COMPARISON OF NATURAL ABUNDANCE OF ^{15}N BETWEEN DIET, RUMEN CONTENTS AND BLOOD PLASMA

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Introduction

Animal bodies tend to have a higher natural abundance of ^{15}N ($\delta^{15}N$) compared with that found in their diets (DeNiro and Epstein, 1981). Therefore, it could be assumed that when there is no or little isotopic discrimination in the process of N absorption, the $\delta^{15}N$ value of digesta would be reflected by the proportion of N derived from diet and endogeneous materials. In our previous study (Sutoh et al., 1987), it was observed that the $\delta^{15}N$ value of the digesta was altered at various points through out the digestive tract, and the $\delta^{15}N$ value of the rumen digesta was higher than that found in the diet. In this study, measurements were made of the $\delta^{15}N$ values in the diet, the rumen bacteria fraction and the blood plasma in order to estimate the relative contributions of each of them to the higher $\delta^{15}N$ in the rumen digesta.

Materials and Methods

Three female Japanese native goats, with an average age of 2 years old, were given alfalfa hay cube once a day for at least 90 d. They were slaughtered at 24 h after the final feeding, and, just prior this feeding, the rumen mucosa and digesta taken from the goats. The rumen bacteria fraction was separated from the other rumen contents by first squeezing through two layers of cotton gauze and they by two steps of centrifugation at 1,000 x *g* for 10 min and 16,000 x g for 25 min. Blood was collected from the jugular vein just prior to the slaughter and the plasma was isolated. Plasma protein was separated using the tangstic acid method. To estimate the change of the $\delta^{15}N$ value in the diet during digestion in the rumen, alfalfa hay cube was digested by a protease (Abe et al., 1979) for 1, 4, and 16 h. A part of the rumen bacteria fraction obtained as described was freeze-dried and digested by the same method for 16 h. All samples were freeze-dried and ground. A small amount of H_2SO_4 was added to the whole rumen digesta before the freeze-drying process in order to prevent a loss of ammonia. The $\delta^{15}N$ value of each sample was determined by using the Carlo Erba 1500 nitrogen analyzer – Finnigan Mat 251 mass spectrometer system. The results were expressed as follows:

$$\delta^{15}N\,(‰) = \left[\frac{R(sample)}{R(air)} - 1\right] \times 1000$$

where R is the $^{15}N/^{14}N$ ratio. The N contents were measured by Kjeldahl method.

Results and Discussion

The $\delta^{15}N$ values of the diet and each sample taken from the goats are shown in figure 1. The $\delta^{15}N$ value of the whole rumen digesta was about 4‰ higher than that of the diet as reported in the previous study (Sutoh et al., 1987). On the other hand, no significant changes were found to exist in the $\delta^{15}N$ values for both the alfalfa hay and the rumen bacteria fraction during digestion

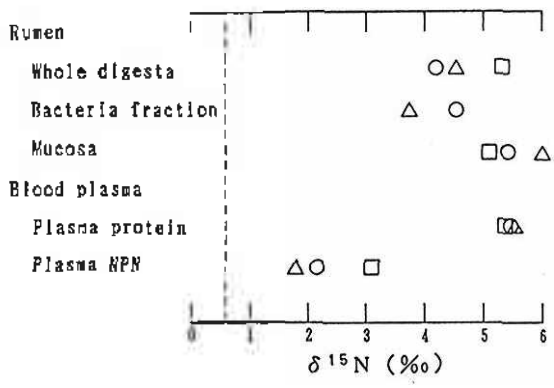

Figure 1. $\delta^{15}N$ of several fractions obtained from three goats (○, △, □) fed alfalfa hay cube: Comparison of natural abundance of ^{15}N between diet, rumen contents and blood plasma.
The dots line show the $\delta^{15}N$ of the diet (alfalfa hay cube), 0.57 ± 0.03‰.

TABLE 1. CHANGES OF NITROGEN CONTENTS AND $\delta^{15}N$ OF ALFALFA HAY CUBE AND RUMEN BACTERIA FRACTION DURING THE PROTEASE DIGESTION

Digestion time (h)	Alfalfa hay cube				Rumen bacteria fraction[1]	
	0	1	4	16	0	16
Remained Nitrogen (%)[2,3]	100	36.0 (0.5)	27.3 (3.2)	24.7 (2.2)	100	3.6 (0.8)
$\delta^{15}N$(‰)[3]	0.57 (0.03)	0.56 (0.05)	0.54 (0.05)	0.56 (0.07)	13.73 (1.22)	14.12 (1.31)

[1] Three samples obtained from goats were mixed and used.
[2] Initial versus residual contents. Initial N concentration was 2.58 ± 0.12 mg/g dry matter.
[3] Mean (S.D.) of three digestions.

with the protease (see table 1). These results indicate that the higher $\delta^{15}N$ values recorded for the rumen digesta over those found in the diet before feeding would not have resulted from the disappearance of the dietary fractions which possesed low $\delta^{15}N$ values.

The $\delta^{15}N$ value of the plasma NPN was significantly lower than that found in other samples (figure 1). Since about a half of the plasma NPN consists of urea under the normal condition, the lower $\delta^{15}N$ value of plasma NPN suggests that the $\delta^{15}N$ value of the urea in the saliva has only a small effect on the high $\delta^{15}N$ value of the rumen digesta. Rumen mucosa which is mainly composed of protein showed a high $\delta^{15}N$ value, and this value was similar to that of plasma protein. The protein N contained in other endogeneous materials such as mucoprotein in saliva may also have high $\delta^{15}N$ values. Although contamination of these endogeneous materials of high $\delta^{15}N$ value can be an explanation of the difference in $\delta^{15}N$ between diet and whole rumen digesta before feeding, the difference seems to be too large to consider the contamination as the sole source of it.

Since it has been reported that isotopic discrimination of low molecular N compounds occurs in the process of assimilation (Wada, 1986), we might speculate that the change observed in the $\delta^{15}N$ value of the rumen ammonia caused by the assimilation in the rumen microbes could also contribute to the increase of $\delta^{15}N$ in the rumen digesta. This discrimination of ammonia may lower the $\delta^{15}N$ value of the rumen bacteria compared to that found in the N source, though the $\delta^{15}N$ value of the rumen bacteria fraction obtained in this study was significantly higher than that of the diet. Nonetheless, $\delta^{15}N$ values of N compounds in the rumen are thought to vary with time based on the absorption of ammonia through the rumen wall and the flow of microbes out of the rumen. The higher $\delta^{15}N$ value of the bacteria fraction observed in this study may be a reflection of the change that occured in the $\delta^{15}N$ value of N compound in the rumen. A detailed explanation is left for a future study.

(**Key Words**: Natural Abundance of ^{15}N, Rumen Digesta, Endogeneous Nitrogen)

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