

A SLOW RELEASE UREA COMPOUND FOR WINTER
SUPPLEMENTATION OF BEEF COWS AND
HEIFERS CONSUMING LOW QUALITY
FORAGES

By

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CHAPTER I

INTRODUCTION

Increased competition from non-ruminants for feed grains and plant seed protein has enhanced the need to utilize low-quality roughage and non-protein nitrogen (NPN), mainly urea, in ruminant rations.

Urea is well utilized in high energy rations today, but little progress has been made in the utilization of urea or other NPN source with feeds low in fermentable energy. Urea is hydrolyzed to ammonia at a rate in excess of the amount which can be converted to microbial protein with low-quality feedstuffs. Consequently, treatment of NPN sources to temper the ruminal concentration of ammonia and imitate that found with natural protein might improve their utilization.

The concept of slow ammonia release is not new. Nevertheless, practical application has been difficult because of effective and applicable methods to regulate release are rare. An ideal slow-release ammonia product (1) would release ammonia gradually but totally, (2) must not produce undesirable side effects or cross-over reactions in the ruminal medium, and (3) should be low in cost.

The purpose of the present study was:

- To evaluate a new slow-release urea (coated urea)¹ for the beef cow grazing a winter range grass.

¹Developed by NIPAK Corporation, Pryor, Oklahoma.

- To compare the effect of level of supplemental energy upon protein and non-protein nitrogen utilization by the range beef cow.

CHAPTER II

REVIEW OF LITERATURE

Introduction

Urea is the most commonly used non-protein nitrogen (NPN) material for ruminant feed supplementation. However, urea has many problems which complicate its use. It is unpalatable, highly hygroscopic, difficult to combine with certain feedstuffs and hydrolyzed to ammonia very rapidly into the rumen which results in ammonia losses and toxicity problems.

The mechanism of urea utilization in the ruminant can be summarized as follows: (1) hydrolysis of urea by microbial urease to ammonia and carbon dioxide in the rumen, (2) fermentation of dietary carbohydrates to volatile fatty acids, (3) amination of keto acids to form amino acids, (4) incorporation of amino acids into microbial protein, (5) passage to the abomasum and small intestine and digestion of microbial cells to amino acids and (6) absorption of the resulting amino acids.

From those steps it is apparent that many factors can affect the utilization of urea. Rapid release of ammonia requires simultaneous rapid fermentation of carbohydrates. The latter is thought to limit urea use by animals grazing low-quality roughages.

Aspects of utilization of urea and other NPN compounds have been thoroughly reviewed by Loosli and McDonald (1968), Chalupa (1968,

1973), Conrad and Hibbs (1968), Tillman and Sidhu (1969), Helmer and Bartley (1971), Goodrich et al. (1972), and more recently by NRC (1976) and UN-ECE (1977). This review will deal with those aspects related to urea utilization on low-quality forage conditions, release of ammonia into the rumen, interaction nitrogen utilization-carbohydrate availability, methods to slow the release of ammonia and slow release urea compounds.

Hydrolysis of Urea in the Rumen

Rate of Hydrolysis

The rapidity of urea hydrolysis in the rumen and the accompanying problem of toxicity has been well documented. Reid (1953) in one of the earliest reviews of urea utilization described such toxicity problems. Although the cause of toxicity was unknown at that time, he stated that the use of less soluble non-protein nitrogen materials could reduce the waste of ammonia. Lewis et al. (1957) studied the relationships between urea feeding, rumen ammonia concentrations, blood ammonia levels and toxicity. In general, improper use of urea can result in rapid accumulation of ammonia in the rumen, elevate rumen pH, and increase absorption of ammonia into the blood beyond the ability of the liver to convert ammonia to urea which results in accumulation of ammonia in the blood and finally, toxicity (NRC, 1976).

Loosli and McDonald (1968) in a review of NPN compounds concluded that methods are needed to reduce the rate of urea breakdown in the rumen and advised incorporating urea into material that would degrade slowly and release urea at a slow rate. Chalupa (1973) pointed out

that the major factor limiting the efficient use of urea nitrogen is obtaining parallel rates for urea hydrolysis and for fixation of liberated ammonia into cellular protein. Helmer and Bartley (1971) in an extensive review agreed that the limiting factor is probably rapid hydrolysis of urea and enumerated several approaches to improve urea utilization, most by minimizing ammonia losses. More recently an updated review (NRC, 1976), a decade after Reid's review, still indicated that feeding practices that provide a continuous intake of NPN or supply of ammonia would be recommended for maximum urea utilization provided that other necessary ingredients are fed.

Ruminal Ammonia Levels Related to Urea Feeding

Pearson and Smith (1943) reported that 100 mg of urea were hydrolyzed to ammonia per hour per 100 ml of rumen fluid. Subsequently, Bloomfield et al. (1960) estimated that urea was hydrolyzed to a rate of 80 mg per hour per 100 ml of rumen fluid while the microbes could utilize only 20 mg per hour. Recently, Prokop et al. (1971) found that the total ruminal urease activity of cattle was capable of hydrolyzing 75 to 125 g of urea per minute, a rate far exceeding the capacity of bacteria to use ammonia.

Such estimates are not exaggerated as they have been seen repeatedly when urea was fed to animals. For instance, Oltjen et al. (1968) when comparing urea, biuret, urea phosphate and uric acid in purified diets for steers found that urea and urea phosphate degradation resulted in similar ruminal ammonia patterns characterized by a rapid rise in ammonia levels after feeding. The peak occurred about one hour after feeding at a level of 50-60 mg/100 ml rumen fluid. Williams

et al. (1969) working with fistulated steers fed with urea and non-urea containing rations and poor quality hay, observed that ruminal ammonia values were greater (8-35 mg/100 ml rumen fluid) in animals fed urea rations for a period of five hours post-feeding as compared to cotton-seed meal fed animals (1-8 mg/100 ml) during the same period.

Bacterial Utilization of Ruminal Ammonia

Estimates of the maximum amounts of ammonia liberated from urea or protein which can be used efficiently for the microbial protein synthesis have been variable. According to Satter and Roffler (1975), maintenance of ruminal ammonia levels in excess of 5 mg/100 ml rumen fluid does not increase microbial protein production. Some in vivo results (Hume, et al., 1970; Miller, 1973) have indicated however that greater microbial yield is achieved between 10 and 20 mg of ammonia 100 ml rumen fluid. Either of these estimated values when compared to the ammonia levels resulting from urea feeding would indicate that a large proportion of the ammonia is wasted when urea is fed. Therefore, the efficient utilization of any NPN product may require ammonia release rates which do not exceed the ability of rumen bacteria to convert the ammonia into protein.

Relationship Between Rumen Ammonia Nitrogen and Carbohydrate Availability

Role of Carbohydrates in Microbial Protein

Synthesis

The conversion of NPN to microbial protein is an energy consuming process (Chalupa, 1973). Optimal cell synthesis requires an adequate

supply of ammonia nitrogen, carbon skeletons, sulfur, possibly free amino acids and other as yet unidentified growth factors or cofactors (Bergen and Yokoyama, 1977). Thus, a deficiency of any one of these components will interfere with the process and decrease the utilization of NPN.

The degradation of carbohydrates to VFA which yields energy is critical to production of microbial cells. In fact, Y_{ATP} value (grams of microorganisms produced per mole of ATP) was initially developed by Bauchop and Elsdon (1960) on the basis that microbial growth is proportional to the amount of ATP generated from the catabolism of energy substrates.

Based on this Y_{ATP} concept several calculations concerning microbial protein production from carbohydrate fermentation have been reported. Hungate (1966) estimated that 100 g of fermented carbohydrate were required to synthesize 6.9 g of microbial protein, while Bloomfield (1964) calculate a value of 11.2 g of microbial protein fixed per 100 g of carbohydrate fermented. Recently Bergen and Yokoyama (1977) cited research showing that microbial synthesis rate is between 15 to 22 g of protein per 100 g of organic matter fermented.

Carbohydrate Availability and NPN Utilization

A balance between nitrogen and energy furnished to the microbial population is essential for good NPN utilization. In fact, new systems for evaluation of proteins (Burroughs et al., 1974; Satter and Roffler, 1975) are based on the premise that nitrogen metabolism in the ruminant cannot be considered separately from carbohydrate digestion.

The Urea Fermentation Potential (UFP) system (Burroughs et al., 1974) calculates the quantity of urea which can be transformed into rumen microbial protein based upon the relative amounts of energy fermented and protein degraded from the feedstuffs. Similarly, Satter and Roffler (1975) consider TDN and protein together in a regression equation to predict rumen ammonia concentrations and add urea to reach that point.

The relationship between dietary carbohydrates and NPN utilization has been discussed in detail by Johnson (1976) who classified the carbohydrates into three general types: (1) cell wall carbohydrates, (2) glucose polymers and (3) simple sugars. These carbohydrates differ in two characteristics: amplitude and time span of fermentation. Cell-wall carbohydrates are digested slowly over a long period of time, simple sugars are digested rapidly over a short period of time and starches are somewhere between. Starches traditionally have been considered as the most desirable carbohydrate source to combine with NPN although still not ideal. Since none of the fermentation curves of the different carbohydrates precisely match the ammonia release curve of urea, Johnson (1976) concluded that the major limitation for a microbial protein synthesis when NPN is fed is timing of energy availability in the rumen and that an ideal source of nitrogen is one which fits the specific carbohydrate source being fed.

Approaches to Match Nitrogen and Energy

Availabilities

Energy availability for range beef animals is limited during the winter season. Energy supplementation increases cost and adversely

affects forage intake (Cook and Harris, 1968) and thereby is undesirable. The problem of matching nitrogen and energy availabilities has usually been approached by attempting to maintain low but constant levels of rumen ammonia. This can be done by frequent feeding, by feeding slow-release urea products and experimentally by the use of continuous ruminal infusions.

As Knight and Owens (1973) have pointed out, it is necessary to differentiate between feeding frequencies (meal frequency) and frequencies of feeding of urea alone, since in the former intake of urea and energy are confounded.

One of the earliest works dealing with meal frequency (Bloomfield et al., 1961) compared wethers fed sixteen or two times per day with rations containing 3.2% urea and determined that more frequent feeding appeared to increase urea utilization. Campbell et al. (1963) utilized dairy heifers fed twice or six times daily with urea or soybean meal supplements and found that with frequent feeding, urea compared favorably with natural protein, but with infrequent feeding, soybean meal supplementation proved superior. Prior (1974) fed lambs either twice daily or hourly with rations containing soy protein or urea as the only dietary nitrogen source. He concluded that frequent feeding of urea rations improved utilization of dietary nitrogen whereas soy nitrogen utilization was not modified by frequency.

Mizwicki et al. (1976) studied the effects of frequency of feeding urea supplements on the performance and supplement consumption of finishing lambs. The rations were 80% concentrate (12% protein) with either 1% urea (basal ration) or 3% urea present and were fed free-choice either 24 hours per day or ad libitum for one hour per day. When fed once daily, the higher level of urea in the ration decreased

intake by 46% and decreased gain by 30%. But when fed ad libitum for 24 hours, urea addition increased the intake 33% and gains by 14% as compared to the basal ration. Therefore, it was concluded that frequency of feeding definitely influenced urea utilization.

Pitzen et al. (1973) supplemented calves with a ration containing 1.8% urea which was fed at 12 hour or 2 hour intervals and with a slow-ammonia release product fed every 12 hours. They found that the amount of total nitrogen leaving the abomasum was greater with the 2 hour interval and with the slow-release product than with the 12 hour interval. Kropp et al. (1977) studied microbial protein synthesis of steers fed low quality grass and supplemented with soybean meal or soybean meal plus urea rations in which urea replaced 25, 50 or 75% of the supplemental nitrogen. Microbial protein production was constant across treatments which indicated good utilization of frequently ingested urea with low-quality forages. However, the amount of nitrogen reaching the abomasum decreased as urea replaced more SBM in the supplement since the amount of soybean meal available for bypass decreased.

When urea has been furnished independently of the rest of the ration, results have not been so consistent. Streeter et al. (1973) working with lambs fed a basal ration (6% crude protein) and infused urea into the rumen at various intervals. They found no difference in the utilization of nitrogen when continuously infused which provided a sustained release of ammonia, than when dosed twice daily. They also observed that nitrogen reaching the abomasum and nitrogen balance was improved by the addition of urea at any frequency to the basal ration. Similarly, Knight and Owens (1973) concluded that ruminal infusions of urea for lambs fed a low protein basal diet

improved nitrogen retention. Efficiency of urea utilization was not related to the infusion time intervals, but was related to the energy content of the diet. With lower energy rations, moderately long infusion (3-hr) slightly improved nitrogen retention. With higher energy rations rapid infusions (1-hr) were superior to gradual ones. Knight and Owens (1973), therefore, advised for synchronous availability of energy and nitrogen to maximize benefits from NPN.

Mizwicki et al. (1978) fed steers urea at different intervals and limit fed low quality hay every hour. Slow and rapid release of ammonia were simulated by feeding urea intermittently at four different rates. Results showed that slow-release of ammonia did not improve ammonia use since digestibility, nitrogen balance and ruminal protein synthesis were not improved.

Feeding studies with slow-release urea products have shown somewhat contradictory results. Torell et al. (1971) fed a coated urea to weaned lambs on range and found a negative correlation between blood urea nitrogen and daily gain which indicated that urea was available in the rumen but was not efficiently utilized, possibly because energy was limited. Huston et al. (1974) demonstrated by using a coated urea product that reducing the rate of release of urea in the rumen increased its value as a supplemental nitrogen source when added to a medium fiber diet fed to sheep. However, the authors emphasized that even though the slow release urea preparation maintained similar ruminal conditions as cottonseed meal, animal performance from urea did not equal that obtained from cottonseed meal but offered no explanation for this difference.

Urea Utilization Under Poor-Quality Forage Conditions

Cattle fed low-quality roughages suffer from both nitrogen and energy deficiencies, the former generally considered as the most limiting factor (Nelson et al, 1954; Clanton and Zimmerman, 1970; Rush et al., 1976). In many cases both nutritional deficiencies could be eliminated by overcoming the nitrogen deficiency since rumen microorganisms with adequate nitrogen supply will increase growth and will degrade feeds more rapidly.

A large and active population of bacteria, then, will increase intake and utilization of poor quality roughages as has been observed repeatedly by researchers supplementing nitrogen to grazing ruminants (Coombe and Tribe, 1962; Campling et al., 1962; Elliot, 1967; Cook and Harris, 1968).

Unfortunately, much research has shown that the combination of low quality forage and supplementary urea does not provide sufficient energy, carbon fragments or other nutrients for adequate utilization of NPN

Nelson and Waller (1962) summarized 16 experiments with about 900 cows under Oklahoma winter range conditions and concluded that cows could not efficiently utilize supplements containing up to half the nitrogen from urea as well as cottonseed meal supplements. Similarly, Perry et al. (1967) in seven fattening and growing trials concluded that high urea supplements were not as satisfactory as those containing natural proteins in growing type rations consisting primarily of roughage. In contrast, urea could replace at least 90% of natural protein in high-energy fattening rations. Williams et al. (1969) in

a trial with 190 Angus cows grazing dry range grasses and supplemented with isonitrogenous feeds with urea or cottonseed meal demonstrated that cows fed urea lost more weight than those fed natural protein.

Rush et al. (1976) in trials with wintering beef cattle reported that cows fed 30% natural protein supplements lost less weight than cows receiving isonitrogenous supplements in which urea or biuret provided 50% of the nitrogen. In subsequent experiments, Rush and Totusek (1976) noted that cows receiving natural protein supplements tended to lose less weight than cows fed NPN supplements.

Clanton and Brown (1971) compared the performance of calves fed 40% protein supplements while grazing native range. Supplements containing 3% urea produced similar gains to natural protein rations (0.25 vs. 0.26 kg, respectively), but those containing 6% urea gave significantly lower gains (0.19 kg). More recently, Clanton (1978) has summarized the Nebraska work with NPN supplements, and concluded that urea supplements are not as effective as all natural protein supplements for meeting protein requirements of growing calves wintered on native range.

In a review of more than 100 beef cattle reports which were classified by the energy level of the basal diets, the NRC (1976) concluded that despite many trials in which urea has been shown to be beneficial, the overall conclusion was that urea nitrogen was not equivalent in feeding value to protein nitrogen.

Methods of Improving Urea Utilization

If the major problem with urea is its rapid hydrolysis in the rumen, improvement in urea utilization should be approached by

reducing ammonia waste. According to Karr et al. (1961) this can be done by: (1) reducing the rate of urea hydrolysis in the rumen or converting urea to a less soluble form, (2) increasing the ability of rumen microorganisms to utilize available ammonia nitrogen, (3) increasing tissue utilization of ammonia nitrogen, and (4) increasing the amounts of urea recycled into the rumen.

The two first points seem to be more easily approached; consequently, the use of coated ureas, carbohydrate treated ureas, less soluble NPN products, urease inhibitors, adsorptive compounds and of ways to encourage more frequent consumption have been considered of primary interest.

Urease Inhibitors

Since rapid hydrolysis of urea in the rumen is the result of activity of bacterial urease, several attempts have been made to reduce urease activity. Clifford et al. (1968) studied the effects of barbituric acid, copper and nitrate ion on growth of lambs fed urea diets and concluded that none had any beneficial effects upon urea utilization. Loper et al. (1967a,b) evaluated several compounds for urease inhibition using in vitro and in vivo techniques. Copper sulfate, neomycin sulfate and bacitracin-MD depressed urea hydrolysis under in vitro conditions, however when tested with animals they failed to decrease rumen ammonia levels. Another compound, acetohydrozamic acid, has shown more effective characteristics in inhibiting urease activity (Streeter et al., 1969).

The use of subcutaneous injections of jack-bean urease (Glimp and Tillman, 1965; Sidhu et al., 1968) resulted in reduced ureolytic

activity and improved animal performance, however research results in this area are still inconclusive. Practical applicability and commercial feasibility of urease inhibitors have been questioned (Coppock, 1973).

Urea Derivatives

Biuret,¹ (U.S. Patent 2,861,886, 1958) a product of urea condensation, is less soluble than urea and consequently less toxic. The slow degradation of biuret in the rumen should theoretically allow a better utilization of ammonia, especially when fed with low-quality roughages. However, biuret requires a long period of adaptation. Johnson and Clemens (1973) working with sheep noticed that biuretolytic activity began six weeks after the initiation of feeding, was high at twelve weeks and disappeared rapidly when biuret was removed from the ration. Oltjen et al. (1969) noticed that biuret-fed-steers required 21 days for adaptation.

Results from biuret supplementation of low-quality forages have been variable. A review of biuret research (Fonnesbeck et al., 1975) has indicated some advantage in using biuret instead of urea under such conditions. The NRC review (1976) noted that biuret supplementation of poor quality roughages produced responses equal to or better than urea. More recently Clanton (1978) has indicated that biuret was superior to urea, especially when dehydrated alfalfa is included in the ration. Nevertheless, other workers (Oltjen et al., 1969, 1974; Rush and Totusek, 1976) have found lower nitrogen utilization with

¹Manufactured by Dow Chemical Company, Midland, Michigan.

biuret than with urea when fed with low quality forages.

Urea phosphate has been another urea compound tested in an effort to find products which are better utilized and less toxic than urea. Urea phosphate is formed by the mole per mole combination of urea and phosphoric acid (U.S. Patent 3,180,735, 1965). Perez et al. (1967) using steers concluded that urea phosphate was not superior to urea but was less toxic. Oltjen et al. (1968) using the purified diet technique found that nitrogen retention of urea phosphate-fed-steers was inferior to those receiving urea, biuret or uric acid.

More recently, Imperial Chemical Industries (England) as mentioned by Mudd in UN-ECE (1977), has developed a product called isobutylidene diurea (IBDU) with a characteristic slow-release ammonia and 32% nitrogen content. It is suggested that during the release of urea, the other component, isobutyraldehyde, is converted to isobutyric acid. Research with the product in different European countries has shown an improved value of IBDU over that of urea as an NPN product in dairy and beef fattening rations.

Urea-Carbohydrate Products

Since one of the prerequisites for a high utilization of urea is the matched availability of carbohydrate to ammonia (Johnson, 1976), several researchers have studied different urea-carbohydrate complexes.

Starea² developed by Kansas workers (Deyoe et al., 1968) is formed by mixing finely ground grains with urea and processing the combination through a cooker-extruder under conditions of moisture, temperature and

²Manufactured by Far-Mar-Co, Hutchinson, Kansas.

pressure that cause starch to gelatinize. The final product generally contains 70% crude protein with 64% protein equivalent from urea. In vitro studies (Helmer et al., 1970) showed that starea markedly reduced the ruminal ammonia concentration when compared with supplements of unprocessed corn plus urea, suggesting improved utilization of ammonia. Bartley et al. (1973) compared several processed grain and urea products and found that starea was the only product that did not produce toxic signs. Helmer et al. (1970) found that starea was approximately equal to soybean meal as a protein supplement for lactating cows. However, Thompson et al. (1975) and Schmidt et al. (1974) compared starea with urea and found little difference in cattle performance or ration palatability. The varying levels of crude protein in the starea products used in these experiments (Helmer, 23%; Thompson, 41%; Schmidt, 50.2%) could explain some of the discrepancy in results.

A product similar to starea, with the difference that no moisture is involved, was developed by Muhrer et al. (1968). They claimed that at a high temperature (170°C) urea was changed to ammonia and cyanic acid which react with starch to form starch carbamate. They also stated that nitrogen from this product was released at a more desirable rate than from urea. No animal performance data was reported.

A similar method, an extrusion-expansion process,³ has also been developed involving the use of friction as the sole source of heat with no water or steam added. The product, called Golden-Pro,⁴ is a combination of sodium bentonite, urea and starch. The heat partially

^{3,4} Insta-Pro method and Golden-Pro product are patented by the Triple F Company, Des Moines, Iowa.

gelatinizes and expands the starch causing encapsulation of the mixture of urea and sodium bentonite. Iowa experiments (Pitzen et al., 1973) with calves showed the product to behave similarly to urea fed every two hours. Males and Johnson (1974) observed that processing of starch increases the rate of starch fermentation and thereby alters both rumen pH and rumen ammonia. The pH peak after feeding urea is lower when urea is fed with molasses or processed forms of starch than when accompanied by ground corn.

Other researchers have attempted to combine urea with less desirable carbohydrates. Ohio workers (Conrad and Hibbs, 1966) developed and tested with dairy cows a combination called Dehy-100⁵ composed of 66% dehydrated alfalfa, 31.6% urea, 2% monosodium phosphate and 0.4% sodium propionate (as a preservative) containing 100% crude protein equivalent. Milk yield of cows fed Dehy-100 were similar to those receiving soybean meal. It was assumed that the urea contained in the pellet was released more slowly in the rumen.

Missouri workers (Daniels et al., 1974) have also reported slow release of ammonia from a urea-cellulose complex. The reaction between urea and the cellulose source (solka-floc, newsprint, wheat straw, etc.) was enhanced by heating at 170°C under pressure. No animal performance data was reported.

Other urea-carbohydrate compounds of interest in European countries have been listed by Szentmihalyi in UN-ECE (1977). Urebetin-I contains sugar beet pulp, molasses, 9% urea and a small amount of ammonia sulphate. Neobetin is similar to the former but with more

⁵Dehy-100 is patented by Ohio State University.

ammonium sulphate and less molasses. Another compound is the so called "Fatty acid-Urea Adduct" which contains distilled fatty acids derived from animal and plant fats, and urea. Less prevalent products are Karbavitid and Furturo1bran. The former is elaborated by mixing urea with wine byproducts allowed to ferment and then dried and granulated whereas the latter is a byproduct of the furfuro1 production from maize cob enriched with urea.

Slow Release Urea Liquid Supplements

Since urea is the main source of nitrogen in liquid supplements for ruminants, several attempts have been made to develop safer and more efficient liquid supplements.

One such compound under the name of Nutrena CLS Controlled Release⁶ is formed by reacting urea with molasses in the presence of phosphoric acid. The reaction is conducted at 70 to 80°C for a 6-8 hr period and then neutralized with sodium hydroxide. It is claimed that the process interlocks molecules of urea and molasses which slow down the release of ammonia (U.S. patent 3,677,767, 1972). The supplement is formulated to a 32% crude protein level and includes mineral mix and vitamins. Trials in vitro and in vivo at the producer's laboratory have indicated that the product is safer, better utilized and easily handled.

Recently, Nebraska workers (Prokop and Klopfenstein, 1977) in cooperation with Liquid Feed Commodities, Inc. have developed a molasses based liquid supplement called SARU, possessing a form of slow release

⁶Nutrena - CLS is patented by Cargill, Inc., Elk Rivers, Minn.

ammonia. The supplement contains 36% crude protein with urea, molasses, water, vitamins and trace minerals. It is different from other liquid supplements in that it contains a small amount of formaldehyde per ton, which react with urea to form methylenediurea. Laboratory studies indicated that the mix has slower ammonia release than conventional urea-molasses liquid supplements but faster than soybean meal. Two feedlot trials showed that SARU was not different from conventional liquid supplements regarding animal performance although reduced toxicity was evident.

Bentonite

Sodium bentonite (an inert colloidal clay) has also been postulated to improve utilization of urea diets because of its great adsorptive capacity for water and certain cations. Martin et al. (1969) reported that when added at the 2% level to a high-roughage ration, bentonite increased nitrogen retention. From an in vitro study they were able to indicate that bentonite might adsorb ammonia when the concentration in rumen fluid is high and then release a portion when the ammonia concentration is decreased.

Coated Ureas

Various forms of coating have been applied to prilled urea in efforts to sustain the release of ammonia. Agronomists have tried to develop slow-release nitrogen fertilizers by using compounds such as polyethylene, plastic, resins, vinyl acetate, asphalt, parafin compounds, waxes and plasticized sulphur (Oertly and Lunt, 1962; Brown et al., 1966). Brown et al. (1966) found that resin coatings were

very effective in controlling the availability of nitrogen from urea applied to moist soil. Release rates were controlled by the coating thickness. Tennessee Valley Authority researchers (Allen et al., 1968; May, 1970) detailed an effective urea coating procedure with elemental sulphur and wax. The coating was produced by placing preheated granular urea in a pan granulator and spraying it with molten sulphur; the coating thickness depended on the length of time that the granules remained at the pan. After coating with sulphur, the granules were sealed with wax.

Johnson et al. (1962) reported one of the first attempts at coating of urea to improve utilization in ruminants. Prilled urea was coated with 20 different fat and waxy type materials and six varying amounts of $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$. Three waxy coating materials appeared to reduce ammonia release in vitro. When offered at low levels in urea supplements, coated urea materials were well accepted but at a 12% urea level animals preferred the uncoated material. No animal performance data with these products was obtained. CuSO_4 coated urea inhibited urease activity but also inhibited rumen microorganisms. Animal acceptance and growth data were not different for coated than regular urea prills.

Kunkle (1970) coated prilled urea with a high-melting point hydrogenated tallow and measured the effects of coating on rumen ammonia, blood ammonia and blood urea of wethers fed the coated material. No performance data was reported. The 24% tallow coated urea produced lower blood ammonia and urea levels and lower rumen ammonia values than the other rations. He concluded that the coating was effective in slowing the hydrolysis of urea. Gutcho (1973) in a description of urea related patents, mentioned two encapsulated ureas. Hansen (U. S. Patent

3,295,984, 1967) multicoated urea with several layers of copolymer of dicyclopentadiene and an ester of unsaturated acid. He claimed a 6-hr NH_3 release of 61% and a 24-hr release of 92%. Kohl (U. S. Patent 3,617,798, 1971) coated various NPN materials including urea with finely divided natural diatomaceous earth particles and a palatability improver such as molasses. No other information is available.

Sulfur and starch coated ureas also have been tested by Umunna and Woods (1970). Rumen ammonia was not reduced significantly by a compacted starch-urea complex as compared to urea alone. Sulfur coatings of urea, varying between 20 and 30% of weight, two of them including 3% of wax, did not affect nitrogen retention and dry matter digestibility, although when compared to urea, the thicker coatings significantly reduced the concentration of ruminal ammonia. Ward and Cullison (1970) used ethyl cellulose to coat urea. As compared to urea, the coated material produced lower blood ammonia values, was less toxic and was consumed more readily. Again, no growth data was reported.

Recently, Huston et al. (1974) studied by in vitro and in vivo evaluations a carboxy resin pelleted urea. Several mixtures containing urea and several combinations of starch and carboxy resin were initially tested for release of ammonia in artificial saliva and rumen fluid. On this basis, pellets for subsequent studies were formed by dry mixing ingredients (50% urea, 40% corn starch and 10% carboxy resin), adding small amounts of water to form a soft dough and extruding it through a die and finally drying and cracking the resulting particles to 3 to 6 mm lengths. After a nitrogen balance trial and two growth trials with lambs fed a medium fiber diet, they concluded that the coated urea reduced the rate of release of urea and improved nitrogen retention. Gains

of animals fed slow-release urea tended to be slightly less than gains of animals fed cottonseed meal but were statistically superior to urea-fed animals.

Criteria for Evaluating Slow-Release Compounds

An ideal slow-release urea compound is a combination of products not harmful to the ruminal environment, with no peak of ammonia accumulation into the rumen but a slow and continued ammonia release over a 12 to 24-hr period (Males and Johnson, 1974a). However, it should be pointed out that a reduced rate of urea hydrolysis is not necessarily synonymous with increased microbial protein synthesis and that stable ammonia levels are simply one of the many mechanisms involved in that.

Since measurement of microbial protein synthesis is difficult, evaluation of the slow-release urea compounds in the laboratory is normally based on rates of ammonia release. Several techniques have been used. Males and Johnson (1974a,b) utilized a buffered urease solution (Johnson et al., 1962) and the Ohio in vitro fermentation system (Karn et al., 1967). Huston et al. (1974) used a modification of artificial saliva (McDougall, 1948) and the artificial rumen procedure (Huh-tanen et al., 1954). Water leaching has also been used in some instances. All these procedures incubate the test compound with the medium and withdraw sequential samples for ammonia or urea analysis.

In vivo evaluation can be done with either fistulated or intact animals. Ruminally cannulated animals can be fed with the compound under study and the rumen fluid sampled in a sequential manner for certain periods after feeding for ammonia content (Owens et al., 1979). The Nylon Bag Technique (Johnson, 1969) has also been utilized. Samples of

the compound have been suspended intraruminally for different periods of time and dry matter and protein disappearance determined (Owens et al., 1979). With intact animals, interval rumen fluid samples can be obtained through stomach tubing for ammonia analysis, after feeding the supplements containing the compound (Lusby et al., 1977).

Besides monitoring ruminal ammonia, other parameters such as digestibility, nitrogen balance and performance have been used as have been reported by Mizwicki et al. (1974), Owens et al. (1979) and Lusby et al. (1977).

Comparative evaluation of different urea compounds has been reported by Males and Johnson (1974a,b). They tested in vitro two commercial gelatinized starch-urea products, a commercial slow-release urea liquid supplement and sulfur coated fertilizers. The first two showed the same ammonia release rate as the corn-urea mix whereas with the liquid supplement only 40% of the nitrogen present as urea was released and with the sulfur coated fertilizers about 30% was released. When the same products plus corn-urea and molasses-urea mixes were tested in vivo, results generally confirmed the in vitro results; therefore, in the authors' opinion, none of these commercial products were in fact, sustained ammonia release products.

CHAPTER III

A SLOW-RELEASE UREA COMPOUND FOR WINTER SUPPLEMENTATION OF LACTATING RANGE COWS

Summary

A new slow-release urea compound (SRU), which previously, under in vitro and in vivo conditions, released ammonia slowly in the rumen and reduced the potential for urea toxicity, was evaluated under range conditions.

In a 92-day winter trial, 85 lactating Hereford cows were individually fed five different supplements. Cows grazed a common pasture of native Oklahoma range and were gathered each morning for individual supplementation. Supplements contained: 15 and 40% protein from soybean meal and corn grain (negative and positive control, respectively), 40% protein (62.5% of the crude protein equivalent from SRU), 40% protein (same as the former but with urea), and a 20% protein supplement with urea but fed at twice the daily rate as the 40% supplements. The first four treatments were fed at a rate of 1.22 kg/head/day and the latter at a rate of 2.44 kg/head/day. The SRU supplement was fed in a meal form.

No supplement refusals were observed in cows fed the natural protein or SRU supplements, whereas cows fed urea supplements (20 or 40%) consumed only about 60% of the amount offered. Ruminal fluid

samples taken by stomach tubing at 1-hr and 4-hr after feeding showed that SRU produced patterns of ammonia release similar to those of soybean meal. Ammonia levels from both urea supplements (20 and 40%) at 1-hr were 2-2.5 times ($P < .05$) that of SRU whereas levels were not different at 4-hr. Forage consumption, estimated with the use of markers, were: 7.0, 9.3, 8.4, 8.5 and 8.0 kg for the 15 and 40% soybean meal, 40% SRU, 40% urea and 20% urea supplements, respectively. Positive control differed ($P < .05$) from the negative control and the 20% urea treatment. Forage digestibilities followed a trend similar to that of intakes.

Cow daily weight change (kg) for the above named treatments were -0.98, -0.39, -0.74, -0.85 and -0.85, respectively. Positive control was different ($P < .05$) from all the other treatments in that regard. Both urea and SRU treatments had similar responses and were different ($P < .05$) from the negative control. Calf daily gains (kg) were: 0.39, 0.50, 0.44, 0.37 and 0.36, respectively. Positive control being different ($P < .05$) from the urea and negative control treatments. Cow rebreeding performance was poorest for the negative control and highest for the positive control with SRU and urea treatments intermediate.

Results of this experiments indicated that although SRU improved palatability of the supplement and effectively slowed ammonia release from urea, its inclusion on range supplements did not improve urea utilization.

Introduction

A necessary condition to improve urea utilization in the rumen is to adapt the rate of NH_3 release from urea to match the capacity

of ruminal bacteria for protein synthesis (Loosli and McDonald, 1968; Chalupa, 1973; NRC, 1976).

Several attempts to achieve slow release have been reported. They have varied widely, not only in method but also in degree of success. To date, no slow release method has received acceptance under practical conditions; prilled urea continues to be the most commonly used NPN source for ruminants around the world (NRC, 1976; UN-ECE, 1977).

Approaches to study and to attenuate ruminal ammonia production from NPN sources, mainly from urea, have ranged from closely controlled experimental techniques such as rumen infusions or continuous feeding techniques, to use of different urea compounds and urease inhibitors (Clifford et al., 1968; Sidhu et al., 1968).

With the use of ruminal infusions or controlled feeding techniques, it has been found that utilization of urea is increased with more frequent supply when it is accompanied by an energy source (Pitzen et al., 1973; Prior, 1974; Mizwicki et al., 1976; Kropp et al., 1977), but when urea is fed or infused more continuously but independently of the energy source, little improvement for limit fed ruminants has been observed (Streeter et al., 1973; Knight and Owens, 1973; Mizwicki et al., 1978).

From this basic research, it is clear that once or twice daily feeding of a supplement containing prilled urea is far from being efficient, especially if the energy consumption is limited and the remainder of the ration consists of low-quality forages, as is the case with wintering range beef cows. Therefore, the attenuation of urea hydrolysis has been considered an obligatory step to improve NPN utilization.

Combinations of urea and starches (Deyoe et al., 1968; Muhrer et al., 1968; Pitzen et al., 1973), urea and cellulose (Conrad and Hibbs, 1966; Danniels et al., 1974), urea and molasses (Cargill Inc., 1977; Prokop and Klopfenstein, 1977); urea and fatty acids (UN-ECE, 1977) are potentially useful. Also, urea derivatives such as biuret, isobutylidene diurea (UN-ECE, 1977), urea phosphate and even the coating of urea with different substances (Johnson et al., 1962; Allen et al., 1968; Kunkle, 1970; Umunna and Woods, 1970; Ward and Cullison, 1970; Huston et al., 1974) have been considered.

Recently, NIPAK Corporation has developed a new coated urea (U.S. patent pending) using a mixture of tung and linseed oils plus talc to cover individual urea granules. Previous work (Owens et al., 1979) had shown that this compound releases ammonia slowly, enhances the acceptability of urea-containing diets, and reduces the toxicity potential of urea.

The objective of the present research was: (1) to evaluate under range conditions, using lactating beef cows, the theory that the slow-release of ammonia from urea will improve NPN utilization, and (2) to test the effect of supplemental energy for lactating beef cows grazing a low-quality forage.

Experimental Procedure

The trial was conducted during the 92-day period from November 15, 1976 to February 15, 1977. Eighty-five fall-calving Hereford cows were randomized by weight and allotted to five treatments. Animals grazed a single pasture of native tallgrass range in central Oklahoma, where the predominant forage species are little bluestem (Schizachyrium

scoparium), switchgrass (Panicum virgatum), big bluestem (Andropogon gerardii) and indiagrass (Sorghastrum nutans). Nutritive characteristics of mid-winter hand-clipped mixed prairie grass are presented in Table I.

Six mornings each week, animals were gathered from the pasture, and individually offered one of the five supplements for approximately 30-45 minutes in covered stalls. Orts were weighed each day. Supplement treatments and amounts (kg) offered per head per day (based on 7 days/week) were: (1) 15% natural protein (negative control), 1.22 kg, (2) 40% natural protein (positive control), 1.22 kg, (3) 40% crude protein (62.5% of the crude protein equivalent from slow release urea (SRU)), 1.22 kg, (4) 40% crude protein (62.5% of the crude protein equivalent from urea), 1.22 kg, and (5) 20% crude protein (62.5% of the crude protein equivalent from urea), 2.44 kg. Treatments 4 and 5 were isonitrogenous but 5 furnished twice the energy of 4. Ingredient composition and actual crude protein content of the supplements are shown in Table II.

The slow release urea compound (coated urea) used in this experiment was manufactured by NIPAK, Inc. Prilled feed grade urea was mixed in a portable cement mixer with 0.5% talc. Subsequently, an oil mixture formed from 10% linseed oil, 89% tung oil, 0.5% manganese octanoate and 0.5% cobalt octanoate was slowly dripped in small quantities onto the prilled urea-talc mixture as the mixer was revolving. During the addition of the oil mixture, heated air was constantly blown into the mixer to facilitate drying.

Since coating thickness could be modified to attain any attenuated release rate desired, the nitrogen content of the product can vary.

TABLE I
 CHEMICAL COMPOSITION AND DRY MATTER DIGESTIBILITY
 OF WINTER RANGE FORAGE^a GRAZED BY
 LACTATING COWS

Items ^b	%
Field dry matter	93.6
Neutral detergent fiber	79.7
Acid detergent fiber	52.8
Cellulose	34.2
Hemicellulose (NDF-ADF)	26.9
Lignin	13.6
Crude Protein	3.8
Ash	9.0
Phosphorus	0.029
Calcium	0.52
Potassium	0.35
<u>In Vitro</u> DMD ^c	30.3

^aAnalysis represent the average of eight samples of the mixed prairie collected in January, 1977. It includes the entire aerial portion of the plants.

^bItems are expressed in dry matter basis.

^c48 hour fermentation and 24 hour pepsin digestion, modified Tilley and Terry (1963) procedure.

TABLE II
INGREDIENT COMPOSITION OF PROTEIN SUPPLEMENTS FED TO LACTATING RANGE COWS

Ingredient (%)	International Reference Number (NRC, 1971)	15% Natural Protein	40% Natural Protein	40% Crude Protein (SRU)	40% Crude Protein (Prilled Urea)	20% Crude Protein (Prilled Urea)
Corn, ground	4-02-915	53.80	---	43.30	43.60	69.50
Alfalfa hay, ground	1-00-118	15.00	5.00	15.00	15.00	7.50
Cottonseed hulls	1-01-599	10.00	5.00	---	---	11.00
Soybean meal	5-04-604	16.90	85.25	19.10	19.40	---
Cane molasses	4-04-696	---	---	6.00	7.00	4.00
Dicalcium phosphate	6-01-080	0.89	0.50	1.00	1.00	1.00
NaH ₂ PO ₄	6-04-287	2.70	2.20	2.70	2.70	1.35
Na ₂ SO ₄	6-04-292	0.75	2.00	2.35	2.35	1.17
Trace mineral premix		0.05	0.05	0.05	0.05	0.05
Urea ^a		---	---	---	8.90	4.50
Coated urea (SRU) ^b		---	---	10.50	---	---
Vitamin A (30,000 IU/g)	7-05-143	0.12	0.12	0.12	0.12	0.12
Actual crude protein content (%) (DM basis)		13.9	39.9	41.5	41.7	20.8
TDN content (%)		63.4	63.9	60.7	61.7	62.8

^a281% crude protein equivalent (45% N)

^b237.5% crude protein equivalent (38% N)

However, NIPAK researchers (1976) observed that only coating thickness, which made the coating between 15 and 20% of total weight, offered adequate release, therefore, the product used in this and subsequent research contained 38% N.

Cows were visually scored for body condition (degree of fatness) at the beginning and end of the trial. A scale 1-10 was used with 1 equal to very thin and 10 being very fat. Cows weights were taken after overnight withdrawal from feed and water. The initial weights of 10 cows that had not calved at the start of the trial were adjusted to a calved basis by using a regression equation (Ewing et al., 1966) derived from previous trials with similar cows. The following equation was used:

$$\text{Adjusted initial weight} = \text{Actual initial weight} - [(\text{Calf birth weight} \times 1.9697) - 19.0].$$

Calves were weighed at birth and at the end of the experimental period, following six hours separation from their dams. Cows were artificially inseminated over a 30-day period (January 2-February 1) and subsequently exposed to a bull for 15 days. Estrus was detected using sterile teaser bulls with chin-ball markers during the A.I. period and with breeding bulls during the natural service period. Pregnancy was determined by rectal palpation approximately 60 days after the end of the breeding season.

At the end of December, on 2 consecutive days, 69 randomly selected cows from each treatment were ruminally sampled 1 hr post-feeding and 64 were sampled 4 hr after feeding. Rumen liquor samples were taken via stomach tube using a modification of the technique proposed by Raun and Burroughs (1962). A metal mouth piece was used to drive a

2 cm diameter transparent polyethylene hose through the mouth into the esophagus and the cranial region of the reticulo-rumen. The hose did not carry any strainer; only 4-6 small holes (ca. 0.3 cms. diameter) were opened in the distal end to facilitate the movement of the liquids into the hose. Suction was generated using a common hand pump.

Microbial action was stopped in ruminal fluid by adding 5 g of meta-phosphoric acid per 50 ml of rumen fluid. Samples were then frozen for later analysis for rumen ammonia nitrogen by a colorimetric method (Chaney and Marbach, 1962).

Forage intake was estimated in January by using chromic oxide as an external marker and acid insoluble ash (Van Keulen and Young, 1977) as an internal marker for determining the digestibility of the forage. Chromic oxide was administered at the rate of 10 g/head/feeding, fed twice daily (8:00 a.m. and 4:00 p.m.). To insure complete consumption of the marker it was mixed with 100 g of ground corn and offered immediately before meals. A seven-day adaptation period was allowed and 200 g fecal "grab samples" were taken from each cow at the time of feeding for the subsequent seven days. Samples were dried at 55°C, prepared according to the method suggested by Williams et al. (1962) and analyzed for chromium content by atomic absorption spectroscopy.

Cow-weight change, cow-condition scoring, calf weight gain, dry matter digestibility and forage intake were statistically analyzed by procedures outlined by Steel and Torrie (1960) for a Randomized Block Design with two missing values. Two cows were removed from the experiment, one due to refusal to eat the supplement, and the second due to an injury not related to the experiment.

Rumen ammonia results were analyzed as a Completely Randomized

Design following the procedure called Unweighted Analysis of Cell Means described by Snedecor and Cochran (1967, page 475). Estrus and pregnancy rates were analyzed by the Chi-square procedure (Steel and Torrie, 1960). Comparison between means in the different analyses were performed by LSD tests (Steel and Torrie, 1960).

Results and Discussion

Results are summarized in Tables III and IV. Analysis of variance tables for each of the parameters estimated in this experiment are presented in Tables XV and XVI of the Appendix.

The supplement containing coated urea proved highly palatable as no refusal of the SRU supplement was observed (Table III). Similarly, all offered feed was consumed by cows fed the soybean meal supplements whereas cows fed the prilled urea ate only 59 and 63% of the offered amounts of the 20 and 20% protein supplements, respectively. Thus, these cows consumed approximately 70 g of urea per day regardless of the energy content of the supplement. Actual supplementary nitrogen intake for each one of the treatments is presented in Table III.

Intake problems with urea-containing supplements have been widely reported (NRC, 1976; UN-ECE, 1977). If these refusals are caused by undesirable taste, odor or any other physiological problem is still a controversial matter. Huber and Cook (1972) suggested that rejection was due to an undesirable taste of urea and not ruminal or post-ruminal effects. Kertz and Everett (1975) also indicated that one of the reasons for limited urea utilization in dairy rations is its unpalatability at higher dietary levels due to flavor and/or odor.

However, other research is contradictory. Wilson et al. (1975)

TABLE III
RUMEN AMMONIA, FORAGE INTAKE AND DRY MATTER DIGESTIBILITY
OF RANGE LACTATING COWS FED FIVE DIFFERENT PROTEIN
SUPPLEMENTS

Item	Protein Source					SE ^a
	Natural	SRU	Urea			
Protein level, %	15	40	40	40	20	
No. cows	16	17	16	17	17	
Supplement consumed, kg/day	1.22	1.22	1.22	0.72	1.54	
Supplemental protein intake, g/day	169.6	486.8	506.3	300.2	320.3	
Forage intake, kg/day	7.0 ^d	9.3 ^b	8.4 ^{bc}	8.5 ^{bc}	8.0 ^c	0.32
Dry matter digestibility, %	33.1	38.3	35.6	34.9	32.1	1.68
Intake of indigestible dry matter, kg/day	4.7 ^c	5.7 ^b	5.4 ^b	5.5 ^b	5.4 ^b	0.13
Rumen ammonia, 1 hour, mg/dl	4.8 ^b	6.2 ^{bc}	10.4 ^c	25.0 ^e	18.3 ^d	1.93
Rumen ammonia, 4 hour, mg/dl	2.7 ^b	7.2 ^c	7.3 ^c	10.3 ^c	9.2 ^c	2.00

^aApproximate standard error.

^{bcd}Means on a line with the same superscript letter do not differ significantly (P<.05)

TABLE IV
PERFORMANCE OF LACTATING COWS FED ONCE DAILY WITH NATURAL PROTEIN,
SLOW RELEASE UREA (SRU) AND UREA SUPPLEMENTS

Item	Protein Source					SE ^a
	Natural	SRU	Urea	Urea	Urea	
Protein level (%)	15	40	40	40	20	
No. cows	16	17	16	17	17	
Supplement offered, kg/day	1.22	1.22	1.22	1.22	2.44	
Supplement intake, kg/day	1.22	1.22	1.22	0.72	1.54	
Initial cow weight, kg	429	429	427	428	424	
Cows daily weight change, kg	-0.98 ^d	-0.39 ^b	-0.74 ^c	-0.85 ^c	-0.85 ^c	0.042
Cow condition change	-3.5 ^c	-1.6 ^b	-3.0 ^c	-3.3 ^c	-3.4 ^c	0.22
Calves daily gain, kg	0.39 ^d	0.50 ^b	0.44 ^c	0.37 ^d	0.36 ^d	0.017
Cows showing estrus	9 ^d	17 ^b	12 ^{bd}	15 ^{bc}	10 ^{cd}	
Cows pregnant	7 ^c	16 ^b	8 ^c	13 ^{bc}	9 ^c	

^aApproximate standard error (assuming 17 observations per treatment).

^{bcd}Means on a line with the same superscript letter do not differ significantly (P<.05).

found that urea infused into the rumen at levels of above 2% depressed total ration consumption. They concluded that some physiological parameter other than taste depressed intake. Conrad et al. (1977) observed that urea infused into the rumen of goats did not modify the total daily feed intake but did shorten the meal length by 20 to 30%. Chalupa et al. (1978) concluded that decreased consumption of urea diets is not due to taste and/or odor, but results from post-ingestional aversion by associating a malaise with the flavor of urea diets.

In this experiment refusals of prilled urea supplements were larger during the last part of the trial, suggesting that the cows were able to "sense" urea levels and adjust intake accordingly, apparently in agreement with Wilson et al. (1975), Conrad et al. (1977) and Chalupa et al. (1978). Feed rejection with urea supplementation adds importance to the fact that SRU did not reduce supplement consumption.

Tables XVII and XVIII of the Appendix show some data used for calculating forage intakes and how the calculations were performed. Acid-insoluble ash (AIA) was chosen because it was suggested (Van Keulen and Young, 1977) that AIA estimates digestibility as accurately as the total collection method. Recent reports have confirmed this finding and also the superiority of AIA over lignin as an internal marker for forage rations (Thonney et al. 1978; Sherrod et al., 1978).

Daily grass intake was 2.3 kg more ($P < .05$) for the positive than the negative controls. This corroborates the generally observed effect of protein supplementation upon low quality forage consumption (Campling et al., 1962; Coombe and Tribe, 1962). This is in opposition to Rittenhouse et al. (1970) who concluded that the amounts of high protein supplements commonly fed in the mixed prairie region are too

small to influence either the intake or the digestibility of forage consumed.

Differences of forage intake between positive control cows and SRU and 40% urea fed cows were not significant. They were only 0.4 and 0.5 kg, respectively. Intakes of positive control treatment differed ($P < .05$) from 20% urea treatment in 1.3 kg.

Intakes of forage were not statistically different between cows fed SRU and those fed urea supplements. The small differences observed between the 20% urea treatment (fed 2.44 kg) and the other isoproteic treatments (fed 1.22 kg) can be attributed to displacement of forage by supplement in the rumen. If intake of low quality forage is limited by bulk fill, the supplement may occupy part of the space allowed to the forage dry matter and would thereby reduce forage intake irrespective of the effect of the supplement in forage digestibility. A comparison between intakes of indigestible dry matter for the different treatments (Table III) seems to corroborate the previous argument. It can be observed that only the negative control treatment differed ($P < .05$) in this regard of the rest of the treatments.

Effects of supplementation on forage digestibility (Table III) followed approximately the same trend that forage intakes took. Although the range of digestibility values was large (32.1 to 38.3%). Differences between treatments were not significant when the F test was effected. However, when an unprotected LSD test was performed it was found that the positive control differed ($P < .05$) from the negative control and the 20% urea treatments but not from the SRU and 40% urea.

Forage intake and digestibility data suggest that poor utilization of urea under range conditions cannot be attributed to deleterious

effects upon forage digestion and intake since feeding isonitrogenous natural protein or slowing the ammonia release of urea did not significantly modify these parameters. Similar results have also been reported by Mizwicki et al. (1978) in trials with fistulated steers.

Although the SRU product had been tested for slow ammonia release characteristics both on in vitro and in vivo conditions (NIPAK, 1976; Owens et al., 1979), such properties were tested again in this experiment for the grazing conditions used.

Rumen ammonia levels are shown in Table III. Results show that sources of nitrogen altered ruminal ammonia levels at both 1 hr and 4 hr after feeding. Ammonia levels of SRU were slightly higher, not statistically different, than those of the positive control at 1 hr, and almost identical at 4 hr.

Rumen ammonia levels of the prilled urea fed cows were about 225% greater ($P < .05$) than those of SRU cows at the 1 hr sampling time and numerically greater at 4 hr post-feeding. The rapid decline in ammonia levels of prilled urea (50-60% in 3 hours) and consequential nitrogen wasted has been frequently reported and is often considered the main problem of urea utilization (Chalupa, 1968, 1973; Helmer and Bartley, 1971; Johnson, 1976).

Energy content of the urea supplements had little effect upon rumen ammonia levels. Nevertheless, slightly lower ruminal ammonia levels among cows fed the high energy (20% urea) treatment at 1 hr post-feeding might suggest more efficient utilization of ammonia immediately after feeding as the amount of nitrogen ingested for these cows and for those fed the 40% urea supplement was the same (Table III).

Lactating cows fed the 40% natural protein supplement lost consi-

derably less ($P < .05$) weight during the 92 day feeding period than those fed the 15% protein supplement or the urea supplements (Table IV). The three urea treatments (SRU, 40% and 20% urea) resulted in less weight losses ($P < .05$) than the negative control but were not statistically different of each other, weight loss of cows fed these three treatments was approximately twice as great as those of cows fed the positive control supplement.

Slow-release urea tended ($P < .10$) to produce less weight loss than urea although performance was still poor. According to previous Oklahoma research with the same type of cows (Turman et al., 1965; Hughes et al., 1978), the amount of weight loss observed with the three urea treatments would be borderline for maintaining adequate reproductive performance. The results obtained then suggest that urea, even in an improved form such as SRU, remained inadequate for lactation and reproduction.

Satter and Roffler (1975) concluded that high producing dairy cows should not be fed non-protein nitrogen during early lactation since their protein requirements greatly exceeds the potential microbial synthesis. Kwan et al. (1977) on the contrary, proposed the use of urea in early lactation, although they stated that urea must be used with high-energy complete rations containing ingredients of low nitrogen solubility. Since range beef cows are generally supplemented with restricted amounts of a high-protein low-energy mixture fed once daily, low milk yields of beef females fed urea might be expected.

Level of energy in the supplement did not affect weight changes among urea fed cows. Although better utilization of urea can be expected with a greater availability of fermentable carbohydrates

(Burroughs et al., 1974; Johnson, 1976), the improvement obtained might be masked by the slight reduction in forage intake caused by the increased supplementary energy as already discussed.

Conformation scoring followed trends observed with cows weight change. Cows fed the positive control supplement lost less condition ($P < .05$) during the experimental period. The remaining treatments were not statistically different in condition score, although the urea treatments tended to be lower than the SRU treatment, in agreement with the weight loss difference.

Calf gain, as expected, followed the same pattern as cow weight change. Mean calving dates for each treatment were all within six days so the groups were uniform. Calves of cows in the positive control group had higher gains ($P < .05$) than any other treatment whereas those of cows fed prilled urea and the negative control had the lowest gains ($P < .05$). Calves of cows fed SRU gained more ($P < .05$) weight than those of dams fed prilled urea or the negative control supplement.

Past research in Oklahoma (Pope et al., 1963) found a high relationship ($r = 0.65$ to 0.80) between the amount of milk produced and the weight of fall born calves; therefore, results would indicate greater milk production of SRU fed cows than urea-fed cows. This was reflected not only in the calf weight but also in the tendency ($P < .10$) of these cows to have less weight losses.

All the cows fed the 40% positive control exhibited estrus and 94% were pregnant at palpation 60 days after breeding. Only 56% of the cows fed the negative control showed estrus and 44% were pregnant.

Among the urea treatments, no statistical differences were

observed, although the 40% urea treatment had slightly greater pregnancy and estrus rates. This treatment did not differ from the positive control while the SRU and the 20% urea were lower ($P < .05$) than the positive control fed cows. Estrus rates were 88, 75 and 59 and pregnancy rates were 76, 50 and 53% for the 40% urea, SRU and 20% urea treatments, respectively. The good performance of the 40% urea fed cows is difficult to explain, especially as related to cow weight loss which did not differ statistically between those three treatments.

Urea feeding per se has no direct deleterious effects upon re-breeding performance (Word et al., 1969; Ryder et al., 1972; Erb et al., 1977). Therefore, differences in post-partum estrus and pregnancy rates between the positive control and the urea treatments observed in this experiment are related to the losses in body condition of the cows and the indirect effects of nitrogen source on energy and protein availability.

Data obtained in this experiment indicate that SRU improved palatability of urea-containing supplements and effectively slow ammonia release from urea. However, SRU inclusion on range supplements did not improve urea utilization which would indicate that other factors rather than merely a steady supply of urea might be limiting use of urea with low-quality forages. The absence in urea diets of certain nutritional factors such as some amino acids, branched chain volatile fatty acids, minerals or vitamins should be considered more important than a continuous ruminal ammonia supply, for adequate bacterial utilization of NPN under range conditions.

CHAPTER IV

A SLOW-RELEASE UREA COMPOUND FOR WINTER SUPPLEMENTATION OF HEIFERS AND PREGNANT COWS CONSUMING LOW-QUALITY FORAGES

Summary

A new slow-release urea compound (SRU), a coated urea, was compared with natural protein and prilled urea in winter supplements for dry, pregnant cows on range and heifers fed mature fescue hay in drylot.

In trial 1, 80 cows were group-fed 15 or 40% protein supplements (from soybean meal), a 40% protein supplement with SRU furnishing 62.5% of the protein equivalent or a 40% protein supplement with urea instead of SRU. Weight gains of cows fed the 40% soybean meal supplement did not differ from that of cows fed SRU. Cows fed the 15% soybean meal and urea had the lowest gains.

In trial 2, 88 cows were individually-fed eight supplements with varying levels of energy, containing soybean meal (S), urea (U) or coated urea (SRU). Crude protein (%), supplement/head/day (kg) and protein equivalent from NPN (%) were: S, 15, 0.9, 0 (negative control); S, 20, 1.8, 0 and S, 40, 0.9, 0 (positive controls); U, 20, 1.8, 62.5; U, 40, 0.9, 62.5; SRU, 20, 1.8, 62.5; SRU, 40, 0.9, 62.5 and SRU, 70, 0.5, 75. Weight gains were greater ($P < .05$) for cows fed 40% natural protein than those fed 40% SRU or urea treatments. Responses to

natural protein were not affected by level of energy supplementation. Prilled urea and SRU produced very similar performance which tended to result in greater gains when fed with higher levels of energy. Intake of SRU supplements was not different from that of natural protein whereas urea fed cows ate 3 to 5% less ($P < .05$) supplement. Ruminal ammonia of cows fed SRU and those fed natural protein were similar but lower ($P < .01$) than from urea fed cows. Added energy reduced ruminal ammonia levels greatly for cows fed urea, slightly for cows fed SRU and very slightly for cows fed natural protein.

A third trial used 56 heifers fed one of four supplements: 20 and 40% natural protein or 40% protein supplements where urea or SRU furnished 62.5% of the crude protein equivalent. Prilled urea and SRU were again poorly utilized as compared to the positive control ($P = .08$). No differences of hay intake ($P = .54$) and feed efficiency ($P = .31$) were observed.

From these experiments it is possible to conclude that the use of SRU improved the palatability of the supplements but did not consistently improve animal performance. It is probable that besides a sustained level of ammonia, rumen bacteria require other factors such as some amino acids, branched VFA's, minerals, and vitamins not present in urea diets.

Introduction

Utilization of urea by ruminants grazing or fed low-quality forages is poor when compared to natural protein sources (Nelson and Waller, 1962; Williams et al., 1969; Rush and Totusek, 1976; NRC, 1976; Clanton, 1978). Under such conditions, not only is energy insuffi-

cient but also the rate of energy availability does not match the release of ammonia from urea (Johnson, 1976).

Since energy supplementation under grazing conditions is limited by cost and by reduced forage intake (Cook and Harris, 1970; Rittenhouse et al., 1970; Lusby et al., 1976), improving urea utilization has been directed toward attenuating ammonia release rate to maintain a more constant level of ammonia into the rumen in order to more closely parallel the cell wall carbohydrate fermentation rate. Although several methods of slowing ammonia release have been proposed, none has been accepted either from a functional or an economical point of view.

Recently, NIPAK Corporation has developed a coated urea product (U.S. Patent pending) using a mixture of tung and linseed oils plus talc. Details of the manufacturing process are given in Chapter III. This compound when tested in laboratory trials (Owens et al., 1979) had shown to produce a slow and sustained release of ammonia in the rumen and to be much safer than urea in toxicity studies.

A previous experiment with the same compound under range conditions (Chapter III) had shown little advantage of slowing the ammonia release from urea to improve its utilization with lactating beef cows. However, since SRU might be utilized more readily by other beef females whose nutritional requirements are lower, the product was tested with pregnant cows and heifers.

The purposes of these experiments were: (1) to evaluate the effects of slow ammonia release under practical conditions by feeding coated urea (SRU) to heifers and pregnant cows eating low-quality forages and (2) to study the effect of the energy level in the supple-

ment on the utilization of urea and coated urea as compared to natural protein.

Experimental Procedure

Trial 1

Eighty mature Hereford, dry-pregnant cows were utilized in this experiment. Animals grazed five pastures of about 60 hectares each with cows rotated among pastures once every two weeks to remove pasture effects.

The trial period was 60 days from November 15, 1976 to January 14, 1977. Although the experiment was scheduled to last 100 days, it was stopped in mid-January because drought conditions the preceeding summer greatly limited forage growth. Main forage species and nutritive quality of the prairies used were described previously (Chapter III).

Cows were randomized by weight and allotted to the following five treatments: (1) 15% protein (soybean meal) supplement, negative control; (2) 40% protein (soybean meal) supplement, positive control; (3) 40% protein SRU supplement (62.5% of the crude protein equivalent from coated urea); (4) same as 3, except the supplement was in meal form; and (5) 40% protein supplement (62.5% of the crude protein equivalent from prilled urea). All supplements were offered in 0.5 cm diameter pellets with the exception of supplement 3. Supplement 3 was unpelleted to study possible damage to the SRU coat during the pelletting process. Composition of the supplements used was identical that of the respective supplements used in the lactating cow trial (Chapter III, Table II).

Cows were group-fed the supplement in bunks located in the respective pastures. Feeding was effected six days per week, prorated to make 7 days effective supplementation equal to 0.9 kg/head/day.

As in other experiments, cow weights were taken approximately every four weeks after overnight feed and water withdrawal. The data obtained were analyzed by the Randomized Block Design with missing data procedure outlined by Steel and Torrie (1960), where animals were considered as replications according to the allotment effected. The analysis of variance table is shown in Table XIX of the Appendix. Two cows were removed from the experiment; one died 10 days after the beginning of the experiment for unknown reasons and the other (a negative control cow) was removed due to sickness. Statistical comparisons between means were performed by LSD tests (Steel and Torrie, 1960). Bartlett's test for homogeneity of variance (Steel and Torrie, 1960, page 347) was performed to study the possible variation in cow weight gain associated with individual acceptance of supplementary sources.

Trial 2

Eighty-eight dry pregnant Hereford cows were randomized by weight and allotted to eight protein treatments. The experiment was designed to further evaluate the response of pregnant cows fed the SRU under an individual feeding scheme and to study the effect of increasing the intake of supplementary energy levels upon utilization of three different protein sources: natural protein (soybean meal), SRU (coated urea) and urea.

The trial period lasted 100 days from December 5, 1977 to

March 15, 1978. Snow partially or completely covered the ground during 47 days in January and February which forced the feeding of 6.8 kg/head/day of good quality (11% CP) prairie grass hay to all the cows for 16 days.

Cows grazed a common pasture of native tall grass similar to that described in Chapter III. Samples of mixed prairie vegetation were collected at the beginning of the trial and nutritive characteristics evaluated (Table V).

The eight treatments included six isoproteic supplements where each protein source was fed at two levels of energy (0.9 kg of 40% crude protein/day supplying approximately 0.6 kg/day of TDN and 1.8 kg of the 20% crude protein/day supplying approximately 1.25 kg/day of TDN). Urea or SRU supplied 62.5% of the crude protein in supplements containing urea.

In addition, a negative control (15% protein) and a very high protein supplement (70% protein) were evaluated. This latter with 75% of the crude protein from SRU. Those supplements were fed at rates of 0.9 and 0.52 kg/head/day, respectively.

The supplements in the present trial were formulated to contain the closest amounts of energy possible and to contain 1, 2 or 3% potassium according to the amount of supplement fed. Potassium has been recently found to be an important factor in the utilization of urea on range supplements and chemical analysis of native dry vegetation grazed for cows in central Oklahoma range has shown very low potassium content (Tables I and V). Ingredient composition of the supplements is presented in Table VI.

Cows were gathered six days each week at 8 a.m. and individually

TABLE V
 CHEMICAL COMPOSITION^{a,b} AND DRY MATTER DIGESTIBILITY
 OF WINTER RANGE FORAGE GRAZED BY
 PREGNANT COWS (TRIAL 2)

Items	%
Field dry matter	91.4
Neutral detergent fiber	78.1
Acid detergent fiber	52.2
Hemicellulose (NDF-ADF)	25.9
Crude Protein	2.5
Ash	7.3
Phosphorus	0.013
Calcium	0.38
Potassium	0.29
<u>In Vitro</u> DMD ^c	26.9

^aAnalysis represent the average of six samples of the mixed prairie collected in the area grazed in December, 1977. Samples were hand-collected and included the entire aerial part of the plants.

^bItems are expressed in dry matter basis.

^c48 hour fermentation and 24 hour pepsin digestion, modified Tilley and Terry (1963) procedure.

TABLE VI
INGREDIENT^a COMPOSITION OF PROTEIN SUPPLEMENTS FED TO PREGNANT RANGE COWS (TRIAL 2)

Ingredient %	International Reference Number	Natural Protein			Urea		Coated Urea		
		Protein %			Protein %		Protein %		
		15	20	40	20	40	20	40	70
Corn, ground	4-02-915	61	49	---	76.8	53.3	77	52.6	33.0
Cottonseed hulls	1-01-599	4	6	---	5.2	---	5.2	---	---
Soybean meal	5-04-604	20	33	87.5	---	21	---	19.9	33.0
Alfalfa hay, ground	1-00-118	6	6	8	6	6	5	6	---
Cane molasses	4-04-696	5	5	2.5	5	6	5	6	5
Sodium sulfate	6-04-292	2	1	2	1.1	2.3	1.2	2.3	3.5
Trace mineral mix		0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.1
Potassium chloride	6-03-756	2	---	---	1.6	2.4	1.5	2.4	4.1
Urea ^b		---	---	---	4.3	8.9	---	---	---
Coated urea ^c		---	---	---	---	---	5.2	10.7	22.0
Vitamin A (30,000 IU/g)	7-05-143	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12
Actual crude protein content (%) (DM Basis)		14.7	18.5	37.2	19.4	39.2	19.4	39.5	64.4
TDN Content (%)		72.8	74.2	74.5	70.3	66.6	70.0	65.1	58.4

^aPhosphorus offered free choice in mineral feeders containing a 1:1 mixture of salt: dicalcium phosphate.

^b281% crude protein equivalent (45% N).

^c237.5% crude protein equivalent (38% N).

fed in a covered stall barn one of the eight different supplements at rates corrected for seven-day per week feeding. Thirty to forty-five minutes were allowed for intake of the supplements with orts weighed daily.

Cow weights were recorded approximately every 28 days after withdrawals of feed and water overnight. At the end of December, ruminal fluid samples for ammonia analysis were taken from all the cows 1 hour and 4 hours after feed consumption in a way similar to that described in Chapter III.

Statistical analyses of the parameters studied (cow weight change, supplement consumption and rumen ammonia levels) were conducted by Least Square Analysis. Two different set of analysis were performed. The first, involved all the eight treatments (Table XX of the Appendix) allotted to a Randomized Block Design whereas the second involved only six treatments (Table XXI of the Appendix) allotted to a Randomized Block Design with a 3 x 2 Factorial arrangement of treatments. Factors were protein source (3 levels) and energy level (2 levels).

Treatment effect was tested by F tests. If significant, treatment sum of squares (7 df) was partitioned into several of its components to test for interaction effect (protein source x energy level). If no interaction was found, preplanned comparisons between treatment effects were made by LSD tests. If interaction was present, the following preplanned comparisons between simple effects were performed: urea (40% supplement) vs urea (20%); natural protein (40%) vs natural protein (20%); urea (40%) vs natural protein (40%); urea (40%) vs SRU (40%) and SRU (20%) vs natural protein (20%). Again, LSD tests

were utilized.

Negative control treatment (15% protein) was compared to the positive control (40%) and SRU (70%) was compared to SRU (40%) by corresponding LSD tests.

Cow weight change and supplement consumption had seven missing data. Seven cows were removed from the experiment. Five of them calved 12 to 20 days before finishing the experiment so an accurate final weight data could not be obtained. One other cow was injured and the other suffered a severe mastitis. Ruminal ammonia levels had two missing data because the ruminal samples were contaminated.

Trial 3

This experiment was conducted in east central Oklahoma at the Kerr Foundation facilities near Poteau. The trial period involved 84 days, from October 4, 1977 to December 27, 1977.

Fifty-six, 8-9 month old crossbred heifers were allotted by weight and breed to four treatments. Breeds involved were Hereford and Charolais with three replications, heavy, medium and light, based on heifers weight. Replications were randomly assigned to twelve pens of approximately 100 sq. meters each and covered partially by a roof. Medium and light replications involved 5 animals (3 Charolais and 2 Hereford) per pen whereas the heavy replication included only 4 animals per pen, two of each breed.

Supplements fed were: (1) 20% natural protein (soybean meal), negative control, (2) 40% natural protein, positive control, (3) 40% protein SRU supplement (62.5% crude protein equivalent from coated urea), and (4) same as 3 with prilled urea instead of coated urea.

Supplements were formulated to be isopotassic and the closest possible in energy content. Ingredient composition and actual crude protein content of the supplements is presented in Table VII.

Supplements were offered once daily, seven days per week at a rate of 0.9 kg/head/day. All supplements were fed as 0.5 cm pellets for the first 28 days. However, pelleting resulted in heavy damage of the coating of the SRU so for the remaining 56 days of the trial, this supplement was fed in meal form.

Heifers were fed mature fescue hay (Festuca arundinacea, IRN 1-08-657). The forage was obtained from two different locations and had some differences in nutritive composition (Table VIII). This difference was balanced by feeding two bales of the lower quality source with each bale of the better quality hay.

Hay was fed ad libitum in portable mangers located in each pen. Amounts offered were recorded daily and refusals were weighed back weekly. Samples of the two hay sources were taken every week throughout the experimental period for chemical analyses. Table VIII presents the average composition of four composite samples for each source.

Animals were weighed every 28 days after 16 hours without feed and water. Statistical analysis of the average daily gain was performed by Least Square Analysis for a Split-Plot (Split-pen) Design. Hay intake and feed/gain data were analyzed as a Randomized Block Design by Least Square Analysis (Table XXII of the Appendix). Means were compared by LSD tests (Steel and Torrie, 1960).

TABLE VII
INGREDIENT^a COMPOSITION OF PROTEIN SUPPLEMENTS FED TO HEIFERS (TRIAL 3)

Ingredient (%)	International Reference Number	Natural Protein		Urea	Coated Urea
		Protein % 20	Protein % 40	Protein % 40	Protein % 40
Soybean meal	5-04-604	33.0	87.5	21.0	19.9
Alfalfa hay, ground	1-00-118	6.0	8.0	6.0	6.0
Cane molasses	4-04-696	5.0	2.5	6.0	6.0
Sodium sulfate	6-04-292	1.0	2.0	2.3	2.3
Corn, ground	4-02-915	49.0	---	53.3	52.6
Cottonseed hulls	1-01-599	6.0	---	---	---
Urea ^b		---	---	8:9	---
Coated urea ^c		---	---	---	10.7
Potassium chloride	6-03-756	---	---	2.4	2.4
Trace mineral mix		0.05	0.05	0.05	0.05
Vitamin A (30,000 IU/g)	7-05-143	0.12	0.12	0.12	0.12
Actual crude protein content (%) (DM basis)		18.5	37.2	39.2	39.5
TDN content (%)		74.2	74.5	66.6	65.1

^aPhosphorus offered free choice in mineral feeders containing a 1:1 mixture (salt: dicalcium phosphate).

^b281% crude protein equivalent (45% N).

^c237.5% crude protein equivalent (38% N).

TABLE VIII
 CHEMICAL COMPOSITION^{a,b} AND DRY MATTER DIGESTIBILITY
 OF FESCUE (*Festuca arundinacea*) HAY FED TO
 HEIFERS (TRIAL 3)

Items	Source A Hay %	Source B Hay %
Neutral detergent fiber	70.8	68.5
Acid detergent fiber	45.9	42.7
Hemicellulose (NDF-ADF)	24.9	25.8
Crude protein	8.4	8.8
Ash	9.2	9.9
Phosphorus	0.24	0.21
Calcium	0.52	0.47
Potassium	1.5	2.36
<u>In Vitro</u> Dry matter digestibility	50.4	59.3

^aAnalyses for each source represents the average of four composite samples collected from the bales throughout the experiment (Oct-Dec, 1977).

^bAll items are expressed in dry matter basis.

Results and Discussion

Trial 1

In this trial SRU was fed in either meal or pelleted supplements to evaluate the effects of pelleting damage to the coating on performance of cows. Weight gains of cows fed SRU in meal or pelleted form were identical (Table IX), suggesting that a possible damage of the coat during pelleting did not affect cow performance.

No feed refusals (Table IX) were noted for any of the supplements during the trial in contrast to observations with lactating cows. No precise explanation can be offered but several factors might have been involved. The amount of supplement offered was smaller (0.9 kg vs 1.22 kg), competition between group fed animals may estimate feed consumption, and individual susceptibility to urea could vary appreciably.

Individual susceptibility to urea supplements have been observed at this station not only with lactating cows (Chapter III) but also with steers fed the same kind of supplements (Forero and Owens, unpublished results). It has been noted that some animals are able to detect or "sense" urea rather easily in the feed and to modify intake accordingly whereas other animals seem not to be affected and they consume the supplements in a more uniform manner.

Therefore, in group-feeding urea supplements it might be possible to find a large individual variation in supplement intake which should be reflected in the performance of the animals. On that basis, a comparison was made of the variation in weight changes within the groups of animals fed the 40% protein pelleted rations (natural pro-

TABLE IX

WINTER PERFORMANCE OF GROUP-FED RANGE PREGNANT COWS SUPPLEMENTED WITH
NATURAL PROTEIN, COATED UREA (SRU) AND UREA (TRIAL 1)

Item	Natural		Protein Source SRU		Urea	SE ^a
	15	40	40	40 (M)		
Protein level	15	40	40	40 (M)	40	
Number of cows	15	16	16	15	16	
Supplement intake, kg/day	0.9	0.9	0.9	0.9	0.9	
Avg. initial cow weight, kg	424	430	428	433	430	
Cow daily weight gain, kg	0.08 ^c	0.50 ^b	0.45 ^b	0.44 ^b	0.11 ^c	0.043

^aStandard error (approximated).

^{bc}Means on a line with different superscript letters differ significantly (P<.01).

M = All rations pelleted except this one.

tein, SRU and prilled urea). The results (Table X) showed no statistically (Bartlett's Test) differences in this respect, however, other factors involved in the weight variation of the cows might have influenced such results.

Weight changes (Table IX) of cows fed the 40% natural protein and the SRU supplements did not differ significantly, although the former produced 10% faster gain. Results suggest that in this trial coated urea was utilized almost as well as soybean meal. However, this conclusion might be weakened by the fact that the experiment was limited to the first half of the winter season omitting a period in which the nutritional demands of the cows are large.

Weight change of cows fed the SRU treatment differed significantly ($P < .01$) from the negative control and prilled urea treatments. This suggests that coating improved urea utilization. The only other compound for which a similar conclusion was reached was by Huston et al. (1975) for sheep fed a carboxy-resin pelleted urea.

The poor performance of cows fed prilled urea again illustrated poor utilization of urea when fed with low quality forages which has been reported by several authors (Nelson and Waller, 1962; Rush and Totusek, 1976; Clanton, 1978).

Trial 2

Statistical analyses of the 3 x 2 factorial indicate that interaction (protein source x energy level) effect was present in the case of supplement intakes, therefore, preplanned simple effect comparisons (see methods) were evaluated by LSD tests. No interaction was present in the case of rumen ammonia levels and cow weight change,

TABLE X
 INDIVIDUAL WEIGHT CHANGE (KG) OF GROUP-FED PREGNANT COWS
 FED 40% PROTEIN PELLETTED SUPPLEMENTS (TRIAL 1)

	Supplements		
	Natural Protein	Coated Urea	Prilled Urea
	16.4	32.7	- 1.8
	21.4	1.4	-24.1
	38.6	66.8	9.5
	29.1	28.6	3.2
	43.2	34.1	- 7.3
	31.8	29.5	14.5
	29.5	30.5	12.3
	22.3	20.5	1.8
	29.5	19.5	3.6
	34.1	30.0	2.3
	34.1	31.4	21.8
	29.5	9.1	31.4
	37.3	18.2	27.3
	26.8	24.1	- 3.6
	4.1	33.6	11.4
	54.5	19.1	- 2.3
Number of cows	16	16	16
Mean	30.1	26.8	6.3
Variance	129.274	198.795	190.0373
St. Error	2.84	3.52	3.45

Bartlett's Test: $\chi^2 = 0.8011$; 2 df

χ^2 table = .50 \leq 0.8011 \leq .75

consequently main effects were used for treatment comparisons. The two other treatments outside of the factorial, the negative control (15% protein) and the SRU (70%) were compared to natural protein (40%) and SRU (40%), respectively.

Rumen ammonia levels (Table XI) for cows fed SRU were lower ($P < .01$) at both 1 and 4 hour post-prandial samplings than for those fed urea and nonsignificantly higher for those fed the soybean meal supplement. These results parallel those seen with lactating cows (Chapter III) and confirm the effectiveness of the coating in preventing the rapid release of ammonia. The change of ruminal ammonia levels between the two sampling times further indicates that SRU produced a sustained ammonia release, similar to that of soybean meal. Ruminal ammonia levels of cows fed prilled urea decreased drastically between 1 and 4 hours post feeding (Figure 1).

Effects of protein source, energy level and source x energy interaction on ruminal ammonia are graphically presented in Figures 1 through 3. Greater intake of energy reduced ($P < .05$) ruminal ammonia of cows fed urea at 4 hours but only slightly in the case of SRU and soybean meal. The comparison between the SRU and soybean meal indicated that ammonia was reduced to a greater extent with SRU than with the natural protein when the diet furnished more energy (Figure 3). Both SRU and soybean meal produced rumen ammonia levels near or above those concentrations considered by Satter and Roffler (1975) as adequate for microbial protein production.

It was again found that SRU supplements (Table XII) were more palatable than those containing prilled urea. Intake differences between SRU and natural protein supplements were not observed whereas

TABLE XI

RUMEN AMMONIA LEVELS 1 AND 4 HOURS AFTER FEEDING DIFFERENT SOURCES OF PROTEIN
AND ENERGY LEVELS TO PREGNANT RANGE COWS (TRIAL 2)

Item	Protein Source								
	Natural Protein			Urea		Slow-release Urea			SE ^a
Crude protein, ^b %	15	20	40	20	40	20	40	70	
Supp. offered/day, kg	0.9	1.8	0.9	1.8	0.9	1.8	0.9	0.52	
Number of cows sampled	11	11	10	11	11	10	11	11	
Rumen ammonia, 1 hr, mg/dl	1.8 ^c	5.0 ^c	6.2 ^c	25.9 ^d	26.1 ^d	4.8 ^c	4.9 ^c	5.6 ^c	1.63
Rumen ammonia, 4 hr, mg/dl	0.5 ^e	3.0 ^c	4.4 ^c	8.8 ^{dx}	14.0 ^{dy}	3.3 ^c	5.6 ^c	7.3 ^e	0.87
Change, %	-72	-40	-28	-66	-46	-30	+13	+23	

^aApproximate standard error (assuming 11 observations per treatment)

^b62.5% crude protein equivalent from urea in NPN supplements, exception the 70% protein where urea furnished 75%.

^{cde}Means on a line with different superscript differ significantly but they refer to selected, pre-planned comparisons (see text p. 51 for explanation).

^{xy}As above but refer to comparison between energy levels (20% vs. 40% protein supplement) within protein source.

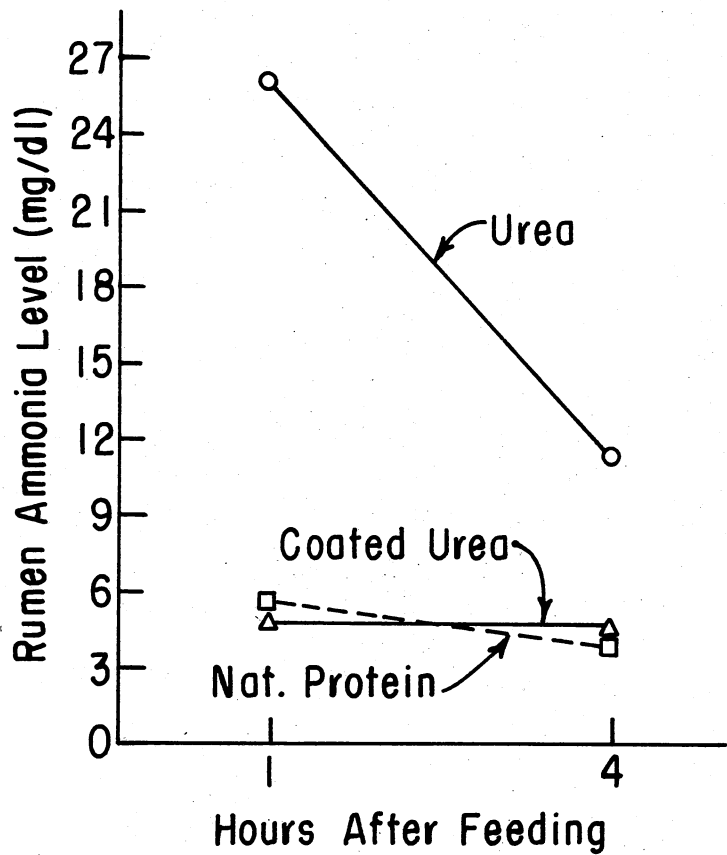


Figure 1. Effect of Protein Source on Postprandial Ruminal Ammonia Change (Trial 2)

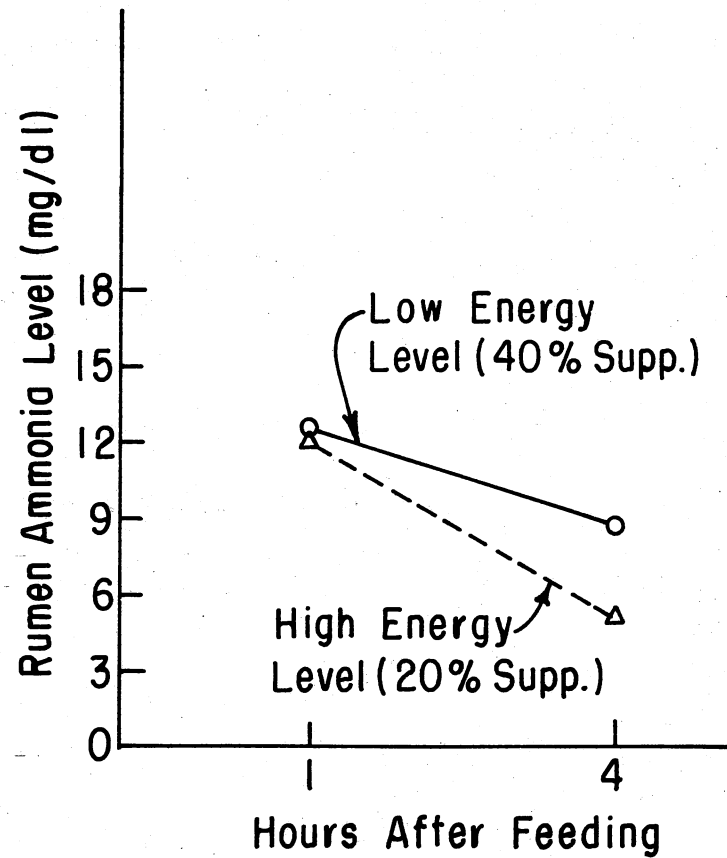


Figure 2. Effect of Energy Level on Postprandial Ruminal Ammonia Change (Trial 2)

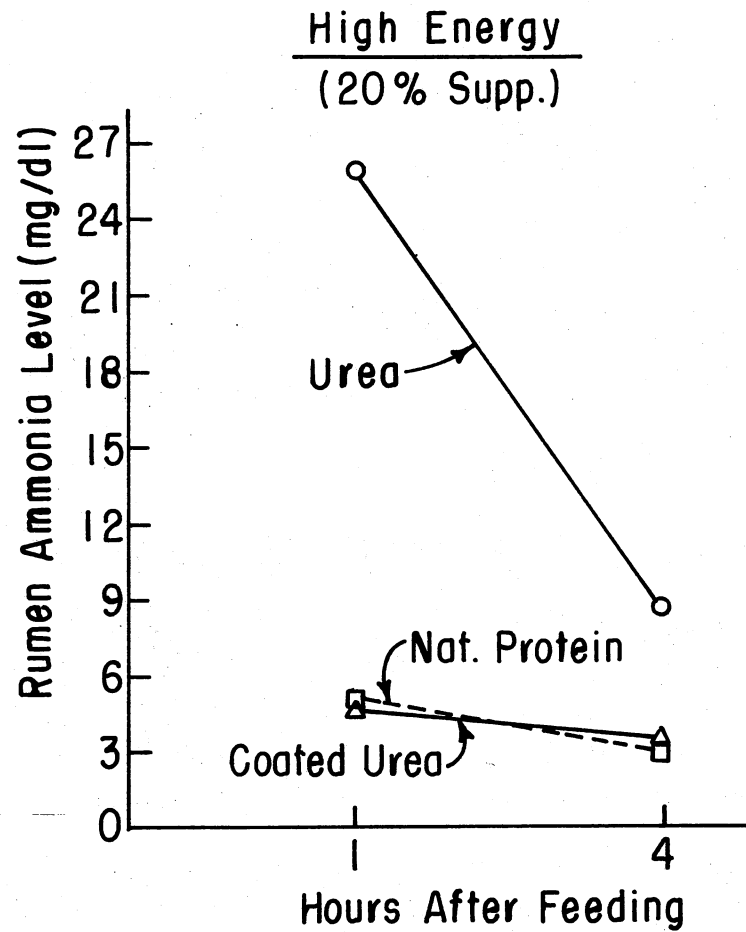
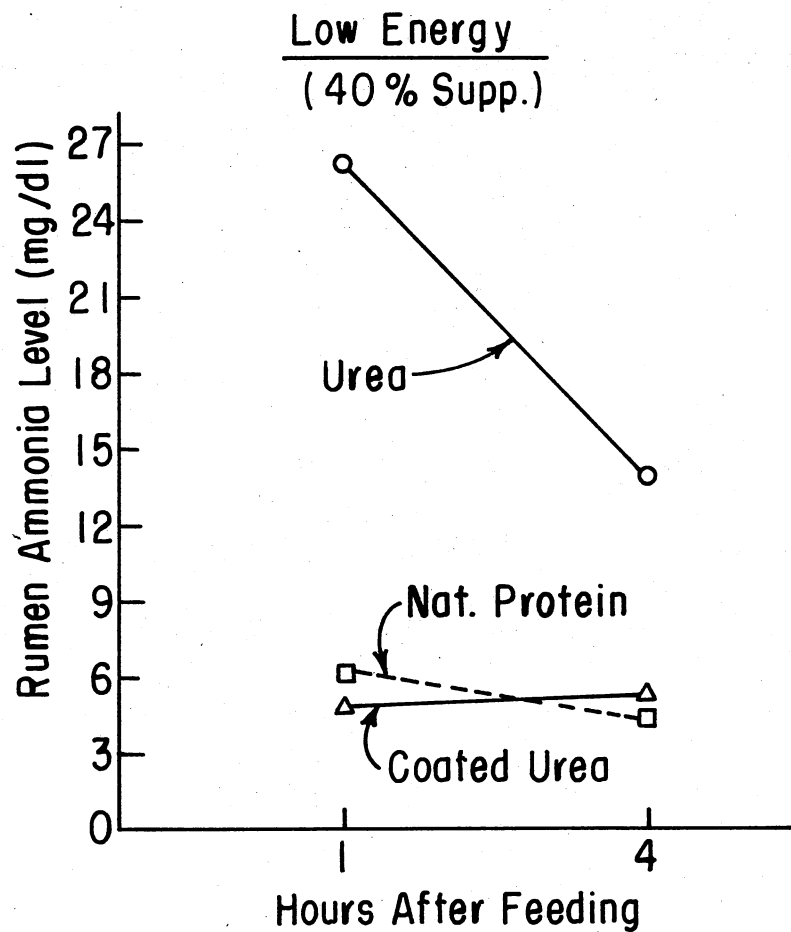


Figure 3. Interaction Effect of Protein Source and Energy Level on Ruminal Ammonia Change (Trial 2)

TABLE XII
WEIGHT CHANGE OF INDIVIDUALLY-FED COWS RECEIVING DIFFERENT SOURCES
OF PROTEIN AND LEVELS OF ENERGY (TRIAL 2)

Item	Protein Source								SE ^a
	Natural Protein			Urea		Slow-release Urea			
Crude protein, ^b %	15	20	40	20	40	20	40	70	
Number of cows per trt.	10	10	9	11	11	10	10	10	
Supp. offered/day, kg	0.9	1.8	0.9	1.8	0.9	1.8	0.9	0.52	
% supp. consumed	99.9 ^c	99.5 ^c	99.4 ^c	94.2 ^{dx}	97.2 ^{dy}	99.8 ^c	99.7 ^c	97.3 ^e	0.79
Initial cow weight, kg	447	446	442	446	445	445	448	440	
Cows daily weight change, kg	-0.31 ^c	-0.20 ^c	-0.19 ^c	-0.30 ^d	-0.42 ^d	-0.34 ^d	-0.42 ^d	-0.46 ^d	0.045

^aStandard error (approximated).

^b62.5% crude protein equivalent from urea in NPN supplements, exception the 70% protein where urea furnished 75%.

^{cde}Means on a line with different superscript differ significantly but they refer to selected preplanned comparisons (see text p. 51 for explanation).

^{xy}As above but refer to comparison between energy levels (20% vs 40% protein supplements) within protein source.

animals fed the 20% prilled urea supplement ate 5% less ($P < .01$) supplement than those fed the 20% protein SRU and soybean supplements. The 40% prilled urea treatment reduced intake approximately 3% ($P < .05$) below that from 40% protein from SRU and soybean meal. Animals fed the urea supplements refused more toward the end of the trial as was previously observed with individually fed lactating cows (Chapter III). This agrees with results from Wilson et al. (1975), Conrad et al. (1977) and Chalupa et al. (1978) who have indicated that the palatability of rations containing urea is attributable to non-taste factors.

Poor palatability of urea is still a matter of interest as Kertz and Everett (1975) has reviewed for dairy cattle. Undoubtedly, SRU can overcome such problem; consequently, this desirable characteristic might become important under conditions where urea can be efficiently utilized or at the time when ways are found to improve urea utilization with low quality forages.

Weight losses for all groups (Table XII) were greater than anticipated due to the unusually severe winter. Cows fed the negative control ration lost more weight than those fed an isocaloric amount of 40% natural protein supplement. This difference approached significance and undoubtedly would have been greater in a "normal" winter. Previous works at the same location (Rush et al., 1976; Lemenager et al., 1978; Forero et al., 1978) has shown that such narrow differences between those two treatments are unusual.

Cold weather and reduced forage availability probably increased the needs for supplementary energy and made energy the factor limiting cow weight retention. Past research at Oklahoma (Nelson et al.,

1954) has demonstrated that this is not normally the case since generally 40% protein supplements proved more beneficial than 20% protein supplements under grazing conditions. Similarly, other work (Cook and Harris, 1968; Rittenhouse et al., 1970; Lusby et al., 1976) has indicated that supplements formulated to furnish more energy to range animals would reduce forage consumption. Since the winter weather in Oklahoma is variable from year to year one must agree with Clanton and Zimmerman (1970) who indicated that under Nebraska conditions the effect of year and range conditions upon forage quality and voluntary intake by cattle makes it difficult to decide which supplement to use.

Urea, prilled or coated, was poorly utilized in this experiment. Weight losses (Table XII) of the cows fed such supplements were as large or larger than those fed the negative control. Cows fed supplements with urea or SRU lost more weight ($P < .05$) than cows fed isoproteic natural protein supplements.

The 70% protein supplement with 75% of the crude protein from SRU was fed to evaluate the effect of feeding a slow-release ammonia source as the primary nitrogen supply to the rumen. Apparently nitrogen in the form of ammonia alone was insufficient to meet ruminal protein needs, since this group lost more weight than any other group.

The results of pooling the weight change data pertaining to the six treatments fed at the 20 or 40% protein levels are presented in Figure 4. The following observations can be made: (1) natural protein treatments were not affected by the level of supplementary energy whereas the urea (coated or not) treatments tended to be better utilized with higher levels of energy, (2) prilled urea and SRU

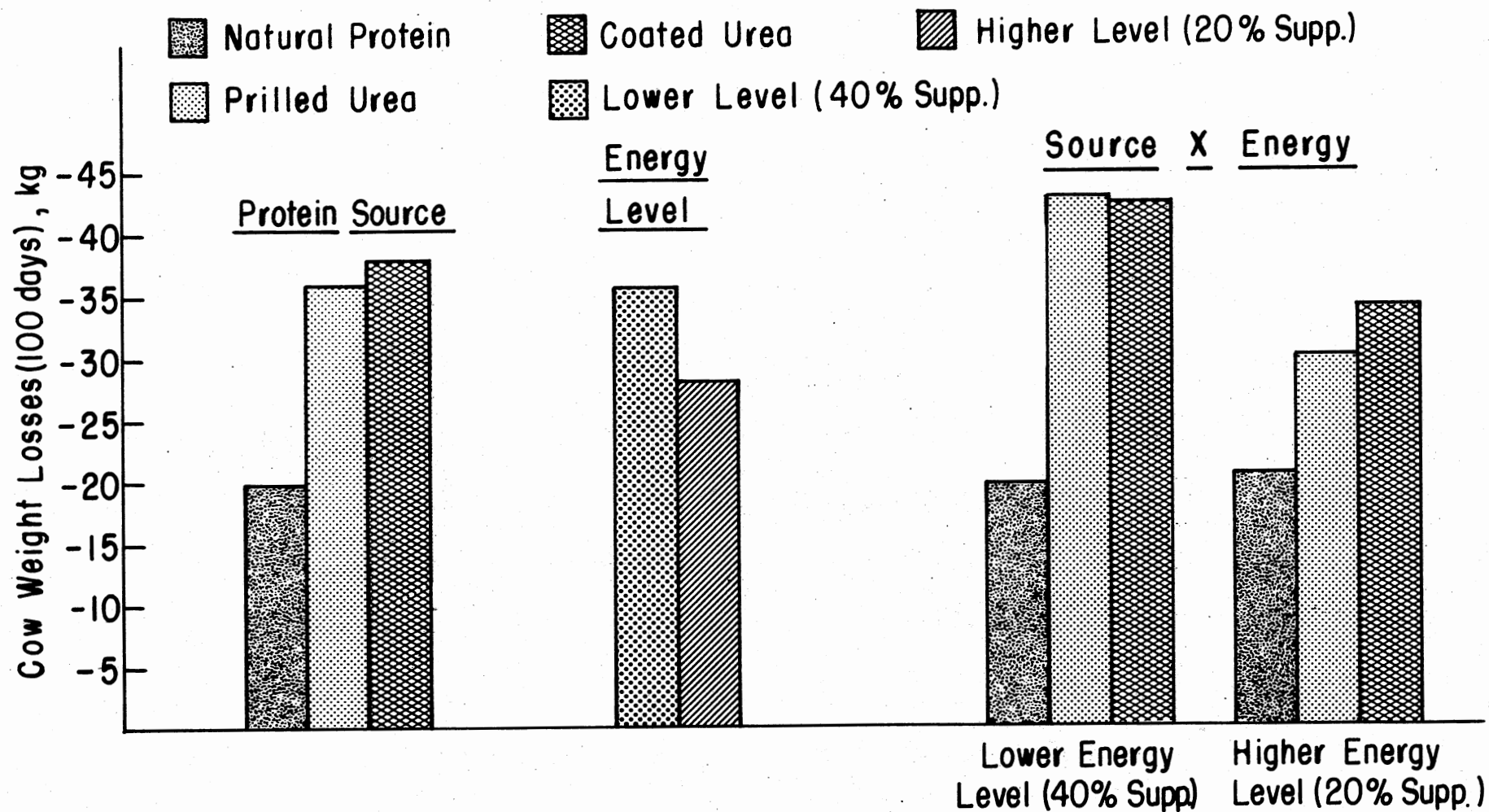


Figure 4. Effect of Protein Source and Energy Level of the Supplement on Weight Change of Pregnant Range Cows. (Trial 2)

treatments behaved almost identically at both energy levels.

Benefits from SRU seen in Trial 1 with dry, pregnant cows were not observed in this trial. The theory of coating is to moderate the release of ammonia to a rate comparable to that of carbohydrate availability. With these rations, carbohydrate would be mainly cell-wall components. Since forage availability was limiting during an appreciable part of the experiment, differential responses to protein source or ammonia release rate might not be expected.

Trial 3

The pelleting process unexpectedly resulted in heavy damage of the coated urea. Therefore, SRU supplement was fed in meal form after day 28. Only the last 56 days of the trial were used to evaluate the effects of slow release of ammonia on heifer performance.

Average daily gains for the last 56 days of the study (Table XIII) indicated differences ($P=0.08$) in heifer response to the treatments. Heifers fed the positive control gained the fastest while the urea fed heifers had the poorest gains. Gains for SRU fed heifers were intermediate between the negative and the positive control. Mean comparisons by LSD tests showed that positive control fed animals gained more ($P<.05$) than those fed urea but not different from those fed SRU or the negative control supplement.

The absence of difference between the positive and the negative controls indicates that protein probably was overfed to all heifers. Previous analysis of the forage fed had shown a protein content of approximately 7%, however, protein content of the forage was found higher in samples taken throughout the experiment (Table VIII). Since

TABLE XIII

DAILY GAIN (ADG), HAY INTAKE AND FEED EFFICIENCY OF HEIFERS
FED FOUR DIFFERENT PROTEIN SUPPLEMENTS (TRIAL 3)

Item	Protein Source				P Value ^a	SE ^b
	Natural Protein	SRU	Urea			
Protein, %	20	40	40	40		
No. heifers	14	14	14	14	---	
<u>84 days</u>						
Ave. initial weight	225	227	227	223		
ADG (kg)	0.295	0.372	0.304	0.277	0.12	0.036
Hay intake (kg)	5.90	5.94	6.15	5.81	0.34	0.11
Kg hay/kg gain	20.0	16.0	20.3	21.1	0.27	0.74
<u>Last 56 days</u>						
Ave. initial weight	238	242	239	237	---	
ADG (kg)	0.204 ^c	0.286 ^c	0.249 ^c	0.163 ^d	0.08	0.041
Hay intake (kg)	6.28	6.38	6.61	6.24	0.54	0.15
Kg hay/kg gain	30.7	22.5	26.6	37.9	0.31	2.79

^aStatistical probability value.

^bStandard error (ADG, 14 observations/treatment; hay intake and feed efficiency, 4 observations/treatment)

^{cd}Means on a line with the same superscript do not differ significantly ($P < .05$).

the supplemental protein levels were based on the first forage analysis which underestimate the actual protein content of the hay, all heifers consumed more total protein than planned.

The blocking of heifers by weight showed to be desirable not only for management but also for studying the animal responses. A comparison of the gains observed during the complete experimental period and during the last 56 days of the trial is presented in Table XIV. It is noted that the urea treatment behaved the poorest in almost any comparison whereas SRU was outperformed only by the positive control. Such ranking can especially be observed in the data corresponding to the last 56 days.

Hay intake (Table XIII) for the different treatments were similar throughout the trial although heifers fed the 40% natural protein and SRU supplements tended to eat more hay than heifers fed the negative control or urea. Feed efficiency differences, although large were not statistically different ($OSL=0.31$) and again the positive controls were the most efficient, urea the least, with SRU and the negative control intermediate. Heifers fed the coated urea showed a 30% improvement in conversion of hay to gain over heifers fed urea. The positive control improved feed efficiency by 15 and 40% in relation to SRU and urea, respectively. Those differences in feed efficiency suggest that the digestibility of the hay was improved by the soybean meal and coated urea supplementation.

This trial appears to indicate that SRU does not produce consistent improvement in heifers performance.

The results of the experiments discussed appear to indicate that SRU overcame intake problems frequently observed with urea-containing

TABLE XIV
DAILY GAINS OF HEIFERS BY TREATMENT AND WEIGHT GROUPING
(TRIAL 3)

Treatments	No. Heifers	84 Days		Last 56 Days	
		ADG ¹	S.E. ²	ADG	S.E.
15% Nat. Protein					
Heavy	4	0.303	0.048	0.202	0.059
Medium	5	0.243	0.054	0.154	0.063
Light	5	0.335	0.075	0.259	0.068
Overall Ave.	14	0.293	---	0.205	---
40% Nat. Protein					
Heavy	4	0.365	0.096	0.202	0.125
Medium	5	0.367	0.053	0.243	0.039
Light	5	0.383	0.045	0.389	0.062
Overall Ave.	14	0.372	---	0.283	---
40% Protein (SRU)					
Heavy	4	0.378	0.035	0.274	0.019
Medium	5	0.248	0.064	0.178	0.083
Light	5	0.297	0.054	0.300	0.043
Overall Ave.	14	0.302	---	0.248	---
40% Protein (UREA)					
Heavy	4	0.249	0.057	0.101	0.094
Medium	5	0.291	0.066	0.178	0.070
Light	5	0.281	0.049	0.202	0.057
Overall Ave.	14	0.275	---	0.165	---

¹ADG = Average daily gain in kilograms.

²SE = Standard error.

supplements and effectively lowered the release of ammonia from urea. Nevertheless, SRU did not consistently improve utilization of urea under range conditions and the slight non-significant advantage of SRU over urea observed in some instances might be due to either increased amounts of supplement ingested or to a small beneficial effect at the ruminal level.

Higher levels of energy in the supplement apparently contributed to a more rapid utilization of urea into the rumen, however, it was not translated into cow weight response. Higher supplementary energy tended to decrease forage digestibility and consumption.

Therefore, data would be indicating that factors other than a steady supply of ammonia are responsible for the low urea utilization with low-quality forages. Factors such as some amino acids, branched chain VFA's or even certain minerals or vitamins present in soybean meal-corn diets and apparently absent in urea diets might be a more direct cause.

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APPENDIX



TABLE XV
ANALYSES OF VARIANCE (LACTATING COWS TRIAL, CHAPTER III)

AOV* TABLE FOR SCORING CONFORMATION			
Source of Variation	df	Mean Squares	P value
Total	82	---	---
Blocks	16	0.822	P>.10
Treatments	4	10.752	P<.005
Error	62	0.835	---

*Two missing data

AOV* TABLE FOR CALF DAILY GAIN			
Source of Variation	df	Mean Squares	P value
Total	82	---	---
Blocks	16	0.0110	P<.02
Treatments	4	0.0562	P<.005
Error	62	0.0050	---

*Two missing data

AOV* TABLE FOR COW TOTAL WEIGHT CHANGE (92 DAYS)			
Source of Variation	df	Mean Squares	P value
Total	82	---	---
Blocks	16	672.626	P<.005
Treatments	4	7304.816	P<.005
Error	62	255.764	---

*Two missing data

AOV* TABLE FORAGE INTAKE			
Source of Variation	df	Mean Squares	P value
Total	82	---	---
Blocks	16	28.2856788	P>.10
Treatments	4	45.0866047	P<.005
Error	62	106.4644153	---

*Two missing data

TABLE XV (CONTINUED)

AOV* TABLE FORAGE DIGESTIBILITY			
Source of Variation	df	Mean Squares	P value
Total	82	---	---
Blocks	16	30.3361242	P>.10
Treatments	4	97.4873794	P<.10
Error	62	48.2398163	---

*Two missing data

AOV* TABLE INTAKE OF INDIGESTIBLE DRY MATTER

Source of Variation	df	Mean Squares	P value
Total	82	---	---
Blocks	16	0.2652364	P>.10
Treatments	4	2.5915856	P<.01
Error	62	0.3023970	---

*Two missing data

AOV* TABLE RUMEN AMMONIA LEVELS

Source of Variation	df	Mean Squares	P value
Total	9	---	---
Treatments	4	61.365	P<.005
Hours	1	78.457	P<.005
Trt x hours	4	19.498	P<.005
Within cells ^a	123	8.8734	---

*Unweighted analysis of cell means (Snedecor and Cochran, 1967, p. 475).

^aWithin cell mean squares = $\left(\frac{\text{sum of squares}}{\text{df}}\right) \left(\frac{1}{\text{Harmonic mean}}\right) = s^2 \times \frac{1}{H}$;
LSD uses s^2 only.

TABLE XVI
 CHI-SQUARE ANALYSES FOR COW REPRODUCTIVE DATA
 (LACTATING COWS TRIAL, CHAPTER III)

ANALYSIS OF POST-PARTUM ESTRUS DATA						
Feeds	Natural Protein		SRU	Urea		Total
% Protein	15	40	40	40	20	
Cows showing estrus	9	17	12	15	10	63
Cows did not show estrus	7	0	4	2	7	20
Total	16	17	16	17	17	83

$$\chi^2 = \sum \frac{(\text{Expected} - \text{Observed})^2}{\text{Expected}} = 12.785$$

$$\text{Expected}_{16} = \frac{63}{83} \times 16 = 12.1; 16 - 12.1 = 3.9$$

$$\text{Expected}_{17} = \frac{63}{83} \times 17 = 12.7; 17 - 12.9 = 4.1$$

$$\chi_{df}^2 = (5-1)(2-1) = 4$$

$\chi_{table}^2 = .025 \leq 12.785 \leq .010$; since the overall χ^2 was significant several χ^2 tests were performed for each pair of comparisons between treatments.

TABLE XVI (CONTINUED)

ANALYSIS OF PREGNANCY DATA						
Feeds	Natural Protein		SRU	Urea		Total
% Protein	15	40	40	40	20	
Cows Pregnant	7	16	8	13	9	53
Cows Open	9	1	8	4	8	30
Total	16	17	16	17	17	83

$$\chi^2 = \left(\frac{E-O}{E}\right)^2 = 12.85$$

$$\text{Expected}_{16} = \frac{52}{83} \times 16 = 10; 16 - 10 = 6$$

$$\text{Expected}_{17} = \frac{52}{83} \times 17 = 10.6; 17 - 10.6 = 6.4$$

$$\chi_{df}^2 = (5-1)(2-1) = 4$$

$\chi_{table}^2 = .025 \leq 12.85 \leq 0.010$; since it is significant, χ^2 analysis are performed for each pair of treatment comparisons.

TABLE XVII

PROCEDURE FOR CALCULATION OF FORAGE INTAKE AND DIGESTIBILITY
(LACTATING COWS TRIAL, CHAPTER III)

(a) Total feces output (g/day)

$$\text{Feces (g/day)} = \frac{\text{Chromium consumed (g/day)}}{\text{Chromium in feces (g/g dry matter)}}$$

Chromium determined in ppm by spectrophotometry, converted to grams and corrected for the dilution effected.

(b) Acid Insoluble Ash (AIA) content in total feces

Cow's feces output x content AIA (%) in that cow's feces

(c) AIA content in the supplement ingested

Supplement intake (g/day) x AIA content (%) in the supplement

(d) AIA in feces corrected for that furnished with the supplement

(b) minus (c)

(e) Percentage of AIA in feces furnished by the forage

(d)/daily feces output

(f) Dry matter digestibility of the forage

$$\frac{\% \text{AIA in forage samples}}{\% \text{AIA in feces furnished by the forage}}$$

(g) Dry matter forage intake

$$\frac{\text{Feces dry matter output}}{\% \text{ dry matter undigestible}}$$

TABLE XVIII

FECES OUTPUT AND AIA CONTENT IN SUPPLEMENT, FORAGE AND FECES
(LACTATING COWS TRIAL, CHAPTER III)

Supplement	Natural Protein		SRU	Urea	
% Protein	15	40	40	20	40
Daily feces output (g)	4,614	5,767	5,734	5,491	5,392
AIA (%) supplements	0.27	0.20	0.49	0.19	0.18
AIA (%) forage	----- 6.97 -----				
AIA (%) feces*	10.58	11.44	10.89	10.78	10.37

AIA = Acid Insoluble Ash

*AIA(%) in feces ranged from 8.21 to 15.58.

TABLE XIX

ANALYSIS OF VARIANCE FOR COW WEIGHT CHANGE (60 DAYS)
(TRIAL 1, CHAPTER IV)

AOV* TABLE			
Source of Variation	df	Mean Squares	P value
Total	77	---	
Blocks	15	241.801	P<.05
Treatments	4	2,862.275	P<.005
Error	58	104.741	

*Two missing data

TABLE XX

ANALYSIS OF VARIANCE (ALL TREATMENTS) FOR THE DIFFERENT
PARAMETERS STUDIED (TRIAL 2, CHAPTER IV)

AOV* TABLE FOR RUMEN AMMONIA LEVELS (1 HR)

Source of Variation	df	Mean Squares	Observed Significance Level (O.S.L.)
Total	85	---	---
Blocks	10	34.4779	0.3251
Diet	7	1,080.7835	0.0001
Error	68	29.4276	---

*Two missing data

AOV* TABLE FOR RUMEN AMMONIA LEVELS (4 HR)

Source of Variation	df	Mean Squares	O.S.L.
Total	85	---	---
Blocks	10	7.6468	0.5295
Diet	7	191.3892	0.0001
Error	68	8.4066	---

*Two missing data

AOV* TABLE FOR COW WEIGHT CHANGE(100 DAYS)

Source of Variation	df	Mean Squares	O.S.L.
Total	80	---	---
Blocks	10	29.3238	0.2348
Diet	7	1,002.0667	0.0004
Error	63	220.5698	---

*Seven missing data

TABLE XX (CONTINUED)

AOV* TABLE FOR SUPPLEMENT CONSUMPTION
(PERCENTAGE BASIS)

Source of Variation	df	Mean Squares	O.S.L.
Total	80	---	---
Blocks	10	78,582.0899	0.3388
Diet	7	429,390.3364	0.0001
Error	63	68,136.8176	---

*Seven missing data

TABLE XXI
ANALYSES OF VARIANCE (SIX TREATMENTS) FOR THE DIFFERENT
PARAMETERS STUDIED (TRIAL 2, CHAPTER IV)

AOV* TABLE FOR RUMEN AMMONIA LEVELS (1 HR)

Source of Variation	df	Mean Squares	Observed Significance Level (O.S.L.)
Total	63	---	---
Blocks	10	47.0397	0.2940
Protein source	2	3,126.3199	0.0001
Energy level	1	3.3924	0.7667
Energy x source	2	1.1149	0.9712
Error	48	38.0995	---

*Two missing data

AOV* TABLE FOR RUMEN AMMONIA LEVELS (4 HR)

Source of Variation	df	Mean Squares	O.S.L.
Total	63	---	---
Blocks	10	8.2411	0.5822
Protein source	2	386.4249	0.0001
Energy level	1	144.1073	0.0003
Energy x source	2	20.8508	0.1268
Error	48	9.6676	---

*Two missing data

AOV* TABLE FOR COW WEIGHT CHANGE

Source of Variation	df	Mean Squares	O.S.L.
Total	60	---	---
Blocks	10	35.2807	0.1480
Protein source	2	1,709.0755	0.0014
Energy level	1	764.0363	0.0721
Energy x source	2	203.2678	0.4127
Error	45	225.2151	---

*Five missing data

TABLE XXI (CONTINUED)

AOV* TABLE FOR SUPPLEMENT CONSUMPTION

Source of Variation	df	Mean Squares	O.S.L.
Total	60	---	---
Blocks	10	44,201.8914	0.5110
Protein source	2	1,079,220.924	0.0001
Energy level	1	136,030.1507	0.0967
Energy x source	2	185,709.317	0.0268
Error	45	47,272.2957	---

*Five missing data

TABLE XXII
ANALYSES OF VARIANCE LAST 56 DAYS OF THE TRIAL
(TRIAL 3, CHAPTER IV)

AOV TABLE FOR AVERAGE DAILY GAIN

Source of Variance	df	Mean Squares	Observed Significance Level (O.S.L.)
Total	55	---	---
Breeds	1	0.00027	0.92
Blocks	2	0.06018	0.09
Block x breed ^a	2	0.02031	---
Supplement	3	0.03742	0.08
Suppl. x Breed ^b	3	0.02209	0.42
Suppl. x Block ^b	6	0.01008	---
Suppl. x Block x Breed ^c	6	0.02002	---
Error	32	0.02308	---

AOV TABLE FOR DAILY HAY INTAKE

Source of Variance	df	Mean Squares	O.S.L.
Total	11	---	---
Block	2	2.688	0.0009
Suppl	3	0.075	0.54
Error	6	0.095	---

AOV TABLE FOR DAILY FEED EFFICIENCY

Source of Variance	df	Mean Squares	O.S.L.
Total	11	---	---
Block	2	101.023	0.11
Suppl	3	46.592	0.31
Error	6	31.161	---

^aBlock x Breed M.S. is the error to test Breeds.

^bSuppl. x Block M.S. is the error to test Supplements.

^cSuppl. x Block x Breed M.S. is the error to test Suppl. x Breed.

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