Effects of Concentrate to Roughage Ratio on the Formation of *cis*-9, *trans*-11 CLA and *trans*-11-Octadecenoic Acid in Rumen Fluid and Plasma of Sheep When Fed High Oleic or High Linoleic Acid Oils*

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ABSTRACT : A metabolism trial with four ruminally fistulated sheep was conducted in a 4×4 Latin square design to examine the effect of concentrate to roughage ratio (70:30 vs. 85:15) and oil source (soybean oil vs. rapeseed oil) on the ruminal fermentation pattern and C_{18}-fatty acids composition including *trans*11-$C_{18:1}$ (*trans*11-ODA) and *cis*9, *trans*11-18:2 (*cis*9, *trans*11-CLA) in the rumen fluid and plasma. Oil was added to the concentrate at 5% level of the total diet (DM basis) and chopped rye grass hay was fed as roughage. An increased level of concentrate (85%) within supplemented oil slightly lowered pH but increased ammonia concentration. Supplementation of rapeseed oil relatively increased pH and ammonia concentration. Higher concentrate level resulted in increased tendencies of total VFA concentration while oil source did not affect the total VFA concentration and VFA proportion. Whole tract digestibilities of DM, CP, EE, NDF and OM in diets slightly increased at higher concentrate level. Proportions of oleic acid ($C_{18:1}$) and linoleic acid ($C_{18:2}$) in the rumen fluid were influenced by the fatty acid composition of oil source but oil source did not affect the *in vitro* formations of *trans*11-ODA and *cis*9, *trans*11-CLA. Slightly increased *trans*11-ODA and *cis*9, *trans*11-CLA proportions, however, were observed from the sheep fed high roughage diet supplemented with both soybean oil and rapeseed oil. The $C_{18:1}$ and $C_{18:2}$ composition in supplemented oils responded to those in plasma of sheep. Effects of concentrate to roughage ratio and oil source on *trans*11-ODA and *cis*9, *trans*11-CLA proportions in plasma were found to be small. Proportion of *cis*9, *trans*11-CLA in plasma tended to be increased from the sheep fed high roughage diet and collection time at 9 h post feeding. *(Asian-Aust. J. Anim. Sci. 2003. Vol 16, No. 11 : 1604-1609)*

Key Words : Concentrate to Roughage Ratio, Oil Source, *Cis*9, *Trans*11-$C_{18:2}$, *Trans*11-$C_{18:1}$, Sheep

INTRODUCTION

The contents of *cis*9, *trans*11-linoleic acid (*cis*9, *trans*11-CLA) as a major conjugated linoleic acid isomer and *trans*11-octadecenoic acid (*trans*11-ODA) in ruminant animal products may be related to factors that are associated with rumen fermentation. These factors include the concentration and type of unsaturated fatty acids (Bateman and Jenkins, 1998), concentrate level in diet (Wang et al., 2002a), supplementation type of oil (Wang et al., 2002b), pH (Kalscheur et al., 1997; Wang and Song, 1999) and ionophores (Sauer et al., 1998) et al. A previous *in vitro* study (Wang et al., 2002a) indicated that percent *cis*9, *trans*11-CLA had a clearly decreasing trend with concentrate level when incubated with linseed or rapeseed. Kelly and Bauman (1996) found that the total CLA levels in milk were halved when fed the high concentrate diet. Santos-Silva et al. (2002) also found that total CLA and *trans*11-ODA contents in intramuscular tissue of lambs on pasture were higher than those in lambs raised mostly with concentrate. One of the influences on these results could be pH in the rumen since pH should be low when feeding high concentrate. Kepler et al. (1970) reported that free carboxyl radical which needs for the production of CLA occurs more at high pH. Romo (1995) also found that lowering the pH below 6 caused an accumulation of *trans*-fatty acids with slow hydrogenation for the CLA formation *in vitro*.

Despite the fact that roughage to concentrates ratio and lipid supplementation influence on the ruminal fermentation their concomitant effects on compositions of fatty acid intermediates in the rumen and blood has been found little, especially when the ruminant animals are fed oils different in fatty acid composition. Therefore, the present study was conducted to determine the changes in the profiles of long-chain fatty acids, especially *cis*9, *trans*11-CLA and *trans*11-ODA in rumen fluid and blood plasma from sheep in response to diets supplemented with high-oleic rapeseed or high-linoleic soybean oil.

MATERIALS AND METHODS

Animals and diets

Four ruminally fistulated Corriedale male sheep (mean body weight, 62±6 kg) were fed the diets consisting of two different roughage to concentrate ratios (85:15 and 70:30, DM basis). Soybean oil (SBO) or rapeseed oil (RSO) was added to the concentrate at 5% level of the total diet (DM basis) prior to feeding. The metabolism trial was conducted in a 4×4 Latin square with 4 dietary treatments and 4 periods. Each period consisted of 7 adjustment days to the

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Table 1. Major fatty acid composition (% of total fatty acid) of oils and concentrate

Items	$C_{16:0}$	$C_{18:0}$	$C_{18:1n-9}$	$C_{18:2n-6}$	$C_{18:3n-3}$
Soybean oil	12.3	6.1	23.8	51.0	5.4
Rapeseed oil	5.1	2.3	61.5	20.0	7.6
Concentrate	25.7	5.9	25.5	31.0	1.7

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

Components	Concentrate	Rye grass hay
Crude protein	5	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

diet and 4 sampling days. Sheep were housed in an individual metabolic cage with quantitative collection of feces and allowed free access to water and mineral block.

Chopped Italian rye grass (*Lolium multiflorum Lam*) hay was selected as the roughage. The sheep were fed the mixed diets of roughage and concentrate twice (08:00 and 18:00 h) a day in an equal amount. Feeding 1.3 kg diets (DM) closely met the daily maintenance requirements of sheep (NRC, 1985). Fatty acid compositions of the SBO, RSO and concentrate were presented in Table 1, and the chemical compositions of concentrate and Italian rye grass hay were presented in Table 2.

Measurements and analyses

On day 10, feed residues and feces were collected at 18:00 h for two consecutive days each period to estimate the whole tract digestibility. Mixed diets were also taken for the proximal analysis. The proximal analysis was made according to AOAC (1984). The neutral detergent fiber (NDF) was estimated by the method of Goering and Van Soest (1970). On day 8, 100 ml rumen fluid was collected through a perforated probe attached to a vacuum pump from various sites of the rumen at 2, 4, 7 and 9.5 h post feeding for two consecutive days each period. pH was immediately measured and the collected rumen fluid was strained through four layers of cheesecloth. Ammonia concentration was determined by the method of Fawcett and Scott (1960) using the spectrophotometer (DU-650). Four ml rumen fluid was mixed with 1 ml 25% phosphoric acid and 0.5 ml pivalic acid solution (2%, w/v) as an internal standard. The mixed solution was centrifuged at 15,000×g for 15 min., and the supernatant was used to determine the concentration and composition of VFA using gas chromatograph (GC, HP 5890II, Hewlett Packard Co.). The remaining rumen fluid was stored frozen (-20°C) and freeze dried (Refrigerant R502), and the lipids were extracted using Folch's solution (Folch et al., 1957). Methylation of the lipids was followed the method of Lepage and Roy (1986) prior to injecting into

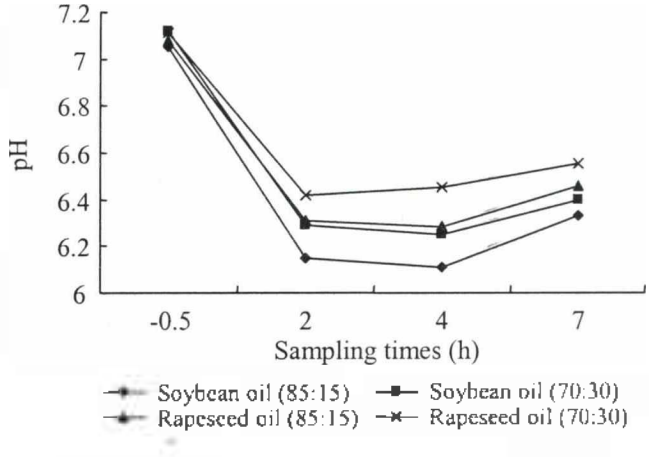

Figure 1. pH of the rumen fluid at various sampling times.

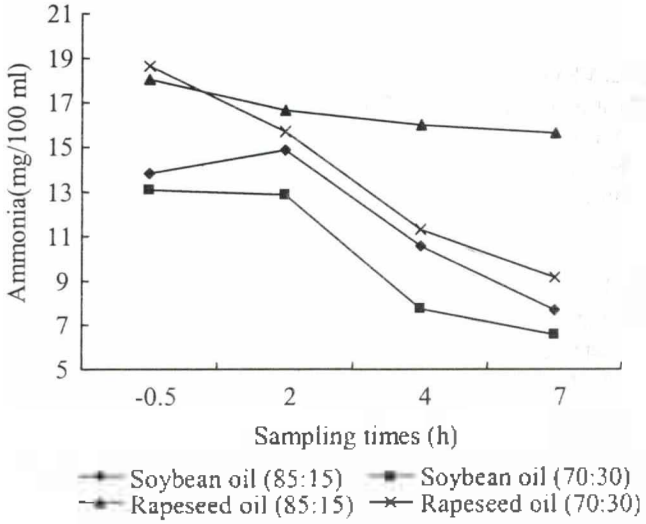

Figure 2. Ammonia concentration in the rumen fluid at various sampling times.

the GC. A fused silica capillary column (100 m×0.25 mm, i.d., 0.20 μm thickness, SPTM-2560, Supelco) was used. The initial column temperature was 175°C (held for 30 min), and then increased at 15°C/min to 220°C (held for 40 min). Ultra pure helium was used as a carrier gas.

On day 10, 30 ml blood was collected from jugular vein at 3 h and 9 h post feeding with vacutainer containing sodium heparin for two consecutive days each period. The blood was centrifuged immediately at 3,000 rpm for 10 min while the other samples were stored in ice box, and the supernatants (plasma) were removed into 30 ml screw-cap tubes and were kept frozen at -40°C until analyzed. After thawed, plasma lipids were extracted and followed by methylation. Analysis of fatty acid of the plasma followed the same procedure as that of the rumen fluid.

Statistical analysis

The results obtained were subjected to least squares

Table 3. Concentration and molar proportion of VFA in the rumen fluid

Items	Concentrate to roughage ratio by oil source				SEM [1]	Pr>F [2]
	Soybean oil		Rapeseed oil			
	85:15	70:30	85:15	70:30		
	2 h post feeding					
Total VFA (mmoles/100 ml)	56.88	48.43	62.51	54.94	6.970	0.602
Molar proportion (mmoles/100 mmoles)						
Acetate (C_2)	39.94	39.19	41.69	40.55	0.492	0.088
Propionate (C_3)	38.18	38.46	33.57	38.30	2.378	0.478
Butyrate	15.94	17.28	18.92	16.35	1.678	0.638
C_2/C_3	1.05	1.03	1.25	1.06	0.066	0.217
	4 h post feeding					
Total VFA (mmoles/100 ml)	62.07	52.69	58.06	41.61	4.304	0.098
Molar proportion (mmoles/100 mmoles)						
Acetate (C_2)	44.22	42.91	40.94	44.14	1.534	0.480
Propionate (C_3)	34.55	36.20	31.89	34.92	2.599	0.711
Butyrate	16.25	16.62	21.39	16.72	1.928	0.329
C_2/C_3	1.28	1.21	1.29	1.27	0.111	0.955
	7 h post feeding					
Total VFA (mmoles/100 ml)	43.62	41.27	41.39	37.04	1.218	0.074
Molar proportion (mmoles/100 mmoles)						
Acetate (C_2)	44.65	44.43	43.55	46.13	1.124	0.511
Propionate (C_3)	31.15	34.03	30.27	31.47	2.236	0.690
Butyrate	18.90	17.45	21.71	17.23	1.620	0.317
C_2/C_3	1.44	1.33	1.45	1.48	0.118	0.819
	9.5 h post feeding					
Total VFA (mmoles/100 ml)	30.79	25.71	30.32	26.72	4.898	0.844
Molar proportion (mmoles/100 mmoles)						
Acetate (C_2)	48.60	50.02	47.42	49.76	2.617	0.886
Propionate (C_3)	28.31	29.66	25.42	28.35	1.323	0.282
Butyrate	17.03	16.93	19.97	18.53	1.485	0.501
C_2/C_3	1.73	1.72	1.97	1.67	0.155	0.592

[1] Standard error of the mean. [2] Probability levels.

Table 4. Whole tract digestibility (%) of dietary components

Items	Concentrate to roughage ratio by oil source				SEM [1]	Pr>F [2]
	Soybean oil		Rapeseed oil			
	85:15	70:30	85:15	70:30		
Dry matter	69.02	64.86	72.19	68.12	2.541	0.263
Crude protein	65.56	59.41	69.93	67.18	2.297	0.116
Ether extracts	90.59	90.01	93.25	92.26	1.527	0.494
Neutral detergent fiber	51.61	48.56	60.20	57.41	5.342	0.382
Organic matter	72.46	66.94	74.42	69.63	2.743	0.260

[1] Standard error of the mean. [2] Probability levels

analysis of variance according to the general linear models procedure of SAS (1985) and significances were compared by S-N-K's Test (Steel and Torrie, 1980).

RESULTS

Fermentation characteristics and digestibility of nutrients

Despite the supplementation of oils to the diets, pH of rumen fluid lowered rapidly up to the 2 h post feeding (Figure 1). Responses of concentrate to roughage ratio to the pH of rumen fluid were found different depending upon the oil source supplemented in which an increased level of concentrate (85%) within supplemented oil slightly lowered pH (Figure 1) but increased ammonia concentration (Figure 2). Supplementation of RSO relatively increased pH and ammonia concentration compared to those of SBO. Higher concentrate level resulted in increased tendencies of total VFA concentration but molar proportion of each VFA was not influenced by the concentrate to roughage ratio (Table 3). Oil source did not affect the total VFA concentration and VFA proportion.

Whole tract digestibilities of DM, CP, EE, NDF and OM in diets slightly increased at higher concentrate level and in sheep fed RSO supplemented diets when concentrate was fed at a same level (Table 4).

Table 5. Composition (%) of C_{18}-fatty acids in rumen fluid

Fatty acids	Concentrate to roughage ratio by oil source				SEM [1]	Pr>F [2]
	Soybean oil		Rapeseed oil			
	85:15	70:30	85:15	70:30		
	2 h post feeding					
$C_{18:0}$	49.73	48.65	57.83	49.51	5.004	0.243
$C_{18:1}$	12.14	10.90	12.99	10.24	2.649	0.184
t-$C_{18:1}$ [3]	7.63	8.45	8.20	8.27	1.620	0.363
CLA [4]	0.24	0.38	0.33	0.39	0.241	0.464
$C_{18:2}$	10.62	7.54	7.76	6.94	2.944	0.330
$C_{18:3}$	0.59	0.89	1.18	0.63	0.352	0.653
	4 h post feeding					
$C_{18:0}$	53.89	50.70	56.71	54.29	3.200	0.102
$C_{18:1}$	11.23	15.37	14.62	15.99	2.088	0.155
t-$C_{18:1}$	9.76	11.14	9.08	10.61	1.738	0.121
CLA	0.32	0.59	0.38	0.49	0.174	0.160
$C_{18:2}$	5.85	3.45	2.69	2.61	1.338	0.291
$C_{18:3}$	0.35	0.47	0.98	0.75	0.177	0.196
	7 h post feeding					
$C_{18:0}$	56.02	58.04	61.70	57.39	2.932	0.229
$C_{18:1}$	9.68	10.43	11.01	14.92	2.347	0.326
t-$C_{18:1}$	10.27	10.17	7.52	8.47	1.653	0.235
CLA	0.29	0.40	0.16	0.30	0.091	0.203
$C_{18:2}$	3.25	3.46	1.74	2.46	0.423	0.131
$C_{18:3}$	0.81	0.60	1.10	1.32	0.377	0.591
	9.5 h post feeding					
$C_{18:0}$	69.28	61.91	65.05	67.09	1.853	0.168
$C_{18:1}$	5.48	5.59	8.50	6.51	1.906	0.697
t-$C_{18:1}$	4.18	6.48	4.97	4.02	0.508	0.079
CLA	0.04	0.36	0.11	0.24	0.271	0.253
$C_{18:2}$	1.92	2.26	1.40	1.49	0.316	0.122
$C_{18:3}$	0.26	1.17	0.93	0.63	0.300	0.229

[1] Standard error of the mean. [2] Probability levels. [3] *Trans*-11 $C_{18:1}$ isomer. [4] Conjugated linoleic acid (*cis*-9, *trans*-11 isomer).

Fatty acid composition of rumen fluid and plasma

While compositions of stearic acid, *trans*11-ODA and *cis*9, *trans*11-CLA in the rumen fluid increased, those of linoleic acid ($C_{18:2}$) and linolenic acid decreased up to 4 h after feeding in the all treatments (Table 5). Proportions of oleic acid ($C_{18:1}$) and $C_{18:2}$ in the rumen fluid were influenced by the fatty acid composition of oil source. But oil source did not affect the *in vitro* formations of *trans*11-ODA and *cis*9, *trans*11-CLA. Slightly increased *trans*11-ODA and *cis*9, *trans*11-CLA proportions, however, were observed from the sheep fed high roughage diet supplemented with both soybean oil and rapeseed oil.

Fatty acids such as $C_{18:1}$ and $C_{18:2}$ in supplemented oils responded to them in plasma of sheep. Effects of concentrate to roughage ratio and oil source on *trans*11-ODA and *cis*9, *trans*11-CLA proportions in plasma were found to be small (Table 6). Relatively increased proportions of *trans*11-ODA and *cis*9, *trans*11-CLA, however, were observed from the plasma collected at 9h post feeding. Proportion of *cis*9, *trans*11-CLA in plasma tended to be increased from the sheep fed high roughage diet and collection time at 9 h post feeding.

DISCUSSION

The present study evaluated the effects of concentrate to roughage ratio and oil source containing high $C_{18:1}$ or $C_{18:2}$ on composition of long-chain fatty acids, especially *trans*11-ODA and *cis*9, *trans*11-CLA in rumen fluid and in the plasma of sheep. Fermentation characteristics (pH, Figure 1; ammonia concentration, Figure 2 and VFA, Table 3) were reflected mainly by the concentrate to roughage ratio of diet. The results was confirmed by the observations from previous *in vitro* study (Wang et al., 2002a) in which pH and total VFA concentration was influenced, to some degree, by the concentrate addition level in culture solution. Effects of oil source on ruminal fermentation, however, were relatively small compared to those of concentrate to roughage despite the considerable difference in fatty acid composition between SBO and RSO (Table 1). But VFA concentration in the present study might be influenced by the fatty acid composition of oils supplemented which are high in unsaturation. Jenkins (1987) indicated that the ruminal fermentation decreases as the degree of unsaturation increase. The narrow ratio in C_2 to C_3 proportion would certainly be due to the oil supple-

Table 6. Composition (%) of C_{18}-fatty acids in plasma

Fatty acids	Concentrate to roughage ratio by oil source				SEM [1]	Pr>F [2]
	Soybean oil		Rapeseed oil			
	85:15	70:30	85:15	70:30		
	3 h post feeding					
$C_{18:0}$	31.98	33.22	36.56	35.99	2.399	0.499
$C_{18:1}$	15.04	15.06	18.32	19.47	2.620	0.243
t-$C_{18:1}$ [3]	2.90	1.82	2.22	1.60	0.372	0.124
CLA [4]	0.38	0.31	0.35	0.32	0.079	0.200
$C_{18:2}$	23.08	20.71	19.92	18.97	2.998	0.194
$C_{18:3}$	1.52	1.07	0.97	1.09	0.144	0.081
	9 h post feeding					
$C_{18:0}$	37.06	36.04	36.94	34.64	2.635	0.907
$C_{18:1}$	15.85	14.69	16.43	19.47	2.489	0.182
t-$C_{18:1}$	1.79	2.06	1.56	2.99	0.474	0.213
CLA	0.44	0.68	0.44	0.80	0.253	0.182
$C_{18:2}$	17.35	19.65	16.20	16.68	2.152	0.110
$C_{18:3}$	1.09	1.05	1.83	1.19	0.265	0.178

[1] Standard error of the mean. [2] Probability levels. [3] Trans-11 $C_{18:1}$ isomer. [4] Conjugated linoleic acid (*cis*-9, *trans*-11 isomer).

mentation as observed by Jenkins and Jenny (1989) and Song et al. (1995). Jenkins and Jenny (1989) indicated that supplementation of lipid to the diet lowered C_2 proportion while increased C_3 due to the reduced degradation of fiber in the rumen.

Increased tendencies of *cis*9, *trans*11-CLA and *trans*11-ODA proportions from high roughage diet supplemented each oil in the rumen fluid might be caused by the increased rumen pH due to the high roughage level. Previous *in vitro* study indicated that higher pH increased significantly the *cis*9, *trans*11-CLA and *trans*11-ODA proportions (Wang et al., 2002c). The relationships between roughage level and CLA production in milk fat have been studied more extensively. An *in vivo* study using supplemental fat by Kelly and Bauman (1996) indicated that the CLA levels in milk were halved when the forage to concentrate ratio of the diet was changed from 50:50 to 20:80. Griinari et al. (1996) also reported that increased forage to concentrate ratios increased milk fat concentrations of CLA. Meanwhile, rumen bacteria produce greater amounts of CLA when the concentration of $C_{18:2}$ in diet is high enough to reduce the bacterial growth (Kim et al., 2000) and an important factor affecting CLA production is dietary $C_{18:2}$ concentration (Chouinard et al., 1998; Kelly et al., 1998). The $C_{18:2}$ proportion (51.0%) in SBO supplemented at the level of 5% of total diet is higher than that (20.0%) in RSO (Table 1). However, difference in *cis*9, *trans*11-CLA proportion between SBO and RSO in rumen fluid was not found in the present study although parts of the fatty acid profiles such as $C_{18:1}$ and $C_{18:2}$ were influenced by those of each oil source.

The trends in $C_{18:1}$ and $C_{18:2}$ proportions of plasma were similar to those of the rumen fluid as reflected by the profiles of each oil source. Oil source, however, did not affect the proportion of *cis*9, *trans*11-CLA in the present study. Loor and Herbein (2002) also observed no difference in *cis*9, *trans*11-CLA proportion of the blood plasma in dairy cattle between canola oil and SBO feeding. But increased tendency of *cis*9, *trans*11-CLA proportion in plasma collected at 9 h post feeding also reflected that of rumen fluid as roughage level in the diet increased.

Based on the results, it might be concluded that the influence of concentrate to roughage ratio in the present metabolism trial with sheep on *cis*9, *trans*11-CLA proportion in the rumen fluid and plasma was small when supplemented with SBO or RSO. Oil source effect on the formation of *cis*9, *trans*11-CLA proportion was not found either under the present experimental condition despite some fatty acids in the rumen fluid and plasma was reflected by those of oil source. Possible reasons for the present results could be due to the narrow ratio of concentrate to roughage in the diet and supplemented level of oil. Thus, broader ratio of concentrate to roughage between treatments and greater amount of oil supplementation to the diet need to be examined.

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