control. No differences were found in the other fatty acid profiles of the ruminal fluid among these cows. However, oleic acid (C18:1n-9) tended to be lower in the ruminal fluid of cows fed SF (P = 0.062; Table 3). The lower ruminal level of C16:0 and C18:1 should be reflected from their dietary level (Table 1). The higher level of ruminal C18:0 in this study was likely the consequence of an increase in the ruminal biohydrogenation process of linoleic acid (C18:2n-6) and linolenic acid (C18:3n-3), which was induced by: 1) untreated fats used in this experiment provided a reduced degree of protection for long chain unsaturated fatty acids from biohydrogenation in the rumen; and 2) an increase in the ruminal pH of cows fed SB and SF (P = 0.007; Table 3) that attempted to promote rumen digestibility instead of the bypass one. In accordance with the current study, a greater concentration of C18:0 in rumen digesta has been reported from cows fed fish oil and extruded soybean (AbuGhazaleh *et al*., 2002). In addition, a study of the biohydrogenation effect in cows fed a high-concentrate diet, rich in SO, LO or fish oil (FO) had shown alterations in the composition of these particular fatty acids in the ruminal fluid (Loor *et al*., 2005). The differences in the biohydrogenation rates was thought to be due to the influence of the amount of supplemented fat, the number of double bonds in fatty acids and the ruminal pH (AbuGhazaleh *et al*., 2003). Moreover, the rates of ruminal biohydrogenation of C18:2 and C18:3 have been reported in the range 80 to 93% in lambs fed fish oil and/or whole linseed (Wachira *et al*., 2000).

Blood composition

Serum NEFA concentrations were lower for cows fed SB (P = 0.054) and SF (P = 0.045) when compared to those of cows fed on the control diet (Table 4). These similar results have been previously reported for cows fed flaxseed (Petit, 2003). In the current investigation, the lower concentrations in the blood NEFA could be explained by: 1) the ruminal biohydrogination that might predominantly occur in this study, as according to the results, ruminal pH significantly increased and blood PUFA levels were not elevated in oil-treated cows; 2) an appropriate proportion of oil supplement in these experimental rations that

Table 3 Temperature (average on day 28), pH (average on day 28) and fatty acid composition (average on day 28, g/100g fatty acid) in the ruminal fluid of cows fed supplements with soybean oil (SB) and sunflower oil (SF).

	Supplement			Pooled	P
	Control	SB	SF	SD	
Temp °C	36.65	36.87	36.82	0.86	0.929
pH	5.77[b]	6.35[a]	6.40[a]	0.36	0.007
Fatty acids	———— % of total fatty acids ————				
C12:0	5.99	5.78	6.69	1.90	0.815
C14:0	5.72	5.70	6.73	1.22	0.548
C16:0	33.98[a]	26.51[b]	23.39[b]	7.10	0.001
C18:0	45.50[b]	55.93[a]	61.23[a]	8.40	0.003
C18:1n-9	7.20	3.77	1.07	4.73	0.162
C18:2n-6	0.50	1.69	0.43	1.60	0.350
C18:3n-3	N/A	N/A	N/A	N/A	N/A
C24:1	1.12	0.31	0.46	0.69	0.221

[a,b] = Means within row with different superscript differ significantly at $P < 0.05$.

did not have a lower DMI resulted in a reduction in fatty acid mobilization from body stores; and 3) fat in the diet can be incorporated into lipoprotein in the intestine and absorbed into the circulation, which can be used directly by tissues (especially the mammary gland) leading to reduced NEFA loads in the circulation (Block and Sanchez, 2000). However, some previous reports demonstrated there was no effect on blood NEFA by feeding treated oil seed (Petit *et al*., 2001; Petit, 2003).

Even though only a small amount of the profile of fatty acids in plasma was observed in this study, there were significantly ($P < 0.05$) lower percentages of C16:0 and C18:1n-9 in cows fed SB or SF compared to the control, and a significantly ($P = 0.027$) higher percentage of C18:0 only in SB-fed cows (Table 4). The pattern of these changes appeared to be similar to that of the ruminal fluid, indicating that an alteration at the ruminal level in these fatty acids was reflected in plasma levels. Greater linoleic acid (C18:2n-6) levels have been reported for cows fed crushed solin seed (linoleic-acid rich) supplement to a fresh forage diet (Ward *et al*., 2003). However, the reports presented inconsistent data regarding whether the oil and oil seed supplementation altered the composition of plasma fatty acids, due to different conditions associated with the oils and the rumen (Petit, 2003; Ward *et al*., 2003; Loor *et al*., 2005). No differences between the plasma linoleic acids of cows fed SB or SF in this study could be attributed to the effect of ruminal biohydrogenation, since higher levels of C18:0 were evident in the ruminal fluid of cows fed SB or SF.

Fatty acid composition of milk fat

The results of the effect of SF and SB on the profile of the fatty acids of milk fat are presented in Table 5. The fatty acid composition in milk fat indicated that supplementary SB and SF significantly ($P < 0.001$) increased the concentration of linoleic acid (C18:2n-6) by 82.86 and 34.86%, respectively, when compared with the control. Moreover, feeding SB diets significantly increased the concentration of linolenic acid (C18:3n-3) in the milk fat when compared with the control (580%; $P = 0.019$) and SF diet (240%; $P = 0.02$). There were no significant changes in other fatty acid profiles of the milk fat in this study; however, the level of heptadecanoic acid (C17:0) in the milk fat of cows fed on the SB diet tended to be higher ($P = 0.067$) than the control.

The increase in the C18:2 level of the milk fat was similar to that in a previous study using an extruded soybean diet; however, this fatty acid and also C18:3 were not altered by feeding a sunflower seed diet (Schingoethe *et al*., 1996). The greater levels of C18:2 and C18:3 in the current

Table 4 Blood composition (average on day 28) of cows fed supplements with soybean oil (SB) and sunflower oil (SF). Fatty acid components of plasma are expressed as g/100g fatty acid.

	Supplement			Pooled	*P*
	Control	SB	SF	SD	
NEFA	0.326[a]	0.303[b]	0.303[b]	0.019	0.087
Fatty acids	———— % of total fatty acids ————				
C16:0	19.40[a]	17.51[b]	16.22[b]	2.03	0.013
C18:0	29.06[b]	34.43[a]	31.71[ab]	3.68	0.076
C18:1n-9	24.01[a]	15.60[b]	18.54[b]	5.42	0.005
C18:2n-6	27.54	31.69	33.19	6.19	0.319
C18:3n-3	N/A	N/A	N/A	N/A	N/A

[a,b] = Means within row with different superscripts differ significantly at $P < 0.05$.

experiment could have resulted from the concentration of PUFA contained in the oils being higher than that in oilseeds. These levels would be supported by evidence of higher levels of C18:2 and other long chain fatty acids produced in the milk from cows fed on a supplement of soybean oil and cottonseed oil (Zheng *et al*., 2005). In addition, the proportion of C18:2 increased in the milk fat of cows fed on a diet of fresh forage plus solin (a new cultivar of flax), when compared to a diet of fresh forage with or without a tallow supplement (Ward *et al*., 2002).

There is evidence that supplementing the diet of Holstein cows with sunflower oilseeds resulted in decreases in the levels of medium chain fatty acids (such as C8:0, C12:0, C14:0 and C16:0) and increases in long chain fatty acids (such as C18:0 and C18:1), without any change in either C18:2n-6 or C18:3n-3 (Bett *et al*., 2004). Another report demonstrated that milk fat from cows supplemented with sunflower and soybean oils resulted in a reduction in the concentration of short and medium chain fatty acids and an increase in the concentration of long chain fatty acids, especially C18:1 and C18:2n-6; however, no effect on C18:3n-3 was found in that experiment (Rego *et al*., 2005). In addition, a higher concentration of C18:3n-3 in milk fat was found by feeding a flaxseed-rich diet (Petit, 2003). Most experiments indicated that feeding vegetable oil-rich diets often showed higher proportions of unsaturated fatty acids and lower amounts of saturated fatty acids in the milk fat (Schingoethe *et al*., 1996; Ashes *et al*., 1997; Bett *et al*., 2004; Rego *et al*., 2005).

As mentioned above, these inconsistent results in milk fatty acid profiles can be explained by the main factors that influence the conversion of dietary fat into milk fat including: 1) the lipid type and its amount of dietary; 2) the degree of inertness or protection; 3) ruminal biohydrogenation; 4) absorption or digestibility; 5) adipose tissue deposition; and 6) mammary

Table 5 Fatty acid composition of milk fat (average on day 28; g/100g fatty acid) of cows fed supplements with soybean oil (SB) and sunflower oil (SF).

	Supplement			Pooled	*P*
	Control	SB	SF	SD	
	———— % of total fatty acids ————				
C8:0	0.39	0.53	0.32	0.18	0.169
C10:0	1.75	1.97	1.03	0.69	0.194
C11:0	0.10	0.07	0.02	0.08	0.342
C12:0	5.30	6.14	4.30	1.90	0.432
C13:0	0.14	0.08	0.04	0.11	0.515
C14:0	12.54	9.33	11.50	4.18	0.594
C14:1	0.73	2.19	1.04	1.22	0.367
C15:0	0.77	1.02	0.62	0.65	0.499
C16:0	35.86	32.17	30.27	6.56	0.614
C16:1	1.87	1.91	0.80	1.11	0.236
C17:0	0.24	0.64	0.39	0.31	0.156
C18:0	9.77	9.41	10.77	6.36	0.410
C18:1n-9	28.46	30.00	30.02	11.57	0.958
C18:2n-6	1.75[b]	3.20[a]	2.36[a]	1.04	0.000
C18:3n-3	0.05[b]	0.34[a]	0.10[b]	0.18	0.027

[a,b] = Means within row with different superscript differ significantly at $P < 0.05$.

gland metabolism (Palmquist *et al*., 1993; Ashes *et al*., 1997). Even though unprotected fat incorporated with ruminal biohydrogenation was thought to be the main factor in this study, increases in C18:2n-6 and C18:3n-3 levels in the milk fat, without any changes in these PUFA in the plasma and ruminal fluid, would have resulted from the absorbed bypass PUFA in the intestine, which was taken up directly by the mammary glands. These long-chain fatty acids that were taken up have been reported to inhibit de novo synthesis of short-chain fatty acids in mammary tissue (Palmquist *et al*., 1993). However, higher C18:2n-6 and C18:3n-3 levels, without any changes in other fatty acids (C10:0, C12:0, C14:0, C16:0 and C18:0), reflected unchange in the milk fat percentage of the current trial. High amounts of trans fatty acids (*trans*-FA), either from dietary sources or from incomplete ruminal biohydrogenation, had been thought to inhibit milk fat or fatty acid synthesis (Palmquist *et al*., 1993). Unfortunately, *trans*-FA were not evaluated in the current study, due to technical limitations with the analysis procedure. However, by using C18:1 as a representative of *trans*-C18:1 (Palmquist *et al*., 1993), lower C18:1 and higher C18:0 levels were observed in the ruminal fluid and plasma of SB- and SF-fed cows, which might indicate that total ruminal biohydrogination led to less of this intermediate compound that inhibits milk fat synthesis.

CONCLUSIONS

The current study indicated that both soybean oil and sunflower oil can be used as dietary fat supplements to increase the proportion of polyunsaturated fatty acids in milk fat, without interfering with either milk production or milk composition. The balance between amounts of long-chain fatty acids taken up by the mammary gland and de novo synthesis of fatty acids derived from ruminal biohydrogenation are thought to play a role in these results. Feeding both SB- and SF-rich diets increased the level of linoleic acid (C18:2n-6), and the SB supplement increased the level of linolenic acid (C18:3n-3) in milk fat, indicating that soybean oil and sunflower oil are appropriated sources for the production of milk high containing omega-3 and omega-6, which would reflect an improvement in the dairy products from Thailand and other tropical countries.

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