

SUPPLEMENTAL VALUE OF UREA, BIURET,
EXTRUDED UREA-GRAIN, AND MHA
FOR RANGE BEEF CATTLE

By

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CHAPTER I

INTRODUCTION

Protein is the first limiting nutrient, when forage is adequate, for cattle production throughout the world. Due to lack of sufficient rainfall, topsoil, or management skills, legumes cannot always be grown, increasing protein deficiencies of ruminants. In many parts of the world, competition from humans for existing natural plant proteins may severely inhibit future use of these natural proteins by ruminants. As world population increases, it is logical to expect the competition to increase in severity. Ruminants are equipped with a unique digestive system that allows the animal to use less readily available sources of protein and energy; however, methods of utilizing this special capability must be improved. The rumen is the site for a large population of microorganisms that is capable of synthesizing high quality microbial protein that is later digested by the animal and used for biological protein synthesis. To synthesize this microbial protein, the microbes must have sources of energy (alpha-keto acids) and nitrogen. The nitrogen can be utilized rather effectively from a non-protein-nitrogen (NPN) compound under proper conditions. The manufacturing of NPN compounds has been economical enough to be very competitive with natural protein until emergence of the energy crisis. However, with the increasing clamor of humans for red meat products, NPN products may soon revert to their previous relative cost position.

Animals consuming low quality roughage diets utilize NPN compounds less favorably than animals consuming concentrate diets. Energy derived from lignocellulose complexes is made available too slowly to unite with ammonia (rapidly hydrolyzed from NPN compounds) to form microbial protein. Research studies have been diverted to searching for other NPN compounds that are hydrolyzed to ammonia more slowly, or to altering structure of NPN compounds now available so that a slower rate of hydrolysis might be obtained. Biuret appears to have some promise in this regard. Its rate of ammonia release more closely resembles the rate of alpha-keto acid production from roughage diets. Little research has been conducted concerning the use of protein supplements, for range cattle on high roughage diets, containing nearly all of the nitrogen in the form of NPN compounds.

The purpose of this study was: (1) to compare the utilization of feed grade biuret, urea and extruded urea-grain mixtures for lactating cows and for replacement heifers; (2) to evaluate the utilization of protein supplements composed of NPN supplying nearly 100% of the total crude protein for weaned heifers; and (3) to evaluate the addition of methionine-hydroxy-analogue (MHA) to natural protein supplements for lactating cows consuming winter range grass.

CHAPTER II

REVIEW OF LITERATURE

Introduction

In 1879, it was discovered that ruminants could convert non-protein-nitrogen (NPN) to protein. It was probably unknown at that time what a profound impact this discovery would have on the future nutrition of ruminant animals. Hart et al. (1939) began American studies of NPN with a report that growing dairy heifers could utilize either urea or ammonium carbonate as a nitrogen source. Another important study was conducted by Loosli et al. (1949). He found that the rumen was capable of synthesizing ten amino acids which are dietarily essential to the rat. Purser (1970) states, in agreement with Maynard and Loosli (1969), Johnson et al. (1942), Oltjen (1969) and other workers, that microbial protein quality is rather constant without regard to the metabolic source of protein. Although the ruminant has at least nine dietary essential amino acids, this animal is capable of producing the required amino acids with a non-specific source of available nitrogen. On the other hand, Lofgreen, Loosli and Maynard (1947) suggested that protein quality may be important at times. Microbial cell material synthesized in the rumen was found to be about 65.6% crude protein by Hungate (1966). McNaught et al. (1954) declared microbial protein, passed on through the digestive tract, to be only 80% digestible. Purser (1970) suggests that an interaction between amino acid utilization and a specific metabolic

energy source is possible. There are several factors to consider concerning the conversion of dietary nitrogen to microbial protein. Rate of passage of nitrogen through the rumen is positively correlated to the conversion of nitrogen to protein. Resistance of dietary nitrogen to deamination in the rumen, the availability of nitrogen for protein synthesis, and the amount of energy available for rumen fermentation are important factors governing the conversion of dietary nitrogen to microbial protein. The population composition of bacterial and protozoal species is a factor in the efficiency of nitrogen utilization. Unknown factors plus certain minerals are necessary for optimum utilization.

NPN Utilization

Utilization of Urea. Johnson et al. (1942) and Briggs et al. (1947) studied the use of urea as a natural protein substitute in rations of ruminants. Johnson rationalized "that a considerable portion of the protein ultimately utilized by the ruminant is microorganismal protein, regardless of the nature of the nitrogenous compounds contained in the ration as consumed." He found, however, that natural protein (soybean meal) was utilized better than urea in the rumen. Supplements, with NPN making up less than 50% crude protein equivalent, were similar to 100% cottonseed meal in a study reported by Briggs et al. (1947). Pellets with 50% urea crude protein equivalent proved palatable in his study at first, but were unpalatable later on in the experiment. He found urea supplements with low protein rations tended to increase feed consumption. Leibholz (1972) found weight gain or feed efficiency to be unaffected by dietary urea in early weaned calves. Lofgreen et al.

(1947) found that a urea supplement, with urea making up 40% crude protein equivalent, plus 0.2% methionine significantly increased nitrogen retained by lambs. Tillman and Swift (1953), Freitag, Theurer and Hale (1970), and Streeter et al. (1973) reported similar results. Urease, an enzyme that hydrolyzes urea to ammonia and carbon dioxide, can also break down other NPN compounds such as amides and nitrates or natural sources such as intact proteins, peptides and amino acids according to Tillman and Sidhu (1969). Brookes et al. (1972), Tillman and Sidhu (1969), and Streeter et al. (1973) agree that the hydrolysis of urea to ammonia is a rapid process, with Tillman suggesting that the rate of urea hydrolysis is four times greater than the rate of nitrogen utilization by the microbial organisms. Several studies have been made on the effects of the addition of various products to an NPN source used for nitrogen utilization in the formation of protein. Van Slyke, Baeson and Perry (1971), Harbers and Tillman (1962), Martin, Clifford and Tillman (1969), and Gil, Shirley and Moore (1973) have tried the addition of dehydrated alfalfa meal, barbituric acid, sodium bentonite, and methionine-hydroxy-analogue (MHA), respectively, to urea diets and have found no significant improvements in nitrogen utilization. Virtanen (1966) reported that dairy cows, fed purified carbohydrates plus urea and ammonium salts (only sources of energy and nitrogen, respectively), maintained body weight and a relatively high level of milk production.

Utilization of Other NPN Sources. Some comparisons of various sources of NPN compounds have been made by Oltjen et al. (1969), Oltjen, Burns and Ammerman (1973), Bond and Rumsey (1973), Ammerman et al. (1972), and Rush (1974). The consensus of these experiments seems to be that while some nitrogen from NPN sources was utilized, all were

inferior to natural protein sources as measured by body weight changes, condition score, weaning weights of calves, and other measurements. Oltjen et al. (1973) and Bond and Rumsey (1973) reported that NPN utilization tended to favor biuret over urea supplements. Because of biuret's slower ruminal hydrolysis, it seems logical that this should prove to be advantageous on the range, under optimum conditions. However, the Oklahoma studies (Ivan G. Rush, personal communication) indicate very similar results from either biuret or urea.

Utilization of Energy. The released ammonia in the rumen can be more efficiently utilized when there is sufficient energy present. Amino acids are produced from ammonia, carbon-chain skeletons, and energy according to Tillman and Sidhu (1969). Gallup, Whitehair and Bell (1954), Bloomfield, Wilson and Thompson (1964), Mizra and Ranhotia (1969), Williams, Whiteman and Tillman (1969), and Potter et al. (1971) found favorable results when an energy source such as molasses, liquid hemicellulose, or sugars were included in the diets. Bloomfield et al. (1964) found that for each gram of nitrogen utilized, the bacteria required 55 grams of carbohydrates. They concluded that the urea level of a diet is not restricted by a fixed percentage, but can be fed as a function of the energy level. It seems that problems begin to develop when NPN compounds are used in a ration with low energy. Mizra and Ranhotia (1969) suggested that wheat straw was not a sufficient energy source. Morris and Gulbransen (1970) could achieve only a small growth increase with a urea supplement and oat or Rhodes grass pastures. Fick et al. (1973) reported that a low quality roughage diet could be enhanced by a NPN supplement, but that supplemental energy did not increase the voluntary intake of hay and it actually depressed cellulose

digestibility. Several studies have been made at the Oklahoma State University Lake Carl Blackwell Range with cattle fed NPN supplements under range conditions. Rush, Sharp and Totusek (1972) found poor utilization of NPN supplements by cows grazing weathered, winter forages. Totusek, Holloway and Sharp (1971) found similar results, but the cows fed prairie hay utilized NPN to a greater degree than those allowed to graze pastures only. Pidgen (1971) reported that the lignocellulose complex accounts for most of the gross energy in mature forages. Tillman (unpublished manuscript) stated "that when lignocellulose is the main energy source, optimum consumption of roughages becomes an important factor. Urea utilization is improved by roughage-processing methods which increase forage consumption by ruminants." This is a possible explanation why harvested forages seem to foster better NPN utilization than do mature range forages. Various authors have seemed to find contrasting results concerning the effect of NPN upon the level of intake of poor quality roughages. Ammerman et al. (1969), Ely et al. (1972), and Messenger, Donald and Brown (1971) reported increased consumption of poor quality roughage with addition of a NPN supplement. Ely et al. (1972) found that a 4% ammonium chloride supplement increased feed intake, but higher levels decreased feed intake. Williams et al. (1969) reported that cattle consumed urea supplements slowly, especially near the completion of the trial. They concluded that the low quality roughage did not furnish sufficient energy for effective nitrogen utilization. Oltjen et al. (1973) stated that hay intakes were not influenced by supplements added to the ration. In another experiment, Ammerman et al. (1972) reported that urea decreased hay intake in contrast to his earlier work (Ammerman et al., 1969). Bond and Rumsey

(1973) compared molasses, molasses plus urea, and molasses plus biuret to timothy hay fed alone as a control. Molasses tended to lower hay consumption, but the total feed intake remained nearly constant.

A number of studies concerning use of NPN supplements with winter range forages indicated that nitrogen utilization was low. Nelson et al. (1957), Nelson and Waller (1962), Williams et al. (1969), Messenger et al. (1971), Rush et al. (1972), Bond and Rumsey (1973), and Oltjen et al. (1973) reported poor utilization of NPN on low quality roughages. Nelson et al. (1957) found that the addition of trace minerals or dehydrated alfalfa meal increased nitrogen utilization a small amount.

Problems of NPN Utilization. As is often the case in the search for new solutions for old problems, new problems are encountered in the process. With an expected future expanded use of natural protein by humans it is important to find ways to use up to 100% NPN supplements on low roughage diets. Raleigh and Wallace (1963), Oltjen et al. (1968), Oltjen et al. (1969), and Tucker and Fontenot (1970) found that growth, feed efficiency and nitrogen retention was reduced, up to 35% in one study, by use of NPN as compared to natural protein. Tillman et al. (unpublished manuscript) suggested that "the rate of protein synthesis might be too slow, the quality of the microorganisms too poor, or a combination of these are limiting growth and performance of ruminants." In the Raleigh and Wallace (1963) study, urea plus hay proved to be highly toxic and killed two animals at a 12% crude protein level. In another study, Briggs et al. (1947) stated that urea had no toxic effects when included as only part of the dietary nitrogen. Hatfield et al. (1959) stated that biuret was not acutely or cumulatively toxic to sheep. Biuret was determined to be a superior supplement when fed only twice a

day as compared to ad libitum feeding by Oltjen et al. (1969). Tillman et al. (unpublished manuscript) discussed at length the toxicity problem, particularly with urea diets where ammonia is rapidly hydrolyzed in the rumen. Factors to be considered include: (1) allowing a time for adaptation of the ruminal microorganisms; (2) prevent fasting prior to urea consumption; (3) use of urea supplements in high roughage, low quality diets; (4) feeding of diets which promote a high pH in ruminal fluid; and (5) low water intake. These factors suggest that special management practices must be maintained. Inhibition of urease has been studied. Streeter et al. (1969), Brent, Adepoju and Portela (1971), and Tillman and Sidhu (1969) have tried acetohydroxamic acid or jackbean urease to limit urease production. Both products were successful in limiting urease production, but they did not improve digestibility of the ration or increase microbial numbers. Knight and Owens (1973) found that nitrogen retention was increased by infusions (one or three hour intervals) of urea rather than continuous infusions with less than high energy diets. Ludwick, Fontenot and Tucker (1971) studied the adaptation phenomena of microorganisms and found it took 30 to 50 days for nitrogen retention of a urea diet to equal that of a soybean diet. Feed intake can be a problem associated with NPN supplements on high roughage diets. Campling, Freer and Balch (1962) stated that intake is directly related to relative disappearance of digesta from the rumen-reticulo. This is supported by Oh, Longhurst, and Jones (1969). Tudor and Morris (1971) reported significantly increased voluntary feed intake when urea was fed two or three times per day as compared to when urea was fed once daily. Martz et al. (1973) found that the addition of urea to low quality roughages significantly decreased feed intake. Bhattacharya and

Pervez (1973) reported that urea did not significantly increase feed intake. It has been found in Oklahoma studies (Rush et al., 1973) that feed intakes were lowered somewhat under range conditions with NPN supplements. This is in agreement with Chalupa (1968) who reported lowered feed intakes as a problem of feeding urea to ruminants.

Influence of Mineral Supplementation. The addition of certain minerals (particularly sulfur) to a NPN supplement has been found to be advantageous to vitamin formation, cellulose digestion, and nitrogen utilization as supported by Hunt et al. (1954), Barton, Bull and Hemken (1971), Chalupa, Oltjen and Dinius (1973), and Gil et al. (1973). In contrast, Leibholz (1972) found no sulfur addition was necessary for young calves in Australia. Rush et al. (1973) reported that MHA decreased palatability of both urea and biuret supplements. However, most of the data suggest there is a need for some sulfur in the diet. Barton et al. (1971) suggested that the optimum level of sulfur was 0.14 to 0.17% of the ration dry matter to achieve efficient digestion of cellulose and lignocellulose. Some data suggest that one should incorporate a nitrogen:sulfur ratio of 8:1 to 15:1. This has become a common practice within the industry.

Griel et al. (1968), Patton, McCarthy and Griel (1970), and Polan, Chandler and Miller (1970) reported an increased milk and/or butterfat production by dairy cows supplemented with MHA. Varner, Bellows and Oltjen (1973) reported an increased milk and butterfat production by MHA-fed beef cows. Rush (1974) found little increase in NPN utilization or calf weaning weights by range cows fed a MHA-NPN supplement as compared to cows fed a NPN supplement without MHA.

Summary

It has been known that NPN can be converted to microbial protein by ruminal microorganisms for nearly a century. Several factors can determine the efficiency of NPN utilization by ruminants. One important factor is the presence of an energy source that is available in a manner compatible with NPN hydrolysis within the rumen. High concentrate diets furnish plenty of energy for the microbial population, but high roughage diets present some problems. Cellulose complexes furnish sufficient energy too slowly to be used efficiently by microbes because of the rapid hydrolysis of NPN. More research needs to be conducted to study methods to improve NPN utilization by beef cattle grazing winter range forages. NPN utilization is considered to be least efficient under these conditions.

CHAPTER III

SUPPLEMENTAL VALUE OF UREA, BIURET, EXTRUDED

UREA-GRAIN, AND MHA FOR

RANGE BEEF CATTLE^{1,2}

Summary

Four winter trials, using 297 cattle, were conducted to evaluate the supplemental value of feed grade biuret, urea, extruded urea-grain mixtures, and methionine-hydroxy-analogue (MHA) for beef cattle grazing low quality winter forage.

Lactating Angus and Hereford cows (104) were allotted to 30 and 15% natural protein (positive and negative controls, respectively), urea [30% crude protein (CP)], starea 44 (30% CP), and starea 70 (30% CP) supplements. Each non-protein-nitrogen (NPN) source furnished one-half of the supplemental nitrogen. The positive control cows sustained the smallest weight loss (128.6 kg) ($P < .05$). The starea 44 cows lost less weight (18.5 kg) than the negative control cows ($P < .05$), but the urea and starea 70 cows did not ($P > .10$). Condition loss was greater for

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the negative control and starea 44 cows than the positive control cows ($P < .025$). Calves raised by cows receiving the positive control and urea supplements gained more weight (15.8 kg) than the calves from cows receiving the negative control ($P < .025$) during the treatment period. Treatment did not affect post-treatment calf gain ($P > .16$) or calf weaning weight ($P > .09$).

Yearling, crossbred replacement heifers (66) were allotted to 30 and 15% natural protein (positive and negative controls, respectively), urea (30% CP), and biuret (30% CP) supplements. Each NPN source furnished one-half of the supplemental nitrogen. The positive control heifers lost less weight (8.5 kg) than the negative control and biuret heifers ($P < .01$). The urea heifers lost weight midway between the positive and negative controls and not significantly different from either ($P > .05$).

Weaned, crossbred replacement heifers (80) were allotted to 30% natural protein (positive control), no supplemental nitrogen (negative control), urea (106.68% CP) and biuret (104.72% CP). Each NPN source furnished about 98% of the supplemental nitrogen. The positive control heifers lost less weight (10.8 kg) than the other treatment groups ($P < .025$). Urea and biuret heifers sustained a weight loss midway from and significantly different from either control ($P < .05$).

Lactating, crossbred cows (47) were fed a 30% natural protein supplement with (avg. 16.8 g daily) or without MHA. Of the cows that calved before treatment began, those fed MHA lost more weight (26.2 kg) than the cows fed no MHA ($P \approx .05$). Post-treatment average daily gain of the calves was greater ($P < .005$) for calves from cows with MHA than calves from cows without MHA. Change in cow condition and average daily gain of the calves were similar for both groups ($P > .10$) for the

treatment period. Treatment did not affect calf weaning weights in either trial ($P > .25$).

Introduction

Low quality forages are used extensively for wintering beef cattle and supplementation with protein is usually needed for satisfactory performance. Nelson and Waller (1962), summarizing 16 experiments involving beef cattle wintered on low quality native range grass in Oklahoma, found that urea-containing supplements were of lower value than supplements containing cottonseed meal. Since poor utilization of urea is caused in part by rapid hydrolysis, interest has developed in biuret (Berry, Riggs and Kunkel, 1956; Ammerman et al., 1972; Oltjen et al., 1973), extruded urea-grain mixtures (Milligan and Robblee, 1969), and other sources of NPN (Ely et al., 1972; Webb, Bartley and Meyer, 1972). Addition of methionine-hydroxy-analogue (MHA) improved milk production in beef cows (Varner et al., 1973) and dairy cows (Griel et al., 1968). Beef cows, wintered on low quality forage, are subjected to stress and lose weight in a pattern similar to high producing dairy cows. Few researchers have studied semi-purified NPN supplements for cattle grazing low quality winter range forage.

All cattle in this study grazed low quality winter range forage. The objectives of this study were: (1) to compare supplements containing high levels of NPN to supplements of natural protein for lactating beef cows and heifers; and (2) to evaluate MHA for lactating beef cows fed a natural protein supplement.

Experimental Procedure

Four winter trials were conducted in Central Oklahoma on native tallgrass range with climax vegetation of little bluestem (Andropogon scoparius), big bluestem (Andropogon gerardi), Indian grass (Sorghastrum nutans), and switch grass (Panicum virgatum). Number and ingredient makeup of experimental supplements fed in the trials are shown in Table 1. The nitrogen:sulfur ratio for all supplements was approximately 12:1. Initial and final weights were obtained after a 12-hour shrink.

Trial 1. One-hundred-four mature Angus and Hereford cows were randomly allotted, after stratification by breed and by actual or expected calving date, to five treatments for a 113-day wintering trial. The five treatments consisted of supplements 1 to 5 in Table 1. Treatments 1 and 2, positive and negative controls, respectively, consisted of 30 and 15% natural protein supplements. Treatments 3, 4, and 5 consisted of 30% crude protein supplements in which one-half of the nitrogen was provided by either urea or the urea within starea 44³ (44% protein equivalent) and starea 70³ (70% protein equivalent), respectively. Urea provides 13 and 22% of the total nitrogen in the two products. Cows, allowed to graze in a common pasture, were gathered to a central feeding area each morning six days per week, placed in 0.91 x 2.44 m stalls located in a shed and individually fed their supplement. Twenty minutes were allowed for consumption of supplement; feed refusals were recorded daily, and minor intake adjustments were made periodically to achieve equal intake of supplement among all treatments. Cows calved

³Gelatinized starch-urea products obtained by processing a mixture of finely ground grains with urea under regulated conditions of temperature, moisture and pressure. Ingredients are ground sorghum grain and urea.

from September 28 to February 16, with an average calving date of November 21. Calving was completed before the trial was ended. Initial and final condition of cows was estimated by scoring each cow on a scale of 1 to 9, with 1 being the thinnest and 9 the fattest.

Since the number of cows which calved previous to the trial was disproportionate among treatments, initial weight of the cows that had calved before the trial was adjusted to a pregnant weight basis. The regression equation used to correct the initial cow weight, derived from data involving similar cattle (Ewing et al., 1966 and unpublished data) wherein calving weight loss and calf birth weight were accurately obtained, was:

$$\text{Adjusted initial weight} = \text{actual initial weight} + [(\text{calf birth weight} \times 1.9697) - 19.0].$$

Data in Trial 1 were analyzed by least squares with a model that included the effects of breed of cow, treatment, and breed of cow x treatment interaction. Dependent variables were cow weight loss expressed in kg and as a percentage of adjusted initial weight (initial weight adjusted as stated in the preceding paragraph), weight gain of the calf, calf weaning weight, and change in cow condition.

Post-treatment calf gain and calf weaning weight were analyzed with 89 observations, because of missing data, rather than the 104 observations used in the analysis for the remainder of the variables studied. An analyses-of-variance table is in the Appendix (Table 6). The student's t test (protected by a preliminary F test) was utilized to test for differences between any two treatments. If the F test was significant ($P < .05$), all treatment means were compared.

Trial 2. Sixty-six crossbred (1/2 Charolais x 1/2 Angus, 1/2 Charolais x 1/2 Hereford, 1/2 Hereford x 1/4 Angus x 1/4 Holstein),

pregnant yearling heifers were used in a 77-day growth trial. After stratification by breed and initial weight, heifers were allotted to four treatments. Treatments 1 and 2, positive and negative controls, consisted of 30 and 15% natural protein supplements (supplements 1 and 2, Table 1). Treatments 3 and 4 consisted of 30% crude protein supplement with one-half of the nitrogen from urea (supplement 6, Table 1) and biuret and urea from feed grade biuret⁴ (supplement 7, Table 1). Alfalfa hay was included at a level of 40% in these supplements. Supplements were supplied ad libitum in mineral feeders with salt added to the supplement to limit intake. Salt, NaCl, comm, (6) IRN 6-04-152 (salt) comprised 30% of the total mixture for treatments 1 and 2 and 20% for treatments 3 and 4. Equal intake of non-salt supplement among the four treatments was achieved. Heifers were rotated among pastures at 14-day intervals.

Since the heifers in Trial 2 varied considerably in initial weight, they were blocked within breed group according to initial weight and treatments were randomly assigned to heifers within block. Body weight loss was analyzed by least squares with a model that included the effects of breed, blocks within breed, treatment, and breed by treatment interaction. An analysis-of-variance table is in the Appendix (Table 7). Tests of significance were made as described in Trial 1.

Trial 3. Eighty crossbred and Hereford weaned heifer calves were used in a 90-day growth trial. After stratification by breed and initial weight, the heifers were randomly allotted to four treatments.

⁴ Approximate chemical composition (dry weight basis): biuret 60%, urea 15%, cyanuric acid 21% and total nitrogen 37%. Available nitrogen (31%) used in ration calculations was considered to be that nitrogen from biuret and urea only.

Treatment 1 served as the positive control and consisted of a 30% natural protein supplement (supplement 1, Table 1); salt was added at an average level of 30% to limit intake. Treatment 2 served as the negative control and consisted only of a mineral mixture of 50% calcium phosphate, dibasic comm, (6) IRN 6-01-080 (dicalcium phosphate) and 50% trace mineral salt with no nitrogen included. Treatments 3 and 4 consisted of supplements with a high crude protein equivalent (106.68 and 104.72%) supplied by urea (supplement 8, Table 1) and biuret and urea from feed grade biuret (supplement 9, Table 1). Approximately 98% of the total nitrogen in these two supplements was supplied by urea, or urea and biuret from feed grade biuret, respectively. Ground corn, dent, grain, gr 2 US mn 54 wt, (4) IRN 4-02-915 (ground corn) (at levels of 20 and 10%) and salt were included in the supplements to encourage intake. It was necessary to add magnesium oxide (2% of the supplement) to lower hygroscopicity of these high NPN-mineral supplements to a satisfactory level. All supplements were fed ad libitum in mineral feeders. Intake of supplement in treatment 1 was limited to equal the nitrogen intake of treatment 3; nitrogen intake of treatment 4 was substantially lower than for treatments 1 and 3. Heifers were rotated among pastures at 14-day intervals.

Data in Trial 3 was subjected to the same analysis as that used in Trial 2. An analysis-of-variance table is in the Appendix (Table 3).

Trial 4. Forty-seven mature Angus x Holstein cows were randomly allotted, after stratification by actual or expected calving date, to two treatment groups for a 134-day wintering trial. Each treatment group was divided into two subclasses, for analysis purposes only. Trial 4a cows calved before treatment began with an average calving date

of October 27; trial 4b cows calved after treatment began with an average calving date of December 25. The calving dates ranged from September 28 to February 11 for all cows. Calving was completed before the trial was ended. Treatment 1 consisted of a 30% natural protein supplement (supplement 1, Table 1). Treatment 2 consisted of the same supplement with methionine-hydroxy-analogue (MHA) added at the rate of 8.33 kg per ton. Supplements were fed at the rate of 1.56 kg per cow daily for 40 days and 1.95 kg for the remaining 94 days of the trial. Intake of MHA was 14.3 and 17.9 g per cow daily, respectively, for the two periods. Initial and final condition of cows was estimated as in Trial 1. The cattle were rotated among pastures at 28-day intervals.

Many of the cows utilized in Trial 4 calved prior to the application of treatments. In addition, there was considerable variation among the average initial weight and calving date for the cows of the various treatments. Therefore, these data were subjected to a preliminary multiple regression analysis to study the relationship of cow weight loss, calf gain, and condition score change with initial cow weight and calving date. Regression coefficients were calculated within treatment separately for cows calving before and during the treatment period. These regression coefficients are presented in Table 10 in the Appendix. The regression coefficients appear to be different for each calving group. This is the basis for making two separate analyses on this data: (1) of cows that calved before treatment began; and (2) of cows that calved during the treatment period. However, the regression coefficients for all trials were very similar within each calving group. Therefore, within treatment regression coefficients were pooled and were used to adjust cows of each calving group to the initial weight and the calving date of cows receiving no MHA (Table 10 of the Appendix).

Post-trial calf average daily gain and calf weaning weight were included as variables when this data became available, but the preceding adjustments were not utilized. These data were then analyzed with a one-way classification for each calving group with treatment being the classification. An analysis-of-variance table is in the Appendix (Table 9).

Results and Discussion

Trial 1. Performance data are presented in Table 2. Average daily supplement intake per cow was 1.13 kg for all groups. Cows fed the 30% natural protein supplement, the positive control, lost less weight than cows fed the 15% natural protein supplement, the negative control ($P < .001$). This indicates that the negative control failed to provide adequate protein, and substantiates the validity of using positive and negative controls as a basis of comparison for the NPN-containing supplements. Weight loss of cows fed the NPN-containing supplements was intermediate between positive and negative controls, but only starea 44-supplemented cows lost significantly ($P < .05$) less weight than the negative control. Weight loss expressed as a percentage of initial weight provides a more valid comparison of supplements due to variation in initial weight among treatments. On this basis none of the NPN-containing supplements were significantly different from the negative control.

Negative control cows lost more condition than positive control cows ($P < .005$), consistent with the difference in weight loss. Condition loss of NPN-supplemented cows, intermediate between the controls, was closer to that of negative controls. However, only starea 44-supplemented cows lost ($P < .025$) more condition than positive controls.

It is not known why starea 44 cows did not lose weight and condition in a consistent pattern.

Weight and condition loss comparisons in this trial indicated a low utilization of the NPN portion of the supplements, consistent with previous results obtained in the same area on similar dry winter range grass (Nelson et al., 1957; Nelson and Waller, 1962; Williams et al., 1969; Rush et al., 1972; Rush et al., 1973). Rush (1974) observed better utilization of urea than an extruded urea-grain supplement, but the products used in this trial (starea 44 and starea 70) were utilized at least as well as the urea supplement.

Calves raised by cows receiving the positive control supplement gained significantly ($P < .025$) more than calves from cows receiving the negative control during the treatment period. However, gains of calves in NPN-supplemented groups were not significantly different from positive controls, and significantly different from negative controls in only one case (urea) ($P < .025$). Treatment did not affect calf gain ($P > .16$) and calf weaning weight ($P > .09$) during the post-treatment period. Rush (1974) previously observed a lack of effect of supplement treatments on calf gain even though weight loss was affected. In short duration trials of this nature cows probably maintain milk production at the expense of body tissues.

Trial 2. Results are presented in Table 3. Daily intake of supplemental protein was the same for all groups. Heifers fed the 30% natural protein supplement (positive control) lost less weight than heifers fed the 15% natural protein supplement (negative control) ($P < .001$), demonstrating the need for more protein than supplied by the negative control. Weight loss of urea-supplemented heifers was midway between positive and

negative controls and not significantly different from either. Weight loss of biuret-supplemented heifers was slightly more than that of urea-supplemented heifers and significantly ($P < .01$) greater than the positive control. The level of apparent urea utilization in this trial, with a self-fed supplement containing a high level of alfalfa, is the highest observed on this experimental winter range. Other workers have reported better NPN utilization, but their trials involved harvested forage rather than dry range grass.

Trial 3. Performance data are presented in Table 4. Heifers fed the 30% natural protein supplement (positive control) lost less weight than those which received no protein supplement (negative control) ($P < .001$). NPN-supplemented heifers sustained weight losses intermediate between the control groups ($P < .05$), but the NPN supplements were not different from each other in weight loss ($P > .50$). Supplemental nitrogen intake by positive control and urea groups was similar; intake of the positive control supplement was restricted to that of the urea supplement. Nitrogen intake by the biuret heifers, on the other hand, was only one-half that of the urea group, so their similar weight loss was somewhat surprising.

The apparent NPN utilization in this trial was approximately 50% based on weight loss. Oltjen (1969) concluded that growth rates are about 65% as good on total NPN diets as on protein diets. In this trial, however, the low palatability of high NPN supplements did not permit sufficient intake of nitrogen to sustain a desirable level of performance by the heifers.

Trial 4. Performance data are presented in Table 5. Among cows that calved before treatment began (Trial 4a), those supplemented with

MHA lost more weight than cows without MHA ($P \approx .05$). Score change of the cows, average daily gain of the calves during the treatment period, and calf weaning weights were not different ($P > .10$). Post-treatment average daily gain of the calves was greater ($P < .005$) for calves from cows with MHA than calves from cows without MHA. Among cows that calved after treatment began (Trial 4b), supplementation with MHA did not affect weight loss of cows, change in condition of cows ($P > .25$), average daily gain of calves ($P > .10$) during the treatment period, average daily gain of the calves ($P > .25$) after the treatment period, and calf weaning weight ($P > .25$).

As treatment did not affect daily gain of calves from birth to end of treatment, milk production of cows was apparently not affected by MHA in either trial. It is not known why cows fed MHA lost more weight. MHA had no apparent affect on palatability of the supplement in this trial, whereas Rush (1974) noted that MHA decreased palatability of NPN-containing supplements. In agreement with results of this trial Rush (1974) observed no improvement in cattle performance. In contrast, (Griel et al., 1968; Patton et al., 1970) increased milk production by dairy cows, and increased calf gain and milk yield with beef cattle (Varner et al., 1973) have been attributed to MHA. Intake of MHA in this trial was 57 to 72% of the level recommended for dairy cows (Polan et al., 1970), but the response with beef cows was noted at 15 g daily (Varner et al., 1973). Perhaps the quality of forage consumed by cows in this trial was not sufficient to support increased milk yield.

TABLE 1. INGREDIENT MAKEUP OF PROTEIN SUPPLEMENTS (PERCENT)

Item	International Reference Number	Supplement Number and Description								
		1 Natural 30	2 Natural 15	3 Urea	4 Starea 44	5 Starea 70	6 Urea- Alfalfa	7 Biuret- Alfalfa	8 Urea- Mineral	9 Biuret- Mineral
Crude protein ^a		30.00	15.00	30.00	30.00	30.00	30.00	30.00	106.68	104.72
Corn, dent, grain gr 2US mn 54 wt, (4)	4-02-915	27.77	68.75	59.35	23.32	41.35	28.96	24.51	20.00	10.00
Soybean, seed, solv-extd grnd, mx 7 fbr, (5)	5-04-604	58.25	17.25	19.25	16.30	18.45	13.05	13.94	--	--
Alfalfa, hay S-C grnd, stemmy, (1)	1-00-118	5.00	5.00	5.00	5.00	5.00	40.00	40.00	--	--
Sugarcane molasses, mn 48 invert sugar mn 79.5 degrees brix, (4)	4-04-696	5.00	5.00	5.00	5.00	5.00	5.00	5.00	--	--
Sodium phosphate, monobasic, NaH ₂ PO ₄ H ₂ O, cp, (6)	6-04-287	2.50	2.75	2.85	2.80	2.80	3.60	3.60	11.79	8.76
Calcium phosphate, dibasic, commercial, (6)	6-01-080	0.75	1.20	1.17	1.18	1.15	--	--	6.97	5.58
Sodium sulfate, ^b Na ₂ SO ₄ 10 H ₂ O, cp, (6)	6-04-292	0.68	--	2.03	2.10	2.05	4.00	4.00	13.80	11.94
Trace mineral mix		0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.17	0.15
Vitamin A palmitate, comm, (7) ^c	7-05-143	+	+	+	+	+	+	+	--	--
Urea, mn 45% nitrogen, (5)	5-05-070	--	--	5.30	--	--	5.34	--	37.27	--
Starea 44 ^d		--	--	--	44.25	--	--	--	--	--
Starea 70 ^d		--	--	--	--	24.15	--	--	--	--
Kedlor 250 ^e		--	--	--	--	--	--	8.90	--	53.57
Salt, NaCl, comm, (6)	6-04-152	--	--	--	--	--	--	--	8.00	8.00
Magnesium oxide, MgO, cp, (6)	6-02-757	--	--	--	--	--	--	--	2.00	2.00

^aApproximate crude protein as determined by feed composition tables, Crampton and Harris (1969).

^bFormulated to supply 12:1 nitrogen:sulfur ratio.

^c22,000 IU per kg of supplement.

^dGelatinized urea-grain mixture.

^eKedlor 250, feed grade biuret, approximate chemical composition (dry weight basis): biuret 60%, urea 15%, cyanuric acid 21% and total nitrogen 37%.

TABLE 2. PERFORMANCE OF COWS AND CALVES DURING WINTER SUPPLEMENTATION PERIOD (TRIAL 1 - 113 DAYS)

Item	Protein supplement, % crude protein					Prob. ^b
	Natural 30	Natural 15	Urea ^a 30	Starea 44 ^a 30	Starea 70 ^a 30	
No. cows	21	21	20	21	21	
Avg. daily supplement, kg	1.13	1.13	1.13	1.13	1.13	
Daily crude protein intake, kg	0.34	0.17	0.34	0.34	0.34	
Cows rebred, % ^c	76.2	71.4	75.0	80.9	71.4	
Initial cow wt., kg	440	476	471	445	450	
Avg. calving date	324	332	326	326	329	
Adjusted cow wt. loss, kg ^d	128.6 ± 6.0 ^f	164.5 ± 6.0 ^g	152.9 ± 6.1 ^{g,h}	146.0 ± 6.1	152.0 ± 6.0 ^{g,h}	.002
Adjusted cow wt. loss, % ^d	27.3 ± 0.9 ^f	32.6 ± 0.9 ^g	30.7 ± 0.9 ^g	30.6 ± 0.9	31.3 ± 0.9	.002
Condition score change, cows ^{d,e}	-1.8 ± 0.25 ^f	-2.8 ± 0.25 ^g	-2.5 ± 0.25 ^{f,g}	-2.6 ± 0.25 ^g	-2.4 ± 0.25 ^{f,g}	.038
Calf weight gain, kg ^d						
Treatment period	48.7 ± 4.5	32.4 ± 4.5	47.6 ± 4.6	43.1 ± 4.6	44.4 ± 4.5	.095
Post-treatment period	107.7 ± 3.1	109.2 ± 3.5	113.0 ± 3.2	119.2 ± 3.6	111.3 ± 3.3	.164
Avg. daily gain, kg ^d						
Treatment period	0.40 ± .03	0.29 ± .03	0.38 ± .03	0.36 ± .03	0.36 ± .03	.168
Post-treatment period	0.94 ± .03	0.95 ± .03	0.98 ± .03	1.0 ± .03	0.97 ± .03	.164
Weaning weight, kg ⁱ	170.7 ± 4.4	168.7 ± 5.0	176.2 ± 4.6	187.4 ± 5.2	175.5 ± 4.8	.094

^aUrea and the urea portion of starea products to furnish 50% of total crude protein.

^bProbability that differences in means are due to chance.

^cPercentage of cows determined pregnant by palpation.

^dValues are least square means ± standard deviation.

^eDifferences in initial and final condition based on a scale of 1 to 9, 1 the thinnest and 9 the fattest.

^{f,g,h}Means with different superscripts are significantly different (P < .05).

ⁱAdjusted to 205-day, steer basis; heifer weights were multiplied by 1.05.

TABLE 3. PERFORMANCE OF YEARLING HEIFERS DURING WINTER SUPPLEMENTATION PERIOD (TRIAL 2--77 DAYS)

Item	Protein supplement, % crude protein				Prob. ^b
	Natural 30	Natural 15	Urea ^a 30	Biuret ^a 30	
No. heifers	16	17	16	17	
Daily non-salt supplement intake, kg	0.93	0.93	0.93	0.93	
Daily crude protein intake, kg	0.28	0.14	0.28	0.28	
Initial wt., kg	329	339	334	335	
Body wt. loss, kg	12.5 ± 1.8 ^d	22.4 ± 1.8 ^e	17.5 ± 1.8 ^{de}	19.5 ± 1.8 ^e	.0043

^aTo furnish 50% of total crude protein.

^bProbability that differences in means are due to chance.

^cValues are least square means ± standard deviation.

^{d,e}Means with different superscripts are significantly different (P < .05).

TABLE 4. PERFORMANCE OF WEANED HEIFERS DURING WINTER SUPPLEMENTATION PERIOD (TRIAL 3--90 DAYS)

Item	Protein supplement, % crude protein				Prob. ^b
	Natural 30	No Supplement	Urea ^a 106.68	Biuret ^a 104.72	
No. heifers	20	20	20	20	
Daily non-salt supplement intake, kg	0.316	--	0.095	0.052	
Daily supplemental crude protein intake, kg	0.095	--	0.101	0.054	
Initial wt., kg	230	229	229	228	
Body wt. loss, kg	39.4 ± 2.1 ^d	55.2 ± 2.4 ^e	46.9 ± 2.4 ^f	48.6 ± 2.4 ^f	.0003

^aTo furnish 98% of total crude protein.

^bProbability that differences in means are due to chance.

^cValues are least square means ± standard deviation.

^{d,e,f}Means with different superscripts are significantly different (P < .05).

TABLE 5. PERFORMANCE OF COWS AND CALVES DURING WINTER SUPPLEMENTATION PERIOD (TRIAL 4 - 134 DAYS)

Item	Supplement, % crude protein			Prob ^a
	Natural 30	Natural + MHA 30		
Trial 4a - cows calving before trial				
No. cows	13	13		
Avg. daily supplement, kg	1.83	1.83		
Avg. calving date	300.1	299.5		
Cows rebred, % ^b	84.6	76.9		
Cow				
Initial wt., kg	463	477		
Body wt. loss, kg	49.8 ± 8.3 ^c	76.0 ± 9.6		P = .05
Score change ^d	-1.8 ± 0.37	-2.5 ± 0.38		.1 < P < .25
Calf				
Avg. daily gain, kg				
Treatment period	0.66 ± 0.05	0.66 ± 0.06		P > .25
Post-treatment period	1.01 ± 0.03	1.17 ± 0.03		P < .005
Weaning wt., kg ^e	206.8 ± 5.26	212.1 ± 6.47		P > .25
Trial 4b - cows calving during trial				
No. cows	11	10		
Avg. daily supplement, kg	1.83	1.83		
Avg. calving date	358.5	374.0		
Cows rebred, % ^b	27.3	40.0		
Cow				
Initial wt., kg	539	523		
Body wt. loss, kg	111.4 ± 13.0	107.6 ± 8.0		P > .25
Score change ^d	-2.5 ± 0.36	-2.6 ± 0.29		P > .25
Calf				
Avg. daily gain, kg				
Treatment period	0.78 ± 0.04	0.88 ± 0.05		.1 < P < .25
Post-treatment period	1.11 ± 0.04	1.11 ± 0.01		P > .25
Weaning wt., kg ^e	230.5 ± 6.14	238.7 ± 5.64		P > .25

^aProbability that differences in means are due to chance.

^bPercentage of cows determined pregnant by palpation.

^cStandard error of mean.

^dDifference in initial and final condition based on a scale of 1 to 9, 1 the thinnest and 9 the fattest.

^eAdjusted to 205-day, steer basis; heifer wts. were multiplied by 1.05.

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APPENDIX

TABLE 6. ANALYSES OF VARIANCE FOR COW AND CALF VARIABLES (TRIAL 1)

Source of variation	df	Mean square	F value
<u>Adjusted cow weight loss, kg</u>			
Breed of cow	1	64,622.7413	17.9012**
Treatment	4	17,131.0778	4.7455**
Breed of cow x treatment	4	5,011.6528	1.3883
Error	94	3,609.9743	-- --
<u>Adjusted cow weight loss, %</u>			
Breed of cow	1	4.1925	25.2283**
Treatment	4	0.7874	4.7382**
Breed of cow x treatment	4	0.2553	1.5360
Error	94	0.1662	-- --
<u>Condition score change of cows</u>			
Breed of cow	1	11.6460	9.4017**
Treatment	4	3.2706	2.6403**
Breed of cow x treatment	4	0.1638	0.1322
Error	94	1.2387	-- --
<u>Weight gain of calves--treatment</u>			
Breed of cow	1	53,595.6639	26.0372**
Treatment	4	4,187.7656	2.0345
Breed of cow x treatment	4	5,553.8640	2.6981*
Error	94	2,058.4288	-- --
<u>Weight gain of calves--post-treatment</u>			
Breed of cow	1	32,231.4544	34.1639**
Treatment	4	6,310.8759	1.6723
Breed of cow x treatment	4	2,626.8227	0.6961
Error	79	943.4363	-- --
<u>ADG of calves--treatment</u>			
Breed of cow	1	2.3608	24.4354**
Treatment	4	0.6359	1.6455
Breed of cow x treatment	4	0.6856	1.7740
Error	94	0.0966	-- --
<u>ADG of calves--post-treatment</u>			
Breed of cow	1	2.4372	34.1639**
Treatment	4	0.4772	1.6723
Breed of cow x treatment	4	0.1986	0.6961
Error	79	0.0713	-- --
<u>Calf weaning weight</u>			
Breed of cow	1	68,719.4890	34.9523**
Treatment	4	16,120.2472	2.0498
Breed of cow x treatment	4	9,417.0385	1.1975
Error	79	1,966.0670	-- --
<u>Cow initial weight adjusted for calving losses</u>			
Breed of cow	1	8,806.5794	1.0116
Treatment	4	2,450.2635	2.29157
Breed of cow x treatment	4	7,505.3149	0.8621
Error	94	8,705.9429	-- --

* Significant at .05 level of probability.

** Significant at .01 level of probability.

TABLE 7. ANALYSIS OF VARIANCE FOR YEARLING HEIFER WEIGHT LOSS (TRIAL 2)

Source of variation	df	Mean square	F value
Breed of heifer	2	3796.7893	16.7221**
Block within breed	15	286.8699	1.2635
Treatment	3	1184.9380	5.2188**
Treatment x breed	6	235.9767	1.0393
Error	39	227.0525	-- --

** Significant at .01 level of probability.

TABLE 8. ANALYSIS OF VARIANCE FOR WEANED
HEIFER WEIGHT LOSS (TRIAL 3)

Source of variation	df	Mean square	F value
Breed of heifer	3	3248.5451	8.5983**
Block within breed	18	1051.6088	2.7834**
Treatment	3	3193.2699	8.4520**
Treatment x breed	9	161.6484	0.4279
Error	46	377.8125	-- --

** Significant at .01 level of probability.

TABLE 9. ANALYSES OF VARIANCE FOR COW AND CALF VARIABLES (TRIAL 4)

Source of Variation	df	Mean square	F value
<u>Cows that calved before trial</u>			
<u>Adjusted cow weight loss</u>			
Treatments	1	21,544.4729	4.2648*
Error	24	5,051.7298	-- --
<u>ADG of calves during treatment</u>			
Treatments	1	0.0001	0.0006
Error	24	0.1766	-- --
<u>Condition score change of cows</u>			
Treatments	1	3.1580	1.7531
Error	24	1.8014	-- --
<u>ADG of calves after treatment**</u>			
Treatments	1	0.8660	12.0613**
Error	24	0.0718	-- --
<u>Calf weaning weight</u>			
Treatments	1	874.2109	0.3850
Error	24	2,270.6045	-- --
<u>Cows that calved during trial</u>			
<u>Adjusted cow weight loss</u>			
Treatments	1	367.0908	0.0310
Error	19	12,210.7988	-- --
<u>Average daily gain of calves</u>			
Treatments	1	0.2103	2.2584
Error	19	0.0931	-- --
<u>Condition score change of cows</u>			
Treatments	1	0.0479	0.0407
Error	19	1.1759	-- --
<u>ADG of calves after treatment</u>			
Treatments	1	0.0014	0.0220
Error	19	0.0636	-- --
<u>Calf weaning weight</u>			
Treatments	1	1,726.2442	0.9677
Error	19	1,783.9312	-- --

* Significant at .05 level of probability.

** Significant at .005 level of probability.

TABLE 10. REGRESSION COEFFICIENTS OF DEPENDENT
VARIABLES MEASURED (TRIAL 4)

Treatment	Cow weight loss	Cow score change	Calf daily gain
	b+S.E.	b+S.E.	b+S.E.
(Initial weight as independent variable)			
<u>Cows that calved before trial-</u>			
30% natural protein	0.2422+ .12 *	0.0037+ .003	0.0015 +.001 ***
30% natural protein + MHA	0.2733+ .20	0.0037+ .003	0.0023 +.001 ***
<u>Cows that calved during trial-</u>			
30% natural protein	0.3691+ .25	-0.0012+ .004	0.0011 +.001
30% natural protein + MHA	0.5817+ .33	-0.0024+ .003	-0.00002+ .001
(Calving date as independent variable)			
<u>Cows that calved before trial-</u>			
30% natural protein	-0.6917+ .66	0.0211+ .01	0.0005 +.003
30% natural protein + MHA	-0.4205+ .90	0.0169+ .01	0.0039 +.004
<u>Cows that calved during trial-</u>			
30% natural protein	-0.1856+1.55	0.0178+ .02	-0.0007 +.005
30% natural protein + MHA	-1.4268+1.30	0.0139+ .01	0.0077 +.002 ***

* Significant at .05 < P < .10 level of probability.

** Significant at .01 < P < .05 level of probability.

*** Significant at .005 < P < .025 level of probability.

TABLE 11. POOLED REGRESSION COEFFICIENTS OF DEPENDENT VARIABLES USED TO ADJUST TO EQUAL INITIAL WEIGHT AND CALVING DATE WITHIN CALVING GROUPS (TRIAL 4)

Item	<u>Cow weight loss</u> b	<u>Cow score change</u> b	<u>Calf daily gain</u> b
		(Initial weight as independent variable) ^b	
Cows that calved before trial ^a	0.2412	0.0051	0.0019
Cows that calved during trial ^a	0.4593	-0.0010	0.0010
		(Calving date as independent variable) ^b	
Cows that calved before trial ^a	-0.3016	0.0254	0.0042
Cows that calved during trial ^a	-0.4198	0.0140	0.0059

^aRegression coefficients represent combined treatments of each calving group.

^bCows receiving MHA were adjusted to the initial weight and calving date of the cows receiving only the 30% natural protein supplement within each calving group.

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VITA

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