UREA UTILIZATION IN HIGH

ROUGHAGE DIETS

By

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CHAPTER I

INTRODUCTION

The use of urea in ruminant rations is increasing. It has been estimated by one industry source that 200,000 tons of feed grade urea were used in the United States in 1966, a figure which authorities in the U.S.D.A. predicted would not be reached until 1970. Urea is used primarily for feedlot cattle, where it has been shown by researchers that it is an excellent substitute for the vegetable protein supplements in rations containing high levels of grain and properly supplemented with necessary minerals and vitamins. However, researchers have not been as successful in developing urea-containing supplements for ruminants consuming poor quality forages during the winter season. The results indicate that the nitrogen-free extract to nitrogen ratio in supplements containing 40-45 percent crude protein is too narrow for good urea utilization and that such supplements should contain lower levels of urea and higher levels of starch or sugars. The objectives of the studies reported herein were: (1) To compare cottonseed meal and urea in isonitrogenous, low protein supplements for beef cows grazing indigenous, dead, and weathered range grasses during the winter season. (2) To determine a nitrogen-free extract to nitrogen (NFE:N) ratio which would promote optimum nitrogen retention in cattle fed urea-containing supplements in combination with poor quality roughage.

CHAPTER II

REVIEW OF LITERATURE

As early as 1891, Zuntz suggested that ruminants could utilize non-protein nitrogen compounds by action of micro-organisms in the rumen. In earlier reviews, Armsby (1911) and Krebs (1937) concluded that synthesis of protein from these compounds by microorganisms in the ruminant animal is apparent, but of little significance in its nutrition. However, the experiments they described were not continued long enough to provide reliable results; also, the nature of the diets to which urea was added influenced the degree to which it was utilized. The results of more recent experiments have demonstrated that urea is converted into protein in amounts that are important in ruminant nutrition.

UREA UTILIZATION

Urea is hydrolyzed in the gastrointestinal tract by the enzyme urease to carbon dioxide and ammonia (Pearson and Smith, 1943; Dintzis and Hastings, 1953; Kornberg, et al, 1954, 1955; Visek, 1962). In ruminants ammonia may follow any of three pathways as follows: (1) used by the bacteria for synthesis of cell constituents; (2) absorbed through the rumen wall into the portal blood; (3) passed out of the rumen into the lower portion of the gut.

The rate at which ammonia passes through the rumen wall depends on its concentration in the rumen (Hogan, 1961). When the ruminal level is high, the rate of absorption is greater than the ability of the liver to convert it to urea. Under these conditions, excess ammonia builds up in the peripheral blood. Absorption of ammonia from the rumen is influenced by the pH of the rumen ingesta since it is transported more readily than NH_4^+ (Coombe *et al*., 1960). Part of the beneficial effect of feeding readily available carbohydrates with urea may be due to the fact that acids produced by their fermentation prevent a rise in pH in the rumen thus reducing the rate at which ammonia passes through the rumen wall.

The ammonia passing into the lower portion of the gut has no beneficial effect on the animal, but indirectly could be recycled as urea. No estimates on the amount of ammonia which may be passed from the rumen to the small intestine has been made; however, it might be considerable when the level in the rumen is high (Head and Rook, 1955).

The amount of ammonia being assimilated into the microbial protein seems to be affected by three or more factors as follows: (1) the level and form of carbohydrates available; (2) the level of protein in the feed; (3) the adequacy of minerals needed for optimum microbial action.

Carbohydrates

The synthesis of amino acids from ammonia by rumen microorganisms requires the presence of necessary "carbon fragments". Various workers have found that many products of rumen fermentation can be used as precursors of necessary carbon skeletons. Some of these are the carbon from carbohydrate (Annison and Lewis, 1959), carbon dioxide (Hutanen et al, 1954; Otagalei et al, 1955), and acetate (Hoover et al, 1963).

It has been demonstrated that for microorganisms to make efficient conversion of urea to protein the ration must contain a readily available source of carbohydrate (Mills and associates, 1942; Hunt et al, 1954). Mills and associates, (1942), have found that the increase in protein content of the rumen ingesta was nearly 57 percent higher when starch and urea were added than when urea alone was added. Using in vitro techniques, Arias et al, (1951), compared dextrose, cane molasses, sucrose, starch, cellulose, and ground corn cobs as energy sources for urea utilization. All sources of energy aided urea utilization with starch promoting the most efficient utilization followed by sucrose, cane molasses, dextrose, corn cobs, and cellulose respectively. Small amounts of readily available carbohydrate improved cellulose digestion which in turn improved urea utilization. Belasco (1956) found that starch was superior to either xylan, pectin, with xylan and pectin promoting more efficient urea utilization than cellulose.

Bell et al, (1953) compared corn to cane molasses and found that protein digestibility was greater in the corn ration. Mills et al, (1944) found that by including a small amount of corn in a urea-molasses mixture, that this diet was superior to a urea molasses mixture alone in stimulating protein synthesis.

It thus appears that starch is superior to simple sugars in improving urea utilization. Schwartz et al, (1964) reported that simple sugars disappear very rapidly from the rumen and were available for only a short time as a carbon and energy source for synthesis of bacterial protein from urea nitrogen. Soluble starch was also rapidly hydrolyzed, but the sugars formed from it remained in the rumen two to three times longer than did glucose, indicating that rumen conditions appear to be more favorable for the conversion of urea to bacterial protein when starch is fed than with sugar.

Few results have been reported regarding the quantity of carbohydrate needed for the synthesis of bacterial protein from urea nitrogen. On a theoretical basis, considering urea to contain 42 percent nitrogen and on the basis of the structure of the molecules 2.2 gm. of carbohydrate is needed for each gram of urea in the ration. As there is much loss in the conversion of starch or sugars to alpha-keto acids, the optimum ratio of nitrogen to soluble carbohydrates is probably many times this. Hungate, (1966) estimates that approximately 100 gm. of carbohydrate is required per gm. of nitrogen fixed while Bloomfield et al, (1964) in in vitro studies reported a requirement of 55 gm. of carbohydrate per gm. of fixed nitrogen.

Proteins

Burroughs et al, (1951) reported the results of a series of in vitro experiments which were designed to determine the influence of proteins on urea utilization and found that urea utilization was highest in the absence of conventional proteins. By adding proteins having widely different amino acids composition to the fermentation flasks, it was noted that a high quality protein (casein) gave the best utilization of urea, a poorer quality protein (gelatin) gave the poorest utilization, while an intermediate quality, hay, fell between casein and gelatin. When adequate or excessive ammonia of urea origin was avilable, the presence of gelatin or casein resulted in large amounts of additional ammonia being formed. These results indicate that in the presence of urea, protein was being used primarily as an energy source. Ammonia liberated from the protein may or may not be utilized later depending upon the needs of the rumen microorganisms. Ammonia from protein sources compete with the ammonia produced by the breakdown of urea and an inefficient use of urea nitrogen results (Pearson and Smith, 1943; McNaught and Smith, 1947-1948). Thus the efficiency of urea utilization is improved when the level of dietary protein is low.

Wegner et al, (1941) in an in vitro experiment found that the level of dietary protein influenced the rate and amount of conversion of urea to microbial protein and concluded that, as protein level in the medium was increased above 2.5 gm. per 100 cc., the rate of ammonia nitrogen to protein was decreased. Using a fistulated heifer, they also found that the conversion of urea nitrogen to protein in the rumen decreased as the protein level in the feed was increased. The results of Grainger et al, (1960) and Tillman and McAleese, (1963) suggested that urea can be used to improve diets when the level of crude protein does not exceed 12 percent.

Minerals

Grain and urea are normally used to replace natural protein supplements. The ratio of 7 units of grain to 1 unit of urea is isonitrogenous with 8 units of cottonseed or soybean meal. The protein supplements, however, are better sources of calcium, phosphorus, and trace minerals than are most grains. Thus the combination of urea plus grain may be deficient in certain minerals.

Burroughs et al, (1952) employing the artificial rumen technique to measure the effect of minerals upon urea utilization, found that water extracts of dehydrated clover meal, rumen ingesta, or manure stimulated urea utilization. Individual additions of iron, phosphorus, magnesium, potassium, and calcium were added to the fermentation flask and only iron and phosphorus improved urea utilization. McNaught et al, (1950) observed an increase in urea utilization when they made small additions of iron, copper, cobalt, and molybdenum to the diets. Copper has also been shown (Goodrich and Tillman, 1966) to increase urea utilization in purified diets when fed to sheep. The results of Bently and Maxon, (1952) indicate that an iron supplement alone had no effect on urea utilization.

Sulfur additions has improved urea utilization (Loosli et al, 1949; Thomas et al, 1951; Williams and Moir, 1951; Starks et al, 1953; Brown, 1960). The increase occured when it was added as elemental sulfur, sulfate sulfur, or sulfur from amino acid sources. Additions to diets containing more than 0.1 percent had not been beneficial in practical feeding trials (Gallup, 1956).

Urea in High Roughage Diets

Nelson and Waller, (1962) summarized the results of sixteen feeding tests involving 879 cattle in which they compared urea and cottonseed meal in isonitrogenous diets. Urea replaced from one-third to one-half of the nitrogen in protein supplements. The performance of animals consuming urea was never as good as those receiving the cottonseed meal. However, the performance of animals receiving urea was greatly improved by the addition of trace minerals. Thomas and co-workers, (1953) found that wintering steers fed rations supplemented with soybean meal made significantly greater gains than steers fed rations supplemented with urea. When adequate phosphorus and trace minerals were added to the urea supplement, there was no difference between gains. Darlow et al, (1945), and Reynolds et al, (1955) wintered steers on weathered range grasses and found vegetable protein

protein supplements are superior to urea in producing gains.

Briggs et al, (1947) found that urea was efficiently utilized by pregnant cows when urea supplied only 25 percent of the dietary nitrogen in the protein supplement. Nelson et al, (1956) fed wintering steers a urea supplement (one-half of the nitrogen from urea) and obtained performance equal to steers consuming a cottonseed meal supplement.

More satisfactory results have been found when urea supplements are fed to animals being fed poor quality roughages in the dry lot. Beeson and Perry, (1952) substituted urea for soybean meal in the Purdue Supplement A and fed it in diets containing corn cobs as the only roughage source. The urea containing supplements gave results as good as did the vegetable protein supplements. Nelson and Waller, (1956) found that steer calves fed a poor quality roughage plus a supplement containing one-third of the nitrogen from urea gave results equivalent to animals receiving an isonitrogenous supplement containing cottonseed meal. Similar results have been reported by Murry and Romyn, (1939); Embry and King, (1953); Bell and associates, (1955) when feeding urea as a partial replacement for vegetable protein supplements. Bell and associates, (1955) wintered pregnant females in the dry lot in which they compared urea and cottonseed meal supplements and found the performance of the animals receiving cottonseed meal to be superior to those receiving urea.

In recent years considerable work has been done using a molasses mixture containing urea. Woods et al, (1962) fed wintering steers a urea-molasses mixture containing 3.5 percent urea and found that a cottonseed meal supplement was superior. Coombe and Trails, (1960) in a series of digestion trials with sheep, fed oat straw and molasses with and without urea. They found that dry matter intake was increased, mean retention in the digestive tract shortened from 59 to 49 hours, and rate of digestion increased when urea was fed. Urea was also found to be effective as casein in stimulating synthesis of B-vitamins. Berry et al, (1960) fed a commercially prepared liquid supplement of urea and molasses containing 4 percent phosphoric acid. The acid pH caused slower consumption and allowed the supplement to be self-fed under a variety of range conditions. They found that the liquid supplement was equal in feeding value to cottonseed cake as a range supplement when fed on a protein equivalent basis.

CHAPTER III

A COMPARISON OF COTTONSEED MEAL AND UREA IN LOW PROTEIN WINTER RANGE SUPPLEMENTS FOR CATTLE

INTRODUCTION

Responses obtained during the years has tended to indicate that the nitrogen-free extract (NFE:N) ratio in supplements containing 40-45 percent crude protein is too narrow for good urea utilization and that supplements containing lower levels of crude protein are needed under range conditions. The purpose of the present experiment is to compare cottonseed meal and urea in isonitrogenous, low protein supplements for beef cows grazing dead and weathered range grasses during the winter season.

EXPERIMENTAL PROCEDURE

Trial 1 One hundred and ninety grade Angus cows, bred for spring parturition, were divided into two equal groups on the basis of initial weight, age, and sire-test group. Each of these groups was then further sub-divided into two groups; the four groups were fed their assigned rations from November 5, 1966, until April 25, 1967. Two locations in a large range area, were utilized with both rations being fed at each location. Animals on the different treatments within a location were rotated among the pasture to minimize the effect of pastures upon animal performance.

Each cow received 1.37 kg. per day of its assigned ration from November 5 until parturition, at which time her ration was increased to 1.82 kg. per day and this level was then fed until April 25, the supplements (Table I) being fed daily. In addition, a poor-quality grass hay, indigenous to the area, was fed when snow covered the forage. Usual managment concerning castration, vaccination, parasite and insect control were followed.

Each cow was weighed and assigned a condition score at the beginning of the experiment, two months after starting of the supplemental feeding, when supplemental feeding was discontinued, and when their calves were weaned. A score of 1 was used to indicate animals in the poorest condition and a score of 9 indicated those in the best condition. Calves were weighed within 24 hours after birth and again when they were weaned.

In addition, five animals were randomly selected from each subgroup on March 1, 1967, and blood samples, via jugular puncture, were taken from each of these animals two hours after feeding. Serum samples were analyzed for calcium, magnesium, copper, and zinc by atomic absorption spectophotomery (Perkin-Elmer, 1964) and phosphorus by the method of Fisk and SubbaRow, (1925). Ammonia nitrogen concentration was determined by the procedure of Conway, (1957) and urea nitrogen was determined by the Hycel, (1960) procedure.

All cows were allowed to graze the green indigenous forage in two different locations during the subsequent spring and summer with allotment to the locations being made on the basis of previous winter treatment and sire-test group. The cows were rebred starting on May 1 and ending August 1. In order to determine the possible effect of previous winter rations upon reproductive performance, the subsequent date of calving as well as the weight of the calf were obtained on all cows.

The birth weights of the calves were corrected for sex by multiplying the heifer weight by 1.048 and weaning weights corrected to 205 day equivalents by the formula

$$\left[\frac{(\text{Actual weaning weight} - \text{birth weight})}{\text{Actual weaning age}} \times 205\right] + \text{birth weight}$$

and corrected for sex by multiplying heifer weights by 1.059 (Smithson, 1966).

Trial II Steers equipped with permanent rumen fistulae, were used to determine the effect of the two rations upon ruminal fluid ammonia levels, (Table I). The trial consisted of two parts: In part 1, six steers, three per treatment, were given their assigned rations for 10 days prior to sampling. Food was removed for 36 hours after which they were fed 1.82 kg. of their assigned supplement and 3.64 kg. of a poor-quality grass hay. Rumen fluid samples were taken at 0, 30, 60, 90, 120, 180, 240, 300, 480, 600, and 720 minutes after feeding and analyzed for their ammonia

TABLE I

COMPOSITION OF RATIONS[a]

Ration	1	2
Treatments	CSM	Urea
Ingredients %		
Ground milo	48.0	74.5
Alfalfa meal	5.0	5.0
Molasses	5.0	5.0
Wheat bran	5.0	5.0
Cottonseed meal	32.0	----
Trace minerals - salt[b]	3.0	3.0
Diammonium phosphate	1.0	1.0
Dicalcium phosphate	1.0	1.5
Vitamin A	----	----
Urea	----	4.0
Sodium phosphate	----	1.0
TOTAL	100.0	100.0
Chemical Analysis		
Crude protein	20.80	21.00
Calcium	0.42	0.45
~~Protein~~ Phosphorus	1.00	1.00

[a]Feeds ground and pelleted into three-quarter inch diameter pellets

[b]To each 45 kg. of diet were added the following mineral salts, in grams: NaCl, 1296.2; $CoSO_4 \cdot 7H_2O$, 0.4; $CuSO_4 \cdot H_2O$, 3.7; $FeSO_4 \cdot H_2O$, 41.3: $MnSO_4 \cdot H_2O$, 5.6; K.I., 0.02; and $ZnSO_4 \cdot H_2O$, 13.6.

nitrogen contents as described in Trial I.

In the second part, four fistulated steers, two per treatment, were used. Feeding, management, and sampling was the same as in Part 1 except no fasting period preceded the feeding and sampling of the rumen contents.

RESULTS AND DISCUSSION

Trial I The performances of the cows are shown in Table II. Treatments had no effect on the average weight change or condition score during the period from November 1, 1966, to January 4, 1967; however, animals receiving the cottonseed meal supplements lost less ($P < .005$) weight during the winter feeding period and were in better ($P < .005$) condition of flesh when the season ended.

Weight changes and condition scores during the total period from November 1, 1966, to September 1, 1967, were small; however, both measurements apparently ($P < .10$) favored the cows which received the cottonseed meal. The cows did not consume the urea-containing supplement readily and on some days they did not consume all of the supplement for several hours after feeding. In contrast those receiving ration 1 consumed the feed within a very short time after feeding. These results appear to be divergent from results reported by Gallup et al. (1953) where they observed that urea containing feeds were as palatable as those containing no urea.

Neither birth weight nor weaning weight of the calves was affected significantly ($P > .05$) by winter feed; however, calves from mothers receiving the cottonseed meal-containing supplement averaged 5.4 kg. heavier at weaning. Winter treatments did not affect the subsequent calving dates or birth weights of the calves.

Blood values on samples taken from five cows in each subgroup are shown in Table III. Since location had no significant effect ($P > .05$) on any mineral, each value in Table III represents the average value from ten cows. Ration did not significantly affect ($P > .05$) serum levels of calcium, magnesium, copper, zinc, or urea. However, the animals receiving the urea-containing supplement contained higher levels ($P < .005$) of both ammonia and phosphorus. The higher blood ammonia values were expected. No explanation is offered for the higher phosphorus level; however, two factors may bear on this observation. (1) The forage from one pasture appeared to contain a higher level of phosphorus than the other and the cows fed the urea-containing diet were in this pasture when blood samples were obtained. (2) The urea-containing diet contained a higher proportion of inorganic phosphorus than the other diet.

Toward the end of the supplemental feeding period animals in one location which were receiving the urea supplement began to lose hair (Figures 1 and 2), a condition commonly found when animals are deficient in protein.

The results of the present experiment agrees with those of Nelson and Waller, (1962) and Darlow, (1945) who found

TABLE II

EFFECT OF WINTER SUPPLEMENTS ON PERFORMANCE OF COWS AND CALVES

Item	Diet		
	Ration 1 CSM	Ration 2 Urea	SE[a]
Weight change, midway[b] (kg.)	21.8	19.9	1.1
Weight change, winter[c] (kg.)	-36.50[e]	-56.50[f]	3.2
Weight change, total[d] (kg.)	4.4	-6.4	5.8
Condition change, midway[b]	-0.43	-0.65	0.15
Condition change, winter[c]	-1.5[g]	-2.1[h]	0.13
Condition change, total[d]	-0.52	-0.83	0.17
Birth weight of calves (kg.)	29.0	28.6	0.64
Weaning weight of calves (kg.)	213.4	208.1	3.2

[a]Standard error of treatment means.

[b]Weights and condition change, from November 5 to January 4.

[c]The winter feeding period, from November 5, 1966 to Apr. 5.

[d]The total period, November 5, 1966 to September 26, 1967.

[e-h]Horizontal values with differing superscripts differ significantly ($P < .005$).

that the performance of animals consuming a urea-containing supplement was never as good as that of animals consuming cottonseed meal. For two months after the cows started receiving supplemental feed, treatments did not seem to affect performance of the cows. However, after calving those receiving urea appeared to lose weight and condition rapidly while the others did not. Explanation could concern the increased need for energy during lactation. Higher blood ammonia levels and the loss of hair in the animals receiving urea indicated that protein synthesis from urea was low. Further evidence is reported in Trial II.

Trial II Ruminal fluid ammonia values are shown in Table IV and Figures 3 and 4. These values were greater ($P < .005$) in animals which had received the urea-containing ration. Urea is readily hydrolyzed by microbial urease present in ruminal fluid to ammonia and carbon dioxide (Pearson and Smith, 1943; Kornberg et al, 1954, 1955; Visek, 1962) If there are sufficient carbon fragments (alpha-keto acids) primarily from carbohydrate origin present (Annison and Lewis, 1959), the ammonia is incorporated into microbial protein. However, if there is a shortage of carbon skeletons, and the pH of the rumen fluid is correct, the ammonia will enter the rumen epithelium and be absorbed into the body (Coombe et al, 1960; Hogan, 1961; Bloomfield et al, 1964). These results indicate that a combination of weathered range and the supplement did not provide enough carbon fragments, and much of the urea nitrogen provided in

TABLE III

EFFECT OF PROTEIN SUPPLEMENTS UPON BLOOD SERUM CONSTITUENTS OF COWS

Item	Diet: Ration 1 CSM	Diet: Ration 2 Urea	SE[a]
Number of cows	10.0	10.0	
Calcium (mg./100 ml.)	11.10	10.75	0.18
Phosphorus (mg./100 ml.)	6.79[b]	8.18[c]	0.23
Magnesium (mg./100 ml.)	2.61	2.59	0.04
Copper (ppm)	0.80	0.77	0.03
Zinc (ppm)	0.80	0.79	0.06
Ammonia (mg./100 ml.)	1.56[d]	2.05[e]	0.09
Urea (mg./100 ml.)	5.14	7.16	1.09

[a]Standard error of treatment means.

[b-e]Horizontal values with differing supercripts differ significantly ($P < .005$).

Figure 1. Animals Consuming a Urea-Containing Supplement, April 15, 1967.

Figure 2. Animals Consuming a Urea-Containing Supplement, April 15, 1967.

Ration 2 was wasted. For this reason greater weight loss and a poorer condition was found in these animals as compared to those fed the cottonseed meal-containing supplement.

As noted in Figures 3 and 4, the ammonia concentration in the rumen at time zero was higher when the steers were fasted for 36 hours, whereas the overall ammonia concentration in the rumen was higher in the animals which were not fasted. The higher ammonia values at time zero follows the work of Sutherland et al, (1962) in which it was found that a greater increase in ammonia occurs before feeding than 11 hours after feeding. This increase could result from a decrease in ammonia utilization and its continued production from feed protein. However, fermentation of feed protein is essentially complete 6-8 hours after feeding, and the increases in ammonia occurs after that. Indogenous metabolism of nongrowing microbes releases ammonia when soluble carbohydrate is scarce (Hendrick, 1960) or cytolytic bacteria may digest and ferment other rumen organisms with the release of ammonia (Hungate, 1966).

The higher overall ammonia concentration in the nonfasted animal can be explained by the work of Clifford et al, (1967) in which it was found that ureolytic activity was decreased when the animals were fasted thus slowing the rate of urea hydrolysis.

These results support the idea that the level of urea in Ration 2 was too high. Bloomfield et al, (1964) has shown by use of artificial rumen studies that a ratio of 55

TABLE IV

EFFECT OF PROTEIN SUPPLEMENTS ON AMMONIA CONCENTRATIONS IN RUMEN FLUID

Item	Diet: Ration 1 CSM	Diet: Ration 2 Urea	SE[a]
Non-fasted (mg./100 ml.)	5.2[b]	7.0[c]	0.30
Fasted 36 hours (mg./100 ml.)	4.0[d]	16.0[e]	1.8

[a]Standard error of treatment means.

[b-e]Horizontal values with differing superscripts differ significantly ($P < .005$).

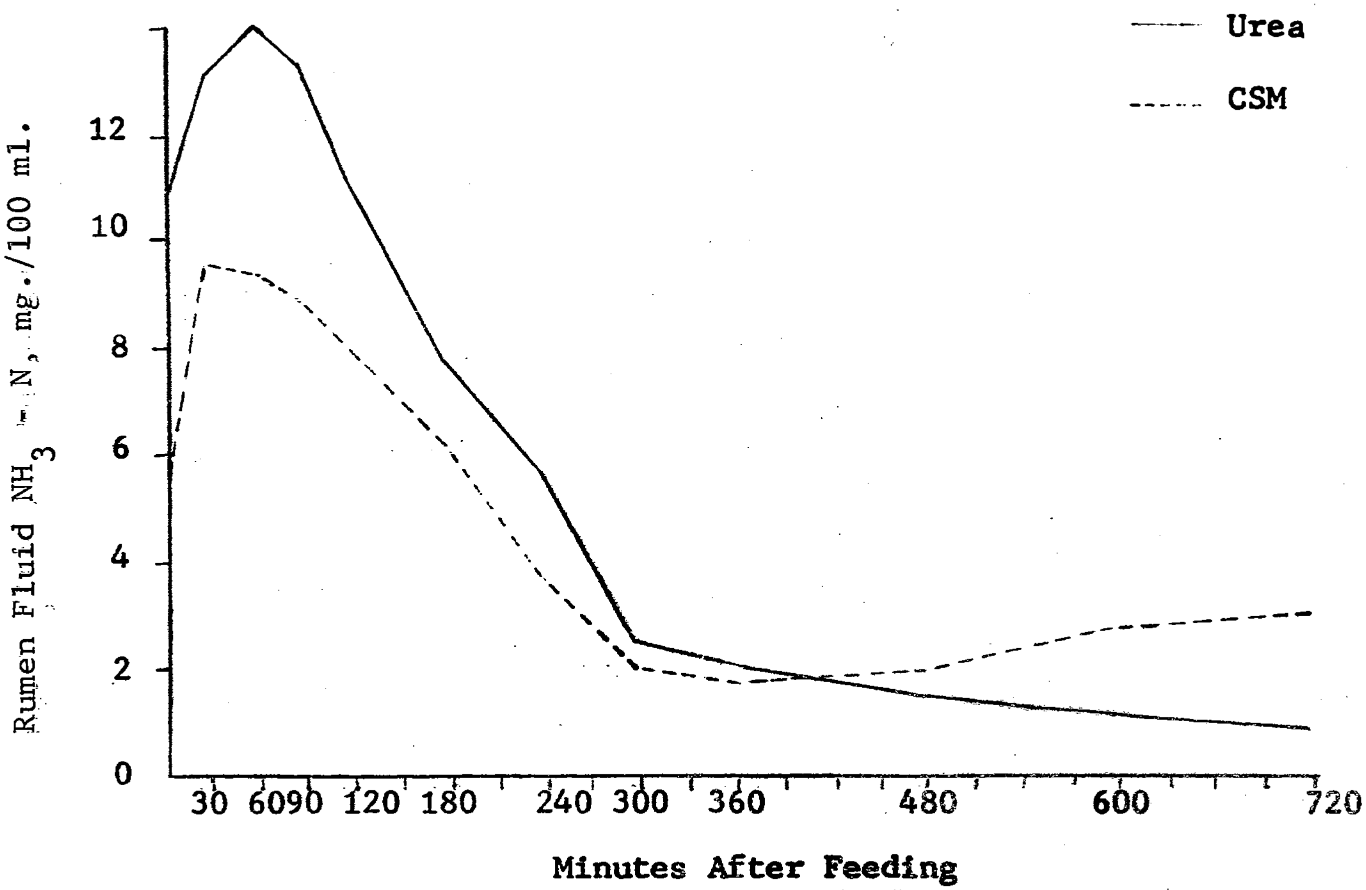

Figure 3. Effect of Protein Supplements on Ammonia Concentration in Rumen Fluid of Steers Fasted 36 Hours Prior to Feeding and Sampling.

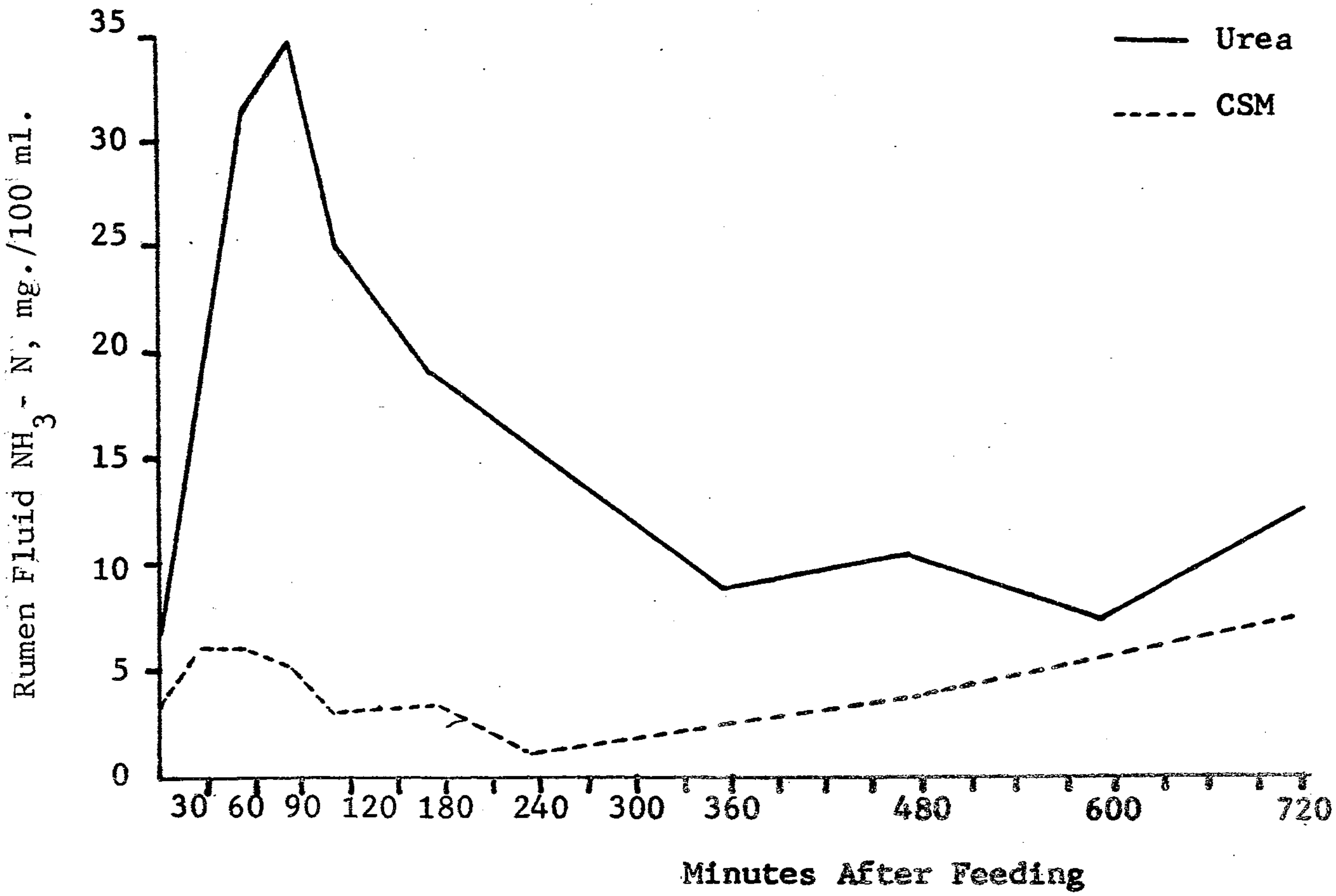

Figure 4. Effect of Protein Supplement on Ammonia Concentration in Rumen Fluid of Steers.

parts of carbohydrate is needed for optimum protein synthesis when urea is the sole source of dietary nitrogen. As feed grade urea contains about 45 percent nitrogen, these results would indicate a ratio of about 25 parts of NFE to one part of urea is needed for optimum urea utilization. More importantly, the NFE of weathered range forage is slowly hydrolyzed, thus it is doubtful if the NFE contained in these feeds is of much value for furnishing the carbon fragments needed for protein synthesis in the rumen. If this is true all of the required NFE for protein synthesis must be included in the urea-containing supplement. This, of course, would place the upper level of urea in most range supplements to around two percent.

SUMMARY

One hundred and ninety grade Angus cows, bred for spring calving, were used to compare urea and cottonseed meal in low protein supplements, when these were fed to beef cows grazing native range forage during the winter season. However, treatments had no significant effect on weight or condition score of cows from November 5, 1966 to January 4, 1967. However, animals fed the cottonseed meal supplements lost less weight and were in better condition at the end of the supplemental feeding period. Differences in weights and condition changes from November 5 to September 26 were small, but tended to favor cows receiving the cottonseed meal. Treatments did not affect birth weights of calves. Dams which had received the cottonseed meal during the winter season weaned calves which averaged 5.4 kg. heavier than those receiving urea.

Treatments did not affect blood levels of calcium, magnesium, copper, zinc, or urea, but animals receiving the urea supplement had higher levels of phosphorus and ammonia. The supplements were also fed to steers equipped with permanent rumen fistulaes which permitted the sampling of rumen contents at various times after the rations were fed. Higher ruminal fluid ammonia levels were found in those fed the urea-containing supplements.

These results indicate that the level of urea was too high for this type of urea-containing ration.

CHAPTER IV

NITROGEN-FREE EXTRACT TO NITROGEN IN UREA SUPPLEMENTS FED RUMINANTS RECEIVING A POOR-QUALITY ROUGHAGE

INTRODUCTION

The *in vitro* work of Bloomfield *et al*, (1964) indicates that approximately 55 gms. of carbohydrates is needed per gram of nitrogen fixed. As carbohydrates are in important part in the effective utilization of urea and is one of the major deficiencies in range grasses, no work has been reported on the amount or ratio of carbohydrates to nitrogen that is needed *in vivo*. The objective of this experiment was to determine the optimum nitrogen-free extract to nitrogen (NFE:N) ratio in urea-containing supplements fed ruminants receiving a poor-quality roughage.

EXPERIMENTAL PROCEDURE

The nitrogen-free extract of grains and molasses was used as an estimate of the soluble carbohydrate portion of the diet and to calculate the NFE:N ratio in the present experiment. As the nitrogen-free extract (NFE) of cottonseed hulls would be expected to be slowly hydrolyzed, it appears doubtful that it would be an important source of soluble carbohydrates for protein synthesis, thus was ignored in calculating the NFE:N ratio.

Trial I Twelve two-year old steers averaging 318 kg. were randomly assigned to four treatments, which involved NFE:N ratios of 11, 17, 22, and 28:1 (Table V). Three steers per treatment were used to determine organic matter, dry matter, and nitrogen digestibilities and nitrogen retention values. All animals were placed in metabolism stalls, which permitted separation of feces and urine. A 4-day adjustment and a 14-day preliminary period preceded a 10-day collection period, during which time feces and urine were collected using standard methods. During the preliminary and collection periods, the animals were fed one-half of their daily feed in the morning and one-half in the afternoon. Each animal received 0.91 kg. of the basal mixture (Table VI) per day plus eight pounds of cottonseed hulls. The increased ratios of NFE:N were obtained by increasing equal proportions of sugar and starch. When this trial was completed, all animals were given a 14-day rest period during which all animals were kept in outdoor pens and fed a common basal diet. Upon completion of the rest period, the animals were reassigned to treatments at random and the experiment was duplicated as before, thus six animals appeared on each treatment. Chemical analysis of feeds and excreta were determined by methods of the A.O.A.C. (1960).

Trial II Results of Trial I indicated that increased ratios of NFE:N upon nitrogen retention were linear and that the highest ratio did not supply the level of NFE needed for optimum protein synthesis. Thus it was decided to extend the ratios up to 55:1. Unfortunately, the animals used in the first trial were not available for this trial because they were sold. Twelve yearling steers, averaging 254 kg., were used in this trial. The NFE:N ratios used in this trial were 28, 37, 46, and 55:1 (Table V). All other details were as described in Trial I with the exception of cottonseed hulls in which the consumption was reduced to 1.82 kg. per day due to the younger and lighter steers.

RESULTS AND DISCUSSION

The effect of increasing the NFE:N ratios from 11:1 to 28:1 are shown in Table VII. Treatment had no significant effect ($P > .05$) upon the digestibility of organic matter, dry matter, or nitrogen. Although not significant, there appeared to be a trend for nitrogen digestibility to decrease with increased NFE intake and agrees with the results of Fontenot et al, (1955). Nitrogen retention increased in a manner not different from linearity ($P < .005$) indicating that a ratio of NFE:N beyond 28:1 could provide rumen conditions allowing greater ruminal protein synthesis.

Effects of further increasing the NFE:N ratios from 28:1 to 55:1 are shown in Table VIII. Level of NFE in the ration did not affect ($P > .05$) organic matter or dry matter digestibility. Animals receiving rations 5, 6, and 7 digested less dietary nitrogen that those receiving ration 4, while those on ration 7 digested less nitrogen ($P < .05$) than

TABLE V

RELATIONSHIP OF BASAL DIET AND STARCH-DEXTROSE MIXTURE IN VARIOUS NFE:N RATIOS

Ration	NFE To N Ratio	Basal Kg.	Starch Dextrose Kg.
I	11:1	0.91	
II	17:1	0.91	0.31
III	22:1	0.91	0.62
IV	28:1	0.91	0.91
V	37:1	0.91	1.30
VI	46:1	0.91	1.70
VII	55:1	0.91	2.10

TABLE VI

COMPOSITION OF THE
BASAL RATION

Ingredients	%	Ingredients	%
Urea (45% N)	11.5	Diammonium phosphate	1.0
Ground milo	67.5	Dicalcium phosphate	2.5
Molasses	5.0	Salt Trace minerals[a]	3.0
Wheat bran	5.0	Vitamin A[b]	

<u>Chemical Analysis</u>

Crude protein	40.6
NFE	55.6
Calcium	0.7
Phosphorus	1.0
NFE:N	11.1

[a]Mixture supplied, in grams, the following mineral salts per 45 kg.: NaCl, 1296.2; $CoSO_4 \cdot 7H_2O$, 0.4; $CuSO_4 \cdot H_2O$, 3.7; $FeSO_4 \cdot 7H_2O$, 41.3; $MnSO_4 \cdot H_2O$, 5.6; K.I., 0.02; and $ZnSO_4 \cdot H_2O$, 13.6.

[b]Vitamin A palmitate at level of 10,000 I.U./lb.

TABLE VII

EFFECT OF DIFFERENT NFE:N RATIOS ON THE DIGESTIBILITY OF RATION CONSTITUENTS AND THE RETENTION OF NITROGEN

Ration NFE:N Ratio	1 11:1	2 17:1	3 22:1	4 28:1	SE[a]
Digestibility %					
Organic Matter	53.5	47.8	49.9	51.1	1.5
Dry Matter	50.7	45.5	47.8	49.2	1.5
Nitrogen	49.8	49.5	49.0	51.7	1.7
Nitrogen Balance					
Intake, gm.	84.2	84.9	83.7	83.7	
Feces, gm.	42.1	42.8	42.7	40.4	
Urine, gm.	51.3	46.3	43.9	40.4	
Retained, % intake	-10.8[b]	-5.0[c]	-3.3[e]	0.5[d]	1.3

[a]Standard error of treatment means.

[b-d]Horizontal values with differing superscripts differ significantly ($P < .05$).

TABLE VIII

EFFECT OF DIFFERENT NFE:N RATIOS ON THE DIGESTIBILITY OF RATION CONSTITUENTS AND THE RETENTION OF NITROGEN

Ration	4	5	6	7	SE[a]
NFE:N Ratios	28:1	37:1	46:1	55:1	
Digestibility %					
Organic Matter	62.1	60.6	62.6	61.2	1.0
Dry Matter	59.5	57.7	59.8	58.0	1.0
Nitrogen	68.9[b]	65.8[c]	64.8[cd]	62.7[d]	1.2
Nitrogen Balance					
Intake, gms.	81.7	79.7	82.2	82.3	
Feces, gms.	25.4	27.1	28.9	30.7	
Urine, gms.	33.3	34.7	30.6	28.5	
Retained, % intake	28.2[e]	22.3[f]	27.7[e]	28.0[e]	1.4

[a]Standard error of treatment means.

[b-f]Horizontal values with differing supercripts differ significantly ($P < .05$).

those on ration 5. Even though the effect of widening the ratio of NFE:N was not linear ($P > .05$) the trend was in that direction ($P < .10$) and agrees with the results of the first trial. Also, these results are in agreement with those of Hamilton, (1926); Titus, (1927) where they found that as the dry matter intake increased the fecal endogenous nitrogen loss was greater. As the ratios of NFE:N were obtained by increasing equal proportions of starch and sugar thus increasing the dry matter intake, the lower digestibility of nitrogen is explained on this basis. As there was little difference in digestible nitrogen, the linear response obtained by increasing the NFE:N ratios from 11:1 to 28:1 is then a function of the exogenous urinary nitrogen; if there are not enough carbon skeletons present in the rumen to combine with the ammonia produced to form microbial protein, the ammonia is absorbed through the rumen wall into the blood then transported to the liver, where it is converted to urea and excreted in the urine. As the NFE levels in the rations were increased, more carbon fragments were available to combine with the ammonia to form microbial protein. There was no trend towards improved nitrogen retained above a NFE:N ratio of 28:1, suggesting that under the conditions of this experiment this ratio supplied enough alpha-keto acids for the most efficient utilization of urea nitrogen.

SUMMARY

Two metabolism studies involving a total of six animals per treatment were used to study the effect of varying NFE:N ratios in urea-containing supplements fed to ruminants receiving a poor-quality roughage. As there were differences between the ages of the animals, levels of cottonseed hulls, and time of year, no attempt was made to combine the results of the two studies. Treatments in neither study significantly affected the digestibility of dry matter nor organic matter. Although not significant, there was a trend for the nitrogen digestibility to decrease with graded increases in the NFE:N ratio from 11:1 to 55:1. Nitrogen retention increased in a manner not different from linearity with NFE:N ratios from 11:1 to 28:1. No further increase in nitrogen retention was found in increasing the NFE:N ratios above 28:1, thus indicating that this ratio provides sufficient carbon fragments for urea utilization.

CHAPTER V

GENERAL DISCUSSION

Nitrogen from urea has not consistently been well utilized when used as a protein supplement for animals grazing dead and weathered range grasses during the winter season. One of the major deficiencies in the forages are carbohydrates; probably the most important component for protein synthesis from urea. Alpha-keto acids, mainly from soluble carbohydrate origin combine with ammonia, produced from the breakdown of urea, to form microbial protein. If the supply of readily fermentable carbohydrates is small, the ammonia produced is absorbed through the rumen wall into the portal blood where it is of little importance to the animal; however, if there is an abundance of carbon fragments, the ammonia will combine with the alpha-keto acids for the formation of microbial protein.

The optimum ratio of NFE:N for the efficient conversion of ammonia nitrogen to microbial protein has not been determined in the ruminant animal; however, investigation has shown in *in vitro* studies that 55 gm. of carbohydrates is needed for each gm. of nitrogen formed.

The results of Experiment II indicates that a NFE:N ratio of 28:1 promoted maximum nitrogen retention, whereas in Experiment I a NFE:N ratio of 31:1 resulted in a greater weight loss and a poorer condition after 2 months on test than animals consuming cottonseed meal. Reasons for the apparent differences are unknown, but several points seem logical: (1) The proportion of concentrates to roughage was higher in the metabolism studies; (2) the dextrose-starch mixture was more rapidly hydrolyzed than the starches of the grain in Experiment 1, thus might promote greater protein synthesis and thereby reduce urinary nitrogen loss; (3) cottonseed hulls, used in the metabolism studies, might be a better source of roughage for urea utilization than weathered range grass when grazed by cattle; (4) a larger amount of energy was needed by the cows during lactation thus reducing the availability of alpha-keto acids for protein synthesis; (5) in Experiment 1, the cows were fed their assigned supplements until April 25; however, several cool season grasses began to appear under the cover of the dead grass around March 1-15. The amount of these grasses was never enough to supply much of the nutrient needs of the cows; however, they do contain high levels of nitrogen, much of it in the form of non-protein nitrogen. If the animals were consuming enough of the grass, which would be readily hydrolyzed to ammonia (Annison and Lewis, 1959), it could be expected to reduce (Johnson et al, 1948) urea utilization.

CHAPTER VI

GENERAL SUMMARY

In the first experiment, one hundred and ninety pregnant cows were divided into two equal groups for comparing a ration containing 4 percent urea to an isonitrogenous one containing 32 percent cottonseed meal. The cows grazed weathered forages from November 5 until April 15 and in addition were fed three pounds per day of their appropriate supplement until parturition and then four pounds was fed until the pasture would supply the nutrients needed by the cow. Rations had no effect on the weight or condition change of the animals from November 5 until January 4, however, animals fed the cottonseed meal diet lost less weight and were in better condition at the end of the supplemental feeding period. Differences in weight and condition changes from November 5 to September 26 were small, but tended to favor the cows receiving the cottonseed meal. Birth weights were not affected by treatments; however, dams receiving cottonseed meal weaned calves which averaged 5.4 kg. heavier. Winter treatments did not affect the subsequent calving dates or birth weights of the calves. The serum levels of calcium, magnesium, copper, zinc, or urea were not affected by treatments, however, animals receiving urea had higher levels of phosphorus and ammonia. Rumen fluid samples obtained from fistulated steers fed the urea were higher in ammonia than animals receiving cottonseed meal.

In Experiment II, two metabolism studies involving a total of six animals per treatment were used to study the effect of variable NFE:N ratios of 11, 17, 22, 28, 37, 46, and 55:1 in urea-containing supplements fed ruminants receiving a poor quality roughage on the digestibility of dry and organic matter and nitrogen balance. Nitrogen digestibility decreased as the intake of dry matter increased, but dry and organic matter digestibilities were not affected. Nitrogen retention increased in a linear manner when the NFE:N ratio increased from 11:1 to 28:1, but no further increases were found in the wider ratios.

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