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Supplementation Effects of $C_{18:2}$ or $C_{18:3}$ Rich-oils on Formations of CLA and TVA, and Lipogenesis in Adipose Tissues of Sheep*

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ABSTRACT : The present study was conducted to investigate the supplementation effects of $C_{18:2}$ rich-soybean oil or $C_{18:3}$ rich-perilla oil (7% of total diet, DM basis) for 12 weeks on plasma metabolites, fatty acid profile, *in vitro* lipogenesis, and activities of LPL and FAS in adipose tissue of sheep. The treatments were basal diet (Control), $C_{18:2}$ rich-soybean oil supplemented diet (SO-D) and $C_{18:3}$ rich-perilla oil supplemented diet (PO-D). All the sheep were fed the diets consisting of roughage to concentrate in the ratio of 40:60 (DM basis). Oil supplemented diets (SO-D and PO-D) slightly increased contents of triglyceride (TG) and total cholesterol (TC), proportions of both *cis*-9 *trans*-11 and *trans*-10 *cis*-12 CLA and TVA, but lowered ($p<0.01$) those of $C_{18:0}$ compared to the control diet. No differences were observed in the contents of TG and TC and proportions of fatty acids in plasma between supplemented oils. Oil supplemented diets slightly increased the proportions of *cis*-9 *trans*-11 and *trans*-10 *cis*-12 types of CLA in subcutaneous adipose tissue of sheep compared to the control diet. The rate of lipogenesis with acetate was higher ($p<0.01$) for intermuscular- and subcutaneous adipose tissues than that for intramuscular adipose tissue, while that with glucose did not differ among fat locations in sheep fed SO-D. No differences were observed in the rate of lipogenesis between substrates in all fat locations. The rates of lipogenesis with glucose increased only in the intermuscular- ($p<0.01$) and subcutaneous adipose tissue ($p<0.005$) compared to those with acetate. The rates of lipogenesis with acetate were the highest in the intermuscular and intramuscular adipose tissue of the sheep fed PO-D. Oil supplemented diets slightly increased the rate of lipogenesis with glucose for all fat locations. Supplementation of oils to the diet numerically increased the fatty acid synthase activity but did not affect the lipoprotein lipase activity in subcutaneous adipose tissue. (**Key Words :** Conjugated Linoleic Acid, Oils, Lipogenesis, Lipogenic Enzymes, Adipose Tissue, Sheep)

INTRODUCTION

Conjugated linoleic acid (CLA) in nature originates from either ruminal *bio*-hydrogenation of dietary linoleic acid ($C_{18:2}$) and linolenic acid ($C_{18:3}$) or endogenous synthesis in ruminants. Endogenous synthesis of *cis*-9, *trans*-11CLA takes place from *trans* 11-$C_{18:1}$, (trans-vaccenic acid, TVA) which is another intermediate of ruminal *bio*-hydrogenation, *via* Δ^9-desaturase (Griinary et al., 2000). The CLA contents in goat milk (Mir et al., 1999), subcutaneous adipose tissue of heifers (Garcia et al., 2003) and lambs (Kott et al., 2003) have been shown to increase mostly by dietary modifications with $C_{18:2}$ rich-oil seeds. It was also found that the CLA proportion could be increased by the addition of $C_{18:3}$ (Choi and Song, 2005; Wang et al., 2005), supplementation of oils to the diets for sheep (Choi et al., 2006a; Choi et al., 2006b) and Korean native (Hanwoo) steers (Wang et al., 2006), and manipulation of environmental factors such as energy level (Wang et al., 2002a, 2002b) or pH in incubation solution (Wang et al., 2003).

Meanwhile, ruminant body fat can be either synthesized *de novo*, mainly in adipose tissues using acetate and glucose, or arises from hydrolysis of circulating plasma triglyceride (Cryer, 1981). Enzymes such as fatty acid synthase (FAS), acetyl-CoA carboxylase (ACC) and lipoprotein lipase (LPL) are necessary for lipogenesis in adipose tissues (Vernon, 1976). Lipogenesis in the ruminant adipose tissues, in general, is dependent upon energy status, but lipid supplementation negatively affects the activities of lipogenic enzymes and *de novo* fatty acid synthesis in ovine

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Table 1. Major fatty acid composition (% of total FA) of oils, concentrate and alfalfa hay

Items	$C_{16:0}$	$C_{18:0}$	$C_{18:1}$	$C_{18:2}$	$C_{18:3}$
Soybean oil	11.67	4.07	21.25	48.84	5.07
Perilla oil	6.82	1.98	17.17	16.31	55.37
Concentrate	15.48	3.27	20.50	44.33	3.91
Alfalfa hay	30.99	6.28	6.74	18.63	29.02

Table 2. Chemical composition of diet (%, DM basis)

Items	Concentrate	Alfalfa hay
Dry matter	86.87	97.63
Crude protein	18.44	16.32
Ether extract	4.29	2.06
Neutral detergent fiber	37.14	37.23
Organic matter	91.03	90.17

abdominal and subcutaneous adipose tissuee (Vernon, 1976). Conjugated linoleic acid also has been known to depress the lipid synthesis, but it seems that it is dependent upon the type of CLA. Feeding a CLA mixture to pigs (Bassaganya-Riera and Hontecillas, 2006) and mice (Tsuboyama-Kasaoka et al., 2000) down regulated the differentiation of adipose tissues but its effect was greater from the *trans*-10 *cis*-12 CLA than from the *cis*-9 *trans*-11 type (Park et al., 1997, 1999; Brown et al., 2001). The TVA could also depress lipogenesis and milk fat synthesis in the udder (Griinary et al., 2000).

Major *bio*-hydrogenation intermediates formed from $C_{18:2}$ and $C_{18:3}$ in the rumen are *cis*-9, *trans*-11 CLA and TVA. Examinations of rate of lipogenesis and activities of major lipogenic enzymes in the adipose tissues of sheep fed oil supplemented diet are rare. The present feeding trial, therefore, was conducted to determine the supplementation effect of $C_{18:2}$ or $C_{18:3}$ rich oil on plasma metabolites, fatty acid profile, *in vitro* lipogenesis, and activities of LPL and FAS in adipose tissue of sheep.

MATERIALS AND METHODS

Animals and diets

Twelve male Corriedale sheep (mean body weight, 60±3.5 kg) were allotted to three groups of 4, each based on body weight. The dietary treatments were basal diet (Control), $C_{18:2}$ rich-soybean oil supplemented diet (SO-D) and $C_{18:3}$ rich-perilla oil supplemented diet (PO-D) feeding groups. All the sheep were fed the diets consisting of roughage to concentrate at the ratio of 40:60 (DM basis). Concentrate was supplemented with soybean oil or perilla oil at 7% of the total diet (DM basis) prior to feeding. Chopped alfalfa hay was fed as the roughage. The sheep were fed the mixed diets twice (08:00 and 18:00 h) a day in an equal quantity for 12 weeks. Feeding 1.2 kg of diets (DM) without oils closely met the daily maintenance requirement of sheep (NRC, 1985). The sheep had free access to water and mineral blocks. Fatty acid compositions of the soybean oil, perilla oil, concentrates and alfalfa hay are presented in Table 1, and the chemical compositions of concentrate and alfalfa hay are presented in Table 2.

Preparation of adipose tissues

Subcutaneous, intermuscular and intramuscular adipose tissues were collected from the sirloin under the 7th to 8th vertebrae into liquid nitrogen immediately after sheep were sacrificed prior to the morning when the feeding trial was terminated, and were later transferred to a -70°C deep freezer until used.

Determination of plasma metabolites

Blood samples were collected from the jugular vein immediately before sheep were sacrificed for the determination of triglyceride (TG) and total cholesterol (TC) content and fatty acid profile in plasma. Samples were stored in ice and centrifuged at 970×g for 10 min, and then the supernatant (plasma) was removed into 30 ml screw-cap tubes and kept frozen at -70°C until analyzed. After thawing, Spotchem^{TM}II cholesterol reagent strip (ARKRAY, Inc. Japan) was used to determine the TC content in plasma, and the TG content in plasma were also measured using the corresponding reagent strip supplied by the Spotchem Analyzer (SP-4410, KAK Corp., Japan).

Analysis of fatty acids

Lipids from tissues and plasma were extracted using Folch's solution (Folch et al., 1957). Methylation of the fatty acids followed 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, SP^{TM}-2560, Supelco, USA) was used. The gas chromatograph was programmed to operate at an oven temperature of 175°C for 5 min, and then the temperature was gradually increased by 15°C/min up to 220°C, then held for 40 min. Ultra-pure helium was used as a carrier gas. Standard fatty acid mixture (Sigma Co., USA) was applied to estimate the fatty acid composition of the samples.

In vitro lipogenesis of adipose tissues

In vitro incubations were performed for 2 h with subcutaneous, intermuscular and intramuscular adipose tissues which were taken immediately after the sheep were sacrificed. Adipose tissue explants (20 to 40 mg) were

incubated in 3 ml of incubation medium containing Krebs-Henseleit bicarbonate buffer (pH 7.4), 5 mM sodium acetate, 5 mM glucose, 10 mM hepes, and 1 μCi [U-^{14}C]acetate (Amersham Life Science, Arlington Heights, IL). Vials were gassed for 1 min with gas mixture of 95% O_2: 5% CO_2, capped and incubated for 2 h in a shaking water bath at 37°C. The incubation was terminated by placing the vials on ice. The incubated adipose tissue was weighed after 30 min. and placed in a new 20 ml vial. Fat was extracted from the adipose tissue in 5 ml Dole's solution (*iso*-propanol 40 : *n*-heptane 10 : 1 N H_2SO_4 1, v/v; Dole, 1956) in a ultrasonic water bath for 30 min. After 3 ml hexane and 3 ml H_2O was added to the vial, the upper layer (hexane) containing fat was transferred to a scintillation vial and diluted with 1.5 ml hexane. A scintillation cocktail was added to the vial and the specific radioactivity of ^{14}C-fat was measured by liquid scintillation counter (β-counter, Beckman LS5801). Specific radio activities of 0.1 μCi 1-^{14}C-acetate and 0.1 μCi 1-^{14}C-glucose were 793,461 cpm and 749,106 cpm, respectively, and background cpm was 26. Lipogenesis was expressed as picoM substrate/mg tissue.1 h.

FAS activity

Preparation of homogenate : Subcutaneous adipose tissues taken from the sheep were chopped very thoroughly and were placed into 50 ml falcon tubes. Portions of the chopped tissue were homogenated with 3-6 volumes/g wet weight of 0.25 M sucrose containing 1 mM dithiothreitol and 1 mM EDTA (final pH of the medium about 6.8). Particulate material was removed from the homogenate by centrifugation at 15,000×g for 45 min. All operations above were conducted at 0-4°C. The particle-free supernatant was stored at -20°C under N_2 until analyzed.

Enzyme assay : The activity of purified FAS was determined by measuring the oxidation of NADPH. Reagent solution was made with 200 mM potassium phosphate buffer (pH 6.6), 1 mM dithiolthreitol, 1 mM EDTA, 0.24 mM NADPH, 30 μM acetyl-CoA and 40-50 μM malonyl-CoA at 37°C, and then 10 μl of homogenate added. Consumption of NADPH was measured by the change in absorbance until it reached 0.05-0.15 unit/min in a final volume of 1.0 ml. After measuring the NADPH oxidation without acetyl-CoA and malonyl-CoA, the reaction was begun by adding 0.1 ml of acetyl-CoA (4.41 mg/ml) and malonyl-CoA (2.61mM/ml).

LPL activity

Preparation of homogenate : The LPL activity of subcutaneous adipose tissue was measured after detergent extraction, essentially as described by Iverius and Brunzell (1985). Tissue fragments (0.5 to 1 g) were homogenized in 3 to 4 ml of a cold (0 to 4°C) extraction buffer (0.2 mg/dl of deoxycholate 0.2 M Tris, 0.25 M sucrose, 1 mg/dl of BSA, 9 IU/ml of heparin, and 0.008 mg/dl of Nonidet P40, pH 8.3) at 15,000 rpm using a Polytron homogenizer (Polytron, Pt 3100) for six periods of 10 sec. Homogenates were centrifuged at 12,000×g for 15 min at 4°C. The clear supernatant was stored at -20°C until used for enzyme assay.

Enzyme assay : The LPL activity was assayed in triplicate after dilution of the supernatants (1:20 or 1:30, according to activities) in detergent-free buffer. A trioleic emulsion was prepared (Nilsson-Ehle and Schotz, 1976) by mixing 600 mg of unlabeled trioleic (Sigma, reference no. 128F-8443), 1.5 μCi of glycerol tri (9, 10(n)-^{3}H) oleic acid (Amersham, Les Ulis France, reference no. TRA-191), and 36 mg of phosphatidylcholine distearoyl (Sigma, reference no. 16F-8350). The mixture was emulsified in 10 ml of glycerol by homogenization at 4°C for 5 min using the ultra sonicator (Ultr-Sonicator, USA). The substrate emulsion for assay was prepared immediately beforehand using mixed trioleic emulsion: Tris-HCl 0.67 mM (pH 8.3) containing 2.4% (w/v) BSA:bovine serum (1:4:1, v/v/v). Heat inactivated (60°C for 45 min) bovine serum was used as a source of apolipoprotein CII activator. The assay was initiated by adding 100 μl of sample to 100 μl of substrate emulsion. At the end of assay (30 to 60 min at 37°C), aliquots were taken for separation of the released fatty acid from non-hydrolyzed triglyceride using the tow-phase system of Belfrage and Vaughan (1969). Blanks were prepared from the complete assay mixture incubated at 37°C for 60 min without added enzyme. Enzyme activity was expressed as nmoles of released fatty acid min^{-1} g^{-1}.

Statistical analysis

The results obtained were subjected to least squares analysis of variance according to the general linear models procedure of SAS (1985) and significances were compared by S-N-K test (Steel and Torrie, 1980).

RESULTS

Mean liveweight gains for 12 weeks of feeding were 7.30, 9.9 and 10.4 kg for the sheep fed control diet, SO-D and PO-D, respectively, and the corresponding daily DM intakes and daily gains were 1.20, 1.28 and 1.27 kg, and 0.09, 0.12 and 0.12 kg, respectively.

Metabolites and fatty acid profile in plasma

Oil supplemented diets (SO-D and PO-D) slightly increased contents of TG and TC compared to the control diet (Table 3). Oil supplemented diets also slightly enhanced the proportion of both *cis*-9 *trans*-11 and *trans*-10 *cis*-12 CLA and TVA, but lowered ($p<0.01$) that of $C_{18:0}$ (Table 3). No differences were observed in the contents of TG and TC and proportions of fatty acids in plasma between supplemented oils.

Table 3. Content of metabolites and fatty acid composition (%) in plasma of sheep as influenced by oil supplementation

Items	Treatments [1]			SEM [2]	p values
	Control	SO-D	PO-D		
TG (mg/dl) [3]	36.00	45.07	39.05	13.517	0.586
TC (mg/dl) [4]	62.67	66.67	72.67	12.970	0.411
Fatty acids :					
$C_{16:0}$	18.84	17.18	18.94	1.111	0.471
$C_{16:1}$	1.23	1.90	2.13	0.362	0.226
$C_{18:0}$	31.61^a	25.15^b	25.40^b	1.401	0.019
trans-11$C_{18:1}$ (TVA)	3.44	4.45	5.77	1.752	0.123
$C_{18:1}$	17.32	17.71	17.39	1.137	0.968
$C_{18:2}$	18.58	20.03	19.81	1.213	0.668
$C_{18:3}$	1.17	1.83	1.62	0.221	0.123
Total CLA	0.28	0.45	0.61		
cis-9, *trans*-11	0.16	0.25	0.34	0.051	0.306
trans-10, *cis*12	0.12	0.20	0.27	0.102	0.266
Others	7.19	8.09	8.17	0.912	0.637

[1] SO-D, Soybean oil supplemented diet; PO-D, perilla oil supplemented diet. Means in the same row with different superscripts differ ($p<0.05$).
[2] Standard error of means. [3] Triglycerides. [4] Total cholesterol.

Table 4. Fatty acid composition (%) of subcutaneous adipose tissue in sheep as influenced by oil supplementation

Items	Treatments [1]			SEM [2]	p value
	Control	SO-D	PO-D		
$C_{16:0}$	26.44	26.91	26.84	1.489	0.971
$C_{16:1}$	2.64^b	4.23^a	3.94^a	0.179	0.001
$C_{18:0}$	27.08^a	20.08^b	21.66^b	1.561	0.043
trans-11$C_{18:1}$ (TVA)	3.59	3.78	4.01	0.780	0.575
$C_{18:1}$	27.73	36.12	33.85	2.046	0.064
$C_{18:2}$	1.71	1.55	2.00	0.325	0.638
$C_{18:3}$	0.52	0.41	0.32	0.053	0.091
Total CLA	0.45	0.89	0.63		
cis-9, *trans*-11	0.45	0.75	0.55	0.098	0.180
trans-10, *cis*-12	0.03	0.14	0.11	0.029	0.146
Others	8.83	6.41	6.67	1.117	0.517

[1] Means in the same row with different superscripts differ ($p<0.05$).
[2] Standard error of means.

Fatty acid profile of adipose tissue

The proportion of $C_{16:1}$ ($p<0.01$) was higher but that of $C_{18:0}$ was lower ($p<0.05$) in subcutaneous fats of sheep fed oil supplemented diets (SO-D and PO-D) than the corresponding values of sheep fed the control diet (Table 4). Oil supplemented diets slightly increased the proportions of $C_{18:1}$, and *cis*-9 *trans*-11 and *trans*-10 *cis*-12 types of CLA compared to those on the control diet.

Lipogenesis

Comparisons of the rate of lipogenesis *in vitro* among substrates for various adipose tissues are shown in Table 5. In sheep fed the control diet, intermuscular preference of glucose was significantly higher ($p<0.005$) than that of acetate. No differences in substrate preference were found among fat locations in both acetate and glucose. In sheep fed SO-D, the rate of lipogenesis with acetate was higher ($p<0.01$) for intermuscular- and subcutaneous adipose tissues than that for intramuscular adipose tissue, while that of lipogenesis with glucose did not differ among fat locations (Table 5). No differences were observed in the rate of lipogenesis between substrates in all fat locations. In sheep fed PO-D, no differences were found in the rates of lipogenesis among fat locations for both substrates, while rates of lipogenesis with glucose increased in the intermuscular- ($p<0.01$) and subcutaneous adipose tissues ($p<0.005$) compared to the rates with acetate (Table 5). There was no difference in the rate of lipogenesis of intermuscular adipose tissue between substrates.

The rates of lipogenesis with acetate were highest in the intermuscular and intramuscular adipose tissues of sheep fed PO-D, while the highest rate was observed from the subcutaneous adipose tissue of sheep fed the control diet, although the differences among the diets were small (Table 5). Oil supplemented diets slightly increased the rate of lipogenesis with glucose for all fat locations.

Activities of FAS and LPL

An estimated FAS activity is shown in Table 6. Supplementation of oils to the diet numerically increased

Table 5. Effects of substrates on lipogenesis (picomol/1 h/g, tissue) of adipose tissue (AT) in sheep as influenced by oil supplementation

Items	Substrates		SEM[1]	p value
	Acetate	Glucose		
Control				
Intramuscular AT	77.2	99.5	13.582	0.374
Intermuscular AT	40.9[B]	95.0[A]	4.066	0.003
Subcutaneous AT	95.9	103.2	16.564	0.771
SEM	14.203	11.767	-	-
p value	0.138	0.888	-	-
Soybean oil				
Intramuscular AT	41.1[b]	70.4	6.229	0.058
Intermuscular AT	107.3[a]	115.1	12.829	0.726
Subcutaneous AT	99.3[a]	97.25	5.567	0.925
SEM	8.465	12.003	-	-
p value	0.013	0.175	-	-
Perilla oil				
Intramuscular AT	50.2	98.5	30.307	0.377
Intermuscular AT	35.2[B]	104.5[A]	9.034	0.016
Subcutaneous AT	42.8[B]	85.5[A]	1.639	0.002
SEM	10.811	17.602	-	-
p value	0.659	0.802	-	-

[a, b] Means in the same column with different superscripts differ ($p<0.05$).
[A, B] Means in the same row with different superscripts differ ($p<0.05$).
[1] Standard error of the means.

the FAS activity but did not affect the LPL activity in subcutaneous adipose tissue of sheep.

DISCUSSION

Slightly increased body weight gain could be due to the increased energy intake by oil supplementation. The responses of supplemented oil were limited to plasma TC (Table 3) and CLA proportions in plasma (Table 3) and adipose tissues (Table 4) of sheep, but the responses were relatively small. Kott et al. (2003) reported that the inclusion of high linoleic safflower seed in the finishing diets of lambs had positive effects on fatty acid profile, and especially on CLA content in meat. Wang et al. (2006) also observed slightly increased contents of TC and HDL-cholesterol, and CLA proportion in intramuscular fat of Korean native (Hanwoo) steers when a soybean oil based mixed oil supplemented (5% of concentrate, DM) diet was fed for up to 5 months.

Changes in lipogenesis and lipolysis regulate the accumulation of triglycerides in adipose tissue. Two major metabolic pathways contribute to fatty acid availability in ruminant adipose tissues. One is the acetate conversion into fatty acids, which depends on lipogenic enzyme activities such as ACC and FAS. Another is the activity of LPL, which is synthesized by adipose tissues and migrates its active form toward the capillary endothelium (Faulconnier et al., 1994). The LPL hydrolyzes circulating triglycerides and allows fatty acids to enter the adipose cells (Faulconnier et al., 1994). Vernon (1979) supposed that acetate was the primary carbon source for fat synthesis in ruminant adipose tissue. Acetate was the preferable substrate whereas the ability to use glucose for the fat synthesis was small in the subcutaneous adipose tissue in beef cattle (Smith and Crouse, 1984). Chung et al. (2000) also observed that the amount of fat synthesized in adipose tissue of Hanwoo bulls was higher from acetate than from glucose in subcutaneous and intermuscular adipose tissues. The rate of fat synthesis from acetate was also higher than from glucose in subcutaneous and intramuscular adipose tissues (Song et al., 2001). In the present study, however, higher rates of fat synthesis with glucose were observed from intermuscular adipose tissue and both intermuscular and subcutaneous adipose tissues when the sheep were fed the control diet and PO-D, respectively (Table 5). Mean preference of acetate was greater in intermuscular and intramuscular adipose tissues from the sheep fed PO-D whereas that of glucose looked higher in all fat locations irrespective of dietary treatments. Oil supplementation also slightly enhanced the FAS activity of subcutaneous fat in *in vitro* lipogenesis but did not affect the LPL activity (Table 6). Thus, the rate of fat synthesis, at least, was not suppressed by the supplementation of oils, but the relationship between the rate of fat synthesis and oil sources which differ in fatty acid composition remains to be examined further.

One of the many beneficial health effects of CLA has been a reduction of body fat levels. Several animal models have indicated the reduced adiposity from CLA feeding (Cook et al., 1999; Ostrowska et al., 1999; Corino et al., 2002). The CLA effect on reduction of fat level, however, depends upon the isomer type. Park et al. (1999) reported that the *trans*-10 *cis*-12 isomer was the more potent anti-obesity agent in mice than other CLA isomers such as *cis*-9 *trans*-11 CLA. The rate of fatty acid synthesis of human adipose tissue from glucose *in vitro* also decreased when only the *trans*-10 *cis*-12 CLA level increased (Brown et al., 2001). However, Bee (2001) indicated that mixed CLA did

Table 6. Effect of oil supplementation on FAS (nmol/min/g, tissue) and LPL (μmol/min/g, tissue) activities of subcutaneous adipose tissues in sheep

Enzymes	Treatments			SEM [1]	p value
	Control	SO-D	PO-D		
FAS	30.90	36.90	40.50	3.414	0.213
LPL	30.45	29.88	31.48	3.973	0.135

[1] Standard error of the means.

not affect the activities of malic enzyme and FAS in pigs. In the present study, both types of CLA existed in the plasma (Table 3) and subcutaneous adipose tissue (Table 4), but the proportion of *cis*-9 *trans*-11 CLA was slightly higher than that of the *trans*-10 *cis*-12 isomer at both sites. Thus, the depressing effect of CLA in the differentiation of adipocytes might not be evident.

Trans vaccenic acid has also been known to depress adiposity and milk fat synthesis in the udder (Griinary et al., 2000). It is also a source of endogenous synthesis of *cis*-9 *trans*-11CLA *via* Δ^9-desaturase (Griinary et al., 2000). Oil supplementation did not increase the TVA proportions of subcutaneous adipose tissue (Table 4) compared to those in plasma (Table 3) in the present study. Thus, the depressing effect of TVA on fat synthesis may not be expected under this experimental circumstance. This result might also be related to the contribution of TVA for the endogenous synthesis of *cis*-9 *trans*-11CLA. Chant et al. (1992) reported that SCD activity in adipose tissue was elevated in steers fed a high-oleic acid sunflower seed.

Based on the results of the present feeding trial with sheep, it might be concluded that supplementation of the $C_{18:2}$ or $C_{18:3}$ rich-oil slightly increased CLA content and, to some extent, enhanced the *in vitro* lipogenesis of subcutaneous adipose tissue. The CLA in subcutaneous adipose tissue, at least, did not suppress the lipogenic enzyme activities of FAS and LPL.

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