Effect of dietary sources of roasted oilseeds on blood parameters and milk fatty acid composition

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ABSTRACT: The aim of this experiment was to investigate the effect of supplementing the basal diet with oilseeds on blood parameters and composition of milk fatty acids, especially conjugated linoleic acid (CLA). Forty-eight lactating Holstein cows in early lactation were used in a randomized block design. The cows in each group were fed the control basal diet (the control diet) or diets containing roasted soybean (RSB), roasted linseed (RLS), roasted sunflower seed (RSS), hulled roasted peanut (HRP) and roasted cottonseed (RCS), respectively. Milk yield and dry matter intake (DMI) were not significantly different. Milk fat percentage and yield decreased ($P < 0.05$) in RLS, RSS and RCS diets compared with the control. Feeding various oilseeds had no effect on plasma parameters, but it tended to increase concentrations of *trans* C18:1 and C18:2 in plasma. In milk fat, the concentrations of short and medium fatty acids decreased while C_{18} unsaturated fatty acids increased when the cows were fed oilseed diets. *Cis*-9, *trans*-11 CLA content increased ($P < 0.01$) in the milk fat of cows fed oilseeds. RSB treatment produced the highest ($P < 0.01$) content of *cis*-9, *trans*-11 CLA, which was a 60% increase compared with the control. The results indicate that the diets supplemented with oilseeds improve the content of C_{18} unsaturated fatty acids and CLA in milk fat, and soybeans seem to be the optimal source to improve the nutritive value of milk compared with other oilseeds.

Keywords: conjugated linoleic acid; milk fat; lactating dairy cattle; plasma parameters; soybean

Conjugated linoleic acid (CLA) is the only fatty acid shown definitively to inhibit carcinogenesis in experimental animals (Lee et al., 2005). It is found predominantly in food products from ruminant animals, especially in milk products (Chin et al., 1992). Besides the anticarcinogenic property, CLA also enhances the immune response, increases the feed efficiency and reduces the body fat (Tricon et al., 2005). Recently, it has been reported that each CLA isomer may have a different pharmacological effect. For example, *cis*-9, *trans*-11 CLA is primarily responsible for the inhibition of the growth of human cancer cells (Ip et al., 1999), while *cis*-12, *trans*-10 CLA apparently regulates fatty acid metabolism (Pariza et al., 2001).

Milk fatty acids are altered by many factors, such as breed (Pešek et al., 2005). However, changing the dietary fat source is the most important factor to influence milk fatty acids, especially the concentration of CLA in milk fat, remarkably. The concentration of CLA in milk fat could be significantly enhanced by the addition to the diet of vegetable oilseeds or oils which are rich in C_{18} unsaturated fatty acids (Komprda et al., 2001; Peterson et al., 2002; Ward et al., 2002), especially oils or oilseeds high in linoleic acid (Chouinard et al., 2001; Collomb et al., 2004). An increase in the proportions of C_{18} unsaturated fatty acids in diets can improve the concentration of vaccenic acid (VA) in the rumen. After VA is absorbed by the tissue, CLA will be synthesized in

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the mammary gland with Δ^9-desaturase enzyme (Corl et al., 2001; Mosley et al., 2006).

Different processing methods for oilseeds in the diet affect the concentration of CLA in milk fat. The concentration of CLA in milk fat was twice to three times higher with extruded or roasted soybean diets than with the control diet of raw ground soybeans (Chouinard et al., 2001). The reason may be that less complete biohydrogenation of polyunsaturated fatty acids was observed in the rumen of cows fed roasted oilseeds. Therefore, we roasted all oilseeds in this experiment. Previous studies did not include the fat sources from different oilseeds in a single study. Therefore, the objective of this study was to examine the effect on blood parameters and CLA content in milk fat after supplementing a control diet with the same dose of roasted oilseeds containing high concentrations of oleic (peanut), linoleic (soybean, sunflower seed and cottonseed) or linolenic acid (linseed), and to explore which one is the most effective to improve the nutritive value of milk.

MATERIAL AND METHODS

Animals and treatments

Twenty primiparous and twenty-eight multiparous Holstein cows, averaging 47 (28 to 63 days) days in milk at the start of the experiment, were used in a randomized block design according to milk yield in the previous lactation. Cows were randomly assigned with six treatments to eight blocks and fed a total mixed ration (TMR) once daily (07:30 h). Diets (their composition and nutrients see Table 1) were a 51:49 blend of forage and concentrate (DM basis). Dietary treatments consisted of either a control diet or a control diet supplemented with roasted soybean, roasted linseed, roasted sunflower seed, hulled roasted peanut and roasted cottonseed replacing a portion of soybean meal as the protein. Diets were formulated to meet energy and protein requirements (NRC, 2001). The experimental period lasted 6 weeks. For

Table 1. Nutrient composition of experimental diets

Composition	Diets[1]					
	control	RSB	RLS	RSS	HRP	RCS
Ingredients (% of DM)						
Lucerne hay	20.2	20.2	20.2	20.2	20.2	20.2
Maize silage	30.8	30.8	30.8	30.8	30.8	30.8
Cracked maize	31.5	31.5	31.5	31.5	31.5	31.5
Soybean meal	15.2	7.7	7.7	7.7	7.7	7.7
Oilseed	0.0	7.5	7.5	7.5	7.5	7.5
Dicalcium phosphate	1.0	1.0	1.0	1.0	1.0	1.0
Limestone	0.3	0.3	0.3	0.3	0.3	0.3
Mineral and vitamin mix[2]	1.0	1.0	1.0	1.0	1.0	1.0
Chemical (% of DM)						
CP	18.20	18.30	17.80	18.00	18.10	17.90
Ether extract	2.51	4.13	4.71	4.52	4.70	4.27
NDF	35.80	35.50	35.00	35.70	35.20	36.40
ADF	19.30	19.00	18.70	19.10	18.90	19.60
Ca	0.85	0.92	0.93	0.87	0.90	0.88
P	0.41	0.43	0.38	0.42	0.38	0.41
DM (% of diet)	54.30	55.70	55.40	56.00	55.10	54.80
NE_L (Mcal/kg)	6.73	6.90	7.15	7.11	7.06	7.02

[1]RSB = roasted soybean; RLS = roasted linseed; RSS = roasted sunflower seed; HRP = hulled roasted peanut; RCS = roasted cottonseed

[2]content (per kilogram): NaCl 400 g; $NaHCO_3$ 200 g; I 0.1 g; Fe 3 g; Mg 20 g; Cu 2 g; Mn 3 g; Zn 6 g; Se 0.06 g; Co 0.02 g; vitamin A 800 000 IU; vitamin D 100 000 IU; and vitamin E 4 000 IU

the first two weeks of the experiment, the cows were adapted to dietary treatments.

Sampling and fatty acid analysis

Cows were milked daily at 07:00 and 19:00 h, with milk yield measured weekly and milk samples collected from consecutive a.m. and p.m. milking during the last two days of each week. The samples from each consecutive two days for each cow were blended, and then split into two portions for analysis. One portion was stored at 4°C and sent to a laboratory to be analyzed for fat, protein, lactose, and solids not fat (SNF) (AOAC, 1990) by infrared spectrophotometry (Bentley 2000; Bentley Instruments, US). The other portion was stored at –20°C until the end of the experiment.

Blood samples were obtained on the last day at 14:00 h by venipuncture of the coccygeal vein. Blood was transferred into heparinized vacutainers and centrifuged at 3 500 × g for 15 min for harvesting the plasma. Plasma concentrations of metabolites and insulin were determined as described by Gagliostro et al. (1991).

The frozen milk samples were rapidly thawed and centrifuged at 12 000 × g at 8°C for 1 h to harvest the fat cake for fatty acid analysis. Lipid extraction of milk fat was performed according to Hara and Radin (1978) in a mixture of hexane and isopropanol (3:2; v/v). Fatty acids in plasma were extracted by using a chloroform/methanol (2:1; v/v) solution. Methyl esters of the fatty acids in milk fat and diets were prepared by transesterification with sodium methoxide according to the method of Christie (1982).

Fatty acid methyl esters in hexane were then injected into a Finnigan Trace Gas Chromatograph (Thermo Electron Co., US) equipped with an electron ionization detector. The separation of fatty acid methyl esters was performed with a fused silica capillary column (HP-88, 100 m × 0.25 mm i.d. × 0.25 μm film thickness; Agilent, US). The analysis of fatty acids in total samples of lipid fractions required an injection of 1 μl of methyl esters in hexane. Helium was the carrier gas and was set at a split ratio of 1:40. The injector and detector were maintained at 230°C. For fatty acids in milk, the column temperature was 70°C for 2 min, increased 8°C/min to 120°C and then increased 15°C/min to 160°C, maintained for 40 min, finally increased 5°C/min to 215°C and maintained for 10 min. For fatty acids in plasma, the column temperature was 140°C for 2 min, increased 8°C/min to 160°C and maintained for 40 min, then increased 5°C/min to 215°C and maintained for 10 min. All fatty acids

Table 2. Milk yield, milk composition and plasma parameters of cows fed different diets

Item	Diet						SEM
	control	RSB	RLS	RSS	HRP	RCS	
DMI (kg/day)	19.2	18.9	19.5	19.4	19.0	18.9	0.87
Milk (kg/day)	22.5	21.9	22.2	22.4	21.9	21.8	0.82
Fat (%)	3.76[a]	3.63[ab]	3.42[b]	3.75[a]	3.52[b]	3.50[b]	0.15
Fat (kg/day)	0.84[a]	0.79[ab]	0.76[b]	0.84[a]	0.77[b]	0.77[b]	0.04
Protein (%)	2.90	2.81	2.80	2.74	2.84	2.84	0.11
Protein (kg/day)	0.65	0.61	0.62	0.61	0.62	0.62	0.03
Lactose (%)	5.04	5.11	5.03	5.08	5.07	5.08	0.09
Lactose (kg/day)	1.13	1.12	1.12	1.14	1.11	1.11	0.05
SNF (%)	8.44	8.48	8.60	8.51	8.58	8.51	0.17
Plasma analysis							
Glucose (mmol/l)	3.40	3.48	3.38	3.42	3.41	3.54	0.05
Triglycerides (mmol/l)	1.59	1.69	1.64	1.71	1.65	1.53	0.13
Cholesterol (mmol/l)	5.13	5.19	5.15	5.16	5.09	5.13	0.05
NEFA (mmol/l)	1.73	1.68	1.75	1.87	1.67	1.79	0.07
Insulin (pg/ml)	375	406	385	417	399	389	14.130

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

were eluted at a flow of 1 ml/min. The methylated fatty acid standards (Matreya, Inc., US) including CLA were used to identify the retention times and to determine correction factors for individual fatty acids.

Calculations and statistical analyses

Data from the experiment were analyzed by analysis of variance (ANOVA), procedure of Statistical Analysis Systems (SAS Version 8.2). Duncan's multiple range method was used to compare the difference in the treatment means.

RESULTS AND DISCUSSION

DMI, milk production and milk composition

There were no significant differences ($P > 0.05$) for milk protein, lactose, and SNF between the cows fed all diets (Table 2). In our study, the cows with oilseed treatment received a diet that was higher in NE_L (Table 1) than in the control cows. But DMI and milk production did not differ ($P > 0.05$) between the cows fed oilseeds and the control diet. The same result was observed before (Dhiman et al., 2000; Karakas Oguz et al., 2006) with cows fed soybeans or cottonseeds. The result can often be traced to the interference of fat with ruminal fermentation or to poor digestibility of fatty acids.

The milk fat percentage was lower ($P < 0.05$) when cows were fed all oilseed diets except RSB and RSS. Because daily milk yields were similar ($P > 0.05$) across all diets, the milk fat yield for cows fed oilseeds diet was also slightly lower ($P < 0.05$). This decrease in milk fat percentage and yield was observed in other trials with cows fed oilseeds (Chichlowski et al., 2005). Diets supplemented with oilseeds can cause a reduction in the milk fat secretion while the reason for a milk fat depression is not clear. Bauman and Griinari (2000) proposed that the typical pathways of rumen biohydrogenation are altered to produce unique fatty acid intermediates such as *trans*-10, *cis*-12 CLA that inhibit the milk fat synthesis under dietary supply of unsaturated fatty acids condition.

Parameters and fatty acids in plasma

The effect of fatty acids on the insulin release is related with both the chain length and the degree of non-saturation in the ration. In our study, the plasma glucose and insulin were not affected ($P > 0.05$) by the treatments (Table 2). Similarly, recent researches reported no differences ($P > 0.05$) for insulin in cows fed oilseeds (Chichlowski et al., 2005). Feeding various oilseeds had no effect on other plasma parameters, either (Table 2). In agreement with our results, Bailoni et al. (2004) reported there were no differences when cows were fed soybean seeds. However, another study showed that oilseed supplementation increased the concentrations of cholesterol and/or non-esterified fatty acids (NEFA) in bovine blood (Gonthier et al., 2005). These differences may be caused by the source and quantity of oilseed. Therefore, we speculated that the addition of 7.5% (of diet DM) oilseeds from roasted oilseeds did not affect plasma parameters.

In our research, feeding oilseed diets tended to increase plasma concentrations of *trans* C18:1 and C18:2 whereas the other fatty acids (C14:0, C16:0, C16:1, C18:0, and *cis*-9 C18:1) did not change (data not shown). Plasma concentrations of *trans* C18:1 increased ($P < 0.05$) when the cows were fed soybean and linseed diets (Table 3). Plasma con-

Table 3. Contents of fatty acids in the blood of cows fed different diets

Blood fatty acid (g/100 g of total fatty acids)	Diet						SEM
	control	RSB	RLS	RSS	HRP	RCS	
C18:1 *t*11	1.07^{b}	1.68^{a}	1.64^{a}	1.43^{ab}	1.42^{ab}	1.33^{ab}	0.15
C18:2 *c*9, *c*12	49.60^{b}	51.94^{a}	50.85^{ab}	51.29^{ab}	50.39^{ab}	50.08^{ab}	0.71
CLA *c*9, *t*11	0.10	0.18	0.14	0.17	0.13	0.13	0.03
α-C18:3	4.86^{B}	4.78^{B}	6.09^{A}	4.95^{B}	4.88^{B}	4.96^{B}	0.16

^{A,B,C}means within the row with different uppercase superscripts differ ($P < 0.01$)

^{a,b,c}means within the row with different lowercase superscripts differ ($P < 0.05$)

Table 4. Contents of fatty acids in the milk fat of cows fed different diets

Fatty acid (g/100 g of total fatty acids)	Diet						SEM
	control	RSB	RLS	RSS	HRP	RCS	
C6:0	2.07^{a}	1.82^{b}	1.93^{ab}	1.88^{ab}	1.96^{ab}	1.89^{ab}	0.14
C8:0	1.47^{a}	1.12^{b}	1.23^{ab}	1.27^{ab}	1.19^{b}	1.19^{b}	0.13
C10:0	3.24^{a}	2.67^{c}	2.82^{bc}	2.72^{bc}	2.92^{b}	2.74^{bc}	0.15
C12:0	2.21^{a}	1.24^{c}	1.58^{b}	1.68^{b}	1.52^{b}	1.69^{b}	0.16
C14:0	11.63^{A}	8.29^{C}	9.59^{B}	10.12^{B}	9.69^{B}	10.38^{B}	0.64
C16:0	27.11^{A}	22.75^{C}	25.24^{B}	24.84^{B}	24.20^{B}	24.28^{B}	0.71
C16:1	2.70^{a}	1.84^{c}	2.22^{b}	2.38^{b}	2.09^{bc}	2.14^{bc}	0.23
C18:0	15.51^{c}	17.60^{a}	16.30^{bc}	16.25^{bc}	16.80^{ab}	16.37^{bc}	0.75
Total C18:1	24.88^{C}	30.02^{A}	27.51^{B}	27.58^{B}	28.46^{AB}	27.39^{B}	0.82
C18:1 *c*9	22.61^{c}	26.51^{a}	24.18^{b}	24.42^{b}	25.32^{ab}	24.37^{b}	0.81
C18:1 *t*6	0.30^{b}	0.42^{a}	0.38^{ab}	0.35^{ab}	0.37^{ab}	0.35^{ab}	0.06
C18:1 *t*9	0.21^{C}	0.43^{A}	0.39^{B}	0.35^{B}	0.33^{B}	0.32^{B}	0.02
C18:1 *t*11	1.41^{C}	2.31^{A}	2.21^{A}	2.10^{B}	2.08^{B}	1.99^{B}	0.06
C18:2 *c*9, *c*12	2.73^{b}	3.46^{a}	3.05^{b}	2.96^{b}	3.03^{b}	2.76^{b}	0.22
CLA *c*9, *t*11	0.53^{C}	0.85^{A}	0.82^{A}	0.72^{B}	0.66^{B}	0.63^{B}	0.02
α-C18:3	0.29^{b}	0.34^{b}	0.41^{a}	0.27^{b}	0.27^{b}	0.29^{b}	0.05

^{A,B,C}means within the row with different uppercase superscripts differ ($P < 0.01$)

^{a,b,c}means within the row with different lowercase superscripts differ ($P < 0.05$)

centrations of C18:2 increased ($P < 0.05$) in RSB treatment. Plasma concentrations of saturated fatty acids were not affected by oilseed supplementation. Interestingly, we observed that concentrations of α-C18:3 in plasma were higher ($P < 0.01$) in cows fed linseed diet than in those fed other diets. This result is similar to those reported by other authors (Gonthier et al., 2005).

It is known that in ruminants the milk long-chain fatty acids originate from the uptake of plasma circulating lipids, which are derived from the hydrolysis of triacylglycerol-rich lipoproteins and direct uptake of plasma NEFA (Bauman and Griinari, 2003). Thereby, increased plasma concentrations of *trans* C18:1 and C18:2 may be related to increased concentrations of long-chain fatty acids in milk fat when cows were fed oilseeds.

Fat acid composition in milk fat

The milk fatty acid composition was affected when diets were supplemented with oilseeds (Table 4).The proportions of C6:0 to C16:1 were reduced in milk fat when the cows were fed oilseeds, compared with the control (Table 4). A similar trend was observed with other oilseeds sources (Chichlowski et al., 2005; Komprda et al., 2005). Short-chain fatty acids (C6:0 to C14:0) are mainly derived from *de novo* synthesis in the mammary gland of the dairy cow, and the content of C16:0 in milk originates both from *de novo* synthesis in the mammary gland and from uptake from blood fat. Therefore, we suggest that high concentrations of long-chain unsaturated fatty acids in diets inhibit *de novo* synthesis of short and medium-chain fatty acids in the mammary gland. In terms of human health, decreased short and medium-chain fatty acids could improve milk fatty acid profiles due to the potential effects of these fatty acids on increased total and LDL cholesterol concentrations in plasma (Kris-Etherton, 1997).

The proportions of the C18:0, C18:1 and C18:2 content in milk fat could increase in cows fed oilseeds (Table 4). This is due to the increase in C_{18} unsaturated fatty acids in the diets and it was already observed previously (Chichlowski et al., 2005; Komprda et al., 2005) .The concentration of α-C18:3 in milk fat was higher ($P < 0.05$) in RLS diet compared to other diets (Table 4). The same

response was observed in a previous experiment when cows were fed linseed or linseed oil (Ward et al., 2002). Collomb et al. (2004) also showed that the feeding of linseed reduced the concentration of C16:0 and increased the concentrations of C18:1, C18:2, C18:3 and CLA in milk fat.

The proportions of individual C18:1 isomers in milk fat were increased by oilseed treatments. The *trans*-11 C18:1 content of milk was increased ($P < 0.01$) by oilseed treatments compared with the control (Table 4). The increase in *trans*-11 C18:1 was 64, 57, 47, 50, and 40% for RSB, RLS, HRP, RSS, and RCS treatments, respectively, compared with the control. This increase was also observed in the proportion of *cis*-9, *trans*-11 CLA in milk fat, which was increased ($P < 0.01$) by all oilseed treatments. The *cis*-9, *trans*-11 CLA of milk fat was increased by 60, 55, 25, 39, and 19% by RSB, RLS, HRP, RSS and RCS treatments, respectively, compared with the control. The results are in agreement with previous studies (Peterson et al., 2002). In Collomb et al. (2004), cows fed 1.0 kg/day rapeseed, sunflower and linseed oil induced increases in the concentration of *cis*-9, *trans*-11 CLA by 22, 61, and 4%, respectively, compared to the control diet.

The majority of milk fat CLA is synthesized endogenously via Δ^9-desaturase from VA, an intermediate in the rumen biohydrogenation of polyunsaturated fatty acids (Griinari et al., 2000). In the rumen, the increase in polyunsaturated fatty acids in diets might be converted to more VA (Kepler et al., 1966; Harfoot and Hazelwood, 1988; Mosley et al., 2002). Therefore, in our study the increased C18:1, C18:2, and C18:3 contents in oilseed diets enhanced *cis*-9, *trans*-11 CLA in milk fat.

There was a significant linear relationship between *trans*-11 C18:1 and *cis*-9, *trans*-11 CLA ($R^2 = 0.591$, $P < 0.01$) (Figure 1), which agrees with another study (Peterson et al., 2002). Several studies showed that *cis*-9, *trans*-11 CLA in milk fat can be synthesized from *trans*-11 C18:1 via the Δ^9-desaturase enzyme in the mammary gland of lactating cows. Supplementation with *trans*-11 18:1 by abomasal infusion led to a 31% increase in the concentration of *cis*-9, *trans*-11 CLA in milk fat (Griinari et al., 2000). Lock and Garnsworthy (2002) investigated the effects of dietary linoleic and linolenic fatty acids on the CLA content in milk fat and concluded that more than 80% of *cis*-9, *trans*-11 CLA in milk fat was desaturased from *trans*-11 18:1 during the endogenous synthesis. Corl et al. (2001) infused an inhibitor (sterculic oil) of Δ^9-desaturase enzyme into the abomasums of lactating cows and revealed that the endogenous synthesis accounted for the estimated 78% of total *cis*-9, *trans*-11 CLA in milk fat. The study published recently used ^{13}C-labelled VA to synthesize *cis*-9, *trans*-11 CLA in lactating cows. This convictive result showed that 80% of milk fat *cis*-9, *trans*-11 CLA originated from VA (Mosley et al., 2006).

Compared with other treatments, the proportions of C14:0 and C16:0 were lower ($P < 0.01$) and the proportions of total C18:1 and C18:2 were higher ($P < 0.05$) in the milk fat of cows fed RSB diet. As mentioned before, decreased short-chain saturated fatty acids and increased long-chain unsaturated fatty acids in milk fat have been shown to be beneficial to human health (Kris-Etherton, 1997; Chilliard et al., 2000). In addition, feeding roasted soybean did not affect the milk fat percentage but feeding other oilseeds depressed the milk

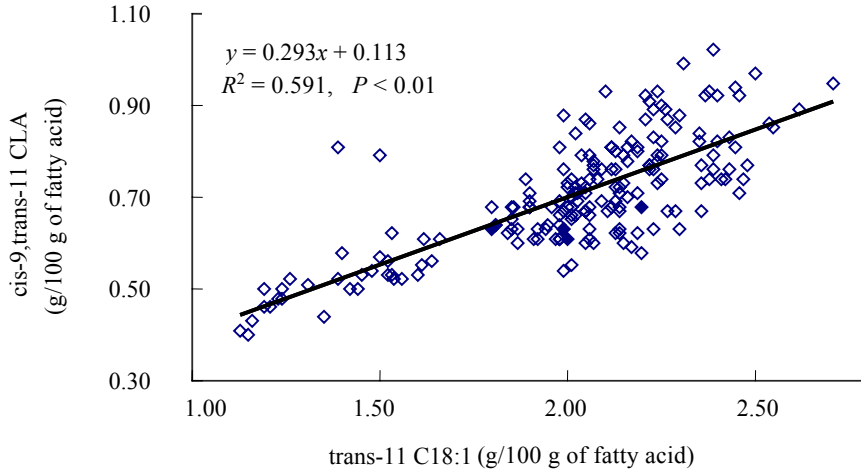

Figure 1. Linear relationship between the concentrations of *trans*-11 C18:1 and *cis*-9, *trans*-11 CLA in the milk fat of cows

fat percentage. According to our data, roasted soybean is an optimum choice in the dairy application of oilseeds to improve the nutritive value of milk which is beneficial to human health and which did not affect the performance of cows.

CONCLUSION

Results from the present research show that the milk yield was not influenced. Feeding oilseeds had no effect on plasma parameters, but tended to increase concentrations of *trans* C18:1 and C18:2 in plasma. The short-chain fatty acids decreased and the contents of C_{18} unsaturated fatty acids, especially CLA, increased in the milk fat of cows fed dietary oilseeds. When dietary oilseeds were fed, the content of C_{18} unsaturated fatty acids and CLA was highest ($P < 0.01$) for RSB diet. Compared with other treatments, roasted soybean may be an optimum choice in the dairy application of oilseeds to improve the quality of milk.

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