Rumen Metabolism of Nonprotein Nitrogen

W. H. HALE

Department of Animal Husbandry, Iowa Agricultural Experiment Station, Ames, Iowa

The role of nonprotein nitrogen in the biochemical processes that occur in the rumen is reviewed, as well as possible mechanisms of toxicity. In vitro and animal studies with rumen microorganisms show useful nonprotein nitrogen compounds, in terms of cellulose digestion and bacterial growth, to be those from which the ammonia nitrogen is easily released. Rumen microorganisms show adaptability in utilizing nitrogen from certain nonprotein nitrogen compounds whose nitrogen is generally considered unavailable. Fifty per cent or more of a natural protein entering the rumen may be degraded or altered by rumen microorganisms with the nitrogen appearing as free ammonia or bacterial protein.

Table I. Activity of Various Nonprotein Nitrogen Compounds as a Nitrogen Source in the Artificial Rumen[a]

Active nonprotein nitrogen compounds	
Propionamide	Ammonium succinate
Butyramide	Ammonium lactate
Glycinamide	Ammonium alpha-ketoglutarate
Formamide	Ammonium formate
Acetamide	Ammonium malate
	Ammonium pyruvate
Guanidine acetate	Ammonium fumarate
Guanidine carbonate	Ammonium citrate
Guanidine hydrochloride	Ammonium adipate
Creatinine	Ammonium acetate
Creatine	Ammonium sulfate
	Ammonium carbonate
Urea	Ammonium sulfamate
	Ammonium nitrilotrisulfonate
	Ammonium triamidodiphosphate
Inactive nonprotein nitrogen compounds	
Methylenediurea	Hydroxyacetamide
n-Butylurea	Succinimide
Biuret	Oxamide
Hydrazoformamide	Malonamide
Ethylurea	Glutaramide
Ethylenediurea	Diglycolamide
Acetylurea	Adipamide
Methylurea	Cyanoacetamide
Allylurea	
Ethyleneurea	
Dimethylolurea	
Semicarbazide HCl	
Dicyandiamide	Ammonium nitrate
Acetamidine	
Guanylurea phosphate	
Aminoguanidine bicarbonate	
Aminoguanidine sulfate	

[a] Adapted from (*1*).

DIETARY PROTEIN for the ruminant may be partially replaced by nonprotein nitrogen such as urea (*18*, *30*). This is possible because rumen microorganisms utilize nonprotein nitrogen to build their body proteins and, as these microorganisms subsequently pass through the intestinal tract of the ruminant, the bacterial protein becomes available to the host animal. Because of its low cost per unit of nitrogen and highly available nitrogen content to rumen microorganisms, urea has become the accepted nonprotein compound to use as a protein substitute in ruminant rations. A large portion of the experimental work with urea has been concerned with the effect of ration composition on the utilization of the added urea. Generally, the criterion used to measure the utilization of the urea is an increase in weight gains of the test animal as compared to suitable controls. Reid (*25*) has written a review on urea as a protein replacement.

Other recent reviews are available on certain aspects of rumen function (*9*, *24*). However, little attention has been given to nonprotein nitrogen compounds other than urea, even though in many instances the nonprotein nitrogen content of certain feedstuffs may represent a large portion of the total nitrogen of that feedstuff. There is a lack of experimental data concerning the exact fate of nonprotein nitrogen or protein after it enters the rumen. This review covers

the recent literature and is restricted to rumen metabolism of nitrogen.

Artificial Rumen Studies

Belasco (*1*) employed the artificial rumen to screen nonprotein nitrogen compounds as possible feed ingredients. Cellulose digestion, residual ammonia, nitrogen utilization, and bacterial growth were observed with urea being used as the control nonprotein nitrogen compound. The general groups of compounds tested were urea derivatives, selected amides and amidines, and certain organic and inorganic ammonium salts. A summary of this work is presented in Table I.

The urea derivatives, except possibly methylenediurea, released little or no nitrogen into the fermentation medium. Bacterial growth was poor and, in some instances, the urea derivatives were toxic to the rumen microorganisms. As might be expected, cellulose digestion was poor with this group of compounds.

The rumen bacteria appeared to have sufficient amidases to hydrolyze the amides of the monocarboxylic acids such as formamide, acetamide, propionamide, butyramide, and glycinamide. These compounds all provided the desired feature of a low ammonia level in the fermentation flask, but the resulting cellulose digestion was not as good as that obtained with urea. The low levels of free ammonia produced by the amides indicated a hydrolysis rate comparable to the rate of bacterial ammonia nitrogen utilization. The diamides of oxalic, glutaric, diglycolic, and adipic acids were inert as nitrogen sources.

Of the amidines tested, the guanidine salts gave good cellulose digestion and excellent bacterial growth together with low levels of ammonia. These compounds on the basis of the artificial rumen would appear to offer excellent possibilities as a nonprotein nitrogen feeding compound.

The artificial rumen experiments with the ammonium salts of the various organic and inorganic acids point to the high availability of their nitrogen to the rumen microorganisms. Both cellulose digestion and bacterial growth were comparable to those obtained with urea. Of the compounds tested, ammonium nitrate proved to be toxic to the rumen microorganisms. The high ammonia release suggests that these compounds have the same limitation for feed ingredients as urea. Ammonium lactate and ammonium succinate proved to be the most interesting of the ammonium salts of the organic acids. Cellulose digestion was high with these compounds and nitrogen utilization was higher than with urea. The organic fragments of these compounds may enter into some metabolic process of the rumen bacteria that stimulates rapid nitrogen fixation.

In artificial rumen studies comparing urea with soybean, linseed, cottonseed, and corn-gluten meals Belasco (*2*) found urea superior to oilmeals as a nitrogen source in promoting cellulose digestion. Urea in 1 to 1 mixtures with various feed proteins improved cellulose digestion above that observed with the feed proteins alone. These data suggest that a certain amount of ammonia is essential for optimum performance of the rumen microorganisms but do not take into consideration the protein needs of the animal itself. It has also been shown from in vitro studies with rumen microorganisms that urea promoted higher levels of propionic acid, and lower levels of butyric and valeric acids, than did the equivalent amounts of nitrogen from conventional plant proteins usually fed ruminants. This is significant in that high levels of propionic acid production in the rumen are probably desirable from the standpoint of the nutrition of the ruminant animal (*3*).

As many bacteria are capable of using ammonia as a sole or part source of nitrogen for growth, and ammonia is the chief nitrogenous end product in the breakdown of proteins by bacteria, ammonia might be expected to be very important in the nitrogen metabolism in the rumen. El-Shazly and Synge (*12*) have demonstrated the marked capacity of ruminal bacteria to deaminate amino acids. When suspensions of washed bacteria were incubated with acid-hydrolyzed casein, up to 35% of the amino acid nitrogen appeared as ammonia. All of the amino acids were attacked at similar relative rates. This is in contrast to the report of Sirotnak and coworkers (*29*), who found only six out of 22 amino acids tested were attacked by washed suspensions of rumen microorganisms: aspartic acid, glutamic acid, serine, arginine, cysteine, and cystine.

Recently it has been demonstrated with techniques using washed suspensions of rumen microorganisms that certain short-chain fatty acids (isobutyric, valeric, isovaleric, and caproic) enhance cellulose digestion in vitro (*5, 6*). Rates of gains by steers and lambs were not increased by the addition of valeric acid to their rations. However, it appeared that the valeric acid supplementation did increase feed consumption (*4*). These acids are found in the rumen ingesta of animals on a natural ration. The source of these acids in rumen ingesta is apparently through the Stickland reaction (*11*) after degradation of the ration proteins by the rumen microorganisms. It would be desirable to determine if the above-mentioned fatty acids are produced in the rumen of an animal receiving all of its dietary nitrogen in the form of nonprotein nitrogen and if supplementation of these fatty acids to a ration of this type would be beneficial.

While the in vitro technique is highly

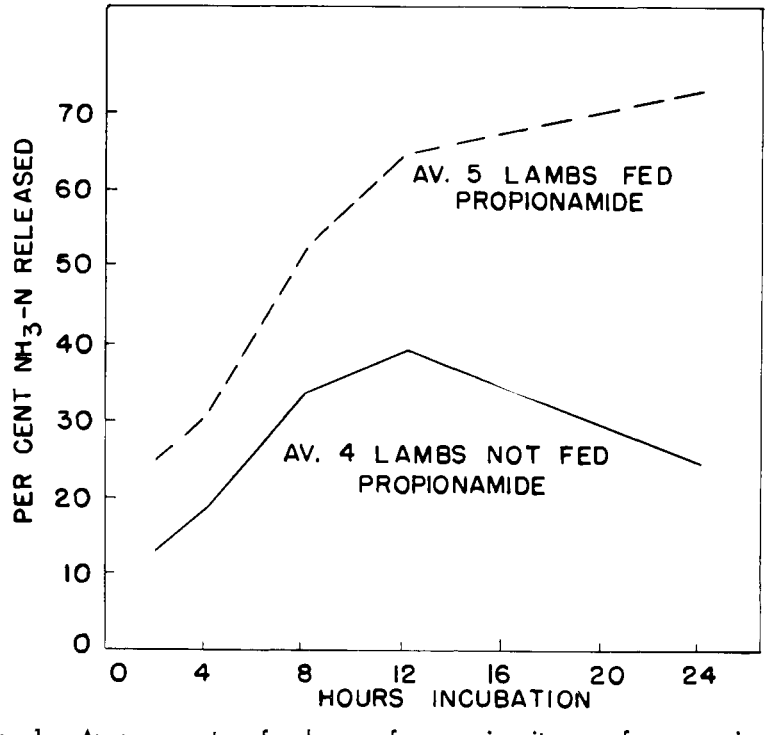

Figure 1. Average rate of release of ammonia nitrogen from propionamide incubated with lamb rumen microorganisms

Table II. Effect of Nonprotein Nitrogen Compounds on Weight Gains of Lambs[a]

Treatment[b]	No. of Lambs	Av. Daily Gains, Lb. First 21 days	Av. Daily Gains, Lb. Last 53 days
Conventional protein	20	0.38	0.41
Urea	17	0.02	0.41
Propionamide	20	0.08	0.37
Ammonium formate	18	0.09	0.46
Ammonium propionate	19	0.13	0.39
Ammonium acetate	18	0.08	0.45

[a] Adapted from (*28*).
[b] In nonprotein nitrogen treatment nonprotein compound supplied 50% of ration nitrogen.

useful in screening nonprotein nitrogen compounds, the results obtained should be confirmed with animal tests. Biuret and dicyandiamide were not active in vitro. Animal studies show that the nitrogen from these compounds may be available to some extent (*22*, *23*), presumably through an adaptation or species change by the rumen microorganisms.

Animal Studies

Nonprotein Nitrogen Compounds. In a series of tests with lambs, Repp, Hale, and Burroughs (*27*) obtained results similar to those of Belasco (*1*) in vitro. In feeding trials with lambs in which 50% of the ration nitrogen was replaced by a nonprotein nitrogen source, they found that the ammonium salts of the organic acids (formate, acetate, and propionate) were equal to urea in promoting growth of the animal. Formamide was definitely inferior to urea and had toxicity problems other than ammonia toxicity. In the first test, propionamide was less satisfactory than urea as a nonprotein nitrogen feeding compound. In later trials when used at the 15, 30, and 50% protein-equivalent replacement level, it was equal to urea.

In vitro studies with rumen microorganisms from lambs not previously fed propionamide showed that the utilization of the nitrogen from propionamide was poor (Figure 1). However, with rumen microorganisms from lambs which were being fed propionamide the utilization of the nitrogen from the propionamide was excellent. Digestibility of cellulose paralleled the ammonia nitrogen release. The longer the lambs had been fed propionamide, the greater the utilization of the propionamide nitrogen. The length of the adaptation period ranged from 0 to 110 days with most of the adaptation occurring the first 20 days (*26*). Subsequent toxicity tests with lambs which had been fed propionamide for 60 days showed that the release of the ammonia was not at a rate which would cause toxicity.

Data in Table II suggest that an adaptation period was required before the rumen microorganisms could satisfactorily utilize the nonprotein nitrogen compound being fed. In the rations fed these lambs 50% of the nitrogen was supplied in the form of nonprotein nitrogen.

When the nitrogen in the diet of the ruminant is supplied as urea, a different rumen population exists than that found on a natural diet (*13*). It may be that many of the ruminal bacterial species are exacting in their requirements for amino acids; and, in a diet which supplies little or no protein nitrogen there is a disappearance of the exacting species with a survival of a species which can use ammonia as the sole source of nitrogen.

Natural Proteins. The loss of ammonia through the rumen wall has not been fully investigated, but nonprotein nitrogen toxicity studies suggest that this loss may attain significant amounts under certain conditions. McDonald has shown sharp increases in rumen ammonia on addition of certain proteins to a hay ration of sheep (*19*). The extent of conversion of zein to microbial protein was shown by McDonald to be about 40% in sheep fed a partially purified diet (*20*). As zein is water-insoluble it may be that a larger percentage of a more soluble protein would be converted to microbial protein. It has also been demonstrated that approximately one half of the nitrogen from hay was converted to microbial protein. While this information is useful, it does not give the amount of plant protein converted to microbial protein (*14*).

Chalmers, Cuthbertson, and Synge (*8*) failed to obtain a satisfactory increase in nitrogen balance with ewes by the addition of casein to a low-protein diet. If the casein was administered by duodenal fistula there was a greater utilization of the nitrogen than when the same amount of casein was fed. Analysis of rumen liquor from the animal fed casein showed it to be high in ammonia, which apparently came from the degradation of the casein. It was assumed that this ammonia was absorbed through the rumen and intestinal wall and excreted in the urine without serving any useful purpose to the animal. Urine analysis showed such urine to be high in nitrogen. Casein was treated with alkali and dried in such a way as to give it a rubbery consistency when wetted. Oral administration of this casein gave higher nitrogen retention than untreated casein administered in a similar way, but very little rise in rumen ammonia. Apparently, treatment of the casein rendered it less labile to the actions of the rumen microorganisms. The casein then presumably passed into the intestinal tract and was digested as in the monogastric animal. These data are not necessarily in conflict with those of Repp and coworkers (*28*), who failed to observe an increase in blood ammonia or urea nitrogen upon administration of large doses of casein. They did not measure urinary nitrogen. It is possible that large amounts of rumen ammonia nitrogen may be absorbed through the rumen wall and pass into the urine as urea nitrogen with very little elevation of blood ammonia or urea nitrogen. The relationship of peripheral blood ammonia and urea nitrogen to rumen ammonia nitrogen is not known. The lambs of Repp and coworkers (*28*) were on a high level of nutrition as compared to a low level for those of Chalmers, Cuthbertson, and Synge (*8*).

These data raise the question as to the advisability of using casein as a standard reference protein in nutritional studies for ruminants. They further suggest the possibility of some type of treatment for proteins to be fed to ruminants in an effort to improve their total usefulness to the animal. However, a certain amount of nitrogen is essential for growth of the rumen microorganisms. Burroughs and coworkers (*7*) demonstrated that a source of nitrogen is essential for cellulose digestion. Another point of consideration is that the quality of the proteins generally fed ruminants is low. If part of this protein is not converted to microbial protein, the animal may require certain amino acid supplements for optimum performance. The biological value of rumen microbial protein for the rat is high (*21*).

Toxicity. Few data are available on reactions by which ammonia may enter into the rumen other than by the metabolic process concerned with protein synthesis by the rumen microorganisms. The early studies on urea toxicity in ruminants led workers in the field to reach the conclusion that urea toxicity was due to the rapid liberation of ammonia from this compound in the rumen and subsequent absorption of the am-

monia through the rumen wall. In effect, the animal died of an ammonia toxicity. Repp and coworkers (*28*) have shown a close correlation between levels of blood ammonia and the degree of urea toxicity observed in sheep. Clark and coworkers (*10*) failed to produce typical symptoms of urea toxicity by intravenous injections of ammonia solutions in this species. They suggested that some toxic compound was produced by the urea in the rumen, and when absorbed, resulted in the death of the animal. Kaishio and coworkers (*17*) reported that urea is converted to ammonium carbamate in the rumen or abomasum and that toxicity is the result of the absorption of this compound. Hale and King (*15*) have also shown that it is toxic to lambs when injected intravenously or administered orally. It may be formed as an intermediate product of urea hydrolysis by urease. If ammonium carbamate is the toxic agent, the toxicity syndrome resulting from administration of the ammonium salts of the organic acids remains unexplained. It is known that in the presence of ammonia the bicarbonate ion exists in equilibrium with the carbamate ion (*31*). As rumen liquor is rich in the bicarbonate ion, it is suggested that because of the rapid liberation of ammonia from certain nonprotein nitrogen compounds, large concentrations of ammonium carbamate may be present. The significance of this compound in rumen metabolism of nonprotein nitrogen and its exact physiological effect upon the animal have yet to be demonstrated.

Although formamide, the guanidine salts, and ammonium sulfate gave excellent results in vitro, subsequent tests with animals showed that formamide and guanidine carbonate had specific animal toxicities which eliminated them as possible feed ingredients (*28*). Ammonium sulfate appeared to have no specific toxicity but feed consumption was poor when this compound was included in the ration (*26*).

Formamide was definitely inferior to urea and had toxicity problems other than ammonia toxicity. In the first test, propionamide was less satisfactory than urea as a nonprotein nitrogen feeding compound. In later trials when used at the 15, 30, and 50% protein-equivalent replacement level, it was equal to urea. Further studies (*28*) with ammonium formate, acetate, and propionate demonstrated that their toxicity was equal to that of urea at the dose levels tested. These compounds also gave high ammonia levels in the artificial rumen. The compounds which failed to produce an ammonia toxicity in lambs also failed to give high free ammonia in the artificial rumen experiments of Belasco (*1*). These were formamide, propionamide, biuret, ammonium succinate, guanidine, and glycine. Apparently, the potentially toxic nonprotein nitrogen compounds released ammonia more rapidly than it could be utilized by rumen microorganisms. With some of the nontoxic nonprotein nitrogen compounds the rate of release of the ammonia was too slow to permit satisfactory bacterial growth.

Nutritional Significance

Two opposing nutritional tendencies are apparent with nitrogen digestion in the rumen. First, such compounds as urea, which are of no nutritional value to the animal, are converted into microbial protein nitrogen which can be later digested by the host animal with a gain in nitrogen. Secondly, natural proteins may be degraded in part to yield ammonia, a portion of which is absorbed through the rumen or intestinal wall. Probably both nonprotein nitrogen compounds and feed proteins are broken down to yield ammonia for use in microbial synthesis. However, the rate of breakdown may far exceed the ability of the microorganisms to convert the nitrogen into microbial protein nitrogen. This excess ammonia for the most part would represent a loss of nitrogen to the host animal. As these two forces are in play at the same time, they probably account for the constant biological value of food nitrogen for ruminants (*16*). As feedstuffs contain varying amounts of nonprotein nitrogen, the magnitude of these two forces cannot be estimated at the present time. The extent of conversion of food nitrogen to microbial protein and factors affecting this conversion need study.

It becomes apparent that relatively little is known of the metabolism of nitrogen in the rumen and the subsequent effects of this metabolism on the nutrition of the ruminant animal. Animal studies with nonprotein nitrogen have received attention at the expense of natural proteins. It may be that the two are of equal importance in rumen metabolism and nutrition of the animal. It will take the combined efforts of the biochemist, microbiologist, physiologist, and nutritionist to extend our knowledge in this field and interpret it in terms of practical feeding, so that the ruminant will retain the position in agricultural economy as a producer of meat, milk, and fiber.

Literature Cited

(1) Belasco, I. J., *J. Animal Sci.* **13,** 601 (1954).
(2) *Ibid.*, p. 739.
(3) *Ibid.*, p. 748.
(4) Bentley, O. G., Cline, G. H., Klosterman, E. W., Johnson, R. R., Moxon, A. L., *Ibid.*, **14,** 1194 (1955).
(5) Bentley, O. G., Johnson, R. R., Hershberger, T. U., Cline, J. H., Moxon, A. L., *J. Nutrition* **57,** 389 (1955).
(6) Bryant, M. P., Doetsch, R. N., *J. Dairy Sci.* **38,** 340 (1955).
(7) Burroughs, W., Arias, C., DePaul, P., Gerlaugh, P., Bethke, R. M., *J. Animal Sci.* **10,** 672 (1951).
(8) Chalmers, M. I., Cuthbertson, D. P., Synge, R. L. M., *J. Agr. Sci.* **44,** 255 (1954).
(9) Chalmers, M. I., Synge, R. L. M., *Advances in Protein Chem.* **9,** (1954).
(10) Clark, R., Oyaert, W., Quin, J. I., *Onderstepoort J. Vet. Research* **25,** 73 (1951).
(11) El-Shazly, K., *Biochem. J. (London)* **51,** 647 (1952).
(12) El-Shazly, K., Synge, R. L. M., *Intern. Congr. Physiol.*, Copenhagen, Abstr. 445 (1950).
(13) Gall, L. S., Thomas, W. E., Loosli, J. K., Huhtanen, C. N., *J. Nutrition* **44,** 113 (1951).
(14) Gray, F. V., Pilgrim, A. F., Weller, R. A., *Nature* **172,** 347 (1953).
(15) Hale, W. H., King, R. P., *Proc. Soc. Exptl. Biol. Med.* **89,** 112 (1955).
(16) Johnson, B. C., Hamilton, T. S., Mitchell, H. H., Robinson, W. B., *J. Animal Sci.* **1,** 236 (1942).
(17) Kaishio, Y., Higaki, S., Harii, S., Awai, Y., *Bull. Natl. Inst. Agr. Sci. (Japan)* **2,** 131 (1951).
(18) Loosli, J. K., Williams, H. H., Thomas, W. E., Ferris, F. H., Maynard, L. A., *Science* **110,** 144 (1949).
(19) McDonald, I. W., *Biochem. J. (London)* **51,** 86 (1952).
(20) *Ibid.*, **56,** 120 (1954).
(21) McNaught, M. L., Smith, J. A. B., Henry, K. M., and Kon, S. K., *Ibid.*, **46,** 32 (1950).
(22) Magruder, N. D., Knodt, C. B., *J. Dairy Sci.* **37,** 657 (1954).
(23) Meiske, J. C., Van Arsdell, W. J., Leucke, R. W., Hoefer, J. A., *J. Animal Sci.* **14,** 941 (1955).
(24) Phillipson, A. T., *Advances in Veterinary Sci.* **2,** (1955).
(25) Reid, J. T., *J. Dairy Sci.* **36,** 955 (1953).
(26) Repp, W. W., Ph.D. thesis, Iowa State College, 1954.
(27) Repp, W. W., Hale, W. H., Burroughs, Wise, *J. Animal Sci.* **14,** 901 (1955).
(28) Repp, W. W., Hale, W. H., Cheng, E. W.-K., Burroughs, Wise, *Ibid.*, p. 118.
(29) Sirotnak, F. M., Doetsch, R. N., Brown, R. E., Shaw, J. C., *J. Dairy Sci.* **36,** 1117 (1953).
(30) Starks, P. B., Hale, W. H., Garrigus, U. S., Forbes, R. M., James, M. F., *J. Animal Sci.* **13,** 249 (1954).
(31) Taylor, T. W. J., "The Organic Chemistry of Nitrogen," Clarendon Press, Oxford, 1937.

Received for review February 6, 1956. Accepted May 10, 1956. Journal paper No. J-2907, Iowa Agricultural Experiment Station, Ames, Iowa. Project No. 1208.