Effect of Soybean Oil Supplementation on the Contents of Plasma Cholesterol and Cis9, trans11-CLA of the Fat Tissues in Sheep*

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ABSTRACT : A feeding trial was conducted with 10 sheep for 12 weeks to examine the effect of soybean oil (SBO) supplementation on long-chain fatty acids composition, especially *cis*9,*trans*11-conjugated linoleic acid (c9,t11-CLA) in fat tissues. Sheep were fed either a SBO supplemented diet (5%, DM basis) or a control diet without SBO. Chopped rye grass hay was fed as roughage. Concomitant increases in contents of total cholesterol (T-C) and HDL-cholesterol (HDL-C) in the plasma of sheep were observed from the SBO supplementation. The supplementation of SBO reduced ($p<0.05$) the proportions of $C_{16:1}$, $C_{17:0}$ and $C_{17:1}$ but increased ($p<0.05$) the proportions of $C_{18:0}$ and octadecenoic acid (t11-$C_{18:1}$) in the intramuscular fat. The $C_{18:0}$ proportion only in the subcutaneous fat was increased ($p<0.05$) by the SBO supplementation. The SBO supplementation slightly increased CLA proportion in the intramuscular fat and subcutaneous fat. ***(Asian-Aust. J. Anim. Sci. 2006. Vol 19, No. 5 : 679-683)***

Key Words : Soybean Oil, Fat Tissue, C9,t11-CLA, Cholesterol, t-FA, Sheep

INTRODUCTION

Research efforts have been intensified to increase the content of *cis*9,*trans*11-conjugated fatty acid (c9,t11-CLA) in meat and dairy products due to its potential health benefits such as anti-carcinogenic effect (Ha et al., 1987) and immune stimulating effect (Michal et al., 1992). While the t10,c12- CLA is known to reduce the total cholesterol (T-C) and the low density lipoprotein-cholesterol (LDL-C), c9,t11-CLA did not have any effect on cholesterol in plasma of mouse (Sugano et al., 1997; de Deckere et al., 1999). Meanwhile, octadecenoic acid (t11-$C_{18:1}$) which is positively correlated with CLA in rumen contents(Bessa et al., 2000) is known to have negative effects on animal performance (Kennelly, 1996) and human health (Erasmus, 1993).

Based on the metabolic process of CLA formation via bio-hydrogenation by rumen microbes, selection of oil source which is rich in linoleic acid ($C_{18:2}$) and linolenic acid ($C_{18:3}$) are very important. While most of the experiments regarding the CLA production has been made with $C_{18:2}$-rich sunflower oil (Ivan et al., 2001) and safflower oil (Mir et al., 2000), $C_{18:3}$-rich linseed oil can be an alternative source (Wang et al., 2002; Wang and Song, 2003).

In fact, supplementation of vegetable oil to the diets has proved the promising effects in CLA content of the ruminant's products. Mir et al. (2000) indicated that dietary supplementation with safflower oil (6% of dietary dry matter) increased the c9,t11-CLA content of all tissues in lamb by more than 200%. Ivan et al. (2001) also released the increased c9,t11-CLA contents in various tissues in sheep fed the sunflower oil (6% of DM). The sunflower and safflower oils, however, are not easily accessed in Korea and expensive, thus an application of soybean oil which is also high in $C_{18:2}$ can be more practical.

The present study was, therefore, conducted to determine the effect of soybean oil (SBO) supplementation on contents of plasma cholesterol and c9,t11-CLA in adipose tissues of sheep.

MATERIALS AND METHODS

Animals and diets

Ten female Corriedale sheep (62.5±4.2 kg) were randomly assigned to two groups in an equal number, based on body weight. Each group of sheep was housed in two pens, 2 or 3 sheep in each pen. The animals in control group were fed the diet (1.3 kg/head/day, DM basis) consisting of commercially produced concentrate (80%) for the growing cattle and rye grass hay (20%). The total diet for the animal of control group met the nutrient requirements of sheep (NRC, 1985). Soybean oil (SBO) was added to the concentrate at 5% level of the total diet (DM basis) prior to feeding for another group of animals (SBO). The animals were fed twice daily (0800 and 1800 h) in an equal amount. The animals were allowed a free access to water and mineral block. The feeding trial was conducted for 12 weeks. Proportions (% of total) of palmitic acid ($C_{16:0}$), stearic acid ($C_{18:0}$), oleic acid ($C_{18:1}$), $C_{18:2}$ and $C_{18:3}$ as major

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Table 1. Chemical composition of the diet (%DM basis)

Components	Concentrate	Ryegrass hay
Crude protein	16.04	5.43
Ether extract	3.75	0.89
Neutral detergent fiber	40.64	74.84
Ash	7.67	3.08
Ca *	0.75	0.16
P *	0.35	0.25

* Contents of Ca and P in concentrate were obtained from the manufacturing company, and book values (NRC, 2001) were used for those of rye grass.

fatty acids for the soybean oil are 12.3, 6.1, 23.8, 51.0 and 5.4%, respectively, and those for the concentrate are 25.7, 5.9, 25.5, 31.0 and 1.7%, respectively. Chemical composition of concentrate and rye grass hay is presented in Table 1.

Feed analysis

Feed intake was measured by weighing the leftover at three-day interval and body weight at three-week interval. Proximal analyses of diets were also made at three-week interval. Contents of dry matter, crud protein and ether extract in the diet were determined according to AOAC (1995). The NDF content was estimated by the method of Van Soest et al. (1991).

Blood sampling and analysis

Blood was collected with vacutainer (Becton Dickinson) containing sodium heparin from jugular vein at three-week interval after initiation of the experiment. Blood samples were centrifuged at 3,000 rpm for 10 min. The supernatant was transferred to 30 ml screw-cap tubes and was kept frozen at -70°C until analyzed. After thawed, approximately 1 ml of the plasma was applied to the Spotchem Analyzer (SP-4410, KAK Corp.) with Spotchem™ II cholesterol reagent strip (ARKRAY, Inc. Japan) to determine the contents of total cholesterol (T-C) and high density lipoprotein-cholesterol (HDL-C).

Tissue sampling and analysis

After feeding for 12 weeks all the sheep were sacrificed to determine the fatty acid (FA) composition of tissues. Some tissues of intramuscular fat of *longissimus dorsi* and subcutaneous fat were removed from carcass at time of processing, and used for FA analysis of muscles and fat tissues. Upon receiving, all samples were packed well and frozen at -40°C until analyzed. The fat tissue samples were homogenized in chloroform/methanol (2:1, v/v) solution using a homogenizer (PT-MR3100, Switzerland) according to the procedure of Folch et al. (1957) to extract the fat. The chloroform from measured aliquots of the extract was evaporated on the Dri-Bath (Type l6500, USA) at 50°C under the nitrogen stream. Methylation of the lipids was conducted by following the method of Lepage and Roy (1986) prior to injecting into the gas chromatograph (GC, HP 5890 II, Hewlett Packard Co.). A fused silica capillary column (100 m×0.25 mm, i.d. ×0.20 μm thickness, Supelco, SP™-2560, USA) was used. The injector and detector temperature was maintained at 250°C, respectively. The initial column temperature was 175°C (held for 30 min), and then increased by 15°C/min to 220°C (held for 40 min). Ultra pure helium was used as the carrier gas. The various FAs were identified using standard FA (Sigma Chemical Co.).

Table 2. Daily feeding of dietary lipids and major fatty acids by treatment

Daily feeding	Control	SBO-diet
Dietary lipids (g)	41.3	106.3
Major fatty acids (g)		
$C_{16:0}$	10.02	18.02
$C_{18:0}$	2.30	6.27
$C_{18:1}$	9.95	25.42
$C_{18:2}$	12.09	45.24
$C_{18:3}$	0.66	4.17
Total	35.01	53.88

Statistical analysis

The results obtained were subjected to least squares analysis of variance according to the general linear models procedure of SAS (1985) and orthogonal contrasts between control vs. SBO supplementation for fat location were applied to compare differences in treatment means by T-Test (Freund et al., 1986).

RESULTS

A sheep of control diet was fed 41 g lipids daily while those of SBO supplemented diet fed 106.3 g (Table 2). But the sheep of SBO group had 102.6 g of lipid daily while the sheep of control group fed all (41.3 g) as given in the diets

Table 3. Growth performance of sheep as influenced by soybean oil supplementation

Items	Control	SBO	SEM[1]	Pr>F[2]
Initial BW (g)	65.3	65.5	0.400	0.757
Final BW (g)	72.3	74.5	1.622	0.447
Total body gain (g)	7.05	9.00	1.285	0.395
Average daily gain (g)	0.08	0.11	0.015	0.394
Daily DMI (g)	1.30	1.29	-	-
Daily lipid intake (g)	41.3	102.6	-	-
DMI/ADG	15.51	13.22	1.909	0.486

[1] Standard error of the means. [2] Probability level.

Table 4. Effect of soybean oil supplementation on total- and HDL-cholesterol (mg/100 ml) in plasma of sheep

Cholesterol	Control	SBO	SEM	Pr>F
	--------- 3 weeks ---------			
T-C (T) [1]	118.5	142.5	22.896	0.535
HDL-C (H) [2]	47.0	83.5	24.405	0.358
H/T	0.56	0.57	0.085	0.957
	--------- 6 weeks ---------			
T-C (T)	111.0	155.5	10.920	0.034
HDL-C (H)	25.5	99.0	6.174	0.013
H/T	0.35	0.64	0.058	0.037
	--------- 9 weeks ---------			
T-C (T)	98.0	143.0	12.529	0.126
HDL-C (H)	49.0	100.5	12.869	0.104
H/T	0.50	0.70	0.062	0.147
	--------- 12 weeks --------			
T-C (T)	97.0	154.0	13.601	0.097
HDL-C (H)	43.0	102.0	13.892	0.095
H/T	0.45	0.66	0.055	0.112

[1] Total cholesterol. [2] High density lipoprotein-cholesterol.

(Table 3). Feeding of the soybean oil supplemented diet slightly increased mean daily gain (0.11 kg) of sheep compared to control diet (0.08 kg) without significant difference (Table 3). Therefore, the feed requirement was slightly lower (13.22) for the sheep fed SBO than for the control sheep (15.51).

Tendency of concomitant increases in contents of both T-C and HDL-C in the plasma of sheep were observed from the sheep fed SBO supplemented diet, resulting in the increased ratio of HDL-C to T-C (Table 4). The differences in T-C ($p<0.034$) and HDL-C ($p<0.013$) were even greater in sheep fed SBO supplemented diet than in sheep in control for 6 weeks.

The FA composition in the tissue lipids of sheep sacrificed at the end of experiment is presented in Table 5. The SBO supplemented diet reduced ($p<0.05$) the proportions of $C_{16:1}$, $C_{17:0}$ and $C_{17:1}$ but increased ($p<0.05$) the proportions of $C_{18:0}$ and t11-$C_{18:1}$ in the intramuscular fat compared to control diets. The $C_{18:0}$ proportion was increased ($p<0.05$) by the SBO supplementation only in the subcutaneous fat. However, SBO supplementation did not significantly increase the proportions of c9,t11-CLA and $C_{18:2}$ in the intramuscular and subcutaneous fat tissues although their proportions in the sheep fed SBO were higher than those from the control.

DISCUSSION

The present study was to see how the sheep respond to the fatty acid such as $C_{18:2}$ in SBO supplemented (5%, of total diets, DM basis), especially in the production of c9,t11-CLA and its deposition. But the SBO supplementation resulted in an increased body gain compared to the control sheep due to the increased energy intake.

Cholesterol content in blood plasma has been used to assess the changes in lipid metabolism by oil feeding. The simultaneous increase in T-C and HDL-C levels in the plasma by SBO supplementation in present study indicates that the oil supplementation was clearly reflective in the plasma cholesterol level in sheep and increased T-C was mostly due to the increased HDL-C (Table 4). The HDL-C has been known to decrease the risk of coronary heart disease (Rudel et al., 1998). But Sugano et al. (1997) and de Deckere et al. (1999) reported that c9,t11-CLA did not have any effect on cholesterol level in plasma of mouse.

Earlier studies by Keys et al. (1965) and Hegsted et al. (1965) indicated that most saturated FA raised T-C levels in blood. Nestel et al. (1992) and Wood et al. (1993) indicated that the *trans*-FA can also increase the T-C concentration in blood plasma. Thus, the increased T-C content in plasma of sheep fed the SBO supplemented diet in the present study could be related with the $C_{18:0}$ and t11-$C_{18:1}$ in fat tissues. The $C_{18:0}$ in fat tissues was highly possible to be originated

Table 5. Effect of SBO supplementation on fatty acid composition (%) in the intramuscular, and subcutaneous fat

Fatty acid	Control		SBO		SEM	Contrast (Control vs. SBO)	
	Intra-muscular	Subcu-taneous	Intra-muscular	Subcu-taneous		Intra-muscular	Subcu-taneous
$C_{14:0}$	3.54	4.95	2.47	4.77	1.21	NS [1]	NS
$C_{15:0}$	0.37	0.37	0.31	0.41	0.12	NS	NS
$C_{16:0}$	25.14	26.71	25.18	28.60	1.78	NS	NS
$C_{16:1}$	2.63	2.50	2.02	1.84	0.46	*	NS
$C_{17:0}$	0.76	1.43	0.63	1.18	0.15	*	NS
$C_{17:1}$	0.59	0.91	0.38	0.54	0.14	*	NS
$C_{18:0}$	11.64	12.99	15.32	23.24	1.98	*	*
t11-$C_{18:1}$	2.36	5.24	4.20	5.52	0.83	*	NS
$C_{18:1}$	37.29	39.54	32.22	30.64	3.62	NS	NS
CLA [2]	0.33	0.23	0.42	0.35	0.03	NS	NS
$C_{18:2}$	6.59	1.28	8.29	1.42	0.81	NS	NS
$C_{18:3}$	0.19	0.15	0.22	0.19	0.04	NS	NS
Others	8.57	3.70	8.34	1.30	-	-	-

[1] Not significant at $p<0.05$. [2] *Cis*9, *trans*11-CLA. * $p<0.05$.

from both SBO supplemented and hydrogenation product of $C_{18:1}$ and $C_{18:2}$ in the rumen (Wang et al., 2003).

The oil supplementation increased the t11-C18:1 proportion but slightly increased the c9,t11-CLA proportion of the intramuscular fat. These results indicated that $C_{18:2}$ in the diets is required to increase the c9,t11-CLA content of the body tissues. Mir et al. (2000) suggested that CLA produced *in situ* by isomerization of $C_{18:2}$ during bio-hydrogenation in the rumen is an effective method to increase the CLA content of tissues. Improved CLA contents in milk (Kelly et al., 1998; Mir et al., 1999) and tissues of lambs (Ivan et al., 2001; Kott et al., 2003) have been observed when the oil sources which are high in $C_{18:2}$ were supplemented. Proportion of c9,t11-CLA was 0.84% in muscle (Mir et al., 2000) and 1.69% in subcutaneous tissue (Banni et al., 1996) of lambs fed safflower oil. The CLA proportion of fat tissues in the current study was lower than those reported values above. The slightly increased CLA proportion by SBO supplementation compared to that by control might be due to the differences in the age of the animals. In the present study, the average age was about 14 months and the sheep had a relatively heavier initial body weight. Mir et al. (2000) began to work with average 3.5 months of lambs, and Banni et al. (1996) carried out the study with one months lamb. Duration of twelve weeks feeding to the 14 months sheep also may not be long enough to deposit the CLA in the body fat.

The present study showed that dietary supplementation of $C_{18:2}$-rich SBO increased the HDL-C concentration and HDL-C to T-C ratio in blood plasma which is desirable in the human health perspective, and was, to some extents, effective in the deposit of c9,t11-CLA in the fat tissues of sheep.

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