

## Nitrogen Utilization in Sheep Given a Low-Quality Hay and Orally Supplemented with Casein<sup>#</sup>

by Tsutomu FUJIHARA\* and Yasuhiko TAKAHASHI\*

低質乾草にカゼインを添加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・高橋 康彦

### INTRODUCTION

It is well known in ruminants that the utilization of low-protein roughage is influenced by several factors, and one of these factors is undoubtedly a shortage of nitrogen to satisfy the requirements for growth of an adequate microbial population in the rumen. The supplementation of nitrogenous compound, such as urea alone to improve the nitrogen utilization of such dietary material has produced obviously variable responses. According to Campling et al.<sup>1)</sup>, the consumption of oat straw by cattle increased markedly, and the amount of chewing during eating and ruminating decreased clearly when small amounts of urea were added, and a response was not affected by the further addition of sucrose. They has also suggested that there are important interactions between the microbial degradation and the chewing during eating and ruminating for comminuting the food particles in the rumen.<sup>2)</sup> Contrary, the experiments with cattle and sheep reported by Minson and Pigden<sup>3)</sup> have shown that roughage intake was reduced when supplemented with urea alone. Coombe and Tribe<sup>4)</sup> also reported an increase in roughage intake only when both urea and molasses were added in sheep. In the nitrogen balance study, Cuthbertson and Chalmers<sup>5)</sup> has reported that casein supplements fed to ewes on a low-plane of nutrition were surprisingly ineffective in improving the nitrogen retention of these animals.

It is also well recognized that there is a depressing effect of readily available carbohydrates on fibre digestion when the ration is insufficient in nitrogen.<sup>6)</sup> Similarly, Lewis<sup>7)</sup> has suggested that the digestion of strach by rumen microbes may also be stimulated by the higher VFAs or peptides derived from protein degradation. It appears that the rate of breakdown of low-quality roughage in the rumen is changed by means, such as the addition of nitrogenous compounds,<sup>1,3,4,8)</sup> certain minerals<sup>9)</sup> or some fatty acids.<sup>8)</sup> These substances added would presumably aid microbial degradation of the roughage feed ingested in the rumen.

In the present experiment, the digestion and utilization of dietary nitrogen were investigated in sheep fed a low-quality hay and orally supplemented with casein. A part of the results in this study was reported previously.<sup>10)</sup>

<sup>#</sup> Studies on the roughage utilization in sheep No. 4.

\* Laboratory of Animal Science.

## EXPERIMENTAL PROCEDURE

Three Japanese Corriedale wethers, each weighing 20-25 kg, were used repeatedly. All sheep were fed a mixed hay at 2% as dry matter of diet per kg body weight per day. Casein was orally supplemented at level 0, 10, 20 and 30% of dietary nitrogen intake from hay. Chemical composition (as % D. M.) of the mixed hay, determined by the AOAC method, were as follows: organic matter, 93.3; crude protein, 10.2; crude fat, 1.6; crude fibre, 38.6; nitrogen free extract, 42.6; crude ash, 7.0.

The experimental animals were kept in the metabolism cages throughout the experimental period, and 7-day sampling periods were preceded by 8-day preliminary periods. Half of the daily ration was given at 09:00 hr and the other half at 18:00 hr. One-half of the daily dose of casein suspended in 100 ml of warm water was directly introduced into the rumen through the stomach tube twice a day after consumption of hay diet. Water and salt licks containing trace minerals were accessible at all times. Faeces and urine were collected just before the morning feed during sampling period. On the final day of each trial, about 100 ml of rumen contents were sampled using a stomach tube, just before the morning feed and at 1, 2, 3, 5 and 7 hours after first sampling, and the pH value and the concentrations of ammonia and VFAs of rumen fluid were measured. Likewise, about 5 ml of jugular blood was collected, just before the morning feed and at 3, 5 and 7 hours after the first sampling on the final day of each trial and blood urea-nitrogen and plasma total protein were determined.

Nitrogen in the diet, faeces and urine were analyzed by Kjeldahl method, and the contents of crude fat, crude fibre and crude ash in the diet and faeces were determined according to AOAC method. Ammonia in the rumen fluid was analyzed by the method of Oser. Ruminal VFAs were determined by the aeration method. Blood urea-nitrogen and plasma total protein were analyzed using the Unitest System (Model 300, Biodynamic, Inc., USA).

## RESULTS and DISCUSSION

The chemical composition of hay used in the present study was almost similar to that of timothy hay used in the previous experiment<sup>14)</sup> apart from the crude protein content. As shown in Table 1, digestibilities of organic matter, crude fibre and nitrogen free extract were not changed with an oral casein supplements, and the figures were almost similar to those reported previously using timothy or Italian ryegrass hay.<sup>14)</sup> These results are in agreement with that reported by Minson and Pigden,<sup>3)</sup> in which urea had no consistent effect on digestibility coefficients for dry matter, energy, crude fibre and nitrogen free extract in sheep and cattle fed on a low-quality forage. The digestibility of crude protein was increased with an increase of casein added, and this may be due to a high digestibility of casein and/or an increase of dietary protein intake as generally accepted.<sup>17)</sup> The values in protein digestibility of hay without casein was fairly low compared with that of timothy hay as reported previously,<sup>14)</sup> and this would be due to the difference of hay quality.

Faecal nitrogen output was almost similar in all the treatments of oral casein supplement, although the nitrogen intake was fairly increased with an increase of casein added. This shows the increase of digestibility of dietary protein as described above. Urinary nitrogen excretion was obviously increased with an increase of oral casein supplement. Consequently,

Table 1. Apparent digestibility and nitrogen balance

Supplemented casein level (%)#	0	10	20	30
Digestibility, (%)				
Organic matter	55.6± 1.9*	55.2±0.5	55.6±0.9	56.5±0.3
Crude protein	47.8± 3.9	52.7±1.0	56.8±1.5	59.3±1.3
Crude fat	48.6±10.5	37.3±2.2	37.2±1.2	33.8±5.3
Crude fibre	61.4± 2.7	60.3±0.9	60.1±0.6	60.6±1.0
NFE**	52.3± 4.5	52.0±0.4	51.5±1.5	52.9±0.5
Nitrogen balance (g/kg BW <sup>0.75</sup> /day)				
Intake	0.71±0.02	0.81±0.01	0.87±0.01	0.96±0.01
Faecal	0.37±0.04	0.38±0.01	0.38±0.01	0.39±0.02
Urinary	0.31±0.05	0.49±0.04	0.54±0.02	0.60±0.05
Retention	0.03±0.05	-0.07±0.03	-0.05±0.03	-0.03±0.04

# % of casein nitrogen to the dietary nitrogen from hay.

\* Mean±S. E. of 3 sheep.

\*\* Nitrogen free extract.

the retained nitrogen was not increased with an increase of oral casein addition, and the negative nitrogen balance was observed in the sheep supplemented casein, though there were considerable variations among individuals. These facts might indicate that the nitrogen from added casein (or more nitrogen) was mostly excreted into urine. The retained nitrogen after feeding of hay without casein was very similar to that reported earlier,<sup>14)</sup> in which the sheep were fed only timothy hay at 2% level of dry matter per kg body weight. All the data indicated by Hemsley and Moir have shown that the basal diet of straw was primarily deficient in nitrogen, and that this deficiency was overcome by the addition of urea, with or without other supplements. In contrast to this, the present study with mixed hay fed to sheep showed that the supplement of casein alone did not increase the retained nitrogen in sheep. The reason why this result differs from other work<sup>8)</sup> is unknown at present. It may be a possible reason, however, that the nitrogen content of basal hay diet was relatively high, so that the utilization of casein nitrogen by rumen microbes was very limited, i. e., a rapid degradation of casein, and absorption of elaborated ammonia through the rumen wall, synthesis to urea in the liver, and excreted into urine.<sup>18)</sup> Cuthbertson and Chalmers also showed a negative nitrogen balance in sheep when they were well-fed and orally supplemented with casein. It would appear that the rate of deamination of the casein in the rumen was in excess of the capacity to synthesis of bacterial protein. From these facts, it may be that the extent to which the nitrogen was retained also depended on the state of nutrition<sup>4)</sup>

Table 2. Ruminal pH and the concentrations of ammonia and VFAs in ruminal fluid of sheep given hay and orally supplemented with casein

Supplemented casein level#	0	10	20	30
pH	6.71±0.06*	6.75±0.04	6.74±0.03	6.77±0.03
Ammonia (mg/100 ml)	8.69±0.89	8.19±0.73	13.10±1.51	14.23±2.28
VFAs (mM/100 ml)	8.37±0.24	7.13±0.13	7.18±0.13	6.80±0.44

# % of casein nitrogen to the dietary nitrogen from hay.

\* Mean±S. E. of 3 sheep.

Table 3. The Ht value and concentrations of blood urea-nitrogen and plasma total protein in sheep given hay and orally supplemented casein

Supplemented casein level#	0	10	20	30
Ht	—	24.7±0.7*	27.0±0.5	24.9±0.4
Blood urea-nitrogen (mg/100 ml)	—	12.50±1.38	14.94±0.8	17.44±1.7
Plasma total protein (g/100 ml)	—	6.93±0.05	7.01±0.14	6.84±0.07

# % of casein nitrogen to the dietary nitrogen from hay.

\* Mean±S. E. of 3 sheep.

of the animal.

Table 2 shows the ruminal pH and the concentrations of ruminal ammonia and VFAs, and their time course changes are shown in Figure 1. Ruminal pH value tended to be higher after feeding of hay with casein than after feeding of hay alone, and the values were very comparable to those after feeding of fresh grass<sup>15)</sup> or the fibrous residue-silage plus hay.<sup>14,19)</sup> The concentration of ammonia was fairly higher after feeding of hay with 20 and 30% casein than after feeding of hay with 0 and 10% casein supplements. The figures after feeding of hay with 20 and 30% casein were almost similar to that reported earlier in which the sheep were given Italian ryegrass hay contained 16.5% crude protein. These high concentrations of ruminal ammonia after feeding hay with casein would be a cause for the increase in ruminal pH value as described above. The VFAs concentration tended to be decrease with an increase of casein supplement, and the values after feeding hay with casein were in agreement with those reported previously, in which the sheep were fed only fresh Italian ryegrass<sup>15)</sup> or broad bean residue-silage plus timothy hay.<sup>14)</sup> The concentration of VFAs after feeding hay alone was very comparable to that after feeding silage made from the fibrous residue of broad bean.<sup>14)</sup> The reason why the ruminal VFAs concentration was fairly lower in feeding hay with casein than in feeding hay alone is at present not clear. A possible reason for this fact may be certain effect of added casein to stimulate the quantitative uptake of VFAs by the rumen microorganisms<sup>20)</sup> or the absorption from rumen wall.

Table 3 shows the Haematocrit value and concentrations of blood urea-nitrogen and plasma total protein in sheep fed hay with oral supplement of casein, though it was not measured in feeding hay alone. Haematocrit value was slightly higher in 20% casein supplement than in 10 and 30% casein supplement, and

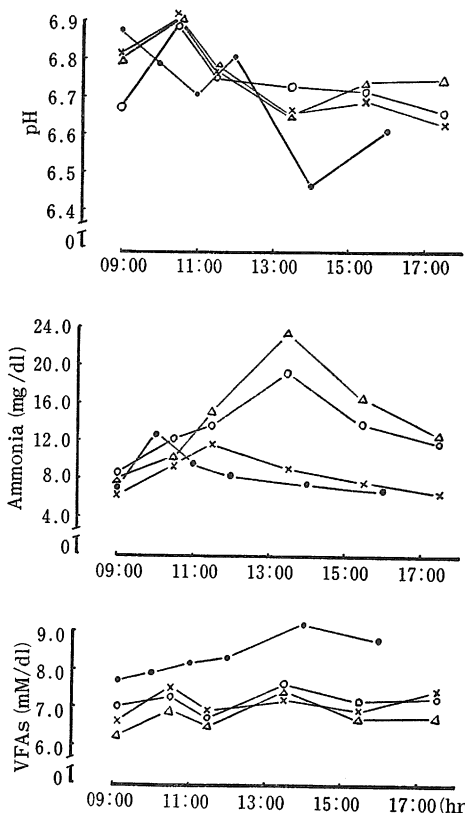


Fig. 1. Time course changes in pH and concentrations of ammonia and VFAs in the rumen fluid of sheep. (—●—: 0, —×—: 10, —○—: 20, —△—: 30% casein supplements).

the figures were in agreement with that in feeding timothy hay alone as reported earlier.<sup>14)</sup> The level of blood urea-nitrogen increased gradually with an increase of casein supplement, and this would be due to the increase of ammonia absorption in the rumen.<sup>19,21)</sup> The values of blood urea-nitrogen levels were fairly higher than that of feeding diet of timothy hay, and were markedly lower than those after feeding the residual silages of legumes, or fresh grass.<sup>14)</sup> The concentration of plasma total protein was not changed by a change of amounts of added casein, and the values were relatively high compared with those observed in our previous experiments,<sup>14,15,22)</sup> showing the plasma total protein level was 5.7–6.3 g/100 ml when the sheep were given only hay diet, although the figures were in a range generally accepted.<sup>23)</sup> The values obtained in this experiment were rather in good agreement with that when the sheep were given fresh grass alone.<sup>15)</sup> According to Fujihara and Tasaki,<sup>24)</sup> the plasma total protein level was 7.2–7.3 g/100 ml in goats sustained by abomasal feeding of purified diet contained 10–20% casein. From these facts, it can be considered that the level of plasma total protein should be fairly varied with the differences in quality and/or quantity of dietary protein. In the present experiment, the plasma total protein level was fairly high compared with those reported previously using sheep,<sup>14,15)</sup> although the nitrogen retention was rather lesser in this study than in previous works. From this, it may be necessary to investigate fundamentally with the relationship between the utilization of dietary protein and the level of plasma total protein in ruminants.

The results obtained in the present experiment show that casein supplements fed to sheep on a roughage feed may be unexpectedly ineffective in improving the nitrogen retention of these animals, and this also indicate that the nitrogen retention should be dependent on the nutritional status of the animal.

### SUMMARY

The present experiment was carried out in order to investigate the digestion and utilization of dietary nitrogen in sheep fed on a low-quality hay and orally supplemented with casein at various levels, and the results obtained were as follows; 1). The digestibility of crude protein was fairly increased with an increase of casein added orally. The digestibilities of organic matter, crude fibre and nitrogen free-extract were not changed with an oral casein supplement. 2). Urinary nitrogen excretion was fairly increased with an increase of oral casein addition, and the retained nitrogen was not increase with an increased of oral casein supplements. It appears that the nitrogen from added casein was mostly excreted into urine. 3). Ruminal ammonia level was clearly increased with an increase of oral casein supplements, and subsequently, ruminal pH tended to be higher after feeding hay with casein than after feeding hay alone. The level of ruminal VFAs tended to be lower in feeding hay with casein than in feeding hay only. 4). The concentration of blood urea-nitrogen increased gradually with an increase of casein added. The level of plasma total protein was not changed by the change of amounts of supplemented casein.

### ACKNOWLEDGEMENTS

We are grateful to Mr. T. Nakao for his kind assistance during the course of experiment. We are also indebted to Dr. T. Harumoto for his valuable advice during the experiment.

## REFERENCES

1. CAMPLING, R. C., FREER, M. and BALCH, C. C. : Brit. J. Nutr. **16** : 115—124. 1962.
2. FREER, M., CAMPLING, R. C. and BALCH, C. C. : Brit. J. Nutr. **16** : 279—295. 1962.
3. MINSON, D. J. and PIGDEN, W. J. : J. Anim. Sci. **20** : 962 (Abstract).
4. COOMBE, J. B. and TRIBE, D. E. : Aust. J. Agr. Res. **14** : 70—82. 1963.
5. CUTHBERTSON, D. P. and CHALMERS, M. I. : Biochem. J. **46** : xvii—xviii. 1950.
6. EL-SHAZLY, K., DEHORITY, B. A. and JOHNSON, R. R. : J. Anim. Sci. **20** : 268—273.
7. LEWIS, D., J. Agr. Sci. **58** : 73—81. 1962.
8. HEMSLEY, J. A. and MOIR, R. J. : Aust. J. Agr. Res. **14** : 509—517. 1963.
9. BLAXTER, K. L. : The energy metabolism of ruminants, Hutchinson & Co. Ltd. London, 1962. pp. 185—216.
10. FUJIHARA, T. and NAKAO, T. : Jpn. J. Zootech. Sci. **55** : (in press).
11. HOITZ, H. (ed.). Official Methods of Analysis, 9th ed. A. O. A. C. Washington D. C. 1960. pp. 283—288.
12. OSER, B. L. (ed.). Hawk's physiological chemistry. 14th ed. McGraw Hill C. New York, pp. 1219—1220. 1965.
13. MORIMOTO, H. (ed.). Dobutsu Eiyo Shikengo. Yokendo. Tokyo. pp. 429—430. 1971. (in Japanese).
14. FUJIHARA, T. and OHSHIMA, M. : J. Japan Grassl. Sci. **28** : 209—216. 1982.
15. FUJIHARA, T. : Bull. Fac. Agr. Shimane Univ. **14** : 16—22. 1980.
16. FUJIHARA, T. and TASAKI, I. : Jpn. J. Zootech. Sci. **44** : 125—127. 1973.
17. GLOVER, J., DUTHIE, D. W. and FRENCH, M. H. : J. Agr. Sci. **48** : 373—378. 1957.
18. McDONALD, I. W. : Biochem. J. **42** : 584—587. 1948.
19. FUJIHARA, T., HANABUSA, Y. and OHSHIMA, M. : J. Japan Grassl. Sci. **30** : (in press).
20. LENG, R. A. : Physiology of digestion and metabolism in the ruminant. (Phillipson, A. T. ed.). Oriel Press. England, pp. 406—421. 1975.
21. FUJIHARA, T. and OHSHIMA, M. : J. Japan Grassl. Sci. **26** : 191—200. 1980.
22. FUJIHARA, T. and OHSHIMA, M. : J. Japan Grassl. Sci. **26** : 94—101. 1980.
23. HARPER, H. A. : A review of physiological chemistry, 15th ed. Maruzen, Tokyo. p. 199. 1975.
24. FUJIHARA, T. and TASAKI, I. : Jpn. J. Zootech. Sci. **50** : 471—478. 1979.

## 摘 要

低質粗飼料のメンヨウにおける利用性改善の目的で添加給与された窒素源の効果について検討するため、比較的低位質の混播乾草を乾物重で1日当たり体重の2%の量をメンヨウに給与し、更に乾物中窒素量の10~30%に相当するカゼインを経口的に投与して代謝試験を行い、次の様な結果を得た。

- 1) 粗蛋白質の消化率はカゼインの投与量が増加するにつれて上昇する傾向を示したが、有機物、粗繊維および可溶無窒素物の消化率はカゼインの添加によってほとんど変化しなかった。
- 2) 尿中への窒素排出量はカゼインの投与量が増加するにつれて増加し、その結果体内への蓄積窒素量はカゼイン無添加の場合とほぼ同程度であった。
- 3) 第1胃内液中のアンモニア濃度はカゼインの投与量の増加につれて高くなり、一方第1胃内液 pH 値はカゼイン投与量の増加によって低下する傾向を示した。  
第1胃内液中の VFA 濃度はカゼイン投与時に低下する傾向を示した。
- 4) 血中尿素態窒素濃度はカゼイン投与量の増加によって明らかに上昇した。血中総蛋白濃度はカゼイン投与量の変化によってほとんど影響をうけないようであった。

これらの事から、粗飼料給与時に補足添加された窒素栄養源のメンヨウにおける蛋白質利用改善に対する効果は、粗飼料の蛋白質含量のみならず、添加窒素源の第1胃内における分解の速度によっても影響をうけるものと推察された。

(メンヨウにおける粗飼料の利用性に関する研究 4)