EFFECT OF BIURET ON LOW ENERGY BEEF CATTLE RATIONS

by

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INTRODUCTION

The increasing demand for nutrients caused by the rapidly growing human population is putting more and more pressure on the livestock producer. Higher efficiency of feed utilization is becoming increasingly more important because of the competition between animals and humans for plant materials formerly thought of only as feedstuffs. This competition is especially important in respect to high protein feeds. As more vegetable protein is utilized in the feeding of man and monogastric livestock, less will be available for ruminant feed.

Harbers (1970) calculated that approximately 8.3 kg of plant protein are required to produce 1 kg of beef protein. This figure includes the protein used through the finishing period and the protein required to maintain the breeding herd. This plant protein can, however, be replaced to a large extent by non-protein nitrogen (NPN). In fact, Virtanen (1966) reported maintaining cows on a ration in which 99.5 percent of the nitrogen was supplied in the form of urea and ammonium nitrogen.

Urea has been by far the most used source of NPN and has been in use for several years. There are, however, some drawbacks to the use of urea. While it has generally performed well on high energy or fattening rations, its use with low energy, high roughage rations has often shown both poor nitrogen utilization and toxicity. There has been considerable interest, therefore, in the development of other

NPN compounds which could be used to replace greater quantities of plant protein. Biuret, a pyrolysis product of urea, has shown promise in this area. According to the Federal Register (1969) the composition of feed grade biuret is: biuret-60 percent minimum, urea-15 percent maximum, cyanuric acid and triuret-21 percent maximum, total nitrogen-35 percent minimum.

Several factors are known to affect the utilization of NPN compounds and the practicality of their use. Some of these factors will be discussed as they apply to NPN compounds in general and to biuret in particular.

LITERATURE REVIEW

Toxicity studies. Toxicity to NPN compounds usually arises when ammonia is released at a rate much higher than the microorganisms are capable of utilizing it to synthesize protein. The excess ammonia can then be absorbed across the rumen wall. This absorption is influenced by concentration gradient and pH (Hogan, 1961). Since ammonia is a weak base with a pK_a of about 8.80 to 9.15, an increase in pH causes the ammonium ion to be converted to ammonia which can then be absorbed (Tillman and Sidhu, 1969).

The absorbed ammonia is then carried to the liver where it is converted to urea. According to Lewis et al. (1957) the liver can handle this conversion until the portal blood ammonia level reaches 0.8 mM, at which point some of the ammonia remains in circulation.

This causes an upset of the acid-base balance of the blood and affects the nervous system. Symptoms usually observed include bloat, incoordination, ataxia, excessive salivation, convulsions, and death. The symptoms depend on the severity of the case.

Biuret. Berry *et al*. (1956) fed rats a ration containing ten percent biuret for 140 days and reported that they observed no symptoms or lesions indicative of toxicity. Daily intraperitoneal injections of 150 mg of biuret into 300 to 400 gm rats caused no adverse effects. Eight-week old chicks were fed a diet containing one percent biuret. In this study they found no significant beneficial effects on gains, nor were toxicity symptoms observed.

Repp *et al*. (1955) found very little increase in either blood urea or ammonia levels after feeding relatively large amounts of biuret. They concluded that ammonia was released very slowly if at all from biuret in the rumen.

Hatfield *et al*. (1959) observed no toxicity symptoms or adverse after-effects when a 66 kg ewe was given 375 gm of biuret in 4.5 kg of feed in three portions at twelve-hour intervals. In another study, two grade wethers (38 kg) were drenched with 175 or 275 gm of biuret. Distress symptoms were observed for thirty-six hours. They also observed that an appreciable amount of material had apparently crystallized from urine and collected on the prepuce hairs of the lamb receiving the higher dosage. The material was found to contain 82 percent biuret positive material. Internal organs appeared normal upon slaughter three months later. On standing, biuret will form a heavy crystalline deposit from urine of sheep drenched with 250 gm of the NPN compound (Clark *et al.*, 1963).

Since feed grade biuret may contain as much as 21 percent cyanuric acid and triuret, it is imperative that these compounds also be non-toxic. Clark *et al.* (1965) found that triuret had no effect on ruminal pH, food intake, or health of sheep given an initial 250 gm dose *per fistula*, then 12 gm per day for 14 days followed by another 250 gm dose. Cyanuric acid has also been observed to be neither an acute nor a cumulative toxin. Altona and Mackenzie (1964) observed no toxic effects and a positive nitrogen balance was obtained when cyanuric acid was given at the rate of 3.3 gm per kg body weight.

Urea. Urea has long been known to be hazardous when fed in large amounts. It is especially dangerous when fed to unadapted animals or those on low energy rations. Davis and Roberts (1959) reported death in cattle given 0.31 gm of urea per kg body weight in a drench, 0.48 gm per kg in a capsule, and 0.45 gm per kg body weight in feed. Repp *et al.* (1955) reported that 0.88 gm urea per kg body weight in sheep resulted in death.

Toxicity symptoms appeared within ten minutes in fasted pregnant cows drenched with urea (0.44 gm per kg body weight). A drench of five percent acetic acid relieved symptoms for about 35 minutes. Symptoms then reappeared and the animals died. Ruminal ammonia and peripheral blood ammonia levels peaked within 15 to 20 minutes in fistulated steers given varying levels of urea (Word *et al.*, 1969). In another study cows fed poor quality hay were given 5 gm of sorghum grain per kg body weight, then four hours later drenched with urea at 0.44 gm per kg body weight. Fifteen minutes later acetic acid was given at a rate of two moles per mole of urea. If no further treatment was given the cows died; however, when an additional dose of acetic acid was given (one mole per mole of urea) 180 minutes later, the cows survived without changes in weight or reproductive performance.

The toxicity studies discussed thus far have been largely concerned with unadapted animals. After an animal has been adapted to the feeding of urea much higher levels can be tolerated. Virtanen (1966) reported that cows weighing 450 kg were given as much as 650 gm of urea per day (1.44 gm per kg body weight) resulting in a positive nitrogen balance and an increase in milk production.

Adaptation. Using in vitro cellulose digestion and bacterial growth parameters, Hale (1956) found that useful NPN compounds were those from which the NH_3-N was easily released. He also observed that rumen microorganisms show adaptability in utilization of certain NPN compounds whose nitrogen is usually considered unavailable.

Urea. In order for a non-protein nitrogen compound to be utilized it must first be broken down to a form which the microorganisms can use in protein synthesis. With urea, this breakdown is made possible by the presence of bacterial urease in the rumen. Rahman and Decker (1966) concluded that the urease activity of the rumen mucosa was of bacterial origin since much of the activity was removed by washing. The optimum pH for ruminal urease was found to be about 8.5 while that of soybean urease was 7.5 to 8.0.

Urease activity in rumen contents of animals fed urea is generally lower than that of animals on a natural protein ration (Merino and Raun, 1964). This lowered activity may be caused by a decrease in bacterial urease synthesis. Magana-Plaza and Ruiz-Herrera (1967) found that ammonium ions caused a decrease in urease synthesis in *Proteus rettgeri*.

The fact that high levels of urease activity are commonly found in unadapted animals may be explained by the fact that urea enters the rumen by way of the saliva and directly from the blood. From urea injection experiments Houpt (1959) concluded that 7.8 to 13.0 moles of urea were transfered from the blood to the rumen. In other experiments it was found that 16 times as much urea entered the rumen via the blood as from saliva. Vercoe (1969) indicated that in his injection experiments, the net amount of urea passing from the blood to the rumen was from 17 to 20 gm nitrogen per day. Since urea is constantly "infused" into the rumen from the blood and saliva, the micro-flora is naturally adapted to it.

Bloomfield *et al.* (1960) reported that the rate of urea hydrolysis was about four times as great as the rate of ammonia uptake by rumen microorganisms. This would indicate that urea utilization would be increased by slowing the rate of urea hydrolysis.

Attempts have been made to slow this hydrolysis by use of urease inhibitors. Harbers *et al.* (1962) reported that the urease inhibitor, barbituric acid, inhibited *in vitro* cellulose digestion and decreased gain and feed efficiency *in vivo*. Clifford *et al*. (1968) found that on high roughage rations, barbituric acid increased fecal nitrogen loss and lowered nitrogen retention.

Acetohydroxamic acid (AHA) was found to inhibit urease both *in vitro* and *in vivo* (Brent and Adepoju, 1967). Streeter *et al*. (1969) indicated that AHA lowered ruminal ammonia and increased nitrogen retention and digestibility of dry matter in sheep. Moore *et al*. (1968) found no improvement in nitrogen retention when steers were fed AHA.

Gastrointestinal urease activity can also be decreased by producing circulating antibodies to urea. Subcutaneous injections of crystalline jackbean urease have been used to stimulate this production. Harbers *et al*. (1965) found that growth rate was related to the level of circulating antiurease in urease immunized calves.

Deyoe *et al*. (1968) took another approach to the problem and developed a product called "Starea". By cooking and extruding a mixture of grain and urea, they developed a product which released ammonia at a slower rate both *in vitro* and *in vivo*. Based on animal performance, the product was reported as being superior to urea and equal to soybean meal.

Biuret. Biuret is not a naturally occurring compound in the rumen; therefore, no "natural" adaptation to it is to be expected as is the case with urea. Unlike urease, the biuretolytic enzymes are apparently absent or present in very small quantities in the unadapted animal. Repp *et al*. (1955) showed very little increase in ruminal ammonia after fairly heavy drenches of biuret. Gilchrist *et al.* (1968) reported that with sheep biuretolytic activity was evoked only when biuret was included in the ration. Schroder and Gilchrist (1969) found measurable activity in only four out of thirty-eight determinations. This activity was described as low and sporadic and was postulated to be due to a temporary increase in numbers of biuretolytic organisms brought on by change of diet.

Wheldon and MacDonald (1962) reported that biuret was hydrolyzed by *Pseudomonas aeruginosa*. Ammonium ions were found to repress synthesis of the biuretolytic enzyme and the inclusion of an organic nitrogen source resulted in preferential utilization of the added material. The enzyme was found to be inducible by these workers. Broken cell preparations were found to hydrolyze biuret with the stoichiometric release of three moles of ammonia per mole of biuret. Malonamide and N-acetyl urea, structural analogues of biuret, induced enzymes capable of acting on the inducer and biuret. Biuret, however, does not induce enzymes capable of hydrolyzing these analogues. Growth of unadapted inocula on a biuret medium was reported to occur near pH 6, however, greatest dissimilation of biuret occurred at about pH 7.5. (Wheldon and MacDonald, 1962).

A coccoid, facultative anaerobic, non-motile, gram-positive organism which was capable of utilizing biuret was recently isolated by Slyter *et al.* (1970). This organism actively degraded biuret but not urea and grew in a medium containing biuret as the only nitrogen source. Biuret could be replaced by ammonia but not by urea in growth of the organism. Further study of the metabolism of this organism should yield information helpful in understanding utilization of biuret by the ruminant animal.

The fact that biuretolytic activity is absent or very low prior to biuret feeding indicates the necessity of an adaptation period for maximum utilization. The reported time required for adaptation has varied among workers. Waite and Wilson (1968) found this period to be five to eight weeks in fistulated cows using concentration of ruminal ammonia as response criteria while Oltjen _et al._ (1969) found that biuret fed steers were not "adapted" until twenty-one days, while those fed urea were adapted after seven days. These workers used fecal nitrogen and nitrogen utilization as criteria of response. Welch _et al._ (1957) reported that biuret nitrogen increased to a maximum after a period of thirty-five days in lambs. Diethylstilbestrol reduced the period of adjustment needed to get maximum utilization to ten days but did not affect apparent digestibility of nitrogen. Farlin _et al._ (1968) substituted biuret in rations of sheep accustomed to urea and found that during the period from day 11 to day 17 after substitution fifty percent of the biuret was excreted in the urine. The amount excreted decreased to about thirty percent after 45 days.

Mackenzie and Altona (1964_a) observed that liveweight response of sheep to a mineral mix containing biuret was immediate and continued for eight weeks. In experiments with cattle it was found that liveweight response became evident only after four weeks on the biuret mineral mix. In other studies (Mackenzie and Altona, 1964_b) a four week lag was observed in liveweight response of sheep fed biuret.

Campbell et al. (1963) noted improvement in growth rate of cattle on rations supplemented with either urea or biuret after three weeks, while there was improvement after two to three weeks in lactation studies. They also reported that post-feeding ruminal ammonia levels (measured thirty minutes after feeding) increased up to five weeks on a biuret ration.

Drenching lambs with 50 to 100 ml of rumen fluid (taken from sheep fed biuret for 20 months) prior to biuret feeding increased nitrogen retention (Ewan et al., 1958).

Schroder and Gilchrist (1969) reported an increase in ruminal biuretolytic activity with time. The time required to reach maximum activity was found to depend upon the crude protein (C.P.) content of the basal ration. With a basal ration containing 3.4% C.P., 12 to 20 days were required to reach maximum activity while 68 to 75 days were necessary when the ration contained 10.3 to 10.4% C.P. Rations containing intermediate levels of C.P. required intermediate times for adaptation. Maximum activities ranged from 187 to 259 mg biuret degraded by 100 ml rumen fluid in 24 hours with the higher rates being recorded for the animals on the higher protein rations.

The withdrawal of biuret from the rations caused a sharp drop in biuretolytic activity. The reintroduction of biuret to the deadapted animals caused an increase in activity at about the same rate as the original adaptation period (Schroder and Gilchrist, 1969).

In vitro studies. There have been considerable differences in the results obtained with in vitro studies. Brent et al. (1966) found an increase in ammonia nitrogen when biuret was added to *in vitro* fermentations. However, it was concluded that the rate of ammonia release was too low to be of practical application.

The fact that biuretase is apparently an inducable enzyme would emphasize the importance of adaptation of the donor animal to biuret before rumen fluid samples are taken. Results of experiments by Schroder and Gilchrist (1969) indicate that straining of rumen fluid prior to use in *in vitro* fermentations caused a great reduction in biuretolytic activity. By use of fermentation flasks operated in parallel they were able to show high activity (187 to 259 mg per 100 ml per 24 hours) with the stoichiometric release of ammonia. At this rate over 200 gm of biuret could be broken down per day in the rumen of a cow. This quantitative conversion, however, could not be demonstrated *in vivo* with sheep (Schroder and Gilchrist, 1969). While there was a rapid decrease in biuret concentration during the first 6.5 hours, there was little increase in ammonia concentration in the rumen. It was, therefore, suggested that ruminal ammonia level constitutes a poor criterion of the availability of biuret.

Gilchrist *et al.* (1968) determined biuretolytic activity of rumen flora by measuring the disappearance of biuret (colorimetric analysis based on the yellow Ni-biuret complex). They reported that addition of maize meal tripled activity. The high activity was sufficient to account for the disappearance of 16.4 gm of biuret per day in sheep.

In vitro studies with ^{14}C labeled biuret showed a decreased recovery of evolved $^{14}CO_2$ for the first nine days after biuret was added to the ration (Farlin et al., 1968). After seventeen days recovery of ^{14}C was higher from rumen inoculum of biuret supplementated lambs than from those supplemented with urea. There was no significant difference in ^{14}C recovery for biuret or urea after fifty-one days.

Rumen and blood studies. Various studies have been conducted to determine the effects of urea and biuret on the contents of the rumen and their effects on the blood. Most of these studies have been concerned with ruminal and blood ammonia levels as affected by feeding, ruminal infusion, or intra-venous injection. The effects on ruminal and blood ammonia have been discussed under the topics of "toxicity" and "adaptation".

In infusion and injection experiments Vercoe (1969) found the increase in plasma urea-nitrogen similar when urea was infused into the rumen and when given intravenously. Oltjen et al. (1969) reported that taurine, ammonia, and histidine were found in greater concentration in the blood of urea fed steers while urea, citrulline, glycine, aspartic acid, glutamic acid, serine, and ornithine were found in lesser amounts than in the blood of biuret fed steers. An increase in butyric acid and a decrease of acetic acid content of rumen fluid was observed when biuret was compared to urea. Concentration of other VFA's did not differ significantly. An increase in the bacterial population and a decrease in the protozoal population was observed with both urea and biuret.

Waite and Wilson (1968) indicated that rumen fluid from urea fed cows contained more total solids, total VFA, total nitrogen, NPN and NH_3-N than that from cows fed biuret or oilcake as a nitrogen source.

Farlin *et al.* (1968) studied the metabolism of urea and biuret by injection of ^{14}C labeled biuret, urea, and $NaHCO_3$ in sheep fed these NPN compounds. When injected intravenously about 95% of the label from biuret was found in the urine, 1.4% in expired CO_2 (8 hours) and about 2.5% in the rumen. With intraruminal injection, the urine content of the label did not exceed 3%, expired CO_2 contained 28.5% and 25.1% for biuret and urea respectively. Negligable amounts were found in the feces. Since less than 50% of the label from either biuret or urea was accounted for including ruminal and blood plasma counts, the authors suggest that the compounds are metabolized without complete hydrolysis to CO_2 and NH_3. Nearly 100% of the label from $NaHCO_3$ was found in expired CO_2 within 6 hours.

Digestion studies. The effects of biuret on digestion of nutrients have been studied by several workers. Belasco (1954) obtained poor results *in vitro* for cellulose digestion and bacterial growth when biuret was compared with urea. However, strained rumen fluid was used in this study and the ration of the donor animal was not mentioned. The results obtained by Schroder and Gilchrist (1969) indicate that these factors may have had a bearing on the outcome of the study.

Hatfield *et al.* (1955) found the digestibility of ether extract and dry matter to be similar when nitrogen in steer rations was supplied as urea, biuret, or soybean meal. The digestibility of nitrogen and nitrogen retention were, however, higher with urea and soybean meal than with biuret. Welch et al. (1956) also found that pure biuret depressed digestibility of organic matter and protein and lowered nitrogen retention in lambs. Crude biuret depressed apparent nitrogen digestibility but did not affect either organic matter digestibility or nitrogen retention.

Campbell et al. (1956) indicated that diethylstilbestrol improved nitrogen utilization when NPN was furnished by urea alone or by 50% urea and 50% biuret. With all NPN from biuret, however, organic matter and apparent protein digestibility were depressed by diethylstilbestrol.

In metabolism studies with lambs, Hatfield et al. (1959) obtained a lower apparent digestion coefficient for nitrogen and higher nitrogen balance for biuret than for urea. At a higher level of feed intake, the apparent digestion coefficient was highest with urea, intermediate with a combination of biuret and urea, and lowest with biuret. The nitrogen balance was significantly lower ($P<.05$) for the biuret ration than the urea-biuret ration but was only slightly lower than the urea ration.

In a similar study with steers (Hatfield et al., 1959) lower digestion coefficient for nitrogen and lower nitrogen balance (expressed as percentage of apparently digested nitrogen) were obtained with biuret than with urea or soybean meal.

Oltjen et al. (1969) indicated a slight (2%) depression in acid detergent fiber digestibility when biuret was compared to urea with steers on 50% NPN diet.

Utilization. Mackenzie and Altona (1964_b) observed no improvement in weight gains when either biuret or urea was added to a ration of good quality hay (7.88% C.P., 29.29% C.F.). However, when biuret was added to a poor quality hay (4.12% C.P., 39.07% C.F.) ration the animals maintained weight while the unsupplemented controls lost weight. Addition of biuret was found to increase hay consumption.

In other studies Mackenzie and Altona (1964_a) observed that a mineral mix containing 50% biuret was consumed voluntarily at levels sufficient to meet nitrogen requirements for maintainance of cattle and sheep on poor quality roughage.

Hatfield et al. (1959) reported satisfactory growth, reproduction, and wool growth with positive nitrogen balances when biuret furnished a major portion of total nitrogen intake. Ewes fed a biuret supplemented ration gave birth to normal lambs and lactated sufficiently to promote normal growth in the lambs. Campbell et al. (1963) reported that biuret promoted slightly (not significant) lower growth and feed efficiency than urea when fed to Holstein heifers. They also observed that biuret was slightly inferior to urea as based on FCM production in Holstein cows.

Oltjen et al. (1969) found urea slightly superior to biuret in growth trials under ab libitum feeding. However, biuret was clearly superior under twice daily feeding. The difference was suggested to be due to wastage of nitrogen caused by high ruminal ammonia levels resulting from consumption of urea in larger quantities in a shorter period as compared to ab libitum feeding.

Meiske et al. (1969) observed that urea, biuret, and soybean meal improved growth in calves over those not given supplemental nitrogen. Calves fed urea gained faster than those fed biuret in the growing phase while in the finishing phase gains were in favor of those fed biuret. Gains over the entire feeding period (278 days) were similar for cattle fed urea or biuret (1.06 kg and 1.07 kg/day, respectively).

EFFECT OF BIURET ON LOW ENERGY BEEF CATTLE RATIONS

The practice of wintering beef cows on pasture or low quality roughages is common throughout Kansas and much of the mid-west. Since these feeds are low in protein, supplementation is necessary if satisfactory weights are to be maintained. The phosphorus content of plants decreases with maturity, making it necessary to supplement minerals under these conditions. In this area soybean meal is the most commonly used source of protein for this supplementation. The increasing demand for soybean protein for use in human and monogastric livestock nutrition may, in the future, limit its availability for use in ruminant nutrition. The use of non-protein nitrogen compounds is, therefore, a subject of considerable interest and research.

Use of urea on low energy rations of the type described above has met with varying degrees of success (Westmeyer, 1965). The usefulness of urea is limited under these conditions by its unpalatability and the hazard of toxicity. In addition limited feeding time may result in poor nitrogen utilization. Oltjen et al. (1969) reported that while urea was slightly superior to biuret under ab libitum feeding, biuret was clearly superior under twice daily feeding. The poorer performance of urea under twice daily feeding was suggested to be due to increased intake of urea in a shorter period of time, resulting in high ruminal ammonia levels and consequent nitrogen wasteage. This would indicate that the utilization of urea-N would be even poorer under the common practice of once daily supplementation of cows on winter pasture. Since biuret-N is released more slowly than urea-N, its use under these conditions should result in improved nitrogen utilization.

The present studies were conducted to compare supplements containing biuret and soybean meal for pregnant cows wintered on bluestem pasture. The feasibility of supplying biuret in a mineral mix was also studied.

EXPERIMENTAL PROCEDURE

Experiment I. Forty-eight five-year-old pregnant Hereford cows were divided into two groups of 28 and 20 to evenly stock two pastures. Each of these groups was subdivided into two equal groups and hand fed either a sorghum grain-soybean or sorghum grain-biuret supplement (Table I). Cows were gathered into corrals, sorted, and fed their assigned supplement each morning. After feeding, they were allowed access to pasture, water, and mineral mix (Table 3). Prairie hay was fed when snow cover prevented grazing.

Weight criteria consisted of monthly weighing of cows (fasted 12 hours) and birthweight of calves. Data were subjected to least squares analysis of variance with unequal subclass numbers, Duncan's multiple range test, and Student's t test.

Experiment II. Twenty pregnant five-year-old Hereford cows were divided into two groups of ten and assigned to two pastures. Each morning they were fed 1.5 kg of sorghum grain supplemented with vitamins A and E (Table 1). They had constant access to water, pasture, and a mineral mix containing biuret (Table 3). Consumption of mineral mix was determined by weigh-back. Weighing procedures were identical to Experiment I. Birthweights of calves were also recorded.

Experiment III. Consumption of biuret mineral mix in Experiment II was a problem at the onset of the trial. A third experiment was conducted to determine whether an addition of fine ground sorghum grain to the mineral mix would hasten acceptance, and if so, at what level it would be required. For this trial twelve cross bred heifers (233 to 290 kg) were divided into four lots. The lots were then randomly assigned to one of four treatments. The control group was given the same mineral mix as was used in Experiment II (Table 3). The remaining three groups received the same mineral mix with fine ground sorghum grain added at the rates of 10, 50, or 100 parts of mineral mix plus 1 part of sorghum grain. Daily consumption was determined by weigh-back. The animals had free access to water and the mineral mix in individual pens. Each animal was given prairie hay *ad libitum* and approximately 1.8 kg sorghum grain daily. Sorghum grain was removed from the test mineral mixtures when consumption was satisfactory. Consumption of mineral mix was followed for seven days following removal of sorghum grain.

RESULTS AND DISCUSSION

<u>Experiment I</u>. Monthly cow weights are shown in Figure 1. Since not all cows had calved by the time supplementation was discontinued in the spring, data obtained in the final weighing period are not included. Calf birthweights (Table 4) include only weights of calves born during the supplementation period. Cow weights for the first four weighing periods were subjected to analysis of variance. Cow weights were not influenced by month, treatment month interaction, or replication treatment interaction. Significance was indicated in the effect of treatment on weight. Subclass means, compared by approximate Duncan's Multiple Range Test, indicated that cows fed the soybean supplement maintained higher ($P<.05$) weights than those supplemented with biuret. Birthweights did not differ significantly ($P<.05$) between treatments, as indicated by Student's <u>t</u> test.

The supplements were isocaloric, so differences were probably due to nitrogen source. The fact that the biuret supplement furnished 97 gm more protein equivalent per day than did the soybean supplement (412 gm protein equiv. versus 315 gm for soybean) indicates that nitrogen from biuret was not utilized nearly as efficiently as that from soybean meal.

The death of one calf from a cow on the biuret supplement was attributed to low milk production. Although a protein deficiency could have caused this low production, it seems more likely that it was due to insufficient energy. The energy levels of both the biuret and the soybean supplements were consequently increased by the addition of 1.36 kg sorghum grain per head per day.

Although the weights maintained by cows receiving the biuret supplement are not considered unsatisfactory, it is evident that the soybean supplement was superior when based on both cost and animal performance (Table 5).

Experiment II. Weights of cows are given in Figure 1. These weights and calf birthweights were compared with those obtained in Experiment I. Statistical methods were the same as used in Experiment I. Both cow and calf weights were lower ($P<.05$) with cows fed biuret mineral mix than those on either treatment in Experiment I. Since supplemental energy level in Experiment II was higher than in Experiment I, the observed results were apparently due to differences in nitrogen utilization.

Although the average intake of nitrogen for biuret-mineral fed animals (380 gm prot. equiv./head/day) was intermediate to those of soybean and biuret supplemented cows in Experiment I (315 and 412 gm prot. equiv./head/day respectively) there was much daily and periodic variation (Figure 2). A wide variation in consumption of a biuret mineral mix by cattle was also reported by Mackenzie and Altona (1964_a). It can be assumed that there was also wide animal variation in mineral mix consumption, as evidenced by standard errors in weight data from cows in Experiment II as compared with data from Experiment I. One reason for the observed variability may be ascertained from the work by Schroder and Gilchrist (1969). They state that adaptation to biuret is lost quickly (1 to 9 days) if it is not constantly present in the diet. The fact that individual animals may have consumed the mineral mix at intermittant intervals may explain the poor utilization of biuret nitrogen in Experiment II. On the other hand, Templeton et al. (1970) reported no significant difference in gains of steers fed a biuret supplement either daily or on alternate days.

The greatest weight losses occurred at the beginning of the trial while mineral mix consumption was lowest. This could have been the result of either low intake or very poor utilization of biuret. Mackenzie and Altona (1964_a) reported similar loss of weight during the first two weeks of their trial even though consumption of the biuret mineral mix was satisfactory.

Since mineral consumption during the first week of the trial was too low to meet nitrogen requirements, attempts were made to increase and regulate intake. Addition of fine ground sorghum grain to the mixture caused an increase in consumption (Figure 2). Once animals accepted the mixture, consumption increased to 454 gm/head/day. Higher levels were not allowed. It can be assumed that consumption would have gone even higher since that amount was consumed in less than one hour on one occasion. Considering the small size (approximately 45 x 91 cm) of the mineral boxes and the temperaments of the animals, it may be assumed that only about 50% of the animals had access to the mineral mix during this period of time. When the mineral mix was made available *ad libitum* consumption again increased, then varied greatly (Figure 2).

Loss of one calf on this treatment was also apparently due to low energy and 1.36 kg sorghum grain was added to the supplement at the same time that energy was increased in Experiment I.

It is apparent that this method of supplementation is inferior to those used in Experiment I. Cost and performance data in Table 5 further substantiate this conclusion.

Experiment III. Average consumption of the biuret mineral mix by control, 10+1, 50+1, and 100+1 groups were 10.270, 10.709, 6.460, and 8.291 kg respectively for the 21 days of the trial. Consumption and weight gain of treatment groups were compared with those of the control group using Student's *t* tests. While there were no significant differences ($P<.05$), in consumption, the 50+1 group was approaching statistical significance at the 5% level of probability. Weight gains for control, 10+1, 50+1, and 100+1 groups were 10.44, 7.87, -2.57, and 9.99 kg respectively. Only the 50+1 group differed significantly ($P<.05$) from the control group.

Consumption of the mineral mix was not hastened by any of the added levels of sorghum grain and considerable lower values for total consumption were obtained with sorghum grain added at the rates of 50 and 100+1. Since consumption in all cases was satisfactory at the end of two weeks, the sorghum grain was removed from the test mixtures on day 15 of the trial. In all groups this removal of sorghum grain was followed by an increase in average consumption of the mineral mix. There was, however, an increase in consumption by the control animals at this time.

While magnitude of changes in daily intakes varied greatly, there appeared to be a trend for the change to be in the same direction for all groups (Figure 3). This would indicate that consumption was in some way influenced by environment. Since environmental records were not kept, no conclusions can be made.

Variations in daily mineral consumption were similar to those observed in Experiment II. The highest consumption (1145 gm) was recorded for an animal on the 10+1 mixture on day 10 of the trial. Values of zero were recorded for animals on 50+1 and 100+1 on days 1, 2, and 3; and for the 10+1 group on day 3 of the trial.

The rates of acceptance of the mineral mix in this experiment were much faster than was observed in Experiment II. Although the reason for this more rapid acceptance is not readily evident it may have been due to differences in age and prior feeding of the animals and the conditions of the trials (individual pens versus pasture feeding).

Since this trial lasted only 21 days there may not have been adequate time for adaptation to biuret by all animals. This may account for some of the differences in weight response observed. This trial was conducted to determine the effects of addition of sorghum grain on consumption of the mineral mix; weight data collected were incidental. While interesting, it may be hazardous to base conclusions on the observed weight changes.

TABLE 1. COMPOSITION OF SUPPLEMENTS USED IN EXPERIMENTS I AND II

Component	Experiment I		Experiment II
	Soybean	Biuret	Energy Supp.
Soybean meal, kg	37.8	----	----
Biuret 1/ kg	----	8.1	----
Sorghum grain, kg	62.2	91.9	100.0
Vitamin A, I.U.	1.474×10^6	1.456×10^6	1.456×10^6
Vitamin E, I.U.	1474	1456	1456
Consumption kg/day	1.36	1.5	1.5

1/ Kedlor 230, furnished through the courtesy of Dow Chemical Company

TABLE 2. ENERGY AND PROTEIN CONTENT OF SUPPLEMENTS

	Experiment I		Experiment II
	Soybean	Biuret	Energy Supp.
Energy Kcal/kg	3154	2857	3139
Prot. Equiv.,%	23.23	27.46	10.00
DE Kcal/day	4289	4286	4708
Prot. Equiv., kg/day	.315	.412	.150

TABLE 3. COMPOSITION OF MINERAL MIXTURES

Mineral	Experiment I	Experiments II&III
Biuret 1/	00.0	50.0
Bone meal	65.3	32.4
Salt	33.3	16.2
Trace minerals	1.2	1.2
Sulfur (as K_2SO_4)	0.2	0.2

1/ Kedlor 230, furnished through the courtesy of Dow Chemical Company

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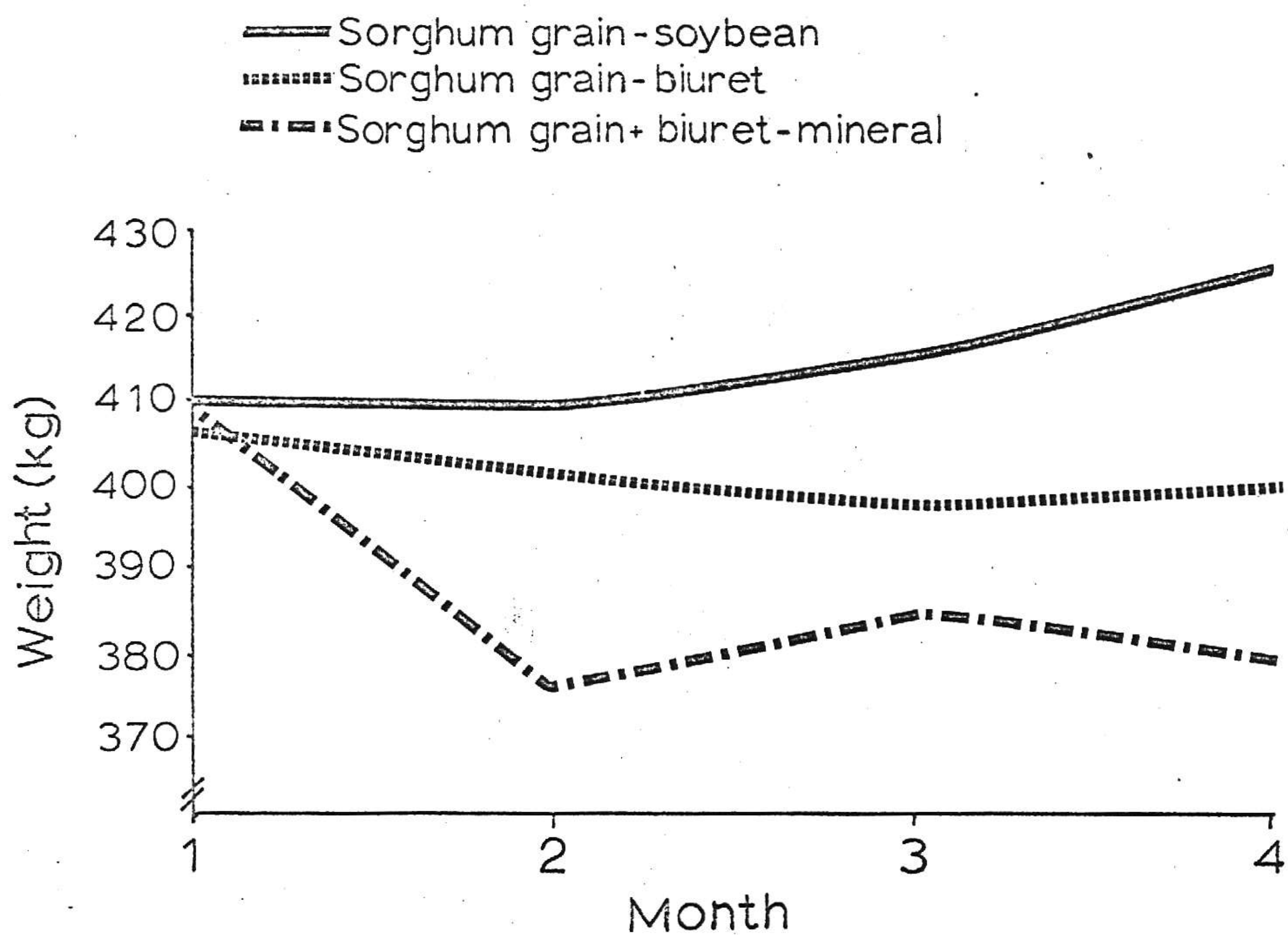
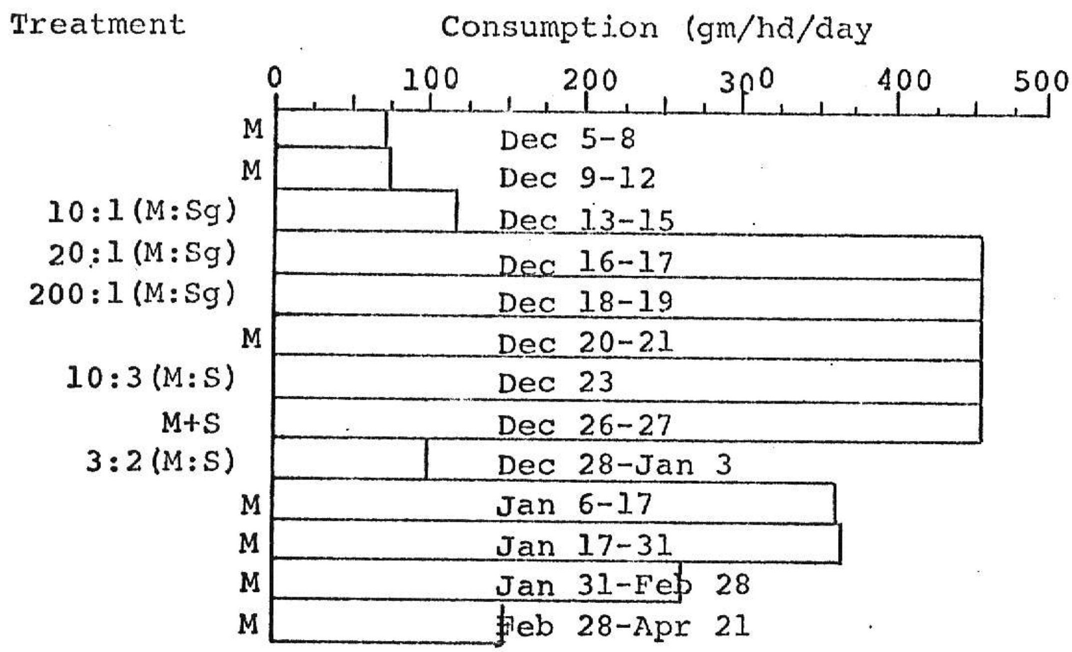
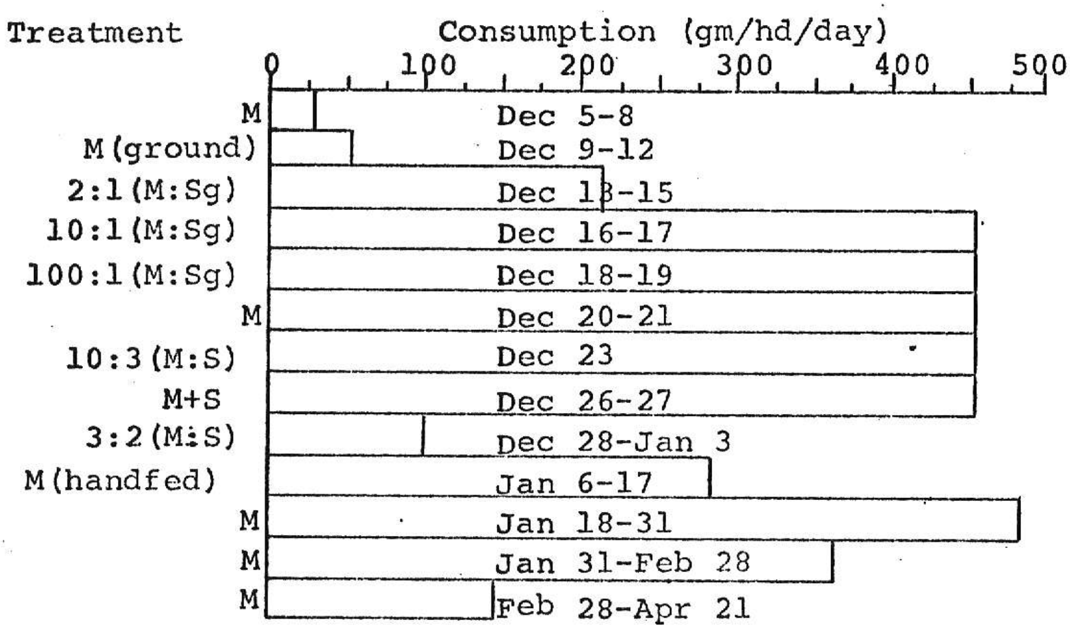


Figure 1 Weight Change of Cows Fed Soybean or Biuret



Group I (10 cows)

M---biuret mineral mix, free choice
M:Sg---ratio of biuret mineral mix to sorghum grain
M:S---ratio of biuret mineral mix to salt
M+S---biuret mineral mix alone, salt offered separately



Group II (10 cows)

Figure 2. Consumption of Biuret-mineral Mixture by cows on Various Treatments During Experiment II

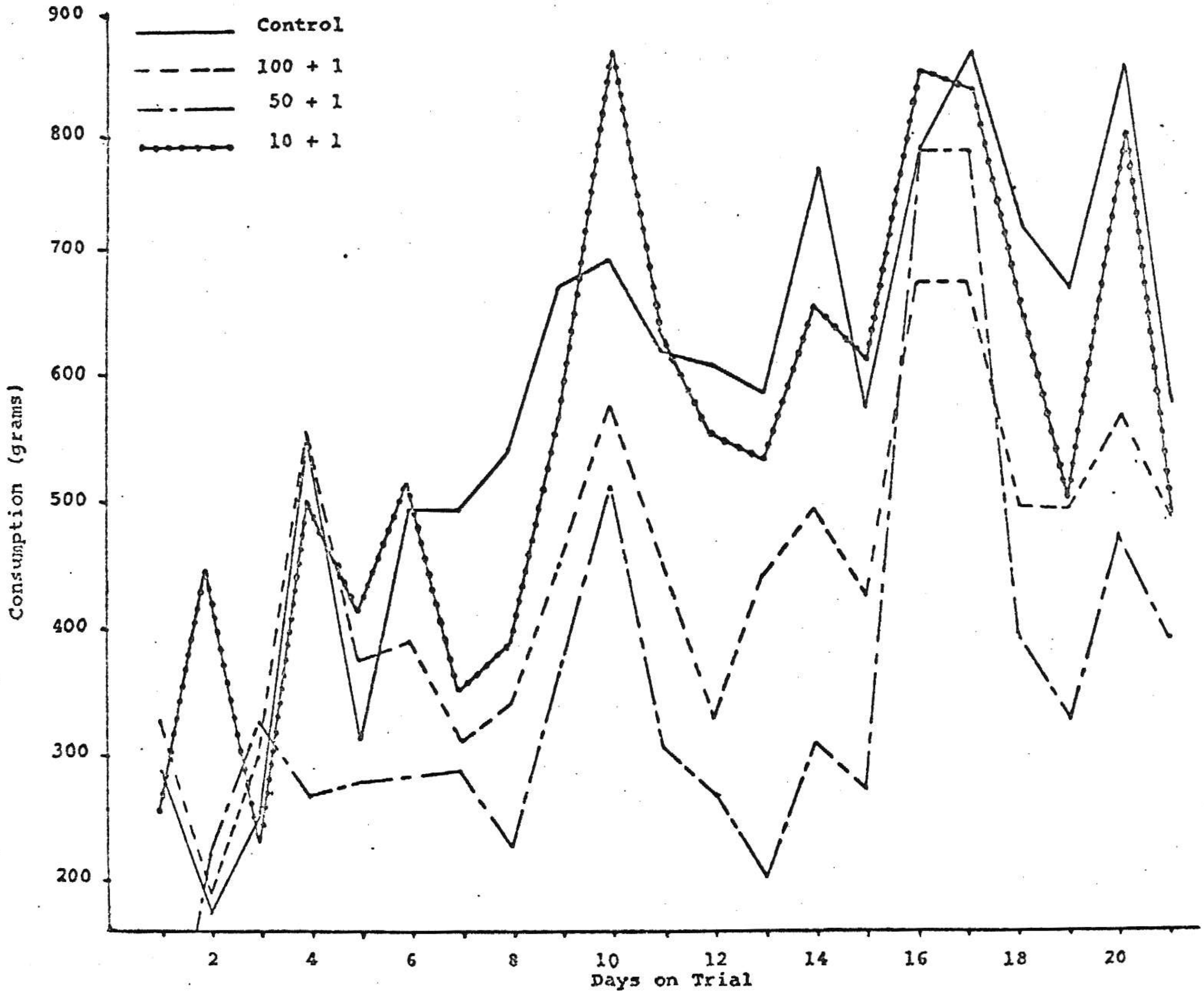


Figure 3. Consumption of Mineral Mix by Heifers on Experiment III

TABLE 4. BIRTHWEIGHT OF CALVES FROM COWS WINTERED ON PASTURE AND SUPPLEMENTED WITH SOYBEAN MEAL OR BIURET

Treatment	No. Males	No. Females	Avg. Birthweight 1/	Adjusted Birthweight 2/
Soybean				3/ 4/
Group 1	2	8	30.69	31.21±2.65
Group 2	5	7	28.79	
Biuret				
Group 1	5	4	30.55	30.92±3.05
Group 2	6	6	29.28	
Biuret-mineral				
Group 1	4	4	28.77	28.68±2.81
Group 2	5	4	26.58	

1/ Birthweight in kg
2/ Birthweight adjusted by adding 2.27 kg to heifer weights
3/ Average for calves in Groups 1 and 2
4/ Standard Error

TABLE 5. COMPARATIVE PERFORMANCE OF COWS SUPPLEMENTED WITH SOYBEAN MEAL OR BIURET

Treatment	Soybean		Biuret		Biuret Min-mix	
Group	1	2	1	2	1	2
No. of cows	10	14	10	14	10	10
Stocking rate (A/head)	6.95	6.78	6.95	6.78	6.0	6.0
Initial wt, kg 1/	410.02±9.21 2/		406.31±9.21		407.23±10.05	
Wt. prior to calving, kg	424.50±9.21		399.88±9.21		378.86±10.05	
Wt. gain	14.48		-6.43		-28.37	
Supp/hd/day(kg)	1.36		1.50		1.50 3/	
Cost of Supp. 4/ (¢/hd/day)	7.71		9.54		9.49 5/	

1/ Average of two groups on each treatment
2/ Standard Error
3/ Inlcudes only Energy Supplement (Table 1)
4/ Based on Kansas City Market (December, 1969) Soybean Meal--$68.50 per Ton, Sorghum grain--$2.00/cwt., Biuret--$260.00/Ton (Dow Chemical Co., Midland, Michigan, Kedlor 230
5/ Considering cost of Sorghum grain and Biuret (with mineral mix consumption at 201.6 gm/hd/day

SUMMARY

Forty-eight pregnant five-year-old cows were used to compare supplements of sorghum grain-soybean meal and sorghum grain-biuret. Supplements were isocaloric and stocking rates were equal, but the biuret supplement supplied 97 gm more protein equivalent per head per day. Significantly ($P<.05$) higher weights were maintained by cows receiving the soybean supplement indicating more efficient utilization of nitrogen. Birthweights of calves were not significantly different ($P<.05$) between treatments. Although the soybean supplement was superior when based on animal performance and cost, performance of animals fed the biuret supplement was considered satisfactory.

A second experiment was conducted with 20 pregnant five-year-old cows to study the feasibility of adding biuret in a mineral mix fed free choice. Stocking rate and supplemental energy level were slightly higher than in the first experiment. Consumption of the biuret mineral mix at the onset of the trial was insufficient to meet nitrogen requirements; however, the addition of fine ground sorghum grain to the mixture increased consumption. The sorghum grain was subsequently removed and satisfactory intake was maintained. The average nitrogen intake for the entire period (140 days) was intermediate to those of the soybean and biuret supplemented cows (Experiment I). Weight of cows on this treatment was maintained at significantly ($P<.05$) lower levels and cows gave birth to smaller ($P<.05$) calves than for either treatment in Experiment I.

The lower performance of cows on the biuret mineral mix than those on the biuret-sorghum grain supplement may have been due to

variation of intake and/or intermittent intake resulting in deadaptation of the rumen flora to biuret.

A third experiment was conducted to determine whether the addition of fine ground sorghum grain to a mineral mix containing biuret would hasten acceptance. None of the rates of added sorghum grain improved acceptance and consumption was considerably lower (non-significant statistically) for the lower rates than for the higher rate and the control group. The lowest intake (obtained on 50 parts mineral mix to 1 part sorghum grain) was accompanied by weight gains statistically ($P<.05$) lower than those of the controls. The length of the study (21 days) was probably not long enough to permit adequate adaptation, therefore weight gains in this trial are not considered reliable indicators of biuret utilization.

From these studies it was concluded that of the three forms of supplementation studied, the soybean supplement was most desirable, a biuret-sorghum grain supplement was intermediate, and supplementation of biuret in a mineral mix was least desirable. Addition of sorghum grain to the mineral mix at the levels studied cannot be relied upon to hasten acceptance or increase consumption. The extreme variability of intake lowers the reliability and therefore the value of this form of supplementation.

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APPENDIX

APPENDIX TABLE A

WEIGHTS OF COWS SUPPLEMENTED WITH SOYBEAN MEAL OR BIURET

Month	Soybean meal[1]	Biuret[1]	Biuret Min-mix[2]
1	410.03[3]±9.21[4]	406.31±9.21	407.24±10.05
2	410.22±9.21	401.59±9.21	375.23±10.05
3	415.80±9.21	397.71±9.21	384.77±10.05
4	424.50±9.21	399.88±9.21	378.86±10.05
Avg.[5]	415.13±4.65	401.37±4.65	386.52± 5.03

1/ Cows in Experiment I, Avg. of two groups on each treatment
2/ Cows in Experiment II, Avg. of two groups
3/ Mean weight in kg
4/ Standard Error
5/ Avg. of first four weigh periods

APPENDIX TABLE B

CONSUMPTION OF BIURET MINERAL MIX BY HEIFERS ON EXPERIMENT III

Treatment	Day	Average Consumption (gm)	Range	Standard Error
Control	1	288.3	9-540	266.6
	2	176.3	14-305	148.4
	3	254.0	48-400	183.5
	4	549.3	325-850	272.1
	5	315.0	215-440	114.6
	6	493.3	265-670	185.1
	7	492.0	235-835	309.1
	8	541.3	265-708	241.0
	9	667.0	462-875	206.5
	10	687.3	288-935	349.1
	11	616.7	380-870	245.4
	12	606.7	530-670	70.9
	13	583.3	540-620	40.4
	14	770.0	370-985	346.7
	15	576.7	180-895	363.9
	16	785.0	460-985	383.9
	17	860.0	640-975	190.6
	18	710.0	410-970	282.1
	19	666.7	390-930	270.2
	20	851.7	580-1000	235.6
	21	575.0	540-630	48.2
100+1	1	332.7	0-957	541.1
	2	191.3	0-444	228.3
	3	300.0	0-500	264.6
	4	555.7	493-626	66.8
	5	376.7	160-492	187.8
	6	391.0	355-441	44.7
	7	310.7	210-447	122.5
	8	340.7	253-507	144.1
	9	450.0	311-535	121.4
	10	575.0	250-915	332.8
	11	453.3	300-750	257.0
	12	330.0	180-570	210.0
	13	440.0	105-935	437.5
	14	495.0	195-960	408.3
	15	425.0	200-780	311.1

APPENDIX TABLE B (continued)

Treatment	Day	Average Consumption (gm)	Range	Standard Error
100+1	16	668.3	525-805	140.1
	17	670.7	565-790	113.1
	18	493.3	220-860	330.1
	19	491.7	290-815	282.9
	20	566.7	315-800	243.0
	21	490.0	360-745	220.9
10+1	1	256.7	29-712	394.3
	2	441.0	316-645	178.2
	3	229.3	0-559	292.7
	4	500.3	375-646	136.6
	5	414.0	255-512	138.9
	6	515.0	468-567	49.7
	7	349.3	235-463	114.0
	8	391.7	265-470	110.7
	9	564.7	450-630	99.6
	10	860.0	553-1145	296.6
	11	628.3	520-700	95.4
	12	553.3	420-690	135.0
	13	531.7	440-680	129.6
	14	653.3	500-850	179.0
	15	610.0	480-800	168.2
	16	845.0	785-950	91.2
	17	829.7	750-934	94.4
	18	666.7	450-880	215.0
	19	500.0	440-600	87.2
	20	796.7	760-840	40.4
	21	490.0	315-700	194.9
50+1	1	18.0	0-54	31.2
	2	222.3	0-605	332.8
	3	328.7	0-669	334.7
	4	270.3	139-526	221.4
	5	280.7	90-437	176.0
	6	281.0	188-380	96.1
	7	285.0	215-317	60.7
	8	229.0	152-302	75.1
	9	370.0	337-400	31.6
	10	513.3	457-584	64.0
	11	311.7	45-520	242.8
	12	275.0	95-450	177.5

APPENDIX TABLE B (continued)

Treatment	Day	Average Consumption (gm)	Range	Standard Error
50+1	13	208.3	195-220	12.6
	14	313.3	240-450	118.5
	15	276.7	180-360	90.7
	16	785.0	730-875	78.6
	17	781.7	765-800	17.6
	18	395.0	150-555	215.5
	19	331.7	260-420	81.3
	20	478.3	220-655	228.7
	21	393.3	300-460	83.3

EFFECT OF BIURET ON LOW ENERGY BEEF CATTLE RATIONS

by

HARLAN A. THYFAULT

B. S., Kansas State University, 1968

AN ABSTRACT OF A MASTER'S THESIS

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1970

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