Effects of Different Concentrate and Roughage Ratios on Ruminal Balance of Long Chain Fatty Acids in Sheep

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ABSTRACT : The effects of different feeding ratios of concentrate (C) and roughage (R) on balance of long chain fatty acids and microbial fatty acids in the rumen of sheep were investigated. The diets were divided into 8:2 (concentrate feeding), 4:6 (middle mixture) and 0:10 (roughage feeding) ratios (C:R). Duodenal digesta was collected through 24 hours after feeding. Biohydrogenation rate, total duodenal flow of fatty acids and microbial fatty acids were measured. Total duodenal flow of fatty acids was significantly ($p<0.05$) increased with increasing concentrate. Total duodenal flow of fatty acid was greater than intake of fatty acid in all diets. In comparison with intake of each fatty acid, duodenal flow of stearic acid ($C_{18\,0}$) remarkably increased in all diets. Biohydrogenation rate for total C18 unsaturated fatty acids in the rumen tended to increase ($p<0.10$) when sheep were fed the middle mixture. In particular, biohydrogenation rate of linoleic acid ($C_{18\,2}$) and linolenic acid ($C_{18\,3}$) with the middle mixture were highest ($p<0.05$) compared with other diets. Duodenal flow of protozoal fatty acids was significantly ($p<0.05$) increased with the increased supply of concentrate. That of bacterial fatty acids was significantly ($p<0.05$) increased with both concentrate diets compared with the roughage feeding diet. $C_{18\,0}$ occupied the greater part of both protozoal and bacterial fatty acids in all treatments. Results indicated that biohydrogenation of free unsaturated fatty acids was actively carried out when the middle mixture diet was supplied, and that microbial uptake and synthesis of fatty acids were accelerated by adding the supply of concentrate. *(Asian-Aust. J. Anim. Sci. 2001. Vol 14, No. 7 : 960-965)*

Key Words : Concentrate and Roughage Ratio, Long Chain Fatty Acids, Biohydrogenation, Microbial Fatty Acids, Sheep

INTRODUCTION

It is known that biohydrogenation of unsaturated fatty acids and lipid synthesis by microorganisms are carried out in the rumen. Microbial lipid in the rumen shifts to the lower digestive tract and contributes an important part of nutrition in the host. In the rumen, lipids taken and biologically synthesized become cell membranes and other microbial components (Allison et al., 1961; Emmanuel, 1974; Gutierrez, 1962; Jenkins, 1993; Patton, 1968). It is reported that about 1/4 of milk lipid is synthesized by rumen microorganisms in dairy cattle (Keeney et al., 1962). The microbial lipid contributes to ruminant lipid metabolism. It is considered that since it is dependent on dietary components and volatile fatty acids, diet affects rumen lipid metabolism. Therefore, quantity and composition of microbial lipid would seem to change according to diet.

On the other hand, it is known that microbial population, concentration, and activity differ when host ruminants are fed diets with differing concentrate and roughage ratios (Grubb and Dehority, 1975; Abe and Iriki, 1978). Sasaki et al. (2000) reported that microbial uptake and synthesis of fatty acids are accelerated with an increased supply of concentrate, and furthermore suggested that biohydrogenation is accelerated with supply of roughage. Accordingly, different concentrate and roughage feeding ratios may affect duodenal flow of fatty acids. However, there is little knowledge on these issues.

Therefore, in this examination, different concentrate and roughage ratios were incorporated into the diet. Ruminal balance of long chain fatty acids and duodenal flow of microbial lipids were then examined with the changed concentrate and roughage feeding ratios.

MATERIALS AND METHODS

Animals and diets

Three sheep (Suffolk, body weight 34.6to39.9 kg) fitted with rumen fistula and T-shaped duodenal cannula were used. Commercial mixture diet (Nihonnousann, Kanagawa, Japan) was used as concentrate and Italian ryegrass hay (2 cm cut length) was used as roughage. Experimental diets were divided into 8:2 (concentrate feeding), 4:6 (middle mixture) and 0:10 (roughage feeding) ratios (Concentrate: Roughage) and sheep were fed once a day (09:00). Dietary ration amount was 2% of body weight on a dry matter basis. Main chemical composition and fatty acids composition of three diets are shown in tables 1 and 2. Water and minerals were freely available to the animals. Each experimental period consisted of a 17 day adaptation phase followed by a 14 day experimental phase. Examination was carried out by a 3×3 Latin square design.

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Table 1. Chemical component of experimental diets

Item	Concentrate:roughage[1]		
	8:2	4:6	0:10
Dry matter (DM %)	83.9	82.6	81.3
Chemical component (% of DM)			
Crude protein	14.3	10.0	5.7
OCC[2]	60.8	46.1	31.4
OCW[3]	31.7	46.8	62.0
Crude ash	7.5	7.1	6.6
Total lipid[4]	5.0	3.3	1.7
Total fatty acid	1.6	0.9	0.2

[1] Calculated on dry matter basis. [2] Organic cellular content. [3] Organic cell wall. [4] Extracted with chloroform-methanol by Folchs'method. (Folch et al., 1967)

Table 2. Fatty acid composition of experimental diets

Fatty acid	Concentrate:Roughage		
	8:2	4:6	0:10
Composition (%)			
$C_{15:0}$*[1]	tr	tr	tr
$C_{16:0}$	27.1	27.1	27.2
$C_{18:0}$	5.5	8.4	11.4
$C_{18:1}$	34.6	24.0	13.5
$C_{18:2}$	24.3	23.9	23.5
$C_{18:3}$	3.7	10.0	16.3
Other fatty acids [2]	5.0	6.5	8.1
SFA[3]	36.9	40.8	44.7
USFA[4]	63.1	59.2	55.4

tr: Trace amounts present. * Involved iso, anteiso and normal $C_{15:0}$. [1] Number of carbon atoms in fatty acids: number of double bonds. [2] Involved $C_{14:0}$, $C_{16:1}$, iso and anteiso $C_{17:0}$. [3] Saturated fatty acid. [4] Unsaturated fatty acid.

Sampling of duodenal digesta

On test-days between day 11 and day 15 of each period, before feeding, each sheep was administered with a 4 g chromium tablet (Cellulose powder: Starch: Chromium oxide=10:10:7) through the rumen fistula. On day 15 of each period, total duodenal digesta were collected during 24 h after feeding. After weighing the total collected duodenal digesta, a portion was freeze dried and prepared as a duodenal digesta sample. Also, a part of the freeze dried sample was used for a dry matter analysis by the dry heat method (135°C, 2 h). Duodenal flow was calculated using the chromium recovery rate in duodenal contents.

Rumen fluid sampling and microorganism fractionation

On test day 17 of each period, 300 ml of rumen fluid was collected at 3 h after feeding through rumen fistula and the collected rumen fluid was filtered through double folded gauze. Centrifugal separation of protozoa (1,000 rpm, 5 min) and bacteria fractions (12,000 rpm, 20 min) was performed as described by Sasaki et al. (2000). Separated protozoa and bacteria fractions underwent 48-hour freeze-drying processing and were used for analysis.

Analytical method

Analysis of main chemical composition of diets was performed as described by Sasaki et al. (2000). The lipid was extracted with chloroform-methanol by Folch's method (Folch et al., 1967). Extracted lipid was methylated with 5% sulfuric acid-methanol solution for 3 h in 95°C water bath. Each fatty acid was determined by gas chromatography (Sasaki et al., 2000). Pentadecanoic acid, $C_{15:0}$; palmitic acid, $C_{16:0}$; stearic acid, $C_{18:0}$; oleic acid, $C_{18:1}$; linoleic acid, $C_{18:2}$; and linolenic acid, $C_{18:3}$ were considered as main fatty acids. Myristic acid, $C_{14:0}$; palmitoleic acid, $C_{16:1}$; and margaric acid, $C_{17:0}$ were defined as other fatty acids. Total of main and other fatty acids were considered as total fatty acids.

Determination of duodenal content chromium was based on the phosphoric acid potassium reagent method (Morimoto, 1971). Phosphachidylecoline (PC) was used as a marker of the protozoa. PC in extracted lipid was separated by thin-layer chromatography. The thin layer plate used a silica gel adsorption aluminum sheet (MERK Co. TLC Aluminium-sheets 20×20 cm silica gel 60); chloroform-methanol-acetic acid-water (25:15:4:2) was used for the development eluent; 10% iodine methanol solution was used as a PC detection reagent. For identification of PC, using the lecithin from soybeans and comparing the sample spots. Next, phosphorus concentration of separated PC was measured by the sulfate molybdic acid method after eliminating silica gel by filtration. For PC, measured values were multiplied by 25 because 4% phosphorus was included (Fujino, 1995), and the PC concentration was required. Purine determination was based on the method described by Zinn and Owens (1986).

Calculation and statistical analysis

Duodenal flow of protozoal and bacterial fatty acids was calculated from equation (Microbial fatty acids concentration/ Microbial markers concentration× Duodenal markers concentration) of Farley (1993). Biohydrogenation rates of C18 unsaturated fatty acid were calculated (100-(Duodenal flow of C18 unsaturated fatty acids/Duodenal flow of C18 fatty acids)×(Intake of C18 fatty acids/Intake of C18 unsaturated fatty acids)×100) as described by Wu et al. (1991). Upon receiving test results, 3×3 Latin square analysis was carried out by the GLM procedure of SAS (Shinjou, 1995), and examined for significance.

RESULTS AND DISCUSSION

Table 1 shows main chemical composition of eeexperimental diets. In particular, OCC was increased by increasing the supply of concentrate. Table 2 shows fatty acid

Table 3. Intake of fatty acids in sheep fed different ratios of concentrate (C) and roughage (R)

Fatty acid	C:R ratio		
	8:2	4:6	0:10
Intake (g/day)			
$C_{15:0}$*[1]	tr	tr	tr
$C_{16:0}$	3.2[a]±0.12	1.9[b]±0.14	0.6[c]±0.04
$C_{18:0}$	0.6[a]±0.02	0.6[a]±0.04	0.3[b]±0.02
$C_{18:1}$	4.1[a]±0.15	1.7[b]±0.12	0.3[c]±0.02
$C_{18:2}$	2.8[a]±0.11	1.7[b]±0.12	0.5[c]±0.04
$C_{18:3}$	0.4[b]±0.02	0.7[a]±0.05	0.3[c]±0.03
Other fatty acids[2]	0.6[a]±0.02	0.5[a]±0.03	0.2[b]±0.01
Total	11.7[a]±0.44	7.0[b]±0.50	2.2[c]±0.16

Means ±SE.

tr: Trace amounts present. [a,b] Different superscripts with same rows are significantly different at $p<0.05$. * Involved iso, anteiso and normal $C_{15:0}$. [1] Number of carbon atoms in fatty acids: number of double bonds. [2] Involved $C_{14:0}$, $C_{16:1}$, iso and anteiso $C_{17:0}$.

Table 4. Duodenal flow of fatty acids in sheep fed different ratios of concentrate(C) and roughage(R)

Fatty acid	C:R ratio		
	8:2	4:6	0:10
Duodenal flow (g/day)			
$C_{15:0}$*[1]	0.4±0.0	0.5±0.1	0.4±0.0
$C_{16:0}$	2.9[a]±0.3	2.2[b]±0.3	0.9[c]±0.1
$C_{18:0}$	12.3[a]±1.2	8.9[b]±1.2	1.6[c]±0.1
$C_{18:1}$	1.9[a]±0.2	1.4[b]±0.1	0.3[c]±0.0
$C_{18:2}$	0.6[a]±0.1	0.3[b]±0.0	0.1[c]±0.0
$C_{18:3}$	tr	tr	0.1±0.0
Other fatty acids[2]	0.4±0.0	0.3±0.0	0.4±0.0
Total	18.5[a]±1.7	13.5[b]±1.9	3.7[c]±0.3

Means±SE.

tr: Trace amounts present. [a,b] Different superscripts with same rows are significantly different at $p<0.05$. * Involved iso, anteiso and normal $C_{15:0}$ [1] Number of carbon atoms in fatty acids: number of double bonds. [2] Involved $C_{14:0}$, $C_{16:1}$, iso and anteiso $C_{17:0}$.

composition of experimental diets. By increasing the supply of concentrate, total unsaturated fatty acid was increased while saturated fatty acid was decreased. Table 3 shows intake of each fatty acid. Intake of $C_{16:0}$, $C_{18:1}$ and $C_{18:2}$ were significantly ($p<0.05$) increased by increasing the supply of concentrate.

The flow of $C_{16:0}$, $C_{18:0}$, $C_{18:1}$, $C_{18:2}$ and total fatty acids to the duodenum was increased ($p<0.05$) by increasing the supply of concentrate (table 4). The flow of fatty acids to the duodenum was greater than their intake for all treatments, suggesting *de novo* synthesis of fatty acids by rumen microbes. There was $C_{15:0}$ in duodenal contents while there was almost none in all diets. From Noble (1981) and Ifkovits and Ragheb (1968), it was reported the branch odd-numbered fatty acids ranging from C13 to C17 are excluded from almost all vegetable oils which are included in the ruminant diet, and that these fatty acids are peculiar for rumen microorganisms. Also, it has been confirmed that the odd-numbered fatty acids such as $C_{15:0}$ in ruminant body fat are derived from rumen microorganisms (Christie, 1978). In this examination, however, fatty acids with carbon numbers lower than 14 were not measured because $C_{15:0}$ and $C_{17:0}$ branched chain fatty acids in duodenal flow were biosynthesized by rumen microorganisms (Sasaki et al., 2000). Furthermore, although $C_{16:0}$ of duodenal flow was significantly ($p<0.05$) increased with increased concentrate, it became 15.6, 16.3 and 24.3% in concentrate feeding, middle mixture and roughage feeding respectively, when these were calculated at the composition ratio; it was significantly ($p<0.05$) high when only roughage was also supplied. It seemed possible that $C_{16:0}$ was biosynthesized when only roughage was supplied to rumen microorganisms, because there was no clear difference in dietary fatty acid composition. It is suggested that both of these fatty acids - $C_{15:0}$ and $C_{16:0}$ - were actively biosynthesized in only roughage supply because it was formed by mainly extending the carbon skeleton of acetic acid by rumen microorganisms (Emmanuel, 1974; Sklan, 1974). $C_{18:0}$ was remarkably increased from 1.6 to 12.3 g/day in duodenal flow compared with $C_{18:0}$ intake ranging from 0.3 to 0.6 g/day. Rumen biohydrogenation occured for C18 unsaturated fatty acids, which was abundant in the diets. Differences between intake and duodenal flow of C18 fatty acid showed biohydrogenation: the C18 biohydrogenation rate for unsaturated fatty acids as an index (Wu et al., 1991) is shown in table 5. High biohydrogenation rates occurred with $C_{18:2}$ and $C_{18:3}$. When $C_{18:1}$ was observed, the concentrate feeding and the middle mixture showed higher biohydrogenation rates than roughage feeding. Also, $C_{18:1}$ biohydrogenation tended to be even lower in all diets of the double bond number than that of the abundant fatty acid. After being isomerized, the $C_{18:3}$ biohydrogenation route shows conversion to $C_{18:2}$, which is then converted into $C_{18:0}$ from $C_{18:1}$ (Harfoot, 1981). Therefore, the greater part of C18 unsaturated fatty acid is converted into $C_{18:0}$ as a biohydrogenation end product. However, with the report of Sasaki et al. (2000), conversion from C18 monoene acid to $C_{18:0}$ is delayed as C18 unsaturated fatty acid content is more abundant in diet and it is accumulated in the rumen (Harfoot et al., 1973). Furthermore, most bacterial groups which are capable of biohydrogenation can biohydrogenate $C_{18:3}$ and $C_{18:2}$ to octadecenoic acids ($C_{18:1}$) but these groups are without having biohydrogenation ability to $C_{18:1}$ and a different part of the bacterial group shoulders the role (Kemp and Lander, 1984). It is considered that biohydrogenation from $C_{18:1}$ to $C_{18:0}$ was not promoted further than the conversion from $C_{18:2}$ and $C_{18:3}$ when much $C_{18:1}$ was formed in the rumen. Also, it became 66.6, 65.5 and 43.8% in

Table 5. Biohydrogenation rate of unsaturated fatty acids of C18 in the rumen of sheep fed different ratios of concentrate (C) and roughage (R)

Fatty acids	C:R ratio		
	8:2	4:6	0:10
Biohydrgenation rate (%)			
$C_{18:1}$	81.2^{a}±3.2	81.6^{a}±2.8	66.5^{b}±1.0
$C_{18:2}$	91.2^{b}±2.7	96.6^{a}±1.3	93.5^{ab}±1.7
$C_{18:3}$	92.7^{b}±4.5	98.7^{a}±1.0	93.0^{b}±1.0
Total C_{18}	83.5^{B}±5.4	86.2^{A}±4.1	77.9^{B}±6.6

Means ±SE.

[a,b] Different superscripts with same rows are significantly different at $p<0.05$.

[A,B] Different superscripts with same rows are significantly different at $p<0.10$.

Table 6. Duodenal flow of protozoal and bacterial total fatty acids in sheep fed different ratios of concentrate (C) and roughage (R)

Item	C:R ratio		
	8:2	4:6	0:10
Protozoal flow (g/day)			
$C_{15:0}$*[1]	0.1±0.0	0.1±0.1	tr
$C_{16:0}$	0.8^{a}±0.1	0.6^{a}±0.3	0.1^{b}±0.0
$C_{18:0}$	2.6^{a}±0.1	1.4^{b}±0.7	0.2^{c}±0.1
$C_{18:1}$	0.6±0.1	0.3±0.2	tr
$C_{18:2}$	0.1±0.0	0.1±0.0	tr
$C_{18:3}$	tr	tr	tr
Other fatty acids[2]	0.2±0.0	0.1±0.1	0.1±0.0
Total	4.3^{a}±0.8	2.5^{b}±1.3	0.5^{c}±0.2
Bacterial flow			
$C_{15:0}$	0.2±0.0	0.2±0.1	0.2±0.1
$C_{16:0}$	1.1^{a}±0.2	0.7^{ab}±0.3	0.3^{b}±0.2
$C_{18:0}$	3.5^{a}±0.8	2.4^{a}±1.2	0.3^{b}±0.2
$C_{18:1}$	0.8^{a}±0.2	0.4^{a}±0.2	0.1^{b}±0.0
$C_{18:2}$	0.2±0.0	tr	tr
$C_{18:3}$	tr	tr	tr
Other fatty acids	0.3±0.1	0.2±0.0	0.1±0.1
Total	6.1^{a}±1.3	3.9^{a}±1.8	1.1^{b}±0.6
Proportion of microbial flow to duodenal flow (%)			
Protozoal TFA	23.9^{a}±4.7	18.9^{ab}±4.3	12.6^{b}±3.5
Bacterial TFA	33.0±5.4	28.7±7.4	21.4±5.9

Means ±SE.

tr: Trace amounts present. [a,b] Different superscripts with same rows are significantly different at $p<0.05$. * Involved iso, anteiso and normal $C_{15:0}$ [1] Number of carbon atoms in fatty acids: number of double bonds. [2] Involved $C_{14:0}$, $C_{16:1}$, iso and anteiso $C_{17:0}$

concentrate feeding, middle mixture, and roughage feeding, respectively, when $C_{18:0}$ which shifted in the duodenum was calculated at the composition ratio. The diet including concentrate was significantly ($p<0.05$) higher than roughage feeding. Under these conditions, abundant microorganisms with biohydrogenation ability seem to exist in the rumen. It is suggested that biohydrogenation to $C_{18:0}$ was actively carried out from $C_{18:1}$. Also, the biohydrogenation rate for all C18 unsaturated fatty acids in middle mixture tended to increase ($p<0.10$). Wu et al. (1991) reported that the biohydrogenation rate rose when plant and animal oil were added to diet. Also, Tanaka and Hayashi (1972) reported that in the $C_{18:3}$ biohydrogenation route, it was competitively processed with the three steps of $C_{18:0}$ from $C_{18:1}$, $C_{18:1}$ from $C_{18:2}$, and $C_{18:2}$ from $C_{18:3}$, and that it is done in order of double bond number. From these facts, it seemed that biohydrogenation was promoted in the diets mainly contained unsaturated fatty acids. And then, hydrogen derived from roughage might accelerate the biohydrogenation.

Microbial fatty acids are also biosynthesized from volatile fatty acids such as acetic acid and glucose as a substrate, as well as from direct dietary fatty acid intake (Jenkins, 1994).

In table 6, duodenal flows of microbial fatty acids are shown. It is evident that $C_{18:0}$, the main component of protozoal fatty acids, was significantly ($p<0.05$) increased with the increased concentrate supply. Furthermore, with C18 unsaturated fatty acids, there were many $C_{18:1}$ fatty acids in each diet. Total duodenal flow of protozoal fatty acids was significantly ($p<0.05$) increased by the increased concentrate supply. The proportion of protozoal fatty acids to the flow of total fatty acids to the duodenum in concentrate feeding was significantly ($p<0.05$) higher than with roughage feeding. It was reported that fatty acid intake of microorganisms and fatty acid content in microorganisms were increased when rapeseed oil was added to the diet (Ferlay, 1993). Also, Bauchart (1990) reported that fatty acid content in microorganisms was increased with increased oil content when rapeseed oil was gradually added *in vitro*. It is known that protozoa ingest particles of feed grains and bacteria (Harfoot, 1981). Also, it is known that some protozoa can selectively take long chain fatty acids (Gutierrez, 1962). From this fact, it seems to raise intake and biosynthetic ability of $C_{18:0}$ and $C_{18:1}$ with increased concentrate. Also, $C_{18:0}$ and $C_{16:0}$ of bacterial fatty acids also occupied the greater part of duodenal fatty acid flow in all diets, and duodenal flow of these saturated fatty acid occupied the greater part. Concentrate feeding significantly ($p<0.05$) increased the duodenal flow of $C_{16:0}$ compared with roughage feeding. The duodenal flow of $C_{18:0}$ and $C_{18:1}$ from bacteria was significantly ($p<0.05$) increased with the increase of the concentrate supply compared with roughage feeding. Total duodenal flow of fatty acids in bacteria was also significantly ($p<0.05$) increased with the increased supply of concentrate. It appears that bacterial fatty acid dynamics differ from those of protozoa. It is estimated that these changes in bacterial

fatty acids in the duodenum did not originate only from increase and decrease of supply of fatty acids in diet. Ieki et al. (1997) reported that the duodenal flow of bacterial fatty acids was increased with supply of cereal. Although the difference in kind of dietary fatty acid seemed to influence the bacterial fatty acid (Bauchart et al., 1987; Zhao et al., 1996), in this report's results it is assumed that the difference of supply amount of fermentable carbohydrate is utilized as an active source of bacteria in the rumen - thus affecting fatty acid intake and biosynthesis abilities. Also, the bacterial fatty acid proportion tended to increase with concentrate supply, though the deviation between sheep could not be recognized as significant. Furthermore, the bacterial fatty acid proportion was 21.4 to 33.0% while the proportion of protozoa occupied for fatty acid which shifts to the duodenum was 12.6 to 23.9%.

From the above results, it was considered that biohydrogenation by rumen microorganisms was actively carried out when the middle mixture was supplied. It was shown that microbial fatty acids increased with greater concentrate supply.

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