

Quantitative Aspects of Appearance of Re-cycled Urea in the Digestive Tract of Goats

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In domestic ruminants, the rumen has been assumed to be the principal site of appearance of re-cycled urea in the digestive tract. This assumption has been supported by the results of experiments which demonstrated that a large quantity of urea is transferred to the rumen^{4,8}). Moreover two pathways, salivary secretion and direct diffusion through the rumen wall, have been considered to be involved in appearance of blood urea in the rumen¹²). Juhasz⁸) and Houpt and Houpt⁴) suggested that direct diffusion through the rumen wall was the principal pathway. Nolan and Leng¹⁰) found that the amount of re-cycled urea that appeared in the rumen was approximately 20% of the total amount of re-cycled urea that appeared in the digestive tract. They suggested that the lower part of the digestive tract was an important site of decomposition of blood urea. Furthermore, they suggested that the main pathway for the appearance of urea in the rumen was not direct diffusion through the rumen wall, but salivary secretion. The latter finding was not in agreement with those of other workers.

The present investigation was designed to elucidate first the significance of the rumen in the utilization of re-cycled urea in goats fed on a normal-, low-, and high-protein diet and secondly the quantitative relationship between salivary secretion and diffusion through the rumen wall at the time of appearance of urea in the rumen. This work was published elsewhere by dividing it into two parts, one related to a low-protein ration¹¹) and the other related to a high-protein ration¹³). In

the present paper, consideration is given on changes of urea metabolism accompanied with the amount of nitrogen intake in the domestic ruminant.

Materials and methods

1) *Experimental animals and diets*

Six female goats were used, each of which had an oesophageal and rumen fistula. They were fed on four rations (ration nos. 1, 2, 3, and 4) once daily at 16:00 and given water ad lib. According to the feeding schedule shown in Table 1, each ration was given for 4 weeks. The chemical composition of ration nos. 1, 2, 3, and 4 is also shown in Table 1. The four rations contained hay, soybean meal, and different proportions of two home-prepared feeds (A and B), details of which are shown in Table 2.

2) *Design of experiment*

Each experiment using two goats was carried out in the period of 17–24 hr after feeding. The animal was placed in a metabolism cage and the oesophageal fistula was removed for saliva collection. The amount of saliva secreted was measured at the hourly interval using a graduated cylinder. A 5 ml portion of each hourly saliva sample was used for analysis and the remainder was returned to the rumen at a given interval. At the same time a single dose of 300–500 mg ¹⁵N-urea (90 Atom%) was injected into the jugular vein. Changes in the concentration of ¹⁵N-urea in jugular blood and ¹⁵NH₃ in the rumen

Table 1. Composition of rations and chemical composition of the constituents of rations fed to goats

(unit: g/kg body weight per day)

Composition	Ration 1	Ration 2	Ration 3	Ration 4
Hay	7.5	7.5	7.5	7.5
Formula feed B	12.0	6.0	—	—
Formula feed A	—	6.0	12.0	6.0
Soybean meal	—	—	—	6.0
Total digestible nutrient	11.1	11.4	11.4	11.4
Digestible crude protein	0.6	1.2	1.9	3.5

Chemical composition	Hay	Formula feed		Soybean meal
		B	A	
Moisture	15.1	15.1	11.0	11.9
Crude protein	8.4	7.4	18.6	46.2
Crude fat	2.3	2.9	4.0	1.3
N-free extract	40.2	59.7	56.8	29.6
Crude fiber	26.7	6.3	4.5	5.0
Crude ash	6.4	8.6	5.1	6.0
Total digestible nutrient	38.0	69.0	71.3	70.9
Digestible crude protein	2.8	3.2	13.7	40.7

(unit: %)

Table 2. Ingredient composition of formula feed

Formula feed A		Formula feed B	
Ingredient	%	Ingredient	%
Soybean meal	10.0	Corn	18.9
Linseed meal	10.0	Molasses	7.6
Barley	25.0	Beat pulp	47.4
Corn	16.0	Corn starch	18.9
Wheat bran	25.0	Soybean oil	1.9
Rice bran	10.0	Mineral mixture ¹⁾	3.8
Calcium carbonate	2.5	Sodium bicarbonate	1.0
Sodium chloride	1.0	Potassium bicarbonate	0.5
Mineral mixture ¹⁾	0.4	Vitamin E supplement ²⁾	0.06
Vitamin A and D mixture ³⁾	0.1	Vitamin A and D mixture ³⁾	0.02

1) : Consisting of 660 mg of Fe, 132 mg of Cu, 132 mg of Co, 132 mg of Mg, 330 mg of Mn, 469 mg of S, 761 mg of Ca, 225 mg of P and 965 mg of NaCl per 100 kg of mixture.

2) : Containing 100 I. U. of vitamin E per gram of supplement.

3) : Containing 1,000 I. U. of vitamin A and 2,000 I. U. of vitamin D per gram of mixture.

were examined at 10 min, 0.5, 1, 2, 3, 5, and 7 hr after injection. After 1 week, goats were injected simultaneously with a single dose of 250–300 mg $^{15}\text{NH}_4\text{Cl}$ (95–96 Atom%) into the rumen. Changes in the concentrations of ^{15}N -urea in jugular blood and $^{15}\text{NH}_3$ in the rumen were examined at the previously-mentioned intervals after injection. For urine

analysis a 7 hr total urine sample was collected into toluene.

3) Chemical analysis

Urea-N in serum, urine and saliva was estimated by a modified diacetyl-monoxime method¹⁾. Ammonia in rumen fluid was measured by a colorimetric method¹⁶⁾. The

determination of ^{15}N in the urea of serum, urine and saliva and NH_3 of rumen content was carried out by the method reported by Obara and Shimbayashi¹¹⁾.

A ^{15}N -analyzer (model NIA-1; Nippon Bunko Co., Ltd. Tokyo) was used for estimation of ^{15}N .

4) Mathematical procedures

The results of the ^{15}N tracer experiment were obtained by mathematic treatment of experimental values using the method reported by Nolan and Leng¹⁰⁾. The pool size and the irreversible loss rate with respect to the pool into which an isotope was injected were calculated from equations for the curves by standard procedure¹⁰⁾. The rate of flow of nitrogen associated with rumen fluid NH_3 and the serum urea pool was obtained by solving a general two-pool open-compartment model reported by Obara and Shimbayashi¹¹⁾.

Results

Measurement of urea and ammonia metabolism determined in goats fed on normal-, low-, and high-protein diets are shown in Table 3. Parameters in urea metabolism are indicated per body weight. The serum urea level was 6.3, 10.9, 17.2, and 36.0 mgN/dl in the groups given ration nos. 1, 2, 3, and 4, respectively. The serum urea level was in proportion to the amounts of nitrogen ingested. The urea pool size in the body and the irreversible loss of urea increased in

proportion to the serum urea level. The amount of urea excreted into the urine was 1.0 mgN/kg/hr when the serum urea level was 6.3 mgN/dl. The amount of urea ex-

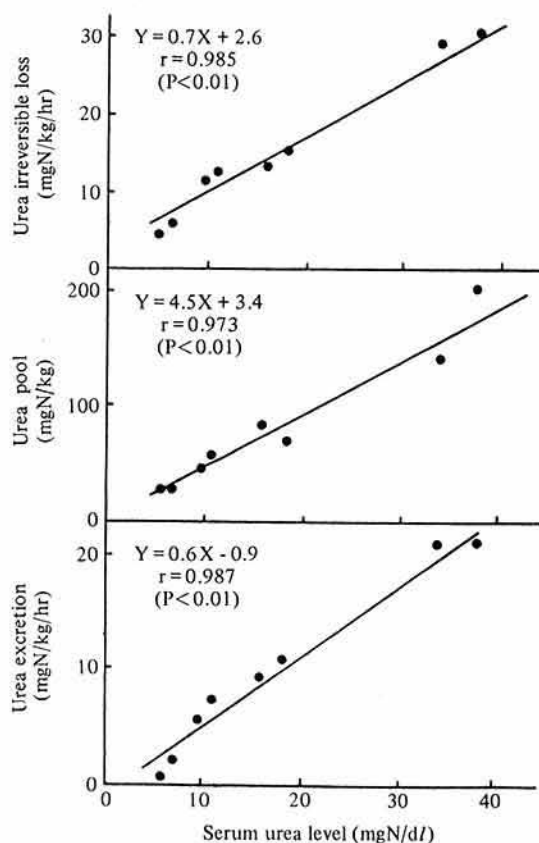


Fig. 1. Relationship between serum urea level and urea pool, urea irreversible loss, or urea excretion rate

Table 3. Measures of urea and ammonia metabolism in goats

	Ration 1	Ration 2	Ration 3	Ration 4
Animal No.	11, 12,	11, 12,	13, 14,	15, 16,
Body weight (kg)	30.5±0.5	30.0±0.5	29.3±0.3	29.3±3.8
Serum urea level (mgN/dl)	6.3±0.6	10.8±1.0	17.2±1.1	36.0±2.1
Saliva urea level (mgN/dl)	3.9±0.5	6.6±0.4	11.3±1.6	22.5±0.5
Rumen NH_3 level (mgN/dl)	5.0±1.0	10.9±0.5	22.3±2.7	32.8±1.9
Body urea pool size (mgN/kg)	30.7±2.1	54.2±7.9	78.1±7.4	169.9±51.3
Rumen NH_3 pool size (mgN/kg)	9.7±0.9	23.8±2.7	31.2±9.0	57.5±7.1
Urea irreversible loss (mgN/kg/hr)	4.9±0.5	11.8±0.5	13.9±0.9	29.6±0.4
Rumen NH_3 irreversible loss (mgN/kg/hr)	10.5±2.4	15.2±3.5	11.4±1.6	19.9±0.5
Urea excretion (mgN/kg/hr)	1.0±0.9	6.4±0.8	9.9±0.7	21.0±2.9

Average value±standard error.

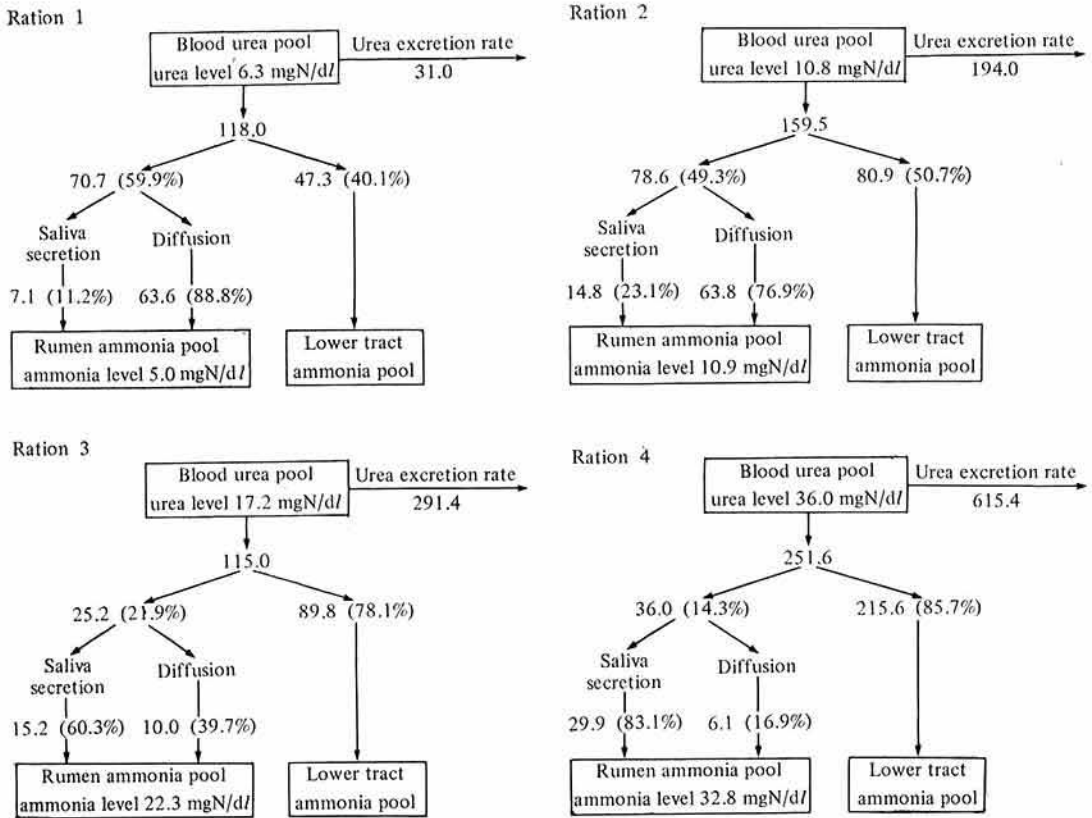


Fig. 2. A model of nitrogen metabolism in goats showing the movement of nitrogen (mgN/hr) between the three nitrogenous pools in the body

creted increased markedly to 21.0 mgN/kg/hr when the serum urea level was increased to 36.0 mgN/dl. The salivary urea level was 60.6–65.7% of the serum urea level in each experimental group. The ruminal NH_3 level was in proportion to the serum urea level. The ruminal NH_3 pool size and ruminal NH_3 irreversible loss were in proportion to the rumen ammonia level in general.

The relationship between the serum urea level and the serum irreversible loss, the serum urea pool size or the urea excretion by the urine is shown in Fig. 1. There are very high correlation between the serum urea level and the body urea pool size ($r=0.973$), the urea irreversible loss ($r=0.985$), and the urea excretion rate by urine ($r=0.987$). Namely, the observation indicates that the parameters of urea metabolism in-

creased in proportion to the increase of serum urea level.

The amounts of nitrogen moving among the rumen, blood, and lower part of the digestive tract were estimated and presented in Fig. 2. The amount of nitrogen transferred from the serum urea pool to the rumen NH_3 pool was 70.7, 78.6, 25.2 and 36.0 mgN/hr in the groups given ration nos. 1, 2, 3, and 4, respectively. The amount of nitrogen transferred to the whole digestive tract was 118.0, 159.5, 115.0 and 251.6 mgN/hr in the groups given ration nos. 1, 2, 3, and 4, respectively. The rate of blood urea transferred to the rumen NH_3 pool relative to that transferred to the NH_3 pool of the whole digestive tract was 59.9, 49.3, 21.9, and 14.3% in the groups given ration nos. 1, 2, 3, and 4, respectively. On the other hand, the quantitative ratio of

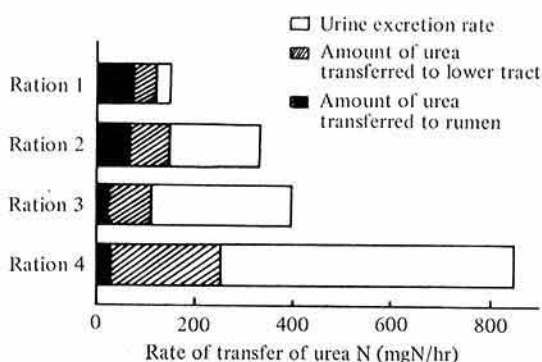


Fig. 3. Rate of transfer of serum urea nitrogen

salivary urea secretion to the amount of blood urea transferred to the rumen NH_3 pool was 11.2, 23.1, 60.3, and 83.1% in the given ration nos. 1, 2, 3, and 4, respectively. Therefore, it was demonstrated that the rate of diffusion in the rumen wall was much higher in goats of which the blood urea level was low (given ration nos. 1 and 2), and conversely, salivary secretion was higher in goats with the high blood urea level (given ration nos. 3 and 4).

Fig. 3 shows the rate of transfer of serum urea to the lower tract, rumen, and urine in each feeding group. As shown in the figure, the amount of urea excreted by urine was suppressed and more than 80% of the blood urea was transferred to the whole digestive tract when the animals were fed on the ration 1 and their blood urea level was 6.3 mgN/dl. However, when the ration 3 as a normal-protein ration and the ration 4 as a high-protein ration were supplied, the amount of blood urea transferred to the whole digestive tract was below 30%, showing the large amount of urea excreted by urine. The amount of blood urea transferred to the rumen relative to that transferred to the whole digestive tract trends to decrease as protein intake increases. From the findings above mentioned, it is thought that feeding on the low protein diet such as the rations 1 and 2 is appropriate, because urea-recycling mechanism can be utilized sufficiently.

Discussion

The present experiment was carried out with goats fed normal-, low-, and high-protein rations. From the experimental results, each parameter in urea metabolism will be discussed when the level of DCP is varied under the condition of constant level of TDN ingested. The serum urea level was closely associated with protein intake in each feeding treatment in which energy requirement was thought to be satisfied. Very high correlation was found out between the serum urea level and the body urea pool size, the urea irreversible loss, or the urea excretion rate in the present experiment. It was indicated by some investigators^{2,5,6,7,9}) that the relationship between the plasma urea level and each parameter in urea metabolism was linearly and positively related to the level of dietary nitrogen intake. These observations indicate that the parameters of urea metabolism increased according to the increase of the serum urea level. Also it is suggested that if energy intake is constant, the urea pool, irreversible loss, and urea excretion rate might be presumed from the serum urea level.

As indicated in Fig. 2 it was estimated that the relative transfer of urea to the rumen was 59.9, 49.3, 21.6, and 14.3% of the amount of urea appearing in the whole digestive tract when the serum urea level was 6.3, 10.6, 17.2, and 36.0 mgN/dl, respectively. Houpt³) estimated that the rate of blood urea transferred to the rumen was 40–67% of that of the whole digestive tract. Nolan and Leng¹⁰) pointed out that the lower part of the digestive tract played a more important role than the rumen in the utilization of re-cycled urea. According to them, the rate of urea transferred into the rumen was less than 20% of that of the whole digestive tract. These results have raised controversy, since they are not in agreement with the hypothesis of Waldo¹⁵) that the decomposition of urea may take place in the rumen and the results previously reported that a large amount of urea transferred to the rumen^{4,8}). It should be noted that Nolan and Leng's result¹⁰) was

obtained from the animal in which the plasma urea level was as high as 22.5 mgN/dl. In the present experiment, the rate was 21.9% when the serum urea level was 17.2 mgN/dl. This result agreed quite well with that of their experiment.

The results in the present experiment pointed out that in ruminants given a low-protein ration re-cycled urea was presumed to be utilized in the rumen. However, in ruminant which had ingested a sufficient or excessive amount of nitrogen, the rumen was assumed to be replaced as the site of appearance of re-cycled urea by the lower part of digestive tract. Urea re-cycling in domestic ruminants seems to vary greatly with the amount of nitrogen ingested and the serum urea level.

Authors¹²⁾ have already carried out the experiment to confirm the pathway of transfer of blood urea into the rumen. Fig. 4 shows a schematic drawing of the experimental arrangement of the animal and the equipment. The rumen contents were withdrawn as much

as possible through the rumen fistula and reticulo-omasal orifice was plugged. Artificial ruminal solution was introduced into the empty rumen and mixed saliva secreted from salivary gland was collected by the intraoesophageal funnel. Then, a single injection of ^{15}N -urea was carried out intravenously. And appearance of ^{15}N in saliva and rumen content was observed. ^{15}N ammonia in the ruminal fluid increased until 2 hr after injection and then decreased. ^{15}N -urea of saliva showed 4 atom % excess in the first one hour and then decreased gradually. It was shown in this experiment that the pathways of transfer of blood urea into the rumen were the salivary secretion and the direct diffusion through the rumen wall.

Since the present authors could actually measure the amount of urea discharged into the saliva, the quantitative relationship between the amount of the two urea fractions appearing in the rumen could be elucidated. The two fractions were the urea discharged in and transferred by the saliva to this or-

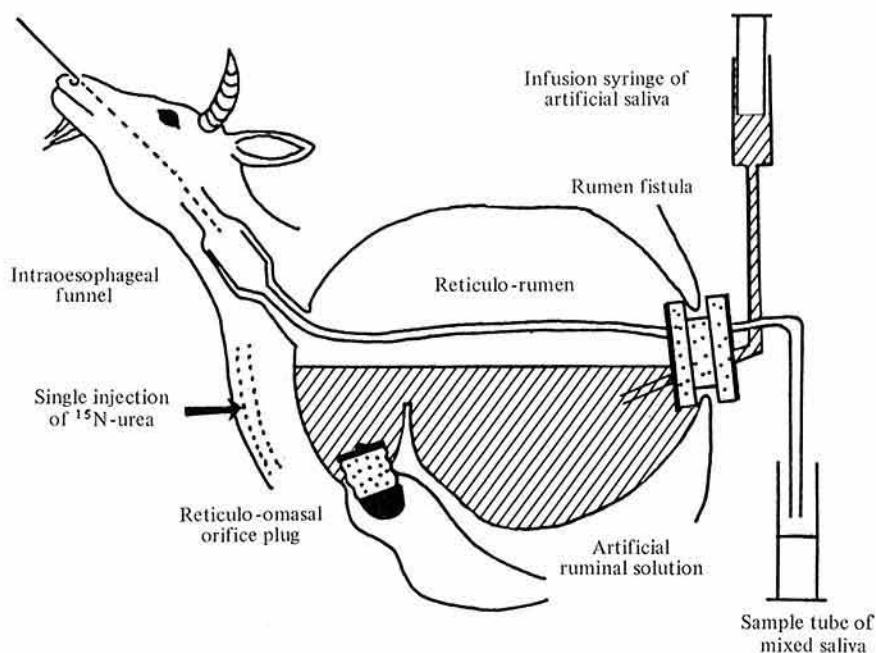


Fig. 4. Schematic drawing of the experimental arrangement of the animal and the equipment

gan and urea diffused in the rumen wall. As a result, the quantitative ratio of salivary urea secretion to the rate of transfer of blood urea to ruminal NH_3 was 11.2, 23.1, 60.3 and 83.1%, when the serum urea level was 6.3, 10.6, 17.2 and 36.0 mgN/dl, respectively. It was assumed that the diffusion in the rumen wall might be a principal pathway involved in the appearance of urea in the rumen of goats fed a low-protein ration. However, it was estimated that more than 80% of the amount of urea appearing in the rumen in goats fed a high protein ration might have been derived from urea discharged in saliva. These results reveal an outstanding fact; that is, the serum urea level varies with the amount of nitrogen ingested, and the reverse occurs in the ratio of salivary secretion vs. urea diffusion in the rumen. Further experiments may be necessary before the mechanism of reversion of this ratio is clarified.

The parameter of urea metabolism in domestic ruminants may be influenced not only by the content of nitrogen but also by the quality of carbohydrate and other nutrients of food contained in the rumen at the time. An improvement in feeding techniques seems to be required to make the rumen display its function sufficiently and to economize the nitrogen content of a ration.

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(Received for publication, October 16, 1987)