Some Aspects of Urea Feeding in Ruminants

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A large amount of urea is held in the mammalian body. Especially, in the body of the ruminant, urea is circulated again by way of saliva and the digestive tract[8]. Such being the case, urea is not a foreign body for the ruminant. Nowadays, such discussion is made on the carcinogenicity and teratogenicity of feed additives. It is assumed, however, that urea may be perfectly free from these properties. Nevertheless, if urea is fed in an inadequate manner of feeding, urea poisoning, or ammonia poisoning, will be induced and occasionally be fatal to the animal. Many suggestions have been made to avoid an accident by a urea diet and to raise healthy animals economically.

We have carried out biochemical and physiological studies to solve basic problems directly and indirectly related to the utilization of urea. The subject of our discussion is the safety and efficiency of utilization of urea.

Ammonia in rumen fluid and ammonia and urea in blood of sheep fed a urea diet

Sheep to which a rumen fistula was attached were fed commercial formula feed and hay at first and then a home-combined low protein diet. The amount of urea to be added was increased by 1% every two weeks. This experiment was performed to study the adaptation of sheep to urea. The blood level of urea increased almost in proportion to an increase in the amount of urea added. These findings are supported by the following fact. Ide et al. demonstrated in dairy cows and goats that when the amount of energy was enough to meet the requirement, the blood level of urea was almost in proportion to the intake of protein[1,2]. The observation was made on the relationship between the ammonia level of the rumen fluid and the ammonia and urea levels of the blood in sheep fed a diet with addition of 5 to 11% of urea (Fig. 1). When 5% of urea was added, the urea level increased soon after the increase in the ammonia level of the rumen fluid. On the other hand, ammonia did not reach such a blood level as to cause

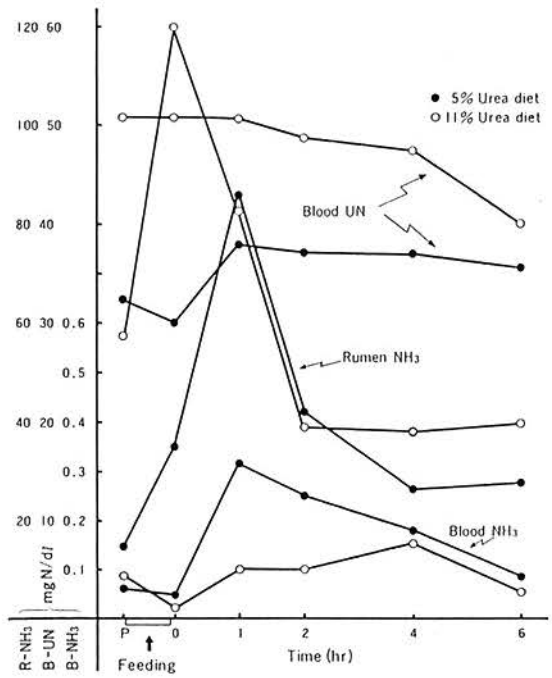

Fig. 1. Ammonia in rumen fluid and ammonia and urea in blood of sheep fed a urea diet

toxic signs, but attained a relatively high blood level. Then, when 11% of urea was added, the blood level of ammonia increased a little 4 hr after ingestion of feed. Since the blood level of urea was low at that time, it was presumed that such increase in the blood level of ammonia might have been induced by a transient reaction to a mild decrease in urea synthesis.

Urea-cycle enzymes of ruminants

The urea cycle enzymes reduce the ammonia concentration to play a role in inhibiting the occurrence of intoxication. They are not so much higher in activity in the goat liver than in the rat liver. As was demonstrated by Schimke in rats[11], their activity increased with an increase in the intake of protein, and was especially clearly demonstrated by the addition of urea to the diet (Tables 1 and 2). Such increase is regarded as a phenomenon of natural adaptation to prevent animals from intoxication with ammonia in the body. Further, as it has been reported that vitamin E may reduce the concentration of ammonia in the blood, studies were made on the effect of vitamin E upon the activity of the urea cycle enzymes. As it is difficult to prepare any experimental diet for ruminants, an experiment was carried out with rats. As a result, it was clarified that carbamyl phosphate synthetase and ornithine carbamoyltransferase (OCT) increased in activity (Table 3). Therefore, it is presumed that vitamin E may serve to eliminate ammonia intoxication, a type of stress. As shown in Table 4, it was elucidated that ammonia exerted a reverse effect upon OCT and upon ornithine ketoacid aminotransferase (OAT)[4], which is assumed to interfere with ornithine. Namely, the activity of OCT was stimulated and that of OAT inhibited by ammonia. When ammonia is produced by the decomposition of urea in the rumen and taken into the liver, its concentration increases transistorily in the liver and it inhibits isocitric dehydrogenase in the TCA cycle and reduces the production of α-ketoglutaric acid. According to Katsunuma[3], a high concentration of ammonia inhibits the reaction in which aspartic acid is converted to glutamic acid, but hardly inhibits the reaction in which glutamic acid is converted to aspartic acid as substrate of the urea cycle enzymes. At the same time ammonia inhibits OAT, as well as carbamyl phosphate does. Therefore, the consumption of ornithine is

Table 1. Chemical composition of diet

Composition	Diet	
	A	C
Moisture	13.2%	12.9%
Crude protein	8.9	20.9
Ether extract	1.6	1.1
Crude fiber	11.0	11.8
N-free extract	61.5	48.3
Crude ash	3.8	5.1

Table 2. Effect of protein level in diet on hepatic activities of urea cycle enzymes

Diet	Serum urea-N (mg/dl)	Liver NH_3-N (mg/100g wet)	Carbamyl phosphate synthetase (units/g)	Ornithine carbamoyl-transferase (units/g)	Arginine synthetase (units/g)	Arginino-succinate cleavage enzyme (units/g)	Arginase (units/g)
A	5.5 ±0.5	22.3 ±6.5	247 ±65	11,860 ±2,283	50 ±11	100 ±13	7,573 ±2,191
A+2% urea	8.2 ±0.2		302 ±97	17,070 ±6,269	99 ±30	166 ±49	8,410 ±829
C	35.6 ±8.5	13.1 ±1.3	283 ±45	15,760 ±3,561	112 ±12	196 ±12	9,959 ±3,912

One unit is defined as one μ mole of product per hour.

Table 3. Effect of vitamin E on hepatic activities of urea cycle enzymes

Treatment	Carbamyl phosphate synthetase (unit)	Ornithine carbamoyl-transferase (unit)	Arginin synthetase (unit)	Arginino-succinate-cleavage enzyme (unit)	Arginase (unit)
Basal diet	1,190 ±770	9,330 ±1,980	73.3 ±9.9	515 ±61	22,300 ±11,100
Basal diet +dl-α-tocopheryl acetate, 100 mg/kg diet	2,020** ±790	17,700* ±7,300	61.3 ±31.1	582 ±83	21,700 ±5,700

Unit is expressed as μ mole per hr per g of the liver and the ± symbol indicates mean standard deviation.

Table 4. Comparison of property between ornithine-ketoacid aminotransferase and ornithine carbamoyltransferase

Ammonia final concentration (mole concn.)	Ornithine-ketoacid aminotransferase+				Ornithine carbamoyltransferase			
	Basal diet +dl-α-tocopheryl acetate (O.D.)	(%)	Basal diet (O.D.)	(%)	Basal diet +dl-α-tocopheryl acetate (unit)	(%)	Basal diet (unit)	(%)
0	26.1 ±14.8	100	31.6* ±19.4	100	17,700 ±7,300	100	9,300* ±1,900	100
5×10^{-2}M	25.1 ±14.2	96	30.0 ±17.9	95	23,000 ±9,400	130	12,600 ±2,300	136
1×10^{-1}M	18.8 ±11.6	72	22.8 ±13.5	72	26,600 ±10,900	150	15,000 ±2,600	161
2×10^{-1}M	13.1 ±7.2	54	17.1 ±9.5	54	18,900 ±7,700	107	10,200 ±1,900	111

+The value is expressed as the absorbancies of the solution derived by reacting pyrroline-5-carboxylate with o-aminobenzaldehyde and they are O. D. per hr per g of the liver.

reduced. On the other hand, the higher the concentration of ornithine, the stronger the activity of OCT. As a result, the urea cycle is intensified and urea production is accelerated.

Relationship between the amount of addition of urea and the components of rumen fluid and blood

A urea diet was prepared from the low protein basal diet shown in Table 5 by adding 3 or 5% of urea. In a feeding experiment, it was given to sheep with hay. In the sheep fed 3 and 5% of urea, the maximal content of ammonia was about 40 and 60 mg/dl, respectively[6]. The increase in this content was not so marked in the sheep fed 3% of urea as in those fed a conventional diet. In the sheep fed 5% of urea, the blood level of ammonia was less than 0.2 mg/dl. On the other hand, there was no distinct difference in the concentration of VFA between the sheep fed 3 and 5% of urea. The VFA concentration, however, was higher in the sheep fed the urea diet than in those fed the basal diet containing no urea. This is probably because the increase in ammonia concentration stimulated the digestion of cellulose and starch by microorganisms and the production of VFA by fermentation. The blood level of urea was almost the same in animals fed the diet containing 3% of urea as in those fed

Table 5. Feeding schedule for animals

(unit: g/day)

Type of feeding	C	U_0	U_3	U_5
Hay	600	300	300	300
Commercial formula feed	300	—	—	—
Experimental formula feed	—	480	480	480
Urea	—	0	14.4	24
TDN	442.2	445.0	445.0	445.0
DCP	57.9	24.0	54.0	74.0
CP	99.9	60.7	102.8	130.8

Table 6. Concentration of plasma free amino acids under various feeding

Amino acid	U_0 (mg/dl)	U_3 (mg/dl)	U_5 (mg/dl)
Lys	3.85±0.38	3.42±0.43	3.63±0.95
His	1.03±0.20	1.56±0.25	1.62±0.19
Arg	1.27±0.18	1.54±0.20	1.22±0.18
Asp	0.31±0.13	0.37±0.04	0.29±0.03
Thr	1.06±0.48	1.34±0.28	1.30±0.27
Ser	1.57±0.54	2.16±0.29	1.95±0.39
Glu	1.38±0.22	1.22±0.26	1.16±0.23
Cit	2.02±0.20	2.86±0.35	2.99±0.34
Gly	5.85±1.22	6.65±1.26	7.66±2.23
Ala	2.67±0.44	1.89±0.22	1.87±0.52
Cys	0.42±0.07	0.38±0.06	0.26±0.05
Val	1.80±0.21	1.79±0.30	1.58±0.16
Met	0.15±0.02	0.17±0.02	0.12±0.03
Ile	0.93±0.08	0.92±0.12	0.80±0.10
Leu	1.38±0.19	1.20±0.12	1.02±0.12
Tyr	1.53±0.09	0.76±0.12	0.65±0.09
Phe	0.75±0.16	0.60±0.07	0.52±0.07

the conventional diet. Therefore, it is presumed that sheep fed the diet containing 3% of urea may be closer to the nutritional conditions of sheep fed the conventional diet in a standard manner than those fed the diet containing 5% of urea.

Virtanen[16] demonstrated that the blood level of free amino acids was low in ruminants fed a diet in which urea was the sole source of nitrogen. In our present experiment[14], the total free amino acids were also very small (Table 6). They increased a little in accordance with an increase in the amount of addition of urea to the diet. The basal diet showed a very low ratio of essential amino acids to nonessential amino acids. In general, when there is a decrease in the amount of protein contained in the diet, there is a reduction in the ratio of EAA to NEAA. When urea was added to it, this ratio became much lower. The decrease of the ratio is presumed to have been induced by the decrease in the amount of EAA and by the enhancement of synthesis of NEAA due to the addition of urea to the diet, while it is natural that not only EAA but also NEAA is used for the synthesis of protein. Like other investigators, we found that of the free amino acids, glycine had shown a high blood level in animals fed a low protein diet. The blood level of glycine was

Table 7. Time course changes in incorporation rate of ^{15}N into amino acids from hydrolyzates of protozoal fraction

Amino acid	Incorporation rate at incubation time indicated below (hr)			
	3	6	12	24
		atom% ^{15}N excess		
Lys	3.90	6.45	7.25	8.31
His	2.73	4.37	5.05	5.80
Arg	3.80	5.35	5.85	6.81
Asp	4.78	8.12	8.79	10.12
Thr	4.00	6.55	7.29	8.39
Ser	4.05	6.81	7.60	8.59
Glu	5.05	8.43	9.57	10.92
Pro	4.52	3.31	1.40	3.15
Gly+Ala	5.40	8.75	9.89	11.34
Cys	1.22	1.78	2.13	2.35
Val	4.43	7.32	8.13	9.17
DAPA	3.42	5.59	6.42	7.19
Met	1.37	2.31	2.52	2.85
Ile	4.43	7.37	8.21	9.28
Leu	4.20	6.95	7.70	8.73
Tyr	3.28	5.89	6.55	7.41
Phe	3.25	5.42	6.03	6.80

Glycine could not be separated from alanine by this column chromatography, so that combined fraction of both amino acids was used for ^{15}N analysis.

especially high in the present experiment. Oltjen[9] reported that plasma glycine had increased in concentration in animals fed a diet containing urea. Accordingly, it is assumed that an increase in glycine concentration indicated that amino acid nutrition does not reach an optimal condition as yet.

In an experiment *in vitro* on rumen fermentation, estimation was made on the incorporation of urea-^{15}N into each amino acid of the hydrolyzate of protozoal fraction (Table 7). This incorporation rate was different from one amino acid to another[15]. It was very small in such sulfur-containing amino acids as cystine and methionine. Since a sufficient amount of sulfur compounds were contained in the culture fluid used, it was made clear that a smaller amount of urea-nitrogen had been used for the synthesis of sulfur-containing amino acids than for that of the other amino acids. This finding suggests that it may be effective to add not only inorganic sulfur but also methionine to a diet.

Caprylohydroxamic acid as urease inhibitor

It is necessary to discuss the fermentation, including the ammonia production, in the rumen before urea is used safely as feed. The experiment was focused on urease of microorganisms in the rumen. It is known that the ingestion of urea feed brings about changes in the rumen microflora. According to Virtanen[16], protozoa disappear and bacteria increase in the rumen microflora after the conventional feed has been switched to an artificial feed containing ammonium sulfate. Yamoor[18] pointed out a decrease in urease activity of microorganisms themselves after urea feeding. It is presumed that the rumen microflora may begin to change in composition after the ingestion of urea feed and adapt itself to this feed by a decrease in activity of the whole enzymatic system controlling the production of ammonia. Studies were made on specific inhibitors against

Table 8. Concentration of ammonia at various levels of caprylohydroxamic acid (CHA) added

Group	CHA	Sampling time			
	mg/kg of body weight	p	0	1	3
Control	0	28.49 ±7.47	61.39 ±2.39	47.85 ±1.19	42.89 ±9.35
CHA	10	6.50 ±0.73	11.77 ±0.61	12.41 ±0.67	7.45 ±0.71
	15	16.86 ±1.54	20.16 ±0.41	17.85 ±1.83	15.99 ±4.07
	20	13.71 ±2.95	15.38 ±0.65	13.84 ±0.96	13.24 ±1.32

All the values are expressed as NH_3-N mg/d*l* in the rumen.

urease. In them, various substances, including chlortetracycline, copper, and barbituric acid, were examined with no marked results. Kobashi and Hase found that hydroxamic acids inhibited urease[5]. Of the many hydroxamic acids, caprylohydroxamic acid (CHA) has been reported to be the most effective. So, we examined CHA for inhibitory effect upon ruminal urease. Moreover, we tried to determine what influence CHA exerted upon the production of ammonia in the rumen fluid of a sheep given urea feed. Rumen urease had a property similar to that of urease of vegetable origin and displayed essentially the same inhibitory effect ($Km=6.3\times10^{-3}$ M) upon rumen urease[12].

When the concentration of CHA surpassed 10^{-4} M, the inhibitory effect became suddenly strong. The inhibition by CHA was parallel to the pH curve of urease activity. Therefore, the stronger urease activity, the higher the degree of inhibition. As the optimal pH of urease was about 8.6, it was revealed that the higher the pH value, the stronger the inhibitory effect. In animals fed a urea diet, the rumen fluid was in bad condition when its pH value went further on the alkaline side. It is expected that CHA will display its effect under such conditions. When the urea diet was mixed with 5 mg of CHA per kg of body weight, urease activity was reduced. Usually, the rapid production of ammonia was seen after the ingestion of a urea diet. It was, however, not so marked in animals fed the diet mixed with CHA (Table 8). It has been demonstrated that CHA exerts no influence upon the production of VFA in the rumen or upon metabolism of any other amino acid[13].

Urea poisoning

Omori and Sato[10] classified the clinical symptoms of urea poisoning into five grades. According to them, the blood level of ammonia ranged from 0.5 to 1.0 mg/d*l* in the stage of depression and fatigue, from 1.2 to 1.9 mg/d*l* at the beginning of the stage of convulsion, and from 4.0 to 7.0 mg/d*l* at the end of this stage. Damage of the brain-stem and paralysis of the respiration center have been pointed out as lethal mechanisms in ammonia poisoning. Yoshida[19] reported on the prevention of urea poisoning that the effect of molasses added to a diet consisted of the action of this substance to reduce the pH value of the rumen fluid. Word[17] mentioned that administration with acetic acid was effective for the prevention and treatment of urea poisoning.

Obara and Shimbayashi[7] found that urea infusion brought about a marked decrease in secretion of saliva. As shown in Fig. 2, when 0.4 to 0.5 g of urea/kg of body weight was infused, saliva secretion was inhibited remarkably. Another experiment was carried

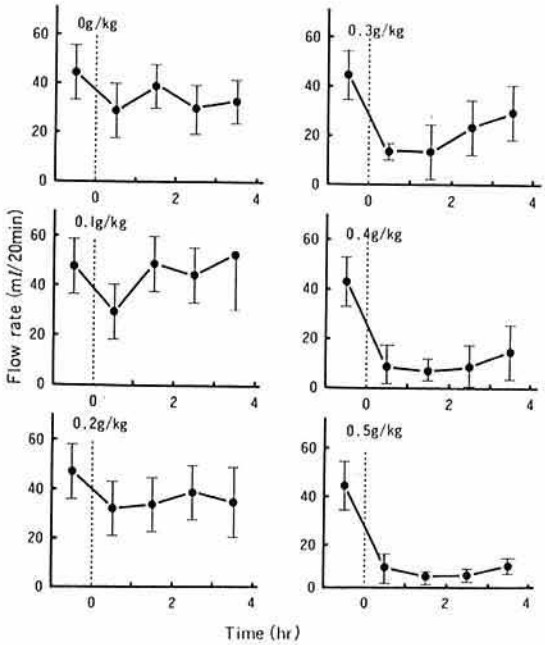

Fig. 2. Changes in salivary secretion (m*l*/20 min) from the unilateral parotid gland of sheep after intraruminal injection of urea dosed (g/kg)
Each point represents the mean value and standard deviations (vertical bars) of the amounts of saliva measured in three sheep. Urea was injected into the rumen at 0 hr.

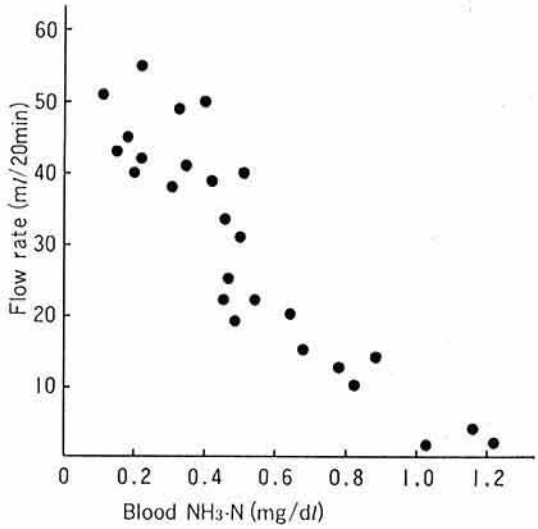

Fig. 3. Relationship between the amount of salivary secretion (m*l*/20 min) and the ammonia concentration of jugular blood of sheep after injection of urea into the rumen ($r=0.889$, $n=26$, $P<0.01$)

out to determine whether this inhibition of saliva secretion was induced by urea infusion or by ammonia produced from urea by decomposition and absorbed. As a result, it was elucidated that saliva decreased suddenly in the amount of secretion when the blood level of ammonia exceeded 0.4 mg/d*l* (Fig. 3). By the way, the concentration 0.4 mg/d*l* mentioned above is the blood level of ammonia regarded by Omori as that which gave rise to early symptoms of the amphibolic state.

Judging from our results, many suggestions which have been made to avoid an accident by a urea diet and raise healthy animals economically are reasonable. They are summarized:

(1) A diet should contain an appropriate amount of starch and the like as a readily usable source of energy. Addition of molasses is effective for the prevention of urea poisoning.

(2) A diet should contain sufficient amounts of phosphorus and calcium and such an amount of sulfur as not less than one-fifteenth the amount of nitrogen. Appropriate amounts of trace elements, especially cobalt and zinc, should be added to it.

(3) Alfalfa meal should be added to a diet as the source of supply of an unknown factor of protein synthesis.

(4) To improve palatability of a diet, sodium chloride with an addition of iodine should be added to the diet at the rate of 3–4%.

(5) Appropriate amounts of vitamin A, E, and some antibiotic (or caprylohydroxamic acid) should be added to a diet.

(6) A low energy and high cellulose diet should be avoided.

(7) Pure protein should be contained in a diet to some extent until a completely-balanced diet is found definitely.

(8) If a diet contains too much protein readily usable, the usable rate of urea will be reduced.

(9) A daily amount of feeding of a urea

diet should be fixed. No excessive amount of the urea diet should be fed.

(10) A urea diet should be used gradually when it replaces the conventional diet.

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(Received for publication, November 2, 1987)