Effects of Dietary Fat Sources on Occurrences of Conjugated Linoleic Acid and *trans* Fatty Acids in Rumen Contents

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ABSTRACT : The effects of dietary sources of C18:2 n-6 or C18:3 n-3 fatty acids on the occurrence of conjugated linoleic acid (CLA) and time-dependent changes of free fatty acid fractions in rumen contents were investigated. Sheep (n=4) fitted with rumen fistula were used in a 4×4 Latin square design with each 14 d period. Sheep were fed one of four diets consisting of grass hay and concentrates in a ratio of 70:30. Dietary treatments were 100% concentrates (served as the control), and concentrates were replaced by safflower seed at 30% (SFS), safflower meal at 18% - safflower oil at 12% (SFO), and safflower meal at 18%-linseed oil at 12% (LNO). At the end of each experimental period, rumen contents from each sheep were collected before feeding and at 1, 3, 6 and 12 h after feeding. The levels of *cis*-9, *trans*-11 CLA in free fatty acid fraction were considerably increased in all treated groups relative to the control, but not significantly. The increase in *cis*-9, *trans*-11 CLA was slightly higher in SFS and SFO groups than group fed diet containing linseed oil. The level of *cis*-9, *trans*-11 CLA in free fatty acid fraction was reached to the maximum value at 1hr after feeding and, thereafter gradually decreased to near the value before feeding. The generation of *trans*-11 C18:1 was significantly higher in all treated groups than that of control. The level of *trans*-11 C18:1 was linearly increased after feeding of experimental diets, reaching the maximum value at 3 h. Feeding of diets containing polyunsaturated fats to sheep resulted in a marked increase in the levels of *trans*-11 C18:1 and a slight increase of CLA in free fatty acid fraction of rumen contents. Our results support that endogenous synthesis of CLA from *trans*-11 C18:1 may be involved the primary source of CLA in dairy product. ***(Asian-Aust. J. Anim. Sci. 2003. Vol 16, No. 2 : 222-226)***

Key Words : Conjugated Linoleic Acid, Free Fatty Acid Fraction, Polyunsaturated Fat Sources, Rumen Content, Sheep, *trans*-11 C18:1

INTRODUCTION

Conjugated linoleic acid (CLA) is an intermediary product in the ruminal biohydrogenation of polyunsaturated fats, such as C18:2 n-6 or C18:3 n-3 fatty acids, presented in concentrates and pasture. It is generally recognized that CLA is formed as a result of the incomplete biohydrogenation of polyunsaturated fat by ruminal bacteria (Sommefeld, 1983; Song, 2000), and thus food products derived from ruminants, especially dairy products are the major dietary source of CLA for human beings.

Recently it has been reported that CLA has a wide range of beneficial health effects in experimental animals (Ip et al., 1999; Yamasaki et al., 2000). The positive effects related to CLA have intensified the research efforts to increase the level of this fatty acid in milk and dairy products (Bauman et al., 2000). Many studies have been suggested that the content of CLA in dairy product, mainly in milk fat, is affected by a number of factors, including the ratio of forage and concentrates, level of intake, and feeding pattern of polyunsaturated fats (Dhiman et al., 2000). In general, CLA incorporation levels in dairy products can be altered by affecting ruminal production of CLA or *trans*-11 C18:1 fatty acid, or by dietary supplement with theses fatty acids (Gulati et al., 2000; Kelly et al., 1998).

But these studies have been focused on the development of dietary regimens to increase the level of CLA in milk and dairy products. Only limited information is available on the occurrence of CLA in rumen, and thus a more fundamental approach is required to support the research effort to enhance the CLA and beneficial *trans*-fatty acids in dairy products. The objectives of present study were to investigate: 1) the occurrence of CLA in rumen contents under the condition of various dietary regimens; and 2) the pattern of time-dependent changes in free fatty acids, including CLA in rumen.

MATERIALS AND METHODS

Four Corridale sheep (71.0±7.3; mean body weight±SD) fitted with rumen fistula were used in a 4×4 Latin square design with each 14 d period. During experimental period, they were maintained on diets consisting of 70% second cut-orchard grass hay and 30% commercial concentrates (TDN, 73.5%; CP, 16%; EE, 4.0%) at 1.5% level of metabolic body weight for all treatments. Fats sources were added at the expense of concentrates at 30% level on weight basis. Dietary treatments were 100% concentrates (served

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as the control), and concentrates were replaced by safflower seed at 30% (SFS), safflower meal at 18%-safflower oil at 12% (SFO), and safflower meal at 18%-linseed oil at 12% (LNO). The C18:2 n-6 fatty acid content in SFO diet was adjusted to the same amount of that in SFS diet. The fatty acid compositions of safflower and linseed oils used in this study are shown in Table 1. Sheep were housed in a tie stall barn and fed individually. Experimental diets were given once daily at 08:30. The concentrates treated or untreated were offered prior to grass hay. Water was provided *ad libitum* through troughs.

At the end of each experimental period, rumen contents from each sheep were collected for determining the fatty acid composition. About 500 g of rumen contents were obtained before feeding and at 1, 3, 6 and 12 h after feeding. An aliquot of these was frozen immediately in liquid nitrogen tank and stored at -80C until use. The total lipids of the rumen contents were extracted with a mixture of chloroform and methanol (2:1, v/v) by the method of Folch et al. (1957). To obtain free fatty acid fraction, the lipid extracts were separated by thin layer chromatography on preformed silica gel plates (20 cm×20 cm Silica gel 60 F254, Merck Ltd.) using hexane:diethylether:acetic acid (105:45:2, v/v) as developing solvents. Free fatty acid extracts were methylated according to the methods of Takenoyama et al. (1999) with some modification. In brief, about a 30 mg of sample was transesterified to fatty acid methyl esters in benzene using 0.5 M KOH/methanol for 10 min at 100°C. After cooling, the turbid preparation was neutralized with HCl/methanol and then reheated. Fatty acid methyl esters were extracted with hexane and measured by gas-liquid chromatography (GL-14B type, Shimadzu Ltd.) using 0.25 mm I.D.×50 m capillary column (FFS HR-SS-10, Shinwa Ltd.). The initial column temperature was programmed at 90°C and increased to 220°C at 2°C/min. The injector and detector were set at 200°C, respectively. The peaks were identified by comparison with standard mixture of fatty acid methyl esters (Lipid standard and Linoleic acid methyl ester, *cis*/*trans*-isomers, Sigma Ltd.). Fatty acid composition of free fatty acid fraction was expressed as a weight percentage of total fatty acids.

Data were analyzed as a 4×4 Latin square design using the general linear models procedure of SAS (1986). The linear model used for the dependent variable was

$$Yijk = \mu + Ti + Pj + Sk + Eijk$$

where Yijk is the observation, μ is the overall mean, Ti is the effect of treatment (i=1, 2, 3 and 4), Pj is the effect of period (j=1, 2, 3 and 4), Sk is the sheep (k=1, 2, 3 and 4) and Eijk is the random error. Significant differences in obtained means were determined using multiple range test at the level of $p<0.05$ (Duncan, 1955). Values in the text were presented as mean±SE.

Table 1. Fatty acid composition of safflower and linseed oils

Fatty acid[1)]	Safflower oil	Linseed oil
	%	
C16:0	7.76	8.07
C18:0	2.44	3.23
C18:1 n-9	16.05	18.25
C18:2 n-6	61.90	14.20
C18:3 n-3	0.35	50.85

[1)] Number of carbon atom: number of double bonds, followed by the position of the first double bond relative to the methyl end.

RESULTS AND DISCUSSION

Sheep fed experimental diets tended to gain body weight during the experiment. Body weights measured at the end of experimental period are slightly increased (from 71.0 to 73.8 kg) as compared to pre-experiment. There were no negative effects on physiological status in experimental animals fed treated diets containing full-fat seed or vegetable oils.

The effects of feeding various fat sources on fatty acid composition of free fatty acid fraction in rumen contents are presented in Table 2. The level of C16:0 did not differ between groups, although the value of the control group was slightly higher than treated groups. The C18:0 fatty acid, that is final product of biohydrogenation of polyunsaturated fats in the rumen, was significantly lowered in control as compared to those of all treated groups before feeding, but this lowering was not consistently observed after feeding. The level of C18:1 n-9 was increased in all treated groups relative to the control. Particularly, the level of C18:1 n-9 in LNO group was much higher than that of the SFS or SFO group. The diet treatments were not influenced the levels of C18:2 n-6 and C18:3 n-3 of free fatty acid fraction in rumen contents (data were not shown) and these fatty acids remained few.

The level of *cis*-9, *trans*-11 CLA in free fatty acid fraction was considerably increased in all treated groups relative to the control, but not significantly. The increase in *cis*-9, *trans*-11 CLA was greater in the SFS and SFO groups than group fed diet containing linseed oil. The generation of *trans*-11 C18:1 was significantly higher in all treated groups than that of the control. But the increase in *trans*-11 C18:1 was greater in the SFS and SFO groups than group fed diet containing linseed oil. The C18:0/*trans*-11 C18:1 ratio in rumen contents indicated marked change in response to dietary fat sources. The reduction in the mean value for C18:0/ *trans*-11 C18:1 ratio was attributed to the variation observed in *trans*-11 C18:1.

The time-dependent changes in *cis*-9, *trans*-11 CLA and *trans*-11 C18:1 of free fatty acid fraction in rumen contents are shown in Figure 1 and 2. The level of *cis*-9, *trans*-11

Table 2. Effects of dietary fat sources on the fatty acid composition of free fatty acid fraction in rumen contents before and after feeding[1)]

Fatty acid[2)]	Experimental diets				
	Control	SFS	SFO	LNO	P[3)]
	%[4)]				
Before feeding					
C16:0	10.78±1.37	8.90±0.80	9.85±1.42	10.80±1.85	NS
C18:0	42.44±6.51^{b}	62.04±2.72^{a}	54.46±4.79^{a}	54.31±0.63^{a}	p<0.005
C18:1 *trans*-11	4.43±0.57^{b}	12.04±2.56^{a}	12.79±1.71^{a}	11.48±1.75^{a}	p<0.05
C18:1 n-9	1.97±0.29^{c}	3.77±0.27^{ab}	3.16±0.20^{b}	5.01±0.52^{a}	p<0.005
C18:2 *cis*-9, *trans*-11	0.53±0.23	0.45±0.17	0.42±0.07	0.70±0.17	NS
C18:0/C18:1 *trans*-11	9.58	5.15	4.26	4.73	
After feeding of 1 h					
C16:0	14.21±3.19	10.64±0.89	11.24±0.52	10.42±0.95	NS
C18:0	41.96±2.92	49.86±2.03	46.93±2.90	45.40±3.74	NS
C18:1 *trans*-11	6.76±0.86^{c}	16.73±1.77^{ab}	18.53±2.21^{a}	13.24±1.17^{b}	p<0.001
C18:1 n-9	4.13±0.51^{c}	5.68±0.66^{b}	6.63±0.25^{ab}	7.55±0.24^{a}	p<0.005
C18:2 *cis*-9, *trans*-11	1.76±0.63	3.37±0.79	3.56±0.67	2.12±0.41	NS
C18:0/C18:1 *trans*-11	6.20	2.98	2.53	3.43	
After feeding of 3 h					
C16:0	13.51±2.15	10.01±1.00	10.14±0.75	10.16±0.94	NS
C18:0	44.40±6.01	45.93±5.04	39.55±4.34	38.39±2.12	NS
C18:1 *trans*-11	7.13±0.62^{c}	21.53±3.77^{ab}	27.03±4.02^{a}	18.62±1.48^{b}	p<0.001
C18:1 n-9	4.06±0.32^{c}	6.18±0.66^{b}	7.33±0.49^{ab}	8.75±0.7^{a}	p<0.001
C18:2 *cis*-9, *trans*-11	1.48±0.25	2.15±0.80	1.88±0.34	1.72±0.38	NS
C18:0/C18:1 *trans*-11	6.23	2.13	1.46	2.06	
After feeding of 6 h					
C16:0	13.55±1.31	10.04±0.80	10.96±1.64	9.52±0.63	NS
C18:0	51.50±4.16^{a}	46.17±7.34^{a}	45.24±5.01^{a}	30.83±1.90^{b}	p<0.05
C18:1 *trans*-11	7.59±0.46^{b}	18.02±1.54^{a}	25.07±6.15^{a}	22.46±4.84^{a}	p<0.005
C18:1 n-9	3.89±0.43^{b}	5.93±1.06^{a}	5.83±0.28^{a}	7.76±0.85^{a}	p<0.05
C18:2 *cis*-9, *trans*-11	0.81±0.15	1.78±0.85	0.80±0.09	0.76±0.01	NS
C18:0/C18:1 *trans*-11	6.79	2.56	1.80	1.37	
After feeding of 12 h					
C16:0	11.84±1.38	9.37±1.29	8.68±0.45	8.96±0.87	NS
C18:0	47.98±5.55	51.99±8.92	51.42±3.34	50.85±3.93	NS
C18:1 *trans*-11	6.03±0.83b	16.05±3.30a	21.08±2.95a	16.30±2.71a	p<0.005
C18:1 n-9	2.47±0.20c	4.55±0.61b	4.42±0.24b	6.89±0.50a	p<0.001
C18:2 *cis*-9, *trans*-11	0.50±0.09	0.96±0.45	0.66±0.09	0.79±0.17	NS
C18:0/C18:1 *trans*-11	7.96	3.24	2.44	3.12	

[1)] SFS, safflower seed; SFO, safflower oil; LNO, linseed oil.
[2)] Number of carbon atom: number of double bonds, followed by the position of the first double bond relative to the methyl end.
[3)] Probability of a significant treatment effect. NS=not significant.
[a-c] Mean values in a row with no common superscript are significantly different (p<0.05).
[4)] Values are expressed as % of total fatty acids.

CLA isomers in free fatty acid fraction was reached to the maximum value at 1 h and, thereafter rapidly decreased to near the value before feeding. A decrease in the SFS group was lower compared to other groups. The generation of *trans*-11 C18:1 was significantly higher in all treated groups than that of the control. The level of *trans*-11 C18:1 was linearly increased after feeding of experimental diets, reaching the maximum value at 3 h, except for the LNO group. In the control, this fatty acid remained smaller and was almost consistent. Furthermore, *trans*-11 C18:1 in three treated groups were considerably higher than CLA contents. This would suggest that the conversion rate of C18:2 n-6 and C18:3 n-3 into *trans*-11 C18:1 seems to be more rapid than that of *trans*-11 C18:1 to C18:0. This result would show that *cis*-9, *trans*-11 CLA produced by the ruminal biohydrogenation of C18:2 n-6 was a transient intermediate, whereas *trans*-11 C18:1 was accumulated in the rumen. The lack of effect of dietary fat sources on the generation of *cis*-9, *trans*-11 CLA of free fatty acid fraction in rumen contents was somewhat unexpected. On the other hand, *trans*-11 C18:1 fatty acid indicated a significant increase in free fatty acid fraction in rumen contents. It has been demonstrated that *cis*-9, *trans*-11 C18:2 CLA can be synthesized endogenously from *trans*-11 C18:1, which also

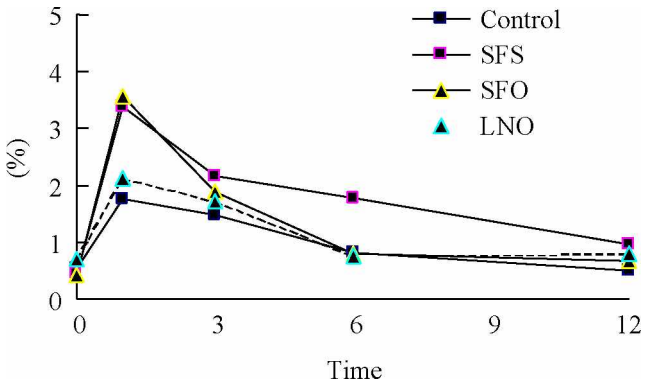

Figure 1. Time-dependent change of *cis*-9, *trans*-11 CLA of free fatty acid fraction in rumen content.

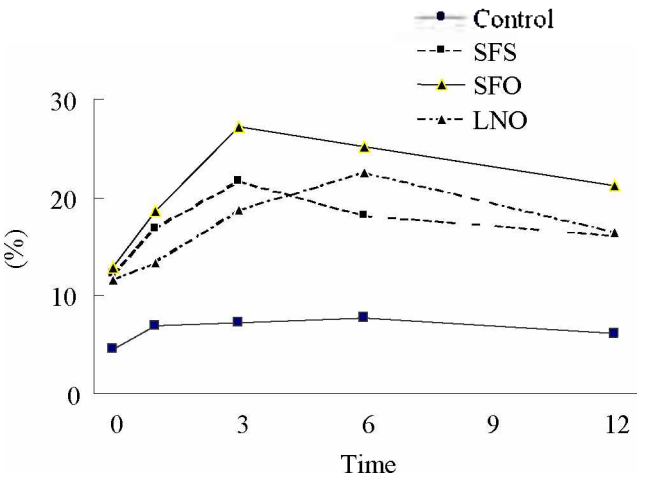

Figure 2. Time-dependent change of *trans*-11 C18:1 of free fatty acid fraction in rumen content.

originates in the rumen from incomplete bio-hydrogenation of unsaturated fatty acids (Chouinard et al., 1999). More recently, Griinari et al. (2000) also suggested that *de novo* synthesis of CLA from *trans*-11 C18:1 represented the primary source of CLA in milk fat of lactating cows supplied by abomasal infusion of *trans*-11 C18:1. Feeding sheep with CLA and unsaturated mixture increased the level of *trans*-11 C18:1 in abomasal digesta, but *trans*-11 C18:1 transferred into milk fat was relatively small in lactating goat fed same diet (Gulati et al., 2000). These would involve Δ-9 desaturase and synthesis from *trans*-11 C18:1, another intermediately product in ruminal biohydrogenation. The larger generation of *trans*-11 C18:1 fatty acid would be expected to increase the output of CLA, mainly *cis*-9 *trans*-11 CLA, into dairy products.

In this study, two groups fed safflower oil and full-fat seed containing rich in linoleic acid resulted in higher *cis*-9, *trans*-11 CLA level in the rumen content relative to the group fed linseed oil containing rich in α-linolenic acid. This would show that *cis*-9, *trans*-11 CLA is the first step of biohydrogenation pathway of linoleic acid, whereas that of α-linolenic acid does not involve this CLA as an intermediate. Furthermore, the level of *trans*-11 C18:1 was markedly higher than that of CLA. Previous study has suggested that the diets containing high level of C18:3 n-3 to sheep resulted in an increase in the levels of *trans*-11 C18:1 fatty acid in rumen contents (Czerkawski et al., 1975). Feeding of diet containing high levels of linseed, fish oil and a mixture of these sources to steers is known to increase the CLA content of beef muscle together with *trans*-11 C18:1 (Enser et al., 1999). Most probably, an increase in the generation of *trans*-11 C18:1 in rumen contents might be responsible for enhancing CLA in dairy product. In this study, the feeding of LNO diet increased the level of *trans*-11 C18:1 in free fatty acid fraction but not to the extent observed when sheep fed SFS or SFO diets.

In conclusion, feeding of diets containing polyunsaturated fatty acids to sheep resulted in a slight increase in the levels of CLA isomers, but not significantly. On the contrary, *trans*-11 C18:1 indicated a significant increase in free fatty acid fraction in rumen contents. CLA from ruminal biohydrogenation of C18:2 n-6 in the diet as well as endogenous synthesis of CLA from *trans*-11 C18:1 involved the major source of CLA in dairy products. But our results support that endogenous synthesis of CLA from *trans*-11 C18:1 could be involved the primary source of CLA in dairy product, and contribution of CLA derived from ruminal biohyrogenation of C18:2 n-6 may be relatively small. It means that one of the feasible condition to enhance CLA content in dairy products may be dietary manipulation of ruminal biohydrogenation to increase the formation of *trans*-11 C18:1.

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