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EVALUATION OF SLOW AMMONIA RELEASE UREA (SARU) AS A LIQUID SUPPLEMENT FOR RUMINANTS

by

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TITLE

EVALUATION OF SLOW AMMONIA RELEASE UREA (SARU)

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INTRODUCTION

Failure of world food production to increase parallel to world human population growth has challenged the moral consciousness of mankind. At issue are two central problems: first, can enough food be produced for everyone, and second, can each person be enabled and persuaded to buy and consume an adequate diet. The ability to produce adequate food stores is dependent upon our agricultural technology and, more important, the transfer of that technology to undeveloped areas. Individual consumption becomes dependent on global distribution. Distribution, a polite name for poverty, is controlled by an international hierarchy of political and economic pressures. Whereas international social justice may depend on future generations, agricultural technology may be advanced continuously. The premise of this new technology is an improved efficiency of agricultural production.

In animal agriculture the ruminant holds a unique role in helping to achieve improved efficiency of production. Under practical ruminant feeding programs, it has been shown feasible and desirable to partially replace expensive natural protein with cheaper nonprotein nitrogen supplements. Numerous nonprotein nitrogen compounds have been tested and shown to support a positive nitrogen balance in ruminants under particular conditions. Undoubtedly, urea has attracted the major portion of experimental attention. As a result, the merit of urea as a protein replacement and its rolein the nitrogen metabolism of the ruminant has been well established.

The ruminant is unique in that it can utilize urea as a source of supplemental protein. The use of urea is mitigated by the rumen microbial population which possess the ability to synthesize amino acids from ammonia. Upon entering the rumen, urea is hydrolyzed to ammonia by action of the bacterial enzyme urease. As ammonia, urea nitrogen is used for the synthesis of relatively high biological value microbial protein. However, there are discrete inefficiencies in the utilization of urea nitrogen. Many factors have been shown to affect the efficiency of urea utilization. Some of these factors are: 1) the rapid hydrolysis of urea to ammonia and 2) the lack of sufficient energy in the rumen at the time of maximum ammonia availability.

The rapid conversion of urea to ammonia results in a loss of the ammonia that is not rapidly used for protein synthesis. Increased absorption rapidly depletes excess rumen ammonia concentrations. Via portal circulation, ammonia is carried to the liver to be converted to urea for subsequent recycling to the rumen or excretion through the kidneys.

Optimum microbial protein synthesis depends on the synchronized availability of energy and ammonia. In high roughage rations peak energy availability occurs 4 to 6 hours postfeeding, while peak ammonia production occurs within the first 2 hours postfeeding. Thus without optimum protein synthesis, much of the ammonia is absorbed and lost through the kidneys as urea.

Excessive dietary levels of urea present the potential for animal toxicity. The absorption of ammonia at both a rate and concentration greater than can be converted to urea by the liver will result in a

"spill over" of ammonia into systemic circulation. Excessive circulating blood ammonia levels can cause a depletion of energy intermediates in brain metabolism and result in neuromuscular disorders and death. This ammonia induced condition is conventionally referred to as "urea toxicity."

A reduction in the rate of urea hydrolysis holds the potential for improving the efficiency of nitrogen utilization. In addition such a reduction will also improve energy utilization and reduce the incidence of urea toxicity. Research efforts to reduce the rate of hydrolysis have centered on the activity of the enzyme urease and on the solubility or availability of urea. Numerous feed additives and processing methods have been tested. Some have shown limited success. Today a successful and economical method of reducing the rate at which urea is converted to ammonia is not available.

Recently researchers have demonstrated that formaldehyde treatment is an effective means of reducing the solubility of proteins within the rumen. In private communication with Liquid Feed Commodities Incorporated, Fremont, Nebraska, it was learned that formaldehyde could be added to a molasses based urea liquid supplement, resulting in an alteration of the nitrogen solubility.

Preliminary experimentation indicated that under stringent conditions formaldehyde could be incorporated into a urea based liquid supplement, resulting in a product which supported a reduced rate of ammonia production within the rumen. After considerable experimentation a single formulation was identified as having commercial value. Prior to commercial application specific data was required for review by the United States Department of Health, Education and Welfare, Food and Drug Administration.

The intent of this report is to outline the product proposed, to describe the chemical assay developed and to detail the studies conducted in assessing its biological impact. These observations constitute a part of the formal Food and Drug Administration review. The specific objectives of the studies herein reported were to: 1) assess the relative toxicity of the product and 2) assess the efficacy of the product as a nonprotein nitrogen supplement in ruminant rations.

For the purpose of this report the product is identified as "SARU" which stands for "slow ammonia release urea." As a commercial product the supplement has been assigned the copyrighted trademark "Soy-rea^{R}".

LITERATURE REVIEW

Nonprotein Nitrogen (NPN) in Ruminant Nutrition

History of NPN

As early as 1891, while the biochemist was extending the knowledge of metabolic nitrogen pathways, Zuntz (1891) and Hageman (1891) recognized the unique role of the rumen in nutrition. They theorized that the microorganisms in the rumen of herbivorous animals synthesize bacterial protein from nonprotein nitrogen compounds such as urea. This work pointed out the protein sparing action of nonprotein nitrogen upon the rumen microflora.

Voltz (1907, 1918, 1919, 1920, 1922, 1924) was one of the first to test the theory that nonprotein nitrogen could be used by the rumen microorganisms to form protein. He obtained growth of lambs on a low protein, semi-purified diet made of starch, alkali washed straw, inorganic salts and urea. Armsby (1911) noted that in the presence of adequate protein, nonprotein nitrogen failed to increase the production of nitrogenous constituents in the animal. He suggested that the limiting factor in the utilization of protein formed from nonprotein nitrogen appeared to be the extent to which it was formed rather than to an inferior nutritive value. It was not until the late 1930's that Hart (1938, 1939) and his coworkers showed conclusively that urea could be used by dairy calves for growth and maintenance.

After 1940 the emphasis on the use of urea in ruminant feeding shifted from proving that urea could be used as a replacement for natural protein to determining the degree to which this replacement

could be made and the nutritive factors necessary to improve the efficiency of urea utilization. It was necessary to define the ideal medium for growth of the microorganism within the rumen and the conversion of nonprotein nitrogen into a form useful to the host animal. Urea research during these years has been reviewed in a monograph by Briggs (1967).

As an alternative to urea various nitrogenous compounds have been used as a source of dietary supplemental nitrogen for the ruminant (Stangel, 1963). Belasco (1954) used *in vitro* cellulose digestion and bacterial growth to evaluate 12 urea derivatives, 23 amides and amidines, 15 ammonium salts, both organic and inorganic, and 19 miscellaneous amines and nitrogen bearing compounds. Hale (1956) identified 25 nonprotein nitrogen compounds that could be used as dietary nitrogen sources for the ruminant. Simpson (1966) and Repp *et al.* (1955a, 1955b) evaluated an additional 30 compounds either *in vitro* or *in vivo*. Recent compounds and products have been reviewed by Gutcho (1973).

Generally, nitrogenous compounds capable of being utilized by the ruminant have an economic disadvantage due to the cost of synthesis or isolation, leaving urea as the main commercial source of nonprotein nitrogen (USDA Ag. Report No. 153, 1969). Two additional nonprotein nitrogen compounds have gained acceptance in roles supportive of urea. Ammonium polyphosphate has been shown by Johnson and McClure (1967) and Peeler (1972) to have a high degree of phosphorus bioavailability. Ammonium polyphosphates, conventionally used in the fertilizer industry, offer the ruminant a source of both phosphorus and nonprotein nitrogen. The three most frequently used feed grade sources, expressed as the percent nitrogen, phosphorus and potassium, are 9-30-0, 10-34-0 and

11-37-0. Compared to urea and phosphoric acid these compounds are relatively low in phosphorus and nitrogen. Thus, ammonium polyphosphate is generally used in combination with urea (Wornick, 1969).

Anhydrous liquid ammonia has found limited application in ruminant feeding when blended with an acidic liquid vehicle. In the acidic media small quantities of the free base are trapped and upon being fed are rendered as available nonprotein nitrogen. Wornick (1969) has outlined such products as ammoniated molasses and ammoniated wood solubles.

Rumen Nitrogen Metabolism

The dietary nitrogen requirement of the ruminant can be met by both natural and nonprotein nitrogenous substances. Some dietary protein passes the rumen, undigested, to the lower digestive tract, where it may be hydrolyzed into amino acids (Wagner et al., 1940, 1941). These amino acids may be absorbed and distributed through the circulatory system to the site of tissue synthesis. Much of the dietary protein is hydrolyzed to amino acids by the proteolytic enzymes of the rumen (Sym, 1938). Some of these amino acids may be directly utilized by the rumen microorganisms in the synthesis of microbial protein, but the major portion is deaminated by the microbial enzymes to ammonia and the corresponding carbon residue (Pearson and Smith, 1943). This ammonia may be passed to the lower tract, be absorbed into the portal blood system through the rumen wall, or be utilized by the rumen microflora in the synthesis of amino acids and subsequently microbial protein. In a similar manner, nonprotein nitrogen sources, converted to ammonia, may be used by the population of rumen microorganisms in the synthesis of a relatively high biological value microbial protein.

Endogenous urea nitrogen is continuously formed by liver enzymes and recycled to the rumen via both the saliva and directly across the rumen wall as part of the normal process of nitrogen metabolism (Annison and Lewis, 1959). This nitrogen conserved and reutilized for the synthesis of microbial protein is of considerable significance to the nitrogen economy of the animal.

The hydrolysis of urea within the rumen by the bacterial enzyme urease proceeds at a rate greater than the ammonia produced can be utilized for microbial protein synthesis (Bloomfield et al., 1960). Excess ammonia is absorbed, converted to urea in the liver, excreted in the urine and lost to the animal (McDonald, 1952). Toxicity may result when the liver is unable to convert all of the absorbed ammonia into urea and the systemic blood ammonia concentration is elevated. Toxicity symptoms may appear when systemic blood ammonia reaches a critical level of 1 to 4 mg per 100 ml (Repp et al., 1955). The toxic level (LD_{50}) of urea depends upon: method of feeding, physiological state of the animal, type of diet, and metabolic state of the rumen (Kromann et al., 1971). Church (1969) suggests that the lethal dose for poorly fed animals is 20 to 25 gm of urea per 45 kg of live weight, while well fed animals succumb to levels of 30 to 35 gm urea per kg live weight. Normally 10 to 40 gm urea per 45 kg of body weight will induce clinical symptoms (Gallup et al., 1952; Oltjen et al., 1963; Nix and Anthony, 1965).

If the rate at which urea is hydrolyzed to ammonia could be reduced, the efficiency of urea utilization would increase and the potential for urea toxicity would be reduced. Numerous experiments designed to reduce the rate of urea hydrolysis have been reviewed by Chalupa (1968).

Generally, two methods have been employed: 1) methods which reduce the concentration and/or the activity of the enzyme urease, and 2) methods which physically or chemically limit the availability of urea as a substrate for urease action. Acetohydroxamic acid (Brent and Adepoju, 1967), barbituric acid (Harpers et al., 1962), antibiotic, diethylstilbestrol (McLaren et al., 1959, 1960) and urease antibodies (Sidhu et al., 1968) have been used to reduce urease action. Wax coating (Johnson et al., 1962), pelleting (Andrec, 1963), starch coating (Deyoe et al., 1968), sulfur coating (Umunna and Woods, 1970) and urea resin complexes (Huston et al., 1974) have been used to reduce the availability of urea as a substrate within the rumen. Some of these methods have shown limited success, yet nonehave proven to be economically feasible.

Nutritional Status of Slow Ammonia Release Urea

With the demonstration by Bloomfield et al. (1960) that urea hydrolysis occurs four times faster than liberated ammonia can be utilized for microbial protein synthesis, numerous control mechanisms were postulated. A partial listing of methods to reduce the rate of urea hydrolysis has been presented in a previous section. Initially Coombe et al. (1960) demonstrated a significant increase in urea utilization when sprayed on a roughage and fed ad libitum compared to an intraruminal drench. Both rumen ammonia and pH remained low compared to the urea drench. Increased frequency of feeding a urea supplemented grain ration was shown by Campbell et al. (1963) to produce a more even rate of urea hydrolysis and to increase the utilization of ammonia nitrogen. Feeding a 3.3% urea ration six times daily compared to twice daily resulted in

growth and feed efficiencies similar to those obtained with animals fed a natural protein supplemented diet.

Perhaps the first assessment of sustained ammonia production was made by Oliver and Cronje (1964). By placing slowly solubilized capsules (pill) of urea into the rumen of sheep, they noted a reduced rate of ammonia absorption and an increase in nitrogen retention. In 1974 Huston et al. made the first practical observation on the utility of slow ammonia release urea. These authors fed a slow release urea pellet produced by extruding a moistened mixture of 50% urea, 40% corn starch and 10% carboxy resin (Carbopol 934). Nitrogen retention, daily gain and feed efficiency were increased compared to a urea control and a basal ration when fed to sheep in a low protein, medium fiber diet.

These data demonstrate that the correct usage of suitable ureolytic inhibitors can improve urea utilization. Supporting metabolic data, although difficult to interpret, is available. In general, researchers have attempted to test the hypothesis by producing a constant or sustained rumen ammonia titer. Frequency of feeding has been used (Campbell et al., 1963) to produce this steady state. Hume et al. (1970) and Hume and Bird (1970) produced this steady state by feeding sheep at two hour intervals and observed a linear increase in rumen protein production when urea was increased from 2 to 9 gm per day. With these urea additions to a virtually protein free medium fiber diet, total nitrogen flowing past the rumen and production of protein per unit of digested organic matter were increased. However, increasing from 9 gm of urea nitrogen (11.4% crude protein) per day to 16 gm (20.6% crude protein) resulted in no further advantage.

Frequency of feeding studies are often interpreted solely on the basis of ammonia release and animal performance, disregarding the confounding effect of simultaneous energy intake. Recent studies have utilized mechanical infusion systems to separate the effect on energy intake and a sustained supply of urea. Knight and Owens (1973) studied the effect of interval infusions on sheep fed either 60, 65 or 75% TDN rations. Urea was infused within each 12 hour interval after feeding a basal diet and resulted in a nitrogen intake equivalent to a 12% protein ration. All supplemental urea was infused within 1 or 3 hours post-feeding. A third continuous infusion schedule supplied all urea evenly over each 12 hour cycle. Results indicate an interaction between level of dietary energy and infusion interval. With the high energy diet, rapid infusion, within 1 hour, proved superior to more gradual infusions. With lower energy diets infusion times of 3 hours improved nitrogen balance. Interestingly, 1 and 3 hour infusions supported greater nitrogen retention than continuous infusion. These data do not support the constant rumen ammonia titer concept, but do suggest a requirement for the synchronous availability of energy and nitrogen.

Streeter et al. (1973), in a similar study, failed to show improved nitrogen economy for the continuous infusion of urea compared to immediate infusion post feeding of a low energy ration. However, both total nitrogen and tungstic acid precipitatable nitrogen reaching the abomasum were numerically greater for the continuously infused animals compared to the animals infused at feeding. In view of the large differences in ruminal ammonia between the two infusion schemes and the failure to detect significant differences in nitrogen balance, these authros have concluded that control of ruminal ammonia levels will not increase the

efficiency of urea utilization. However, these data outline the extremes of fast and slow ammonia release and fail to recognize the interval nitrogen requirement of a low energy diet as demonstrated by Knight and Owens (1973).

Owens et al. (1973) superimposed three levels of continuous urea infusion upon a 8.75% protein high energy ration when fed twice daily to lambs. Treatments resulted in dietary protein equivalents of 8.75, 11.88, 14.06 and 18.50%. Nitrogen balance was improved over the corn based control diet when urea was infused to provide a 11.88% protein equivalent. However, additional urea, 14.06 and 18.50% protein equivalent diets, depressed nitrogen retention. Compared to the basal diet, the low level of urea infusion had little effect upon the entry of tungstic acid precipitable nitrogen into the abomasum. In view of these findings an explanation for improved nitrogen balance is uncertain. Although nitrogen retention was depressed with the two higher levels of urea infusion, total tungstic acid precipitable nitrogen passing into the abomasum increased linearly with each successive level of urea. These observations support the proposed early interval requirement of ammonia by sheep fed a high energy diet (Knight and Owens, 1973). Higher levels of continuous infusion dictate that higher levels of ammonia will be available for protein synthesis, regardless of the interval of energy availability.

Burroughs et al. (1971) have proposed new concepts regarding protein nutrition in the ruminant. Each feedstuff is assigned a urea fermentation potential (UFP) which is expressed as a positive or negative number. A positive value expresses the grams of urea per kilogram of feed dry matter which can be utilized for microbial protein synthesis. This