Effect of Kinds of Hay Given to Sheep on Rumen Degradability of Protein of Various Hays

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Abstract Four kinds of hay (Italian ryegrass, IR ; bermudagrass, BG ; Sudangrass, SG and alfalfa, AL) were given *ad libitum* to wethers in a Latin square design in order to determine the effects of diet on the degradability (dg) of hay protein. Ruminal degradation of hay protein was determined by the *in situ* technique. The passage rate of ruminal digesta was measured using a chromium-mordanted neutral detergent fiber. The dg values did not differ significantly when they were determined under the feeding of different hays as a sole diet. Mean dg values showed relatively large variation under the feeding of different hays. The dg value of hay protein appeared to show smaller variation under feeding of the same hay as that of the incubated sample. AL feeding tended to cause larger variation in the dg values of grass hays. A relatively high correlation was found between dg values and crude protein (CP) digestibility and dry matter degradability. The approximate dg value of forage protein (dg_{CP}) may be estimated by the following equation, with CP digestibility (CP_{DIG}) as a variable ; $dg_{CP} = 0.195 + 0.86 (\pm 0.14) CP_{DIG}$, $r^2 = 0.669$, $s.e. \pm 0.012$.

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Roughage as a basal feed for ruminants must be estimated for its rumen degradability of protein, even though the concentration of protein is low compared with protein supplement feedstuffs. *In situ* ruminal digestion of protein has been routinely performed to evaluate the protein degradability (dg) of feedstuffs. The dg values of dietary protein have exclusively evaluated in protein supplement feedstuffs^{2,7,10)} or in protein in concentrates¹¹⁾. The evaluation of protein dg values for roughage has not been well established. ZHAO and FUJITA¹⁴⁾ claimed that the estimation of dg values of protein in roughage is needed for diverse kinds

of roughage in relation to the establishment of dietary factors affecting the estimation of dg values. IRIKI *et al.*⁷⁾ also pointed out that the effects of the physical and chemical characteristics of feed should be elucidated to establish a technique to determine the dg value of protein. It has been suggested that the chemical composition of conserved forages influences the determination of the dg value³⁾. Fibrous components have been assessed to be the most effective in the determination of the dg value⁹⁾. Differences in the chemical and physical characteristics of feed to be determined by the *in situ* technique may influence the determination

of the dg value of diverse kinds of feed as pointed out by IRIKI *et al.*⁷⁾. A ration for a test animal may affect the determination of the dg value of feed to be evaluated^{2,7,9,11)}. There is a little information about the effects at a ration given to an animal on the dg value of a feedstuff determined by the *in situ* technique. Thus, the effects of a ration should be elucidated for the determination of protein dg in feedstuffs.

The present study aimed to determine the effects of various kinds of hay given to sheep on the dg values of protein in those hays.

Materials and Methods

The animals used were 4 Suffolk × Corriedale wethers fitted with rumen cannula and weighing 40 kg on average. They were individually kept in a metabolism crate. The diets used were Italian ryegrass 1st cut hay (IR), bermudagrass hay (BG), Sudangrass hay (SG) and alfalfa hay (AL). The experimental design was a 4 × 4 Latin square with a 28-day period. Diets chopped to 4 cm and drinking water were given *ad libitum* in each period with free access to mineralized salt blocks.

Digestion trials were carried out for 5 days from the 15th to the 19th day of the experimental period using the total collection method. Diets, feed refusals and feces were weighed daily and aliquot samples were made in a composite sample for each animal. Samples were dried in a forced-air oven at 55 °C and were then ground through a 1 mm screen for further analysis. Dry matter (DM) and nitrogen were analyzed by the methods of AOAC¹⁾. Neutral detergent fiber (NDF) was determined via the method described by GOERING and VAN SOEST⁴⁾. The live weight of each animal was measured at the beginning and at the end of each experimental period.

On the 20th day of each period, 20 g of chromium oxide mordanted NDF were administered in the rumen through the cannula at 7:00 in the morning. Feces were

then collected at the interval of 4 hours for 2 days and thereafter at 8 hour intervals for 3 days. The feces collected were stored at -20°C for further analysis. The chromium concentration in feces was determined by the method described by YOSHIDA *et al.*¹²⁾. The rate constant of ruminal passage (k_p , h⁻¹) was estimated by the method presented by GROVUM and WILLIAMS⁵⁾.

The rumen degradation of DM and crude protein (CP) was determined using the *in situ* technique on days 25 to 28 of each experimental period. Four kinds of hay given to animals were ground through a 2 mm screen and 2 g of each hay were placed in a nylon bag. The bag used was 5 × 10 cm in size with a pore size of 0.045 mm. The bags were soaked in running tap water for 2 hours just before incubation started. Duplicated samples were incubated in the rumen for 6, 12, 24 and 48 hours. After incubation, the bags were retrieved from the rumen and were vigorously washed with tap water to eliminate the ruminal digesta on their surface and then in running water until the wash-out water had become clear for several hours. After being washed, the residues in the bag were dried in a forced-air oven at 55°C and then to a constant weight at 105°C to determine residual DM. Residual CP was then determined by the Kjeldahl method. To determine the quantity of DM invaded in the bag in the course of ruminal incubation, the empty bags with weight (11 g) were also incubated in the rumen in the same manner as described above. The sample residues in the bag were corrected for the quantities of DM invaded in the bag at a corresponding incubation time.

The kinetics of CP degradation in the rumen were calculated using the following model of a first order kinetic equation⁸⁾.

$$P(t) = D \cdot e^{-kt} + U,$$

where $P(t)$ = residual CP at a given incubation time (t), D = slowly degradable fraction of CP, k = degradation rate constant, U = indigestible fraction of CP. The calculation was pro-

secuted by the nonlinear iterative least square method. Readily degradable fraction (R) was then calculated by subtracting the sum of D and U from I.

Statistical analyses were made according to the Latin square method presented by YOSHIDA¹³⁾.

Results

Table 1 shows the mean DM intake and mean live weight of animals used in the present study together with DM, NDF and CP content and corresponding digestibility for hay given to animals. Mean DM intake for AL and BG were significantly greater than that of IR ($P < 0.05$). Alfalfa hay contained significantly less NDF and more CP than grass hays ($P < 0.01$). Mean NDF content was not significantly different among grass hays. BG and SG contained significantly more CP than did IR ($P < 0.01$) and the digestibility of CP for BG and SG was higher than that for IR. The digestibility of CP for AL was significantly higher than those for grass hays ($P < 0.05$).

Mean values with standard deviation for dg of CP are presented in Table 2 for IR, BG, SG

and AL determined via the *in situ* technique when feeding each hay. The dg values of hay protein did not differ significantly among measurements when feeding a single hay diet. The dg values determined when feeding DG and SG, however, tended to be higher than those determined when feeding IR and AL as shown in Fig. 1. The dg value of CP of AL was significantly higher than those of grass hays when compared using pooled mean values ($P < 0.01$). There was no statistically significant difference in dg values among grass hays. The standard deviation showed a relatively wide margin for each mean value determined when feeding any single hay. When feeding AL, the dg values of grass hays showed a greater standard deviation than those determined when feeding grass hays. The dg value of each hay protein appeared to have a smaller standard deviation when it was determined via feeding of the same hay as the *in situ* sample.

The dg values of CP (dg_{CP}) were significantly and positively correlated with the digestibilities of CP (CP_{dig}) of corresponding hay ($r = 0.818$, $P < 0.01$). The following regression equation was obtained; $dg_{CP} = 0.195 + 0.86(\pm 0.14)$

Table 1. Mean dry-matter intake and mean live weight of animals used, and composition and digestibility of Italian ryegrass hay, bermudagrass hay, Sudangrass hay and alfalfa hay

	Diets			
	IR ¹⁾	BG	SG	AL
DM intake, kg/day	0.83 ^{a2)} $\pm$ 0.25 ³⁾	1.30 ^b $\pm$ 0.23	0.86 ^{ab} $\pm$ 0.50	1.40 ^b $\pm$ 0.30
Live weight, kg	40.0 $\pm$ 4.0	40.0 $\pm$ 4.0	39.3 $\pm$ 2.6	40.0 $\pm$ 4.0
Composition of hay				
Dry matter, %	85.7 $\pm$ 2.0	88.4 $\pm$ 4.0	87.9 $\pm$ 3.0	87.0 $\pm$ 3.0
NDF, %DM	65.8 ^a $\pm$ 3.0	71.5 ^a $\pm$ 5.0	69.7 ^a $\pm$ 3.0	45.0 ^b $\pm$ 5.0
Crude protein, %DM	6.7 ^a $\pm$ 0.5	8.6 ^b $\pm$ 0.4	9.0 ^b $\pm$ 0.8	18.7 ^c $\pm$ 2.8
Digestibility				
Dry matter, %	63.0 ^a $\pm$ 5.0	59.1 ^{ab} $\pm$ 1.9	55.0 ^b $\pm$ 3.0	64.6 ^a $\pm$ 0.9
NDF, %	65.0 ^a $\pm$ 7.0	58.0 ^{ab} $\pm$ 5.0	57.0 ^{ab} $\pm$ 4.0	51.0 ^b $\pm$ 8.0
Crude protein, %	53.0 ^a $\pm$ 6.0	58.0 ^{ab} $\pm$ 3.0	62.0 ^b $\pm$ 4.0	76.0 ^c $\pm$ 5.0

¹⁾ Abbreviated notations are as follows: IR, Italian ryegrass hay; BG, bermudagrass hay; SG, Sudangrass hay; AL, alfalfa hay; DM, dry matter and NDF, neutral detergent fiber.

²⁾ Figures in the same row with different superscripts differ significantly ($P < 0.05$).

³⁾ Mean $\pm$ standard deviation

Dietary Influence on Protein Degradability Determination

Table 2. The rumen degradability (dg) of crude protein for Italian ryegrass hay (IR), bermudagrass hay (BG), Sudangrass hay (SG) and alfalfa hay (AL) determined under feeding of various hays by *in situ* technique

Hay given to animals	Hay incubated in the rumen			
	IR	BG	SG	AL
IR	0.68 ±0.04 ¹⁾	0.60 ±0.11	0.66 ±0.09	0.82 ±0.08
BG	0.77 ±0.08	0.65 ±0.05	0.75 ±0.08	0.90 ±0.03
SG	0.71 ±0.09	0.71 ±0.07	0.72 ±0.09	0.89 ±0.06
AL	0.66 ±0.13	0.67 ±0.21	0.64 ±0.11	0.86 ±0.06
Pooled	0.70 ^{a2)} ±0.09	0.66 ^a ±0.11	0.69 ^a ±0.09	0.87 ^b ±0.06

Abbreviated notations are the same as Table 1.

¹⁾ Mean±standard deviation

²⁾ Mean values of pooled results with different superscripts differ significantly ($P<0.01$).

Fig. 1. Mean dg values of hay protein determined under feeding of Italian ryegrass hay (IR), bermudagrass hay (BG), Sudangrass hay (SG) and alfalfa hay (AL), and pooled mean (P).

Open circles (○) show the values determined under feeding of the same hay as incubated samples and solid circles (●) show those determined under feeding of different hays. Open squares (□) are the pooled mean for each hay protein.

Vertical lines show standard deviation of mean.

CP_{dig} , $r^2=0.669$, $s.e.\pm 0.012$. The dg values of CP (dg_{CP}) also correlated significantly with the dg values of DM (dg_{DM}) calculated in the same manner as CP ($r=0.729$, $P<0.01$). The regres-

sion equation obtained was as follows ;

$$dg_{CP}=0.15+0.94(\pm 0.23)dg_{DM}, \quad r^2=0.531, \quad s.e.\pm 0.016.$$

Table 3 shows the passage rate of ruminal digesta (k_p , h^{-1}) for IR, BG, SG and AL. No significant difference was found for k_p among any hays. The k_p values of BG and SG, however, tended to be smaller than those of AL and IR, although the difference was very minute.

Discussion

Although diets were offered *ad libitum*, sheep ate less IR than AL or BG. It has been well documented that the quality of roughage is one of the most important determinants of roughage intake⁶⁾. The quality of roughage is generally assessed by CP and fiber content. A lower CP content in IR may be responsible for lower DM consumption. Bermudagrass hay and SG contained lower CP and higher NDF than did AL. No significant difference was found among DM intake for BG, SG and AL. CP and NDF content alone did not appear to be a limiting factor for feed intake.

The dg values of hay protein were found to show no significant difference when determined via the feeding of different hays. FUJITA *et al.*²⁾ observed no significant influence of the type of forage offered to animals for

Table 3. The passage rate of rumen digesta (k_p , h^{-1}) of sheep given Italian ryegrass hay, bermudagrass hay, Sudangrass hay and alfalfa hay determined by chromium mordanted neutral detergent fiber

	IR	BG	SG	AL
k_p h^{-1}	$0.014 \pm 0.008^{1)}$	0.013 ± 0.006	0.013 ± 0.009	0.018 ± 0.007

Abbreviated notations are the same as Table 1.

¹⁾Mean $\pm$ standard deviation

determinating the dg of forage protein, when wethers were given either grass hay, grass silage or corn silage. Similar results were observed when feeding different kinds of hays in the present study. Therefore, dg values of hay protein appear to be determined when feeding different types of hay as a sole diet. Mean values, however, showed a relatively large variation when determined under different hay feeding condition (Fig. 1). The dg values showed a relatively smaller standard deviation when determined via feeding of the same hay. The dg value of a hay protein should be determined under feeding of the same hay to minimize variation. The dg value of CP in grass hay showed greater variation when determined via the feeding of AL than via grass hays. When mean dg values for grass-hay protein were estimated without results obtained via AL feeding, the magnitude of standard deviation was lowered to 50% of the pooled values shown in Table 2 without any significant change in mean values. It is suggested that the determination of dg values for grass hay should not be carried out via the feeding of a legume hay with high CP content.

A relatively large standard deviation for each mean dg calculated suggests that variation due to other effects such as animal or replication may have concealed the precise effect of the diet. The dg of hay protein determined when feeding BG and SG tended to be higher than that of AL and IR (Table 2). This phenomenon suggests that there are effects of passage rate⁷⁾, chemical composition³⁾ and the physical characteristics of forage⁷⁾. A slower rate of passage for BG or SG may have caused

a higher dg estimation than that determined via feeding of other varieties, although the results of the present study show no clear evidence. The results of our other experiment indicated that brittleness with abrasion or cutting appeared to be lower in BG or SG than in AL or IR (unpublished data). This difference in brittleness among hays may undermine the ruminal passage rate of corresponding forage, resulting in a biased determination of dg. The bias is assumed to range from 7 to 17% when the coefficients of variation of the pooled mean dg values presented in Table 2 are considered.

Some attempts have been made to facilitate the estimation of dg values for dietary protein by using the results of ruminal incubation⁷⁾ and the chemical composition of a diet³⁾. In the present study, an attempt to estimate the dg value of forage protein was made by using the digestibility of CP and degradability of DM in the rumen. This estimation appears feasible by using the two parameters studied in the present study. The coefficients of determination, however, revealed that the equation may not estimate the dg value with high precision by using the digestibility of CP or degradability of DM. The equations may be utilized for an approximation of the dg value of protein in hay that has not been determined.

From the results and discussion, it is concluded that feeding different kinds of hay has no considerable effect on the determination of the dg value of hay protein, even though there is a possible 7 to 17% variation. To minimize the variation in the dg value of hay protein, it is suggested that the determination be carried out when feeding the same hay or hay with

similar CP content. An approximate dg value for hay protein may be possible by using the digestibility of crude protein determined when feeding the corresponding forage as a sole diet.

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乾草蛋白質の第一胃内分解率に及ぼす給与乾草の影響

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乾草蛋白質の第一胃内分解率 (dg_{cp}) に及ぼす給与乾草の影響を検討するため, 4 種類の乾草 (イタリアンライグラス, IR; パーミューダグラス, BG; スーダングラス, SG およびアルファルファ, AL) をラテン方格法によりめん羊に自由採食させた. 乾草蛋白質の第一胃内分解は, in situ 法により測定した. 第一胃内容物の通過速度は, クロムで媒染した中性デタージェント繊維により測定した. 乾草単独給与時に乾草蛋白質の第一胃内分解率を測定した場合, 給与乾草による測定値の有意な違いは認められなかった. 各乾草給与の測定の平均値には, 比較的大きな変動が認められた. 測定試料と同じ乾草を給与して測定した場合には, 測定値の変動が小さい傾向にあった. AL 給与時にイネ科乾草の測定を行なうと, 測定値の変動が大きくなる傾向にあった. 粗蛋白質の消化率および乾物の第一胃内分解率と dg 値との間に比較的高い相関関係が認められた. 推定精度は低い, 乾草蛋白質の第一胃内分解率 (dg_{cp}) の概数値を粗蛋白質の消化率 (CP_{dig}) から以下の式で算定できる.

$$dg_{cp} = 0.195 + 0.86 (\pm 0.14) CP_{dig}, \quad r^2 = 0.669, \quad s.e. \pm 0.012.$$

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