Milk performance of dairy cows supplemented with rapeseed oil, peanut oil and sunflower seed oil

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ABSTRACT: The objective of the study was to investigate the effects of supplementing different plant oils to the basal diet on milk yield and milk composition in mid-lactating dairy cows. Forty Chinese Holstein dairy cows averaging 120 days in milk (DIM) at the start of the experiment (body weight = 580 ± 18.2 kg; milk yield = 33.0 ± 2.00 kg/day) were used in a completely randomized block design. The animals were assigned to four dietary treatments according to DIM and milk yield, and supplemented with no oil (control), 2% rapeseed oil (RSO), 2% peanut oil (PNO) and 2% sunflower seed oil (SFO). Milk yield and milk composition (fat, protein, and lactose) were measured. Dry matter intake was similar in all treatments. The supplementation of plant oil increased milk yield, with the highest milk yield in RSO group. Percentages of milk fat, lactose, solids-not-fat and SCC were not affected by treatments except for an increase in milk protein content in oil supplemented groups. The fatty acid (FA) profile of milk was altered by fat supplementation. Feeding plant oils reduced the proportion of both short-chain (C4:0 to C12:0) and medium-chain (C14:0 to C16:1) fatty acids, and increased the proportion of long-chain (≥ C18:0) fatty acids in milk fat. The inclusion of vegetable oils increased the concentration of *cis*-9, *trans*-11 CLA. The *cis*-9, *trans*-11 CLA content in milk fat was higher from RSO to PNO and SFO was higher than the control. The TVA concentration was higher in the SFO diet, followed by PNO, RSO, and control diets. The results of this study indicated that linoleic acid was more effective in enhancing contents of TVA and CLA in milk fat than oleic acid. No significant effects of week and treatment by week interaction were found out in this study. Overall, feeding plant oils increased monounsaturated and polyunsaturated fatty acids and decreased saturated fatty acids in milk fat. In conclusion, dietary supplementation of RSO increases milk yield the most, while SFO enhances the *cis*-9, *trans*-11 CLA content in milk fat more effectively.

Keywords: conjugated linoleic acid; oleic acid; fatty acid; milk performance

One of the main limiting factors for milk production of the high-yielding dairy cow is the intake of energy. It has been shown that energy and protein are the most significant factors affecting milk performance (NRC, 2001). Fat supplements such as oilseeds are commonly added to ruminant diets to increase caloric density and to enhance the proportions of desirable unsaturated fatty acids in edible products (Raes et al., 2004).

On the other hand, the term "functional foods" is increasingly used as a generic description for the beneficial effects of ingested foods that go beyond their traditional nutritive value. Milk containing functional factors is more and more favourable and conjugated linoleic acid (CLA) is a hot topic. Previous studies have shown that it is possible to alter the fatty acid composition of milk fat and the CLA content (Zheng et al., 2005; Ye et al., 2009). Interest in CLA is increasing because it may be beneficial to health, including the potent anticarcinogenic activity. CLA refers to a group of positional and geometrical isomers of linoleic acid (*cis*-9, *cis*-12 octadecadienoic acid) with conjugated double bonds. It is derived from endogenous desaturation of 18:1 *trans*-11 and produced by the reaction involving the microorganism *Butyrivibrio fibrisolvens* in the ru-

men (Kim et al., 2008). There are many CLA isomers, such as *cis*-9, *trans*-11 and *trans*-10, *cis*-12, but the main isomer in milk fat is the *cis*-9, *trans*-11 CLA (Bauman et al., 1999; Shingfield et al., 2006).

Diet is by far the most influential factor determining the concentration of CLA in milk fat. In the rumen, CLA is formed primarily from isomerization of dietary linoleic acid. Linoleic and linolenic acids are converted to several monoene and diene intermediates containing *trans*-11 bonds during bio-hydrogenation (e.g. trans-vaccenic acid, TVA). But oleic acid is also very common in ruminant diets, which is usually described as being hydrogenated directly to stearic acid without the formation of *trans* intermediates. However, some researches demonstrated that the bio-hydrogenation of oleic acid by mixed ruminal microbes involves the formation of several positional isomers of *trans* monoenes rather than only direct bio-hydrogenation to form stearic acid (Harfoot and Hazelwood, 1988; Mosley et al., 2002). So, oleic acid may be another important precursor for the synthesis of CLA. Rapeseed oil, peanut oil and sunflower seed oil were chosen because they are readily available to dairy producers in southern China. Rapeseed oil and peanut oil were chosen in this experiment for their high content of C18:1 and sunflower seed oil was chosen because of its high C18:2, which are precursors of CLA.

In previous research (Zheng et al., 2005; Ye et al., 2009), supplementing vegetable oil led to significant increases in the concentration of CLA in milk fat of dairy cows and feeding oils rich in linoleic acid was more effective in enhancing *cis*-9, *trans*-11 CLA in milk fat than oils containing linolenic acid. However, few studies were focused on how oleic affected the milk fat composition. Although peanut oil contains similar oleic and linoleic acid compared to rapeseed oil, limited data is available on the effect of peanut oil on dairy cows especially on milk FA profiles.

Therefore, the objective of this study was to determine the effects of rapeseed oil, peanut oil and sunflower seed oil on milk production, milk composition and FA profiles in Holstein dairy cows.

MATERIAL AND METHODS

Animals, diets and experimental design

Forty multiparous Chinese Holstein dairy cows averaging 120 DIM (BW = 580 ± 18.2 kg; milk yield = 33.0 ± 2.00 kg/day) were used. Animals were divided into 10 groups according to DIM and milk production and assigned to 4 treatments randomly within groups to evaluate the response to oil supplementation. Cows were housed in a tie-stall barn, and fed and milked at 06:00, 14:00, and 20:00 h. All animals had free access to drinking water. Feed was offered to result in 10% orts. The experiment lasted 66 days with 10 days for adaptation and 56 days for data collection.

The ingredients and composition of experimental diets are presented in Table 1. Diets were a 45:55 blend of forage and concentrate (DM basis). The basal diet was formulated to meet energy and protein requirements of Chinese Holstein dairy cows (Wang et al., 2007) (Table 1) and supplemented with none (control), 2% rapeseed oil (RSO), 2% peanut oil (PNO) or 2% sunflower seed oil (SFO). Supplemented oil increased the ether extract (EE) content of diets in the RSO, PNO, and SFO diets compared with the control diet (3.4% vs. 5.0% of DM).

Sampling, measurement, and analyses

The forage and concentrates were sampled weekly to determine the DM content and the diets were adjusted to account for changes in DM content. Diets offered and refused were recorded for two consecutive days weekly throughout the trial. One aliquot of samples was dried in an air-forced oven at 55°C for 48 h and stored in sealed plastic containers at room temperature until analysed. When prepared for analyses, dried samples of forages and concentrates were ground first through a 2 mm screen (Thomas-Wiley Laboratory Mill, Arthur H. Thomas, Philadelphia, USA), then through a 1 mm screen in a Cyclotec mill (Tecator 1093, Hoganas, Sweden). Dry matter (DM) was determined by drying a subsample at 105°C for 24 h. All samples were analysed for DM, crude protein (CP), ether extract (EE) (AOAC, 1990) and neutral detergent fibre (NDF) (Van Soest et al., 1991). The other aliquot of samples (kept at –20°C) was pooled at the end of the experiment and freeze-dried, ground and analysed for FA content.

Milk weight was recorded at each milking (3 times daily). Two 50-ml aliquots of milk were collected weekly at each milking proportionally to yield (4:3:3, composite). One aliquot containing Bromopol (milk preservative; D&F Control Systems, San Ramon, USA) was stored at 4°C for

Table 1. Ingredients and composition of experimental diets

Item*	Diet[1]			
	control	RSO	PNO	SFO
Ingredients (% of DM)				
Forage[2]	45.7	45.7	45.7	45.7
Ground maize grain	21.2	20.2	20.2	20.2
Soybean meal 42.5% CP	9.2	9.0	9.0	9.0
Extruded soybeans	1.3	1.3	1.3	1.3
Cottonseed meal	1.7	1.7	1.7	1.7
Barley	9.1	8.5	8.5	8.5
Wheat bran	2.0	2.0	2.0	2.0
DDGS	4.0	4.0	4.0	4.0
Beet pulp pellets	1.5	1.5	1.5	1.5
Oils[1]	–	2.0	2.0	2.0
Macro mineral[3]	3.2	3.2	3.2	3.2
Premix[4]	0.9	0.9	0.9	0.9
Composition (% of DM)				
CP	16.3	16.4	16.5	16.5
RUP[5]	6.7	6.7	6.8	6.7
RDP[6]	9.8	9.7	9.7	9.8
Ether extract	3.5	5.6	5.5	5.6
NDF	36.8	36.9	36.6	36.5

*DDGS = dried distillers grains plus soluble; DM = dry matter; CP = crude protein; RUP = rumen undegradable protein; RDP = rumen degradable protein; NDF = neutral detergent fibre

[1]control = no supplemental oil; RSO = 2% rapeseed oil; PNO = 2% peanut oil; SFO = 2% sunflower seed oil

[2]consiting of lucerne meal (18.0%), maize silage (17.7%) and Chinese wildrye (10.0%)

[3]macro mineral: dicalcium phosphate (1.2%), limestone (0.8%), saleratus (0.7%), and salt (0.5%)

[4]supplement contained (per kg of DM): 1 000 000 IU vitamin A, 200 000 IU vitamin D, 1250 IU vitamin E, 14 000 mg Zn, 100 mg Se, 180 mg I, 3000 mg Fe, 40 mg Co, 3000 mg Mn, 3000 mg Cu

[5,6]calculated based on individual feedstuffs in CNSAPH (2000)

the later analysis of fat, protein, and lactose by infrared analysis (Laporte and Paquin, 1999) with a four-channel spectrophotometer (Milko-Scan, Foss Electric, Hillerød, Denmark) and somatic cell counts (SCC) using a cell counter (Fossmatic 400; Foss Electric, Hillerød, Denmark). The aliquot was stored at –20°C for the analysis of fatty acids.

Diets and the second aliquot of milk samples were analysed for fatty acids. Milk samples were thawed and then centrifuged at 10 000 × g for 1 h to harvest milk fat. Methylation was performed by *in situ* transesterification with 0.5N methanolic NaOH followed by 14% boron trifluoride in methanol (Loor and Herbein, 2001). Fatty acid methyl esters (FAME) were separated by gas chromatograph (Agilent GC6890N) equipped with a flame ionization detector using a fused silica capillary column [100 m × 0.25 mm (*i.d.*) with 0.2 μm thickness; Varian, Inc., Walnut Creek, USA]. The identification of fatty acids was carried out by comparison of gas chromatography peaks with peaks of known standards (GLC-60; Nu Chek Prep Inc., Elysian, USA and Matreya, Inc., Pleasant Gap, USA). Results for each fatty acid were expressed as percentage of the sum of total fatty acids.

Blood samples (10 ml) were collected biweekly approximately 3 h after feeding. Blood sample was drawn from the coccygeal vein and centrifuged at

3000 × g for 15 min (Wang et al., 2008). Serum was frozen at –10°C and later thawed for the analysis of blood urea nitrogen (Wang et al., 2007), nonesterified FA (NEFA; McCutcheon and Bauman, 1986), total protein (Thomas et al., 1998), cholesterol (Deeg and Ziegenhorm, 1983), HDL-cholesterol (HDL; Nauck et al., 1998), LDL-cholesterol (LDL; Lichtenstein et al., 1993), triglyceride (Cole et al., 1997) and glucose (Barham et al., 1972) by an automatic biochemistry analyzer (HITACHI 7020). Test kits were purchased from DiaSys Diagnostic Systems (Shanghai, P.R. China).

Statistics

All data were analyzed using the MIXED procedure of SAS software system (SAS, 2000) with the cow as the repeated subject using the covariance type AR (1). The statistical model included the effects of treatment, week and treatment × week interaction. Results are reported as the least squares means. The least squares means were estimated and separated using the pdiff option when fixed effects were significant. Probability values of $P < 0.05$ were used to define statistically significant results, with statistical trends being defined at $P < 0.10$.

RESULTS AND DISCUSSION

Diet characteristics

The chemical composition of diets is shown in Table 1. Diets were formulated to be isonitrogenous and to meet minimum rumen degradable protein (RDP) and rumen undegradable protein (RUP) requirements according to NRC (2001). The control

Table 2. Fatty acid composition of experimental rations (g/100 g total fatty acid basis)

Fatty acids	Diet[1]			
	control	RSO	PNO	SFO
C12:0	0.25	0.30	0.26	0.25
C14:0	0.32	0.45	0.38	0.30
C14:1	0.87	0.66	0.32	0.56
C15:0	1.19	0.79	0.64	1.00
C16:0	15.20	12.83	17.00	12.10
C16:1	1.54	1.62	1.50	1.11
C18:0	13.50	10.10	8.28	9.90
C18:1 *cis*-9	19.98	30.12	27.40	19.80
C18:1 *cis*-11	0.61	1.12	1.04	0.71
C18:2	23.90	26.87	31.60	41.46
C18:3	3.06	4.67	2.21	2.20
C20:0	0.20	0.13	0.20	0.16
C20:1	0.07	0.06	0.11	0.05
SFA[2]	30.66	24.60	26.76	23.71
MUFA[3]	23.07	33.58	30.37	22.23
PUFA[4]	26.96	31.54	33.81	43.66
Total unsaturated FA	50.03	65.12	64.18	65.89
Others[5]	19.31	10.28	9.06	10.40

[1]control = no supplemental oil; RSO = 2% rapeseed oil; PNO = 2% peanut oil; SFO = 2% sunflower seed oil

[2]SFA = saturated fatty acids

[3]MUFA = monounsaturated fatty acids

[4]PUFA = polyunsaturated fatty acids

[5]unidentified peaks

diet contained 3.5% EE (DM basis), and the content increased to 5.6 as a result of the addition of plant oil to the diet. Consequently, the diets containing supplemental oils had a higher NE_L content. The fatty acid profile of feed was changed by RSO, PNO, and SFO compared to the control group (Table 2). The diets with RSO and PNO contained the highest concentrations of *cis*-9 C18:1 (30.1 and 27.4 g per 100 g of FA, respectively) while the diet with SFO had the highest concentrations of C18:2 (41.5 g per 100 g of FA). Oil supplemented diets had higher monounsaturated FA (MUFA) and polyunsaturated FA (PUFA) and lower saturated FA (SFA) compared to the control. Total unsaturated FA (MUFA + PUFA) were increased by 30, 28, and 31% for RSO, PNO, and SFO diets, respectively, compared with the control. No *trans* FA was detected in any of the diets.

Feed intake, milk yield and milk composition

DM intake, milk yield, and milk composition are presented in Table 3. The DM intake was not different among treatments ($P > 0.1$, average 21.3 kg per day), consistently with previous studies (Loor et al., 2005; Zheng et al., 2005; Ye et al., 2009). The examination of previously published literature indicates that the effect of supplemental oils on DMI varied among experiments (Eugčne et al., 2008; Hess et al., 2008; Ye et al., 2009). Previous researches (AbuGhazaleh et al., 2002; Bell et al., 2006) reported that DMI was decreased by fat supplements when replacing the concentrate with fish oil or 6% of DM plant oil. Bremmer et al. (1998) reported that unsaturated FA are more likely to depress DMI than are saturated FA. However, in the present study DMI was not affected by fat supplementation, which may be due to the lower proportion of supplemented oil, which is consistent with the results of Gozho et al. (2008) when DMI was similar in the cows fed a diet that contained canola or flax as a supplemental fat source. Also, Onetti et al. (2001) reported that there was a trend of lower DMI in cows receiving 4% supplemental fat compared to those applied 2% supplemental fat, which may be due to a decrease in the ration digestibility (Martin et al., 2008).

Daily milk yield of cows fed RSO, PNO and SFO was significantly ($P < 0.05$) higher than that of the control cows, while the milk fat percentage was similar among treatments, resulting in higher FCM in oil supplemented groups. Yields of milk, milk protein and fat of cows remained relatively con-

Table 3. Effects of oil sources on dry matter intake, milk yield and milk composition in dairy cows in mid-lactation

Item	Diet[1]				SEM	*P*-value
	control	RSO	PNO	SFO		
Dry matter intake (kg/day)	21.3	21.3	21.0	21.4	0.21	0.68
Milk yield (kg/day)						
Milk	25.5^{c}	27.8^{a}	26.3^{b}	26.4^{b}	0.20	< 0.05
4% FCM	23.4^{c}	26.0^{a}	25.0^{b}	25.2^{b}	0.18	< 0.05
Protein	0.76^{b}	0.87^{a}	0.85^{a}	0.85^{a}	0.02	< 0.05
Fat	0.88^{b}	1.00^{a}	0.97^{a}	0.98^{a}	0.03	< 0.05
Milk composition (%)						
Protein	3.02^{b}	3.12^{ab}	3.24^{a}	3.23^{a}	0.05	< 0.05
Fat	3.57	3.60	3.65	3.67	0.09	0.34
Lactose	5.04	5.08	5.10	5.12	0.09	0.61
Solids-not-fat	8.80	8.92	9.00	9.05	0.09	0.67
SCC × 10^4/ml	30.2	31.3	32.3	32.5	0.80	0.84

[1]control = no supplemental oil; RSO = 2% rapeseed oil; PNO = 2% peanut oil; SFO = 2% sunflower seed oil FCM = fat-corrected milk; SCC = somatic cell count

[a,b,c]means within the same row with different superscripts differ ($P < 0.05$)

stant throughout the experiment and no interaction of treatment by week was found out ($P > 0.05$), which may be due to the mid-lactation period. An increase in milk yield caused by fat supplementation has been reported (Loor et al., 2005, using 3% oil with hay-based diet; Bu et al., 2007, using 4% oil with hay/maize silage-based diet). When estimated using the NRC (2001) model, a predicted 2.0 kg/day increase in net energy for lactation (NE_L) allowable milk from feeding fat vs. control. Therefore, the increased milk production was consistent with the increased dietary energy density. In contrast, some researchers reported increased or similar milk yield of cows receiving fat supplementation (Onetti et al., 2001). The decreased milk yield may be due to decreased DMI or ruminal fermentation changes (e.g. 6% vs. 2% fat supplementation).

The milk fat content was not affected by fat supplementation (Table 3). However, fat supplementation at 2% of dietary DM resulted in an average 11.7% increase in milk fat yield compared with that of the control treatment. Some researchers (Bell et al., 2006) reported that fat supplementation resulted in a decrease in milk fat content while others reported no effect (Ward et al., 2002; Bu et al., 2007). The reduction of milk fat content may be due to the fact that too much unsaturated FA destroy the membrane of bacteria and further decrease the fibre digestion, which may cause decreased acetate production (Onetti et al., 2001; Ye et al., 2009). However, in this study the higher milk yield of cows supplemented with 2% fat indicated no negative effect on rumen fermentation and DMI. Neither did the chemical composition of diets (Table 1) reveal anything that might contribute to the effect on the rumen environment. Milk protein content and yield of PNO and SFO were higher than those of the control ($P < 0.05$). Fat supplementation increases the efficiency of ruminal bacterial protein synthesis associated with the increased degree of unsaturation of dietary fat (Oldick and Firkins, 2000). And this increased ruminal bacterial efficiency may be due to the decline in protozoal counts that leads to decreased intraruminal bacterial recycling (Jenkins, 1993) or to an increase in urea-N transfer from blood to rumen (Gozho et al., 2008). However, a summary of the literature on fat supplementation showed a 0.15% decrease in milk protein when tallow was fed to dairy cows (Shaver, 1990). These differences may be due to different saturation of fat sources. Percentage of milk fat, total solids, solids-not-fat and SCC did not vary among treatments and no effect of week and treatment by week interaction was found out in any production parameters ($P > 0.05$).

Fatty acid composition of milk fat

The FA profile of milk was altered by oil supplementation (Table 4). Supplementing plant oil reduced ($P < 0.05$) the proportion of both short-chain (C4:0 to C12:0) and medium-chain FA (C14:0 to C16:1), and increased ($P < 0.05$) the proportion of long-chain FA (≥ C18:0) in milk fat. The addition of fat through seeds or as a free oil reduced the proportions of short-chain and medium-chain FA in milk. This apparent reduction in *de novo* synthesis of FA (≤ 16:0) in the mammary gland has been reported in diets that increase the supply of long-chain FA (Grummer, 1991). As expected, feeding RSO and PNO increased the proportion of C18:1 in milk fat compared with the control and SFO, with the lowest proportion in the control. SFO group had the highest C18:2 content in milk fat. The inclusion of vegetable oils increased the concentration of *cis*-9, *trans*-11 CLA. And the *cis*-9, *trans*-11 CLA content in milk fat was higher from RSO to PNO and SFO was higher than the control. The *cis*-9, *trans*-11 CLA content in milk fat was highest ($P < 0.05$) in response to SFO, although sunflower seed oil had the lowest C18:1, compared with the other two vegetable oils. The fact that the sunflower seed oil was rich in C18:2 may explain this observation. The TVA concentration was higher for the SFO diet, followed by PNO, RSO, and control diets. The higher TVA concentration in milk may be due to an increase in TVA concentration in the rumen (Varadyova et al., 2010). It is reported that little *cis*-9, *trans*-11 CLA actually accumulates in the rumen and the main milk CLA is derived from TVA in the mammary gland through the action of Δ-9 desaturase (Griinari et al., 2000). Thus, an increase in the level of *trans*-11 C18:1 in the rumen would be expected to increase milk CLA. In the present study feeding SFO increased the TVA concentration to the greatest extent (three fold compared to the control milk), which was in agreement with Loor et al. (2002). Rego et al. (2009) reported that compared to other plant oils rapeseed oil supplied mostly *cis*-9C18:1, resulting in lower total bio-hydrogenation intermediates, which may be due to less 18:1 isomers. Mosley et al. (2002) demonstrat-

Table 4. Effect of oil source on fatty acid composition in milk (% total milk FA measured)

Fatty acids	Diet[1]				SEM	*P*-value
	control	RSO	PNO	SFO		
C4:0	3.41	3.15	3.10	3.20	0.090	0.31
C6:0	1.99	1.87	1.93	1.89	0.060	0.29
C8:0	1.50	1.40	1.47	1.41	0.030	0.32
C10:0	3.37^{a}	2.62^{b}	2.71^{b}	2.67^{b}	0.041	< 0.05
C12:0	3.47^{a}	2.05^{c}	2.26^{b}	2.36^{b}	0.020	< 0.05
C14:0	11.71^{a}	8.13^{b}	7.97^{b}	7.87^{b}	0.018	< 0.05
C14:1	0.82^{b}	0.65^{b}	0.71^{b}	0.67^{b}	0.017	< 0.05
C15:0	1.10^{a}	0.90^{b}	0.89^{b}	0.86^{b}	0.007	< 0.05
C16:0	26.00^{a}	20.25^{b}	20.60^{b}	20.40^{b}	0.330	< 0.05
C16:1	1.19^{a}	0.97^{b}	0.94^{bc}	0.91^{c}	0.015	< 0.05
C18:0	10.26^{c}	12.93^{a}	12.54^{b}	12.82^{a}	0.115	< 0.05
C18:1	23.24^{d}	31.71^{a}	29.10^{b}	27.64^{c}	0.223	< 0.05
C18:1 *trans*-11	1.81^{d}	3.19^{c}	4.16^{b}	5.40^{a}	0.017	< 0.05
C18:2	1.99^{d}	2.40^{c}	2.73^{b}	3.17^{a}	0.010	< 0.05
C18:3 *cis*-9,12,15	0.41^{a}	0.38^{ab}	0.33^{b}	0.32^{b}	0.020	< 0.05
C18:2 *cis*-9, *trans*-11	0.94^{d}	1.06^{c}	1.26^{b}	1.47^{a}	0.023	< 0.05
C20:0	0.23^{b}	0.29^{a}	0.23^{b}	0.20^{b}	0.009	< 0.05
C20:1(n-9)	0.14^{b}	0.21^{a}	0.12^{b}	0.13^{b}	0.003	< 0.05
C20:4(n-6)	0.81^{d}	0.67^{b}	0.70^{a}	0.66^{c}	0.009	< 0.05
C20:5(n-3)	0.05	0.06	0.06	0.06	0.003	0.49
C22:1(n-9)	0.02	0.04	0.03	0.03	0.001	0.30
C22:6(n-3)	0.05	0.04	0.06	0.05	0.010	0.72
Unidentified	5.49	5.03	6.10	5.81	0.420	0.45
SFA[2]	63.04^{a}	53.59^{b}	53.70^{b}	53.68^{b}	1.020	0.47
MUFA[3]	27.22^{b}	36.77^{a}	35.06^{a}	34.78^{a}	0.780	< 0.05
PUFA[4]	4.25^{c}	4.61^{ab}	5.14^{a}	5.73^{a}	0.200	< 0.05
UFA[5]	31.47^{b}	41.38^{a}	40.20^{a}	40.51^{a}	0.890	< 0.05

[1]control = no supplemental oil; RSO = 2% rapeseed oil; PNO = 2% peanut oil; SFO = 2% sunflower seed oil

[2]SFA = saturated fatty acids

[3]MUFA = monounsaturated fatty acids

[4]PUFA = polyunsaturated fatty acids

[5]UFA = total unsaturated fatty acids (MUFA + PUFA)

[a,b,c]means within the same row with different superscripts differ ($P < 0.05$)

ed that oleic acid, which was previously thought to be bio-hydrogenated only into stearic acid, may also be a precursor for several *trans* fatty acid isomers, including TVA. However, the result in this study indicated that linoleic acid was more effective in enhancing contents of TVA and CLA in milk fat than oleic acid. Overall, the cows fed RSO, PNO and SFO produced the milk with higher content of MUFA and PUFA and lower content of saturated FA ($P < 0.05$) in milk FA than those fed the control diet. Similar differences were found in previous researches (Donovan et al., 2000; AbuGhazaleh et al., 2002; Whitlock et al., 2002). Consistently with other studies, the C16:0 content was decreased in

oil-fed groups (Liu et al., 2008; Vesely et al., 2009). In terms of human health, these changes may represent an improvement in the FA profile of milk due to the fact that medium-chain and saturated FA have been reported to constitute the hypercholesterolaemic portion of milk fat (Ney, 1991).

Results concerning the continuous effect of supplemental vegetable oils on the milk fat composition have varied among researchers (AbuGhazaleh et al., 2004; Ryhanen et al., 2005; Shingfield et al., 2006). There are reports that the inclusion of vegetable oils in diets raises the *trans*-11C18:1 and *cis*-9, *trans*-11C18:2 content in milk fat, but the initial increase in the first week was shown to be transient (AbuGhazaleh et al., 2004; Shingfield et al., 2006). AbuGhazaleh et al. (2004) found out that *cis*-9, *trans*-11 CLA increased in the first week of fat supplementation and then declined and remained relatively constant, being approximately by 230% higher throughout the experiment than the control. But others found out that cows fed grass silage supplemented with a concentrate containing rapeseed oil which increased the levels of *trans*-11C18:1 and *cis*-9, *trans*-11C18:2 that appeared to be retained for 7 weeks (Ryhanen et al., 2005). In the present study, no effect of week and treatment by week interaction was observed ($P > 0.05$), which may suggest that in the present study the diet did not change the rumen environment and the persistency of milk FA composition responses to these three plant oils.

The effect of lipid supplements on the processing and manufacturing properties of milk was less well documented in previous research. Ryhanen et al. (2005) reported that cheese manufactured from CLA enriched milk required a long ripening period. The longer ripening time required to attain satisfactory grading score may arise because of the high concentration of unsaturated FA in cheese made from high CLA milk which due to that oil supplement alter the saturation of the FA in milk fat and reduce the starter culture activity. Butter produced form CLA enriched milk was also softer than the control (Ryhanen et al., 2005). The increase in softness is attributed to the high proportion of unsaturated FA, since increases in the ratio of oleic to palmitic acid result in a softer fat (Plamquist et al., 1993). In the present study, the higher oleic acid in RSO may result in a softer fat compared with the control and other oil supplemented groups.

Plasma metabolites

Supplementing fat did not affect the concentrations of glucose, NEFA, total protein, LDL-cholesterol, triglyceride, and blood urea nitrogen in blood compared with feeding the control diet (Table 5). However, daily supplementation of plant oils increased serum cholesterol and HDL-cholesterol ($P < 0.05$). Similar increases in cholesterol and/or NEFA in the blood plasma of cows fed fat have been reported by others (Petit et al., 2002; Gonthier et al., 2005). Drackley (1999) found that a 1 kg/day increase in dietary FA intake resulted in an increase in NEFA concentration by 0.08mM in lactating

Table 5. Changes in plasma variables of cows fed different oil sources

Item	Diet[1]				SEM	*P*-value
	control	RSO	PNO	SFO		
Glucose (mmol/l)	3.35	3.18	3.37	3.41	0.09	0.70
NEFA[2] (mmol/l)	0.09	0.11	0.14	0.12	0.06	0.35
Total protein (g/l)	76.0	74.9	72.9	75.4	1.00	0.37
Cholesterol (mmol/l)	6.11[b]	6.43[a]	6.33[b]	5.97[c]	0.09	< 0.05
LDL-cholesterol (mmol/l)	1.22	1.37	1.25	1.30	0.05	0.56
HDL-cholesterol (mmol/l)	2.48[b]	2.73[ab]	2.83 [a]	2.62[ab]	0.11	< 0.05
Triglyceride (mmol/l)	0.36	0.36	0.35	0.30	0.03	0.80
Blood urea nitrogen (mmol/l)	5.47	5.14	5.30	5.21	0.11	0.52

[1]control = no supplemental oil; RSO = 2% rapeseed oil; PNO = 2% peanut oil; SFO = 2% sunflower seed oil

[2]NEFA = nonesterified fatty acid

[a,b,c]means within the same row with different superscripts differ ($P < 0.05$)

cows. Advantages of adding oil to the cow's diet include increased energy density of the diet and energy intake by the animals. And NEFA can be used as an energy source by many tissues, including skeletal muscle and hepatocytes, i.e. they can be used for energy production, re-packaged into triglycerides and exported as very LDL-cholesterol or stored within the liver, or converted to ketones. The higher NEFA in cows supplemented with fat may be associated with their greater nutrient demands for milking. However, although there was a numerical increase of NEFA in oil supplemented diets, it did not reach significance ($P > 0.05$). The higher HDL and numerically lower LDL in cows consuming PNO may indicate an improvement of the blood lipid profile. And this may be due to the fact that PNO contains proportionally more MUFA than PUFA, which needs further research.

CONCLUSIONS

Dietary oil sources at a total of 2% fat (DM basis) supplemented to lactating cow diets improved the milk yield and FCM of dairy cows. Oil supplementation did not affect the milk composition except for an increase in milk protein content. The concentrations of CLA and *trans*-vaccenic acid were highest in the milk of cows supplemented with SFO compared with the other groups. Under similar feeding conditions, oils rich in linoleic acid (SFO) are more effective in enhancing the content of milk CLA than oils containing oleic acid (RSO and PNO). In addition to the increase of CLA, diets including plant oils resulted in other favourable changes in the FA profile such as a decrease in saturation.

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REFERENCES

AbuGhazaleh A.A., Schingoethe D.J., Hippen A.R., Kalscheur K.F., Whitlock L.A. (2002): Fatty acid profiles of milk and rumen digesta from cows fed fish oil, extruded soybeans or their blend. Journal of Dairy Science, 85, 2266–2276.

AbuGhazaleh A.A., Schingoethe D.L., Hippen A.R., Kalscheur K.F. (2004): Conjugated linoleic acid increases in milk when fed fish meal and extruded soybeans for an extended period of time. Journal of Dairy Science, 87, 1758–1766.

AOAC (1990): Official Methods of Analysis. In: Herlick K. (ed.): Association of of Official Agriculture Chemists. 15th ed., Arlington, USA.

Barham D., Trinder P. (1972): An improved color reagent for the determination of blood glucose by the oxidase system. Analyst, 97, 142–145.

Bauman D.E., Baumgard L.H., Corl B.A., Griinari J.M. (1999): Biosynthesis of conjugated linoleic acid in ruminants. Proceedings of the American Society of Animal Science Online. Available from http://www.asas.org/jas/symposia/proceedings/0937.pdf (accessed Nov. 1, 2003)

Bell J.A., Griinari J.M., Kennelly J.J. (2006): Effect of safflower oil, flaxseed oil, monensin, and vitamin E on concentration of conjugated linoleic acid in bovine milk fat. Journal of Dairy Science, 89, 733–748.

Bremmer D.R., Ruppert L.D., Clark J.H., Drackley J.K. (1998): Effects of chain length and unsaturation of fatty acid mixtures infused into the abomasum of lactating dairy cows. Journal of Dairy Science, 81, 176–188.

Bu D.P., Wang J.Q., Dhiman T.R., Liu S.J. (2007): Effectiveness of oils rich in linoleic and linolenic acids to enhance conjugated linoleic acid in milk from dairy cows. Journal of Dairy Science, 90, 998–1007.

CNSAPH (2000): Nutrient Requirement and Feeding Standard of Dairy Cattle. 2rd Revision. Chinese National Station of Animal Production and Health. China Agricultural University Press, Beijing.

Cole T.G., Klotzsch S.G., McNamara J. (1997): Measurement of triglyceride concentration. In: Rifai N., Warnick G.R., Dominicazk M.H. (eds): Handbook of Lipoprotein Testing. AACC Press, Washington: 115–126.

Deeg R., Ziegenhorm J. (1983): Kinetic enzymatic method for automated determination of total cholesterol in serum. Clinical Chemistry, 29, 1798–1802.

Donovan C.D., Schingoethe D.J., Baer R.J., Ryali J., Hippen A.R., Franklin S.T. (2000): Influence of dietary fish oil on conjugated linoleic acid and other fatty acids in milk fat from lactating dairy cows. Journal of Dairy Science, 83, 2620–2628.

Drackley J.K. (1999): Biology of dairy cows during the transition period: The final frontier. Journal of Dairy Science, 82, 2259–2273.

Eugčne M., Massé D., Chiquette J., Benchaar C. (2008): Meta-analysis on the effects of lipid supplementation on methane production in lactating dairy cows. Canadian Journal of Animal Science, 88, 331–334.

Gonthier C., Mustafa A.F., Ouellet D.R., Chouinard P.Y., Berthiaume R., Petit H.V. (2005): Feeding micronized and extruded flaxseed to dairy cows: effects on blood parameters and milk fatty acid composition. Journal of Dairy Science, 88, 748–756.

Gozho G.N., Hobin M.R. Mutsvangwa T. (2008): Interactions between barley grain processing and source of supplemental dietary fat on nitrogen metabolism and urea-nitrogen recycling in dairy cows. Journal of Dairy Science, 91, 247–259.

Griinari J.M., Corl B.A., Lacy S.H., Chouinard P.Y., Nurmela K.V.V., Bauman D.E. (2000): Conjugated linoleic acid is synthesized endogenously in lactating cows by Δ-9 desaturase. Journal of Nutrition, 130, 2285–2291.

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

Harfoot C.G., Hazelwood G.P. (1988): Lipid metabolism in the rumen. In: Hobson P.N. (ed.): The Rumen Microbial Ecosystem. Elsevier Applied Science Publishers, London, 285–322.

Hess B.W., Moss G.E., Rule D.C. (2008): A decade of developments in the area of fat supplementation research with beef cattle and sheep. Journal of Animal Science, 86 (E-Suppl. 1), E188–E204.

Jenkins T.C. (1993): Lipid metabolism in the rumen. Journal of Dairy Science, 76, 3851–3863.

Kim E.J., Huws S.A., Lee M.R.F., Wood J.D., Muetzel S.M., Wallace R.J., Scollan N.D. (2008): Fish oil increases the duodenal flow of long chain polyunsaturated fatty acids and *trans*-11 18:1 and decreases 18:0 in steers via changes in the rumen bacterial community. Journal of Nutrition, 138, 889–896.

Laporte M.F., Paquin P. (1999): Near-infrared analysis of fat, protein, and casein in cow's milk. Journal of Agricultural and Food Chemistry, 47, 2600–2605.

Lichtenstein A.H., Ausman L M., Carrasco W., Jenner J.L., Gualtieri L.J., Goldin B.R., Ordovas J.M., Schaefer E.J. (1993): Effects of canola, corn, and olive oils on fasting and postprandial plasma lipoproteins in humans as a part of a National Cholesterol Education Program step 2 diet. Arteriosclerosis, Thrombosis, and Vascular Biology, 13, 1533–1542.

Liu Z.L., Yang D.P., Chen P., Lin S.B., Jiang X.Y., Zhao W.S., Li J.M., Dong W.X. (2008): Effect of dietary sources of roasted oilseeds on blood parameters and milk fatty acid composition. Czech Journal of Animal Science, 53, 219–226.

Loor J.J., Herbein J.H. (2001): Alterations in blood plasma and milk fatty acid profiles of lactating Holstein cows in response to ruminal infusion of a conjugated linoleic acid mixture. Animal Research, 51, 119–134.

Loor J.J., Quinlan L.E., Bandara A.B.P.A., Herbein J.H. (2002): Distribution of *trans*-vaccenic acid and *cis*9, *trans*11-conjugated linoleic acid (rumenic acid) in blood plasma lipid fractions and secretion into milk fat of Jersey cows fed canola or soybean oil. Animal Research, 51, 119–134.

Loor J.J., Ferlay A., Ollier A., Doreau M., Chilliard Y. (2005): Relationship among trans and conjuaged fatty acids and bovine milk fat yield due to dietary concentrate and linseed oil. Journal of Dairy Science, 88, 726–740.

Martin C., Rouel J., Jouany J.P., Doreau M., Chilliard Y. (2008): Methane output and diet digestibility in response to feeding dairy cows crude linseed, extruded linseed, or linseed oil. Journal of Animal Science, 86, 2642–2650.

McCutcheon S.N., Bauman D.E. (1986): Effect of chronic growth hormone treatment on responses to epinephrine and thyrotropin-releasing hormone in lactating cows. Journal of Dairy Science, 69, 44–51.

Mosley E.E., Powell G.L., Riley M.B., Jenkins T.C. (2002): Microbial biohydrogenation of oleic acid to trans isomers in vitro. Journal of Lipid Research, 43, 290–296.

NRC (2001): Nutrient Requirements of Dairy Cattle. 7th Rev. Ed. National Research Council. National Academy of Science, Washington, DC.

Nauck M., Maerz W., Wieland H. (1998): New immunoseparation-base homogenous assay for HDL-cholesterol compared with three homogenous and two heterogeneous methods for HDL-cholesterol. Clinical Chemistry, 44, 1443–1451.

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

Oldick B.S., Firkins J.L. (2000): Effects of degree of fat saturation on fiber digestion and microbial protein synthesis when diets are fed twelve times daily. Journal of Animal Science, 78, 2412–2420.

Onetti S.G., Shaver R.D., McGuire M.A., Grummer R. R. (2001): Effect of type and level of dietary fat on rumen fermentation and performance of dairy cows fed corn silage-based diets. Journal of Dairy Science, 84, 2751–2759.

Petit H.V., Dewhurst R.J., Scollan N.D., Proulx J.G., Khalid M., Haresign W., Twagiramungu H., Mann G.E. (2002): Milk production and composition, ovarian function, and prostaglandin secretion of dairy cows fed omega-3 fats. Journal of Dairy Science, 85, 889–899.

Raes K., S. De Smet, Demeyer D. (2004): Effect of dietary fatty acids on incorporation of long chain polyunsaturated fatty acids and conjugated linoleic acid in lamb, beef and pork meat: a review. Animal Feed Science and Technology, 113, 199–221.

Rego O.A., Alves S.P., Antunes M.S., Rosa H.J.D., Alfaia C.F., Prates J.A.M., Cabrita A.R.J., Fonseca A.J.M., Bessa R.J.B. (2009): Rumen biohydrogenation-derived fatty acids in milk fat from grazing dairy cows supplemented with rapeseed, sunflower, or linseed oils. Journal of Dairy Science, 92, 4530–4540.

Ryhanen E.-L., Tallavaara K., Griinari J.M., Jaakkola S., Mant-ere-Alhonen S., Shingfield K.J. (2005): Production of conjugated linoleic acid enriched milk and dairy products from cows receiving grass silage supplemented with a cereal-based concentrate containing rapeseed oil. International Dairy Journal, 15, 207–217.

Shaver R.D. (1990): Fat sources for high producing dairy cows. In: Proc. 51st Minnesota Nutrition Conference, Bloomington, 13–42.

Shingfield K.J., Reynolds C.K., Hervas G., Griinari J.M., Grandison A.S., Beever D.E. (2006): Examination of the persistency of milk fatty acid composition responses to fish oil and sunflower oil in the diet of dairy cows. Journal of Dairy Science, 89, 714–732.

Thomas L. (ed.) (1998): Clinical Laboratory Diagnostics. 1st ed. TH-Books Verlagsgesellschaft, Frankfurt, 644–647.

Van Soest P.J., Bobertson J.B., Lewis B.A. (1991): Methods of dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. Journal of Dairy Science, 74, 3583–3597.

Varadyová Z., Kišidayová S., Lauková A., Jalč D. (2010): Influence of inoculated maize silage and sunflower oil on the *in vitro* fermentation, ciliate population and fatty acid outpouts in the rumen fluid collected from sheep. Czech Journal of Animal Science, 55, 105–115.

Veselý A., Křížová L., Třináctý J., Hardová S., Navrátilová M., Herzig I., Fišera M. (2009): Changes in fatty acid profile and iodine content in milk as influenced by the inclusion of extruded rapeseed cake in the diet of dairy cows. Czech Journal of Animal Science, 54, 201–209.

Wang C., Liu J.X., Yuan Z.P., Wu Y.M., Zhai S.W., Ye H. W. (2007): Effect of level of metabolizable protein on milk production and nitrogen utilization in lactating dairy cows. Journal of Dairy Science, 90, 2960–2965.

Wang C., Liu J.X., Zhai S. W., Lai J.L., Wu Y.M. (2008): Effect of ratio of rumen degradable protein to rumen undegradable protein on nitrogen conversion of lactating dairy cow. Acta Agriculturae Scandinavica, Section A – Animal Science, 58, 100–103.

Ward A.T., Wittenberg K.M., Przybylski R. (2002): Bovine milk fatty acid profiles produced by feeding diets containing solin, flax and canola. Journal of Dairy Science, 85, 1191–1196.

Whitlock L., Schingoethe D.J., Hippen A.R., Kalscheur K.F., Baer R.J., Ramaswamy N., Kasperson K.M. (2002): Fish oil and extruded soybeans fed in combination increase CLA in milk of dairy cows more than when fed separately. Journal of Dairy Science, 85, 234–243.

Ye J.A., Wang C., Wang H.F., Ye H.W., Wang B.X., Liu H.Y., Wang Y.M., Yang Z.Q., Liu J.X. (2009): Milk production and fatty acid profile of dairy cows supplemented with flaxseed oil, soybean oil, or extruded soybeans. Acta Agriculturae Scandinavica, Section A – Animal Science, 59: 121–129.

Zheng H.C., Liu J.X., Yao J.H., Yuan Q., Ye H.W., Ye J.A., Wu Y.M. (2005): Effects of dietary sources of vegetable oils on performance of high-yielding lactating cows and conjugated linoleic acids in milk. Journal of Dairy Science, 88, 2037–2042.

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