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ARTICLE

Cis-9, *trans*-11-Conjugated Linoleic Acid in Dairy Goat Milk was Increased by High Linoleic (Soybean Oil) or Linolenic (Linseed Oil) Acid Diet

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Abstract

The objective of this study was to examine the effect of supplementation of high linoleic ($C_{18:2}$) oil or high linolenic ($C_{18:3}$) oil to the diet on milking performance and content of conjugated linoleic acid (CLA) isomers in goat milk fat. Forty five dairy goats (Sumnen, 25 d post-partum, 62.5±1.2 kg) were randomly assigned to three treatment groups with each group of 15 dairy goats. The goats were fed a basal diet (CON) consisting 1.2 kg concentrate and 1.2 kg chopped hay (0.6 chopped alfalfa and 0.6 kg hay) daily with 4% soybean oil (SO) or 4% linseed oil (LO). Daily feed intake was not influenced (p>0.05) but daily milk yield (p<0.001) and milk fat yield (p<0.001) were significantly increased by supplementation of oils. Supplementation of oils decreased the short chain fatty acid, medium-chain fatty acid and saturated fatty acid in goat milk fat while increased *trans* vaccenic acid (*trans*-11-$C_{18:1}$, TVA), oleic acid ($C_{18:1}$), $C_{18:2}$, $C_{18:3}$, *cis*-9, *trans*-11-CLA (*c*9, *t*11-CLA), *trans*-10, *cis*-12-conjugated linoleic acid (*t*10, *c*12-CLA), unsaturated fatty acids, mono unsaturated fatty acid and long-chain fatty acid in goat milk fat (p<0.001). Especially, *c*9, *t*11-CLA, *t*10, *c*12-CLA and ω-3 fatty acid ($C_{18:3\ n-3}$) in milk fat were highest when goat fed LO diet. Based on the result, it is suggested that supplementation of linseed oil should be an effective method to increase CLA isomers and ω-3 fatty acid in goat milk fat without negative effect on lactating performance.

Key words: goat milk, conjugated linoleic acid, linoleic acid, linolenic acid, atherogenicitiy index

Introduction

The nutritional and health benefits of dairy goat milk are related to number of medical problems of people, being food allergies with cow milk protein (Walker, 1964). The consumption of goat milk reduces total cholesterol levels and the low density lipoprotein (LDL) fraction because of the presence of high medium chain triglycerides (MCT, 36% in dairy goat milk *vs* 21% in cow milk), which decreases the synthesis of endogenous cholesterol.

Over the past twenty years, conjugated linoleic acid (CLA) has been received an attention. Beginning from anti-carcinogenic effect (Ha *et al.*, 1987), a number of studies have been conducted with special emphasis on the CLA content in animal products and its effect on human health. Ruminant meat or milk is the primary natural source of CLA in the food. The content of CLA isomers in milk fat is mainly result from incomplete bio-hydrogenation of unsaturated fatty acid by rumen microbes. Supplementing plant oils containing a lot of unsaturated C_{18}-fatty acids as a CLA precursor is adopted as the most effective method (Bourattour *et al.*, 2008, Li *et al.*, 2009; Wang *et al.*, 2002a, 2002b, 2005).

Most previous experiments with lactating goats have examined the CLA in milk fat in response to plant oil, especially high linoleic acid ($C_{18:2}$) supplemented diet. Supplementation of high linoleic acid ($C_{18:3}$) or linolenic acid oil or oil seed to the diet has been shown to increase the CLA content in the rumen fluid (Dhiman *et al.*, 2000; Li *et al.*, 2009), goat milk (Bouattour *et al.*, 2008; Li *et al.*, 2009) and beef cattle (Song *et al.*, 2010; Wang *et al.*, 2006). Therefore, the objective of the current study was to examine the supplementation effects of high $C_{18:2}$ or $C_{18:3}$ oil on lactating performance and healthy fatty acid profile including CLAs content in dairy goat milk.

Materials and Methods

Animals and diets

Forty five dairy goats (Sumnen, 25 d post-partum, 62.5

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±1.2 kg) were randomly assigned to three treatment groups based on mainly milk yield, with each group of 15, 3 pens, 5 dairy goats per pen in each treatment. Dietary treatments were basal concentrate and hay without oil supplementation (CON), CON diet supplemented oil with high $C_{18:2}$ soybean oil (SO, 0.4 g/kg of concentrate) or CON diet supplemented with high $C_{18:3}$ linseed oil (LO, 0.4 g/kg of concentrate) on a DM basis (Table 1). The dairy goat were fed 1.2 kg basal concentrate and 1.2 kg hay (0.6 kg chopped alfalfa and 0.6 kg oat hay) per head on a DM basis twice daily (06:00 and 18:00) in an equal amount prior to milking. The ingredient and chemical composition of the diets, and fatty acid content (g/100 g samples) were shown in Table 1 and Table 2, respectively. The current experiment was conducted at private farm of lactating goat for 60 d including 15 d adjustment period to the diets, and followed the farm's conventional management procedures. The feeding level of the basal diet (CON) satisfied with nutrient requirements of lactating dairy goats (AFRC, 1993).

Table 1. Formulae and chemical composition of the diets (%, DM basis)

Items	Diets[1)]		
	CON	SO	LO
Ingredients :			
Oat hay	25	25	25
Alfalfa hay	25	25	25
Corn	11	10	10
Corn gluten feed	12	12	12
Soybean meal	14	15	15
Rice hull	4	4	4
Soybean hull	7	3	3
Soybean oil	-	4	-
Linseed oil	-	-	4
Calcium phosphate	1.5	1.5	1.5
Mineral-vitamin mix	0.5	0.5	0.5
Chemical composition:			
DM	93.88	94.35	94.35
CP	17.65	17.65	17.65
EE	2.55	6.42	6.42
NEF	32.61	30.08	30.08
CF	18.83	17.39	17.39
TDN	66.82	72.37	72.37
Ca	1.19	1.17	1.17
P	0.61	0.60	0.60

[1)]CON, the lactating goats were fed basal concentrate without oil supplementation; SO, CON diet supplemented with 4% of soybean oil to the concentrate; LO, CON diet supplemented with 4% of linseed oil the concentrate.

Measurements and analysis

Feed residues were collected daily at 30 min prior to morning feeding to estimate the feed intake. Diet samples were taken once a week for the proximal analysis by the methods of AOAC (1995). Neutral detergent fiber (NDF) content in the feeds was estimated by the method of Van Soest *et al.* (1991).

Milk yield was recorded daily and an aliquot of goat milk was freeze dried and contents of fat and protein were determined by the method of AOAC (1995). Dried milk was defatted using soxhlet apparatus. Five grams of the defatted milk powder was dissolved in 100 mL of distilled water, deproteininzed with 10% sulfosalicylic acid (Whiting *et al.*, 1971), and centrifuged (20,000 *g*, 20 min). The supernatant was filtered with a Millipore syringe driven filter unit (Hydrophilic PTEE 0.45 μm) and then milk lactose content was measured by a high performance liquid chromatograph (HPLC, Acme 9000, Yonglin Co., Korea) equipped with an ELS detector. A column (Luna 5 μ NH_2 100 A, 250×4.6 mm) was used, and effluent of acetonitrile:water (80:20) was chosen as the mobile phase

Table 2. Fatty acid content (g/100g) of oils and feeds

Fatty acids	Linseed oil	Soybean oil	Concentrate	Alfalfa hay	Oat hay
$C_{14:0}$	0.07	0.08	0.03	0.02	0.02
$C_{15:0}$	0.03	0.02	0.01	0.02	0.01
$C_{16:0}$	4.79	8.45	0.61	0.25	0.02
$C_{16:1}$	0.06	0.08	0.01	0.01	0.25
$C_{17:0}$	0.06	0.08	0.01	0.01	0.01
$C_{18:0}$	3.51	3.09	0.11	0.05	0.01
cis-9 -$C_{18:1}$	17.85	15.48	0.01	N.D.	N.D.
cis-11 -$C_{18:1}$	0.62	1.14	0.76	0.04	0.07
$C_{18:2\text{-}n3}$	0.05	0.29	0.04	0.01	0.18
$C_{18:2\text{ - }n6}$	15.11	43.80	1.16	0.21	0.01
$C_{20:0}$	0.10	0.22	0.01	0.01	N.D.
$C_{18:3}$-n6	0.21	0.50	N.D.	N.D.	0.20
$C_{18:3}$-n3	55.27	7.05	0.10	0.29	0.01

N.D.: Not detected

at a flow rate of 0.7 mL/min.

Fatty acid analysis

Lipids in milk powder, supplemented oils, and feeds for fatty acid analysis were extracted using Folch's solution (Folch *et al.*, 1957). Preparation of FA methyl esters of milk fat followed the method of ISO 15884 (2002) prior to injecting into a gas chromatograph (GC, Agilent 6890N). Injector and detector temperatures were 270 and 280°C, respectively. The oven temperature was multi-step programmed as follows: initial oven temperature was maintained at 40°C for 2 min, increased to 130°C at a rate of 10°C/min. and held for 1 min, then increased at a rate of 6.5°C/min to 170°C, followed by a rate of 2.75°C/min to 215°C and held there for 12 min, then increased at 40°C/min to 230°C and held for 10 min, and finally increased to 240°C and maintained for 5 min. *Cis*-9, *trans*-11-CLA (*c*9, *t*11-CLA) and *trans*-10, *cis*-12-CLA (*t*10, *c*12-CLA) isomers (Sigma, USA) were used to identify and quantify each CLA isomer. Other fatty acid (FA) standards were obtained from Supelco Co. (18919, Sigma, USA). Tridecanoic acid ($C_{13:0}$) was used as an internal standard and all CLA isomers and other FAs in dairy goat milk were quantified using FA standards.

Statistical analysis

The results were analyzed using the general linear procedure of SAS (2008) with a model that included diet effect. The data were analyzed using the following mixed linear model according to a repeated measures design (Littell *et al.,* 1998):

$$Y_{ijk} = \mu + D_i + a_{ij} + \varepsilon_{ij}$$

Y_{ij} : Observation
μ : Overall mean
D_i : Fixed effect of diet 1^{th} (I – 1^{st} to 3^{rd})
a_{ij} : Random effect of the animal K^{th} nested within diet i^{th}
ε : Random residual NID ~ (0, δ^2)

The effect of the diet was tested with the variance associated with the animal within treatment as the error them. Pair-wise comparisons among the means were performed using the Tukey test. Differences were declared significant at $p \leq 0.05$.

Results

Feed intake and milk production

Daily feed intake was not influenced by supplementation of oil to diets ($p>0.05$, Table 2). Daily long-chain fatty acid (LCFA) intake was lowest in CON group (33.00 g/d) among dietary treatment (SO, 91.01 g/d; LO, 97.85 g/d, Table 3). Daily intake of $C_{18:2}$ was higher in SO (40.22 g/d) feeding group than those in LO (20.89 g/d) and CON (11.67 g/d) feeding groups. Also, daily intake of $C_{18:3}$ was greater in LO (39.58 g/d) feeding group then those in SO (7.99 g/d) and CON (3.02 g/d) feeding groups.

Despite of similar daily dry matter (DM) intake between treatments ($p>0.05$), milk yield from SO (2.31 kg/d) and LO (2.15 kg/d) feeding groups was significantly higher than those from CON (1.90 kg/d) feeding group ($p<0.001$, Table 4). The milk DM composition (%) was tended to decrease by supplementation of oils (SO: 19.31%, LO: 19.22%) compared with that by CON (20.62%) diet ($p=0.08$, Table 4). But total Milk DM was significantly increased by feeding the SO diet. Milk protein content (g/d) and composition (%) were not influenced by supplementation of oil ($p>0.05$). Milk fat composition and daily milk fat production were enhanced by supplementation of SO (4.97%, 111.44 g/d) and LO (5.02%, 100.83 g/d) compared those by CON (3.42%, 52.17 g/d) ($p<0.001$, Table 4).

Fatty acid composition in milk fat

Compositions of butyric acid ($C_{4:0}$) and caproic acid

Table 3. Mean daily intake of major fatty acids (g) by lactating goats

Fatty acids	Diets[1)]		
	CON	SO	LO
Total fatty acids	33.00	91.01	97.85
Palmitic acid, $C_{16:0}$	7.10	12.35	9.86
Oleic acid, $C_{18:1}$	7.36	17.92	19.04
Linoleic acid, $C_{18:2}$	11.67	40.22	20.89
Linolenic acid, $C_{18:3}$	3.02	7.99	39.58

[1)]Referred to the Table 1.

Table 4. Feed intake and milk production by lactating goats, and major constituents of the milk

Items	Diets[1)]			SEM[2)]	*P*-value
	CON	SO	LO		
Dry matter intake, kg/d	1.65	1.66	1.65	1.561	0.94
Milk yield, kg/d	1.90^b	2.31^a	2.15^a	1.146	< 0.001
Milk dry matter :					
%	20.62	19.31	19.22	0.130	0.08
g/d	324.85^b	427.09^a	363.17^b	7.630	0.04
Milk protein :					
%	5.47	4.97	5.02	0.041	0.57
g/d	85.73	110.02	96.16	2.090	0.10
Milk Fat :					
%	3.42^b	4.97^a	5.02^a	0.067	<0.001
g/d	52.17^b	111.44^a	100.83^a	2.666	<0.001

[1)]Referred to the Table 1.; [2)]Standard error of the means.

($C_{6:0}$) in milk fat were not influenced by supplemented oil (Table 5). The compositions of medium-chain fatty acids (MCFA, $C_{8:0}$, $C_{10:0}$, $C_{12:0}$, $C_{14:0}$, $C_{14:1}$, $C_{15:0}$, $C_{15:1}$, $C_{16:0}$, $C_{16:1}$ and $C_{17:0}$) were decreased by feeding LO diets compared with respective composition by SO diet and CON diet feeding (p<0.001 ~p=0.036, Table 5). Oil supplementation increased stearic acid ($C_{18:0}$) proportion in milk fat compared with CON diet (p<0.001). *Trans* vaccenic acid (*trans*-11-$C_{18:1}$, TVA) in milk fat was strongly increased by feeding LO (7.30 g/100g milk fat) supplemented diet compared those by SO (5.26 g/100g milk fat and CON (1.17 g/100g milk fat) (p<0.001, Table 5). Supplementation of SO increased $C_{18:2}$ composition in milk fat (p=0.001) while the high $C_{18:3}$ (LO) diet strongly (p<0.001) stimulated c9, t11-CLA content (2.10 g/100g milk fatty acid) in milk fat compared with the high $C_{18:2}$ (SO) diet (1.71 g/100g milk fatty acid) and CON diet (0.40 g/100g milk fatty acid). Feeding the oil supplemented diets to milking goats increased unsaturated fatty acid (USFA, p<0.001), mono unsaturated fatty acid (MUFA, p<0.001) and the ratio of USFA and saturated fatty acid (SFA) (p<0.001) ratio (p< 0.001) but decreased SFA (p<0.001) contents in milk fat (Table 5).

Table 5. Effect of supplementation of oil on fatty acid content in goat milk fat (g/100 g of fatty acid)

Fatty acids	Diets[1)]			SEM[2)]	*P*- value
	CON	SO	LO		
$C_{4:0}$	2.28	2.34	2.26	0.049	0.952
$C_{6:0}$	2.48	2.34	2.12	0.029	0.079
$C_{8:0}$	2.89^{a}	2.56^{b}	2.22^{c}	0.030	<0.001
$C_{10:0}$	10.67^{a}	8.41^{b}	6.74^{c}	0.139	<0.001
$C_{12:0}$	5.39^{a}	3.63^{b}	2.71^{c}	0.099	<0.001
$C_{14:0}$	12.18^{a}	9.32^{b}	7.39^{c}	0.014	<0.001
$C_{14:1}$	0.23^{a}	0.17^{b}	0.12^{c}	0.005	<0.001
$C_{15:0}$	1.25^{a}	0.97^{b}	0.89^{b}	0.014	<0.001
$C_{15:1}$	0.06^{b}	0.05^{b}	0.15^{a}	0.006	0.004
$C_{16:0}$	29.56^{a}	24.10^{b}	20.97^{c}	0.312	<0.001
$C_{16:1}$	0.72^{a}	0.56^{b}	0.61^{ab}	0.014	0.036
$C_{17:0}$	0.86^{a}	0.63^{b}	0.38^{c}	0.022	<0.001
$C_{18:0}$	7.56^{b}	10.93^{a}	12.17^{a}	0.190	<0.001
trans-11- $C_{18:1}$	1.17^{c}	5.26^{b}	7.30^{a}	0.211	<0.001
cis-9 -$C_{18:1}$	17.65^{c}	21.83^{b}	24.49^{a}	0.267	<0.001
$C_{18:2\ n6}$	0.06^{b}	0.08^{b}	0.25^{a}	0.009	<0.001
$C_{18:2\ n3}$	2.71^{b}	3.47^{a}	3.05^{a}	0.043	<0.001
cis-9, tran-11 CLA	0.40^{c}	1.71^{b}	2.10^{a}	0.064	<0.001
trans-10, cis-12 CLA	0.01^{c}	0.05^{b}	0.12^{a}	0.004	<0.001
$C_{18:3n6}$	0.05	0.05	0.07	0.001	0.079
$C_{18:3n3}$	0.30^{b}	0.26^{b}	1.36^{a}	0.052	<0.001
UFA	23.77^{c}	34.07^{b}	40.24^{a}	0.553	<0.001
SFA	76.23^{a}	65.93^{b}	59.76^{c}	0.553	<0.001
MUFA	20.17^{c}	28.13^{b}	32.99^{a}	0.439	<0.001
UFA/SFA	0.31^{c}	0.52^{b}	0.68^{a}	0.003	<0.001

[1)]Referred to the Table 1.; [2)]Standard error of mean.; a, b, c: Means in the same row with different superscripts differ (p<0.05).

Fig. 1 showed the comparison in compositions of short-chain fatty acid (SCFA, $C_{4:0}$ to $C_{10:0}$), MCFA ($C_{12:0}$ to $C_{16:0}$) and LCFA ($C_{18:0}$ to $C_{24:0}$) contents in milk fat as influenced by the supplementation of oil. The SCFA (p<0.001) and MCFA (p<0.001) in milk fat were dramatically decreased by feeding the oil supplemented diets compared with those by feeding the basal (CON) diet. Especially, the SCFA and MCFA contents in milk fat were lowest in lactating dairy goats fed the LO diet in comparison with those in goats fed the SO and CON diets (Fig. 1). But, the LCFA content in milk fat was highest in goats fed the high $C_{18:3}$ LO diet compared with those in dairy goats fed high $C_{18:2}$ and basal diets (p<0.001, Fig. 1). The atherogenicity index was significantly reduced in all goats fed oil-supplemented diets due to the decreased total SFA level and an increased USFA level (Fig. 2).

Discussion

Milking performance

The current study was conducted to examine CLA production in the milk fat of lactating goat when goats fed supplemented with $C_{18:2}$-rich or $C_{18:3}$-rich oil. Supplementation of soybean or linseed oil to the concentrate should increase energy density and has been useful as a source of $C_{18:2}$ or $C_{18:3}$, which are major precursors of CLA isomers in milk. High fat diet generally reduces dairy feed intake and digestibility, depending upon level of fat in diets (Song *et al.*, 2010). Reduced DM intake has been widely reported after the supplementation of high levels of oils to

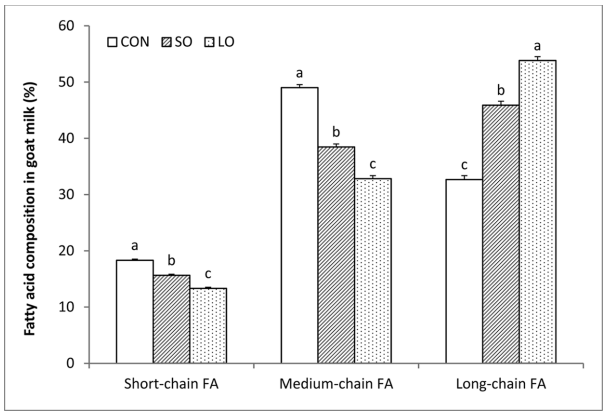

Fig. 1. Effect on supplemented soybean oil or linseed oil in diets on short-chain fatty acid, medium-chain fatty acid and long chain fatty acid composition in goat milk. (Short-chain FA: C_4 to C_{10}; Medium-chain FA: C_{12} to C_{16}; Long-chain FA: C_{18}-C_{24}; a, b, c: Means with different superscripts differ (p<0.001).

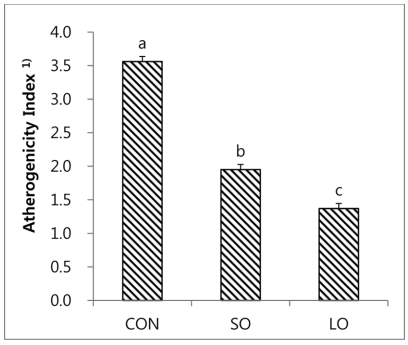

Fig. 2. Effect on supplemented soybean oil or linseed oil in diets on atherogenicity index in goat milk (Atherogenicity index: $(C_{12:0} + 4 \times C_{14:0} + C_{16:0})$/USFA; a, b, c: Means with different superscripts differ ($p<0.001$).

ruminant diets (Huang *et al*., 2008; Jordan *et al.*, 2006; Li *et al*., 2009; Song *et al.*, 2010, Sutter *et al.*, 2000; Sutton *et al*., 1983). In the current study, however, DM intake was not affected by addition of soybean oil or linseed oil at 4% level (DM basis) to the concentrate (Table 4). This result might be due to the low level of oil addition. Daily LCFA intake was greatly increased by addition of soybean oil (78.48 g/d) or linseed oil (89.37 g/d) compared with that from basal diet (CON, 29.15 g/d, Table 3).

In the present study, daily milk yield (kg/d) was markedly increased when goats fed SO diet or LO diet. The feed efficiencies in CON, SO and LO diets group were 1.15, 1.36 and 1.30, respectively. Feeding the concentrate supplemented with soybean oil or linseed oil improved feed efficiency 20% or 13%, respectively, compared with feeding basal (CON) diet. It might be due to increased energy density by oil addition. Looer *et al.* (2005) reported that feeding the high fat diet, including 3% of linseed oil, did not influenced on daily DM intake in dairy cow, but daily milk yield and milk fat compositions were greatly increased by feeding the linseed oil supplemented diet. Increased milk fat content of lactating goats as influenced by plant oil supplementation was similar to the results reported by Daccord (1987) and Li *et al.* (2009). However, Chillared *et al.* (2006) reported that goat milk fat content was not increased by feeding the high poly-unsaturated fatty acid in high concentrate diet.

Fatty acid composition in milk fat

Supplementing the diet with 4% soybean oil or 4% linseed oil decreased the content of SCFA and MCFA ($C_{4:0}$-$C_{14:0}$), except for $C_{4:0}$, $C_{6:0}$ and $C_{16:0}$ in the milk fat compared with the CON diet. The decreases in $C_{4:0}$-$C_{14:0}$ and $C_{16:0}$ contents in milk caused by oil supplementation are consistent with the results reported by others (Chouinard *et al.,* 1998; Dhiman *et al.,* 2000; Li *et al*., 2009). Because the most of $C_{4:0}$-$C_{14:0}$ and $C_{16:0}$ in milk are synthesized *de novo* by the mammary epithelial cells, these changes suggest that supplementing LCFA inhibit *de novo* fatty acid synthesis. In the present study, daily intakes of C_{18}-LCFA from soybean oil and linseed oil were 91.01 g and 97.87 g, respectively. It might be influenced by inhibiting the *de novo* synthesis of SCFA or MCFA in the mammary gland of goats. In the current study, the composition of C_{18}-fatty acids in goat milk was significantly enhanced by high LCFA diets.

Bouattour *et al.* (2008) and Li *et al.* (2009) suggested that feeding a moderate dose of soybean oil to lactating goats would be a useful way to increase CLA content in milk without negative effects on intake, milk yield, and protein content. In the current study, soybean oil diet enhanced CLA isomers composition in goat milk fat compared with basal diet. Also, the high linolenic diet (LO) produced more CLA isomers in goat milk than the high linoleic acid diet (SO). The atherogenicity index was dramatically reduced in both oil supplemented diets (Fig. 2) due to the decreased total SFA and increased USFA level (Fig. 1). This index is regarded to the supposed unhealthy saturated fatty acid risk factor for coronary heart disease (Ulbricht and Southgate, 1991).

The $C_{18:3}$ in dairy goat milk was greatly enhanced by high $C_{18:3}$ diet compared with other diets. The TVA and *c*9, *t*11-CLA contents in dairy goat milk were dramatically increased by supplementation of soybean or linseed oil in diets. Our unpublished data indicated that the mRNA levels of stearoyl Co-A desaturase (SCD), which is make a double bond at 9th carbon in LCFA, in mammary gland of milking dairy goats was greater when dairy goats were fed soybean oil or linseed oil compared with when goats were fed control diet. Fatty acid content in the plasma was not measured, but dietary unsaturated LCFA, especially $C_{18:3}$ or $C_{18:3}$, might enhance TVA and *c*9, *t*11-CLA in the rumen and plasma, then these fatty acids were delivered to mammary gland. Finally, *c*9, *t*11-CLA might be directly incorporated into milk fat or produced with TVA by SCD in the mammary gland.

Feeding a soybean or linseed oil to lactating goats was a useful way to increase CLA isomers in milk. Based on result from the current study, it is suggested that feeding the high $C_{18:3}$ diet, supplementation of linseed oil, to lactating goat is more effectual way to produce increased potentially healthy fatty acid profile such as high CLA isomers and ω_3 fatty acid in goat milk without any detrimental effects on goat milk production than feeding the

high linoleic acid diet.

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