STUDIES ON THE METABOLISM OF NITROGENOUS COMPOUNDS IN THE RUMEN II. WEIGHT CHANGE OF THE NITROGEN DISTRIBUTION IN THE RUMEN OF THE SHEEP UNDER THE RETICULO-OMASAL ORIFICE PLUGGING

STUDIES ON THE METABOLISM OF NITROGENOUS COMPOUNDS IN THE RUMEN
II. WEIGHT CHANGE OF THE NITROGEN DISTRIBUTION IN THE RUMEN OF THE SHEEP UNDER THE RETICULO-OMASAL ORIFICE PLUGGING

By

Yasukuni WATANABE and Motoyoshi UMEZU

Department of Animal Hucsbndry, Faculty of Agriculture, Tohoku University, Sendai Japan

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In a previous paper we reported on the method of weighing the whole rumen contents and the sampling of the rumen contents, under the reticulo-omasal orifice plugging condition (1). By these procedures, the weight change of the rumen contents and sampling could be performed under preventation of the flow of the rumen contents into the omasum.

The experiments described here provide the weight change of nitrogenous compounds in the rumen of the sheep under various feeding conditions by using previously mentioned methods.

Experimental

Animal. Observations were made on one sheep weighing 35 kg in body weight in the nine experiments and the sheep was fitted with cannula into the rumen by the procedures previously described (1).

Food. The experiments were performed on the sheep subjected to nine different feeding conditions. The foods used during these experiments were orchard hay in Expt. (A), orchard hay and soybean oil meal in Expt. (B), ladino clover green fodder in Expt. (C), corn ensilage in Expt. (D), soybean oil meal and rice bran in Expt. (E), ladino clover, soybean oil meal and rice bran in Expt. (F), ladino clover, soybean oil meal, rice bran and urea in Expt. (G), orchard hay and sweet potato in Expt. (H), orchard hay, sweet potato and urea in Expt. (I). The chemical composition of each foods is shown in Table 1.

Feeding. In all of the experiments each diet was offered once daily at 9.00 hours and the sheep were trained to eat quickly. The precise time spent was not measured, but the maximum time allowed was two hours. The sheep

Table 1. Chemical composition of experimental diets (per cent).

	Moisture	Crude protein	Ether extract	N.F.E.	Crude fiber	Ash
Orchard hay	12.5	6.4	2.6	32.1	38.9	6.5
Soybean oil meal	12.0	44.5	6.0	25.0	7.2	5.3
Rice bran	14.5	14.0	17.2	36.0	9.5	8.8
Ladino clover	82.0	3.6	0.8	7.0	5.6	1.0
Corn ensilage	75.0	1.5	0.8	11.0	10.1	1.6
Sweet potato	74.0	2.5	0.3	21.5	0.7	1.0

received each diet continuously for 10 days before sampling of the rumen contents. The intake weight of each foods during 2 hours is shown in Table 2.

Table 2. Compositition of experimental ration.

Expreimental ration / Ingredient	(A)	(B)	(C)	(D)	(E)	(F)	(G)	(H)	(I)
	(g)	(g)	(g)	(g)	(g)	(g)	(g)	(g)	(g)
Orchard hay	380	240	—	—	—	—	—	260	260
Soybean oil meal	—	300	—	—	300	180	180	—	—
Rice bran	—	—	—	—	250	270	270	—	—
Ladino clover	—	—	1500	—	—	1350	1350	—	—
Corn ensilage	—	—	—	1500	—	—	—	—	—
Sweet potato	—	—	—	—	—	—	—	1000	1000
Urea	—	—	—	—	—	—	13	—	13

Sampling of rumen contents. The whole rumen contents were removed, sampled and returned by the procedures previously described (1). In each experiments the weighing and sampling of the rumen contents were made at 2, 5, and 11 hours after the beginning of feeding. About 200g of the rumen contents were removed and prepared for analysis. Two hours after the beginning of feeding, the whole rumen contens were removed completely through the rumen fistula, and the reticulo-omasal orifice was plugged by the reticulo-omasal orifice plug, and the removed rumen contents were returned into the rumen. In each experimental periods of 9 hours the sheep was maintained in fasting and no water was given. After the sampling of 9 hours the reticulo-omasal orifice plug was removed.

Method for analysis. The methods for determination of the total nitrogen, non protein nitrogen and NH_3-nitrogen in the rumen contents were according to Pearson and Smith (2). Total nitrogen of the rumen contents was estimated by the Kjeldahl method. Non protein nitrogen was estimated as the total nitrogen in protein free filtrate by tungstate precipitate. NH_3-nitrogen was estimated by steam distillation of protein free fitrate from the tungstate precipitate. Protein

nitrogen was calculated as total nitrogen minus non protein nitrogen. Volatile fatty acids was estimated by the method of steam distillation technique of Friedmann (3). Gas production volume of the rumen contents was estimated by the syringe incubation method of Shibata *et al.* (4).

Results

Weight change of nitrogen distribution in the rumen.

Total nitrogen.

The weight change of the total nitrogen in the rumen at the various times after the feeding in nine different feeding conditions are shown in Table 3. The weight of total nitrogen decreased at 11 hours after the beginning of feeding in the rumen in every feeding condition, except for Expt. (A). The weight of decreasing ranged from 1.00 g in Expt. (H) to 9.58 g in Expt. (G). The higher concentration of the nitrogen in the foods showed the more decreasing of total nitrogen from the rumen. Particularly, the high protein food plus urea feeding showed the highest decrease of total nitrogen from the rumen. Urea was also provided in Expt. (I) but nitrogen decrease was only 1.67 g. Table 2 shows the slight increase of the total nitrogen Expt. (A), (B), (C) and (I) at 5 hours, and in Expt. (A) at 11 hours. This increase of total nitrogen is due to the inflow of salivary nitrogen into the rumen.

Table 3. The weight change of total nitrogen in the reticulo-rumen under reticulo-omasal orifice plugging

Time after beginning of feeding (hr)	Experimental ration (g and (%))								
	(A)	(B)	(C)	(D)	(E)	(F)	(G)	(H)	(I)
2	9.25	27.30	25.71	7.74	31.30	42.26	48.84	12.76	13.20
5	9.64 (104.2)	27.41 (100.4)	25.62 (99.6)	7.78 (100.5)	29.70 (94.9)	40.14 (95.0)	42.74 (87.5)	12.77 (100.1)	12.72 (96.4)
11	10.03 (108.4)	25.62 (93.8)	24.20 (94.1)	6.50 (84.0)	27.70 (88.5)	38.70 (91.6)	39.26 (80.4)	11.76 (92.2)	11.53 (87.3)

Protein nitrogen.

The weight change of the protein nitrogen is shown in Table 4. The protein nitrogen increased in Expt. (A) ,(D), (H) and (I), but decreased in Expt. (B), (C), (E), (F) and (G) at 11 hours after the beginning of feeding. Generally, the increase rate of the protein nitrogen in the rumen paralleled the rate of the total nitrogen in Expt. from (A) to (G). However, Expt. (H) and (I) showed an increase of the protein nitrogen in the rumen inspite of a decrease of the total nitrogen. It appeared that these foods indicated an excess volume

of resynthesis of bacterial body protein from the non protein nitrogen than the degradation volume of food protein from the rumen. Especially, in Expt. (I) of a low protein and urea feeding condition, the protein nitrogen increased 4.44 g in 9 hours. Slight increase of the protein nitrogen also appeared in Expt. (A) and (D), which were roughage only and very low in protein feeding condition. On the other hand, Expt. (B), (C), (E), (F) and (G) showed decreased protein nitrogen in the rumen. These experiments included soybean oil meal in the foods, that was high in protein feeding conditions, with the exception of Expt. (C) which was of flesh legume feeding. Especially, in Expt. (G) urea was added to Expt. (F) food, the protein decreasing rate in the rumen exceeded that of Expt. (F).

Table 4. The weight change of protein nitrogen in the reticulo-rumen under reticulo-omasal orifice plugging.

Time after beginning of feeding (hr)	Experimental ration (g and (%))								
	(A)	(B)	(C)	(D)	(E)	(F)	(G)	(H)	(I)
2	8.57	26.00	22.95	5.55	28.50	36.67	35.93	10.07	6.16
5	9.12	25.90	21.56	5.45	25.50	34.16	32.72	11.78	9.38
	(106.4)	(9.96)	(93.9)	(98.2)	(89.5)	(93.2)	(91.1)	(117.0)	(152.4)
11	9.41	23.60	21.25	5.60	23.60	33.53	30.46	11.37	10.60
	(109.8)	(90.8)	(92.7)	(100.9)	(82.8)	(91.4)	(84.8)	(112.9)	(172.2)

Non protein nitrogen.

The weight changes of the non protein nitrogen are shown in Table 5. The non protein nitrogen increased in Expt. (B), (C) and (E), but decreased in Expt. (A), (D), (F), (H) and (I). In Expt. (B), (C) and (E), the total nitrogen and the protein nitrogen decreased in contrast with the increasing of the non protein nitrogen. Namely, the protein degradation volume exceeded the microbial body protein synthesis from non protein nitrogen in the rumen in these experiments. Further, it can be seen that the increasing volume of the protein nitrogen in the rumen exceeded the absorption volume of the non protein nitrogen through the rumen epithelium so that the non protein nitrogen was accumulated in the rumen in these experiments. On the other hand, non protein nitrogen decreased in (A), (D), (F), (H) and (I), and the total nitrogen decreased in the rumen in these experiments with the exception of a slight increase in Expt. (A). However, the protein nitrogen was increased in Expt. (A), (D), (H) and (I), but was decreased in Expt. (F) and (G) in spite of the decreasing of the non protein nitrogen in the rumen. In the case of Expt. (E) and (G) it can be seen that the absorption volume of non protein nitrogen through the rumen epithelium exceeded the protein degradation volume in the rumen.

Table 5. The weight change of non protein nitrogen in the reticulo-rumen under reticulo-omasal orifice plugging.

Time after beginning of feeding (hr)	Experimental ration (g)								
	(A)	(B)	(C)	(D)	(E)	(F)	(G)	(H)	(I)
2	0.68	1.30	2.76	2.19	2.80	5.59	12.91	2.69	7.04
5	0.52	1.50	4.06	2.34	4.20	5.98	10.02	0.99	3.34
11	0.62	2.00	2.95	0.90	4.10	5.17	8.80	0.39	0.75

Ammonia nitrogen.

The weight change and the concentration of ammonia nitrogen in the rumen are shown in Table 6. Expt. (A), (D), (H) and (I), showed the increasing of protein nitrogen, ammonia was decreased with the exception of slight increase in Expt. (D), and the concentrations of the ammonia in the rumen were lower than 14.3 mg/*dl* at 11 hours after the beginning of feeding. On the other hand, in Expt. (B), (C), (E), (F) and (G), ammonia was accumulated in the rumen and the concentrates of ammonia ranged from 31.9 to 118.0 mg/*dl* at 11 hours after the beginning of the feeding. An addition of urea to the high protein food, Expt. (G), showed the highest ammonia concentrates in all experiments which indicated 136.4 mg/*dl* at 5 hours. However, with the low protein food plus urea experiment, Expt. (I), the ammonia concentrates do not exceeded 46.0 mg/*dl*.

Table 6. The weight change of NH_3-nitrogen in the reticulo-rumen under reticulo-omasal orifice plugging.

Time after beginning of feeding (hr)		Experimental ration								
		(A)	(B)	(C)	(D)	(E)	(F)	(G)	(H)	(I)
2	(g)	0.40	0.70	1.95	0.57	1.80	4.05	6.90	0.89	0.60
	(mg/*dl*)	8.9	16.8	27.9	12.9	37.0	60.5	107.3	17.9	15.1
5	(g)	0.20	1.20	2.90	1.25	2.70	4.86	8.52	0.68	2.08
	(mg/*dl*)	3.9	26.1	42.1	28.0	52.9	77.4	136.4	13.0	46.0
11	(g)	0.31	1.50	2.18	0.62	3.50	4.37	7.27	0.21	0.41
	(mg/*dl*)	6.3	33.8	31.9	14.3	74.1	72.4	118.0	1.5	4.2

Absorption of nitrogen through the rumen.

The calculated values by the method of 2 hours value of total nitrogen minus 11 hours value of total nitrogen are listed in Table 7, as the absorption volume of nitrogen from the rumen epithelium in nine hours. The total nitrogen was decreased in the rumen in every experiment in the nine hours experimental period with the only exception of Expt. (A). The decrease of total nitrogen

may be seen from the total sum of the absorption volume of the nitrogenous compounds through the rumen epithelium minus inflow volume of salivary nitrogen volume. The only case of increasing of the total nitrogen, Expt. (A), may be due to the more inflow of salivary nitrogen volume into the rumen than the absorption volume of the nitrogen through the rumen epithelium. The maximum absorption of the nitrogen through the rumen was observed in Expt. (G). In this experiment 6.1 g of urea nitrogen was added to Expt. (F), but the calculated value showed that almost all of the added urea nitrogen was absorbed through the rumen within nine hours. On the other hand, in Expt. (I), which showed a high conversion of urea nitrogen to the protein nitrogen, absorption of the nitrogen was only 1.67 g.

Table 7. Absorption of total nitrogen from the reticulo-rumen under reticulo-omasal orifice plugging.

period (hr)	Experimental ration (g and (%))								
	(A)	(B)	(C)	(D)	(E)	(F)	(G)	(H)	(I)
9	−0.78	1.68	1.51	1.24	3.60	3.56	9.58	1.00	1.67
	(−8.43)	(6.15)	(5.87)	(16.02)	(11.50)	(8.42)	(19.62)	(7.84)	(12.65)

Volatile fatty acids.

The concentrates of the VFA in the rumen are shown in Table 8. VFA were not so markedly increased within 11 hours after the beginning of feeding in Expt. (A), (H) and (I). In these experiments protein nitrogen increased. On the other hand, VFA increased in Expt. (B), (C), (D), (E), (F) and (G). In these experiments protein nitrogen decreased within nine hours only with the exception of Expt. (D), which was given corn ensilage. VFA were markedly increased by the addition of urea to the high protein feed in Expt. (G), in contrast

Table 8. The weight change of VFA in the reticulo-rumen under reticulo-omasal orifice plugging.

Time after beginning of feeding (hr)		Experimental ration								
		(A)	(B)	(C)	(D)	(E)	(F)	(G)	(H)	(I)
2	(*M*)	0.30	0.29	0.48	0.28	0.26	0.38	0.66	0.27	0.27
	(m*M*/*l*)	67	70	69	63	55	56	102	53	69
5	(*M*)	0.35	0.44	0.90	0.54	0.66	0.59	0.96	0.37	0.41
	(m*M*/*l*)	71	98	133	122	126	95	155	70	89
11	(*M*)	0.30	0.49	0.78	0.57	0.69	0.62	1.00	0.20	0.27
	(m*M*/*l*)	59	107	114	142	147	105	168	29	55

with Expt. (F). However, VFA were slightly increased by the addition of urea to low protein food in Expt. (I), in contrast with Expt. (H). The concentrations of VFA ranged from 53 to 70 m*M*/*l* at two hours after the beginning of feeding in every experiments with the only exception of Expt. (G). However, 5 and 11 hours values varied respectively.

Gas production volume.

The results of this experiments are presented in Table 9. The gas production volume of the rumen contents was lower and not varied during the experimental period when hay was given in Expt. (A). Namely, this suggested that slow fermentation occured in this experiment. In the Expt. (B), (E), (F), (G), (H) and (I), the gas production volume of the rumen contents showed highest value at two hours after the beginning of feeding and lowered with the increase of time after the feeding. Expt. (G) showed that gas production volume was markedly increased by the addition of urea to the high protein food. However, a slight increase could be observed by the addition of urea to the low protein food in Expt. (I).

Table 9. The gas production volume of reticulo-rumen contents under reticulo-omasal orifice plugging*.

Time after beginning of feeding (hr)	Experimental ralion (*ml*)								
	(A)	(B)	(C)	(D)	(E)	(F)	(G)	(H)	(I)
2	6.4	12.8	7.0	—	11.4	24.0	40.0	22.5	25.5
5	7.6	9.2	24.5	—	7.6	16.0	26.0	15.0	17.0
11	7.8	4.8	9.5	—	5.6	12.0	24.5	9.8	7.0

* Gas production volume from 10 g rumen contents in 37°C, 4 hr.

Discussion

Pearson and Smith pointed out that sampling difficulties and gave precise information as to the nitrogen distribution throughout the whole rumen contents at any given time unobtainable (2). They pointed out that another difficulty exists in the portion of the rumen contents that are periodically passing from the rumen into the omasum and the material passing on at any time may not be representative of the rumen contents as a whole.

The technique used for our experiments give a solution to these difficulties and the methods were previously described (1).

Kameoke and Morimoto demonstrated that the absorption of digestible protein in the forestomach ranged from −20.0 to 52.1 per cent by using the forestomachs separation technique (5). The available evidence suggests that the rate of breakdown of the protein in the rumen is related to the quantity and quality of the protein in the diet.

When a diet rich in protein like soybean oil meal was fed, the protein rapidly broke down and ammonia accumulated in the rumen. A large quantity of the ammonia accumulated in the rumen and became absorption through the rumen epithelium under higher protein and urea feeding. However, the ammmonia was not so largely accumulated by urea feeding when available carbohydrate were presented simultaneously in the rumen. Since the majority of the absorption of the nitrogen through the rumen epithelium exists as ammonia (6, 7, 8), it can be regarded that the decreased quantity of the total nitroge from the rumen were absorbed as ammonia nitrogen. The maximum absorption volume of the nitrogen through the rumen epithelium was 9.58 g within 9 hours in higher protein and urea feeding. Except for this diet, the absorption of the nitrogen ranged from −0.78 g to 3.60 g in 9 hours. Utilization rate of the urea nitrogen for synthesis of microbial body protein was affected by the quantity and quality of carbohydrates.

VFA production in the rumen have some relation with the degradation of protein and the increase of ammonia nitrogen. The protein was not decreased and the ammonia not accumulated when hay was supplied. In a such condition VFA were not increased in the rumen at any time after feeding.

Namely, slow fermentation could have occured in the rumen in such feeding condition. On the other hand, when the concentrates were supplied, the protein decreased and the ammonia accumulated and the VFA increased after feeding. It can be concluded that the rapid fermentation could occur in such diets in the rumen. VFA were markedly increased by urea addtion to the higher protein diets, and the protein degradation improved by the addition of urea to the higher protein degradation improved by the addition of urea to the higher protein diets. On the other hand, VFA were not so markedly increased by the addition of urea to low protein diets. However, protein became increased in the rumen by the synthesis of the microbial body protein. It can be assumed that the ammonia will improve the degradation of the protein in high protein and readily fermentable condition and on the other hand the ammonia will be utilized by the rumen micro-organisms to microbial body protein synthesis under low protein condition in the rumen. These assumptions will be proved by gas production ability experiments. Gas production volume of the rumen contents were markedly increased by the addition of urea to the high protein diets but a slight increase could occur with urea addition to the low protein diets. In genral, a high protein condition provides readily fermentable conditions and the protein degraded, and a low protein conditon will provide the hardly fermentable condition. So that the weight change of the protein nitrogen in the rumen are according to the quantity and quality of protein in the diets and the influence of the diets to the rumen fermentation conditions.

Summary

The weight change of the total nitrogen, protein nitrogen, non protein nitrogen and ammonia nitrogen in the whole rumen contents under nine different feeding conditions were determined. VFA concentrates and gas production ability of the whole rumen contents were examined simultaneously.

The protein decreased and the ammonia increased in the rumen under higher protein diets condition in the rumen, and the protein increased by low protein and urea diets in the rumen.

The protein decreasing, ammonia production, VFA procuction and gas production ability in the whole rumen contents were nearly in proportional relations.

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