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Nitrogen Utilization in Sheep Fed a Low-Quality Hay with Oral Supplement of Urea*

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Abstract In order to investigate the effect of orally supplemented urea on utilization of low-quality roughage in sheep, digestion and nitrogen balance trials were conducted using 3 Japanese Corriedale rams fed a mixed hay supplemented with urea at 3 levels, *i. e.*, 0, 15 and 30% of dietary nitrogen from hay, according to a 3×3 Latin square design. The results obtained were as follows; 1) The digestibility of crude protein was clearly ($P<0.05$) increased with an increase of urea supplemented orally. 2) Urinary nitrogen excretion was fairly increased with an increase of oral urea supplement, and thus, the retained nitrogen increased a little after supplementing urea at a level of 30% dietary nitrogen. 3) Ruminal ammonia level clearly ($P<0.05$) increased with an increase of oral urea supplement. On the contrary, the level of ruminal VFAs tended to be lower after feeding hay with urea than after feeding hay alone. 4) The concentration of blood urea-nitrogen tended to increase with increase in supplemented urea, and the plasma total protein did not change with an oral urea supplement.

Key words: Roughage utilization; urea supplement; sheep.

Introduction

In ruminant animals, the most important factor controlling utilization of low-quality roughage is thought to be on inadequacy of dietary nitrogen to meet the requirement for growth of microbes in the rumen. There are several works, in which nitrogenous compounds have been used as supplements for improving the utilization of low-quality roughage in cattle (Campling *et al.*, 1962; Minson and Pigden, 1961) or sheep (Hemsley and Moir, 1963; Fujihara and Takahashi, 1983). It has been already shown that the consumption of oat straw by cattle was increased markedly with the supplement of small amounts of urea, and the response was not affected by further addition of sucrose (Campling *et al.*, 1962). Contrary, Minson and Pigden (1961) have shown in cattle and sheep that roughage intake was increased when supplemented with urea alone. It has also been suggested that digestibilies of organic matter, crude fibre and nitrogen free-extract (NFE) seemed not to be changed with an oral supplement of casein in sheep fed a low-quality hay (Fujihara and Takahashi, 1983). Coombe and Tribe (1963) also reported that there was increased in roughage intake only when both urea and molasses were added in sheep. In the balance experiment, it has also been reported that casein supplements fed to ewes

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on a low-plane of nutrition were amazingly ineffective in improving the nitrogen retention of those animals (Cuthbertson and Chalmers, 1950). It appears that the rate of breakdown of a low-quality roughage in the rumen can be changed by the addition of nitrogenous materials (Campling *et al.*, 1962; Minson and Pigden, 1961; Coombe and Tribe, 1963), certain minerals (Blaxter, 1962) or some fatty acids (Hemsley and Moir, 1963). These materials supplemented would presumably be able to aid the microbial degradation of roughage feed ingested in the rumen. In the present experiment, digestion and utilization of the dietary nitrogen were investigated in sheep fed a low-quality hay with oral urea supplement. A part of the results obtained in this study was reported previously (Fujihara *et al.*, 1993).

Materials and Methods

Animals and Diet: Three Japanese Corriedale rams, weighing 35-47 kg, were used. The mixed hay was mainly composed of Italian ryegrass and ladino clover, and its chemical composition (as % of dry matter: DM), determined by the AOAC method (Hoitz, 1963), were as follows; organic matter, 93.9; crude protein, 8.2; crude fat, 2.0; crude fibre, 39.1; NFE, 44.6; crude ash, 6.1. Urea was orally supple-mented at 0, 15 and 30% of dietary nitrogen intake from hay.

Experimental procedure: The experimental animals were kept in metabolism cages throughout the experimental period. Each ram was fed 2.0% as DM of diet per kg body weight per day, and water and salt licks containing trace minerals were available at all times. One half of the daily ration and added urea were given at 09:00 hr and the other half at 17:00 hr. Half of the daily dose of urea was orally given by drenching just after finishing the morning and evening feeds, respectively. The three rams were allotted to a 3×3 Latin square design to examine the effect of 3 levels of urea administration, *i. e.*, 0, 15 and 30% of dietary nitrogen from hay. A five-day sampling period was preceded by a 7-day preliminary period. 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 at 09:00, 11:00, 12:00, 14:00 and 16:00 hrs, the pH and the concentrations of ammonia and volatile fatty acids (VFAs) in rumen fluid were measured. Similarly, about 5 ml of jugular blood was collected at 09:00, 12:00, 14:00 and 16:00 hrs on the final day of each trial, and the haematocrit value, urea-nitrogen and plasma total protein were determinded.

Chemical analysis: Nitrogen in the diet, faeces and urine were analyzed by the Kjeldahl method, and the contents of crude fat, crude fibre and crude ash in the diet and faeces were determined according to AOAC method (Hoitz, 1963). Ammonia in the rumen fluid was analyzed by the method of Hawk *et al.* (1954), and the ruminal VFAs were determined by gas chromatography. Blood urea-nitrogen and plasma total protein were analyzed using the Unitest System (Model 300, Biodynamics, Inc., USA).

Results and Discussion

The chemical composition of hay used in this study was almost the same as that of timothy hay used in the previous experiment (Fujihara and Ohshima, 1982), and the crude protein content was a little lower than that of mixed hay also used in the previous study, in which an effect of oral supplement of casein was checked in sheep fed a low-quality hay (Fujihara and Takahashi, 1983).

Table 1. Apparent digestibility and nitrogen balance.

Supplemented urea level (%)[1]	0	15	30
Digestibility (%)			
Organic matter	55.4±2.4[2]	55.2±0.7	54.1±2.0
Crude protein	55.8±0.7[a]	59.5±1.1[b]	63.7±0.6[c]
Crude fat	56.0±1.7	52.6±3.9	51.4±4.5
Crude fibre	61.7±2.9	61.8±0.7	62.3±1.8
Nitrogen free extract	50.4±2.4	51.4±0.8	47.9±3.0
Nitrogen balance (g/kg $BW^{0.75}$/d)			
Intake	0.64±0.0	0.74±0.0	0.83±0.0
Faecal	0.26±0.0	0.28±0.0	0.28±0.0
Urinary	0.24±0.1[a]	0.32±0.1[ab]	0.36±0.0[b]
Retention	0.14±0.1	0.16±0.1	0.19±0.0
	(36.4±8.3)[3]	(33.6±6.0)	(34.7±1.5)

[1] % of urea nitrogen to the dietary nitrogen from hay.

[2] Mean ± S. E. of 3 sheep.

[3] % of digested nitrogen.

a-c Mean not having the same superscript letters are significantry different at 5% level.

As shown in Table 1, the digestibilities of organic matter, crude fat, crude fibre and NFE were not largely affected by an oral supplement of urea and the values were very similar to that reported earlier using mixed hay (Fujihara and Takahashi, 1983). This result is in good agreement with that reported by other workers, in which urea had no consistent effect on digestibility coefficients for DM, energy, crude fibre and NFE in sheep (Hemsley and Moir, 1963) or cattle (Minson and Pigden, 1961) fed a low-quality forage. The digestibility of crude protein was significantly ($P<0.05$) high in feeding hay with urea compared with feeding hay alone, and this could be mainly due to a high digestibility of urea (Campling *et al.*, 1962) and/or an increase of dietary nitrogen intake as generally accepted (Glover *et al.*, 1957). The figures in protein digestibility of hay alone was very comparable to that of timothy hay (Fujihara and Ohshima, 1982), and was fairly high compared with that of mixed hay as reported earlier (Fujihara and Takahashi, 1983). This could be mainly due to the difference of hay quality and/or species of original herbages (Fujihara and Ohshima, 1982).

Faecal nitrogen output did not increase with an increase of oral urea

supplement, and this shows an increase in digestibility of dietary crude protein as indicated above. On the other hand, urinary nitrogen excretion was obviously increased with an increase of oral urea supplement, and the difference in urinary nitrogen excretion after feeding hay with 30% urea supplement and that of control (without urea) was significant ($P<0.05$). Consequently, retained nitrogen did not largely increase with an increase of oral urea supplement, and the ratio of retained nitrogen to digested nitrogen was rather small in urea supplemented groups than in the control group. These facts might indicate that most of the nitrogen from urea added was excreted into urine. The retained nitrogen in feeding hay alone was fairly high as compared with that reported earlier using timothy hay (Fujihara and Ohshima, 1982) or mixed hay (Fujihara and Takahashi, 1983), and was very comparable to that with Italian ryegrass hay (Fujihara, 1980) or cocksfoot hay (Fujihara and Ohshima, 1980), in which sheep were fed a hay diet at 1.8-2.0% level of DM per kg body weight per day. According to Hemsley and Moir (1963), when the basal diet of straw was primarily deficient in nitrogen, this defficiency is usually overcomed by the addition of urea, with or without other supplements in sheep. In contrast to this, in the present study with sheep fed low-quality hay, the increase of retained nitrogen due to urea supplement was not so great. The reason why the discrepancy between the two works was observed is not known yet. There may be a possible reason, however, that the nitrogen content of the basal diet (hay) was relatively high, so that the ammonia nitrogen produced from supplemented urea was much higher than what microbes in the rumen require for their growth. There should therefore be a rapid degradation of urea in the rumen, absorption of ammonia through the rumen wall, synthesis to urea in the liver, and excretion into urine (McDonald, 1948). It has been also shown in sheep that there was a negative nitrogen balance when they were well-fed and further orally supplemented with casein (Cuthbertson and Chalmers, 1950). It would appear, therefore, that the rate of deamination of casein in the rumen was in excess of the capacity to synthesize bacterial protein. From these findings, it may be clear that the extent to which nitrogen is retained in the body also depended on the nutritional status of animals.

Table 2 shows the ruminal pH value and the concentrations of ammonia and VFAs in the rumen fluid, and their time-course changes are shown in Fig. 1. The

Table 2. Ruminal pH and the concentrations of ammonia and VFAs in ruminal fluid of sheep fed and orally supplemented with urea.

Supplemented urea level[1)]	0	15	30
pH	7.0±0.1[2)]	7.2±0.1	7.1±0.1
Ammonia (mg/100ml)	10.1±0.9[a]	14.6±2.5[ab]	18.5±4.7[b]
VFAs (mM/100ml)	8.1±0.3	7.0±0.8	7.4±0.3

1) % of urea nitrogen to the dietary nitrogen from hay.

2) Mean ± S.E. of 3 sheep.

a-b Mean not having the same superscript letters are significantly different at 5% level.

ruminal pH value did not change with urea supplement, and the figures were a little higher than those reported earlier (Fujihara and Takahashi, 1983), in which the sheep were fed a mixed hay diet with casein supplements (10-30% of N from basal diet). This could be due to a high ammonia concentration in the present study as compared to the previous study (Fujihara and Takahashi, 1983), *i. e.*, 10.2-18.5 mg/100 ml in the former and 8.2-14.2mg/100 ml in the latter. The ammonia level was significantly ($P<0.05$) increased with urea supplement, and as shown in Fig. 1. the ammonia level markedly increased 1-2 hours after the morning feed of hay with urea, and quickly decreased 3-5 hours after feeding. The ammonia level after feeding hay alone did not change so greatly, although The levels at 1-2 hours after morning feed was a little higher than that before feeding. These findings clearly show that a ruminal degradation of urea supplemented was very quick after being

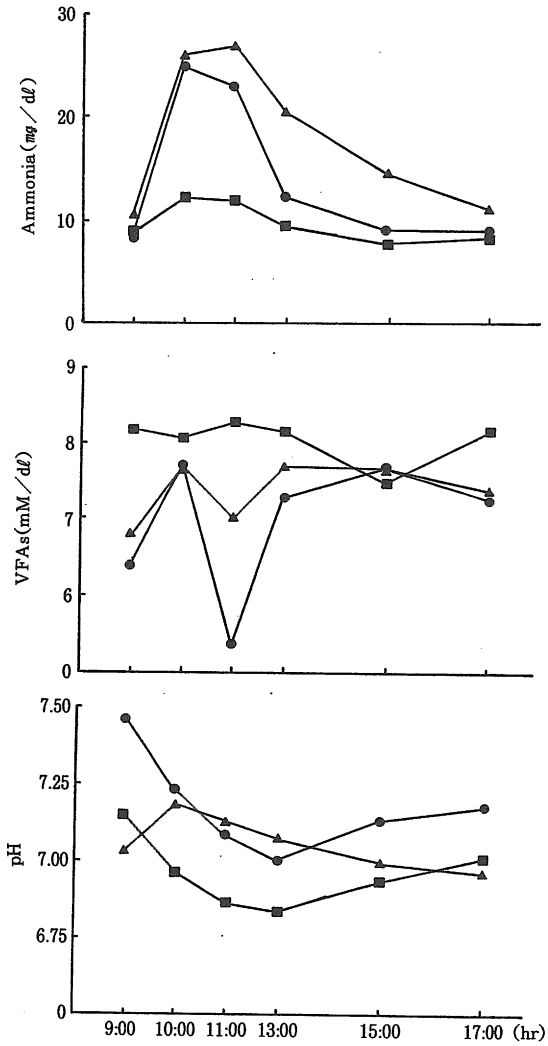

Fig. 1. Time course changes in pH and concentrations of ammonia and VFAs in the rumen fluid of sheep.
(−■− : 0, −●− : 15, −▲− : 30% urea supplements)

given orally. It has been shown that degradation rate of urea by rumen microbes was clearly faster than the incorporation of ammonia to microbial protein in the rumen (Nakamura, 1981). The figures shown in Table 2 was a little higher than those reported earlier (Fujihara and Takahashi, 1983), in which the sheep were fed a hay diet with an oral supplement of casein. VFA concentration was a little lower with urea supplement than without urea, but not significantly ($P>0.05$), and this was very comparable to that reported previously (Fujihara and Takahashi, 1983), in which the sheep were fed a hay diet with an oral casein supplementation. This finding show that the urea supplemented was not consistently used efficiently to stimulate ruminal fermentation in the present study. According to Suto (1970), an addition of urea to artificial saliva in *in vitro* continuous culture using an artificial rumen increased VFA production as compared with no urea supplement. The reason why the ruminal VFA level was rather low in feeding hay with urea than in feeding hay alone in the present study is not clear. Some possible reason are that the supplemented urea might have had an effect to stimulate the uptake of VFAs by rumen microbes (Leng, 1975) or an absorption of VFAs through the rumen wall in those circumstances. It can also be considerd that the lack of increase of ruminal VFAs by urea addition could be partly due to the absence of easily fermentatable carbohydrates in the diet, although there was a relatively high level of nitrogen for microbes in the rumen.

Table 3. The haematocrit value (Ht) and concentrations of blood urea-nitrogen and plasma total protein in sheep fed hay and orally supplemented urea.

Supplemented urea level[1)]	0	15	30
Ht	22.4±1.3[2)]	22.5±1.3	22.9±0.6
Blood urea-nitrogen (mg/100ml)	11.7±2.3[a]	13.7±2.4[ab]	17.2±0.6[b]
Plasma total protein (g/100ml)	6.0±0.4	5.6±0.6	5.8±0.3

1) % of urea nitrogen to the dietary nitrogen from hay.

2) Mean ± S.E. of 3 sheep.

a-b Mean not having the same superscript letters are significantly different at 5% level.

The haematocrit value and the concentrations of blood urea-nitrogen and plasma total protein in sheep fed hay diet with or without oral urea supplement are shown in the Table 3. Haematocrit values were almost the same in all the dietary treatments, and were within a range accepted as the normal level in sheep (Kaneko, 1989). The concentration of blood urea-nitrogen tended to increase clearly with an increase of urea supplementation, and the difference in values between 30% urea supplementation and control was statistically significant ($P<0.05$). This might clearly reflect an increase in ruminal ammonia level after feeding hay with urea added orally as mentioned above. The values indicated in Table 3 were very comparable to that reported earlier (Fujihara and Takahashi, 1983), in which the sheep were fed hay diet with oral supplements of casein. These findings clearly show that the supplemented urea-nitrogen had been retained in the blood stream as urea

resynthesized in the liver from ammonia produced in and absorbed from the rumen. Ide *et al.* (1967) have shown in goats that the blood urea-nitrogen was increased proportionally with an increase in nitrogen intake and of ammonia level in the rumen, when their energy intake was equal, and they concluded that the level of blood urea-nitrogen can be used as an index of the utilizability of dietary protein in ruminants. Fujihara and Tasaki (1973) have also shown a similar evidence in goats sustained by an abomasal feeding of various levels of dietary protein. The result obtained in this experiment should strongly support the results described by other works (Ide *et al*, 1967; Fujihara and Tasaki, 1973), which stressed an important role of blood urea-nitrogen in the protein nutrition of ruminants. From these facts, it could be concluded that supplemented urea was not used efficiently as a nitrogen source in ruminal fermentation, although it should play an important role as recycled nitrogen source which is a peculiar pathway in nitrogen metabolism of ruminant animals (Annison and Lewis, 1959). The level of plasma total protein did not change by an oral urea supplement, and the figures in Table 3 were a little lower than that observed in the previous experiment (Fujihara and Takahashi, 1983), showing the level of 6.8-7.0 g/100 ml, when the sheep were fed hay with oral casein supplements (10-30% N of dietary N), although the values shown in Table 3 were within a range accepted generally (Harper, 1975). The level of plasma total protein in this experiment was rather in good agreement with that when the sheep were fed hay diet alone (5.7-6.3 g/100 ml) described in the previous papers (Fujihara, 1980; Fujihara and Ohshima, 1980; 1982).

From the results obtained in this study, it might be concluded that the urea orally administered by drenching to sheep fed a hay diet will not always be used effectively for stimulating ruminal fermentation, although it could clearly play an important role as a recycled nitrogen source in the metabolism of ruminants.

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