method in measuring the color developed by the test solution. No. 56 is closer to the wave-length peak for the violet-blue color, but with the Klett-Summerson colorimeter it can measure only from 0.5 to 4.0 γ of metaldehyde. A standard curve based on the color readings with No. 54 filter was also constructed, as with this filter amounts of metaldehyde up to 8 γ may be measured.

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RUMINANT NUTRITION

Review of Utilization of Nonprotein Nitrogen in the Ruminant

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The utilization of nonprotein nitrogen, and the relative value of such nitrogen in meeting part of the protein requirement of ruminants, were successfully demonstrated in a series of nitrogen balance trials and practical feeding tests. Urea in amounts to supply up to 50% of the total supplemental nitrogen in fattening rations and 25% in wintering rations was efficiently utilized by beef cattle. Carbohydrate feeds were essential for efficient utilization, and methionine supplements brought further improvement. Chronic toxicity was not encountered. Acute toxicity was produced by drenching with solutions of urea and by fasting the animals before offering urea-containing feeds.

The domestic ruminant has been described, with emphasis on rumen function, as a processor of plant material in the production of food for man—a processor that uses as raw material a high proportion of coarse, fibrous plants not suited to the diet of other animals and employs fermentation in breaking down the structural parts of plants to liberate and concentrate the more valuable nutrients contained therein, adding to these nutrients growth factors not originally present, and utilizing from the over-all processes appreciable amounts of the by-products as a source of energy. With respect to nitrogen metabolism, processing costs to the animal approach the equivalent of 3.5 grams of body protein per 100 grams of dry matter intake, the exact amount depending on the nature and composition of the intake; and, for maintenance, there is a further nitrogen cost related to body size. These costs are made apparent in the fecal and urinary nitrogen excretion of ruminants fed low-nitrogen rations.

The possibility that the nitrogen costs involved in digestion and body maintenance and even including production might be covered in part by supplying as a substitute for protein in the ration nonprotein nitrogen in the form of urea was put to critical test with sheep and dairy heifers about 15 years ago (*7, 8, 11*). The original concepts developed in the interpretation of the results of these tests have not changed. Together with other chemical and bacteriological studies of rumen processes, these tests demonstrated with reasonable certainty the formation of protein from nonprotein nitrogen through the intervention of microorganisms inhabiting the rumen. Practical application of the results to livestock production has encouraged further investigation of the nutritional requirements of rumen bacteria as related to those of the host. Nitrogen as a requirement, however, and the compounds of nitrogen that are most efficient in meeting this requirement, continue to occupy first place among the list of nutrients being studied by most investigators. No less than 15 reviews of the subject have appeared in the past decade.

Protein supplements play a double role in the nitrogen nutrition of the ruminant. First of all, and perhaps of greater importance, they supply in a natural form the nitrogen needed for growth and multiplication of those rumen organisms responsible for the fermentation and disintegration of the ration as a whole. This role of protein has been observed time and again, and it was effectively demonstrated a few years ago by Burroughs and his associates (*2*) in a series of studies dealing with the enhanced nutritive value of corn cob rations effected by protein and mineral supplementation. Much of the recent research on nonprotein nitrogen as a protein substitute has stemmed from, or been influenced by, observations made in those studies and later ones which employed the artificial rumen technique (*1*).

Further, protein supplements ultimately supply in the form of amino acids the nitrogen needs of the animal itself. Protein that is insoluble, is bound into the structure of plant cells by inert material, or has been so altered by physical and chemical means that it resists breakdown by bacterial enzymes of the rumen, is probably resistant to enzymatic breakdown in the gastrointestinal tract as well and therefore is of limited nutritive value (*12*). The low digestibility of heat-treated protein attests to this fact. On the other hand, easily soluble protein such as casein is extensively degraded in the rumen at so rapid a rate that ammonia and other simple compounds of nitrogen accumulate in amounts in excess of the immediate needs of the rumen organisms (*10*). Such excess of ammonia, although some of it is absorbed and returned to the digestive tract by way of the saliva, must be considered for the most part as wasted nitrogen. There lies between these two extremes of solubility the bulk of common protein-containing feeds.

The principal protein of corn, zein, is relatively insoluble; yet, according to measurements made by McDonald (*9*), from 40 to 50% of its nitrogen is liberated and incorporated into the protoplasm of rumen organisms preceding its true digestion by the host. McDonald's studies were designed to answer the questions: Approximately what proportion of feed protein is degraded and built into the bodies of bacteria during its passage through the rumen and reticulum? How much reaches the gastrointestinal tract in the form in which it was fed?

These few comments taken from a voluminous literature on protein nitrogen and its metabolism in the ruminant may apply equally well to nonprotein nitrogen—to such compounds, among others, as urea, biuret, various amides, ammoniated agricultural products, and the common ammonium salts. Among these, only urea has been thoroughly tested. The essence of the problem as it appears to the chemist is this: To prepare a nitrogen compound that can compete with vegetable proteins in ruminant rations, with full consideration being given to the cost of such a product, its stability and handling and mixing properties, its palatability, solubility, and possible toxicity, and its ability to liberate in the rumen metabolizable nitrogen at a rate commensurate with the requirement of rumen organisms. Whatever other conditions are necessary, they probably can be met by specific supplementation.

Although partial replacement of natural feed protein by simpler nitrogen compounds is practicable, the fact that as yet such protein cannot be replaced completely may very well mean that for optimal performance the ruminant requires a certain amount of unaltered protein—certain chemical groupings present in feed protein but absent in microbial protein or stimulatory growth factors associated with high protein feeds are needed, or, as seems most likely, formation of protein in the rumen from nonprotein nitrogen does not proceed at a rate conducive to optimal utilization.

After initial reports of the successful use of urea as a substitute for part of the protein in ruminant rations, the Oklahoma Agricultural Experiment Station (*5*) began a series of feeding tests and balance studies with beef cattle and sheep. The purpose of the tests was to determine the value of urea under practical conditions of maintenance and fattening and to study some of the ration factors influencing urea nitrogen utilization. In some tests attention was given to the possible effect of urea on metabolism of other ration nutrients—for example, phosphorus and vitamin A which, along with protein, oftentimes are limiting factors in wintering rations of beef cattle.

In the first studies of nitrogen balance, urea was incorporated in pelleted feed-mixture supplements in such amounts as to supply 25, 50, 75, and up to about 90% of the total nitrogen in the pellet. The pellets, which contained the equivalent of about 43% protein, were fed as supplements to hay in maintenance and wintering-type rations and as supplements to corn and hay in fattening rations. Later, only the 25 and 50% pellets were fed as supplements. The results demonstrated the superiority of the low-urea pellets over the high-urea ones in promoting nitrogen retentions of an order similar to that obtained by feeding an equivalent amount of protein as oil meal (*4*). Indirectly, the results confirmed earlier work, showing that rumen organisms require readily available carbohydrate as a source of energy for the efficient utilization of nonprotein nitrogen—that is, for protein synthesis—and that a further amount of carbohydrate is necessary for efficient utilization by the animal of the protein so formed.

Extension of this work with urea rations based on corn, barley, milo, dehydrated sweet potatoes, and combinations of corn and molasses gave results indicating that these feeds as sources of energy for protein synthesis were of similar value; milo was especially valuable as measured by the increase in nitrogen retention when urea was added, and corn proved superior to molasses. Similar studies with unusual rations showed that the three simple carbohydrates, sucrose, glucose, and lactose, were equal in value as energy sources (*6*).

The results of further balance trials (*5*) indicated that rations based on limited amounts of any one of the common protein supplements, cottonseed meal, soybean meal, and corn gluten meal, and supplied with adequate energy, could be equally well supplemented with urea. Probably, however, a similar ration based on easily soluble protein would be less effectively supplemented because of rapid ammonia production from both nitrogen sources.

Although sulfur, phosphorus, and an unknown number of mineral elements are required for ruminal synthesis of protein from nonprotein nitrogen, the sulfur requirement has been given the most thorough study, especially by Thomas and associates (*13*). As a source of sulfur and perhaps as a stimulatory substance in urea rations, methionine has been shown to be superior to either inorganic sulfate or elemental sulfur. In the Oklahoma work, methionine supplements improved nitrogen retention of animals on urea rations only slightly. As methionine failed to improve protein utilization on both low- and high-protein rations, its favorable effect appears to be associated with nonprotein nitrogen use.

In spite of the consistent results obtained in these nitrogen balance trials demonstrating the utilization of nonprotein nitrogen, urea when put to practical test did not generally prove to be a satisfactory source of supplemental nitrogen for growing and fattening lambs in the feed lot. Pregnant and lactating ewes, however, were benefited by urea supplements. The problem is one of nutrition and may involve some specific factor which in the presence of nonprotein nitrogen affects efficiency of feed conversion. Mineral and vitamin studies failed to show any adverse effect of urea supplements on the metabolism of calcium, phosphorus, or vitamin A (*5*).

In feed lot trials conducted over a period of 8 years with fattening beef calves full-fed on corn, urea was used successfully to replace up to 50% of the total nitrogen in protein supplements. Where the amount of urea was increased to supply 85% of the total nitrogen in the supplement, feed intake and gains in body weight were reduced. Experiments in wintering beef cattle on dry native grass supplemented with 2 to 3 pounds of a pelleted urea feed gave inconsistent but generally favorable results. The small amount of readily available carbohydrate in such rations and the practice of feeding the supplements on alternate days, as compared to twice daily, produced conditions unfavorable for most efficient utilization of urea nitrogen. Yearling and 2-year-old cattle were wintered more successfully than heifer calves under these conditions; in fact, the older cattle made gains similar to those of cattle fed an equivalent amount of protein supplied as oil meal (*5*).

Information on other organic compounds of nitrogen which have been adequately tested in livestock rations is lacking. The value of some ammoniated products and the specific problems associated with the use of ammonium compounds in ruminant rations have been stated in a brief but effective manner in a recent paper by Davis and others (*3*) at Cornell. Apparently the availability of nitrogen of ammoniated molasses and similar products suggested as livestock feed is conditioned by methods of processing. Low digestibility of nitrogen as measured in a digestion trial with such products has indicated low nitrogen availability.

Because ammonia is the common end product in the degradation of most, if not all, of these compounds, problems of toxicity may arise. Toxicity produced by drenching steers with solutions of urea, by forced feeding, and by withholding feed for 24 to 48 hours before offering urea feeds has been reported from a few laboratories. Field reports of so called "urea toxicity" are misleading because of difficulty of diagnosis. At the Oklahoma Station chronic toxicity could not be produced by feeding 500-pound steers as much as 1 pound of urea per day. The urea was fed in increasing amounts in a ration of 4 pounds of prairie hay, 3 pounds of corn, 6 pounds of molasses, and minerals (bone meal and salt) over a period of 125 days. Acute toxicity was produced by withholding feed for 48 hours and then offering a mixture of corn, molasses, and urea in such amounts (2 to 3 pounds) that the animals consumed about 20 grams of urea per 100 pounds of body weight. Staggering, prostration, convulsions, and bloating were common symptoms. In these cases, as in previous ones (*5*), blood ammonia values were elevated and the animals either died within a few hours or completely recovered.

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RUMINANT NUTRITION

In Vivo and in Vitro Nutritional Requirements of Rumen Microorganisms

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The composition of the ration has an important influence on the digestive and synthetic processes of rumen microorganisms. Both rumen inoculations and changes in the ratio of roughage to grain fed to dairy calves altered the establishment and proportion of microorganisms typically found in the rumens of adult dairy cattle. Ruminal synthesis of vitamin B_{12} can be affected by lack of cobalt. In vitro studies indicate that rumen microorganisms require nutritional factors present in natural feedstuffs, in addition to energy, nitrogen, and minerals. Addition of rumen liquor, extracts of roughage and protein concentrate, or fermentation by-products to the in vitro fermentation medium increases the rate of cellulose digestion. Several laboratories have obtained a cellulolytic response with B vitamins, C_5 to C_6 fatty acids, amino acids, and partial hydrolyzates of proteins.

STUDIES ON RUMINANT NUTRITION conventionally involve evaluating and investigating the effect of various feeds and levels of nutrition on performance—i.e., rate of gain, maintenance, reproduction, and carcass quality of animals. Some early investigators realized the possible importance of the contribution of the bacteria within the rumen to the utilization of feeds by the animal. Tappenheimer in 1884 [cited by Phillipson (*33*)] used ox rumen bacteria to study the fermentation of cellulose and found that large amounts of volatile fatty acids were produced. Zuntz and Hagemann in 1891 [cited by McNaught and Smith (*29*)] postulated that nonprotein nitrogen might be converted to protein by the microbes in the rumen and that subsequent digestion of the microbes would contribute to the protein requirement of the host animal. However, it was not until 1922 that Henneberg [cited by Baker and Harriss (*2*)] first used microscopy to study microorganisms in relation to cellulose digestion.

In recent years, the importance of the rumen microflora population in the nutrition of the ruminant animal has been stressed. Consequently, many studies on ruminant nutrition now emphasize the nutrition of the rumen bacteria themselves. The development of in vitro techniques for studying their nutrition has assisted these investigations greatly.

Basically, the ruminant is a roughage consumer—i.e., it utilizes high fiber feeds, and it finds its greatest economic utility in that role. However, with the increased desire for rapid production of not only more quantity but better quality of meat, many cattle and sheep are now given rations high in starch and low in fiber. Changes in the proportion of fiber to starch affect not only the energy content of the ration but many processes in the rumen as well.

Burroughs and coworkers (*8*) showed that cellulose digestion was depressed by feeding starch to steers, while the presence of starch often improves synthesis of B vitamins (*25, 28*) and urea utilization (*30*). Using in vitro rumen fermentations, Arias and coworkers (*1*) found that small amounts of readily available sources of energy promoted cellulose digestion but larger amounts inhibited it. Again, these energy sources increased urea utili-