UTILIZATION OF NON-PROTEIN-NITROGEN BY RUMINANTS CONSUMING A LOW-QUALITY FORAGE

By

Lethal Conrad Martin

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Chairman: Dr. C.B. Ammerman
Major Department: Animal Science

Seven experiments were conducted: three were undertaken to evaluate urea and biuret as sources of supplemental nitrogen for beef cows being wintered under practical range conditions; three to study the utilization of low-quality forage and nitrogen by sheep when supplemented with varying forms and levels of NPN (biuret and urea) and varying forms and levels of energy (molasses and corn); and one for comparison of formaldehyde treated soybean meal with untreated soybean meal and biuret as sources of supplemental nitrogen for sheep consuming a low-quality forage.

Wintering studies were conducted over 1-,2- and 3-year periods. During the first year mature Angus cows received supplemental nitrogen as cottonseed meal cubes (CSM) (44.9% crude protein) or cubes containing cottonseed meal, citrus pulp and urea (50% of the nitrogen from urea) (43.0% crude protein). During the following 2-year study supplemental nitrogen was provided as either CSM cubes (44.8% crude protein) or citrus pulp cubes (41.3% crude protein) containing feed

grade biuret. Supplemental forage was provided as either Pangola digitgrass (Digitaria decumbens) hay or sorghum (Sorghum vulgare) silage in both studies. During the third study cows were provided supplemental nitrogen as either CSM cubes (41.0% crude protein) or citrus pulp cubes with biuret (CPB) (38.0% crude protein). In addition, a third group of animals received citrus pulp cubes (CP) without biuret (7.0% crude protein) and served as a negative control.

Performance of cows receiving urea cubes was equivalent to that of cows receiving CSM cubes during the first study. However, it was suggested that over-all nutritional stress was not sufficient during this study for animals to derive benefit from either source of supplemental nitrogen. Cows receiving CPB during the following 2- and 3-year studies had greater winter weight loss than did cows receiving CSM supplement. Weight losses for animals receiving CPB were not as great during the final 3-year period, however, as those for cows receiving negative control supplement (CP). Conception rate was 86 and 93% ($P<.07$) for cows receiving CPB cubes and CSM cubes respectively during the 2-year study and cows receiving biuret cubes weaned lighter calves during the 3 years of the third study. Final cow (August) weights were lower for cows receiving biuret cubes in only one of the 5 years. It was concluded that biuret was utilized by wintering beef cows. Performance of cows receiving CPB cubes was not equal, however, to that of cows receiving CSM cubes.

Three voluntary intake and metabolism studies were conducted to compare varying levels and forms of NPN (urea and biuret) and varying levels and forms of energy (corn and molasses) as supplements for sheep consuming a low-quality forage. No combination of supplemental nitrogen or energy increased hay intake above that of control animals receiving no supplemental energy or nitrogen. In general increasing nitrogen levels as either biuret or urea (with equal supplemental energy levels) tended to improve hay and organic matter intake; digestibility of cellulose, organic matter and nitrogen; and nitrogen balance. Increasing energy levels tended to decrease hay intake and cellulose digestibility and increase organic matter digestibility. When supplemented with urea, 80 g of molasses promoted better hay utilization and greater nitrogen balance than did 160 g of molasses. Supplemental energy as corn resulted in a more positive nitrogen balance than did molasses. Results when 0, 5 or 10 g of biuret nitrogen was fed with 0, 80 or 160 g of molasses were highly variable.

A voluntary intake and metabolism study was conducted to compare formaldehyde treated soybean meal (F-SBM) to normal soybean meal (N-SBM) and biuret as sources of supplemental nitrogen for sheep consuming a low-quality forage. A negative control treatment was also utilized. All nitrogen supplements improved hay intake, organic matter and cellulose digestibility and nitrogen balance. The greatest improvements were with F-SBM.

CHAPTER I

INTRODUCTION

The ruminant animal is an important resource to the food production industry. The ability of cattle, sheep and other ruminants to harvest forages and other products that are of little nutritive value to man and other monogastrics and to convert these products into a nutritious and appealing source of food for man has, for many years, been very important.

The world population is presently estimated to be approximately 4.5 billion and is predicted to approach 7 billion by the year 2000 (Carter, 1974). With this increase in world population there is the prospect of the enormous task of producing the food to maintain these numbers of people. As this task of food production grows it is apparent that our highly productive lands must be fully utilized for the production of grains and other plant products that will provide, directly to the population, energy, protein and other required nutrients. However, there are large areas of the world's land masses that are suited only for forage production and these areas, as well as the by-products of row crop production and industry, must be harvested in order to attempt to keep up with the demand for food. The ruminant animal is our most ideally suited tool for this task.

A specific area of importance is the ability of ruminant animals to consume and degrade many non-protein-nitrogen (NPN) compounds and to convert the nitrogen from these compounds into animal protein. The practice of meeting at least part of the crude protein requirement of the ruminant with NPN is widely accepted. Oltjen (1972) indicated that 680,000 tons of urea, the principal commercial source of NPN, was utilized in 1970 in ruminant diets and suggested that the nitrogen provided by this urea was equivalent to the nitrogen provided by approximately 4,000,000 tons of 44% protein soybean meal.

In the past years the greatest use of NPN has been by ruminants in feedlot-type situations where they received high energy diets containing relatively large amounts of grain. However, with the prospects of grain, as well as many natural proteins, becoming less available to the livestock industry, the increased use of NPN with lower energy diets consisting primarily of forages and by-products can be anticipated.

In many areas of the world where ruminants are utilized to harvest available forages there are seasonal periods of growth which provide abundant natural feedstuff as forages alone to support adequate to good animal performance. However, these periods are often followed by dormant seasons during which time the available forages are of a sufficiently low quality as to require the use of supplemental feeds to maintain the animal. These are the conditions under which the use of NPN can be adopted with great benefit.

Natural proteins are, at present, used to supplement animals consuming low quality forages. However, on consumption these highly soluble natural proteins are degraded and in part converted into a lower quality microbial protein with an accompanying loss of a portion of the protein nitrogen. Treatment of natural proteins with formaldehyde has been shown to decrease the solubility of these proteins, decrease their degradation in the rumen, and increase their efficiency of utilization. This offers an additional possibility of increasing the efficiency of protein utilization, and may well enhance the productivity of animals consuming low quality forages.

The studies reported here were undertaken to investigate the utilization of NPN and formaldehyde treated soybean meal by sheep and NPN by cattle consuming low quality roughage under maintenance conditions. Urea and biuret were studied as sources of supplemental nitrogen for wintering brood cows over a six year period. The effect of providing supplemental nitrogen as biuret or urea at varying dietary levels along with different sources and levels of energy to sheep receiving a low quality hay was also studied. In addition, a study was conducted to evaluate formaldehyde treated soybean meal as a source of supplemental nitrogen for sheep consuming a low quality hay.

CHAPTER II

REVIEW OF LITERATURE

The ability of the ruminant animal to utilize non-protein nitrogen (NPN) has long been established. Stangel (1967), in a review of the history of the use of nitrogen, amino acids and urea in ruminant nutrition, indicated that in 1891, both Zuntz and Hagemann reported that the microorganisms of the rumen played an important role in the nutrition of the ruminant animal. The authors suggested that these microbes were important in the processes of cellulose digestion and that the protein sparing action of asparagine and other amides was observed only with ruminants. Morgen and his associates in Germany, during the period of 1907 to 1924, showed that as much as 40% of the protein in the diets of sheep could be replaced with urea. The actual mechanism by which urea replaced natural proteins, however, was still widely debated (Stangel, 1967).

The shortages and necessities brought about by World War I further stimulated the investigation of NPN use, especially in Germany. Undoubtedly the development of a commercial plant for the direct manufacture of ammonia from atmospheric nitrogen in that country in 1913 also encouraged additional research into the nutritional utilization of NPN. In 1935 the commercial production of urea began in the United States. This event made urea available to feed manufacturers of this country at an acceptable price and greatly stimulated both commercial use of urea and further research on NPN as a nutrient.

In 1939 Hart et al. reported work demonstrating that dairy heifers could utilize nitrogen from urea to make satisfactory gains. These workers also concluded that the animals were able to utilize the nitrogen from urea via the action of the rumen microorganisms. The work of this group was extended by Wegner et al. (1941). These researchers used an in vitro investigative system and reported that the nitrogen from both urea and ammonium bicarbonate was incorporated into bacterial protein. They also indicated that this incorporation was increased by the addition of a source of readily available energy and decreased in the presence of higher levels of natural proteins. Loosli et al. (1949) removed all question as to the conversion of urea nitrogen into protein when they fed sheep and goats a purified diet with urea providing the only source of nitrogen. The authors reported that the rumen content of these animals contained from 9 to 20 times more amino acids than did the diet and that all ten essential amino acids were synthesized in large amounts.

In the last twenty-five years the use of NPN, primarily urea, in ruminant diets has grown fantastically. Oltjen (1972) estimated that 465,000 tons of urea were used in ruminant diets in 1965 and that that amount had increased to 680,000 tons by 1970. Simultaneously, the literature pertaining to the use of non-protein nitrogen in ruminant diets has also grown enormously. With this in mind, no attempt will be, or could be made to review the entire field of NPN literature. Rather, the remainder of this review will be confined to those aspects of the literature closely related to the work conducted in these studies. For more complete reviews the reader is referred to publications by Briggs (1967), Chalupa (1968), Helmer and Bartley (1971), Fonnesbeck et al. (1975) or any of the many other excellent reviews presently available.

Urea and Biuret as Sources of NPN

As indicated previously urea is the principle commercial source of NPN for use in ruminant diets. Its utilization is well documented and may be confirmed by many of the publications cited in this review. Its importance to ruminant production may be again confirmed by referring to the magnitude of its use.

Biuret, which is a condensation product of urea, has also been subjected to much attention recently as a potential source of NPN. This increased attention has been brought about by the physical properties of biuret which may result in its being a more highly desirable source of NPN for animals receiving low energy maintenance-type diets consisting of such feeds as weathered forages. A brief review follows on the properties of biuret and urea as they may relate to their use as sources of supplemental nitrogen (N) for animals which are maintained on low-quality forages.

A comparison of the properties of pure urea and biuret are shown in table 1.

It should be noted that the commercial biuret product available for use is not a pure product but is termed "feed grade biuret" and is defined by the 1972 Federal register as follows: minimum biuret, 55%; maximum urea, 15%; maximum cyanuric acid and triuret, 30%; minimum nitrogen, 35%.

TABLE 1

CHEMICAL AND PHYSICAL PROPERTIES OF PURE UREA AND BIURET[a]

Property	Urea	Biuret
Molecular weight g/mol	60.06	103.09
Density g/cm^3	1.32	1.47
Solubility in water g/100ml @ 37°C	>200.00	2.2
Nitrogen content, %	46.65	40.77
Protein equivalent, % (Nx6.25)	291.56	254.81

[a]Fonnesbeck, et al., 1975.

Several differences between urea and biuret are of importance when comparing the two compounds. Solubility is one of these factors and may be seen in table 1. Urea is the more soluble of the two compounds. The comparatively low solubility of biuret in water gives it distinct advantages when feeds or supplements containing biuret are to be exposed to rain and snow or fed in a humid climate (Fonnesbeck et al., 1975). In general this low solubility improves the handling qualities of biuret under any conditions where moisture may present a problem. However, the high solubility of urea does enhance its suitability for use in liquid supplements formulated to contain relatively high concentrations of crude protein. Such liquid supplements are presently popular for use with both high energy diets for dairy and beef cattle and with beef animals under wintering type conditions.

The toxicity and safety of feeding urea and biuret must also be considered when comparing the two compounds. The toxicity of urea has been studied by many workers (Dinning et al., 1948; Repp et al., 1955; Davis and Roberts, 1959; and Word et al., 1969) and it is agreed that the consumption of large amounts of urea over a short period of time may result in the death of ruminant animals. The problem of toxicity becomes of even greater concern when animals are kept under maintenance conditions, and consume diets low in readily digestible carbohydrates. These animals may be considered in a somewhat fasted condition at all times. Davis and Roberts (1959) have both reported that low energy nutritional status enhances the possibility of urea toxicity.

In contrast, Hatfield et al. (1959) drenched sheep with as much as 275 gm of biuret at one time and observed no symptoms of acute toxicity. In more extensive studies, Berry et al. (1956) indicated that biuret produced neither acute nor chronic toxicity when included in the diets of rats, poultry, lambs or steers on relatively high levels. The difference in toxicity of the two compounds is most certainly a result of the differences in their solubility and rate of hydrolysis. Bloomfield et al. (1960) have studied the kinetics of urea hydrolysis and confirmed its rapid breakdown in the media of the rumen. Tiwari et al. (1973a) have studied the metabolism of biuret and have reported data indicating that biuret is hydrolyzed quite slowly from the rumen (5 to 20 mg/100 ml rumen fluid per hour). The rapid breakdown of urea will result in elevated rumen ammonia concentration. High levels of ruminal ammonia, under the influence of proper pH and concentration gradient, will be absorbed into the peripheral blood in quantities greater than can be detoxified by the animal body and result in ammonia toxicity, the symptoms of which have been discussed by Repp et al. (1955) and Davis and Roberts (1959).

Palatibility is also a factor to be considered when dealing with any feed ingredient that is to be included in an animal's diet at a substantial level. This is especially true when we refer to supplemental type feeds such as those that may include urea or biuret and that would be expected to provide major portions of the nitrogen for animals such as brood cows under range conditions. Obviously the nutritive value of any feed or feed ingredient will not be realized unless it is consumed.

Urea can limit the voluntary intake of a feed in which it is contained. Van Horn et al. (1967) fed dairy cattle a concentrate mixture containing 1.7% urea and reduced animal intake when compared to that of animals receiving the same diet without urea. Oltjen et al. (1969) fed a diet containing 85% hay and either 2.3 or 2.8% urea or buiret respectively and found that, when steers were offered these diets for one hour twice daily, consumption of the biuret containing diet was 15% greater than consumption of the urea containing diet. These results were interpreted to indicate that biuret was more palatable to the ruminant. Verde (1971) offered 150 gm of a supplement containing either 7.12% urea or 8.44% biuret for two hours per day to sheep receiving Pangola digitgrass hay *ad libitum* and found that sheep offered the urea supplement refused approximately 25%. All the biuret supplement was consumed. Fonnesbeck *et al*. (1971) reported work indicating that cows would not consume sufficient supplements containing 40% feedgrade biuret to balance the protein deficiency of a stem-cured range grass but concluded that the factor limiting consumption was the presence of urea in the biuret. MacKenzie and Altona (1964), Tollett *et al*. (1969) and Templeton (1970) have reported greater consumption of supplements containing biuret than of supplements containing urea. Huber and Cook (1969) showed that refusal of cows to eat high urea diets was due to undesirable taste of the diet.

The rate of hydrolysis of urea and biuret is also an important factor to consider when discussing these two compounds as possible sources of NPN for ruminants consuming low-quality forages. Both of these compounds are broken down into ammonia and carbon dioxide, but by two different microbial enzymes (Tiwari *et al*., 1973a). The ammonia resulting from the breakdown of these two compounds is utilized by the rumen microbes to combine with α-keto acids which results in the synthesis of amino acids that are in turn incorporated into microbial proteins (Hungate, 1966). A key factor in the efficient synthesis of microbial protein is the presence and availability of ammonia and α-keto acids at the same time and at appropriate levels.

The rapid hydrolysis of urea to carbon dioxide and ammonia by the enzyme urease, which Jones et al. (1964) indicated is produced by about 35% of the viable bacteria in strained rumen fluid, is well extablished (Chalupa, 1968). In fact, one of the major problems in efficient utilization of urea is the rapid release of ammonia (Chalupa, 1968). Bloomfield et al. (1960) has reported that the rate of ammonia production from urea hydrolysis is approximately four timesgreater than the rate of utilization of ammonia nitrogen by the rumen microbes. These workers further indicated that under the conditions of their study urea was broken down at a rate of approximately 80 mg of urea nitrogen/100 ml rumen fluid per hour. This rate of hydrolysis resulted in an eventual loss of nitrogen available to the microbes for protein synthesis. This problem increases in magnitude as the energy concentrations of the over-all diet decreases and brings about a corresponding decrease in the availability of appropriate carbon structures for union with the released ammonia present. Knight and Owens(1973) investigated this relationship by infusing urea into the rumen of lambs receiving either a 60, 65 or 75% TDN ration over varying time periods. These researchers observed that with twice daily feeding nitrogen retention was increased when urea was infused over a period of three hours with the two lower energy level diets. With the higher energy diet nitrogen retention progressively decreased as infusion time was increased from 1 to 3 to 12 hours. The 12 hours infusion time decreased nitrogen retention with all diets as compared to the 1 hour infusion. These workers concluded that providing a moderately slow release of ammonia with high fiber diets improved the utilization of that ammonia.

Many additional studies have been undertaken with the objective of improving urea utilization by retarding its hydrolysis. These attempts have included the processing of urea with cereal grains through an extruder under proper conditions to yield a gelatinized product which releases ammonia more slowly and is more palatable (Meyer et al., 1967; Deyoe et al., 1968; Helmer et al., 1970). Attempts have also been made to reduce the rate of urea hydrolysis with depression of the activity of urease by establishing immunity to the enzyme (Harbers et al., 1965; Glimp and Tillman, 1965). All of these attempts to slow the release of ammonia have met with some degree of success.

In contrast to urea, the hydrolysis and utilization of biuret by the microbes of the rumen were for some time questionable. Belasco (1954) and Johnson and McClure (1964) have reported little or no utilization of biuret by rumen bacteria in vitro, using cellulose digestion as an index of nitrogen utilization. However, other workers have reported satisfactory animal performance when biuret was utilized as a source of nitrogen (Mieske et al., 1955; Gaither et al., 1955; Hatfield, 1959) indicating a certain degree of hydrolysis of the compound. The question concerning biuret hydrolysis has been more fully answered by more recent work. Bauriedel et al. (1969) studied the hydrolysis of biuret utilizing ^{14}C and ^{15}N under in vitro conditions with microorganisms from sheep and cattle which were adapted to biuret diets. They reported that biuret appears to be hydrolyzed completely to carbon dioxide and ammonia, both of which may then be utilized by the bacteria of the rumen for anabolic purposes. They, in fact, reported a broad incorporation of ^{15}N into amino acids of bacterial protein. These workers also reported that the enzyme system for hydrolyzing biuret is distinctly different from urease, but that urease participates in the complete breakdown of biuret and that the organisms associated with biuretase production appeared to be tightly bound to the plant debris of the rumen fluid.

Tiwari et al. (1973a, b) have further studied the degradation of biuret and suggested that biuret is broken down into urea, ammonia and carbon dioxide via biuretase and that the urea resulting from this reaction is then converted to carbon dioxide and ammonia by the action of rumen urease. These workers also studied the rate of disappearance of biuret from the rumen, by measuring both the hydrolysis of the compound and its passage from the rumen. Biuret (8.5 gm) was infused into a ruminally fistulated sheep and samples were taken hourly for the following 6 hours for biuret determination. The disappearance of biuret from the rumen was almost linear and occurred at a rate of 20 mg of biuret/100 ml of rumen fluid per hour. Polythylene glycol (PEG) was also infused with the biuret as a marker and, assuming that biuret passed with the PEG, it was calculated that some 65% of the infused biuret was degraded in the rumen and that approximately 35% passed undegraded from the rumen. These workers also suggested that the rate limiting factor in the degradation of biuret is the catalyzed conversion of biuret to urea and ammonia by the enzyme and not the solubility of biuret. This is in agreement with the observations of Schröder and Gilchrist (1969).

By relating the reported disappearance rate of biuret (20 mg/100 ml rumen fluid per hour) (Tiwari et al., 1973a or b) to a corresponding rate of nitrogen disappearance and accounting for the suggested 35% of the biuret that passes through the rumen, one may obtain a somewhat general value for the rate of biuret nitrogen hydrolysis. This calculation would indicate that approximately 5 mg of biuret nitrogen/100 ml of rumen fluid per hour would be released. A corresponding figure of approximately 80 mg of urea nitrogen has been reported (Bloomfield et al., 1960). Bloomfield et al. (1960) also reported that under the conditions of their study ammonia nitrogen was utilized by rumen microbes at a rate of 20 mg/100 ml of rumen fluid per hour. Using these values as a general guide line (and it must be pointed out that these values would be expected to be highly variable and dependent on many factors) one may conclude that the rate of hydrolysis of urea is too rapid to result in its maximum utilization and that the hydrolysis of biuret occurs at too slow a rate to provide for its maximum utilization. The rate of hydrolysis of both biuret and urea appear to be completely unrelated to the subsequent utilization of ammonia produced by their breakdown (Bauriedel, 1971; Bauriedel et al., 1971; Bauriedel et al., 1969; and Bloomfield et al., 1960).

A period of adaptation has been suggested by many investigators to enhance the utilization of non-protein nitrogen compounds.

In the case of urea, however, requirement of an adaptation period may be questionable. Repp et al. (1955), Campbell et al. (1963), Smith et al. (1960), McLaren et al. (1959) and Anderson et al. (1959) have observed improved performance with this adaptation period. Other workers (Ewan et al., 1958; Oltjen et al., 1969; Caffrey et al., 1967; Schaadt et al., 1966; and Miller and Morrison, 1942) have failed to obtain positive responses with an adaptation period. Pearson and Smith (1943) have demonstrated that urease is present in the rumen at all times and Caffrey et al. (1967) have shown that the hydrolysis of urea occurred at a greater rate in rumen fluid from sheep receiving a diet without urea than in rumen fluid from sheep receiving a diet with urea. If an adaptation response occurs then it would seem that adaptation is to the utilization of ammonia from urea and not to the breakdown of urea. Caffrey et al. (1967) have suggested that the improved response with time noted by some workers is an adjustment to the nutritional regimen rather than an adjustment to urea.

Biuretase, unlike urease, is an induceable enzyme that is not found in significant amounts in the rumen when biuret is absent from the diet. The establishment of this enzyme which enables the animal to utilize biuret does require an adaptation period (Ewan et al., 1958; Hatfield et al., 1959; Tomlin et al., 1967; and Johnson and Clemens, 1973). The mechanism of this adaptation seems to involve an increase in the number of biuretolytic microorganisms present in the rumen (Ewan et al., 1958; Gilchrist et al., 1968; Schröder and Gilchrist, 1969; and Slyter et al., 1968). The time required for an animal to adapt to a biuret containing diet has been reported to vary widely. Clemens and Johnson (1972) have reported that lambs receiving a 60% concentrate diet showed maximum biuretolytic activity within 10 days. In contrast Clark et al. (1963) reported that 6 to 8 weeks were required to obtain maximum nitrogen retention in sheep fed a low protein roughage diet. In general, the time required for adaptation to biuret appears to be related to nitrogen status of the animal and the level of protein in the basal diet to which biuret is added. Schröder and Gilchrist (1969) fed sheep forage diets that were deemed to be low, medium or high in protein quality (crude protein ranged from 3.4 to 10.3%) and reported that biuret adaptation with these diets was apparent at 15, 30 and 70 days, respectively. Johnson and Clemens (1973) and Gilchrist et al. (1968) have reported similar results which support the conclusion that biuret adaptation is more rapid with basal diets containing low levels of crude protein.

A de-adaptation problem has been observed in animals which were fed biuret. Johnson and Clemens (1973) removed biuret from the diet of steers adapted to the diet and measured the disappearance of biuret *in vitro* using rumen fluid from the steers during the following 10 day period. They reported that the ability of the steers to hydrolyze biuret was markedly decreased in 1 day and lost completely in 4 days. Schröder and Gilchrist (1969) have reported similar results. These workers also suggested that a normal length of adaptation must follow biuret withdrawal in order to re-establish the animal's ability to utilize biuret.

In recent years the practice of range supplementation to grazing animals on an every other day to weekly basis has become popular. This practice has been successful when natural proteins were utilized. However, if supplements were to contain biuret, a more frequent feeding schedule would seem desirable considering the de-adaptation occurrence with biuret feeding.

NPN Supplementation for Animals Receiving Low-Quality Roughages

As stated previously, ruminant animals are often required to graze and maintain themselves on weathered forages during the winter or dry periods of the year. These forages may be sufficiently low in protein as to require nitrogen supplementation to prevent excessive weight loss or even death. Supplementation of nitrogen under conditions such as these has, in this country, generally been by provision of natural proteins. In the future, the availability of natural proteins may not permit their use in this manner. The increased use of NPN will be called for under these conditions.

The utilization of low-quality fibrous forages by the ruminant requires an active and functional rumen microflora and to maintain the microorganisms, their requirement for nitrogen must be met. Moir and Harris (1962) varied the nitrogen intake of sheep receiving a purified diet and observed that the microbial population of the rumen decreased as the daily nitrogen intake decreased from 12 g to 2.2 g. Digestibility and intake of the diet declined as nitrogen intake dropped below a level of approximately 1% of the diet.

Blaxter and Wilson (1963) have shown that with temperate forages, voluntary intake increases when the protein level increases, until the protein content reaches about 8.5%. Beyond this level no further increase was noted. With tropical forages, Milford and Minson (1965) have demonstrated that a decrease in voluntary intake is not observed until the crude protein level falls below 7%.

Briggs et al. (1960) used survival of sheep on low-quality roughage as the criterion for evaluating NPN supplements and reported that sheep could not survive on roughages containing 2.6 or 4.2% crude protein. Supplementing the roughages with wheat was of no value, but supplements of wheat and urea improved the survival rate and increased the roughage intake. Elliott and Topps (1963) fed 16 diets based on low-quality roughages and found that, in general, as the nitrogen content of the diet increased, voluntary intake increased. Clark and Quin (1951) showed that supplementation of low-quality grass hay with 4% urea or sodium nitrate and 12% molasses resulted in increased feed intake and much smaller loss of body weight.

The supplementation of low nitrogen forages with urea or biuret does not always prove beneficial. Minson and Pigden (1961) infused urea into the rumen of sheep consuming either wheat or oat straw (.6 or .5% nitrogen respectively) and found a 12% decrease in consumption of the forage. The digestibility of the diet did not change. Knight and Owens (1973) infused urea for 0, 1, 3 or 12 hrs after feeding a 60% TDN diet (5.8% crude protein) to sheep and found no increase in digestibility of dry or organic matter and very little increase in nitrogen retention when the 12 hour infusion was compared to the basal diet without supplemental N. The amount of urea infused was that amount required to raise the crude protein level of the diet to approximately 12% and it was suggested that this did not raise rumen ammonia levels sufficiently at a time when energy was available for its utilization. Some beneficial effects were observed, however, when the same amount of urea was infused over a 1 or 3 hr period.

In contrast, Campling et al. (1962) continuously infused urea at levels of 25 or 150 g per cow per day and reported an increase of 9% and 11% in the digestibility of crude fiber and nitrogen free extract (NFE) of straw containing 3% crude protein. Consumption of the straw was increased 40%. Daily infusion of 25 g of urea resulted in a 26% increase in voluntary intake. These workers also observed a six to seven-fold increase in the rate of cotton fiber disappearance as well as a decrease in mean retention time (from 104 to 83 hr) when urea was infused.

Coombe and Tribe (1963) conducted two experiments with oat straw containing 3% crude protein. In the first experiment, voluntary intake of the straw was increased by supplementation of urea and molasses. The dietary urea also increased the rate of cellulose digestion as measured by the rate of disappearance of cotton thread. The author cautioned that cotton thread differs from roughage cellulose, particularly with regard to lignification. However, an increased rate of cotton thread disappearance does indicate a stimulation of the cellulose-digesting microbial population in the rumen and this was supported by an increased concentration of volatile fatty acids in the rumen contents. Therefore, there is good evidence that an increase in the cellulolytic activity of rumen contents results when urea is added to such diets. In the second experiment 0, 8, 16, 24 or 32 g of urea per day were fed to sheep consuming oat straw. Mean daily dry matter intake was increased significantly, except at the highest level of supplementation (32 g). Highest levels of response occurred with the smaller amounts of urea (8-16 g), and the rate of passage was depressed particularly by increasing the level of urea above these amounts. The depression in the rate of passage was not accompanied by significant changes in the rate of cellulose digestion, which led the authors to conclude that some other factors were involved in the decreased rate of passage. They have suggested two possible causes. First, the decreased rate of passage may have been caused by the reduced intake, which occurred with the high dietary level of urea. The second explanation for the depression of rate of passage at higher levels of urea feeding suggested that rumen motility, or at least rumination, was inhibited. No evidence to support either hypotheses was obtained.

Oh et al. (1969) studied the ruminal microbial activity in sheep consuming a low-quality (4.34% crude protein) range grass. The range grass was supplemented with urea (10 g per kg of feed), urea plus volatile fatty acids (VFA), urea plus caproic acid or casein. All supplements increased voluntary intake, dry matter digestibility, rumen concentrations of VFA's and microbial protein. These workers suggested that the reduced consumption by the sheep receiving the unsupplemented grass was due to a nitrogen deficient ration.

Beames (1959) fed 3.5% crude protein hay alone, with .68 kg of molasses per day or with .68 kg of molasses and 100 g of urea per head daily to heifers. Animals receiving hay or hay plus molasses lost .54 and .39 kg per head daily, respectively, while heifers receiving hay with molasses and urea lost only .04 kg per head daily. Urea and molasses supplementation also increased intake of the hay by approximately 38%.

Verde (1971) fed a low-quality Pangola digitgrass hay (2.7 or 4.63% crude protein) to sheep in a set of three experiments and observed the effect of supplementation with cottonseed meal (CSM), CSM and urea, urea, biuret or soybean meal (SBM). In the first experiment, CSM and the supplement containing about 50% of its nitrogen as CSM and 50% as NPN (urea and diammonium phosphate) resulted in a 28 and 22% increase in the intake of the 2.57% crude protein hay and an over-all increase in the organic matter digestibility of the complete diet with either supplement. Nitrogen balance was positive when animals received either of the two supplements and negative when animals received the hay alone. In a second study, nitrogen was supplemented as either CSM or biuret. Both sources of nitrogen increased consumption of 4.63% crude protein hay (17 and 11% respectively) and over-all digestibility of organic matter again was improved. In experiment three, supplemental nitrogen was supplied from SBM, urea or biuret. The SBM, urea and biuret rations increased voluntary intake of the hay by 27, 27 and 19% respectively. Organic matter digestibility of the complete diet was greater for supplemented animals. Supplemental nitrogen as urea, biuret, CSM or SBM in the latter two studies resulted in positive nitrogen balance but a negative balance resulted when hay was fed alone. Supplemental nitrogen from all sources studied by this worker resulted in improved intake and nitrogen balance and none of the nitrogen sources were significantly different. There was not an over-all significant increase in cellulose digestion but in each trial supplemented animals tended to digest cellulose to a greater extent. The author suggested that cellulose digestibility was not sufficiently increased to account for the increased intake and indicated that a general improvement in physiological status of the animal should account for a portion of the increase in intake.

Fick (1971) fed steers a 4.6% crude protein hay along with: (1) a mineral mix, (2) mineral mix plus 454 g of corn meal, (3) as in (2) plus 25 g biuret and (4) as in (2) plus 50 g biuret. Over a 63 day period steers gained an average of -1.5, 1.1, 10.2 and 14.5 kg respectively while consuming an average of 4.25, 4.1, 4.8 and 4.8 kg of hay per head daily, respectively. In a second study, Fick (1971) supplemented a 4.58% crude protein hay with 0 or 10 g of nitrogen as biuret along with 0, 50, 100 or 200 g of an energy supplement consisting primarily of corn. Nitrogen supplementation resulted in a 23% increase in voluntary intake of the hay as well as an increased nitrogen balance and cellulose digestibility. Increasing energy levels with biuret supplementation tended to decrease the digestibility of cellulose while energy supplied at 0, 50, 100 and 200 g without biuret resulted in 44, 53, 54 and 35% cellulose digestion respectively.

Chicco et al. (1971) fed steers green-chopped elephant grass (6.7% crude protein) (<u>Pennisetum purpureum</u>) supplemented with 1 kg per head/day of a basal concentrate containing 10% crude protein, 1 kg of basal plus 120 g biuret or 1 kg of basal plus 100 g urea for 120 days, and measured voluntary intake, gain, nitrogen retention and digestibility of cellulose and organic matter. Average daily gains were not significantly altered by the treatments but the animals fed biuret gained 335 g per head daily compared with 284 g and 244 g for those fed urea and no supplemental NPN respectively. Voluntary intake was not affected by addition of biuret or urea to the diet. In addition, no effect was observed on digestibility of cellulose or organic matter. However, significantly more nitrogen was retained by steers receiving biuret than urea. Both groups receiving supplemental nitrogen retained more nitrogen than control steers.

In an additional study, (Chicco <u>et al</u>., 1972) steers were fed diets of varying levels of mature, chopped guinea grass (<u>Panicum maximum</u>) (5.9% crude protein) with and without 3 kg of sugarcane molasses and 150 g of urea per head daily for 112 days. Average daily gain, nitrogen balance and digestibility of cellulose and organic matter were measured. Animals which received the molasses-urea supplement gained more weight, retained more nitrogen and, when intake was 50 and 75% of <u>ad libitum</u>, had higher cellulose and organic matter digestibilities.

The performance of animals receiving supplemental nitrogen from various NPN sources under practical range conditions has been observed and reported by several investigators. Ewes on mature range were supplemented (Slen and Whiting, 1954) with natural protein supplements or with a supplement containing urea and consumption of the urea supplement was less than that of natural proteins. Ewes consuming urea also produced less wool and lambs which were lighter at both birth and 8 weeks than did animals consuming natural proteins. When range cows were supplemented with CSM pellets or pellets containing 50% of the nitrogen from urea, very little utilization of urea nitrogen was observed (Nelson et al., 1957). Utilization was improved to some extent by including a mineral mix with the urea containing pellet. In a later report (Nelson and Waller, 1962) 16 experiments were summarized and involved 879 cows under winter range conditions in Oklahoma. The authors found that urea-containing supplements, where urea supplied 1/3 to 1/2 the dietary nitrogen, were always inferior to iso-nitrogenous ones containing CSM as the supplemental nitrogen source.

Under Oklahoma range conditions, 190 brood cows were wintered and supplemented with a 21% crude protein range cube containing either CSM or urea (Williams et al., 1969). Cows receiving CSM cubes lost less weight during the winter feeding period (-36.5 vs. -56.5 kg respectively) and were in better condition at the end of the period than were cows receiving cubes with urea. This excessive loss of weight and condition was still apparent the following September. Neither birth weight nor weaning weights of the calves were affected by winter feed and the wintering treatment did not affect the subsequent calving dates or weights of the cows at parturition.

Thomas (1972) reported the results of wintering studies over a 4-year period with cows receiving .91 kg of a 20% crude protein supplement per head daily. The supplement contained 0, 12.5, 25, 37.5 or 50% crude protein equivalent from either biuret or urea with soybean meal providing the remainder of the nitrogen. No difference was observed in birth weights, weaning weights, or adjusted weaning weights, for calves of the treated cows over the 4-year period. Cow weight changes over the winter period were not reported. Three trials were conducted (Utley and McCormick, 1973) with cows or heifers which were provided supplemental nitrogen as CSM or a mixture of biuret and corn. No differences were reported in the performance of these animals. DeRouen et al. (1974) have reported similar results.

It should be emphasized that the studies reported above with wintering cows have compared NPN supplements with natural protein supplements. None have included comparisons of animals which received no supplemental nitrogen; thus, interpretation of the results is difficult. For example, in studies where animals receiving NPN performed as well as animals receiving natural proteins the possibility exists that a negative control group also would have performed equally well and would indicate that there was little need for supplemental nitrogen. In studies where inferior performance was reported with NPN, a negative control group might have shown an even greater reduction in performance and in turn would have indicated a relative benefit for NPN supplementation.

Wintering steers, grazing dormant grass pasture and fed weathered bermuda grass hay, were supplemented (Tollett et al., 1969) with the following treatments: (1) CSM; (2) CSM and biuret (1/2 of crude protein equivalent); (3) CSM and biuret (3/4 crude protein equivalent); (4) CSM and urea (1/2 crude protein equivalent); (5) CSM and urea (3/4 crude protein equivalent); and (6) negative control. Average daily gains (kg) were: (1) .33; (2) .26; (3) .20; (4) .15; (5) .09; and (6) .08 respectively. Wintering trials were conducted for 112 days (Oltjen et al., 1974) with 160 growing steers and supplemental CSM, urea and biuret were compared when low quality chopped grass hay was fed ad libitum. A negative control group was used in each trial. Feeding biuret in a mineral mixture improved animal performance with hay containing 4.5% crude protein but not with a 6% crude protein hay. Feeding iso-nitrogenous and iso-caloric quantities of biuret-corn meal or urea-corn meal supplement improved animal performance over the negative control, but neither supplement was as good as CSM. Feeding either the biuret-corn meal or the urea-corn meal supplement on a daily basis was superior to feeding an equivalent amount three times per week.

In summary the consensus of reported work would at present indicate that, under most conditions, supplementation of forage diets containing less than 7% crude protein with nitrogen from NPN sources would benefit the animal by maintaining an active rumen population which would result in better utilization of the diet and in turn improve the over-all nutritional status of the animal. One would expect, however, to observe less benefit with NPN supplementation than with supplementation of natural proteins. There also appears to be a great deal of variation in the degree of NPN utilization which could be expected. There is a need for additional work on the degree of utilization of NPN as well as other dietary factors which may improve its utilization.

Effects of Energy Supplementation on the Utilization of NPN with Roughages

It has been established that NPN is utilized more efficiently with high energy diets containing adequate sources of readily fermentable carbohydrates than with diets consisting of roughages with considerably less readily available energy. When the object of supplementation with NPN is to aid in maintenance of the animal, to prevent excessive weight loss and to provide for maximum utilization of roughages by the ruminant, the cost of providing large amounts of supplemental energy may be both too high and unnecessary. However, provision of some supplemental energy may be necessary to act as a carrier for the NPN, and to assure proper consumption and thus promote the general performance of the animal.

The influence of carbohydrates on the conversion of urea nitrogen to protein was studied (Mills et al., 1942) by measuring the amounts of protein and ammonia nitrogen at different times in the rumen after feeding various rations. When timothy hay was fed with urea, the inclusion of starch reduced the rumen ammonia level and increased the amount of protein in the rumen ingesta. Williams et al. (1969) studied the effect of varying the NFE to nitrogen ratio (NFE:N) of a supplement provided to steers receiving cotton seed hulls as a basal ration. Nitrogen retention increased as the NFE:N progressed from 11:1 to 28:1 with no further benefit with ratios up to 55:1. Digestibility of dry matter and organic matter did not differ as the NFE:N ratio increased. Further in vitro studies on nitrogen to carbohydrate ratios (Bloomfield et al., 1964) demonstrated that fixation of urea nitrogen by rumen microorganisms was a quantitative function of energy level and that bacterial assimilation required 55 g of carbohydrates for each gram of nitrogen fixed. Briggs (1967) reported that when glucose or starch was added, the uptake of ammonia by bacteria is much more rapid than when roughages alone are present.

In long term studies with Merino sheep (Pierce, 1951) a roughage diet was supplemented with urea and varying levels of starch from potatoes. The amount of starch in 50 g of dried potatoes was too small to give a wool-growth response with urea. With greater quantities of starch, the response to urea was proportional to the amount fed, up to 300 g per day. The response to starch was not due to energy alone. Replacing the starch with appropriate amounts of corn oil did not influence the wool-growth response.

A low-quality Pangola digitgrass hay (4.58% crude protein) was supplemented with 0 or 10 g of biuret nitrogen along with 0, 50, 100 and 200 g of an energy supplement containing 50% cornmeal, 25% corn starch and 25% sucrose per day (Fick, 1971). Hay intake was greatest when 50 g of energy supplement was provided without biuret N, but 100 g of energy supplement stimulated the greatest hay intake when supplemental N was provided. The lowest intake of hay was observed when 200 g of energy supplements were provided with or without supplemental N. The highest level of energy supplementation also depressed cellulose digestion. The depression was greater without N supplementation. However, the higher levels of energy did support the most positive nitrogen balance. It was suggested that the depression in hay intake at higher energy levels was a substitution effect (energy supplement for hay). The substitution of concentrate for hay intake by sheep has been previously shown (Blaxter et al., 1961 and Houser, 1970). Voluntary hay intake also was depressed by providing 116 g of an energy supplement consisting mainly of citrus pulp (Verde, 1971). Hay consumed was 587 g/head daily with the energy supplement and 653 g/head daily without energy supplement. Energy supplementation did, however, increase total dry matter intake.

A decrease in the digestion of cellulose was reported (el-Shazly et al., 1961) as starch was added to an in vitro system containing a known amount of cellulose. The inhibition of cellulose digestion was suggested to be due to competition for nutrients between cellulose digesting and starch digesting bacteria. Further, it was concluded that nitrogen was the limiting factor under the conditions of the study and cellulose digestion was improved with the addition of nitrogen to the system.

The inclusion of sucrose in a urea-straw diet for sheep (Faichney, 1965) had no significant effect on dry matter intake or digestibility, crude fiber digestibility, rate of passage of ingesta, rate of cellulose digestion in the rumen, nitrogen balance, body weight, rumen pH or the concentration of volatile fatty acids or ammonia in the rumen. The lack of readily available energy was not a limiting factor to the utilization of the diets used in this study.

It is frequently stated that starch is more effective in promoting ammonia utilization than are soluble sugars. The levels of true protein in the rumen contents were measured at a uniform time after feeding rations equal in nitrogen and containing different sources of carbohydrates (Mills et al., 1944). The rumen dry matter ingesta contained 6.5 to 7.7% true protein in a ration of grass hay and molasses, 9.3% when urea was added and 11.0% when starch and urea were included. Growth responses of dairy heifers fed these rations were measured. The ration of grass hay, molasses and urea, having 11.3% crude protein, resulted in an average daily weight gain of 0.33 kg compared with 0.64 kg when 0.14 kg of starch replaced the molasses in the supplement. Similar results were reported by Lewis and McDonald (1958).

In nitrogen balance experiments with steers (Bell et al., 1951 and Gallup et al., 1952), greater nitrogen retention occurred on a ration (11% crude protein) consisting of grass hay supplemented with urea and either ground maize, dehydrated sweet potatoes, milo or barley than with cane molasses.

The superiority of starch is attributed to the fact that it is fermented less rapidly than sugars, which disappear from the rumen so quickly that they are unavailable to the bacteria except for a very short time. The results of McNaught (1951) show that more protein was synthesized from starch than from maltose, arabinose or xylose, even though excesses of these sugars were present throughout the incubation period.

Schwartz et al. (1955) also showed that the addition in vitro of different amounts of glucose, in a range of 0.5-3.0 g per 100 ml of ingesta, had no effect on the amount of ammonia taken up by the bacteria. This occurred in spite of the fact that with the smallest addition of glucose there was no sugar in the ruminal fluid after 1 1/2 hr, whereas, with the highest addition, abundant glucose was found throughout the 6 hr duration of the experiment. Further work showed that the bacteria stored carbohydrates in the form of a glycogen-like material which stained blue with iodine. This material persisted long after the free glucose had disappeared. Thus, although no free glucose could be detected after 90 min when 0.5 g glucose was added per 100 ml of ingesta, the bacteria still contained reserve carbohydrates up to 4 hr. With 1 g glucose, reserve carbohydrate was present through the experiment. The author concluded that disappearance of free sugars from the rumen does not necessarily indicate that the bacteria lack carbohydrate for protein synthesis from ammonia.

The use of molasses as an energy source and carrier for NPN compounds for supplementation of range animals is presently gaining wide acceptance even though evidence of the inferiority of molasses as an energy source with NPN has been shown. The increase in popularity of this type of liquid supplementation has been stimulated by the acceptance of urea for use in ruminant rations (Klett, 1971) and by the development of liquid tanks which control intake of supplement and reduce the labor required to feed the supplement. These liquid supplements contain not only energy from the molasses and nitrogen from some NPN source but may also provide phosphorus, vitamins, various macro and micro elements as well as antibiotics. The primary requirement for the use of these materials in liquid supplements is solubility. However, recent innovations have been developed where insoluble materials are added to form suspension mixes (Klett, 1971).

Few controlled studies are available comparing liquid and dry protein supplements for range cattle. In a review (Klett, 1971), comparative studies conducted in Texas, Georgia, Mississippi and Arkansas were summarized and in four of the five studies, cows receiving the liquid supplement lost more or gained less weight than did animals receiving a dry protein meal supplement. It was pointed out, however, that in each case, the performance of cattle which received liquid supplement was acceptable. Additional work is needed to compare the liquid and dry methods of supplementation of both energy and nitrogen for range animals.

In view of the reported studies (Williams et al., 1969; Fick, 1971; and Beames, 1960), the latter reporting that the ratio of molasses to urea could be decreased to as low as 2.1 and still obtain an effective response, there is a need for additional research into the optimum ratios of energy to nitrogen, especially for molasses.

Nitrogen Status, Protected Protein and Utilization of Low-Quality Roughage Diets

It has been suggested (Egan, 1965a, b, c and Egan and Moir, 1965) that the protein status of the animal is a component of a chemoregulatory mechanism which may control intake. A further suggestion is that beneficial responses in the intake and digestion of low-quality forages associated with the supplementation of nitrogen with these forages may not be due entirely to an increase in rumen microbial activity (Verde, 1971). Beneficial responses may have resulted in part from a general improvement in animal nitrogen status and in turn the over-all, nutritional status of the animal.

Others (Moir and Harris, 1962; Egan, 1965a, b, c; and Egan and Moir, 1965) also suggested that the positive response in the rate of intake was not entirely due to an increase in activity of ruminal microorganisms. Egan (1965a) provided 10 g of nitrogen as either urea or casein by duodenal infusion along with a forage containing 3.5% crude protein. Both treatments elevated blood urea and ruminal ammonia concentrations. The blood urea rise was greater and more rapid with the urea treatment but persisted longer with casein treatment. Rate of cellulose digestion increased with both treatments. In a second experiment (Egan and Moir, 1965) the casein infusion produced an increase in the intake of hay of low nitrogen content on the same day of treatment and it was concluded that casein must act directly within the duodenum. In one experiment, a positive response in intake was observed with urea infusion while in another no such response occurred, despite an increase in cellulose digestion. The effect of casein upon cellulose digestion was less marked but the increased intake did occur. Egan (1965a) suggested that casein appeared to act independently of the digestion rate in the rumen, and that the delay in intake response after urea infusion could be due to the time needed for the recycled nitrogen to influence microbial activity.

Egan (1965b) and Egan and Moir (1965) suggested that an improvement in the nitrogen status of the animal resulted in an immediate positive response in intake. Casein infused into the duodenum increased the intake of low quality hay without altering the retention time of the food-particles, but depressed the digestibility of the dry matter and the rate of cotton thread digestion (Egan, 1965b, c). Urea increased intake with a shorter mean retention time of residue in the alimentary tract and with a greater rate of cellulose digestion. The author concluded that, irrespective of physical or microbiological factors, when nitrogen balance was improved the intake of dry matter increased and suggested that nitrogen status of the animal was a component of a chemoregulatory mechanism.

In similar work (Weston, 1967) urea and casein were infused into the abomasum of sheep consuming a 4.4% crude protein hay. The casein infusion resulted in an increase in intake of the hay from 8 to 13% and when urea was infused intake of the hay was improved in one of two trials. Oral supplementation of the hay, which raised the crude protein content of the diet to 7 or 15%, also improved intake.

Treatment of natural protein with formaldehyde has been shown to reduce the ruminal solubility of that protein (Martinez, 1975 and Schmidt et al., 1974). Other workers (Faichney and Weston, 1971; Faichney, 1972; and MacRae et al., 1972) have established that formaldehyde treatment of dietary protein reduces its degradation in the rumen and leads to an increase in the amount of crude protein reaching the intestine in a manner similar to infusion of the protein into the abomasum. Improvements have been reported in the rate of wool growth (Ferguson et al., 1967; Reis and Tunks, 1969; Barry, 1970; and Hemsley et al., 1973) and in the rate of live weight gain of lambs (Faichney, 1971; Peter et al., 1971; and Wright, 1971) and calves (Faichney and Davies, 1973) when dietary protein has been treated for protection against ruminal degradation. Such improvements in performance in response to increased digestion of crude protein in the intestine probably represent responses to an increase in the supply of amino acids to the tissue (Faichney, 1974).

Ewes receiving a low-quality forage were supplemented with formalin treated casein and an increase in birth weight and growth rate of lambs produced by these ewes was reported (Barry, 1969). There was also a small increase in wool produced by these ewes. At a higher level of nutrition there was no effect on lamb production with treated protein. However, increased wool production was observed. Langlands (1971) supplemented grazing sheep with treated or untreated (formaldehyde) casein and reported depression of herbage intake and this depression was proportional to the level of supplementation. Both types of supplementation did improve wool production and the treated casein was more effective.

Other studies with protected proteins generally have been with animals receiving relatively high-quality diets. In one such study, 120 lambs were fed finishing type diets containing soybean meal treated with varying levels of formaldehyde (Martinez, 1974). An increased rate of gain was observed when treated animals were compared to controls and soybean meal treated at a level of .6% formaldehyde supported maximum performance.

In view of all of the aforementioned studies it is reasonable to speculate that a more direct supplementation of amino acids to the animal via protected proteins may be beneficial in improving the general nutritional status of the animal and in turn enhance the utilization of low-quality diets. Additional work in this area would seem justified.

Summary

Literature has been reviewed which related to the physical properties of biuret and urea, as well as the effect of supplementation with NPN, energy and protected proteins on nitrogen utilization and the performance of ruminant animals fed low quality roughages. The low solubility, greater palatibility and lack of toxicity of biuret are factors which enhance its potential as a source of NPN for supplementation of animals which must be maintained on low-quality roughages. However, there are feeding conditions for which the high solubility of urea may prove beneficial. Necessity for adaptation to biuret and the extremely low rate of hydrolysis may also be factors which could limit its use.

In general, supplementation of low-quality forages (7% crude protein or less) with NPN has been shown to improve forage utilization. However, the use of urea and biuret with different dietary forms of energy has provided widely varying results which suggest a need for further work to provide additional information on the utilization of NPN from urea and biuret with different forms and sources of energy. Supplementation with urea and biuret for animals under practical range conditions have yielded such inconclusive estimates of nitrogen utilization, ranging from 0 to 100% when compared to natural proteins, that it is felt that the future need for use of NPN under these conditions most surely dictates the need for additional work.

The use of protected proteins for more direct supplementation of animals consuming low-quality forages would appear to have beneficial effects but work directed toward this point appears limited. The use of protected proteins with NPN, as an attempt to improve the performance of animals by supplementing the rumen microbes with the NPN and the animal directly with the protected protein, would appear to offer reasonable possibilities and justify additional research.

CHAPTER III

NON-PROTEIN NITROGEN FOR WINTERING BEEF COWS

As the continuing expansion of the world's population places an increasing demand on the world's agricultural industry to produce additional food, the importance of the ability of the ruminant animal to utilize feedstuffs other than those required by non-ruminants will become increasingly important. It is presently an accepted practice to supplement brood cows which are being wintered under range conditions with natural proteins in order that sufficient nitrogen is available to meet the minimum needs of the animals. However, the projected future demands for natural proteins for use other than in the cattle industry may make this practice economically unsound. Consequently, the use of non-protein nitrogen (NPN) should increase.

Urea is presently the most available and widely used source of NPN and many workers (Coombe, 1959; Beames, 1959, 1960; and Briggs et al., 1960) have reported favorable results when the compound was used as a source of supplemental nitrogen for animals receiving low-quality roughages. However, other workers (Nelson et al., 1957; Nelson and Waller, 1962) have reported little benefit with supplementation of range cattle with urea.

Much interest of late has been expressed in biuret as a source of supplemental nitrogen for wintering brood cows. This interest has been stimulated by the physical properties of biuret which render it non-toxic and more highly palatable than urea. Tomlin et al. (1967), Tollett et al. (1969) and DeRouen et al. (1974) have indicated that biuret is an effective source of supplemental nitrogen for promoting the performance of ruminants consuming low-quality forages. However, other researchers (Oltjen et al. 1969; Oltjen et al., 1974; Rush and Totusek, 1973) have reported variable results when using biuret with or in place of natural proteins.

The following three studies were conducted during a 6 year period from August, 1967 to August, 1973, at the Beef Cattle Research Station, A.R.S., U.S.D.A., Brooksville, Florida, to evaluate urea or biuret as sources of supplemental nitrogen for cows and heifers being wintered under pasture conditions.

Experiment 1

Urea as a Source of Supplemental Nitrogen for Wintering Beef Cows

Procedure

A herd of 66 mature Angus cows (4 years old and older) were utilized in a 2x2 factorial design from August, 1967 until August, 1968. The factors studied were source of supplemental winter nitrogen and source of supplemental forage. Supplemental nitrogen was supplied as either a cottonseed meal (CSM) supplement or as a CSM, citrus pulp and urea supplement (urea) in which 50% of the nitrogen was provided by either urea or diammonium phosphate. The supplements were processed into range cubes 1.9 cm in diameter and approximately 3.8 cm long.

The composition of these cubes is shown in table 2. The supplemental forage was either Pangola digitgrass (Digitaria decumbens) hay or sorghum (Sorghum vulgare) silage (table 3).

Pregnant cows were assigned at random to one of the four treatments in August. Body weights were recorded in August, November, March and June. Calves were weaned and cows were palpated for pregnancy at the August weighing. Cows were exposed to bulls from March through June of each year.

Supplemental range cubes and forages were fed on the ground from the first of December until approximately April 15th. Cubes and forages were fed on Mondays, Wednesdays and Fridays of each week. CSM and urea cubes were provided in amounts of .91 and .94 kg per head daily, respectively. The feeding levels were chosen to provide equal intakes of nitrogen and energy from supplemental cubes. Forages were provided basically at an ad libitum level. The amounts offered reflected the availability of pasture and in turn the animals' appetite for additional forage. All animals were maintained on similarly managed Argentine bahiagrass pastures (Paspalum notatum) throughout the year and a complete mineral mixture[a] was provided at all time.

Samples of supplements, supplemental forages and available winter pasture were taken at monthly intervals for analysis. Crude protein of these samples was determined by the Kjeldahl method and dry matter by standard procedure (A.O.A.C., 1970). Calcium and

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[a]Lakeland Cash Feed Company, Lakeland, Fl 33802, listed minimum analysis in percent: NaCl, 20.0; Ca, 15.0; P, 6.0; Fe, .60; Mg, .50; Mn, .27; F, .14; Cu, .06; I, .02; Zn, .02.

TABLE 2: COMPOSITION OF SUPPLEMENTAL RANGE CUBES (%), EXPERIMENT 1.

Ingredient	International Reference No.	Cottonseed Meal Cubes	Urea Cubes
Cottonseed w/o hulls, mech extd grnd, mn 43% protein mx 13% fiber mn 2% fat, (5)	5-01-627	100.00	43.00
Citrus pulp w/o fines shredded dehy (4)	4-01-237	------	41.30
Sugarcane, molasses, mn 48% invert sugar mn 79.5 degree brix (4)	4-04-696	------	7.00
Urea 281	--------	------	6.50
Ammonium phosphate dibasic $(NH_4)_2HPO_4$, cp (6)	6-00-371	------	2.00
Sulfur, commercial (6)	--------	------	.20
TOTAL		100.00	100.00

magnesium were determined by atomic absorption spectrophotometry (Anonymous, 1973) and phosphorous by the method of Fiske and Subbarow (1925). The analyses of available winter forage as well as the analyses of supplemental forages and cubes are shown in table 3.

TABLE 3

CHEMICAL COMPOSITION OF SUPPLEMENTS, SUPPLEMENTAL FORAGES AND AVAILABLE WINTER PASTURE, EXPERIMENT 1

	% of Dry Matter			
Feed	Protein	P	Ca	Mg
Cottonseed meal cubes	44.87	1.38	.13	.75
Urea cubes	42.96	1.24	1.16	.37
Sorghum, arial part, ensiled (3) Int. Ref. 3-04-323	10.22	.28	.45	.43
Pangola digitgrass hay	7.29	.25	.30	.22
Winter pasture[a]	3.30	.10	.36	.18

[a]Argentine bahiagrass

Response criteria for cow performance included cow weight change from November to March, August weight, calf survival, 205-day adjusted weaning weight and conception rate. Calf survival was expressed as the percentage of pregnant cows assigned to each treatment which weaned a calf. Conception rate was expressed as the percentage of those cows which had been exposed to bulls and were determined by palpation to be pregnant in August at the end of the production year.

Cow weight data were analyzed statistically by analysis of covariance (Steele and Torrie, 1960) with initial weight serving as the covariate. Winter weight change (November to March) was determined by subtraction on the basis of adjusted body weights. Winter weight changes as well as 205-day adjusted weaning weights were analyzed by analysis of variance and conception rates and calf survival data were analyzed by the Chi Square procedure (Steele and Torrie, 1960).

Results

Cow winter weight change, final cow weight, conception rate, calf survival and 205-day adjusted weaning weight, as affected by individual wintering treatment and main effects, are shown in table 4. There were no differences (P>.10) in response for any of the observed criteria associated with supplemental winter nitrogen source, supplemental winter forage or any combinations of these two factors. Average initial cow weight was 389 kg while average final cow weight was 425 kg representing a 36 kg or approximately 10% increase in average cow weight over the period of the experiment.

Experiment 2

Biuret as a Source of Supplemental Nitrogen for Wintering Beef Cows, I

Procedure

A herd consisting of 78 or 106 mature Angus cows was utilized during a 2 year period (August, 1968 to August, 1970) to compare CSM cubes to citrus pulp cubes containing biuret (CPB) as sources of

TABLE 4: EFFECT OF WINTERING TREATMENTS ON COW WINTER WEIGHT CHANGE, FINAL COW WEIGHTS, CONCEPTION RATE, CALF SURVIVAL, AND 205-DAY ADJUSTED WEANING WEIGHT, EXPERIMENT 1.

Treatment[a]	Cow winter wt. Change kg[b]	Final Cow wt. kg[b,c]	Conception rate %	Calf Survival %	205-Day Adjt. Weaning Wt. kg
Silage + CSM	-34	424	81	100	193
Silage + Urea	-26	432	84	92	188
Hay + CSM	-31	425	100	89	190
Hay + Urea	-44	420	88	89	192
Average:	-34	425	88	92	191
Main Effects					
CSM	-32	425	91	94	192
Urea	-35	426	87	90	190
Silage	-30	428	83	97	191
Hay	-37	423	94	89	191

[a] Cows per treatment, silage + CSM, 16; silage + urea, 13; hay + CSM, 17; hay + urea, 17.

[b] Only those cows weaning calves considered.

[c] Adjusted for initial weight

supplemental nitrogen and to compare sorghum silage to Pangola digitgrass hay as supplemental sources of forages for wintering beef cows. The experimental design was 2x2 factorial. Pregnant cows were assigned at random to one of the four individual treatments (table 5) in August of the first year of the study and randomly reassigned without regard to previous treatment in August of the second year. Cows which were determined to be open by palpation in August were removed from the study. The numbers of cows per individual treatments, main effects and years are shown in table 5.

The CSM and CPB cubes were fed as described in experiment 1 in amounts of .9 and 1.0 kg per head daily, respectively. Composition of the cubes is shown in table 6. The feeding of supplemental forages and other details of this study were as described in experiment 1. Analysis of supplemental range cubes, supplemental winter forages and available winter pasture are shown in table 7.

Results

Table 8 shows cow weight changes as affected by individual wintering treatments and main effects. A great deal of variation was observed in weight losses for individual treatments. During the first year, cows receiving CSM and silage lost the least amount of weight, -43 kg, ($P<.01$) while animals receiving hay and CPB cubes lost the greatest amount of weight (-85 kg). This group of animals (hay and CPB) also had the greatest weight loss during the second year (-91 kg) ($P<.01$) while cows receiving hay and CSM had the smallest weight loss (-68 kg). Cows which received citrus pulp cubes with

TABLE 5

EXPERIMENTAL DESIGN AND ANIMALS PER OBSERVATION, EXPERIMENT 2

	Cows Per Treatment		
Treatment	Year 1	Year 2	Total
Silage CSM[a]	20	26	46
Silage CPB[b]	18	27	45
Hay CSM	20	27	47
Hay CPB	20	26	46
Total	78	106	184
Main Effects			
Nitrogen			
CSM	40	53	93
CPB	38	53	91
Forage			
Silage	38	53	91
Hay	40	53	93

[a]Cottonseed meal cubes.

[b]Citrus pulp cubes w/ biuret.

TABLE 6: COMPOSITION OF SUPPLEMENTAL RANGE CUBES (kg), EXPERIMENT 2.

Ingredient	International Reference No.	Cottonseed Meal Cubes	Cubes w Biuret
Cottonseed w/o hulls, mech extd grnd, mn 43% protein mx 13% fiber mn 2% fat, (5)	5-01-627	100.00	-----
Citrus, pulp w/o fines shredded dehy (4)	4-01-237	------	75.60
Sugarcane, molasses, mn 48% invert sugar mn 79.5 degree brix (4)	4-04-696	------	7.00
Biuret[a]	--------	------	13.75
Sodium phosphate, monobasic (6)	6-04-287	------	3.30
Sulfur, commercial (6)	--------	------	.35
TOTAL		100.00	100.00

[a] Kedlor 230, Feedgrade.

TABLE 7: AVERAGE CHEMICAL COMPOSITION OF SUPPLEMENTS, SUPPLEMENTAL FORAGES AND AVAILABLE WINTER PASTURE, EXPERIMENT 2.

Feed	% of Dry Matter			
	Protein	P	Ca	Mg
Cottonseed meal cubes	44.8	1.10	.20	.74
Cubes w/ biuret	41.3	1.40	2.56	.18
Pangola digitgrass hay	3.2	.17	.26	.12
Sorghum silage	8.0	.24	.60	.23
Winter Pasture[a]	3.0	.05	.62	.15

[a] Argentine bahiagrass.

TABLE 8

EFFECT OF WINTERING TREATMENT AND YEAR ON AVERAGE WEIGHT CHANGE OF COW FROM NOVEMBER TO MARCH (kg), EXPERIMENT 2

Treatment	Year 1	Year 2	Average
Silage CSM	-43^{a}	-74^{ab}	-58.5^{a}
Silage CPB	-76^{bc}	-83^{bc}	-79.5^{b}
Hay CSM	-61^{ab}	-68^{a}	-64.5^{a}
Hay CPB	-85^{c}	-91^{c}	-88.0^{b}
Average:	-66^{x}	-79^{y}	-72.5
Main Effects			
Nitrogen			
CSM	-52^{d}	-71^{d}	-61.5^{d}
CPB	-81^{e}	-87^{e}	-84.0^{e}
Forage			
Silage	-59^{f}	-78^{f}	-68.5^{f}
Hay	-74^{g}	-79^{f}	-76.5^{f}

a,b,c Values in same column with different superscripts differ. ($P<.01$)

d,e Values in same column with different superscripts differ. ($P<.01$)

f,g Values in same column with different superscripts differ. ($P<.05$)

x,y Values in same row with different superscripts differ. ($P<.05$)

biuret as supplemental nitrogen had a greater winter weight loss ($P<.01$) during both years than did animals receiving CSM cubes. This effect was shown by the averages for main effects and for individual treatments. During the first year, cows receiving hay had a greater winter weight loss ($P<.05$) than did animals receiving silage. Average weight loss across all treatments was greater ($P<.05$) during the second year of the study.

Final cow weights (August) as influenced by individual wintering treatment and main effects are shown in table 9. Individual wintering treatments resulted in no difference ($P>.10$) in final cow weight during either year. However, cows receiving supplemental nitrogen as biuret were 15 kg lighter ($P<.05$) at the termination of the first year of the study than were those cows receiving CSM cubes. Cows were lighter ($P<.01$) at the end of the second year than at the end of the first. Conception rate is shown in table 10 and there were no differences due to individual treatment. Cows receiving the CSM supplement did have a numerically higher conception rate during both years of the study (6 and 9% respectively) and this difference was significant ($P<.07$) when these values were combined for the two years. Calf survival was 93% for the study and there were no differences according to individual wintering treatment, source of supplemental forage or source of supplemental nitrogen.

Table 11 shows 205-day adjusted weaning weights as affected by wintering treatment and year. Cows receiving hay and CSM cubes weaned the heaviest calves ($P<.05$) and cows receiving silage and CSM cubes weaned the lightest calves the first year. Cows receiving

TABLE 9

EFFECT OF WINTERING TREATMENT AND YEAR ON FINAL COW WEIGHT[a] (kg), AUGUST, EXPERIMENT 2

Treatment	Year 1	Year 2	Average
Silage CSM	430	405	418
Silage CPB	415	405	410
Hay CSM	426	400	413
Hay CPB	411	398	404
Average:	420[e]	402[f]	411
Main Effects			
Nitrogen			
CSM	428[b]	403	416
CPB	413[c]	402	408
Forage			
Silage	422	405	414
Hay	418	400	409

[a]Adjusted for initial weight.

[b,c]Values in same column with different superscripts differ. $(P<.05)$

[e,f]Values in same row with different superscripts differ. $(P<.01)$

TABLE 10

EFFECTS OF WINTERING TREATMENT AND YEAR
ON CONCEPTION RATE (%), EXPERIMENT 2

Treatment	Year 1	Year 2	Average
Silage CSM	94	96	95
Silage CPB	82	89	86
Hay CSM	89	93	91
Hay CPB	89	81	85
Average:	89	90	89
Main Effects			
Nitrogen			
CSM	92	94	93[a]
CPB	86	85	86[b]
Forage			
Silage	89	92	90
Hay	89	87	88

[a,b]Values in same column with different superscripts differ. (P<.07)

TABLE 11

EFFECT OF WINTERING TREATMENT AND YEAR
ON 205-DAY ADJUSTED WEANING WEIGHT (kg), EXPERIMENT 2

Treatment	205-Day Adjusted Weaning Wt. Year 1	Year 2	Average
Silage CSM	176[a]	172	174
Silage CPB	180[ab]	164	172
Hay CSM	192[b]	167	180
Hay CPB	182[ab]	172	176
Average:	182[x]	169[y]	176
Main Effects			
Nitrogen			
CSM	185	169	177
CPB	182	168	175
Forage			
Silage	178[c]	169	174
Hay	187[d]	168	178

[a,b]Values in same column with different superscripts differ. ($P<.05$)

[c,d]Values in same column with different superscripts differ. ($P<.05$)

[x,y]Values in same row with different superscripts differ. ($P<.05$)

silage weaned lighter calves than did the cows receiving hay during the first year. There were no differences (P>.10) during the second year nor were there differences according to supplemental nitrogen source either year. Calves were heavier (P<.01) the first year than the second year.

Experiment 3

Biuret as a Source of Supplemental Nitrogen for Wintering Beef Cows, II

This study was undertaken to compare the effects of iso-caloric supplements containing no supplemental nitrogen, nitrogen in the form of biuret and nitrogen in the form of cottonseed meal upon cows and heifers wintered under pasture conditions.

Procedure

A herd consisting of 87, 111 or 97 mature Angus cows (table 12) was studied for 3 years, and two successive groups of 47 and 49 yearling Angus heifers (table 12) were studied for one year each. The experiment was initiated in August, 1970, and terminated in August, 1973. The heifers were studied during the first 2 years of this period.

Supplemental cubes for winter feeding were formulated as shown in table 13. Cubes were as described in experiment 2 with the addition of a citrus pulp cube without biuret (CP) which served as a negative control. Feeding levels were again .9 and 1.0 kg per head daily for CSM and CPB cubes and citrus pulp cubes were fed at a level of .86 kg per head daily. These feeding levels were chosen to provide an equal nitrogen intake for animals receiving CSM cubes

TABLE 12

EXPERIMENTAL DESIGN AND
ANIMALS PER TREATMENT, EXPERIMENT 3

	Animals Per Treatment			
Treatment	Year 1	Year 2	Year 3	Total
Cows				
Cottonseed meal	29	35	33	97
Cubes w biuret	31	42	30	103
Cubes w/o biuret	27	34	34	95
Total:	87	111	97	295
Heifers				
Cottonseed meal	16	18	--	34
Cubes w biuret	16	16	--	32
Cubes w/o biuret	15	15	--	30
Total:	47	49	--	96

TABLE 13: COMPOSITION OF SUPPLEMENTAL RANGE CUBES (kg), EXPERIMENT 3.

Ingredient	International Reference No.	Cottonseed Meal Cubes	Cubes w Biuret	Cubes w/o Biuret
Cottonseed w/o huls, mech extd grnd, mn 43% protein mx 13% fiber mn 2% Fat (5)	5-01-627	100.00	------	------
Citrus, pulp w/o fines Shredded dehy (4)	4-01-237	------	75.60	75.60
Sugarcane, molasses, mn 48% invert sugar mn 79.5 degree brix (4)	4-04-696	------	7.00	7.00
Biuret[a]	--------	------	13.75	------
Sodium phosphate, monobasic (6)	6-04-287	------	3.30	------
Sulfur, commercial (6)	--------	------	.35	.35
TOTAL		100.00	100.00	100.00

[a]Kedlor 230, Feedgrade.

and CPB cubes (table 14) and an equal digestible energy intake from supplements for all treatments. Cows and heifers were provided comparable amounts of supplemental forages primarily in the form of low-quality Pangola digitgrass hay (table 14). Sorghum silage and a mixed Pangola digitgrass-alyceclover (Alysicarpus vaginalis) hay were offered on limited occasions when supplies of Pangola hay were depleted.

The analyses of available winter forage from these pastures as well as the analyses of supplemental forages and cubes as determined from samples taken at monthly intervals are shown in table 14.

Cow response criteria was as described in experiment 1 and, in addition, the time required for cows to reconceive was estimated. This estimation was obtained using the calving date for the year after the treatment involved; a gestation period of 282 days was assumed.

Open, 18-month old heifers were randomly assigned to one of the three treatments in August of the first two years of this study. Heifers were wintered in the same manner as were cows and body weights also were taken in August, November, March and June. Heifers were either artificially bred or exposed to bulls from March through June of each year and were palpated for pregnancy at the final August weighing. Heifer performance criteria included weight change from November to March, August weight and conception rate.

Statistical analysis of all weight data as well as time required to reconceive was performed using the method of least squares (Harvey, 1972). Differences among treatment means were tested with a multiple range test (Duncan, 1955). Calf survival and conception rate were analyzed by the Chi Square procedure (Steel and Torrie, 1960).

TABLE 14: AVERAGE CHEMICAL COMPOSITION OF SUPPLEMENTS, SUPPLEMENTAL FORAGES AND AVAILABLE WINTER PASTER, EXPERIMENT 3.

	% of Dry Matter			
Feed	Protein	P	Ca	Mg
Cottonseed meal cubes	45.5	1.28	.22	.66
Cubes w/ biuret	43.6	1.18	1.94	.14
Cubes w/o biuret	7.6	1.16	2.07	.18
Pangola digitgrass hay	3.7	.15	.28	.12
Mixed clover-grass hay	7.1	.22	.60	.37
Sorghum silage	7.6	.17	.45	.29
Winter pasture[a]	3.9	.18	.55	.26

[a]Argentina bahiagrass.

Results

The average November to March weight changes for cows and heifers are shown in table 15. During the first winter (1970-71) cows receiving CSM supplement lost less weight ($P<.01$) than animals receiving either CPB or CP supplements. Weight changes were 36 kg vs 74 kg and 68 kg, respectively. During the second (1971-72) and third (1972-73) winters, cows receiving CSM cubes lost slightly less weight per year (5 kg and 12 kg respectively) than did animals receiving CPB cubes, but differences were non-significant ($P>.05$). However, weight losses for cows receiving CP cubes were greater ($P<.01$) than were weight losses for either of the other two treatments. Over the 3-year period, cows receiving CSM cubes lost the least amount of weight during the winter period; cows receiving CP cubes lost the most weight and cows receiving CPB cubes lost an intermediate amount. The value for each treatment differed ($P<.01$) from those of the other two. Effects for years and year x treatment interaction were significant ($P<.05$).

Heifer weight changes are also shown in table 15. There were no differences ($P>.05$) in winter weight gains due to treatment during the first year. However, heifers receiving CP cubes gained the most weight ($P<.01$) during the second year and this effect was also found when gains were averaged for the 2 year period ($P<.05$). Heifers gained more weight during the first year ($P<.01$) of the study than during the second year.

Cow and heifer weights at the end of each treatment year (August) are shown in table 16. There were no difference in final

TABLE 15

EFFECT OF WINTERING TREATMENT AND YEAR ON AVERAGE WEIGHT CHANGE FOR COWS AND HEIFERS FROM NOVEMBER TO MARCH (kg), EXPERIMENT 3

Treatment	Year 1	Year 2	Year 3	Average
Cows				
Cottonseed meal	-36^{a}	-34^{a}	-54^{a}	-49^{a}
Cubes w biuret	-74^{b}	-39^{a}	-66^{a}	-60^{b}
Cubes w/o biuret	-68^{b}	-58^{b}	-84^{b}	-70^{c}
Average:	-60^{x}	-44^{y}	-68^{z}	-57
Heifers				
Cottonseed meal	41	7^{d}	----	24^{f}
Cubes w biuret	44	10^{d}	----	27^{f}
Cubes w/o biuret	40	26^{e}	----	33^{g}
Average:	42^{x}	14^{y}	----	28

^{a,b,c}Values in same column with different superscripts differ. (P<.01)

^{d,e} Values in same column with different superscripts differ. (P<.01)

^{f,g} Values in same column with different superscripts differ. (P<.05)

^{x,y,z}Values in same row with different superscripts differ. (P<.01)

TABLE 16

FINAL COW AND HEIFER WEIGHTS (AUGUST) AS AFFECTED BY YEAR AND WINTERING TREATMENT (kg)[1], EXPERIMENT 3

Treatment	Year 1	Year 2	Year 3	Average
Cows				
Cottonseed meal	425	403	416	413
Cubes w biuret	417	406	406	410
Cubes w/o biuret	418	409	408	412
Average:	420^{x}	406^{y}	408^{y}	411
Heifers				
Cottonseed meal	421	370^{a}	---	395^{a}
Cubes w biuret	423	376^{a}	---	400^{a}
Cubes w/o biuret	429	408^{b}	---	418^{b}
Average:	424^{x}	384^{y}	---	404

[1]Adjusted for initial weight.

[a,b]Values in same column with different superscripts differ. (P<.01)

[x,y]Values in same row with different superscripts differ. (P<.01)

cow weights due to treatment; however, cows were heavier at the end of the first year ($P<.01$) than at the end of the next 2 years. Heifers were also heavier at the end of the first year ($P<.01$) than at the end of the second year. Heifer weights did not differ according to treatment the first year, but heifers receiving no supplemental nitrogen were heavier at the end of the second year ($P<.01$), an effect that also influenced the two year averages in the same manner ($P<.01$).

Neither conception rate as determined by palpation nor calf survival was affected ($P>.05$) by either year or wintering treatments. The average conception rate for cows and heifers was 86 and 84%, respectively, with calf survival being 93%.

Calf weaning weights are shown in table 17. In the first year, cows which received CP cubes weaned the heaviest calves. Cows which received CPB cubes weaned the lightest calves ($P<.01$) and cows receiving CSM weaned intermediate weight calves ($P>.05$). During the second year of the study, cows receiving CPB cubes weaned lighter calves than cows which received the other two treatments ($P<.01$). There were no differences ($P>.05$) during the third year. When data for the 3 years were combined, calves weaned from cows receiving CPB cubes were lighter ($P<.01$) than calves weaned from cows receiving either of the other treatments. Weanings weights were influenced also by year ($P<.01$).

The average time required for cows to reconceive is shown in table 18. There were no differences ($P>.05$) in conception time as affected by wintering treatment. However, cows required longer ($P<.01$) to reconceive during the first year of the study than in subsequent years.

TABLE 17

EFFECT OF WINTERING PROGRAM AND YEAR ON 205-DAY ADJUSTED WEANING WEIGHT (kg), EXPERIMENT 3

Treatment	Year 1	Year 2	Year 3	Average
Cottonseed meal	171^{ab}	164^{a}	166	167^{a}
Cubes w biuret	166^{b}	154^{b}	166	162^{b}
Cubes w/o biuret	176^{a}	163^{a}	165	168^{a}
Average:	171^{x}	160^{y}	166^{z}	166

^{a,b}Values in same column with different superscripts differ. $(P<.01)$

^{x,y,z}Values in same row with different superscripts differ. $(P<.01)$

TABLE 18

EFFECT OF WINTERING TREATMENT AND YEAR ON AVERAGE TIME REQUIRED FOR COWS TO RECONCEIVE (DAYS) AFTER BEING EXPOSED TO BULLS: AS DETERMINED BY SUBSEQUENT CALVING DATE, EXPERIMENT 3

Treatment	Year 1	Year 2	Year 3	Average
Cottonseed meal	23.2	19.0	15.2	19.4
Cubes w biuret	24.2	19.6	19.7	21.4
Cubes w/o biuret	28.0	18.0	22.4	22.4
Average:	24.9^{x}	18.8^{y}	19.0^{y}	21.0

^{x,y}Values in same row with different superscripts differ. $(P<.01)$

Discussion

In experiment 1, cows which received wintering supplements containing 50% nitrogen from NPN sources (urea and diammonium phosphate) performed as well as did cows receiving supplemental nitrogen from natural protein (CSM). These results are in contrast to those reported by others (Nelson et al., 1957; Nelson and Waller, 1962; Williams et al., 1969; and Rush and Totusek, 1973) who have reported inferior performance of cows wintered on supplements containing urea when compared to cows which received natural proteins.

Over-all cow performance during the year of this experiment was considered to be excellent. Weaning weights, across all treatments for example, were 191 kg which was substantially above the weaning weights (176 and 166 kg) (table 11 and 17) reported for the following 2 and 3 year studies with basically the same herd of cattle. Cow winter weight loss (which averaged -34 kg, or approximately 8% of body weight) as well as an over-all increase in cow weight of 39 kg during the year was also indicative of the good performance.

The good performance of this cow herd under the conditions of this study and the failure to observe treatment differences such as those reported by other workers, in all probability, may be attributed to two factors. First, it is suggested that the quality of the supplemental forages provided during the winter feeding period was sufficiently high to preclude beneficial responses from supplemental nitrogen in either of the forms provided. This suggestion would seem to be supported when one considers reports (Blaxter and Wilson, 1963; Milford and Minson, 1965; and Oltjen et al., 1974) which indicated no beneficial effect from supplementing forages containing crude protein levels of greater than 7 to 8%. The forages utilized in this study contained 7.3 and 10.2% (table 3) crude protein and amounts offered were limited only by the amounts the animals would consume without excessive wastage. It is secondly suggested that environmental conditions during the period of this study were favorable to the point of stimulating pasture growth which was considered to be above normal. These grazing conditions could account in part for the 39 kg increase in cow weight over the year period. Although winter pasture was found to be low in quality (3.30% crude protein, table 14), it is suggested that some new growth of pasture could have been available during the winter months. This fresh pasture would have been high in quality and selectively grazed by the animals.

Although a negative control group of animals which received no supplemental nitrogen was not available for comparison, it is felt that there was sufficient data to suggest that the animals in this study were not under sufficient nutritional stress to derive measurable benefits from either of the supplements provided.

No direct comparison can be made, however, greater winter weight losses, declining body weights and lesser weaning weights in experiment 2, relative to those in experiment 1, would suggest that animals were under greater nutritional stress during experiment 2. With this assumption, the data of experiment 2 did indicate that cubes containing biuret were not utilized as well as cottonseed meal cubes. Cow weight change is the most direct measurement of this utilization and distinctly favored the animals receiving CSM cubes during both years of the study. The magnitude of this difference was greater, however, during the first year. Differences between years were observed for cow winter weight losses ($P<.05$), final cow weights ($P<.01$) and weaning weights ($P<.05$). These differences would be expected due to yearly differences in environmental conditions which would result in differences in availability of pasture.

Animals wintered on silage lost less weight ($P<.05$) than did animals wintered on hay during the first year of this study. This difference in weight loss may be attributed to differences in quality of the two supplemental roughages (table 7) and might have been expected during both years of the study. However, no such differences were observed during the second year.

Final cow weights were different ($P<.05$) in only one of the 2 years of this study. This difference occurred during the first year of the study following a maximal difference which occurred in winter weight losses (29 kg). Final cow weights also indicated an inverse relationship between winter weight loss and summer weight gain. That is, cows that lost the greatest amount of weight in the winter gained the greatest amount of weight in the summer. This is in agreement with data reported by Pinney et al. (1972) and is an example of compensatory gain.

Conception rate favored the cows wintered on CSM cubes during both years of this study (6 and 9%, $P>.10$) and when combined over the 2 - year period were different ($P<.07$). This result suggested that the 19 and 21% winter weight losses observed with animals wintered on

CPB cubes during the first and second years of this study had approached a point where the animals were in a physical condition sufficiently poor to interfere with reproductive performance. Calf survival was 94% for the two years of this study and was not affected by wintering treatment. Weaning weights did vary among individual treatments during the first year of the study. However, no differences were attributed to winter nitrogen source. Both calf survival and weaning data are in agreement with data reported by Pinney et al. (1972).

Excessive winter weight loss and in one case lower final weight for cows being wintered on CPB cubes indicated that biuret nitrogen was not utilized as well by wintering brood cows as was cottonseed meal nitrogen. Conception data indicated that this decreased utilization approached the point of economic significance.

It should be pointed out that the data give no absolute estimation of the degree of utilization of biuret nitrogen and it is suggested that animals which might have received no supplemental nitrogen under these conditions might have performed much more poorly than biuret supplemented animals in terms of both weight changes and calf production.

The results of experiment 3 suggested that biuret nitrogen was effective during two of the 3 years of this study in reducing winter weight loss in mature cows when compared with animals receiving no supplemental nitrogen. These data are in agreement with those of Tollett et al. (1969) and DeRouen et al. (1974) and suggest that biuret can be utilized as a source of nitrogen for cattle under range conditions. During the first year of the study, however, cows receiving biuret lost more weight than did animals receiving no supplemental nitrogen. The reason for the between-year variation in biuret utilization was not readily apparent, although others (Rush and Totusek, 1973; Oltjen et al., 1974) have observed variability in the degree of biuret utilization with low-quality forages. Oltjen et al. (1974) suggested the best utilization of biuret occurred when crude protein content of the forage fed was 4.5% or less. Johnson and Clemens (1973) and Schroder and Gilchrist (1960) suggested that rumen microbes adapted to biuret as a source of nitrogen more rapidly when a forage with low levels of crude protein was provided. The available winter forage was Pangola digitgrass hay and winter pasture with average crude protein contents of 3.7 and 3.9%, respectively. Little variation was found between samples within years or among years. The higher quality forages, mixed hay and sorghum silage, were provided only for short periods at the end of the winter feeding period each year and, in fact, were fed for a greater period of time during the second year of the study than during the first year. Thus, the quality of supplemental winter feed apparently should have been sufficiently low in each of the 3 years to result in effective biuret utilization.

Differences ($P<.01$) in winter gains for heifers were not considered an effect of treatment in spite of the statistical evaluation. The differences observed during the second year of the study can perhaps be attributed to variation among animals and among pastures.

In addition, nitrogen may not have been the factor limiting growth in either of these two groups of heifers.

The lack of differences in cow weights among treatment groups at the end of each treatment year indicates that cows, regardless of winter weight loss, compensated for that weight loss during the months of abundant natural pasture. The data agreed with those of Pinney et al. (1972), which showed a highly significant inverse relationship between winter weight loss and summer weight gain and also suggested that cows can compensate for large winter weight losses during times of abundant natural feed supply.

Yearly and over-all conception rate and calf survival showed no alteration due to wintering treatment and suggested that there was insufficient nutritional stress to affect these parameters. General management of these animals, however, produced conditions that were highly conducive to good reproductive performance, in that cows were exposed to bulls during April, May and June. During this period lush green pasture was available and the physical condition of all animals was improving rapidly, a condition that is highly conducive to conception. This was especially true for those animals which had experienced the greatest winter weight loss. Time required to reconceive likewise was not affected ($P>.05$) by wintering treatment. However, there was an over-all increase between each of the treatments in average time required to reconceive. This observation suggested that some additional time was required for animals with greater winter weight loss to obtain a physical status conducive to conception.

The actual importance of differences observed in 205-day weaning weights, as affected by wintering treatments, is questionable in the present study. Observed cow weight losses during the winter period suggested an over-all greater stress on animals receiving no supplemental nitrogen and one would have expected these animals to produce lighter calves. Unsupplemented animals, however, weaned calves as heavy as those weaned by cows receiving CSM over the 3-year period. The capacity of the cows to compensate for excessive winter weight loss during the summer months and the fact that the wintering treatment was ended about 120 days prior to weaning suggested that the observed difference in weaning weight may have been influenced by factors other than wintering treatment.

The data suggest that biuret was effective, but to a lesser extent than CSM, in reducing winter weight loss by cows. The importance of the reduced winter weight loss is questionable, however, in view of results with a long-term study reported by Pinney _et al_. (1972), which indicated that cows wintered on a relatively low plane of nutrition have a longer productive life. No other positive responses were obtained with supplemental nitrogen. Under the environmental and management conditions of this particular study, a need for supplemental nitrogen for grazing brood cows during the winter season was not established.

CHAPTER IV

UTILIZATION OF LOW-QUALITY FORAGE BY SHEEP AS AFFECTED BY VARYING LEVELS AND FORMS OF ENERGY AND NITROGEN

Houser (1970) and Fick (1971) have suggested that the nutritive value of any forage is determined by (1) the intake of that forage and (2) by its digestibility. Intake of low-quality forage (less than 7% crude protein) apparently is limited by an insufficiency of nitrogen (Milford and Minson, 1965; Minson, 1967). Nitrogen containing supplements are often fed to animals consuming low-quality forage to: (1) improve the nitrogen status of the animal; and (2) to increase the voluntary intake of the roughage (Houser, 1970; and Fick, 1971). Fick (1970) and Verde (1970) have demonstrated positive responses when sheep consuming low-quality forage were supplemented with nitrogen from natural proteins, urea or biuret.

Energy supplementation may also be important when animals consuming low-quality forage are provided NPN. The energy supplement often serves as a carrier for the nitrogen compound and may also serve to improve the utilization of the diet. Fick (1970) observed a decrease in intake and digestibility of low-quality forage as the level of supplement, primarily of corn, was increased above 100 g per day to sheep receiving 10 g of nitrogen from urea. Other workers (Mills _et al_., 1944; Lewis and McDonald, 1958; Bell _et al_., 1951; and

Gallup et al., 1952) have suggested that starch energy supplements are more effective than molasses when fed with NPN to animals consuming low-quality forages. However, molasses does offer many economical and labor saving advantages when used under practical conditions and because of these, then, its use continues to grow.

The following three studies were undertaken to define further the role of urea and biuret as sources of supplemental nitrogen for sheep consuming low-quality forage and to study the effects of varying the forms and levels of energy which were provided with the nitrogen supplements.

Experiment 4

Varying Levels of Urea and Molasses for Sheep Consuming a Low-Quality Forage

Procedure

Twenty-eight Florida native wethers approximately 18 months in age and ranging in weight from 26.8 to 44.1 kg (average 39.7 kg) were utilized to study the effect of supplementing a low-quality Pangola digitgrass hay with three levels of urea nitrogen (0, 5 and 10 g per head daily) and two levels of energy in the form of sugarcane molasses (80 and 160 g per head daily). Composition of supplements, animal weights and animals per treatment are shown in table 19. The experiment was basically a 2 x 3 factorial with the addition of a control treatment receiving neither supplemental nitrogen nor energy. Sheep were shorn and treated with an antihelminth approximately 2 weeks prior to being randomly assigned to one of the seven treatments.

TABLE 19: COMPOSITION OF SUPPLEMENTS, FEEDING LEVELS, ANIMALS PER TREATMENT AND AVERAGE ANIMAL WEIGHT PER TREATMENT, EXPERIMENT 4.

	Treatment						
	1	2	3	4	5	6	7
Supplement, g/day							
Urea	-----	-----	-----	10.7	10.7	21.4	21.4
Molasses[a]	-----	80.0	160.0	80.0	160.0	80.0	160.0
Mineral & Vitamin[b]	25.0	25.0	25.0	25.0	25.0	25.0	25.0
Sodium Sulfate	2.2	2.2	2.2	2.2	2.2	4.4	4.4
Total Supplement:	27.2	107.2	87.2	117.9	197.9	128.6	208.6
Hay, g/day[c]	500	500	500	500	500	500	500
Dietary crude protein, %[d]	3.7	3.6	3.5	8.6	8.3	12.6	12.9
Animals/Treatment	4	4	4	4	4	4	4
Average Animal Weight, kg	39.4	40.3	39.4	39.5	39.9	39.0	40.2

[a] Sugarcane, Molasses, Mn 48% invert sugar, Mn 79.5 degree brix (4); 2.39% crude protein and 79.8% dry matter, as fed, 89.8% organic matter as % of dry matter.

[b] Minimum analysis in percent listed as: Ca, 16.0; P, 8.0; NaCl, 23.0; Mg, 2.0; Fe, 1.0; Cu, .10; Mn, .20; Zn, .45; Co, .03; I, .01; Vitamins in USP units per kg: A, 68,182; D3, 6,820.

[c] Hay, Pangola digitgrass, provided ad libitum during voluntary intake period; 87.5% dry matter, 3.9% crude protein, 38.1% cellulose, as fed, 95.6% organic matter as % of dry matter.

[d] Dietary crude protein, as fed during period of voluntary intake determination.

Animals were housed in individual, elevated wooden pens (1 x 1.3 m) with expanded metal floors for a period of 29 days. During the first 7 days of this period dietary urea levels were slowly increased until appropriate intake was obtained. The sheep were then allowed a 14-day preliminary period followed by an 8-day period during which voluntary intake of the 3.9% crude protein Pangola digitgrass hay was determined. Hay was chopped into 3-5 cm lengths through a forage harvester to facilitate feeding and was offered daily in amounts representing a minimum of 20% more than the previous day's consumption. Water was provided *ad libitum* and supplements were provided daily in individual dishes separate from the hay. In addition to appropriate levels of urea and molasses, all animals received 25 g of a complete vitamin A, D and mineral mix and .5 or 1 g of sulfur as sodium sulfate was provided for sheep receiving 0 and 5 or 10 g of urea-nitrogen respectively (table 19). Minerals, sulfur and urea were mixed with molasses in feeding dishes on a daily basis. Control animals receiving no molasses were offered mineral and sulfur supplements dry in individual dishes and consumed these supplements readily. The time required to consume the supplements was observed on two successive days at the end of the voluntary intake period.

Following the voluntary intake period, sheep were moved to metal metabolism cages (1 x .4m) for 14 days and were fitted with canvas fecal collection bags to allow for the total collection of feces and urine. During the period hay was offered at a level of 500 g daily and supplements, as described previously, were offered in separate dishes with the hay until consumed. This procedure allowed the animals to consume the supplement or hay at will. The first 6 days of this period were allocated for adaptation of sheep to crates and collection bags. During the following 7 days a total collection of feces and urine was made.

Feces were stored frozen until the end of the collection at which time daily collections were composited, mixed and sampled for analysis. Fecal nitrogen was determined on wet fecal material after thorough mixing in a blender. The remainder of the fecal sample was dried at 50 C and the feces and hay were ground through a Wiley mill containing a 1 mm screen. Urine volume was measured and recorded daily with 10% of the measured volume retained as a aliquot and aliquots were composited over the 7-day period. Approximately 50 ml of a 25% hydrochloric acid solution and 10 ml of toluene were added daily to urine collection containers to prevent loss of nitrogen.

Feed and fecal samples were analyzed for nitrogen, dry matter, and ash and urine samples for nitrogen only according to A.O.A.C. (1970). Cellulose in hay and feces was determined by the method of Crampton and Maynard (1938). Nutrient digestibility coefficients and nitrogen balance data were calculated for total diets (hay plus supplements).

Following completion of the fecal and urinary collection, jugular blood samples were obtained prior to and at 4, 8, 12 and 24 hr after feeding the supplement. Blood urea-nitrogen was determined by the diacetyl monoxime-ferric ammonium sulfate colorimetric method (Richter and Lapoinie, 1962).

Criteria observed were: voluntary hay intake when hay was offered *ad libitum*; digestibility of organic matter, nitrogen and cellulose, nitrogen balance and blood urea-nitrogen levels when hay was restricted to 500 g. All data were analyzed by analysis of variance and significant differences between treatment means were determined by Duncan's multiple range test (1955).

Results

Crude protein of the entire diet, as fed, increased from 3.6 to 8.4 to 12.8% with nitrogen supplementations of 0, 5 and 10 g respectively (table 19).

The effects of levels of supplemental molasses and urea on the voluntary intake of hay and organic matter from hay, molasses and urea are shown in table 20. Voluntary intake of hay was different ($P<.05$) only according to level of molasses fed. Sheep receiving 160 g of molasses daily consumed less hay (480 g) ($P<.05$) than did sheep receiving no supplemental molasses (597 g). Sheep receiving 80 g molasses consumed an intermediate amount (520 g) of hay. There was a non-significant ($P>.10$) increase in hay consumption (468, 514 and 578 g) with increasing levels of nitrogen (0, 5 and 10 g/head) for sheep receiving 80 g of molasses. This trend was not evident with sheep consuming 160 g of molasses but was reflected to some extent in the average of both molasses levels. Voluntary hay intake of animals receiving any combination of urea and molasses never numerically equalled or exceeded the intake of control animals receiving no supplemental energy or nitrogen. Organic matter intake

TABLE 20: VOLUNTARY INTAKE OF HAY (g/day) AND ORGANIC MATTER (g/day) AS AFFECTED BY VARYING LEVELS OF SUPPLEMENTAL ENERGY (MOLASSES) AND NITROGEN (UREA), EXPERIMENT 4.

Molasses g/head Daily	Urea-N, g/head daily						Average	
	0		5		10			
	Hay[a]	Organic Matter[b]	Hay	Organic Matter	Hay	Organic Matter	Hay	Organic Matter
0	597	500	---	---	---	---	597[c]	500
80	468	442	514	488	578	549	520[cd]	493
160	442	470	510	534	489	525	480[d]	509
Average	502	471	512	511	535	537	514[e]	501[f]

[a]Hay consumption as fed.

[b]Hay plus supplement.

[c,d]Values in same column with different superscripts differ. ($P<.05$)

[e]Hay intake per kg of body weight equals 13.6 g.

[f]Organic matter intake per kg of body weight equals 13.2 g.

did not differ according to molasses or urea level and ranged from 442 to 549 g/head daily and an average of 501 g of organic dry matter was consumed per head daily.

Time required for animals to consume supplements is shown in table 21. Increasing nitrogen (urea) content of the supplements increased ($P<.01$) consumption time from .3 to 3.1 to 5.2 hr respectively for animals receiving 0, 5 and 10 g of supplemental nitrogen daily and reflects the low palatibility of urea. A difference in consumption time due to molasses level was observed ($P<.05$) at the 5 g nitrogen level. Sheep receiving 80 and 160 g of molasses required 1.7 and 4.5 hr respectively to consume supplements.

The effects of levels of molasses and urea on the digestibility of organic matter and cellulose are shown in table 22. Cellulose digestibility ranged from a high of 61.8% for sheep receiving 10 g of urea nitrogen and 80 g of molasses per head daily to a low of 50.2% for animals receiving 160 g of molasses with no supplemental nitrogen. These values differed ($P<.05$) while values for other treatments were intermediate and did not differ ($P>.05$). The effect of nitrogen level on cellulose digestibility at 80 and 160 g of molasses is represented in figure 1. Increasing levels of nitrogen supplementation resulted in a linear increase in cellulose digestibility at both 80 g ($P<.05$) and 160 g ($P<.10$) of molasses per head daily.

The increase in cellulose digestibility with increasing nitrogen levels for sheep consuming 80 g of molasses/day is represented by the equation $y = 54.78 + .74x$ while the same values for

TABLE 21

EFFECT OF LEVEL OF ENERGY (MOLASSES) AND NITROGEN (UREA) SUPPLEMENTATION ON TIME REQUIRED (hr) TO CONSUME SUPPLEMENTS, EXPERIMENT 4

Sugarcane Molasses g/head daily	Urea-N, g/head Daily			
	0	5	10	Average
80	$.3^{a}$	1.7^{ab}	5.0^{c}	2.3
160	$.4^{a}$	4.5^{c}	5.5^{c}	3.5
Average	$.3^{d}$	3.1^{e}	5.2^{f}	2.9

[a,b,c]Values with different superscripts differ. (P<.01)

[d,e,f]Values with different superscripts differ. (P<.01)

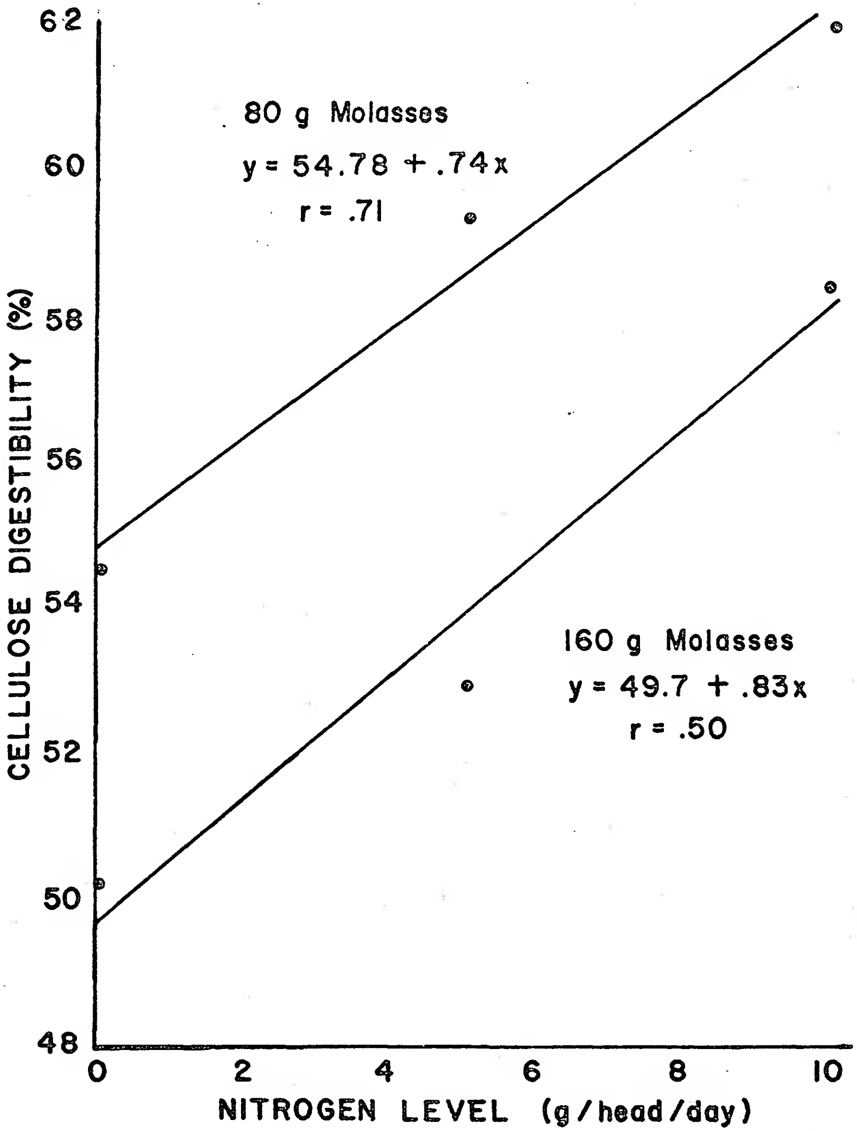

Figure 1. Effect of dietary nitrogen level on digestibility of cellulose at 80 and 160 g/day of supplemental energy (molasses), Experiment 4.

sheep consuming 160 g of molasses/day is represented by y = 49.7 + .83x; where x is supplemental nitrogen level and y is cellulose digestibility. Correlation coefficients of the respective equations were .71 and .50.

Organic matter digestibility, table 22, was greater (P<.05) for all combinations of supplemental molasses and urea than for the control diet where animals received neither supplemental nitrogen nor energy. This effect might have been expected, due to the addition of the more readily digestible supplements. Increasing nitrogen levels also resulted in an increase in the digestibility of organic matter in a manner which did not differ (P<.05) from linearity. This increase is represented by the equation y = 49.43 + .68x; where x and y represent nitrogen level and organic matter digestibility respectively. The coefficient of correlation for this equation was .58.

Nitrogen digestibility and balance data are shown in table 23. Nitrogen digestibility was -18.1, 50.9 and 69.9% (P<.05) for animals receiving 0, 5 and 10 g of supplemental nitrogen per day but did not differ (P>.10) according to energy level. Increasing nitrogen supplementation from 0 to 5 to 10 g/day increased total daily nitrogen intake from 2.75 to 8.19 to 13.39 g respectively. Fecal nitrogen did not increase (P>.10) with increasing nitrogen intake while absorbed nitrogen values increased (P<.05) with progressive 5 g increases in supplemental nitrogen intake. Urinary nitrogen levels were 1.39, 4.57 and 8.57 g/day (P<.05) while total nitrogen balance was negative for 0 and 5 g supplementation levels (-1.88 and -.40 g/day respectively) (P<.05) and positive for the highest level of nitrogen

TABLE 22: DIGESTIBILITY (%) OF CELLULOSE AND ORGANIC MATTER AS AFFECTED BY LEVEL OF ENERGY (MOLASSES) AND NITROGEN (UREA) SUPPLEMENTATION, EXPERIMENT 4.

Sugarcane Molasses g/head daily	Urea-N, g/head daily: 0		5		10		Average	
	Cellulose	Organic Matter	Cellulose	Organic Matter	Cellulose	Organic Matter	Cellulose	Organic Matter
0	59.7^{ab}	45.1^{c}	-----	-----	-----	-----	59.7	45.1^{e}
80	54.4^{ab}	50.7^{d}	59.2^{ab}	52.2^{d}	61.8^{b}	54.9^{d}	58.5	52.6^{f}
160	50.2^{a}	52.2^{d}	52.9^{ab}	53.9^{d}	58.3^{ab}	55.8^{d}	53.9	54.0^{f}
Average	54.8	49.3^{g}	56.1	53.0^{gh}	60.2	55.4^{h}	56.7	52.1

[a,b]Values with different superscripts differ. $(P<.05)$

[c,d]Values with different superscripts differ. $(P<.05)$

[e,f]Values with different superscripts differ. $(P<.01)$

[g,h]Values with different superscripts differ. $(P<.05)$

TABLE 23: EFFECT OF LEVEL OF ENERGY (MOLASSES) AND NITROGEN (UREA) SUPPLEMENTATION ON DIGESTIBILITY AND UTILIZATION OF NITROGEN, EXPERIMENT 4.

	Urea-Nitrogen, g/day									
	0				5			10		
Molasses, g/day	0	80	160	Av.	80	160	Av.	80	160	Av.
Digestibility %	-21.4^{a}	-16.7^{a}	-16.3^{a}	-18.1^{d}	54.6^{bc}	47.2^{b}	50.9^{e}	71.9^{c}	67.9^{bc}	69.9^{f}
Nitrogen Balance g/day										
Intake	2.94	2.37	2.93	2.75	8.04	8.34	8.19	13.32	13.45	13.39
In feces[g]	3.55	2.78	3.38	3.24	3.63	4.40	4.02	3.74	4.32	4.03
Absorbed	------	------	------	------	4.41	3.94	4.17^{d}	9.58	9.13	9.36^{e}
In Urine	1.62	1.32	1.24	1.39^{d}	4.48	4.66	4.57^{e}	8.29	8.86	8.57^{f}
Retained[h]	-2.23^{a}	-1.72^{ab}	-1.70^{ab}	-1.88^{d}	$-.07^{c}$	$-.72^{bc}$	$-.40^{e}$	1.29^{d}	$.28^{cd}$	$.79^{f}$
Retained, % Intake	------	------	------	------	------	------	------	9.7	2.1	5.9

[a,b,c] Values in same row with different superscripts differ. ($P<.05$)

[d,e,f] Values in same row with different superscripts differ. ($P<.05$)

[g] Fecal nitrogen for 80 and 160 g/day of Molasses = 3.38 and 4.03 g/day, respectively. ($P<.10$)

[h] Nitrogen retention for 80 and 160 g/day of Molasses = -.17 and -.71 g/day, respectively. ($P<.10$)

supplementation (.79 g/day) ($P<.05$). Fecal nitrogen excretion was greater ($P<.10$) for sheep receiving 160 g of molasses (4.03 g/day) than for sheep receiving 80 g of molasses per day (3.38 g/day). Nitrogen retention was -.17 g/day and -.71 g/day ($P<.10$) for animals receiving 80 and 160 g of molasses/day respectively.

Blood urea-nitrogen (BUN) levels are shown in figure 2 and appendix table 34. Increasing levels of nitrogen supplementation increased BUN levels over the 24 hr period ($P<.01$) with maximum increases occurring between 4 and 8 hr with 5 g and between 8 and 12 hr with 10 g of nitrogen supplementation. Levels of BUN were in general lower in sheep receiving 160 g of molasses with either 5 or 10 g of supplemental nitrogen. The magnitude of this difference was greater with the 5 g level of nitrogen supplementation and reached significance ($P<.05$) at the 4 and 8 hr bleedings.

Experiment 5

Biuret vs Urea and Corn vs Molasses as Sources of Supplemental Nitrogen and Energy for Sheep Consuming a Low-Quality Forage

Procedure

Twenty-eight Florida native wethers approximately 22 months of age and ranging in weight from 41.4 to 50.4 kg (average 45.4 kg) were utilized to compare the performance of animals receiving a low-quality Pangola hay with no supplemental nitrogen or with 7 g of supplemental nitrogen daily as either urea or feed grade biuret (table 24). The nitrogen treatments (none, urea or biuret) were fed in combination with either 80 g of molasses or 60 g of corn (table 24)

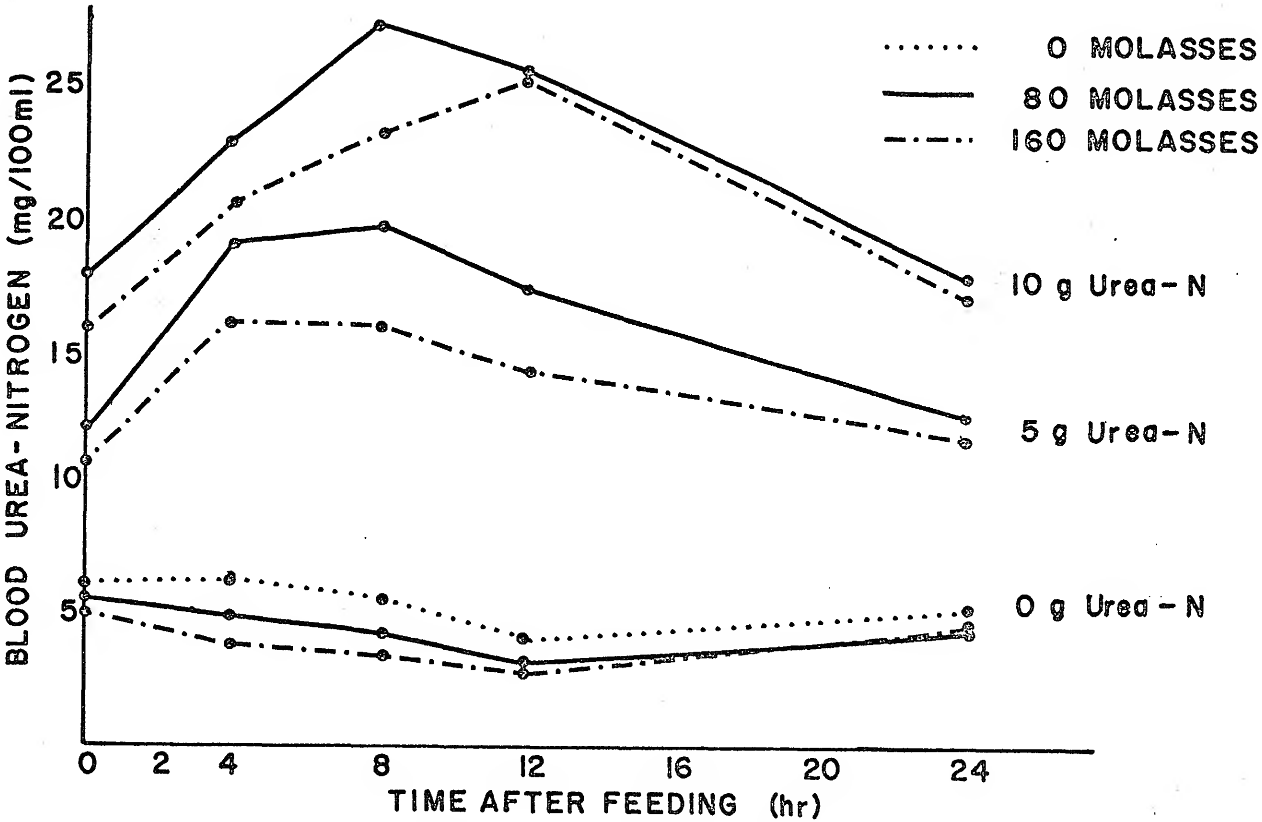

Figure 2. Effect of level of energy (molasses) and nitrogen (urea) on blood urea-nitrogen with increasing times after feeding, Experiment 4.

TABLE 24: COMPOSITION AND DAILY FEEDING LEVELS OF SUPPLEMENTS AND HAY; NUMBER AND AVERAGE WEIGHT OF ANIMALS PER TREATMENT AND DIETARY CRUDE PROTEIN LEVEL, EXPERIMENT 5.

	Treatment						
	1	2	3	4	5	6	7
Supplement, g/day							
Molasses[a]	-----	80.0	-----	80.0	-----	80.0	-----
Corn[b]	-----	-----	60.0	-----	60.0	-----	60.0
Urea	-----	-----	-----	15.5	15.5	-----	-----
Biuret	-----	-----	-----	-----	-----	17.5	17.5
Mineral & Vitamin Mix[c]	25.0	25.0	25.0	25.0	25.0	25.0	25.0
Sodium Sulfate	3.2	3.2	3.2	3.2	3.2	3.2	3.2
Total Supplement:	28.2	108.2	88.2	123.7	103.7	125.7	105.7
Hay, g/day[d]	700	700	700	700	700	700	700
Dietary Crude Protein, %[e]	3.5	3.6	3.4	7.5	7.8	7.6	8.0
Animals/Treatment	4	4	4	4	4	4	4
Average Animal Weight, kg	45.7	45.7	45.8	45.0	45.1	44.8	44.9

[a]Sugarcane, Molasses, Mn 48% invert sugar, Mn 79.5 degree brix (4); 2.39% crude protein and 79.8% dry matter, as fed, 88.1% organic matter as % of dry matter.

[b]Corn meal 50%, corn starch 25%, sucrose 25%; 87.8% dry matter, 3.85% crude protein, and 1.1% cellulose, as fed, 99.4% organic matter as a % of dry matter.

[c]See table 19.

[d]Hay, Pangola digitgrass, provided ad libitum during voluntary intake period; 90.0% dry matter, 3.6% crude protein, 85.5% organic matter and 35.3% cellulose, as fed, 95.5% organic matter as % of dry matter.

[e]Dietary crude protein, as fed during period of voluntary intake determination.

per head daily. In addition, one group of animals received neither supplemental nitrogen nor energy.

Animals in this study were prepared and cared for as in experiment 4. Voluntary intake of the hay was determined during an 8-day period following a 21-day adaptation period. Supplements containing molasses were prepared individually on a daily basis while supplements containing corn were mixed and weighed into plastic bags for individual feedings at the beginning of the study. In addition to energy and nitrogen supplements, each animal received 25 g per day of a complete mineral mix (experiment 4) and .75 g per day of sulfur from sodiem sulfate. Supplements were fed as described in the previous study. Following the voluntary intake phase of the study, sheep were moved to metabolism crates and fitted with canvas collection bags. Details for the adaptation and collection periods were as described in experiment 4. Animals were offered 700 g of Pangola hay daily during the trial. Blood was collected for determination of blood urea-nitrogen as in the previous study.

Chemical analysis of samples and statistical analysis of data as well as criteria observed were as described previously (experiment 4).

Results

The intake of hay or organic matter in this experiment was not affected (P>.10) by source of supplemental energy or nitrogen or by any combination of these factors (table 25). The digestibility of cellulose (table 26) was not altered (P>.10) by source of supplemental energy. However, when no supplemental nitrogen was provided, energy as either corn or molasses resulted in a numerical decrease in cellulose digestibility when compared to sheep receiving

TABLE 25: VOLUNTARY INTAKE OF HAY (g/day) AND ORGANIC MATTER (g/day) AS AFFECTED BY SOURCE OF SUPPLEMENTAL ENERGY AND NITROGEN, EXPERIMENT 5.

	Nitrogen Source						Average	
	None		Urea		Biuret			
Energy	Hay[a]	Organic Matter[b]	Hay	Organic Matter	Hay	Organic Matter	Hay	Organic Matter
None	793	681	---	---	---	---	793	681
Corn	804	743	788	730	735	683	776	719
Molasses	690	654	808	756	768	722	756	711
Average	762	693	798	743	752	703	770[c]	710[d]

[a] Hay consumption as fed.

[b] Hay plus supplement.

[c] Hay intake per kg of body weight equals 17.0 g.

[d] Organic matter intake per kg of body weight equals 15.7 g.

no energy supplement (52.7, 52.3 and 55.8% for corn, molasses and no energy, respectively). Cellulose digestibility was improved ($P<.05$) by 5.2 and 3.6 percentage units with the supplementation of 7 g of urea or biuret nitrogen respectively and did not differ ($P>.05$) according to nitrogen source (58.8 and 57.2% for urea and biuret, respectively).

Organic matter digestibility (table 26) was improved ($P<.05$) by both sources of supplemental energy as would be expected with the relatively high digestibility of both energy sources. Supplemental nitrogen as urea also improved ($P<.05$) organic matter digestibility when compared with animals receiving no supplemental nitrogen (54.9 and 50.7% respectively). The addition of biuret resulted in 53.2% organic matter digestibility which was not different from either ($P>.05$) of the other two values.

Nitrogen digestibility (table 27) was greater ($P<.05$) with supplemental nitrogen as either urea (66.0%) or biuret (64.4%) than with no supplemental nitrogen (14.2%). Supplemental energy as corn resulted in a greater ($P<.05$) nitrogen digestibility than as molasses (50.9 vs 46.5%). Supplemental nitrogen as either urea or biuret resulted in positive nitrogen balances (1.02 and .55 g/day) while animals receiving no supplemental nitrogen were in negative balance (-.95 g/day; $P<.05$). Sheep receiving supplemental energy as corn maintained greater nitrogen balance across all nitrogen treatments than did sheep receiving molasses. This difference was significant ($P<.05$) when biuret was the nitrogen source and when combined across all nitrogen sources (.07 vs .53 g/day for molasses vs corn) ($P<.05$).

TABLE 26: DIGESTIBILITY (%) OF CELLULOSE AND ORGANIC MATTER AS AFFECTED BY SOURCE OF SUPPLEMENTAL ENERGY AND NITROGEN, EXPERIMENT 5.

	Nitrogen Source						Average	
	None		Urea		Biuret			
Energy	Cellulose	Organic Matter	Cellulose	Organic Matter	Cellulose	Organic Matter	Cellulose	Organic Matter
None	55.8	48.4	-----	-----	-----	-----	55.8	48.4^{e}
Corn	52.7	52.2	60.7	56.7	55.8	51.8	56.4	53.6^{f}
Molasses	52.3	51.5	56.8	53.0	54.6	54.6	56.0	53.1^{f}
Average	53.6^{a}	50.7^{c}	58.8^{b}	54.9^{d}	57.2^{b}	53.2^{cd}	56.1	52.6

[a,b]Values in same row with different superscripts differ. ($P<.05$)

[c,d]Values in same row with different superscripts differ. ($P<.05$)

[e,f]Values in same column with different superscripts differ. ($P<.05$)

TABLE 27: EFFECT OF SOURCE OF SUPPLEMENTAL ENERGY AND NITROGEN ON DIGESTIBILITY AND UTILIZATION OF NITROGEN, EXPERIMENT 5.

	Nitrogen Source									
	None				Urea			Biuret		
Energy	None	Corn	Molasses	Av.	Corn	Molasses	Av.	Corn	Molasses	Av.
N Digestibility[d], %	10.9	17.7	14.0	14.2[x]	69.7	62.2	66.0[y]	65.3	63.4	64.4[y]
N Balance g/day										
Intake	4.02	4.78	4.22	4.34	11.14	11.73	11.43	11.43	11.44	11.44
In feces	3.58	3.93	3.63	3.71	3.37	4.43	3.90	3.96	4.19	4.08
Absorbed	.44	.85	.59	.63	7.77	7.30	7.53	7.47	7.25	7.36
In Urine	1.53	1.45	1.74	1.57	6.70	6.33	6.51	6.35	7.27	6.81
Retained[e]	-1.09[a]	- .60[ab]	-1.15[a]	- .95[x]	1.06[c]	.97[c]	1.02[y]	1.12[c]	- .02[b]	.55[y]
Retained, % Intake	------	------	------	------	9.5	8.3	8.9	9.8	------	4.8

[a,b,c]Values in same row with different superscripts differ. (P<.05)

[x,y]Values in same row with different superscripts differ. (P<.05)

[d]N-Digestibility for all sheep receiving Molasses = 46.5; Corn = 50.9 (P<.05).

[e]N-Retained for all sheep receiving Molasses = .07 g/day; Corn = .53 g/day (P<.05).

Blood urea-nitrogen (BUN) levels at various times after feeding are illustrated according to nitrogen treatment in figure 3 and according to individual treatment in appendix table 35. Supplemental energy source had no effect ($P > .10$) on BUN levels and values were therefore pooled according to nitrogen treatment. Levels of BUN were greater ($P < .01$) for sheep receiving either urea or biuret than for sheep receiving no supplemental nitrogen at each bleeding. Sheep receiving urea had lower BUN ($P < .05$) at 0 and 24 hr than sheep receiving biuret. Values at 4, 8 and 12 hr were not different; however, sheep receiving urea did have a numerically higher BUN peak at 4 and 8 hr. This peak is assumed to represent the more rapid breakdown of urea and absorption of ammonia.

Experiment 6

Varying Levels of Biuret and Molasses for Sheep Consuming a Low-Quality Forage

Procedure

Thirty-six Florida native wethers approximately 30 months of age and ranging in weight from 40.0 to 51.4 kg (average 45.6 kg) (table 28) were utilized in a 3 x 3 factorially designed experiment to observe the effect of supplementing a low-quality hay with 0, 5 or 10 g of biuret nitrogen in combination with 0, 80 or 160 g of molasses.

Sheep were prepared as in experiment 4 and housed in 1 x .4 m expanded metal metabolism crates for the duration of the study. Supplements were mixed daily and included additional minerals and sulfur (table 28). The molasses utilized in this study contained

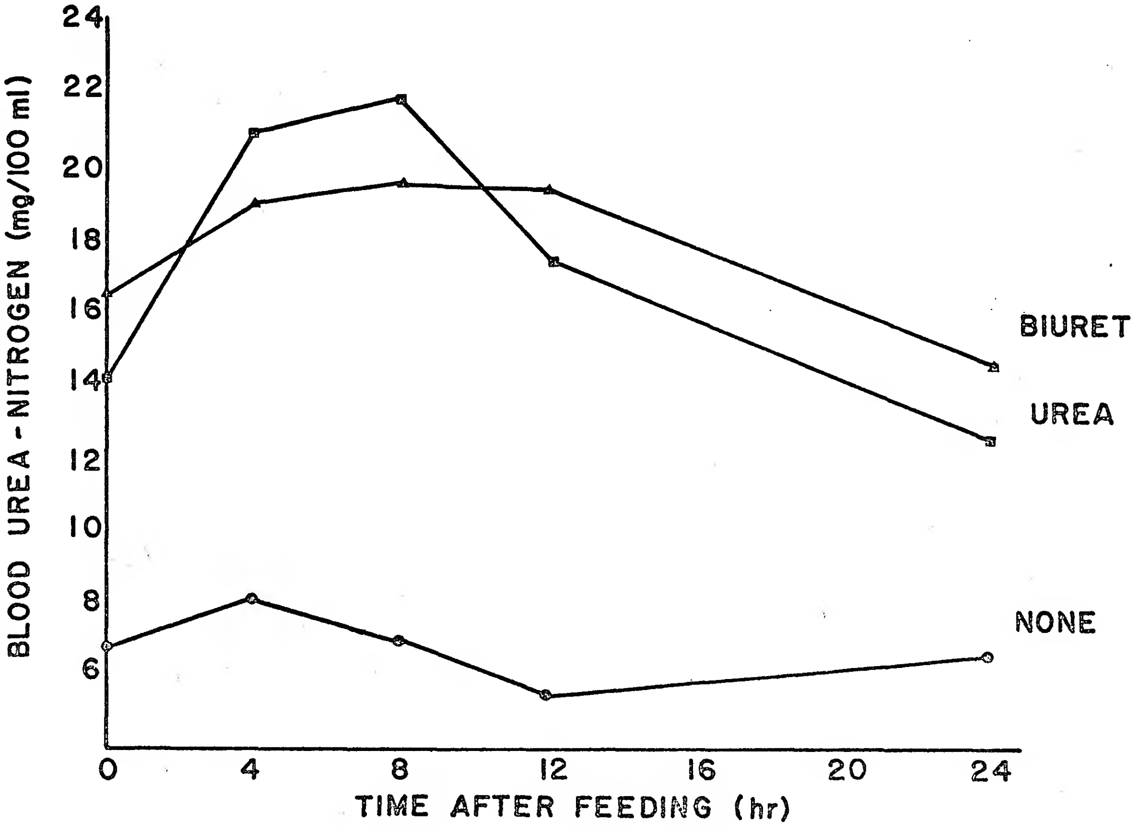

Figure 3. The effect of source of supplemental nitrogen on blood urea-nitrogen levels, Experiment 5.

TABLE 28: COMPOSITION AND DAILY FEEDING LEVELS OF SUPPLEMENTS AND HAY; NUMBER AND AVERAGE WEIGHT OF ANIMALS PER TREATMENT AND DIETARY CRUDE PROTEIN LEVEL, EXPERIMENT 6.

	Treatment								
	1	2	3	4	5	6	7	8	9
Supplement, g/day									
Biuret	-----	13.5	27.0	-----	13.5	27.0	-----	13.5	27.0
Molasses[a]	-----	-----	-----	80.0	80.0	80.0	160.0	160.0	160.0
Mineral & Vitamin Mix[b]	25.0	25.0	25.0	25.0	25.0	25.0	25.0	25.0	25.0
Sodium Sulfate	2.2	2.2	4.4	2.2	2.2	4.4	2.2	2.2	4.4
Total Supplement:	27.2	40.7	56.4	107.2	120.7	136.4	187.2	200.7	216.4
Hay, g/day[c]	500	500	500	500	500	500	500	500	500
Dietary Crude Protein, %[d]	3.3	7.4	11.7	3.3	6.5	11.0	3.1	6.9	9.8
Animals/Treatments	4	4	4	4	4	4	4	4	4
Average Animal Weight, kg	46.1	46.1	44.0	45.1	44.4	47.1	43.8	45.1	48.5

[a]Molasses, Sugarcane, 82.0% dry matter, 2.63% crude protein and 73.3% organic matter, as fed.

[b]See table 19.

[c]Hay, Pangola digitgrass, provided <u>ad libitum</u> during voluntary intake period; 90.5% dry matter, 3.35% crude protein, and 35.4% cellulose, as fed, 96.4% organic matter as a % of dry matter.

[d]Dietary crude protein, as fed during period of voluntary intake determination.

2.63% crude protein and 82% dry matter on an as fed basis. Pangola digitgrass hay, which contained 3.35% crude protein, 90.5% dry matter and 35.4% cellulose, as fed, was ground through a hammer mill with a 12 mm screen. Prilled feed grade biuret was provided to sheep receiving no supplemental molasses and a powdered (ground through a 100 mesh screen) feed grade biuret was mixed with molasses when sheep were supplemented with molasses. Provision of supplements, hay and water was as described in experiment 4. A 21-day adaptation period was followed by an 8-day period during which voluntary intake was established. Sheep were then fitted with canvas collection bags, and allowed 5 days to adapt to the bags prior to the beginning of a 7-day total collection period. Hay intake was limited to 500 g per head daily following the voluntary intake period. Blood samples were taken on the day following the last day of the collection period for determination of blood urea-nitrogen. Sheep were bled as described previously with an additional bleeding 2 hr after feeding.

Details of the collection procedure and chemical analysis of samples were as described in experiment 4. All data were submitted to analysis of variance and significant differences between treatment means were determined by Duncan's multiple range test (Duncan, 1955).

Results

Voluntary intake of hay and organic matter (table 29) were not affected ($P > .10$) by level of supplemental biuret-nitrogen nor by level of energy as sugarcane molasses. Hay intake ranged from a high of 682 g/day for sheep receiving 5 g of biuret-nitrogen and 80 g of

TABLE 29: VOLUNTARY INTAKE OF HAY (g/day) AND ORGANIC MATTER (g/day) AS AFFECTED BY LEVEL OF SUPPLEMENTAL ENERGY (MOLASSES) AND NITROGEN (BIURET), EXPERIMENT 6.

Molasses g/head Daily	Biuret-N, g/day: 0		5		10		Average	
	Hay[a]	Organic Matter[b]	Hay	Organic Matter	Hay	Organic Matter	Hay	Organic Matter
0	614	535	575	515	569	523	586	524
80	449	445	682	660	556	565	562	556
160	486	530	518	570	612	666	539	589
Average	517	503	592	583	579	585	562[c]	557[d]

[a] Hay consumption as fed.

[b] Hay plus supplement.

[c] Hay intake per kg of body weight equals 12.3 g.

[d] Organic matter intake per kg of body weight equals 12.2 g.

molasses to a low of 449 g/day for animals receiving no supplemental nitrogen and 80 g of molasses. Average hay intake for all treatments was 562 g/day (12.3 g of hay/kg body weight). Increasing molasses levels (0, 80 and 160 g) across all nitrogen levels did result in a non-significant (P>.10) decrease in hay consumption (586, 562 and 539 g/head, respectively) and a non-significant (P>.10) increase in organic matter intake (524, 556 and 589 g/day, respectively). Supplementation with 5 and 10 g of biuret-nitrogen resulted in non-significant increases (P>.10) of 75 or 62 g/day for hay intake and 80 or 83 g/day for organic matter intake, respectively.

Cellulose digestibility (table 30) did not differ (P>.10) according to individual treatment but was greater (P<.05) for animals receiving no supplemental molasses (56.7%) than for animals receiving 160 g/day of supplemental molasses (53.6%). Animals receiving 80 g of supplemental molasses had an intermediate cellulose digestibility (56.3%). Cellulose digestibility was non-significantly (P>.10) greater for animals receiving 5 and 10 g/day of biuret-nitrogen (57.8 and 55.2% respectively) than for animals receiving no supplemental nitrogen (53.6%). Organic matter digestibility was greatest (P<.05) for treatments of 160 g of supplemental molasses (58.6%) and least (51.4%) for animals receiving no supplemental molasses. Animals receiving 80 g of supplemental molasses had an intermediate organic matter digestibility (56.1%). The digestibility values again reflect the inclusion of a more readily digestible substance in the diet. No differences in organic matter digestibility were observed according to nitrogen level (P>.10).

TABLE 30: DIGESTIBILITY (%) OF CELLULOSE AND ORGANIC MATTER AS AFFECTED BY LEVEL OF ENERGY (MOLASSES) AND NITROGEN (BIURET) SUPPLEMENTATION, EXPERIMENT 6.

Sugarcane Molasses g/day	Biuret Nitrogen, g/day: 0		5		10		Average	
	Cellulose	Organic Matter	Cellulose	Organic Matter	Cellulose	Organic Matter	Cellulose	Organic Matter
0	55.2	49.6	58.8	52.2	56.1	52.3	56.7[a]	51.4[a]
80	55.2	55.5	59.0	57.2	54.9	55.6	56.3[ab]	56.1[ab]
160	50.6	57.3	55.5	59.4	54.8	59.4	53.6[b]	58.6[b]
Average	53.6	54.1	57.8	56.2	55.2	55.8	55.5	55.4

[a,b]Values in same column with different superscripts differ. ($P<.05$)

Increasing levels of biuret-nitrogen (0, 5 and 10 g/day) resulted in respective increases for: nitrogen digestibility (-6.8, 58.8 and 71.2%) ($P<.05$), nitrogen intake (2.66, 7.90 and 12.81 g/day) ($P<.05$), nitrogen absorbed (negative, 4.64 and 9.11 g/day) ($P<.05$), nitrogen in urine (1.08, 3.91 and 6.75 g/day) ($P<.05$) and nitrogen retention (-1.27, .73 and 2.36 g/day) ($P<.05$) (table 31). Nitrogen retained as a percentage of intake was greater ($P<.05$) for animals receiving 10 g of biuret-nitrogen per day (18.65%) than for animals receiving 5 g of biuret-nitrogen per day (9.43%). Nitrogen intake was also affected by energy level; animals receiving 0, 80 and 160 g of molasses/day consumed 7.84, 7.79 and 8.11 g of nitrogen/day. All values differed ($P<.05$) and this effect apparently was due to a corresponding increase in organic matter intake with increasing levels of molasses. Fecal-nitrogen excretion was greatest (3.56 g/day) ($P<.05$) for sheep receiving the high level of molasses supplementation and least (2.86 g/day) for sheep receiving no supplemental molasses. The animals receiving 80 g/day of molasses excreted an intermediate amount of fecal nitrogen (3.39 g/day).

Blood urea-nitrogen levels, as affected by level of supplemental biuret-nitrogen, are shown in figure 4 and appendix table 36. Blood urea-nitrogen levels increased with increasing levels of biuret-nitrogen supplementation and values for each of the nitrogen levels differed ($P<.05$) from the other two levels at all bleeding times. However, in contrast to the previous experiments of this study, there was very little elevation of blood urea values after feeding. The effect of energy on BUN is indicated in figure 5

TABLE 31: EFFECT OF LEVEL OF ENERGY (MOLASSES) AND NITROGEN (BIURET) SUPPLEMENTATION ON DIGESTIBILITY AND UTILIZATION OF NITROGEN, EXPERIMENT 6.

	Biuret-Nitrogen g/day			
	0			
Molasses g/day	0	80	160	Av.
Digestibility %	-2.0	-9.2	-9.2	-6.8[x]
N Balance, g/day				
Intake[e]	2.52	2.51	2.94	2.66[x]
In feces[f]	2.57	2.74	3.21	2.84[x]
Absorbed	------	------	------	------
In urine	.86[a]	1.18[ab]	1.22[ab]	1.08[x]
Retained	- .91[a]	-1.41[a]	-1.48[a]	-1.27[x]
Retained, % Intake	------	------	------	------

[a,b,c,d]Values in same row with different superscripts differ. ($P<.05$)

[e]Nitrogen intake for 0, 80 and 160 g of molasses/day was 7.48, 7.79 and 8.11 g/day; all differed. ($P<.05$)

[f]Fecal nitrogen for 0, 80 and 160 g of molasses/day was 2.86, 3.39 and 3.56 g/day; extreme values differ. ($P<.05$)

[x,y,z]Values in same row with different superscripts differ. ($P<.05$)

Biuret-Nitrogen g/day							
5				10			
0	80	160	Av.	0	80	160	Av.
62.7	57.7	55.9	58.8^{y}	74.3	68.6	70.6	71.2^{z}
7.59	8.01	8.12	7.90^{y}	12.33	12.83	13.28	12.81^{z}
2.83	3.39	3.58	3.27^{xy}	3.17	4.03	3.90	3.70^{y}
4.76	4.61^{c}	4.54	4.64^{x}	9.16	8.80	9.38	9.11^{y}
3.76^{bc}	4.16	3.81^{bc}	3.91^{y}	6.23^{cd}	8.68^{d}	5.35^{c}	6.75z
1.00^{ab}	$.46^{ab}$	$.74^{ab}$	$.73^{y}$	2.93^{bc}	$.12^{ab}$	4.03^{c}	2.36^{z}
13.15	5.76	9.39	9.43^{x}	24.55	1.03	30.38	18.65^{y}

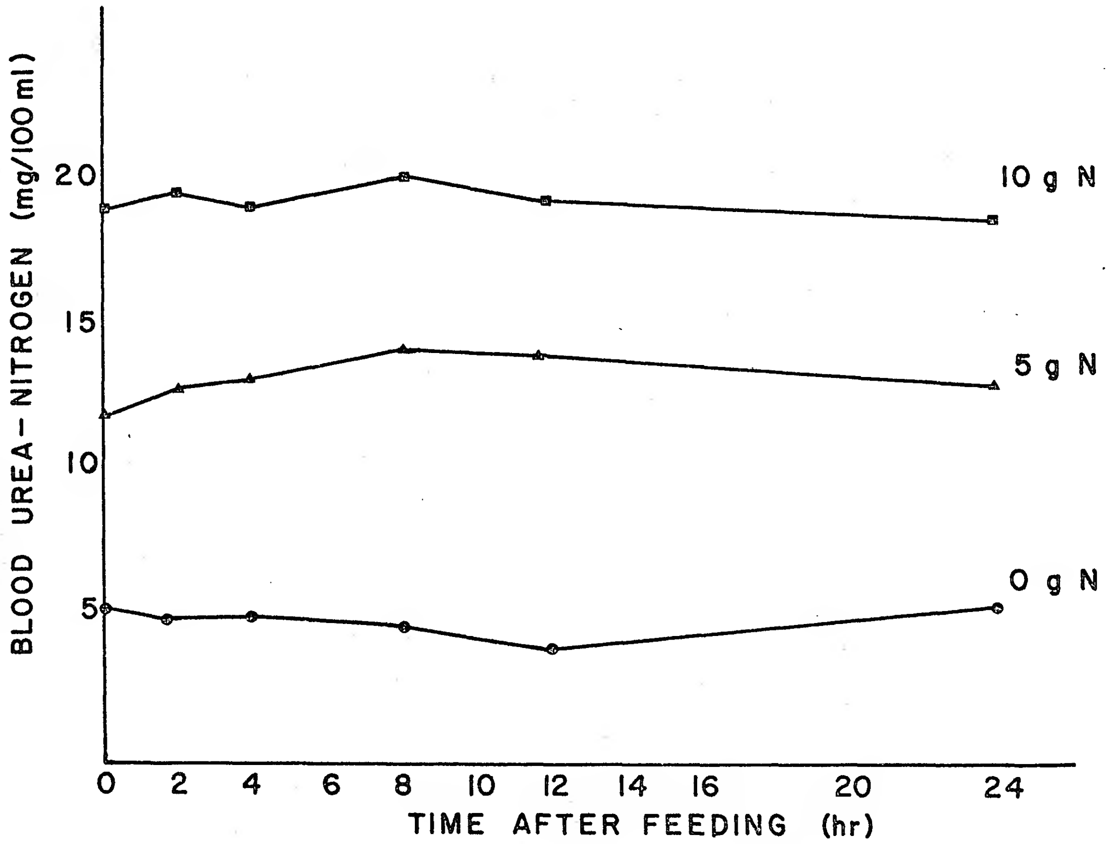

Figure 4. Effect of level of supplemental biuret-nitrogen on blood urea nitrogen levels at various times after feeding, Experiment 6.

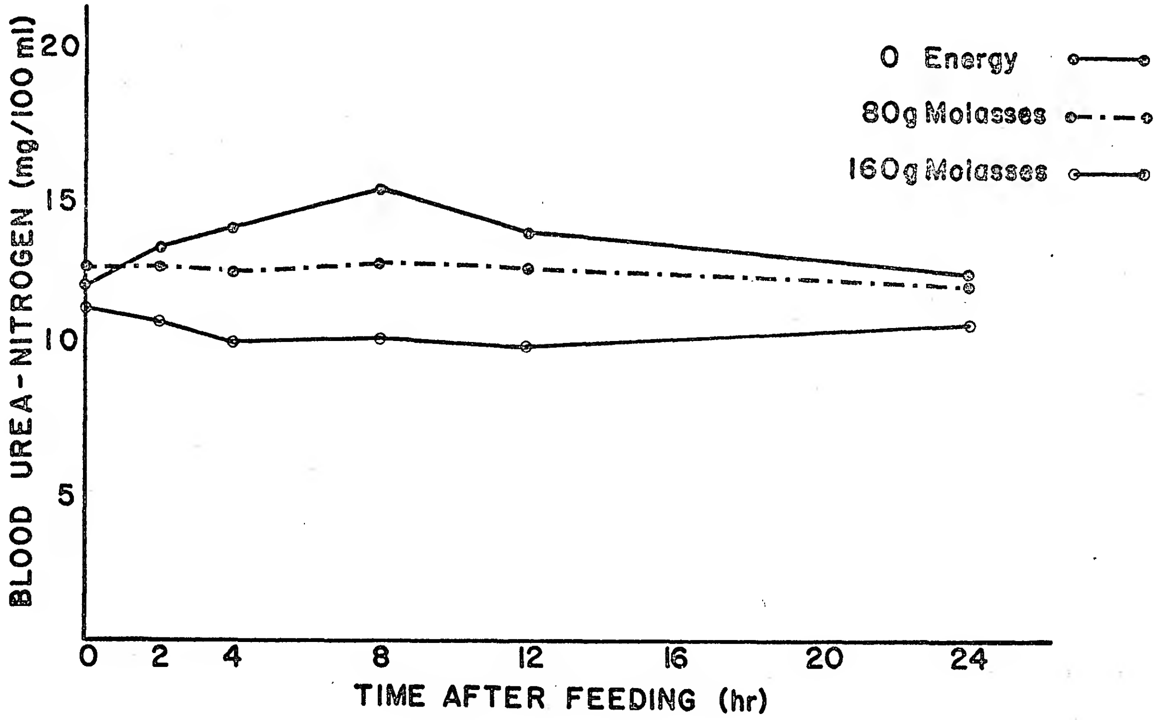

Figure 5. Effect of varying levels of supplemental molasses on blood urea-nitrogen levels at various times after feeding, Experiment 6.

and appendix table 36. There were no differences according to level of molasses fed. However, BUN levels were numerically highest for sheep receiving no molasses and lowest for sheep receiving 160 g of molasses at all post feeding times.

Discussion

Several authors (Hemsley and Moir, 1963; Blaxter and Wilson, 1963; Milford and Minson, 1965; and Minson, 1967) have suggested that the intake and utilization of forages containing less than 7% crude protein are enhanced by nitrogen supplementation. The Pangola digitgrass hay offered in these experiments contained from 3.35 to 3.90% crude protein (air dry basis) and in each case nitrogen supplementation raised the protein level of the diet to above 7%. Under these conditions nitrogen supplementation would have been expected to improve voluntary intake and utilization of the hay and, in fact, Fick et al. (1973) and Ammerman et al. (1972) have reported increased consumption and utilization of a similar hay under conditions approximating those of this study. However, voluntary intake of hay in these experiments was never improved by providing supplemental nitrogen from either urea or biuret or by providing supplemental energy from either corn or varying levels of molasses when compared to control animals receiving neither supplemental nitrogen nor energy. Reasons for this failure to improve hay intake are not obvious.

Five or 10 g of nitrogen supplementation from either urea (experiment 4) or biuret (experiment 6) did tend to produce an expected trend of increased hay consumption across the supplemental 80 and

160 g molasses treatments. This trend was also evident when 7 g of urea or biuret-nitrogen was provided with 80 g of molasses in experiment 5.

The utilization of hay, as measured by cellulose digestibility, was consistently improved (although often not significantly) across given energy levels by providing supplemental nitrogen.

Voluntary intake of low-quality forages has been related to slow digestion and slow passage of digesta through the digestive tract (Campling et al., 1962). Campling et al. (1962), Coombe and Tribe (1963) and Hemsley and Moir (1963) indicated that supplementation of low-quality forages would increase rumen microbial activity and the rate of digestion of the forages and, in turn, the rate of passage and intake of the forage would increase. In general, the present data support these suggestions when considered across a given level of molasses supplementation. Supplemental nitrogen also consistently improved organic matter digestibility and intake, suggesting that microbial activity of the rumen was improved by supplemental nitrogen.

Provision of supplemental energy as molasses decreased cellulose digestibility and hay intake in all three of the experiments reported here. However, provision of supplemental nitrogen (urea or biuret) with the molasses levels used tended to improve intake and digestibility values to levels equivalent to animals receiving no supplemental energy. Urea appeared to be more effective in this respect than was biuret.

The nitrogen status of animals was improved by providing supplemental nitrogen as either urea or biuret. In experiments 4 and

6, 10 g of supplemental nitrogen provided greater nitrogen balance than did 5 g of supplemental nitrogen. Increasing levels of supplemental nitrogen increased nitrogen intake, digestibility, absorption, urinary excretion and nitrogen retention in all three experiments. Animals receiving 5 g of urea-nitrogen in experiment 4 were in negative nitrogen balance while animals receiving 5 g of biuret-nitrogen in experiment 6 were in positive nitrogen balance. Animals used in experiment 4 were lighter weight and younger (39.7 kg and approximately 18 mo) than were animals used in experiment 6 (45.6 kg and approximately 30 mo). These factors would result in a greater nitrogen requirement for sheep in experiment 4.

Nitrogen retention in experiment 4 was greater (-.17 g/day) (P<.10) with sheep receiving 80 g of molasses daily than for sheep receiving 160 g of molasses daily (-.71 g/day). The difference is a reflection of less nitrogen absorbed (7.0 and 6.5 g/day for 80 and 160 g molasses, non-significant) and greater urinary nitrogen excretion (6.38 and 6.76 g/day for 80 and 160 g molasses, non-significant) for animals consuming the higher level of molasses. Increasing the relative amounts of nitrogen in the diet from molasses would lower the amount of nitrogen absorbed because of relatively low (28%, N.R.C., 1968) digestibility of the nitrogen from molasses and would, in part, account for the lower absorption observed. The molasses used in experiment 4 and 6 of this study was selected for its low crude protein content (2.39 and 2.65%) which could have resulted in an even lower crude protein digestibility. The reason for the greater urinary nitrogen in experiment 4 is not apparent and increasing urinary nitrogen levels with increasing molasses supplementation were not observed in experiment 6.

Increased levels of fecal nitrogen ($P<.05$) were also observed with increasing levels of supplemental molasses in experiment 6 and fecal nitrogen was greater ($P<.05$) when energy was provided as molasses in experiment 5. It is suggested that increased fecal nitrogen levels associated with the higher level of molasses supplementation was a result of increasing levels of non-digestible nitrogen provided by the molasses.

Fecal nitrogen levels in experiment 6 were also increased ($P<.05$) by increasing levels of biuret-nitrogen. Tiwari et al. (1973) reported that about 35% of the biuret fed passed out of the rumen undegraded and that a large portion of this may escape further breakdown or absorption in the lower gut and be excreted in the feces. Analysis of fecal samples of biuret fed (adapted) sheep used in their studies revealed that 10 to 14% of the biuret fed per day was excreted in the feces. In experiment 6, fecal nitrogen increased by .43 g/day when biuret-nitrogen supplementation increased from 0 to 5 and from 5 to 10 g/day. This increase in fecal nitrogen represents 8.6% of the increased intake of biuret-nitrogen.

Levels of BUN in experiment 4 and 6 reflect directly the level of supplemental nitrogen fed. Peak BUN levels in experiment 4 occurred at 4 and 8 hr after feeding for the 5 g nitrogen supplementation level and at 8 and 12 hr for the 10 g nitrogen supplementation level. Figure 2 suggests a somewhat later peak for sheep receiving 10 g of urea-nitrogen. The late peak reflects the slower consumption of supplements containing higher levels of urea (3.1 vs 5.2 hr) and might have been expected.

Levels of BUN for sheep receiving urea were lower at 0 and 24 hr than for sheep receiving biuret. However, peak BUN values were higher for urea and reflected the more rapid hydrolysis of urea and absorption of ammonia into the blood system. Post feeding increases in BUN levels in experiment 6 were not of the same magnitude as those observed with biuret in experiment 5. Reasons for these differences are not apparent although the data may suggest that hydrolysis of biuret in the latter experiment was occurring at a slower rate than in experiment 5.

Increasing energy levels depressed BUN levels in both experiment 4 and 6 (figure 2 and 5) and suggest that the presence of more readily available energy could have resulted in a greater incorporation of NPN into microbial protein and thus lowered the amount of ammonia absorbed from the rumen and converted into blood urea. The data may also represent a greater utilization of absorbed ammonia by the body as a result of more available energy. Fick (1970) reported similar results.

Under the conditions of experiment 4, maximum performance appears to have been supported by supplementing sheep consuming a low-quality hay with a combination of 10 g of urea-nitrogen and 80 g of molasses per head daily. This treatment was consumed over a 5 hr period and resulted in the greatest organic matter intake, cellulose digestibility, nitrogen retention and BUN values. The voluntary intake of hay and organic matter digestibility supported by this treatment was not different from the highest values observed for these criteria. These results are in agreement with data reported by Beames (1960) which indicated that the ratio of urea to molasses could be reduced from 1:8 to 1:2 and still maintain the effectiveness of the supplementation. Urea to molasses ratios, where relevant in these experiments, ranged from approximately 1:16 to 1:4 with the latter supporting maximum results.

In experiment 5 and 6, animal variation was large and this, in combination with small numbers per treatment, resulted in few significant differences. Energy, supplemented as corn in experiment 5, did support greater ($P<.05$) nitrogen digestibility and retention than did energy supplemented as molasses. The National Research Council (1968) suggested a crude protein digestibility of 28% for molasses and 78% for corn and these differences in digestibility of the crude protein of the energy supplement would account for the magnitude of difference in nitrogen digestibility and retention of entire diets.

Animals receiving biuret and corn were in greater positive ($P<.05$) (1.12 g/day) nitrogen balance than animals consuming biuret and molasses (.02 g/day). However, it should be emphasized that variation within each treatment was high and especially so for animals receiving biuret.

Individual animal variation within treatments was also large during experiment 6 and no combination of biuret and molasses appears to have promoted over-all improved performance. The combination of 10 g of biuret-nitrogen and 160 g of molasses resulted in the greatest nitrogen retention and voluntary intake of organic matter. Hay intake and digestibility of cellulose and organic matter for animals receiving this treatment were also equivalent to the highest levels observed. Clemens and Johnson (1972) have reported increased hydrolysis and utilization of biuret with increasing levels of dietary energy.

The point of importance in experiments 5 and 6 may well be the variation between animals in utilization of biuret. Johnson and Clemems (1973) and Schröder and Gilchrist (1969) have reported that adaptation to biuret may require from 10 to 70 days. In general this variation in adaptation time has been reported to result from differences in the diet with which biuret is fed. These studies have also indicated relatively wide individual variation in adaptation and utilization of biuret, as was the case in this study. Other workers who investigated the utilization of biuret (Johnson and McClure, 1964; Karr et al., 1965a,b; Oltjen et al., 1969; Oltjen et al., 1974; Rush and Totusek, 1973; and Schaadt et al., 1966) also reported greater individual variation in utilization of biuret than would be expected for studies under the reported conditions.

Animals utilized in the present studies were from a uniform group and were handled in a manner that had resulted previously in uniform results and there were no other apparent reasons for observed variability. It is therefore suggested that individual animal response to and utilization of biuret is highly variable and that significant differences might have been observed in this study, if the number of animal observations had been much larger.

CHAPTER V

FORMALDEHYDE TREATED SOYBEAN MEAL AS A SOURCE OF SUPPLEMENTAL NITROGEN FOR SHEEP CONSUMING A LOW-QUALITY FORAGE

Experiment 7

Natural protein supplements, provided to ruminants consuming low-quality forage diets, are degraded extensively by the rumen microbes. The nitrogen of these proteins is released as ammonia in the rumen and in turn is either utilized by the rumen microbes for synthesis of microbial protein or lost from the rumen into the animal's blood system. This process decreases the efficiency with which natural proteins are utilized because the nitrogen is either lost or converted into microbial proteins which may be of lower quality than the natural protein itself.

Treatment of natural proteins with formaldehyde has been shown to reduce their solubility (Martinez, 1975; and Schmidt et al., 1974) and decrease their degradation in the rumen (Faichney and Weston, 1971; and MacRae et al., 1972) which leads to an increased amount of crude protein reaching the intestine. Improvements have been reported in the rate of wool growth (Ferguson et al., 1967; and Reis and Tunks, 1969) and live weight gain for lambs (Faichney, 1971; and Peter et al., 1971) and calves (Faichney and Davies, 1973) when dietary protein has been treated for protection against ruminal degradation. The improvements in performance probably represent a response to increased amounts of crude proteins and amino acids reaching and being digested in the intestine (Faichney, 1971).

Other workers (Moir and Harris, 1962; Egan, 1965a,b,c; and Egan and Moir, 1965) have suggested that over-all nutritional status of a ruminant animal is an important factor in influencing the intake and utilization of low-quality forages. Supplementation of animals consuming low-quality forages with protected proteins may produce a more positive general nutritional status and in turn enhance the consumption and utilization of low-quality diets.

This study was undertaken to compare formaldehyde treated soybean meal (F-SBM) to untreated soybean meal (N-SBM) and biuret as sources of supplemental nitrogen for sheep consuming a low-quality Pangola digitgrass hay.

Procedure

Thirty Florida native mature wethers were utilized to evaluate soybean meal treated with .6% formaldehyde as a source of supplemental nitrogen for sheep consuming a low-quality hay. The animals ranged in weight from 54.1 to 65.9 kg with an average initial weight of 60.5 kg (table 32). Sheep were shorn and treated with an antihelmentic approximately 1 week prior to random assignment to one of the five treatments shown in table 32. Sheep were housed in elevated wooden cages (1 x 1.3 m) with expanded metal floors for a 34-day voluntary intake period. The first 26 days of this period were allowed for animals to adapt to the diets and voluntary intake of the

TABLE 32

COMPOSITION, DAILY FEEDING LEVEL AND PROXIMATE ANALYSIS OF SUPPLEMENTS AND HAY, AND INITIAL SHEEP WEIGHTS, EXPERIMENT 7

Item	Treatment				
	1	2	3	4	5
Ingredient, g/day					
Corn meal	45.0	45.0	22.5	-----	-----
Corn starch	22.5	22.5	11.2	-----	-----
Sucrose	22.5	22.5	11.2	-----	-----
Treated soybean meal[a]	-----	-----	45.0	90.0	-----
Biuret[b]	-----	16.0	8.0	-----	-----
Soybean meal	-----	-----	-----	-----	90.0
Mineral and vitamin mix[c]	25.0	25.0	25.0	25.0	25.0
Sodium sulfate	3.2	3.2	3.2	3.2	3.2
Total supplement fed:	118.2	134.2	125.1	118.2	118.2
Analysis, %					
Crude protein	4.5	31.3	25.9	35.5	35.2
Dry matter	91.4	91.0	91.0	90.4	91.2
Ash	24.2	18.8	23.2	26.4	26.8
Hay[d], g/day	700	700	700	700	700
Initial sheep weights, kg	59.2	61.0	60.5	60.1	61.6

[a] 44% crude protein soybean meal treated with .6% formaldehyde, Dow Chemical Company, Midland, Michigan.

[b] Kedlor.

[c] Minimum analysis in %: Ca, 16.0; P, 8.0; NaCl, 23.0; Mg, 2.0; Fe, 1.0; Cu, .1; Mn, .2; Zn, .25; Co, .03; I, .01; F, .08; vitamin A-USP units, 68,182 per kg; vitamin D_3 -USP units 6,818 per kg.

[d] Hay, Pangola digitgrass, provided _ad libitum_ during voluntary intake period; 90.5% dry matter, 3.9% crude protein, 37.5% cellulose (values as fed), 96.5% organic matter as a % of dry matter.

3.9% crude protein hay was determined during the last 8 days. Supplements, as shown in table 32, were mixed at two week intervals and weighed into individual plastic containers according to daily allotment. The Pangola hay was ground through a hammer mill with a 12 mm screen prior to feeding. Methods of feeding both hay and supplements, as well as other experimental details were as described in experiment 4.

Following determination of voluntary intake, sheep were placed in metabolism cages and fitted with canvas collection bags to permit total collection of feces and urine. Following a 4-day adaptation period, feces and urine were collected for 7 days. Details of the collection procedure were as described in experiment 4. Blood samples were taken immediately prior to and 2, 4, 8, 12 and 24 hr after feeding for determination of blood urea-nitrogen (BUN) levels as described in experiment 4.

Chemical analysis of samples and statistical analysis of data as well as criteria observed were as described previously (experiment 4).

Results

The composition, daily feeding levels and proximate analysis of supplements utilized in this experiment are shown in table 32. An apparent error in mixing supplement 3, which was to have provided 3 g of supplemental nitrogen daily from biuret and 3 g from treated soybean meal, resulted in a lower concentration of crude protein in that supplement and in turn a decrease in daily nitrogen intake for sheep consuming the supplement (table 33).

Dietary crude protein, voluntary intake of hay and organic matter; digestibility of cellulose; organic matter and nitrogen; and nitrogen balance data are shown in table 33. All sources of supplemental nitrogen (treatments 2-5) resulted in dietary crude protein levels in excess of 7%, while the crude protein level of the control treatment (1) was 4%. Hay consumption for animals receiving both F-SBM and N-SBM (treatments 4 and 5) was greater (P<.05) (1002 and 874 g/day respectively) than was hay consumption for animals receiving no supplemental nitrogen (treatment 1, 672 g/day). Hay consumption for treatments 2 and 3 (biuret and biuret plus F-SBM) was 750 and 814 g/day respectively. These values differed from neither the control (treatment 1) nor N-SBM (treatment 5). Organic matter intake was affected in a manner similar to hay intake and might have been anticipated in that a relatively constant amount of organic matter was consumed by each treatment group from supplements in addition to hay.

Cellulose and organic matter digestibility did not differ (P>.10) according to treatment. Both criteria, however, were improved by providing supplemental nitrogen (treatments 2-5). The highest numerical cellulose digestibility (55.1%) was observed with F-SBM.

Nitrogen digestibility was greatest (P<.05) for animals receiving supplemental nitrogen as biuret (57.3%) (treatment 2) or N-SBM (58.1%) (treatment 5) and least for animals receiving no supplemental nitrogen (11.1%) (treatment 1). Sheep receiving biuret and F-SBM or F-SBM (treatments 3 and 4) had intermediate nitrogen digestibility (P<.05) (45.4 and 47.5% respectively).

TABLE 33

EFFECT OF SUPPLEMENTAL SOURCE OF NITROGEN ON VOLUNTARY INTAKE OF ORGANIC MATTER AND HAY; DIGESTIBILITY OF CELLULOSE, ORGANIC MATTER AND NITROGEN AND NITROGEN UTILIZATION, EXPERIMENT 7

	Treatment				
Item	1	2	3	4	5
Nitrogen Supplement	none	biuret	biuret + F-SBM	F-SBM	N-SBM
Dietary crude protein, %	4.0	8.1	7.9	7.2	7.6
Voluntary intake, g/day					
Hay[a]	672[e]	750[de]	814[cde]	1002[c]	874[cd]
Organic matter[b]	676[e]	764[de]	808[cde]	961[c]	850[cd]
Digestibility %					
Cellulose	46.8	52.9	53.9	55.1	52.4
Organic matter	48.0	52.1	52.1	51.9	50.5
Nitrogen	11.1[c]	57.3[e]	45.4[d]	47.5[d]	58.1[e]
Nitrogen balance, g/day					
Intake	4.12[c]	10.38[e]	9.12[d]	10.60[e]	10.65[e]
Feces	3.66[c]	4.52[d]	4.98[de]	5.55[e]	4.45[d]
Absorbed	.46[c]	6.06[f]	4.14[d]	5.04[e]	6.19[f]
Urine	1.78[c]	6.04[f]	4.66[d]	4.21[d]	5.60[e]
Retained	-1.32[c]	.02[cde]	-.52[cd]	.84[e]	.60[de]
Retained % intake	-------	.2	-------	7.9	5.6

[a]As fed.

[b]Dry basis.

[c,d,e,f]Values in same row with different superscripts differ. (P<.05)

Nitrogen intake was lower for treatment 1 ($P<.05$) (4.12 g/day) than for animals receiving supplemental nitrogen in any form (treatments 2-5). However, due to the lower concentration of nitrogen in the supplemental mixture, animals receiving biuret and F-SBM (treatment 3) had a lower daily nitrogen intake (9.12 g/day) ($P<.05$) than did animals on other supplemental nitrogen treatments (10.38, 10.60 and 10.65 g/day for treatments 2, 4 and 5 respectively). Fecal nitrogen excretion was greatest ($P<.05$) for animals receiving F-SBM (treatment 4, 5.55 g/day) and lowest for control animals (3.66 g/day). Other treatments were intermediate and did not differ ($P>.05$). Animals receiving biuret (treatment 2) and N-SBM (treatment 5) absorbed the greatest amount of nitrogen (6.09 and 6.19 g/day respectively) ($P<.05$) while control animals (treatment 1) absorbed the least ($P<.05$) amount of nitrogen (.46 g/day). Animals receiving F-SBM and biuret or F-SBM (treatments 3 and 4) absorbed 4.14 and 5.04 g nitrogen/day and these values differed ($P<.05$) from each other and all other treatments.

Urinary nitrogen excretion was lowest for control animals (1.78 g/day) ($P<.05$). Of the nitrogen supplemental treatments, the greatest urinary nitrogen loss was by sheep receiving biuret (treatment 2, 6.04 g/day) ($P<.05$) followed by animals receiving N-SBM (treatment 5, 5.6 g/day) ($P<.05$). Treatments 3 and 4 (biuret plus F-SBM and F-SBM) had the lowest urinary nitrogen loss (4.66 and 4.21 g/day respectively) ($P<.05$). Values for treatments 3 and 4 did not differ ($P>.05$). Nitrogen retention for animals receiving either F-SBM or N-SBM (treatments 4 and 5) was greater (.84 and .60 g/day respectively) (P<.05) than that of the control treatment (1) (-1.32 g/day). Values for other treatments were intermediate and did not differ (P>.05).

Blood urea-nitrogen levels are shown in figure 6 and appendix table 37. Control animals had the lowest BUN levels (P<.05) during the 24 hr period and might have been anticipated. The values agree with those observed in experiments 4, 5 and 6. Animals receiving N-SBM (treatment 5) had consistently the highest BUN levels and BUN with other supplemental nitrogen treatments was intermediate.

Discussion

The treatment of natural proteins with formaldehyde results in a cross-linkage of protein chains. The cross-linkage is stable at neutral or alkaline pH conditions and results in reduced solubility of the protein (Fraenkel-Conrat et al., 1945). However, the formaldehyde cross-linkage breaks down as the pH becomes more acid and in turn the protein increases in solubility. The rumen pH of animals consuming a high roughage diet is 6 or above (Martin et al., 1969) while the pH of the abomasum is approximately 3. The change from near basicity to high acidity should allow formaldehyde treated protein to pass through the rumen undegraded and into the abomasum where it should be digested.

The protected SBM used as a supplement in this study had been utilized previously in a growth study with lambs receiving a high energy diet (Martinez, 1975). Lamb responses to formaldehyde treatment levels were compared with 0, .2, .4, .6 and .8% formaldehyde

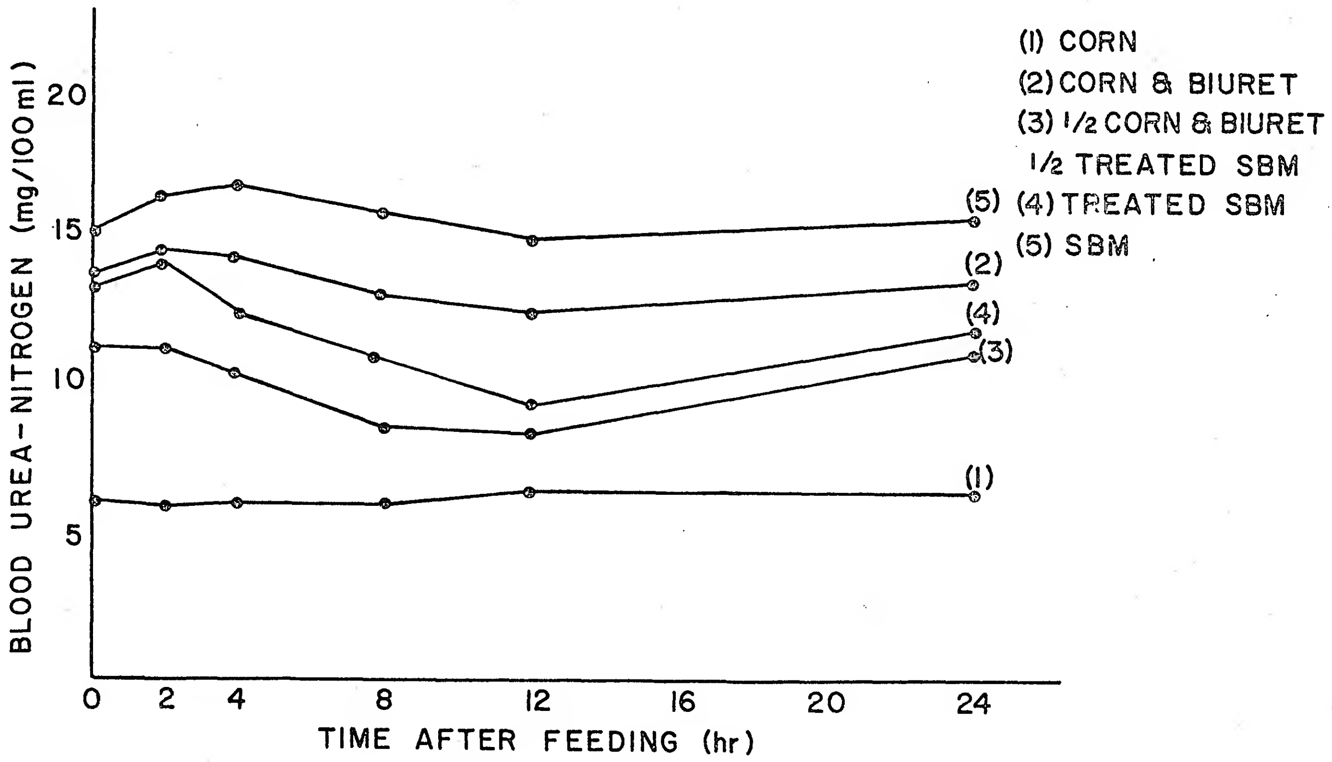

Figure 6. Effect of source of supplemental nitrogen on blood urea-nitrogen levels at various times after feeding, Experiment 7.

treatment. Lambs receiving the .6% treatment level, which was also utilized in the present study, had the greatest (non-significant, P>.10) weight gain of the 5 treatment levels. Martinez (1975) also reported that the nitrogen solubility of the F-SBM, which was utilized in both studies, was greatly reduced (4.81%) when compared with the N-SBM (77.56%). The solubility values were determined in .02N NaOH.

In this study F-SBM did result in the greatest numerical intake of hay and organic matter and the most positive nitrogen balance.

Hay intake for animals receiving F-SBM was approximately 50% greater (P<.05) than that of control animals and approximately 15% greater than that of animals receiving N-SBM. Clark (1951) and Donefer et al. (1960) have attributed increases in intake of low-quality hay, associated with increasing levels of dietary nitrogen, to changes in the rate of cellulose digestion which produces a more rapid rate of passage of food through the intestinal tract. In this study, all forms of supplemental dietary nitrogen resulted in numerically greater intakes of hay and cellulose digestibilities. Assuming that intake would improve in a manner directly related to the improvement in cellulose digestibility only the improved hay intake observed with animals receiving biuret (treatment 2) could be accounted for by the increase in cellulose digestibility. Improved cellulose digestibility of animals receiving F-SBM (treatment 4) could account only for approximately 1/3 of the observed increased hay intake. These data would support implications by Egan (1965a,b,c), Egan and Moir (1965) and Verde (1971) that, with improvement of the nitrogen status and in turn the over-all physiological status of the animal, the intake of a low-quality forage improves.

Decreased nitrogen digestibility, which reflects the somewhat elevated fecal nitrogen and depressed absorbed nitrogen values, was observed for both groups of animals receiving F-SBM (treatments 3 and 4) and is in agreement with data reported by other workers (Nishimuta et al., 1973; Rodriguez et al., 1974; and Schmidt et al., 1973, 1974). Apparently some decrease in solubility of the protein occurred even in the acid medium of the abomasum, which in turn decreased the digestibility of the protein.

A combination of factors suggest that F-SBM was degraded to a lesser extent than N-SBM in the rumen. These factors include: (1) a greater amount of nitrogen absorbed, (2) a greater amount of nitrogen excreted in the urine and (3) higher BUN levels associated with N-SBM when compared to those for F-SBM. The greater nitrogen absorption and higher BUN levels suggest that more N-SBM nitrogen was absorbed as NPN and in turn converted to BUN and eventually was excreted in the urine. Lower urinary nitrogen levels and greater nitrogen retention which were associated with F-SBM suggest that more nitrogen was absorbed as amino acids and retained by the body. The negative nitrogen balance, as well as other criteria associated with treatment 3, are difficult to evaluate due to the lower daily intake of nitrogen. However, it is felt that the decreased nitrogen intake, due to an error in mixing supplement, was responsible for negative nitrogen balances.

Overall, it is concluded that protected protein, under the conditions of this study, was very effective as a source of supplemental nitrogen for animals consuming a low-quality roughage and that further work under more practical conditions is needed to determine the value of this type of nitrogen supplementation under other conditions.

CHAPTER VI

GENERAL SUMMARY

Seven experiments were conducted: three were undertaken to evaluate urea and biuret as sources of supplemental nitrogen for brood cows being wintered under practical range conditions; three to study the utilization of a low-quality roughage and nitrogen by sheep when supplemented with varying forms and levels of NPN (biuret and urea) and varying forms and levels of energy (molasses and corn); and one for comparison of formaldehyde treated soybean meal with untreated soybean meal and biuret as sources of supplemental nitrogen for sheep consuming a low-quality forage. A summary of the results of these studies is as follows.

Urea as a Source of Supplemental Nitrogen for Wintering Beef Cows

A herd consisting of 66 mature Angus cows was utilized in a 2 x 2 factorial experiment to compare cottonseed meal cubes (CSM) (44.9% crude protein) to cubes containing CSM, citrus pulp and urea (43.0% crude protein), where 50% of the nitrogen was supplied by urea, as sources of supplemental nitrogen. In addition, Pangola digitgrass (Digitaria decumbens) hay was compared with sorghum (Sorghum vulgare) silage as a source of supplemental forage during the winter months.

Cows were randomly assigned to treatment in August and remained on Argentine bahiagrass (Paspalum notatum) pastures throughout the winter. Supplements and supplemental forages were fed three times weekly. CSM and urea cubes were provided in amounts of .91 and .94 kg/head daily, respectively, and forages were provided *ad libitum*.

Source of supplemental winter nitrogen or forage resulted in no differences among observed criteria. Average weight change (November to March) for all cows was -34 kg, while conception rate was 88%, calf survival was 92% and 205-day adjusted weaning weight of calves was 191 kg. Cow body weights increased 10% during this study and over-all performance of all cows was considered excellent. It was suggested that cows were not subjected to sufficient nutritional stress, due to favorable environmental conditions, to demonstrate differences between the supplemental nitrogen sources provided.

Biuret as a Source of Supplemental Nitrogen for Wintering Beef Cows, I

A herd consisting of 78 and 106 mature Angus cows was used during a 2-year period to compare CSM cubes (44.8% crude protein) with citrus pulp cubes containing feed grade biuret (CPB) (41.3% crude protein) as sources of supplemental nitrogen and to compare Pangola digitgrass hay to sorghum silage as sources of supplemental forages for wintering beef cows. The experiment was a 2 x 2 factorial in design. CSM and CPB cubes were fed three times weekly in amounts equal to .9 and 1.0 kg/head daily. Supplemental forage were fed *ad libitum* three times weekly.

Over the 2-year period, cows receiving supplemental nitrogen from CSM cubes lost less weight ($P<.01$) during the winter period (-61.5 kg) than did animals receiving CPB cubes (-84.0 kg). Cows receiving silage lost less weight (-59 kg) ($P<.05$) during the first winter than did cows receiving hay (-74 kg). Final cow weights (August) were less (413 vs 428 kg) ($P<.05$) for cows wintered on CPB cubes during one of the two years of the study. Over-all conception rate was 93% and 86% ($P<.07$) for cows wintered on CSM and CPB supplements respectively. Adjusted weaning weights for calves from all treatments was 176 kg and did not differ according to winter nitrogen supplement. However, cows receiving hay weaned a 9 kg ($P<.05$) heavier calf during the first year. Performance of cows receiving cubes with biuret was not equivalent to that of cows receiving cottonseed meal.

Biuret as a Source of Supplemental Nitrogen for Wintering Beef Cows, II

Mature Angus cows and yearling Angus heifers were used during a 3-year period to compare the performance of animals wintered (December to March) with supplements of (1) cottonseed meal cubes (CSM) containing 41% crude protein, (2) citrus pulp cubes with feed grade biuret (CPB) containing 38% crude protein or (3) citrus pulp cubes without biuret (CP) containing 7% crude protein. The supplements were provided three time weekly at rates of .9, 1.0 and .86 kg per head daily, respectively. All animals remained on Argentine bahiagrass pasture and Pangola digitgrass hay was provided as the main supplemental energy feed during the winter. Cattle were randomly assigned to treatments in August of each year. For the 3-year period, mature cows receiving CSM cubes lost 49 kg, those receiving CPB cubes lost 60 kg and those receiving CP cubes lost 70 kg per cow during the winter period. All values differed significantly ($P<.01$). Winter weight gain by heifers was not affected ($P>.05$) by treatment during the first year, but during the second year, heifers receiving CP cubes gained more weight. Conception rate, calf survival and time required to reconceive were not influenced by wintering treatment. Cows receiving CPB cubes weaned lighter weight ($P<.01$) calves (162 kg) than did cows receiving CP cubes (166 kg) or CSM cubes (167 kg).

Varying Levels of Urea and Molasses for Sheep Consuming a Low-Quality Forage

A voluntary intake and metabolism study was conducted to determine the effect of supplementing sheep consuming a 3.9% crude protein Pangola digitgrass hay with 0, 5 or 10 g of urea-nitrogen in combination with 80 or 160 g of molasses per day. In addition a control treatment received no supplemental energy or nitrogen. Blood samples were taken for blood urea-nitrogen determination just prior to and 4, 8, 12 and 24 hr after feeding.

Hay and organic matter intakes were; 502, 471; 512, 511; and 535, 537 g/day, respectively ($P>.10$) for sheep receiving 0, 5 and 10 g of urea-nitrogen/day. Hay intake was less (480 g/day) ($P<.05$) for sheep receiving 160 g of molasses than for the control treatment (597 g/day). Sheep receiving 80 g of molasses consumed 520 g/day of hay which was not different from either of the other treatments.

Voluntary intake of hay for control animals (597 g/day) was not exceeded by animals receiving any supplemental combination. Increasing nitrogen levels improved cellulose digestibility in a manner which did not differ from linearity at both the 80 g ($P<.05$) and 160 g ($P<.10$) level of molasses supplementation. Organic matter digestibility was greater (55.4%) ($P<.05$) when 10 g of urea-nitrogen was provided than when no supplemental nitrogen was fed (49.3%). Organic matter digestibility was intermediate with 5 g of supplemental nitrogen (53.0%) ($P<.05$).

Nitrogen retention was -1.88, -.40 and .79 g/day ($P<.05$) for sheep receiving 0, 5 and 10 g of urea-nitrogen daily. Nitrogen retention was greater with 80 g of molasses, -.17 g/day ($P<.10$), than with 160 g molasses, -.71 g/day. Increasing the urea-nitrogen intake from 0 to 5 to 10 g/day increased time required to consume supplements from .3 to 3.1 to 5.2 hr ($P<.01$). Blood urea-nitrogen levels increased according to increasing supplemental nitrogen levels and were consistently less (non-significant, $P>.10$) with 160 g of molasses than with 80 g of molasses at each level of nitrogen supplementation. BUN levels were highest at 8 and 12 hr after feeding.

Biuret vs Urea and Corn vs Molasses as Sources of Supplemental Nitrogen and Energy for Sheep Consuming a Low-Quality Forage

A voluntary intake and metabolism trial was conducted to study the effect of supplementing sheep consuming a 3.6% crude protein hay with no supplemental nitrogen or with 7 g of nitrogen daily from urea or biuret in combination with 60 g of corn (50% corn meal, 25%

cornstarch, 25% sucrose, 4.8% crude protein) or 80 g of molasses daily. In addition, one group of animals received neither supplemental nitrogen nor energy. Blood samples were taken for BUN determination just prior to and 4, 8, 12 and 24 hr after feeding.

Hay and organic matter intakes averaged 770 and 710 g/day respectively during this study and did not differ according to source of supplemental energy or nitrogen or any combination of these two factors. Cellulose digestibility was improved when nitrogen was supplemented from either urea or biuret ($P<.05$) (53.6, 58.8 and 57.2% for no supplemental nitrogen, urea or biuret respectively). Organic matter digestibility was least ($P<.05$) with no supplemental nitrogen (50.7%), greatest with urea (54.9%) and intermediate (53.2%) ($P>.05$) with biuret. Supplemental energy as either corn or molasses improved organic matter digestibility ($P<.05$) by 5.2 and 4.7% respectively.

Nitrogen balance was positive with both urea and biuret supplements and negative when no supplemental nitrogen was provided. Sheep receiving supplemental energy as corn retained more nitrogen (.53 g/day) ($P<.05$) than did sheep receiving molasses (.07 g/day). BUN levels were higher for sheep receiving supplemental urea or biuret over the 24 hr period after feeding than for sheep receiving no supplemental nitrogen. BUN values for sheep consuming urea were lower than those for sheep consuming biuret at 0 and 24 hr ($P<.05$) but reached higher (non-significant, $P>.05$) peak levels at 4 and 8 hr.

Varying Levels of Biuret and Molasses for Sheep Consuming a Low-Quality Forage

A 3 x 3 factorially designed voluntary intake and metabolism study was conducted to determine the effects of supplementing sheep consuming a 3.35% crude protein hay with 0, 5 or 10 g of biuret nitrogen in combination with 0, 80 or 160 g of molasses per day. Blood samples were taken for BUN determination just prior to and at 2, 4, 8, 12 and 24 hr after feeding.

Voluntary intake of hay and organic matter averaged 562 and 557 g for all treatments and was not different according to level of biuret-nitrogen or molasses. Digestibility of cellulose and organic matter was not affected by supplemental nitrogen level. Cellulose digestibility was greatest with animals receiving no molasses (56.7%) ($P<.05$), least with animals receiving 160 g/day of molasses (53.6%) and intermediate (56.3%) with animals receiving 80 g/day of molasses ($P>.05$). In contrast, organic matter digestibility was highest with 160 g molasses (58.6%) ($P<.05$), lowest with no supplemental molasses (51.4%) and intermediate with 80 g of molasses ($P>.05$).

Nitrogen retention was -1.27, .73 and 2.36 g/day ($P<.05$) for animals receiving 0, 5 and 10 g per head daily of biuret-nitrogen. BUN values over the 24 hr period after feeding were progressively greater for 0, 5 and 10 g nitrogen levels ($P<.05$) and did not differ according to molasses level. However, BUN levels were numerically highest for animals receiving no supplemental energy and lowest for animals receiving 160 g of molasses daily at all bleedings.

Formaldehyde Treated Soybean Meal as a Source of Supplemental Nitrogen for Sheep Consuming a Low-Quality Forage

A voluntary intake and metabolism trial was conducted to study the effect of supplementing sheep consuming a 3.9% crude protein hay with 6 g of nitrogen per day from biuret plus energy supplement, soybean meal (N-SBM), soybean meal treated with .6% formaldehyde (F-SBM) or a combination of biuret, energy supplement and F-SBM (3 g of nitrogen from each source). In addition, a control group of animals received an equivalent amount of energy from the energy supplement (50% corn meal, 25% corn starch and 25% sucrose, 4.5% crude protein). Blood samples were taken just prior to and at 2, 4, 8, 12 and 24 hr after feeding for determination of BUN levels.

Voluntary intake was greater for sheep receiving either N-SBM or F-SBM (874 and 1002 g/day respectively) ($P<.05$) than for animals receiving only the energy supplement (672 g/day). Other treatments were intermediate (750 and 814 g/day for biuret and biuret + F-SBM respectively). Cellulose digestibility and organic matter digestibility were not different according to treatment but were non-significantly higher for all animals receiving supplemental nitrogen. Nitrogen retention was -1.32, .02, -.52, .84 and .60 g/day for animals receiving energy, energy + biuret, energy + biuret and F-SBM, F-SBM and N-SBM respectively. The negative value (-.52 g/day) associated with the energy + biuret and F-SBM was apparently due to decreased nitrogen intake resulting from an error in mixing this supplement. BUN levels were higher for animals receiving supplemental nitrogen than for control animals ($P<.05$) over the 24 hr period. There were no significant differences in BUN levels between treatments receiving supplemental nitrogen. However, values were numerically higher at all times for animals receiving N-SBM.

Over-all performance of animals receiving F-SBM appeared to be superior to that of animals receiving other nitrogen supplements.

APPENDIX

TABLE 34

EFFECT OF LEVEL OF ENERGY (MOLASSES) AND NITROGEN (UREA) ON BLOOD UREA-NITROGEN (Mg/100 ml) LEVELS AT VARIOUS TIMES AFTER FEEDING, EXPERIMENT 4

Hours After Feeding	Urea-N, g/head daily						
	0			5		10	
	Molasses, g/head daily						
0	6.08[a]	5.55[a]	5.38[a]	13.08[bc]	11.55[b]	17.60[d]	15.62[cd]
4	6.20[a]	5.00[a]	3.90[a]	19.20[c]	16.18[b]	23.30[d]	21.62[cd]
8	5.65[a]	4.35[a]	3.70[a]	19.58[c]	15.82[b]	27.40[d]	23.30[d]
12	4.30[a]	3.25[a]	3.00[a]	17.52[b]	14.18[b]	25.80[c]	25.32[c]
24	5.33[a]	4.48[a]	4.98[a]	13.65[b]	12.15[b]	17.75[c]	16.82[c]

[a,b,c,d]Values in same row with different superscripts differ. ($P<.05$).

TABLE 35: EFFECT OF SOURCE OF SUPPLEMENTAL NITROGEN AND ENERGY ON BLOOD UREA-NITROGEN LEVELS (Mg/100 ml) AT VARIOUS TIMES AFTER FEEDING, EXPERIMENT 5.

	Nitrogen Source									
	None				Urea			Biuret		
Energy	None	Corn	Molasses	Av.	Corn	Molasses	Av.	Corn	Molasses	Av.
Hours After Feeding										
0	6.7	7.0	6.0	6.5^{a}	14.1	13.7	13.9^{b}	15.5	17.0	16.3^{c}
4	8.3	9.0	6.6	8.0^{a}	20.6	21.0	20.8^{b}	19.7	18.0	18.9^{b}
8	7.2	6.9	6.7	6.9^{a}	21.1	22.4	21.7^{b}	19.4	19.5	19.4^{b}
12	5.1	5.7	5.5	5.4^{a}	17.2	17.7	17.4^{b}	19.6	19.2	19.4^{b}
24	6.2	7.8	5.9	6.6^{a}	11.6	13.2	12.4^{b}	13.6	15.4	14.5^{c}

[a,b,c]Values in same row with different superscripts differ. ($P<.05$).

TABLE 36

EFFECT OF LEVEL OF SUPPLEMENTAL ENERGY (MOLASSES) AND NITROGEN (BIURET) ON BLOOD UREA-NITROGEN LEVELS (Mg/100 ml) AT VARIOUS TIMES AFTER FEEDING, EXPERIMENT 6

Hours After Feeding	Molasses g/day			Biuret-N g/day		
	0	80	160	0	5	10
0	12.04	12.56	11.25	5.19[a]	11.76[b]	18.91[c]
2	13.45	12.55	10.95	4.81[a]	12.69[b]	19.46[c]
4	14.16	12.19	10.08	4.71[a]	12.79[b]	18.92[c]
8	15.35	12.78	10.26	4.43[a]	13.97[b]	19.97[c]
12	13.91	12.62	10.01	3.80[a]	13.81[b]	18.92[c]
24	12.49	12.07	10.85	4.99[a]	12.61[b]	17.81[c]

[a,b,c]Values in same row with different superscripts differ. ($P<.05$).

TABLE 37

EFFECT OF SUPPLEMENTAL NITROGEN ON BLOOD UREA-NITROGEN LEVELS (mg/100 ml) AT VARIOUS TIMES AFTER FEEDING, EXPERIMENT 7

Hours After Feeding	Treatment				
	1	2	3	4	5
0	6.1^{a}	13.8^{bc}	11.2^{b}	13.4^{bc}	15.3^{c}
2	5.9^{a}	14.6^{c}	11.2^{b}	13.7^{bc}	16.5^{c}
4	6.1^{a}	14.4^{cd}	10.2^{b}	12.6^{bc}	16.9^{d}
8	6.0^{a}	13.1^{c}	8.6^{b}	11.0^{bc}	15.9^{d}
12	6.4^{a}	12.6^{b}	7.9^{a}	9.4^{a}	15.0^{b}
24	6.3^{a}	13.6^{bc}	11.0^{b}	12.0^{b}	15.7^{c}

[a,b,c,d]Values in same row with different superscripts differ. ($P<.05$)

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BIOGRAPHICAL SKETCH

Lethal Conrad Martin was born on March 25, 1945 in Cadiz, Kentucky, the son of Nila C. and Lethal Martin, Jr. He was raised on a general farm in Western Kentucky and was graduated from Trigg County High School, Cadiz, Kentucky in May, 1963. He attended the University of Kentucky, Lexington and was granted a Bachelor of Science degree in Agriculture with a major in Animal Science from that institution in August, 1967.

In September, 1967, he was granted a research assistantship at Oklahoma State University, Stillwater, and was awarded a Master of Science degree in Animal Science from that institution in May, 1969.

He joined the United States Army in February, 1969 and was commissioned a 2nd Lieutenant USAR in January, 1970 upon graduation from U.S. Army Field Artillary Officer Candidate School, Fort Sill, Oklahoma. He then served as an instructor with the U.S. Army Institute for Military Assistance, Fort Bragg, North Carolina and as a foward observer, fire director officer and executive officer with D. Battery, 319th Airborne Artillery, 173rd Airbore Brigade, Republic of Vietnam. While serving in the Republic of Vietnam, he was awarded the Bronze Star Metal with Oak Leaf Cluster and the Vietnamese Cross of Gallantry with Palm Leaf.

He was granted a research assistantship and enrolled in the Graduate School of the University of Florida, Gainesville, and is now a candidate for the degree of Doctor of Philosophy in Animal Science.

He is a member of Alpha Zeta, Sigma Xi, Gamma Sigma Delta and the American Society of Animal Science.

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

C. B. Ammerman

C. B. Ammerman, Chairman
Professor of Animal Science

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

Marvin Koger

Marvin Koger
Professor of Animal Science

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

John E. Moore

John E. Moore
Professor of Animal Science

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

P. E. Loggins

P. E. Loggins
Professor of Animal Science

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

James A. Himes

James A. Himes
Associate Professor of Veterinary Science

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

Eugene G. Sander

Eugene G. Sander
Professor of Biochemistry

This dissertation was submitted to the Graduate Faculty of the College of Agriculture and to the Graduate Council, and was accepted as partial fulfillment of the requirements for the degree of Doctor of Philosophy.

December, 1975

Jack L. Fry

Dean, College of Agriculture

Dean, Graduate School