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The Nitrogen Utilization in Sheep Fed Only Grass Diets in Either the Fresh or Dried Form†

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生草又は乾草を給与されたメンヨウにおける
窒素の利用について

藤　原　　勉

INTRODUCTION

The singularity in digestion of ruminant is a function of microbes inhabited in the rumen, which should affect on the digestion and absorption of nutrients in the lower digestive tract. It is generally recognized that the utilization of food given was markedly influenced by the degree of microbial fermentation in the rumen when the food was changed in physical form as well as chemical composition. Especially, the factor affecting the utilization of dietary protein as same as other nutrients may be a difference of physical form, such as solubility in the rumen ; that is a degree of easiness for microbial degradation in the rumen.[1] According to Chalmers et al.[2] and Chalmers and Synge,[3] ruminal ammonia formation was reduced by treatment to reduce the solubility of dietary protein, and consequently, the utilization of nitrogen in the diet was improved remarkably.

In general, it is known that the solubility of protein in forage is changed by drying treatment.[4,5] It may be assumed that the forage protein has a different property compared with other vegetable proteins used as a dietary supplement. Kameoka and Morimoto[6] reported that the direct absorption of nitrogen in the rumen of goats was smaller in feeding of dried orchardgrass than in feeding of fresh orchardgrass. Relatively little work has been reported on the comparison of nitrogen utilization in sheep fed only the fresh grass or the dried grass prepared from the same origin.

In the present experiment, the nitrogen utilization of grass diets in sheep was investigated when they were given only the fresh or hay, which would be clearly different in dietary physical property. The daily ration of fresh grass or hay was harvested at similar growth stage from the same pasture in order to equalize the dietary chemical composition. A part of the results in this study was reported previously.[7]

MATERIALS and METHODS

Animals and experimental diets

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Three Japanese Corriedale wethers, each weighing 22—30 kg, were used repeatedly. The fresh grass was harvested each morning from a predominantly Italian rygrass pasture (2nd cut). The sun-cured hay was made from the herbage harvested at the same time when harvested the fresh grass. Each grass diets was cut about 10 cm long before feeding. Chemical compositions of the fresh grass and hay are shown in Table 1.

Table 1. Chemical composition of diet

Diet	Organic matter	Crude protein	Crude fat	Crude fiber	N F E[a]	Crude ash
Fresh grass	89.3[b]	17.9	3.9	23.5	44.1	10.7
Hay	87.7	16.5	3.4	24.2	43.7	12.3

a Nitrogen free extract.
b % of dry matter.

Experimental procedure

The experimental animals were kept in the metabolism cages throughout the experimental period. Five-day sampling period was preceded by 7-day preliminary period. Each wether was fed 2.25 % as dry matter of diet per kg body weight per day. Water was freely available, and each sheep had access to a mineralized salt lick at all time. One-half of the daily ration was given at 9 : 00 hour and another half at 17 : 00 hour. Feces and urine were collected just before the morning feed. Feces were dried for 48 hours in forced air drier at 55°C. Urine was collected daily in bottle added a few drops of 10% H_2SO_4 to prevent a loss of nitrogen. On the final day of each trial, about 50 ml of rumen contents was sampled using a stomach tube at 9 : 00, 10 : 00, 11 : 00, 12 : 00, 14 : 00 and 16 . 00 hours, and the pH value and the concentrations of ammonia, and VFAs of lumen fluid were measured. Similarly, about 5 ml of jugular blood was collected at 9 : 00, 12 : 00, 14 : 00 and 16 : 00 hours on the final day of each trial, and urea-nitrogen, plasma total protein and plasma free amino acid were determined.

Chemical analysis

Nitrogen in the diet, faces and urine were analyzed by the Kjeldahl method, and the contents of crude fat, crude fiber and crude ash in the diet and feces were determined according to AOAC method.[8] Ammonia in the rumen fluid was analyzed by the method of Oser.[9] Ruminal VFAs were determined by the aeration method.[10] Blood urea-nitrogen and plasma total protein were analyzed using the Unitest System (Model 300, Biodynamics, Inc., USA). The plasma was deproteinized by treating with picric acid according to the procedure described by Stein and Moore,[11] and the free amino acids were determined by the ion exchange chromatography on automatic amino acid analyzer (Model KLA-5, Hitachi Co. Tokyo).

RESULTS and DISCUSSION

As shown in Table 1, the chemical composition of hay was almost similar to that of the fresh grass. Slight decrease of crude protein in the hay might be due to autolysis of grass during dry process,[5] and it was in the range of changes generally accepted.[12,13] Table 2 shows the apparent digestibility and nitrogen balance in sheep fed the fresh grass and hay. Digestibilities of organic matter, crude protein and NFE were almost the same in sheep after feeding the fresh grass and hay. If green forage is dried without waste and in a way as to prevent fermentation, this process does not appreciably lower its digestibility. However, there are usually losses of some of nutrients parts while drying is taking place and in handling afterward. Thus green forage may be sometimes more somewhat more

Table 2. Apparent digestibility and nitrogen balance

	Fresh grass	Hay
Apparent digestibility (%)		
Organic matter	59.0±2.8[a]	59.9±0.8
Crude protein	66.1±2.4	66.4±0.2
Crude fat	50.7±3.6	59.0±0.4
Crude fiber	50.4±3.1	56.6±0.5
N E F[b]	61.4±2.8	60.8±0.6
Nitrogen balance (g/kgBW$^{0.75}$/day)		
Intake	1.50±0.04	1.37±0.03
Fecal nitrogen	0.51±0.04	0.46±0.01
Urinary nitrogen	0.70±0.02	0.66±0.03
Retention	0.30±0.05	0.25±0.04
	(29.7±4.1)[c]	(27.0±3.8)

a Mean±S.E. of 3 sheep.
b Nitrogen free extract.
c Percent of digested nitrogen.

digestible and give better results than dry forage.[14] Comparing the digestibility of alfalfa fed fresh and alfalfa hay with steers, it was concluded that the fresh green alfalfa was digested better than the hay, although the difference was not great.[14] Holter and Reid[15] also observed that the apparent digestibility of the protein of green forage was slightly higher than that of hay. On the other hand, there was the opposite conclusion following digestion trials with sheep fed the mixture hay.[14] In the present experiment, the result showing similar digestibilities of organic matter, crude protein and NFE in the fresh grass and hay might indicate that the hay used was a good quality as same as the fresh grass. In general, it is accepted that the apparent digestibilities of nutrients in feed are considerably affected by the level of feed intake. Furthermore, it is also known in ruminants that the digestibility of crude protein of roughage diet is considerably affected by the nitrogen contents and its solubility.[1] Digestibility of organic matter obtained in the present study was very comparable with the results of Yahata et al.[16] and of Fujihara and Ohshima[17] using sheep fed only orchardgrass hay at 1.8−2.0% level of dry matter per kg body weight. Digestibility of crude protein was also comparable with that reported by Ishiguri[18] and Fujihara and Ohshima,[17] and was about 10% higher than that reported by Yahata et al.[16] using sheep fed orchardgrass hay at 1.8−2.0% level of dry matter per kg body weight. Recently, Harumoto and Kato[19] demonstrated that the digestibilities of crude protein were 76.0 and 72.4% in sheep fed only the fresh grass and hay at about 1.2% level of dry matter per kg body weight. These values were higher than those obtained in the present experiment, and the discrepancy might be due to the difference of level of feed intake. Digestibility of NFE obtained in the present study was very in agreement with that of Ishiguri[18] using sheep fed only orchardgrass hay. Digestibilities of crude fiber and crude fat were slightly higher, but not significantly, in the hay feeding than in the fresh grass feeding. Digestibility of crude fat in this study was very comparable with those reported by Yahata et al.[16] and Ishiguriin[18] sheep fed only the orchardgrass hay (1st or 2nd cut). According to Harumoto

Table 3. Ruminal pH, and the concentrations of ruminal ammonia, VFA, blood urea nitrogen and plasma total protein in sheep fed the fresh grass or hay

	Fresh grass	Hay	Significance
Ruminal ammonia (mg/100 ml)			
0[a]	8.7±1.6[b]	9.8±1.9	NS ($p<0.25$)
1	30.2±3.7	22.5±4.4	NS ($p<0.10$)
2	34.9±5.6	21.2±1.0	NS ($p<0.10$)
3	33.8±3.5	18.2±1.9	*
5	20.9±4.7	17.4±0.4	NS ($p<0.25$)
7	16.2±4.3	14.5±0.5	NS ($p<0.25$)
Ave	24.1±4.3	17.3±1.9	NS ($p<0.25$)
Ruminal pH	6.83±0.09	6.53±0.05	*
Ruminal VFA (mM/100 ml)			
	8.52±0.18	7.66±0.25	*
Ht	28.4±0.3	25.5±0.6	**
Blood urea-mitrogen (mg/100 ml)			
	24.8±0.9	18.4±1.0	**
Plasma total protein (g/100 ml)			
	6.9±0.1	5.8±0.1	**

a Hours after morning feeding.
b Mean±S.E. of 3 sheep.
Significance of difference between means within diets (*$p<0.05$, **$p<0.01$).

and Kato[19], digestibility of crude fat of fresh grass was significantly higher than that of hay in sheep fed at about 1.2% level of dry matter per kg body weight. In the present study, however, digestibility of crude fat of fresh grass was slightly lower than that of hay, this might indicate that the quality of hay used was good as the fresh grass. Crude fiber digestibility of hay was considerably lower in this study than those of Harumoto and Kato (1.2% D.M./B.W.)[19] and Fujihara and Ohshima (1.8% D.M./B.W.)[17], this may be due to the high level of feed intake in the present experiment (2.25% D.M./B.W.).

As shown in Table 2, fecal nitrogen output was slightly more in fresh grass feeding than in hay feeding, and this may be due to high level of nitrogen intake in fresh grass feeding. Urinary nitrogen excretion tended to increase in fresh grass feeding. Fujihara and Ohshima[17] showed a high nitrogen into urine of sheep fed only high-moisture silage, and they suggested that the increase of urinary nitrogen after silage feeding should be caused by large amount of water intake and increase of urine volume afterward. In the present study, slight increase of urinary nitrogen excretion after feeding of fresh grass may be explained by the high moisture of fresh grass. As a whole, retained nitrogen per kg of metabolic body size was calculated as 0.30 and 0.25 g after feedings of fresh grass and hay, respectively. These figures were corresponded to 29.1 and 27.0% of digested nitrogen, and this indicates that the nitrogen in fresh grass was more utilized than that of hay in sheep when both diets were prepared from the same origin. The high utilizability of nitrogen in fresh grass feeding was closely related to an efficient rumination after feeding of fresh grass as described previously[7]. The utilization of digested nitrogen was almost the same as that reported by Ishiguri[18] and was slightly higher than that of Fujihara and Ohshima[17], in which sheep were fed only orchaıdgrass hay at 1.8−2.0% of dry matter per kg body weight.

Table 3 shows the ruminal pH and the concentrations of ruminal ammonia, ruminal VFAs and some blood constituents in sheep after feedings of the fresh grass and hay. Ruminal ammonia was considerably higher after feeding of the fresh grass than after hay feeding. Ohyama[20] also observed in sheep that high level of ruminal ammonia was occurred in fresh grass feeding compared with the level after dried grass feeding (orchardgrass). The level of ruminal ammonia after fresh grass feeding in the present study was somewhat lower than that reported by Johns[21] with sheep grazed on a high producing pasture. The concentration of ruminal ammonia in hay feeding was slightly higher in the present experiment than those reported by Fujihara and Tasaki[22,23] using goats fed only the mixed hay. Ruminal pH and ruminal VFAs concentrations were significantly higher in feeding of the fresh grass than in feeding of hay. These results were similar to those genrally recognized in sheep or goats.[1)]

Haematocrit value was significantly higher in fresh grass feeding than in hay feeding. The concentration of blood urea-nitrogen after fresh grass feeding was significantly higher than that after hay feeding, and the value in fresh grass feeding was very in agreement with that of Fujihara and Ohshima[17)] in sheep after silage feeding. McIntyre[24)] showed the interrelationships between the concentration of blood urea-nitrogen and the ruminal ammonia concentration. Fujihara and Ohshima[25)] also reported that the high level of ruminal ammonia results in high urea-nitrogen level in the blood of sheep fed only silage. From these facts, a significant difference of blood urea-nitrogen concentration between fresh grass feeding and hay feeding may have been due to the high level of ruminal ammonia as shown in Table 3. Waldo[26)] also described that a considerable amount of ammonia is directly absorbed from the rumen wall when ammonia concentration becomes high in the rumen. The plasma protein level was significantly higher in feeding of the fresh grass than in feeding of hay, and the value after fresh grass feeding was very in agreement with that generally accepted in mammals.[27)] These results might show that a synthesis of reserve protein is more stimulated in fresh grass feeding than in hay feeding. The figure in hay feeding was comparable with that reported by Fujihara and Ohshima[17)] using sheep fed only silage or orchardgrass hay. The difference in plasma protein level after both feedings in the present study may be related to the difference in concentration of plasma free amino acid as described later.

Table 4. Plasma amino acid concentrations in sheep fed the fresh grass or hay (μm/100 ml)

Amino acid	Fresh grass	Hay
Thr	10.0±1.5[a]	13.0±1.1
Val	17.1±1.1	20.8±1.5
Met	0.5±0.1	0.5±0.1
Ile	6.5±0.5	8.0±0.8
Leu	8.8±0.8	12.0±1.1
Phe	3.2±0.3	3.8±0.5
Lys	11.7±1.3	16.9±1.3
His	5.8±0.5	8.1±0.6
Arg	10.7±2.6	11.0±2.1
Trp	Trace	Trace
Asp+Asn	3.6±0.5	4.0±0.4
Ser	10.4±1.5	12.4±0.9
Glu+Gln	24.8±1.9	21.9±3.1
Gly	45.7±8.0	47.3±2.9
Ala	15.3±1.3	17.0±0.6
Tyr	3.7±0.4	3.8±0.5
Orn	8.2±0.7	9.7±0.6
TAA	182.3±15.7	207.3±12.6
EAA	73.6± 7.7	93.0± 7.9
NEAA	108.8±11.2	114.3± 5.9
EAA/NEAA	0.70±0.05	0.81±0.05

a Mean±S.E. of 3 sheep.

As shown in Table 4, the concentration of total free amino acids in plasma was considerably lower, but not significantly, in fresh grass feeding than in hay feeding. The value after feeding of the fresh grass was similar to the result of Fujihara and Ohshima[17)] using sheep fed only silage. The value in hay feeding was vary comparable with that reported by Cross et al.,[28)] in which wethers were freely given alfalfa hay, corn grain and soybean meal. The ratio of essential to non-essential amino acid concentrations was slightly lower in fresh grass feeding than in hay feeding. The values obtained in the present study were considerably higher than that reported by Fujihara and Ohshima,[17)] in which sheep were fed only silage or orchardgrass hay. The fact that a relatively low value in the concentration of total free amino acid in plasma was observed after the feeding of fresh grass may be a result induced by the stimulated synthesis of reserve protein as plasma protein after fresh grass feeding. There is a suggestion that the plasma protein may represent a vehicle of limited capacity for amino acid transport between the liver and other tissues.[29)]

SUMMARY

In order to investigate the nitrogen utilization of grass diet in sheep, the present experiment was carried out using wethers fed only the fresh grass or hay prepared from the same origin. The results obtained were as follows ;

1) The digestibilities of crude protein were almost the same in sheep after feedings of fresh grass and hay. The retained nitrogen per kg of metabolic body size was calculated as 0.30 and 0.25 g in feedings of fresh grass and hay, and these figures were corresponded to 29.1 and 27.0% of digested nitrogen.

2) The concentration of ruminal ammonia was higher, but not significantly, after fresh grass feeding than after hay feeding. Ruminal pH and the concentration of ruminal VFAs were significantly higher in feeding of fresh grass than in feeding of hay.

3) The concentrations of blood urea-nitrogen and plasma total protein were significantly higher in fresh grass feeding than in hay feeding. The concentration of total free amino acids in plasma was slightly lower in fresh grass feeding than in hay feeding.

From the results obtained in the present experiment, it is concluded that the dietary nitrogen is more efficiently utilized in fresh grass feeding than in hay feeding.

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摘　　要

メンヨウにおける草の蛋白質の利用性について検討するため，同一圃場から同時に収穫した生草と乾草を３頭の去勢メンヨウに各々乾物で１日１頭当り体重の2.25%の量を給与して代謝試験を行い，次の結果を得た．

1. 粗蛋白質の消化率は生草給与時と乾草給与時でほとんど同様であった．窒素出納では摂取量に若干差はあったが，体謝体重 kg 当りの窒素の体内蓄積量は生草給与時に 0.30g と乾草給与時に 0.25g であり，これらは各々吸収窒素量の29.1%と27.0%に相当した．
2. 第１胃内のアンモニア濃度は乾草給与時に比べ生草給与時に高くなり，また第１胃内の pH 値と低級脂肪酸濃度は生草給与時で有意に高くなった．
3. 血中尿素態窒素濃度と血漿中総蛋白濃度は生草給与時に有意に高くなったが，血漿中総遊離アミノ酸濃度は乾草給与時に比べ生草給与時に若干低くなった．

これらの結果から，草の蛋白質は乾草として給与した場合よりも生草のまま給与した方がメンヨウにおいてより効率良く利用されることが示された．

（メンヨウにおける粗飼料の利用性に関する研究１）