Effect of Different Ratios of Concentrate and Roughage on Lipid Synthesis by Rumen Microorganisms *In Vitro*

H. Sasaki[1], K. Horiguchi and T. Takahashi*
Faculty of Agriculture, Yamagata University, Tsuruoka-shi 997-8555, Japan

ABSTRACT : The effect of different feeding ratios of concentrate to roughage on ruminal lipid synthesis *in vitro* was examined. Three sheep fitted with a rumen fistula were fed three different ratios (8:2, 4:6 and 0:10) of concentrate and roughage, and their rumen liquor were used for incubation. ^{13}C-labeled glucose or sodium acetate as substrate was added to cultures of rumen liquor, and they were incubated for 6 h. The total lipid in the culture of the rumen liquor was extracted, and the percentage of ^{13}C excess was analyzed. The percentage of ^{13}C excess recovered when incubated with glucose increased with increased ratio of concentrate in the diet. The values of cultures incubated with glucose were higher than those incubated with sodium acetate except the roughage-only feeding. In the roughage-only diet, the percentage of ^{13}C excess when incubated with sodium acetate was highest of all diets. The recovery percentage of ^{13}C from glucose increased with increased ratio of concentrate. The recovery percentage of ^{13}C from sodium acetate addition in only roughage feeding was highest among the three diets. The recovery percentage of ^{13}C from glucose was markedly higher than that of sodium acetate addition in all feedings. The results indicate that high concentrate feeding facilitates lipid synthesis by rumen microorganisms, and that glucose may be the precursor for lipid synthesis rather than acetic acid. ***(Asian-Aust. J. Anim. Sci. 2003. Vol 16, No. 1 : 36-38)***

Key Words : Concentrate and Roughage, Ruminal Lipid Synthesis, Rumen Microorganism

INTRODUCTION

Rumen microorganisms are known to synthesize long chain fatty acids (Harfoot, 1981; Wu et al., 1991; Jenkins, 1993, 1994). Lipids are synthesized from glucose, acetic acid and amino acids by rumen microorganisms (Patton et al., 1968, 1970). These substrates used by rumen microorganisms for lipid synthesis are either the end products of dietary fermentation or an intermediate substance of fermentation in the rumen. Therefore, dietary constituents may affect lipid synthesis by rumen microorganisms.

On the other hand, when host ruminants are fed diets with different ratios of concentrate and roughage, microbial concentrations and their activity change (Abe et al., 1973; Grubb and Dehority, 1975; Abe and Iriki, 1978). Accordingly, ratios of concentrate and roughage may affect lipid synthesis in the rumen. Sasaki et al. (2000, 2001) reported that microbial uptake and biosynthesis of fatty acids were accelerated with increased supply of concentrate. Studies of microbially synthesized protein, carbohydrate and vitamins in relation to diet have been undertaken in recent years, but the relationship between lipid synthesis in the rumen and ratios of concentrate to roughage has not yet been properly studied.

In the present study, the effect of ratios of concentrate to roughage on lipid synthesis in the rumen was investigated using ^{13}C-labeled glucose and sodium acetate as incubation substrates *in vitro*.

* Corresponding Author: T. Takahashi. Faculty of Agriculture, Yamagata University, Tsuruoka-shi, 997-8555, Japan. Tel: +81-235-28-2827, Fax: +81-235-28-2827, E-mail: ttoshi@tdsl.tr.yamagata-u.ac.jp
[1] The United Graduate School of Agricultural Science, Iwate University, Morioka-shi, 020-8550, Japan.
Received July 15, 2002; Accepted October 16, 2002

MATERIALS AND METHODS

Animals and diets

Three sheep (Suffolk) fitted with rumen fistulas were used. A commercial diet mixture (Nihonnosan, Japan) was used as the concentrate, and Italian rye grass hay (2 cm cut length) was used as the roughage. Experimental diets were divided into 8:2, 4:6 and 0:10 ratios (concentrate:roughage), and sheep were fed once a day. The dietary ration was 2% of body weight on a dry matter basis. Sampling of rumen liquor was carried out after adaptation phase of each dietary treatment for 14 days. The chemical compositions of the three diets were reported in previous paper (Sasaki et al., 2001).

Incubation

Rumen liquor was collected through the rumen fistula before feeding and strained through a double layer of cheesecloth. Forty milliliter buffer solution (Morimoto, 1971) was immediately added to 10 ml of the rumen liquor, and it was maintained at 39°C in a water bath. Half gram of [1-^{13}C] glucose (36 mg ^{13}C) or 1-^{13}C] sodium acetate (78 mg ^{13}C) as a substrate was added separately to the culture of each rumen liquor, and these were incubated for

6 h. Culture examination was carried out at 3 replicates for each treatment.

Analysis

Total lipid in the cultivated rumen liquor was extracted by Folchs' method (1967). The extracted lipids were dissolved in 50 μl of chloroform and immediately dropped onto aluminum sheets coated with silica gel (Merck, Germany). Percentages of ^{13}C atoms in the silica gel, in which the total lipid was adsorbed, were analyzed by using EX-130S ^{13}C analyzer (Nihonbunko, Japan). Analyses were carried out 4 replicates for each incubated sample.

Calculation and statistical analysis

The percentage of ^{13}C excesses were calculated from the measured value (^{13}C atom %) of the sample and the ^{13}C naturally abundant in glycine. The recovery percentages of ^{13}C from each substrate were calculated from percentage of ^{13}C excesses of the sample and each substrate. Upon obtaining test results, analysis of variance was carried out. The significance in dietary treatment and incubation substrate, and interaction effect between dietary treatment and incubation substrate were examined.

RESULTS AND DISCUSSION

Table 1 shows the percentage of ^{13}C excess in the total lipid of rumen liquor produced by the three different ratios of concentrate and roughage. The percentage of ^{13}C showed the amounts of lipid synthesized from each substrate by rumen microorganisms. The percentage of ^{13}C excesses increased with increased ratio of concentrate when rumen liquors were incubated with glucose. When incubated with sodium acetate, the percentage of ^{13}C excess in 0:10 ratio of roughage was higher than in combination with the concentrate. The percentage of ^{13}C excesses for glucose substrate were higher ($p<0.001$) than that with sodium acetate in incubation substrate. Furthermore, the difference of interaction effect between diet and incubation substrate was significant ($p<0.01$). The percentages of ^{13}C excesses in total lipid were less than 1%; nevertheless, there are distinct differences between diet and substrate.

It is known that feeding ratios of concentrate and roughage affect microbial strains and their concentration in the rumen (Grubb and Dehority, 1975; Nakamura, 1985). Patton et al. (1970) showed that specific activity was highly sensitive in the phospholipid fraction when ^{14}C-labeled glucose as a substrate was added to a culture of mixed rumen protozoa. In addition, Dawson and Kemp (1967) reported that phospholipid was actively biosynthesized by Entodinium. Latham et al. (1971) observed that the number of *Selenomonas ruminantium*, which can synthesize long chain fatty acids, was increased with increased feeding ratios of cereals rather than roughage. These microorganisms, which participate in lipid synthesis, might increase by feeding increased amounts of concentrate, and they might be able to actively synthesize lipid from glucose in the rumen environment under high concentrate feeding. Moreover, changes in dominant species of rumen microorganisms might affect amount of lipid synthesis. The percentage of ^{13}C excess was highest in high ratios of roughage when rumen liquors were incubated with sodium acetate. Microorganisms with high ratios of roughage in the rumen have the characteristics of both the affinity and adaptability to synthesize lipid from acetic acid.

Table 2 shows the recovery percentage of ^{13}C from each substrate in total lipid in rumen liquor with different ratios of concentrate and roughage. Recovery percentage of ^{13}C from glucose in total lipid increased with the increase of concentrate. On the contrary, when rumen liquor was incubated with sodium acetate, recovery percentage of ^{13}C from sodium acetate in high ratios of roughage was higher than in combination with concentrate. Comparison between glucose and sodium acetate showed that recovery percentage of ^{13}C from glucose was markedly higher ($p<0.001$) than that of sodium acetate. Also, difference of interaction effect between dietary treatment and incubation substrate was significant ($p<0.01$). Russell and Hespell (1979) observed that *Butyrivibrio fibrisolvens* existed under various feeding circumstances; however, this strain was particularly capable of growth when host ruminants were fed low quality roughage. It might indicate that microorganisms utilize the substrate for optimal growth (Russell and Hespell, 1981).

As the above results show, high concentrate feeding facilitates lipid synthesis by rumen microorganisms. Further,

Table 1. Percentage of ^{13}Carbon atom excess in total lipid of rumen liquor with three different ratios of concentrate (C) and roughage (R)

Substrate	C:R		
	8:2	4:6	0:10
	^{13}C atom % excess		
1-^{13}C-$C_6H_{12}O_6$	0.86±0.21	0.68±0.09	0.50±0.20
1-^{13}C-CH_3COONa	0.21±0.06	0.27±0.11	0.48±0.12

Differences were significant in substrate ($p<0.001$) and in substrate×diet ($p<0.01$), not significant in diet ($p\geqq0.05$). (Mean±standard deviation)

Table 2. Recovery percentage of ^{13}C from each substrate in to total lipid in rumen liquor with three different ratios of concentrate (C) and roughage (R)

Substrate	C:R		
	8:2	4:6	0:10
	^{13}C recovery %		
1-^{13}C-$C_6H_{12}O_6$	5.53±1.19	4.36±0.07	3.21±1.26
1-^{13}C-CH_3COONa	0.43±0.12	0.56±0.22	0.98±0.25

Differences were significant in substrate ($p<0.001$) and in substrate×diet ($p<0.01$), not significant in diet ($p\geqq0.05$). (Mean±standard deviation)

glucose may be the precursor for lipid synthesis rather than acetic acid.

ACKNOWLEDGMENT

The authors are grateful to Dr. T. Yamamoto and Dr. T. Kayaba of the Faculty of Agriculture, Yamagata University for his kind advice on the isotope analysis and statistical analysis. We also thank Dr. M. Kanou and Mr. H. Matsuyama of Graduate School of Agricultural Science, Iwate University for technical advice.

REFERENCES

Abe, M. and T. Iriki. 1978. Effects of diet on the protozoa population in permeable continuous cultures of rumen contents. Br. J. Nutr. 39:255-264.

Abe, M., H. Shibui, T. Iriki and F. Kumeno. 1973. Relation between diet and protozoal population in the rumen. Br. J. Nutr. 29:197-202.

Dawson, R. M. C. and P. Kemp. 1967. The aminoethylphosphonate-containing lipids of rumen protozoa. Biochem. J. 105:837-842.

Folch, J., M. Lees and G. H. Sloan-Stanley. 1967. A simple method for the isolation and purification of total lipids from animal tissues. J. Biol. Chem. 226:497-509.

Grubb, J. A. and B. A. Dehority. 1975. Effects of an abrupt change in ration from all roughage to high concentrate upon rumen microbial numbers in sheep. Appl. Microbiol. 30:404-412.

Harfoot, C. G. 1981. Lipid metabolism in the rumen. In: Lipid Metabolism in Ruminant Animals (Ed. W. W. Christie). Oxford. Pergamon Press. pp. 21-55.

Jenkins, T. C. 1993. Lipid metabolism in the rumen. J. Dairy Sci. 76:3851-3863.

Jenkins, T. C. 1994. Regulation of lipid metabolism in the rumen. J. Nutr. 124:1372-1376.

Latham, M. J., E. Sharpe and J. D. Sutton. 1971. The microbial flora of the rumen of cows fed hay and high cereal rations and its relationship to the rumen fermentation. J. Appl. Bacteriol. 34:425-434.

Morimoto, H. 1971. Doubutsueiyoushikenhou. Tokyo. Japan. Yokendo. pp. 448-450.

Nakamura, K. 1985. Rumen senmouchu no seitai. In: Rumen No Sekai (Ed. M. Kandatsu and K. Suto). Tokyo. Japan. Nozan gyoson bunkakyokai. pp. 141-166.

Patton, R. A., R. D. McCarthy and L. C. Jr. Griel, 1970. Lipid synthesis by rumen microorganisms. II. Further characterization of the effects of methionine. J. Dairy Sci. 53:460-465.

Patton, R. A., R. D. McCarthy, L. G. Keske and L. C. Jr. Griel. 1968. Lipid synthesis by rumen microorganisms. I. Stimulation by methionine *in vitro*. J. Dairy Sci. 51:1310-1311.

Polan, C. E., J. J. McNeil and S. B. Tove. 1964. Biohydrogenation of unsaturated fatty acids by rumen bacteria. J. Bacteriol. 88:1056-1063.

Russell, J. B. and R. B. Hespell. 1981. Microbial rumen fermentation. J. Dairy Sci. 64:1153-1169.

Sasaki, H., K. Horiguchi and T. Takahashi. 2000. Effects of the different ratios of concentrate and roughage on the concentration and composition of long chain fatty acids of rumen microorganisms and microorganisms-free-liquor in sheep. Anim. Sci. J. 71:J26-38.

Sasaki, H., K. Horiguchi and T. Takahashi. 2001. Effects of different concentrate and roughage ratios on ruminal balance of long chain fatty acids in sheep. Asian-Aust. J. Anim. Sci. 14:960-965.

Viviani, R. 1970. Metabolism of long-chain fatty acids in the rumen. Adv. Lipid Res. 8:267-346.

Wallace, R. J. 1980. Cytoplasmic reserve polysaccharide of *Selenomonas ruminantium*. Appl. Environ. Microbiol. 39:630-634.

Wu, Z., O. A. Ohajuruka and D. L. Palmquist. 1991. Ruminal synthesis, biohydrogenation and digestibility of fatty acids by dairy cows. J. Dairy Sci. 74:3025-3034.