EVALUATION OF LEVELS OF Ca, P, NPN SOURCES AND HEMICELLULOSE IN PURIFIED DIETS FOR SHEEP

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CHAPTER I

INTRODUCTION

The study reported here can be divided into three parts. The first part was designed to study the possibility of improving the performance of lambs fed purified diets. The need for this is apparent when it is considered that lambs fed purified diets, in which urea serves as the sole source of nitrogen, gain only about 70 percent as rapidly as do lambs fed a natural protein source. An evaluation of feeding high levels of urea and high levels of calcium and phosphorus was made in this regard.

The second part of the work was designed to evaluate a wood hemicellulose extract as a possible substitute energy source in the purified diet. If such a product could be demonstrated to serve as an efficient energy source for ruminants it might have important implications in terms of the world food shortage by helping remove the ruminant from competition with man for cereal grains.

The third part of this work was designed to contribute toward the evaluation of biuret and cyanuric acid as sources of non-protein nitrogen for ruminants. Urea has been used for several years as a substitute for natural protein in the rations of ruminants, however due to its toxicity under certain conditions and poor utilization with high fiber diets there is a need for other NPN sources which are less toxic and more efficiently utilized by ruminants fed low energy diets.

CHAPTER II

REVIEW OF LITERATURE

Introduction

In order to study the dietary effects of calcium, phosphorus, nitrogen source and availability, and energy source on the growth and performance of ruminants it is necessary to understand the factors involved in rumen metabolism. The performance of the animals is dependent on meeting the dietary needs of the rumen microorganisms which influence what subsequently becomes available to the animal's tissue.

The following review will cover some of the factors affecting utilization of purified diets by ruminants. Special emphasis will be placed on calcium and phosphorus, nitrogen level, and energy source. The review will also include the use of biuret and cyanuric acid as nitrogen sources, and factors regulating feed intake by ruminants.

Purified Diets

Growth rates of ruminants fed purified or semipurified diets have been found to be suboptimum (Oltjen, Sirny and Tillman, 1962a; Clifford, Goodrich and Tillman, 1967; Clifford, Bourdette and Tillman, 1968) as has milk production in cows (Virtanen, 1966). This has been particularly true when urea was the sole source of dietary nitrogen. Trials in which preformed protein sources have been included in the ration have indicated that part of the low performance is due to the inability

of the animals to utilize the nitrogen from urea efficiently (Oltjen, Sirny and Tillman, 1962a).

Studies have been conducted to ascertain factors that might be included in a purified diet to improve performance of animals fed such diets. Oltjen, Sirny and Tillman (1962b) found that a level of 30 percent cellulose was superior to levels of 40 and 50 percent cellulose in a purified diet, probably because a more readily available source of energy is needed for the utilization of urea (Mills, et al., 1942; Arias, et al., 1951; Belasco, 1956). An alkaline mineral mixture gave the same performance when fed at levels of 3.5, 5 and 6.5 percent of the diet. Other factors such as molybdenum, vitamins of the B-complex and amino acids have not proven to be of significant value in improving growth performance of ruminants fed a purified diet (Sheriha, Sirny and Tillman, 1962; Clifford, Goodrich and Tillman, 1967; Clifford, Bourdette and Tillman, 1968).

Calcium and Phosphorus

The essential role of calcium and phosphorus in the nutrition of ruminants is well known. Although the recommended level of calcium in the rations of lambs weighing 27 kg is 0.23% (N.R.C., 1968), Stuedemann (1970) obtained the best performance from lambs fed a purified diet containing 1.6% calcium followed by 0.58, 2.79, and 4.46%, respectively. Blaxter (1952) estimated the phosphorus requirement of sheep at 0.15-0.2% of dry matter intake and Wise (1958) estimated the requirement at 0.22%. Phosphorus performs a vital role in ruminants through its influence on the metabolism of microorganisms. The phosphorus requirements of microorganisms are fairly high, the cells containing 2 to 6%

phosphorus on a dry weight basis (Hungate, 1966). It is especially important that adequate phosphorus be available when animals are fed high levels of non-protein nitrogen because of their dependency on the rumen microorganisms to synthesize protein.

Urea Levels

Price and Smith (1968) reported that the performance of lambs on a purified diet, in which all of the dietary nitrogen was supplied by urea, could be improved when the urea was increased to supply an equivalent of 24 percent crude protein. Research has not been done to determine the optimum level of NPN which is needed in the purified diet for the greatest animal performance. The currently used crude protein equivalent levels are based on protein requirements which were established with natural diets (Oltjen, 1969). Virtanen (1966) found that milk production and the percent protein in the milk of cows fed purified diets, increased when the crude protein equivalent of the diet (from urea and ammonium salts) was raised from 12.8 to 15.0 percent.

Ellis, et al. (1956) reported the biological value of urea to be 54 for lambs. McNaught, et al. (1954) reported and Bergen, Purser and Cline (1968) confirmed that rumen bacterial protein had a true digestibility of 74, biological value of 81 and net protein utilization of 60 when fed to rats. True digestibility, biological value, and net utilization of protozoal protein were 90, 80 and 73, respectively.

Purser (1970) concluded that there is strong similarity between the amino acid composition of microbial protein from animals receiving different diets. These include low protein rations with most of the nitrogen as urea, a ration consisting of mostly alfalfa and shelled

corn, a ration containing alfalfa and corn cob, a ration containing mostly shelled corn and soybean meal and a semipurified diet. Attempts to explain differences between different rations based on bulk amino acid composition of rumen bacteria or protozoa appear to be futile.

The failure of animals fed diets high in urea or other NPN sources to perform as well as those fed preformed protein can be explained by one or more of the following factors that affect ruminant nitrogen metabolism (Purser, 1970):

> (1) nitrogen metabolism within the rumen, e.g., protein solubility; (2) the composition of the microbial population; (3) nutrient availability within the rumen, including relative availability and rate of availability of specific nutrients, growth factors included; (4) time of nutrient availability to the animal's metabolic system; (5) digestibility of specific amino acids from proteins in the lower alimentary tract, in particular from bacterial protein; (6) the possible influence of specific free amino acid patterns in the gut upon the rate of absorption of specific amino acids and upon the composition of amino acids being absorbed at any one time; and (7) a possible interaction between amino acid metabolism (utilization) and available energy source, i.e., the distribution of volatile acids available for metabolism.

Work reported by Friegat, Theurer and Hale (1970) indicates that when urea is used as a nitrogen supplement compared to cottonseed meal, the percentage of soluble rumen protein of bacterial origin is high.

Based on the low biological value of urea and the factors enumerated by Purser it is apparent that there is not necessarily any relation between the percentage crude protein needed in a ration when the nitrogen is supplied by natural protein and when it is supplied by urea or some other NPN source.

Wood Hemicellulose Extract as an Energy Source

The synthesis of protein by bacteria in the rumen proceeds in two major steps: (1) the urea is broken down to ammonia; and (2) the

ammonia is then combined with carbon fragments from carbohydrates to form protein in the bacterial cells. For the most efficient use of the nitrogen in the ration the second step must keep pace with the first to prevent the ammonia from being carried in the blood to the liver, converted to urea and excreted by the kidney (Gallup, Pope and Whitehair, 1953).

It is recommended that urea be combined with a carbohydrate feed when it is used to replace one of the common high protein supplements such as cottonseed meal. The usual recommendation is six parts of grain and one part of urea to replace seven parts of 41% meal (Gallup, Pope and Whitehair, 1953).

It has been shown that rumen organisms require readily available carbohydrate as a source of energy for the efficient utilization of non-protein nitrogen and that a further amount of carbohydrate is necessary for efficient utilization by the animal of protein so formed. Work with urea rations based on corn, barley, milo, dehydrated sweet potatoes, and combinations of corn and molasses gave results indicating that these feeds, as sources of energy for protein synthesis, were of similar value. Milo was especially valuable as measured by the increased nitrogen retention when urea was added. Corn proved superior to molasses. Similar studies with other rations showed that the three simple carbohydrates, sucrose, glucose and lactose, were equal in value as energy sources (Gallup, Whitehair and Bell, 1954). However, a comparison between the length of time which starch and glucose remained in the rumen showed that they were both broken down rather rapidly but that simple sugars such as maltose, produced from starch, remained in the rumen two to three times as long as did the glucose. These results indicate that

starch promotes more favorable conditions for the conversion of urea to bacterial protein in the rumen than sugar, in so far as the simultaneous availability of nitrogen, a suitable carbon source and energy is concerned (Schwartz, Schoeman and Faber, 1964).

The rumen fermentation rates supported by various added sugars are not all alike (Hungate, 1966). Glucose, mannose and L-(+)-arabinose are rapidly fermented (McNaught and Smith, 1947) but D-(-)-arabinose is not readily used (McNaught, 1951). Arabinose, xylose and maltose produce end products typical of the rumen fermentation (McNaught, Smith and Black, 1954). Carbohydrate source does have an effect on the proportion of volatile fatty acids produced in the rumen. Starch and glucose produce a greater percentage of propionate than cellulose. Sucrose and glucose increase butyric acid (Caffrey, et al., 1967). Sutton (1969) found an effect on VFAs produced in studies involving the fermentation of D-glucose, D-fructose, D-galactose, D-xylose, L-arabinose and sucrose by rumen contents of cows. The net recovery of carbon from the fermented carbohydrate in the volatile fatty acids was about 35-45 percent. A further 1-6 percent of fermented glucose and sucrose was recovered in lactic acid. These results indicate that the type of sugar available to the rumen microorganisms can have a definite effect on the fermentation process in the rumen.

McLaren, Jett and Britton (1968) reported that when a non-dialyzable, acid-resistant hemicellulose from corn cobs was included in a semipurified diet in which 90 percent of the nitrogen was urea and 10 percent was enzymatically hydrolyzed casein, the retention of absorbed nitrogen was increased in lambs. Other studies by McLaren, Smith and Peters (1968) showed that the inclusion of this product in in vitro

fermentation mixtures resulted in increased microbial protein synthesis when urea furnished 100 percent of the nitrogen.

These results indicate that the type of carbohydrate available for microbial fermentation might influence the utilization of urea as a nitrogen source. A liquid wood hemicellulose extract manufactured by the Masonite Corporation was reported to contain the following: glucose, 15 percent; galactose, 6 percent; mannose, 25 percent, arabinose, 5 percent; and xylose, 49 percent. About 10 percent of the carbohydrate exists as simple sugars and the balance as heavier molecular weight sugars. Gross energy content is approximately 4.03 kcal per gm of dry matter (Masonite Corporation, 1965).

Williams, et al. (1969) have shown that the liquid wood hemicellulose product can serve as a useful energy source for lambs when fed with urea.

Biuret

Urea and other non-protein nitrogen sources have been fed to ruminants since the late 1800's (Hendrickx, 1967). Urea is rapidly hydrolyzed in the rumen and if fed in large quantities may produce toxicity in the animal. The danger of toxicity is increased when urea is fed with low energy diets or when the main energy source is slowly hydrolyzed fibrous material. For maximum utilization of urea a readily available carbohydrate source is needed (Pearson and Smith, 1943; Drori and Loosli, 1961).

Biuret, a condensation product of urea, is less toxic than urea (Berry, Riggs and Kunkel, 1956; Hatfield, et al., 1959) possibly due to its lower solubility in the rumen contents (Meiske, et al., 1955) or

because of its slow rate of hydrolysis in the rumen (Gilchrist, Potgieter and Voss, 1968). Because of these facts biuret would seem to hold some promise as a substitute for urea in ruminant rations and especially in low energy wintering rations.

The use of biuret as a nitrogen supplement depressed weight gains in steers and feed consumption in sheep and cattle when compared to urea (Berry, Riggs and Kunkel, 1956). Campbell, et al. (1963) found that biuret promoted slightly lower growth and feed efficiency than urea in growing Holstein heifers. Biuret was slightly inferior to urea in support of the production of fat corrected milk with Holstein cows. Waite, et al. (1968) found no difference in milk yield between cows supplemented with biuret or urea.

There are numberous studies that indicate that biuret is not well utilized by ruminants as measured in metabolism trials. Nutrient digestibility and nitrogen utilization were depressed when biuret was substituted for urea as a nitrogen supplement (Anderson, et al., 1959). McLaren, et al. (1959) also found that biuret caused a decrease in organic matter digestibility, crude fiber digestibility (which agrees with Oltjen, et al., 1968) and apparent digestibility of protein when it was substituted for urea. There is an apparent reduction in the concentration of cellulolytic bacteria in the rumen of sheep fed biuret as compared to urea (Slyter, et al., 1968). A similar depression in nitrogen digestibility has been found by others (Hatfield, et al., 1959; Schaadt, Johnson and McClure, 1966). Johnson and McClure (1964) found that biuret was not utilized as well as urea by sheep when digestion trials were used to compare the two. Blood urea-nitrogen was higher when the animals were fed urea than when fed biuret. Biuret feeding

had no appreciable effect on rumen ammonia or blood urea whereas urea raised both considerably. Similar results were found by McLaren, et al. (1960) and Oltjen, et al. (1968). Microorganisms taken from urea or biuret adapted animals failed to release ammonia from biuret when incubated in vitro (Johnson and McClure, 1964). Urinary nitrogen losses were greater for biuret fed animals than for urea, indicating that the utilization of the absorbed nitrogen was not as complete for biuret as for urea. Biuret accounts for a large portion of the urinary nitrogen (Oltjen, et al., 1968) but this tends to decrease with time (Farlin, Garrigus and Hatfield, 1968). Nitrogen retention decreased sharply when biuret was substituted for urea in the diet of sheep (Farlin, Garrigus and Hatfield, 1968). Nitrogen retention increased with time but was not as high as for urea even after the sheep had received biuret for 36 days. A second experiment did not support these findings. In this experiment maintaining feed intake was difficult. Nitrogen balance failed to show an advantage for urea over biuret.

Ewan, Hatfield and Garrigus (1958) found that although the apparent digestion coefficients for organic matter and dry matter were depressed when biuret was substituted for urea and nitrogen balance was higher for the diet containing urea, nitrogen balance was higher in animals fed biuret when they were inoculated from lambs already adapted to biuret.

An adaptation is needed when biuret is fed before rumen microbes are able to utilize the nitrogen efficiently (Gaither, et al., 1955; Ewan, Hatfield and Garrigus, 1958; McLaren, et al., 1959; Campbell, et al., 1963; Johnson and McClure, 1964; Mackenzie and Altona, 1964; Karr, et al., 1965a,b; Schaadt, Johnson and McClure, 1966; Farlin, Garrigus and Hatfield, 1968; Gilchrist, Potgieter and Voss, 1968; Waite and

Wilson, 1968; Oltjen, et al., 1969; Schroeder and Gilchrist, 1969). The length of time for this adaptation response has been reported to vary with maximum retnetion being reached after 15 days up to 70 days (Schroeder and Gilchrist, 1969) depending upon the level of nitrogen in the diet and the energy content of the diet. Farlin, Garrigus and Hatfield (1968) showed that the microflora in the rumen of urea fed sheep had the ability to hydrolyze a significant amount of biuret without any adaptation period.

There have been several studies which indicate that the adaptation response seen in ruminants being fed urea, biuret and possibly to other NPN products is due to the adjustment of the animal to a lower plane of nutrition rather than a response to the nitrogen source specifically. Schaadt, Johnson and McClure (1966) reported that lambs on rations with protein as low as 6.5 percent did not stay in negative nitrogen balance, even though they were in negative nitrogen balance at the start of the test. Caffrey, et al. (1967) infused urea intravenously into lambs. This resulted in increased nitrogen retention, apparently because part of the infused N was transferred to the rumen and synthesized into protein by rumen microorganisms. The utilization of the infused urea did not appear to be influenced by previous dietary treatment. Thus Caffrey, et al. (1967) suggested that the adaptation response reported by McLaren, et al. (1960) was not due to increased recycling of blood urea but due to the fact that lambs subjected to a low nitrogen diet for a prolonged period adapt to that diet. It was also suggested that high dietary urea leads to a reduction in ureolytic activity of bacteria thus accounting for part of the adaptation response. Adaptation to NPN sources reported in the literature may also be partly due to adaptation

to changes in feed components other than the nitrogen source or level (Oltjen, et al., 1969).

Schaadt, Johnson and McClure (1966) suggested the possibility that adaptation to biuret occurs not in the rumen but within the body tissue. Karr, et al. (1965a) had previously come to a different conclusion based on the fact that they had found that diethylstilbestrol (DES) markedly reduced the adaptation response time to urea-nitrogen but it had little effect on the adaptation time to biuret. Since the effect of DES apparently takes place at the tissue level it was concluded that the adaptation to urea and biuret are different. Oltjen, et al. (1969) found no such response to DES in improving nitrogen retention or on adaptation. Farlin, Brown and Garrigus (1968) injected ^{14}C labeled biuret intravenously and intrarumenly to sheep adapted to biuret and not adapted to biuret. They recovered 95 percent of the ^{14}C in the urine when it was given intravenously, thus concluding that biuret was not utilized directly by animal tissue.

Gilchrist, Potgieter and Voss (1968) and Schroder and Gilchrist (1969) demonstrated the adaptation of sheep to biuret by measuring biuret disappearance from in vitro flasks and demonstrated the stoichiometric conversion of biuret-nitrogen to ammonia-nitrogen by active ingesta in vitro. This conversion was not demonstrated in vivo. The activity of the ingesta was greatly reduced by straining, indicating that the organisms responsible for breaking down biuret are closely associated with the fibrous portion of the ingesta.

There is evidence that a mixture of urea and biuret may be superior to biuret alone as a nitrogen supplement (Hatfield, et al., 1959; Karr, et al., 1965a). Anderson, et al. (1959) found that nitrogen utilization

and nitrogen digestion were not changed when crude biuret containing 40 percent biuret, 45.5 percent urea, 6.9 percent triuret, 6.7 percent cyanuric acid and 0.3 percent H_2O was used to replace 50 percent of the urea in a ration. When pure biuret supplied the nitrogen instead of urea, nitrogen digestion and utilization were depressed.

Cyanuric Acid

Cyanuric acid (2,4,6-trihydroxyl-1,3,5-S-triazine) is a safe nitrogen source for ruminants (Altona and Mackenzie, 1964). Mackenzie (1965) demonstrated that cyanuric acid was effective in the supplementation of low energy forage rations when fed to sheep. Cyanuric acid was shown to produce higher levels of nitrogen balance in adult sheep than urea, biuret and triuret (Clark, Barratt and Kellerman, 1965).

Volatile Fatty Acids

The nitrogen source included in ruminant rations influences the total concentration and relative percentage of the various volatile fatty acids (VFA) found in the rumen (Hungate, 1966). A problem exists in making measurements of the volatile fatty acids in rumen contents which is representative of the concentration found when different rations are fed (Oltjen, et al., 1969) especially when the rumen is sampled only once after feeding (Waite and Wilson, 1968). Annison (1965) reported that when samples of rumen fluid were taken as quickly as possible from different regions of the sheep rumen after feeding, they differed as much as 30 percent in VFA content.

Karr, et al. (1965a) found that supplementation of urea and biuret containing rations with dehydrated alfalfa meal increased average daily

gain, feed efficiency and nitrogen retention. Rumen fluid taken four hours after feeding contained an increased total VFA concentration. Orskov and Oltjen (1967) found that the total concentration of VFA in the rumen fluid was greater for the cattle receiving purified diets containing urea than cattle receiving natural diets two hours after feeding. Acetic acid proportion was lower in the rumen when urea was fed than when the natural diet was fed. The valeric acid proportion was greater with the urea diet than with the natural diet. Comparisons of urea and biuret as nitrogen sources failed to reveal any difference in total concentration of VFA in the rumen. The proportion of propionic acid in the rumen fluid was higher when urea was the N source than when biuret was used as the N source. The valeric acid proportion was greater with biuret than urea.

When urea or biuret replaced oilcake in the concentrate portion of cows' rations, there was not any marked difference in the concentration of total VFA in the rumen fluid or in the proportion of acetic, propionic and butyric acids. A decrease in percentage of acetic acid and an increased percentage of propionic acid occurred during the first four hours after feeding followed by a slow reversal. The percentage of butyric acid rose slightly during the first one to two hours after feeding but changed only slowly for the next few hours.

Orskov, et al. (1969) demonstrated that the urinary loss of nitrogen was increased when acetic acid was infused and decreased when propionic acid was infused, thus indicating the proportion of VFA produced in the rumen may influence nitrogen utilization.

Sutton (1969) showed that the simple sugars present in the rumen can have decided effects on the proportion of VFA in the rumen.

Diets containing 85 percent roughage and supplemented with urea or biuret showed that seven hours after the steers consumed the morning feeding the molar percentage of acetic, isobutyric and isovaleric acid decreased while the molar percentage of propionic and butyric acid and total concentration of VFA increased (Oltjen, et al., 1969). The molar percentage of butyric acid was greater when biuret was the source of supplemental nitrogen compared to urea.

Previous work had indicated that the branched chain VFAs were undetectable four hours after feeding. Waite and Wilson (1968) feeding natural diets with NPN supplements found isovaleric and N-valeric acid accounted for only 1 to 3% of the total VFA and isobutyric acid was often absent or present in only traces. It was reported by el-Shazly (1952a,b) that there is a positive correlation between level of protein in the diet and level of branched chain acids in the rumen fluid. He concluded that proteins were the primary source of branched chain fatty acids. Orskov and Oltjen (1967) feeding purified diets supported el-Shazly's conclusions and suggested that the small quantities of branched chain VFA found when NPN is fed may be the result of breakdown of microbial protein.

Some cellulolytic bacteria are known to require the straight and branched chain fatty acids to synthesize the corresponding amino acids and lipids for their growth. Oh, Longhurst and Jones (1969) found that sheep fed low quality range grass and urea performed as well as those given VFA and casein. This indicates that rumen microorganisms are capable of synthesizing their essential cellular components utilizing urea alone as a source of nitrogen.

Regulation of Feed Intake

The regulation of feed intake has been studied from several different viewpoints. Thermostatic regulation, chemostatic regulation and lipostatic regulation have been proposed as regulating mechanisms (Mayer, 1955; Egan, 1965a,b; Egan and Moir, 1965). These factors were proposed to be regulated through the hypothalamus.

Rate of passage through the digestive tract of ruminants has also been studied as a factor regulating feed intake (Blaxter, Wainman and Wilson, 1961; Campling, Freer and Balch, 1962; Freer and Campling, 1963; Faichney, 1965). Involved in the rate of passage of ingesta through the tract are such things as rate of digestion, capacity of the rumen and chemical and physical nature of the diet.

Undoubtedly many or all of these factors are involved in the regulation of food intake by ruminants (Conrad, Pratt and Hibbs, 1964; Montgomery and Boumgardt, 1965). It appears that with a high quality ration, intake is primarily regulated by metabolic body size and energy expenditure. With low quality rations physical factors are probably the first to limit intake. Feed intake is also limited by protein deficiency (N.R.C., 1968). In the case of ruminants a nitrogen deficiency leads to reduced microbial activity in the rumen and thus to low consumption (Oh, Longhurst and Jones, 1969).

The fact that microbial fermentation activity is critical in controlling appetite is well documented (Hungate, 1966). Bartley, et al. (1961) observed that animals provided with autoclaved rumen fluid exhibited less appetite than those receiving unautoclaved fluid. This indicates that the activity of the rumen microorganisms is necessary and more important than the presence of already formed VFA or other

nutrients.

Retardation of rumen fermentation could occur if individual acids accumulated in the rumen (Lee and Moore, 1959) probably partially due to the lowering of rumen pH. It has been reported that addition of $NaHCO_3$ to the ration of steers fed high concentrate diets improved weight gains.

Dry matter disappearance from the rumen by absorption of fermentation products, liberation of fermentation gases and passage to the lower tract may influence the rate of fermentation in the rumen. With highly digestible feeds the quantity leaving the rumen can be rapid and large thus causing considerable fluctuation in dry matter content in the rumen. Based on this it is apparent that frequent feeding would tend to maintain a more steady rate of fermentation when animals are fed diets whose physical form would allow rapid fermentation and passage out of the rumen. This is supported by Putnam, Gutierrez and Davis (1961) who found that feeding ten times daily instead of twice resulted in a 25 percent increased gain. The steady supply of ammonia was probably responsible for the increased feed consumption seen under frequent feeding conditions (Rakes, Lister and Reid, 1961).

Fistulation of the rumen apparently does not adversely effect the fermentation process provided it is kept closed. The small amount of oxygen entering such a fistula can be absorbed by the rumen contents without decreasing the activity of the anarobic organisms (Drori and Loosli, 1959).

CHAPTER III

MATERIALS AND METHODS

Introduction

A series of five trials were conducted using purified diets fed to lambs. Trial 1 was conducted to evaluate the possibility of improving performance of lambs fed purified diets. Treatments included increased calcium and phosphorus content of the diet, increasing the nitrogen content and utilization of a wood hemicellulose extract as an energy source.

Trial 2 was a growth study conducted to evaluate urea, a 1:1 mixture of urea and biuret, biuret, a 1:1 mixture of urea and cyanuric acid and cyanuric acid as nitrogen sources for lambs.

Trial 3 was a biuret metabolism trial designed to compare nitrogen balance, and organic matter digestibility of lambs fed the urea, the 1:1 mixture of urea and biuret and the biuret rations.

Trial 4 was an _in vitro_ biuret trial designed to study the adaptation of lambs to biuret feeding as measured by biuret disappearance and ammonia-nitrogen release in an _in vitro_ system.

Lambs used in the _in vitro_ biuret trial were used simultaneously for an _in vivo_ trial in which the decreasing concentration of biuret in the rumen contents after feeding was measured. Ruminal ammonia-nitrogen concentration and volatile fatty acid (VFA) concentrations were measured simultaneously.

Trial 5 was an attempt to study the possible effect of biuret on palatability of the diet by feeding a diet containing essentially no nitrogen and administering the biuret to the animal through a rumen cannula for 19 days. Following this, biuret was discontinued and urea was administered to the animals through the rumen fistual for 30 days in an attempt to determine if lack of availability of the biuret was restricting voluntary feed intake.

Experimental Procedure

Trial 1

Fifty wether lambs (Hampshire x Western and Dorset x Western) approximately four months of age and weighing approximately 25 kg were placed at random in individual holding pens (140 cm x 84 cm) with slotted floors. Movement within the pens was unrestricted. Lambs were drenched with phenothiazine as an anthelmintic at the time they were placed in the pens.

Five lambs were assigned to each of ten experimental rations (Table I) in a completely randomized experimental design. During a seven day preliminary adjustment period all lambs were fed a basal purified diet similar to one which has been fed at Oklahoma State University previously (Ration 1).

Lambs were fed 895 gm of ration 1 on the day the experimental growth period was started. One blood and rumen fluid sample were obtained from two lambs randomly chosen from each of the ten treatment groups four to six hours after feeding. Blood samples for ammonia-nitrogen and urea-nitrogen analysis were obtained by jugular vein puncture. Rumen fluid samples were obtained by inserting a stomach tube

TABLE I

COMPOSITION OF EXPERIMENTAL RATIONS USED IN TRIAL 1

Ingredients	Group (%)									
	1	2	3	4	5	6	7	8	9	10
Corn Starch	29.89	29.89	29.89	29.89	29.89	29.89	29.89	-----	29.89	29.89
Glucose Monohydrate[1]	29.89	29.89	29.89	29.89	29.89	29.89	29.89	29.89	-----	29.89
Cellulose[2]	30.00	29.75	29.47	29.21	29.00	28.00	27.00	30.00	30.00	-----
Urea[3]	4.20	4.20	4.20	4.20	5.20	6.20	7.20	4.20	4.20	4.20
Corn Oil[4]	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Choline Chloride	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
Vitamin A and D[5]	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02
K_2CO_3	2.21	2.21	2.21	2.21	2.21	2.21	2.21	2.21	2.21	2.21
$CaHPO_4$	1.32	1.58	1.85	2.11	1.32	1.32	1.32	1.32	1.32	1.32
$MgSO_4$	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12
$MgCO_3 \cdot Mg(OH)_2 \cdot 3H_2O$	0.27	0.27	0.27	0.27	0.27	0.27	0.27	0.27	0.27	0.27
Na_2SO_4	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
NaCl	0.62	0.62	0.62	0.62	0.62	0.62	0.62	0.62	0.62	0.62
Trace Minerals[6]	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
Hemicellulose[7]	-----	-----	-----	-----	-----	-----	-----	29.90	29.90	30.00

[1]Cerelose. Corn Products Company.

[2]Solka Floc. B-W20 Brown Co., Berlin, New Hampshire.

[3]Urea. 45% N. Courtesy Nipak, Inc., Pryor, Oklahoma.

[4]Mazola. 1,2-dihydroxy-6-ethoxy,2,2,4,trimethyl quinoline (ethoxyquin) added to provide 0.0125% in total diet and Vitamin E (1 IU/mg) added to give 0.00044%.

[5]Containing 20,000 IU and 2,500 USP units/gm of Vitamin A and D, respectively.

[6]Composition of the trace mineral mixture (mg/100 gm of basal diet): $FeSO_4$, 42.51; $MnSO_4 \cdot H_2O$, 15.37; $Na_2B_4O_7$, 12.56; $ZnSO_4 \cdot 7H_2O$, 26.35; $CuCO_3 \cdot Cu(OH)_2$, 1.97; KI, 0.07; $CoCl_2 \cdot 6H_2O$, 0.05; CaF_2, 0.20; $Na_2MoO_4 \cdot 2H_2O$, 0.50; $Cr_2(SO_4)_3$, 0.04; Na_2SeO_4, 0.12.

[7]Dried Masonex. Courtesy Masonite Corporation, Chicago, Illinois.

into the rumen and applying suction. Rumen ingesta was immediately strained through four layers of cheesecloth and pH measurement was made immediately thereafter. Samples were prepared for urea-nitrogen, ammonia-nitrogen and VFA analysis.

Upon completion of collecting the initial rumen fluid samples and blood samples, lambs were fed one of the rations shown in Table I. Lambs remained on the experimental rations during a 98 day growth trial. Feed consumption was essentially ad libitum with the quantity fed each day adjusted so that a small amount remained in the feed boxes from one feeding to the next but did not accumulate.

Weights were taken on each animal at the start of the feeding experiment and every two weeks thereafter. Animals had access to water at all times.

Two animals on each ration were bled and rumen fluid samples taken for analysis for the second time approximately 90 days after the feeding of the experimental rations began.

All rations used in this and subsequent studies were mixed in batches of approximately 90 kg. Samples of the rations were taken periodically and analyzed for nitrogen.

Trial 2

Fifty lambs approximately five months of age, weighing approximately 25 kg and of similar breeding as those described before were randomly placed in individual pens. A complete randomized design was used. Ten lambs were assigned to each of the experimental rations (Table II). Due to limitations of space nine lambs on each treatment were housed on the ground floor of the barn and one animal on each

TABLE II

COMPOSITION OF EXPERIMENTAL RATIONS USED IN TRIALS 2, 3, 4 AND 5

Ingredients	Ration						
	Non-nitrogenous	Preliminary	1	11	12	13	14
Corn Starch	31.50	17.90	29.77	29.77	29.77	29.77	29.77
Glucose Monohydrate[1]	31.50	17.90	29.77	29.77	29.77	29.77	29.77
Cellulose[2]	31.00	29.60	30.00	29.53	29.06	29.35	28.70
Urea[3]	-----	-----	4.50	2.25	-----	2.25	-----
Biuret[4]	-----	-----	-----	2.72	5.44	-----	-----
Cyanuric Acid[5]	-----	-----	-----	-----	-----	2.90	5.80
Cottonseed Meal (41%)	-----	28.60	-----	-----	-----	-----	-----
Corn Oil[6]	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Choline Chloride	0.10	0.10	0.10	0.10	0.10	0.10	0.10
Vitamin A and D[7]	0.02	0.02	0.02	0.02	0.02	0.02	0.02
K_2CO_3	2.21	2.21	2.21	2.21	2.21	2.21	2.21
$CaHPO_4$	1.32	1.32	1.32	1.32	1.32	1.32	1.32
$MgSO_4$	0.12	0.12	0.12	0.12	0.12	0.12	0.12
$MgCO_3 \cdot Mg(OH)_2 \cdot 3H_2O$	0.27	0.27	0.27	0.27	0.27	0.27	0.27
Na_2SO_4	0.25	0.25	0.25	0.25	0.25	0.25	0.25
NaCl	0.62	0.62	0.62	0.62	0.62	0.62	0.62
Trace Minerals[8]	0.10	0.10	0.10	0.10	0.10	0.10	0.10

[1]Cerelose. Corn Products Company, Argo, Illinois.

[2]Solka-Floc. B-W20. Brown Co., Berlin, New Hampshire.

[3]Feed grade urea. 42% N. Courtesy Nipak, Inc., Pryor, Oklahoma.

[4]Containing 87.10% biuret, 10.30% water, 0.70% urea and 1.91% cyanuric acid. Courtesy Nipak, Inc., Pryor, Oklahoma.

[5]Containing 2.2% biuret and 97.8% cyanuric acid. Courtesy Nipak, Inc., Pryor, Oklahoma.

[6]Mazola. 1,2-dihydroxy-6-ethoxy,2,2,4,trimethyl quinoline (ethoxyquin) added to provide 0.0125% in total diet and Vitamin E (1 IU/mg) added to give 0.00044%.

[7]Containing 20,000 IU and 2,500 USP units/gm of Vitamin A and D, respectively.

[8]Composition of trace mineral mixture (mg/100 gm of basal diet): $FeSO_4$, 42.51; $MnSO_4 \cdot H_2O$, 15.37; $Na_2B_4O_7$, 12.56; $ZnSO_4 \cdot 7H_2O$, 26.35; $CuCO_3 \cdot Cu(OH)_2$, 1.97; KI, 0.07; $CoCl_2 \cdot 6H_2O$, 0.05; CaF_2, 0.20; $Na_2MoO_4 \cdot 2H_2O$, 0.50; $Cr_2(SO_4)_3$, 0.04; Na_2SeO_4, 0.12.

treatment in the loft. All animals were fed the ration designated as the preliminary ration in Table II for a seven day adjustment period.

At the end of the preliminary feeding period two lambs previously designated to be fed each of the experimental rations were bled and rumen fluid samples taken, as described for the study with purified diets. Blood and rumen fluid samples were again taken from the same animals after the experimental rations had been fed for 60 days.

Each lamb was weighed weekly. Lambs had access to water at all times during the experiment.

It was not possible to obtain sufficient numbers of animals of the same sex for this experiment. The distribution of wethers and ewes in each treatment group is shown in Table III.

Trial 3

Sixteen growing wether lambs of similar breeding as lambs in previous trials were randomly divided into two groups of five and one group of six. Five lambs were assigned to ration 1 (Table II), five to ration 11 and six to ration 12 (nitrogen supplied by urea, urea and biuret and biuret, respectively). Two lambs from the group assigned to ration 1, two assigned to ration 11 and three assigned to ration 12 had rumen fistuals installed.

After a period of approximately 30 days for recovery from the fistulation all lambs were fed the preliminary ration shown in Table II.

The lambs were handled as outlined in Table IV during the course of the metabolism trial. The period during which all of the lambs were fed the preliminary ration is designated as "P" under days. The collection periods were of seven day duration during which time a total

TABLE III

NUMBER OF WETHER AND EWE LAMBS IN TRIAL 2

Item	Wethers	Ewes
Ration 1 (Urea Control)	4	6
Ration 11 (Urea-Biuret)	1	9
Ration 12 (Biuret)	3	7
Ration 13 (Urea-Cyanuric Acid)	3	7
Ration 14 (Cyanuric Acid)	2	8

TABLE IV

SCHEDULE OF TREATMENTS FOR TRIAL 3

Day	Location	Operation	Ration
P 1-19	Holding Pen	Adjustment	Preliminary, Ad lib
P 20-23	Holding Pen	Adjustment	Preliminary, 700 gm
P 24-30	Holding Pen	Adjustment	Preliminary, 500 gm
P 31-37	Metabolism Stalls	Adjustment	Preliminary
P 37-44	Metabolism Stalls	Collection	Preliminary
P 45-46	Metabolism Stalls	None	Preliminary
0 - 8	Holding Pens	Adjustment	Experimental, 500 gm
9 - 11	Metabolism Stalls	Adjustment	Experimental
12 - 19	Metabolism Stalls	Collection	Experimental
20 - 31	Holding Pens	None	Experimental
32 - 39	Metabolism Stalls	Collection	Experimental
39 - 66	Holding Pens	None	Experimental

collection of urine and feces was made.

Trial 4

Two fistulated lambs from each of the three groups in the metabolism trial were used in this trial which was conducted simultaneously with the metabolism trial. The day that the collection period was concluded the six lambs used in the in vitro trial were fed 500 gm of the non-nitrogenous diet (Table II) and allowed to eat for 50 minutes. Ten minutes later a 1000 ml sample of rumen fluid was collected from each of the six fistulated lambs and immediately transferred to the laboratory where 375 ml of the unstrained rumen fluid were placed in wide mouth 500 ml flasks containing 125 ml of an aqueous solution of 1.25% (w/v) biuret. The flasks were equipped with a one-way escape valve and an inlet to allow continuous gassing with CO_2. The incubation procedure was as described by Schwartz, Schoeman and Forber (1964) and Gilchrist, Potgieter and Voss (1968). The pH was adjusted to 6.8 with 30% Na_2CO_3 each time the flasks were sampled.

Each flask was sampled as soon as the rumen ingesta and biuret was mixed and again at 4, 6, 12, and 24 hours after the start of incubation. Similar incubation of rumen contents from the same animals was made on day 19 after the start of feeding of the urea, urea and biuret, and biuret supplemented rations (Table II). The experiment was repeated on day 45 and 64 as described with the exception that the flasks were sampled at 0, 12 and 24 hours.

Simultaneously with the metabolism trial and with the in vitro biuret trials described before rumen fluid samples were obtained from the fistulated lambs for measurement of biuret disappearance from the

rumen.

The first sampling period was on the second day after the conclusion of the first metabolism trial. A zero time sample was obtained from the rumen of two of the fistulated lambs which had been assigned to each of the experimental rations. The animals were fed 500 gm of the preliminary ration and allowed to eat for 50 minutes. Ten minutes after the rations were removed the lambs were sampled again. Subsequent samples were obtained at 2, 4, 7 and 12 hours after feeding. Rumen ingesta were obtained by opening the rumen fistula and allowing approximately 280 ml to flow freely into a flask. The rumen contents were strained through two layers of cheesecloth. Samples were prepared for biuret, ammonia-nitrogen and VFA analysis.

The experiment was repeated with the same lambs on days 20 and 41 after the beginning of feeding of the experimental rations.

Trial 5

Two fistulated lambs which had been fed the urea ration (ration 1) and the two lambs which had been fed the biuret ration (ration 12) for 66 days were used to determine the effect on voluntary feed intake of supplementing biuret by way of the fistula. The non-nitrogenous purified diet (Table II) was fed ad libitum and feed intake was recorded daily. Biuret was administered into the rumen through the fistula at the rate of six percent of the feed consumed.

At the conclusion of the above study one lamb from each of the rations was continued on the non-nitrogenous ration. Four gm of feed grade urea (42% N) were placed into the rumen of each lamb once daily for 21 days. Subsequently six gm of urea was administered for eight

days. Feed intake was recorded.

Preparation of Samples and Chemical Analysis

Blood samples were prepared for ammonia-nitrogen analysis according to the procedure recommended for the Oxford[1] ammonia-nitrogen analysis. Blood samples for urea-nitrogen analysis were prepared in a similar manner.

Samples of rumen fluid strained through four layers of cheesecloth were used for pH measurements, ammonia-nitrogen, urea-nitrogen and VFA analysis. A 19.5 ml sample of rumen fluid was pipetted into a centrifuge tube and mixed with 0.5 ml of saturated $HgCl_3$. The tubes were placed on ice, taken to the laboratory and centrifuged[2] at 19,000 x g for 20 minutes. The supernate was saved for urea-nitrogen and ammonia-nitrogen analysis. Strained rumen fluid was prepared for VFA analysis as described by Erwin, et al. (1961). All samples were stored at -4° C until analyzed.

For biuret analysis, 100 ml samples of in vitro and in vivo rumen fluid were collected and centrifuged at 800 x g for ten minutes. A 30 ml sample of the supernate was mixed with 0.7 ml of 10 N H_2SO_4 as a preservative and stored at -4° C until analyzed.

Urine collected for nitrogen balance studies was collected in plastic containers to which 10 ml of 50% HCl had been added. The volume of urine excreted was measured daily and 10% aliquots taken, composited and stored at 10° C. At the conclusion of each collection

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[1]Oxford Laboratories, San Mateo, California.

[2]International High-Speed Refrigerated Centrifuge Model HR-1, International Equipment Company, Neeham Hts., Massachusetts.

period the composite samples were stored at -4° C until analyzed.

Feces collected for nitrogen balance were weighed daily and 20% aliquots were composited and stored at -4° C until analyzed. Wet fecal samples were used for nitrogen analysis. Eight gm of wet feces were mixed with 400 ml of distilled water in a Waring blender. An aliquot of this was analyzed for N by the Kjeldahl procedure. The fecal sample was then dried at 55° C in a forced air oven for 24 hours, allowed to cool to room temperature, and then ground through a Wiley mill. Air dry samples were stored in sealed containers.

Measurements for pH were made using a Beckman Zeromatic pH meter.[3] Analysis for ammonia-nitrogen was made by the Oxford microdiffusion procedure. Urea-nitrogen analysis was made by the Hycel[4] method.

Nitrogen analysis and ash determination was by standard A.O.A.C. (1960) methods. Dry matter was determined by drying for six hours at 60° C under vacuum. VFA analysis was by the method of Erwin, et al. (1961) on an Aerograph Hy-Fi Model 600-D[5] gas chromatograph. All colorimetric readings were made on a Gilford Model 240[6] spectrophotometer.

Biuret analysis was made using a modification of the procedure described by Gilchrist, Potgieter and Voss (1968). The color reagent was prepared by dissolving 110 gm of NaOH pellets in 500 ml of distilled H_2O, then adding 100 gm of $NaKC_4H_4O_6 \cdot 4H_2O$ and dissolving. This alkaline sodium potassium tartrate solution was diluted to 1.0 liter. A copper

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[3]Beckman Instruments, Inc., Fulberton, California.

[4]Hycel, Inc., Houston, Texas.

[5]Varian Aerograph, Houston, Texas.

[6]Gilford Instrument Labs., Oberlin, Ohio.

sulfate solution was prepared by dissolving 35 gm of $CuSO_4 \cdot 5H_2O$ in distilled H_2O and diluting to 1.0 liter. The copper sulfate and alkaline sodium potassium tartrate solutions were mixed by carefully adding the copper solution to the tartrate solution. The color reagent was then allowed to stand for 48 hours before using.

The samples to be analyzed were centrifuged at 14,000 x g for 15 minutes. Ten milliliters of supernatant fluid were pipetted into a centrifuge tube and 5 ml of distilled H_2O, 0.8 ml of 10% NaOH, 7.0 ml of 7% (w/v) $ZnSO_4$ and 10.0 ml of 5% (w/v) $Ba(OH)_2$ were pipetted into the tube. The tubes were stoppered and let stand for 10 minutes. The tubes were then centrifuged at 14,000 x g for 15 minutes and the supernatant fluid was decanted into a beaker containing approximately 0.3 gm of charcoal[7], swirled and let stand for 10 minutes. The sample was then filtered through Whatman number 40 filter paper. Two pellets of NaOH were added to the filtrate, dissolved and let stand 20 minutes. The sample was then filtered through number 42 Whatman filter paper. Fifteen milliliters of filtrate were pipetted into a tube containing 5 ml of color reagent, mixed and allowed to stand for 15 minutes. Optical density was read on spectrophotometer in a 1 cm cuvette at 555 nm.

A blank was prepared by using 10 ml of rumen fluid obtained from lambs fed ration 1 and handling as described for the sample beginning with the addition of 5 ml of distilled water.

Standards of appropriate concentration were prepared by mixing 10 ml of acidified rumen fluid and distilled water and a stock 1% (w/v) standard biuret solution.

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[7]Pyramid Brand Powered Willow Charcoal, Ehrmann-Strauss Co., New York.

Statistical Analysis

All statistical analyses were made by a computer system entitled "Statistical Analysis System" developed by Anthony J. Barr and James Howard Goodnight of the Department of Statistics, North Carolina State University, Raleigh, North Carolina.

The analysis of variance suboption was used to test for differences in response for the ten rations fed in Trial 1. The regression suboption was used to test for linear and quadratic effects using the model $Y = B_0 + B_1\chi + B_2\chi^2 + \varepsilon$ for each of the response criteria, feed consumption, weight gain and feed conversion where χ was the three levels of calcium and phosphorus, the three levels of urea or the three rations containing wood hemicellulose.

The weight gain, feed intake and feed conversion for lambs on Trial 2 were compared by the analysis of variance suboption. Differences between individual rations were tested by use of the least significant difference test.

Grams of N retained, percentage of N retained and organic matter digestibility were compared by use of analysis of variance. Regression analysis was used to test for differences in VFA concentrations in the *in vivo* portion of Trial 4. The F-test was used to test for significant differences.

CHAPTER IV

RESULTS AND DISCUSSION

Trial 1

Average daily feed consumption, average daily gain, and feed conversion for each of the treatment groups are shown in Table V. There was no statistically significant effect due to treatment except for feed conversion when rations 5, 6, and 7, containing 13.7%, 16.3% and 18.9% crude protein, respectively, were fed. There was a large amount of variation in the data as shown by the standard deviations.

The largest average daily feed consumption was 1.66 kg for the basal purified diet (ration 1) and this ration also produced the largest daily gain of 0.181 kg.

Increasing the calcium content of the ration from 0.48 to 0.56 and 0.64% and increasing the phosphorus content from 0.36 to 0.42 and 0.48% (rations 2, 3 and 4) had no significant effect on any of the three response criteria measured. The average gain and feed intake tended to be lower than that of ration 1. Feed conversion seemed to be slightly superior for ration 2 when compared to the control ration but rations 3 and 4 were inferior.

The results obtained when additional calcium and phosphorus were added to the purified diet are in agreement with Oltjen, Sirny and Tillman (1962b) where they showed no significant effect on feed efficiency due to the level of alkaline mineral mixture in the ration.

TABLE V

EFFECT OF ADDITION OF CALCIUM AND PHOSPHORUS AND UREA TO PURIFIED DIETS AND SUBSTITUTION OF WOOD HEMICELLULOSE FOR STARCH, GLUCOSE AND WOOD FIBER

Ration	Average Daily Feed Consumption		Average Daily Gain		Average Kg Feed/Kg Gain	S.D.
	Kg	S.D.[1]	Kg	S.D.		
1	1.66	0.20	0.181	0.03	9.26	0.93
2	1.30	0.19	0.146	0.02	8.78	0.50
3	1.48	0.23	0.156	0.04	9.76	1.31
4	1.52	0.35	0.154	0.03	9.88	1.65
5	1.41	0.20	0.146	0.03	9.70	1.22
6	1.39	0.18	0.149	0.02	9.32	0.84
7	1.38	0.31	0.117	0.05	13.02	3.79
8	1.32	0.32	0.101	0.05	14.86	4.67
9	1.39	0.26	0.116	0.06	20.58	21.98
10	1.02	0.06	0.124	0.03	8.44	1.47

[1]Standard deviation.

These results do differ from their finding that 6.5% mineral mixture tended to produce the fastest gain. Stuedemann (1970) obtained the fastest gains from lambs fed a diet containing 1.6% calcium followed by 0.58, 2.79 and 4.46%, respectively. The difference between the results obtained in this trial and those of other workers can be partially explained by the fact that their indications of response to varying mineral levels have been small and apparently largely due to change.

Increasing the crude protein equivalent of the ration from 11% in the basal ration (ration 1) to 13.7% in ration 5, 16.3% in ration 6 and 18.9% in ration 7 resulted in no improved performance. There was no statistically significant difference between rations 5, 6 and 7 in voluntary feed intake or average daily gain although the averages for feed consumption showed a tendency for feed consumption to decrease with increasing levels of urea in the ration. A significant ($p < .05$) linear effect was detected in feed efficiency for rations 5, 6 and 7 with feed efficiency decreasing with increasing levels of urea in the rations ($b = -22.9$).

The results of adding increased levels of urea to the purified diet are not in agreement with Price and Smith (1968) who found that performance was increased by supplying a crude protein equivalent of 24% or that of Virtanen (1966) who increased milk production from cows fed a purified diet by increasing the crude protein equivalent from 12.8% to 15%. The reason for this discrepancy in results is not readily apparent.

Substitution of wood hemicellulose for glucose, corn starch or purified wood fiber had a tendency to reduce feed consumption and rate of gain. Feed conversion was 14.86 when wood hemicellulose replaced

starch (ration 8), 20.58 when it replaced glucose (ration 9), and 8.44 when it replaced wood fiber (ration 10). There were no significant differences in any response criteria between rations 8, 9 and 10. Those results indicate that wood hemicellulose did not support the fermentation process in the rumen as well as the other carbohydrate sources. The fact that there are a variety of simple and short carbohydrate chains present in the wood hemicellulose extract (Masonite Corporation, 1965) was apparently of no advantage in these diets. Hemicellulose separated from natural forage fiber is intermediate in its digestibility between soluble sugars and cellulose (Hungate, 1966). The substitution of the wood hemicellulose extract for the starch and glucose in these rations may have had the effect of reducing the available energy in the diets and thus reduced the performance of the animals. Schwartz, Schoeman and Faber (1964) have shown that starch promotes more favorable conditions for the utilization of urea than simple sugars because it provides a longer lasting source of energy. If the hemicellulose extract had been used in smaller quantities it might have furnished an energy source which would have been available to compliment the rather rapidly metabolized starch and glucose and the slowly released energy from the wood fiber.

Substitution of wood hemicellulose extract for cellulose in the ration apparently tended to decrease performance due to the physical effect of removing all roughage from the ration. Williams, et al. (1969) showed that removing all roughage from a ration has a detrimental effect on performance of ruminants.

Attempts to relate growth performance with blood and rumen fluid ammonia-nitrogen and urea-nitrogen levels proved futile. The blood and

rumen fluid ammonia and urea-nitrogen concentrations are given in Table VI. Obtaining representative samples from the rumen is difficult (Annison, 1965). The problem of comparing the makeup of rumen contents is especially difficult if the rations fed differ in their rate of fermentation and if only one sample is drawn to make comparisons (Waite and Wilson, 1965; Oltjen, et al., 1969). In this experiment the first rumen fluid and blood samples were taken four hours after feeding the basal ration. The next samples, 90 days later, were taken four hours after they were fed the experimental rations. The possible difference in rate of fermentation of the various rations could account for the results obtained. In addition, difficulty was encountered in getting the animals to consume their rations in the time allotted on the days samples were taken. This led to differences between animals in fill at the time of sampling. If an animal consumed its ration soon after it was placed before him, the animal would be expected to have a different rumen and blood ammonia and urea concentration than one that had not eaten its ration until just before sampling.

Rumen fluid pH values are given in Table VII.

Rumen fluid volatile fatty acid concentrations are shown in Table VIII. The problem of obtaining representative samples for analysis and the loss of several samples makes interpretation of these data difficult. The same problem in obtaining samples was encountered here as in sampling for the ammonia data.

Trial 2

The average daily feed consumption, average daily gain and feed conversion for Trial 2 are shown in Table IX. There was a significant

TABLE VI

TRIAL 1 CONCENTRATION OF AMMONIA-NITROGEN AND UREA-NITROGEN IN BLOOD AND RUMEN FLUID OF LAMBS

Ration	Animal Number	Day[1]	Blood		Rumen	
			NH_3-N	Urea-N	NH_3-N	Urea-N
			µg/ml	mg/100 ml	µg/ml	mg/100 ml
1	2	0	4.8	30.6	200.0	5.1
		90	6.2	14.0	440.0	14.1
1	19	0	2.2	18.0	720.0	27.8
		90	5.4	16.0	1415.0	5.2
2	38	0	7.0	37.0	-----	26.5
2	52	0	-----	-----	240.0	6.3
		90	1.8	25.3	220.0	12.0
3	17	0	2.8	14.2	110.0	16.1
3	42	0	2.6	9.6	285.0	7.8
		90	2.7	21.6	150.0	9.7
4	7	0	6.7	17.6	-----	-----
4	50	0	2.5	40.0	575.0	26.0
		90	2.8	26.8	120.0	16.8
5	5	0	3.6	25.4	550.0	9.3
		90	3.8	20.0	70.0	17.1
5	25	0	3.4	21.4	1170.0	6.2
		90	15.0	25.0	250.0	32.0
6	39	0	4.5	-----	305.0	-----
		90	2.5	26.5	830.0	23.1
6	47	0	-----	-----	370.0	4.6
		90	18.9	17.0	500.0	4.3
7	18	0	15.8	14.0	100.0	5.4
		90	5.1	40.0	380.0	10.1
7	49	0	4.0	16.0	375.0	2.2
		90	15.5	22.6	520.0	21.5
8	11	0	1.3	20.0	100.0	9.2
		90	4.0	14.0	90.0	17.6
8	27	0	2.9	15.0	275.0	8.0
9	15	0	3.9	-----	130.0	72.0
		90	5.0	-----	450.0	17.0
9	16	0	4.0	29.2	240.0	4.4
		90	4.6	12.0	160.0	13.8
10	20	0	3.0	25.2	-----	-----
		90	4.8	12.0	90.0	-----

[1]Samples taken on day 90 were taken from the same lamb used on day "0". Missing data due to inability to obtain sample from animal or loss of sample.

TABLE VII

RUMEN FLUID pH FOR TRIAL 1

Ration	Animal Number	Day[1]	pH	Ration	Animal Number	Day	pH
1	2	0	6.3	7	18	0	6.7
		90	6.5			90	6.6
1	19	0	6.2	7	49	0	5.6
		90	6.6			90	6.6
2	52	0	5.6	8	11	0	5.5
		90	6.5			90	7.1
				8	27	0	6.8
3	42	0	6.8			90	6.5
		90	6.6				
				9	15	0	6.5
4	50	0	5.9			90	6.4
		90	7.2	9	16	0	6.2
						90	7.1
5	5	0	6.5				
		90	6.6	10	43	0	5.4
5	25	0	6.2			90	5.7
		90	6.9	10	20	0	5.5
						90	6.0
6	39	0	5.9				
		90	6.3				
6	47	0	5.9				
		90	6.2				

[1]Samples taken on day 90 were taken from the same lamb used on day "0". Missing data due to inability to obtain samples from animals or loss of samples.

TABLE VIII

RUMEN FLUID VOLATILE FATTY ACID FOR TRIAL 1

Ration	Animal Number	Day	Acetic		Propionic		Butyric		Total Moles
			Moles/ Liter	%	Moles/ Liter	%	Moles/ Liter	%	
1	2	0	0.0807	61.5	0.0214	16.3	0.0292	22.2	0.1313
		90	0.0241	61.6	0.0090	23.0	0.0060	15.3	0.0391
1	19	0	0.0735	61.7	0.0266	22.3	0.0191	16.0	0.1192
		90	0.0581	53.8	0.0259	24.0	0.0239	22.2	0.1079
2	52	0	0.0572	55.4	0.0294	28.5	0.0167	16.2	0.1033
		90	0.0474	54.8	0.0177	20.5	0.0214	24.7	0.0865
3	42	0	0.0190	61.3	0.0062	20.0	0.0058	18.7	0.0310
		90	0.0742	56.9	0.0391	30.0	0.0170	13.0	0.1303
4	50	0	0.0288	62.3	0.0104	22.5	0.0070	15.2	0.0462
		90	0.0548	61.2	0.0204	22.8	0.0144	16.1	0.0896
5	5	0	0.0371	52.6	0.0267	37.9	0.0067	9.5	0.0705
		90	0.0479	50.4	0.0395	41.6	0.0076	8.0	0.0950
5	25	0	0.0626	54.0	0.0396	34.2	0.0137	11.8	0.1159
		90	0.0832	59.0	0.0319	22.6	0.0259	18.4	0.1410
6	39	0	0.0752	50.8	0.0486	32.9	0.0241	16.3	0.1479
		90	0.1122	63.6	0.0382	21.6	0.0261	14.8	0.1765
6	47	0	0.0377	54.8	0.0250	36.3	0.0061	8.9	0.0688
		90	0.0451	47.9	0.0275	29.2	0.0215	22.8	0.0941
7	18	0	0.0600	59.8	0.0175	17.4	0.0228	22.7	0.1003
		90	0.0482	39.0	0.0172	13.9	0.0580	47.0	0.1234
7	49	0	0.0351	57.9	0.0212	35.0	0.0043	7.1	0.0606
8	27	0	0.0188	48.4	0.0106	27.3	0.0094	24.2	0.0388
		90	0.0500	59.6	0.0133	15.8	0.0206	24.6	0.0839
8	11	0	0.0317	66.6	0.0079	16.6	0.0080	16.8	0.0476
		90	0.0408	65.9	0.0085	13.7	0.0126	20.4	0.0619
9	15	0	0.0175	54.5	0.0065	20.2	0.0081	25.2	0.0312
		90	0.0627	83.3	0.0067	8.9	0.0059	7.8	0.0753
9	16	0	0.0796	60.8	0.0217	16.6	0.0296	22.6	0.1309
		90	0.0301	77.2	0.0058	14.9	0.0031	7.9	0.0390
10	20	0	0.0886	53.2	0.0463	27.8	0.0316	19.0	0.1665
		90	0.0772	66.3	0.0191	16.4	0.0202	17.3	0.1165
10	43	0	0.0531	54.2	0.0280	28.6	0.0169	17.2	0.0980
		90	0.0684	68.5	0.0083	8.3	0.0232	23.2	0.0999

TABLE IX

FEED CONSUMPTION, GAIN AND FEED CONVERSION OF GROWING LAMBS FED PURIFIED DIETS CONTAINING UREA, UREA-BIURET, BIURET, UREA-CYANURIC ACID OR CYANURIC ACID AS THE NITROGEN SOURCE

Ration	Average Daily Feed Consumption Kg	Average Daily Gain Kg	Average Feed Conversion Kg Feed/Kg Gain
1 (Urea)	1.39^{1}	0.140^{1}	9.96^{1}
11 (Urea-Biuret)	$1.27^{1,2}$	0.141^{1}	8.97^{1}
12 (Biuret)	0.77^{3}	0.028^{2}	27.10^{2}
13 (Urea-Cyanuric Acid)	1.10^{2}	0.113^{1}	9.73^{1}
14 (Cyanuric Acid)	0.79^{3}	0.036^{2}	22.10^{2}

$^{1-3}$Values in the same column with different superscripts differ ($p < .01$).

difference between treatments in feed consumption. The urea supplemented ration (ration 1) was consumed in significantly ($p < .01$) greater quantity than the biuret (ration 12), urea-cyanuric acid (ration 13), and cyanuric acid (ration 14) supplemented rations. The feed consumption was slightly less for the urea-biuret supplemented ration (ration 11) than for the urea supplemented ration but not significantly so. The ration containing a 1:1 mixture of urea and biuret was consumed in significantly ($p < .01$) larger amounts than was the ration supplemented with biuret alone. A 1:1 mixture of urea and cyanuric acid was consumed in significantly ($p < .01$) greater amounts than was the ration supplemented with cyanuric acid alone. The consumption of the urea-biuret ration and cyanuric acid ration tended to be larger than the urea-cyanuric acid ration and biuret ration, respectively, but not significantly larger. The consumption of the cyanuric acid ration tended to be larger than the biuret ration but not significantly so.

Gain in body weight followed a pattern similar to feed consumption. Ration 1 promoted significantly ($p < .01$) faster gain than did the biuret ration and the cyanuric acid ration. Gain was faster for ration 1 than the urea-biuret ration, and the urea-cyanuric acid ration but not significantly faster. The urea-biuret ration promoted faster gain ($p < .01$) than did the biuret ration and the animals on the urea-cyanuric acid ration gained faster than did the animals on the cyanuric acid ration ($p < .01$). The gain on the urea-biuret ration tended to be greater than that of the lambs on the urea-cyanuric acid ration but not significantly so.

Based on average daily gain there was no apparent improvement in utilization of any of the rations with time (Figure 1).

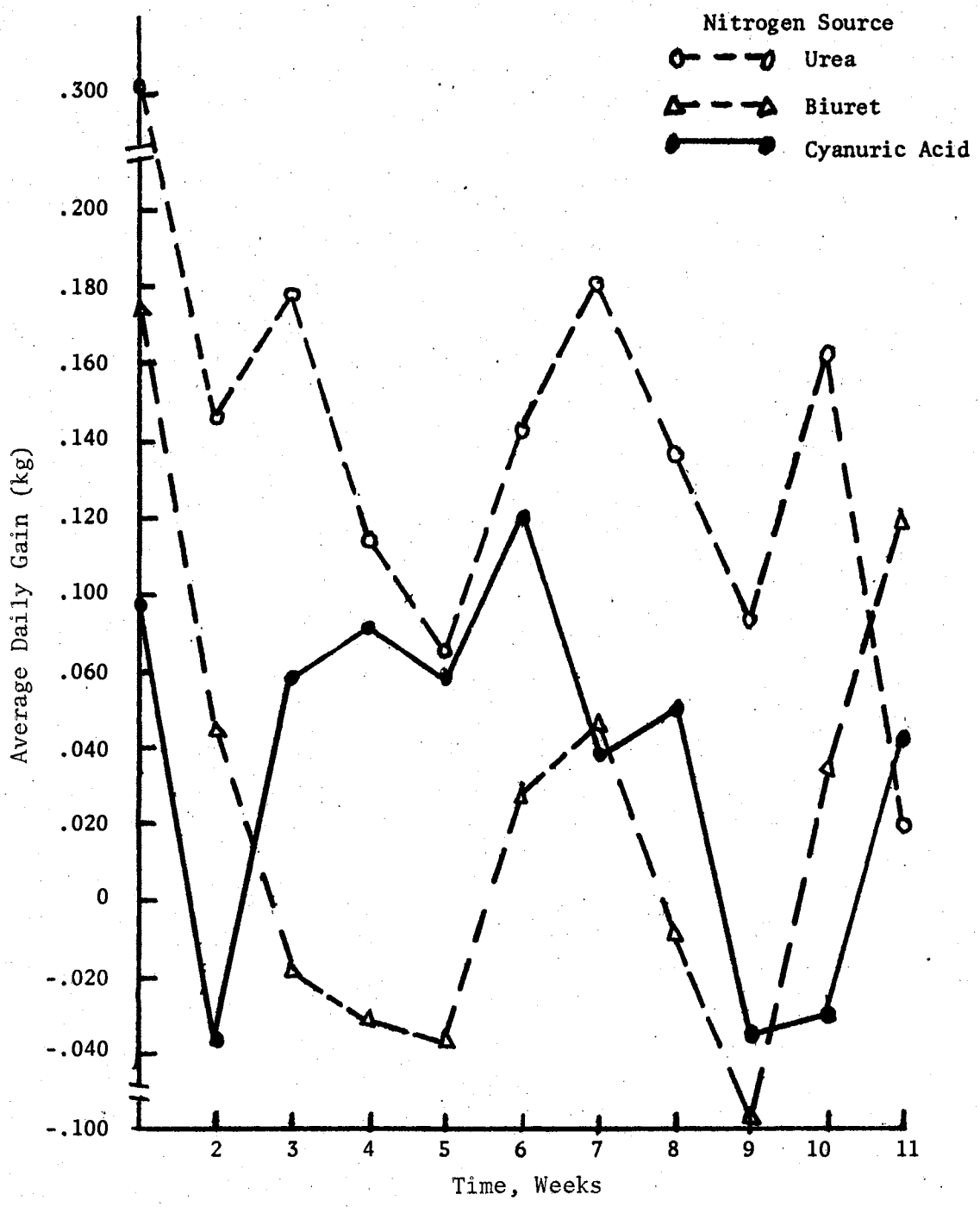

Figure 1. Average Daily Gain of Lambs Fed Various Nitrogen Sources

Feed efficiency showed essentially the same trend as feed consumption and rate of gain. Ration 1 was significantly ($p < .01$) more efficiently used for gain in body weight than were the biuret ration and cyanuric acid supplemented rations. The feeds containing a mixture of urea and biuret and a mixture of urea and cyanuric acid were significantly ($p < .01$) more efficiently utilized than were the biuret or cyanuric acid supplemented rations, respectively. The cyanuric acid supplemented ration tended to be used more efficiently than the biuret supplemented ration but the difference was not significant.

There were no significant differences in any response criteria due to location of the animals on the ground or loft of the barn.

The acetic, propionic, and butyric acid concentration and molar percents in the rumen contents for one or two lambs on each of the experimental rations are shown in Table X. There was no consistent change in the concentration or proportion of any of the three acids apparent with time. Due to the limited number of samples obtained and due to the loss of some of the samples these data were not subjected to statistical analysis. The branched chained fatty acids were present in very small concentrations or were not detected at all in the rumen fluid from these animals.

The concentration of urea-nitrogen and ammonia-nitrogen in blood and rumen fluid samples taken from lambs on the various treatments is presented in Table XI. There was no apparent relationship between the experimental treatment given a lamb and the concentration of either blood or rumen fluid ammonia-nitrogen and urea-nitrogen concentration.

The depressing effect of biuret on weight gains and feed efficiency found in this trial are in agreement with work reported by Berry, Riggs

TABLE X

VOLATILE FATTY ACIDS IN RUMEN FLUID OF LAMBS IN TRIAL 2

Ration	Animal Number	Day	Acetic		Propionic		Butyric		Total Moles
			Moles/ Liter	%	Moles/ Liter	%	Moles/ Liter	%	
1	69	0	0.1091	55.7	0.0733	37.4	0.0134	6.8	0.1958
		60	0.0675	55.9	0.0216	17.9	0.0316	26.2	0.1207
1	92	0	0.0723	59.6	0.0351	29.0	0.0138	11.4	0.1212
		60	0.0759	35.6	0.0193	9.0	0.1183	55.4	0.2135
11	63	0	0.0549	60.0	0.0216	23.6	0.0150	16.4	0.0915
		60	0.0663	61.2	0.0205	18.9	0.0215	19.8	0.1083
11	98	0	0.0509	58.2	0.0196	22.4	0.0169	19.3	0.0874
		60	0.0410	55.8	0.0180	24.5	0.0144	19.6	0.0734
12	82	0	0.0583	63.1	0.0230	24.9	0.0111	12.0	0.0924
		60	0.0379	66.8	0.0121	21.3	0.0067	11.8	0.0567
13	72	0	0.0402	49.6	0.0233	28.7	0.0176	21.7	0.0811
		60	0.0320	64.0	0.0106	21.2	0.0074	14.8	0.0500
14	74	0	0.0494	53.9	0.0220	24.0	0.0202	22.0	0.9160
		60	0.0268	55.8	0.0106	22.1	0.0106	22.1	0.0480
14	97	0	0.0736	55.4	0.0538	40.5	0.0055	4.1	0.1329
		60	0.0221	51.0	0.0157	36.2	0.0055	12.7	0.0433

TABLE XI

CONCENTRATION OF AMMONIA-NITROGEN AND UREA-NITROGEN IN BLOOD AND RUMEN FLUID OF LAMBS IN TRIAL 2

Ration	Animal Number	Day	Blood		Rumen	
			NH_3-N	Urea-N	NH_3-N	Urea-N
			µg/ml	mg/100 ml	µg/ml	mg/100 ml
1	69	0	2.7	23.0	500	4.5
		60	3.4	20.0	110	46.1
1	92	0	4.8	18.4	---	4.1
		60	4.1	9.8	200	10.7
11	63	0	3.6	30.0	450	5.1
		60	1.9	15.4	60	11.2
11	98	0	---	19.6	480	10.8
		60	---	12.0	30	1.6
12	82	0	2.1	18.4	405	---
		60	4.2	15.6	30	---
12	101	0	3.7	14.0	260	3.1
		60	3.9	12.0	10	5.1
13	72	0	2.7	23.8	440	2.6
		60	2.8	27.3	70	10.3
13	94	0	9.4	16.0	---	3.6
		60	9.4	11.0	---	15.3
14	74	0	3.5	---	350	2.6
		60	2.2	---	30	14.9
14	97	0	2.0	---	300	8.0
		60	3.4	---	500	9.4

and Kunkel (1956) and Campbell, et al. (1963). The fact that sheep fed biuret as the sole nitrogen source in their ration continued to gain weight for two weeks after they were switched from a diet containing cottonseed meal as the only nitrogen source indicates that an adaptation period is not needed. This is in agreement with the work of Hatfield (1968) in which he showed that the microflora in the rumen of urea fed sheep had the ability to hydrolyze a significant amount of biuret without any adaptation period. The data from this trial is in disagreement with that of Gilchrist, Potgieter and Voss (1968) who found that a two week adaptation period was needed before biuret breakdown could be demonstrated in an in vitro system. Based on this the possibility exists that utilization of biuret may occur at the tissue level as suggested by Schaadt, Johnson and McClure (1966) as well as in the rumen of sheep.

In contrast to the poor growth, low feed consumption and poor feed efficiency of lambs fed biuret as the sole nitrogen source, a mixture of biuret and urea gave as good or better performance as did urea alone. This is in agreement with work by Hatfield, et al. (1959) and Anderson, et al. (1959).

The performance of the lambs fed cyanuric acid as the sole nitrogen source was inferior to that of the animals supplemented with urea but about equal to those supplemented with biuret alone. The data from this trial does not agree with that of Clark, Barratt and Kellerman (1965) who found that cyanuric acid produced higher nitrogen balance in sheep than either urea or biuret. This difference may be due to the fact that these workers were feeding a low energy forage ration whereas the purified diets used in this trial would be classed as equivalent to a moderate concentrate ration.

A mixture of cyanuric acid and urea gave performance superior to cyanuric acid alone but not as good as the urea and biuret mixture. The reason for the improved growth and performance of lambs fed a mixture of urea and biuret or urea and cyanuric acid over those fed biuret or cyanuric acid as the sole nitrogen source is not readily apparent. It appears that the urea, which makes up half of the nitrogen in the urea-biuret supplemented ration, would not support the performance obtained when the mixture was fed. This is based on the performance obtained when all of the nitrogen was supplied as urea. Thus it appears that there could be some synergistic effect between the urea and biuret. This is further supported by the fact that the lambs fed the urea-cyanuric acid supplemented ration received the same amount of urea in the ration as did those fed the urea-biuret ration yet the feed consumption and weight gain for the urea-cyanuric acid fed animals tended to be less. These results suggest the possibilities that (1) while urea is utilized in the rumen for microbial protein synthesis biuret may be at least partially utilized at the tissue level as suggested by Schaadt, Johnson and McClure (1966); (2) that the level of urea used in the control ration was excessive and that the level of urea in the urea-biuret supplemented ration and in the urea-cyanuric acid ration approached or reached a level necessary to sustain the level of production obtained in this trial; or (3) urea is rapidly hydrolyzed in the rumen thus supplying a rapid but relatively short lived source of ammonia while biuret is less rapidly hydrolyzed but may give a longer lived supply of ammonia, thus the two sources may compliment each other as nitrogen sources.

McLaren, et al. (1960), Johnson and McClure (1964) and Oltjen, et al. (1968) have shown that biuret and urea do not have the same effect

on blood urea and rumen ammonia. In this trial it was not possible to detect any effect on rumen and blood urea or ammonia concentration due to treatment. This probably was partially due to the limited number of samples taken. A similar problem was encountered when an attempt was made to relate rumen VFA levels to dietary treatment. Hungate (1966) has pointed out that the nitrogen source fed to ruminants will have an effect on the concentration of VFA in the rumen. The problem is to make meaningful measurements to detect these differences. This is most difficult when only one sample is taken after feeding (Annison, 1965; Waite and Wilson, 1968; Oltjen, et al., 1969).

Trial 3

The results of the biuret metabolism trial are presented in Table XII. Data for individual animals are given in Tables XIII, XIV, and XV. There were no significant differences between animals in the amount or percentage of nitrogen retained during the first period when all lambs were fed a purified diet supplemented with cottonseed meal. When organic matter digestibility was measured the fistulated lambs showed a significantly ($p < .05$) higher digestibility than the non-fistulated lambs. Statistical analysis showed no difference due to treatment or period when the lambs were fed urea, biuret or a mixture of urea and biuret as the nitrogen source. There was no treatment by period interaction.

These results are not in agreement with Anderson, et al. (1959) who showed that pure biuret depressed nutrient digestibility and nitrogen utilization when substituted for urea. Similar depressions in nutrient utilization have been observed by several other workers

TABLE XII

NITROGEN RETENTION AND ORGANIC MATTER DIGESTIBILITY FOR LAMBS FED PURIFIED DIETS SUPPLEMENTED WITH UREA, BIURET, OR UREA AND BIURET[1]

Period[2]	Ration	Nitrogen Retained (gm)[3]	S.D.[4]	Percent Nitrogen Retained	S.D.	Organic Matter Digestibility	S.D.
1	Urea	16.18	4.74	22.56	6.60	64.69	5.16
	Urea-Biuret	16.38	8.10	22.81	11.29	66.68	4.92
	Biuret	20.84	5.74	29.02	8.07	63.52	7.40
2	Urea	3.36	3.58	5.63	5.93	75.73	1.57
	Urea-Biuret	10.80	6.96	16.33	10.76	75.96	3.50
	Biuret	7.20	7.48	10.49	11.08	73.76	3.49
3	Urea	7.10	4.68	10.80	6.70	73.98	3.62
	Urea-Biuret	5.72	5.10	8.26	7.36	77.52	3.89
	Biuret	10.45	13.10	14.57	18.09	76.09	2.84

[1]All lambs were fed the basal purified diet supplemented with cottonseed meal during period 1. Averages for lambs that were subsequently fed the three experimental rations are listed.

[2]Period 1 was a uniformity trial, collection for period 2 and 3 began 13 and 34 days after the start of feeding of the experimental rations, respectively.

[3]Seven day totals.

[4]Standard deviation.

TABLE XIII

NITROGEN RETENTION AND ORGANIC MATTER DIGESTIBILITY FOR LAMBS FED PURIFIED DIETS SUPPLEMENTED WITH UREA[1]

Period[2]	Animal Number	Nitrogen Retained (gm)[3]	Percent Nitrogen Retained	Organic Matter Digestibility
1	16	16.47	22.94	67.07
	14	11.54	16.08	71.24
	82	11.24	15.66	63.44
	91	20.86	29.06	64.52
	89	20.87	29.07	57.19
2	16	1.84	2.92	73.41
	14	5.48	10.14	75.56
	82	0.11	0.18	75.44
	91	8.60	13.64	76.68
	89	0.79	1.25	77.58
3	16	---	---	---
	14	11.54	17.26	76.09
	82	5.50	8.23	76.53
	91	10.11	15.12	74.59
	89	1.23	2.58	68.70

[1]All lambs were fed the basal purified diet supplemented with cottonseed meal during period 1.

[2]Period 1 was a uniformity trial, period 2 and 3 began 13 and 34 days after the start of feeding the experimental rations, respectively.

[3]Seven day totals except for animal 14, in period 2 which was collected from for 6 days and animal 89, period 3 which was collected from for 5 days.

TABLE XIV

NITROGEN RETENTION AND ORGANIC MATTER DIGESTIBILITY FOR LAMBS FED PURIFIED DIETS SUPPLEMENTED WITH UREA AND BIURET[1]

Period[2]	Animal Number	Nitrogen Retained (gm)[3]	Percent Nitrogen Retained	Organic Matter Digestibility
1	13	10.81	15.06	65.63
	20	25.96	36.17	74.50
	94	23.92	33.32	61.28
	93	13.42	18.70	67.51
	88	7.77	10.82	64.48
2	13	3.76	5.66	79.74
	20	---	---	---
	94	23.92	33.32	61.28
	93	6.50	9.36	73.22
	88	19.03	29.02	78.13
3	13	-0.35	-0.50	76.70
	20	---	---	---
	94	8.42	12.15	80.61
	93	3.66	5.28	72.36
	88	11.16	16.11	80.43

[1]All lambs were fed the basal purified diet supplemented with cottonseed meal during period 1.

[2]Period 1 was a uniformity trial, periods 2 and 3 began 13 and 34 days after the start of feeding the experimental rations, respectively.

[3]Seven day totals.

TABLE XV

NITROGEN RETENTION AND ORGANIC MATTER DIGESTIBILITY FOR LAMBS FED PURIFIED DIETS SUPPLEMENTED WITH BIURET[1]

Period[2]	Animal Number	Nitrogen Retained (gm)[3]	Percent Nitrogen Retained	Organic Matter Digestibility
1	12	19.78	27.56	50.21
	18	17.88	24.91	67.26
	19	17.03	23.72	69.24
	84	32.45	45.21	63.63
	83	18.16	25.30	70.04
	90	19.71	27.45	60.76
2	12	14.91	21.69	77.22
	18	7.95	11.86	76.65
	19	8.93	12.14	73.43
	84	-3.11	-4.74	72.96
	83	-0.16	-0.22	74.74
	90	14.73	22.21	67.53
3	12	10.44	16.91	78.78
	18	-2.41	-4.20	78.31
	19	---	---	---
	84	6.84	9.30	73.99
	83	5.06	6.88	77.13
	90	32.33	43.97	72.26

[1]All lambs were fed the basal purified diet supplemented with cottonseed meal during period 1.

[2]Period 1 was a uniformity trial, period 2 and 3 began 13 and 34 days after the start of feeding the experimental rations, respectively.

[3]Seven day totals.

(Hatfield, et al., 1959; McLaren, et al., 1959; Schaadt, Johnson and McClure, 1966; Oltjen, et al., 1968).

Considerable difficulty was encountered in maintaining constant feed intake in this trial. The energy and protein intake were approximately at maintenance levels for the size sheep used in this trial. Those factors may have had an adverse effect on any attempt to detect differences in nitrogen utilization by the lambs due to treatment. Farlin, Garrigus and Hatfield (1968) showed that when sheep were fed biuret there was an increase in nitrogen retention with time but that urea promoted higher nitrogen retention than biuret. However, in another trial when difficulty was experienced in maintaining feed intake the results were variable and no advantage could be shown for urea. Johnson and McClure (1966) have shown that lambs fed low nitrogen ration would adjust to the low nitrogen and go into positive nitrogen balance even though they were initially in negative nitrogen balance.

Those results do not support the results obtained on the growth trials in which similar rations were fed.

Trial 4

The concentration of biuret in the in vitro flasks at various hours on days 0, 19, 45 and 64 are shown in Table XVI. The results do not show any indication of biuret disappearance from the flasks. The biuret concentrations are quite variable between duplicate flasks and within the same flasks at various sampling times. This variability may be due to the lack of a reliable analytical procedure for determining biuret or due to the fact that the rumen ingesta and biuret solution added to the flasks were not mixed thoroughly before samples were taken

TABLE XVI

BIURET CONCENTRATION IN IN VITRO FERMENTATION FLASKS AT VARIOUS TIMES ON DAYS 0, 19, 45 AND 64 AFTER START OF FEEDING EXPERIMENTAL DIETS

Ration	Animal	Flask	Hour	Day			
				0	19	45	64
				Biuret mg%			
Urea	16	1	0	284	173	293	569
			4	-	207	-	-
			6	318	-	-	-
			12	-	-	276	471
			24	317	250	300	430
		2	0	-	288	339	329
			4	-	269	-	-
			6	-	-	-	-
			12	-	-	346	365
			24	-	209	346	376
Urea	14	1	0	302	214	366	411
			4	-	194	-	-
			6	402	-	-	-
			12	-	-	396	784
			24	593	201	495	483
		2	0	-	215	348	392
			4	-	185	-	-
			6	-	-	-	-
			12	-	-	411	338
			24	-	190	455	321
Urea-Biuret	13	1	0	373	604	428	424
			4	-	375	-	-
			6	201	-	-	-
			12	-	-	378	476
			24	457	388	424	481
		2	0	-	368	407	439
			4	-	400	-	-
			6	-	-	-	-
			12	-	-	422	529
			24	-	407	405	450

TABLE XVI (Continued)

Ration	Animal	Flask	Hour	Day 0	Day 19	Day 45	Day 64
				Biuret mg%			
Urea-Biuret	20	1	0	396	102	355	574
			4	-	277	-	-
			6	425	-	-	-
			12	-	-	343	481
			24	472	138	363	518
		2	0	-	306	395	371
			4	-	297	-	-
			6	-	-	-	-
			12	-	-	383	344
			24	-	353	383	338
Biuret	19	1	0	396	177	-	-
			4	-	146	-	-
			6	540	-	-	-
			12	-	-	-	-
			24	599	84	-	-
		2	0	-	220	-	-
			4	-	206	-	-
			6	-	-	-	-
			12	-	-	-	-
			24	-	90	-	-
Biuret	18	1	0	358	208	385	385
			4	-	280	-	-
			6	450	-	-	-
			12	-	-	349	350
			24	457	286	316	371
		2	0	264	183	442	291
			4	407	244	-	-
			6	435	-	-	-
			12	-	-	390	332
			24	538	-	327	300

for analysis. The sheep on this trial had a tendency to eat wool. The rumen contents from those sheep contained appreciable wool which may have interfered with the ability to mix the rumen contents and to sample the digestion flasks properly.

Those results do not support those of Gilchrist, Potgieter and Voss (1968) and Schroder and Gilchrist (1969) where they demonstrated biuret disappearance in an in vitro system such as used here. One difference between this experiment and that reported by the above workers was that they used natural diets containing some natural protein whereas this work was done with purified diets in which all of the dietary nitrogen supplied to the sheep was from urea, urea and biuret or biuret.

Ammonia-nitrogen concentration in the in vitro flasks is shown in Table XVII. There was no apparent relationship between nitrogen source or day of sampling and ammonia concentration in the flasks. These data support the above data in that the failure to detect increased concentration of ammonia indicates that biuret was not broken down to ammonia.

The results of the in vivo portion of the biuret trial are shown in Tables XVIII, XIX, XX, XXI, XXII and XXIII. Rumen fluid samples taken from lambs fed the cottonseed meal supplemented ration and those subsequently fed the urea supplemented ration showed very low biuret concentrations. Since these animals were not fed any biuret, any biuret detected in these samples should be due to other compounds such as short peptides and amino acids which would react with the color reagent to give some absorbance at the wave length used to measure biuret concentration. Gilchrist, Potgieter and Voss (1968) also reported that rumen fluid contains some color pigments, which interfere with the analysis for biuret. The ammonia concentration generally decreased for the first

TABLE XVII

AMMONIA NITROGEN CONCENTRATION IN IN VITRO FERMENTATION FLASKS AT VARIOUS TIMES ON DAYS 0, 19, 45 AND 64 AFTER START OF FEEDING EXPERIMENTAL DIETS

Ration	Animal	Flask	Hour	Day			
				0	19	45	64
				NH_3-N (µg/ml)			
Urea	16	1	0	45.0	8.3	48.0	24.0
			4	51.0	9.0	---	---
			6	45.0	---	---	---
			12	---	---	31.5	17.0
			24	32.0	8.3	30.0	42.5
		2	0	24.0	7.2	34.0	11.5
			4	53.0	3.5	---	---
			6	64.0	---	---	---
			12	---	---	23.2	8.0
			24	117.0	9.1	29.0	69.5
	14	1	0	10.5	9.0	31.0	25.5
			4	17.5	7.7	---	---
			6	33.0	---	---	---
			12	---	---	74.0	23.5
			24	81.0	3.6	199.0	---
		2	0	4.6	5.2	51.0	30.0
			4	72.0	9.2	---	---
			6	8.5	---	---	---
			12	---	---	103.5	14.0
			24	114.0	4.7	224.0	66.5
Urea-Biuret	13	1	0	19.0	9.0	9.0	11.8
			4	26.0	2.1	---	---
			6	31.0	---	---	---
			12	---	---	22.0	36.5
			24	287.0	97.0	52.0	111.0
		2	0	16.0	8.8	31.0	18.0
			4	25.0	2.3	---	---
			6	29.0	---	---	---
			12	---	---	22.0	39.0
			24	301.0	75.0	37.0	114.0

TABLE XVII (Continued)

Ration	Animal	Flask	Hour	Day 0	Day 19	Day 45	Day 64
				NH_3-N (µg/ml)			
Urea-Biuret	20	1	0	71.0	8.3	26.0	22.5
			4	106.0	1.4	---	---
			6	119.0	---	---	---
			12	---	---	12.0	19.5
			24	233.0	8.2	49.0	25.0
		2	0	69.0	4.9	44.5	15.5
			4	114.0	7.0	---	---
			6	140.0	---	---	---
			12	---	---	36.5	23.0
			24	360.0	5.1	48.0	23.5
Biuret	19	1	0	20.0	110.5	---	---
			4	28.0	196.0	---	---
			6	89.0	---	---	---
			12	---	---	---	---
			24	492.0	910.0	---	---
		2	0	23.0	84.0	---	---
			4	110.0	173.0	---	---
			6	80.0	---	---	---
			12	---	---	---	---
			24	745.0	910.0	---	---
Biuret	18	1	0	26.5	2.6	278.0	11.5
			4	30.5	450.0	---	---
			6	15.0	---	---	---
			12	---	---	58.5	45.0
			24	144.0	81.0	244.0	212.0
		2	0	5.3	3.6	58.0	8.2
			4	5.8	148.0	---	---
			6	10.8	---	---	---
			12	---	---	60.5	57.5
			24	124.0	---	250.0	244.0

TABLE XVIII

IN VIVO BIURET CONCENTRATION IN RUMEN FLUID OF LAMBS FED PURIFIED DIETS

Ration	Animal	Hour	Day		
			0	20	41
				Biuret Mg%	
Urea	16	0	--	21	53
		1	--	17	21
		2	--	0	0
		4	--	0	0
		7	--	21	44
		12	--	26	14
	14	0	0	30	10
		1	0	0	12
		2	7	0	20
		4	14	0	10
		7	2	34	12
		12	5	14	15
Urea-Biuret	13	0	9	107	250
		1	9	186	491
		2	2	195	374
		4	0	186	335
		7	0	193	293
		12	9	155	221
	20	0	9	36	10
		1	25	141	118
		2	27	127	62
		4	27	100	62
		7	34	114	47
		12	45	114	5
Biuret	18	0	11	62	111
		1	54	138	529
		2	57	209	464
		4	14	69	304
		7	0	10	213
		12	2	10	104
	19	0	19	251	---
		1	14	322	---
		2	17	264	---
		4	5	164	---
		7	2	78	---
		12	0	29	---

TABLE XIX

IN VIVO AMMONIA-NITROGEN IN RUMEN FLUID OF LAMBS FED PURIFIED DIETS

Ration	Animal	Hour	Day		
			0	20	41
			NH_3-N (µg/ml)		
Urea	16	0	121.0	26.0	74.0
		1	67.0	191.0	252.0
		2	68.0	345.0	436.0
		4	16.0	64.0	120.0
		7	26.0	21.0	12.5
		12	87.0	18.0	28.5
	14	0	187.0	24.0	108.0
		1	30.0	475.0	284.0
		2	22.8	228.0	320.0
		4	20.2	41.0	102.0
		7	65.0	55.0	9.0
		12	97.0	33.8	61.5
Urea-Biuret	13	0	190.0	2.2	36.0
		1	101.0	202.0	370.0
		2	26.0	126.0	266.0
		4	16.0	2.2	56.5
		7	63.0	0.7	5.5
		12	134.0	2.7	6.0
	20	0	208.0	0.5	59.5
		1	100.0	144.0	379.0
		2	77.0	110.0	40.0
		4	16.0	14.0	9.5
		7	70.0	6.8	28.0
		12	88.0	4.5	46.0
Biuret	19	0	202.0	18.0	---
		1	73.0	26.0	---
		2	36.0	58.0	---
		4	29.0	52.0	---
		7	34.0	72.0	---
		12	34.0	3.1	---
	18	0	107.0	2.4	57.0
		1	91.0	2.9	24.5
		2	104.0	5.2	41.0
		4	159.0	3.2	4.0
		7	106.0	34.2	13.5
		12	82.0	20.0	18.0

TABLE XX

AVERAGE ACETIC, PROPIONIC AND BUTYRIC ACID CONCENTRATION IN RUMEN FLUID OF LAMBS FED PURIFIED DIETS CONTAINING COTTONSEED MEAL AS THE NITROGEN SOURCE

Hours	Acetic Acid		Propionic Acid		Butyric Acid	
	gm/liter	Mole %	gm/liter	Mole %	gm/liter	Mole %
0	1.98	66.4	0.98	22.9	0.50	10.7
1	1.95	57.0	1.12	23.1	1.08	19.9
2	2.18	62.0	0.85	18.2	1.00	19.8
4	1.86	67.2	0.63	15.9	0.71	16.9
7	1.82	69.4	0.58	16.5	0.47	14.1
12	1.56	71.5	0.59	18.7	0.36	9.6

TABLE XXI

AVERAGE ACETIC, PROPIONIC AND BUTYRIC ACID CONCENTRATIONS IN RUMEN FLUID OF LAMBS FED PURIFIED DIETS SUPPLEMENTED WITH UREA

Days on Ration	Hours	Acetic Acid		Propionic Acid		Butyric Acid	
		gm/ liter	Mole %	gm/ liter	Mole %	gm/ liter	Mole %
20	0	1.46	68.9	0.60	22.2	0.28	9.0
	1	1.99	52.6	1.68	34.4	0.71	13.0
	2	2.36	47.8	2.20	36.2	1.13	16.0
	4	2.57	58.0	1.54	28.2	0.89	13.7
	7	1.46	60.5	0.84	28.8	0.36	10.7
	12	1.82	73.8	0.70	19.8	0.24	6.4
41	0	1.11	62.6	0.56	23.1	0.40	14.2
	1	1.58	54.2	0.88	28.2	0.87	17.5
	2	1.72	55.0	1.00	29.0	0.81	15.9
	4	1.98	58.1	1.00	24.6	0.88	17.3
	7	1.80	63.9	0.68	21.6	0.65	14.6
	12	1.45	68.4	0.44	19.6	0.42	12.0

TABLE XXII

AVERAGE ACETIC, PROPIONIC AND BUTYRIC ACID CONCENTRATIONS IN RUMEN FLUID OF LAMBS FED PURIFIED DIETS SUPPLEMENTED WITH UREA AND BIURET

Days on Ration	Hours	Acetic Acid		Propionic Acid		Butyric Acid	
		gm/ liter	Mole %	gm/ liter	Mole %	gm/ liter	Mole %
20	0	1.38	61.2	0.45	21.2	0.48	17.5
	1	1.26	56.2	0.72	26.5	0.57	17.2
	2	1.27	57.6	0.66	26.4	0.57	16.0
	4	2.20	58.7	1.01	23.9	0.95	17.4
	7	1.20	61.0	0.50	23.3	0.44	15.7
	12	1.04	68.2	0.26	20.0	0.24	11.9
41	0	1.07	73.0	0.31	16.4	0.26	10.6
	1	1.48	52.8	1.00	29.3	0.74	18.0
	2	2.05	54.2	1.28	28.0	0.98	17.8
	4	1.77	62.6	0.72	20.3	0.73	17.2
	7	1.24	69.4	0.36	17.2	0.38	13.5
	12	1.00	64.4	0.32	18.9	0.48	16.6

TABLE XXIII

AVERAGE ACETIC, PROPIONIC AND BUTYRIC ACID CONCENTRATIONS IN RUMEN FLUID OF LAMBS FED PURIFIED DIETS SUPPLEMENTED WITH BIURET

Days on Ration	Hours	Acetic Acid		Propionic Acid		Butyric Acid	
		gm/ liter	Mole %	gm/ liter	Mole %	gm/ liter	Mole %
20	0	1.06	51.1	0.57	24.0	0.79	24.8
	1	1.00	58.6	0.44	20.8	0.54	20.7
	2	0.91	62.6	0.36	20.0	0.38	17.3
	4	0.92	59.0	0.49	24.6	0.38	16.4
	7	0.63	54.0	0.38	24.4	0.40	21.5
	12	1.12	62.3	0.56	22.9	0.44	14.8
41	0	1.33	65.6	0.53	21.4	0.39	13.0
	1	0.70	42.0	0.56	27.5	0.74	30.4
	2	1.25	55.6	0.62	22.4	0.72	21.9
	4	2.29	65.2	0.43	9.9	1.28	24.8
	7	1.48	71.1	0.32	12.4	0.50	16.5
	12	0.75	70.2	0.20	15.2	0.23	14.6

four hours and then began a gradual rise in concentration for the next six hours when the cottonseed meal supplement was fed.

Biuret concentrations were at their highest levels at one hour in lambs fed a combination of urea and biuret and then decreased slightly through the 12 hour sampling time. The rumen ammonia concentration reached its peak at one to two hours after feeding in lambs fed urea and urea plus biuret. The ammonia concentration generally returned to the pre-feeding level by the time the four or seven hour samples were taken. There was no apparent difference in the concentration due to feeding the urea or urea plus biuret supplements.

The lambs fed the biuret supplement showed peak biuret concentrations at one to two hours after feeding. The biuret concentration then decreased to a low level at seven to 12 hours, indicating that the biuret was broken down in the rumen, absorbed from the rumen, passed down the digestive tract, diluted with water or tied up by some means in the rumen. The rumen ammonia concentrations found when biuret was fed resemble those seen when cottonseed meal was fed. The ammonia concentration remained near the pre-feeding concentration or else decreased slightly. Based on this no conclusion can be drawn as to whether ammonia was released from biuret or not. It may be that ammonia was released from the biuret at such a slow rate that it was immediately utilized by the microorganisms in the rumen and thus did not accumulate.

The results are in agreement with those of Johnson and McClure (1964) who found that biuret feeding had no appreciable effect on rumen ammonia concentration, whereas urea did. The fact that ammonia concentration did not increase *in vivo* is in agreement with the fact that there was little increase in ammonia concentration in the *in vitro*

fermentation flask reported above. These results are in direct contrast to those of Gilchrist, Potgieter and Voss (1968) and Schroder and Gilchrist (1969) who demonstrated the stoichiometric conversion of biuret-nitrogen to ammonia-nitrogen.

Concentrations of acetic acid and butyric acids were not affected by the feeding of urea, urea and biuret or biuret and did not change from day 20 to day 41. Propionic acid concentration was higher ($p < .05$) on the urea ration than on the other two rations on day 20 and 41 two hours after feeding. Also the urea and biuret ration produced higher concentrations of propionic acid than the biuret ration ($p < .05$) and ($p < .01$), respectively. Again at four hours the propionic acid concentration was higher on the urea ration than on the biuret ration ($p < .05$).

These results are in general agreement with those of Orskov and Oltjen (1967) who showed that there was no difference in total concentration of VFA in the rumen when urea and biuret were compared as nitrogen sources. This work did not indicate any differences in concentration of VFA or difference in molar percent of acetic, propionic and butyric acids when a natural protein source was fed or when NPN sources were used. This is in contrast to what Orskov and Oltjen (1967) found. Those workers reported that the total concentration of VFA in the rumen fluid was greater for cattle receiving purified diets containing urea than cattle receiving natural diets two hours after feeding.

The concentration of branched chain VFA was low on all diets and in general made up a very small percentage of the total VFA. This is in agreement with Waite and Wilson (1968) and el-Shazly (1952a,b) who reported that the branched chained fatty acids are derived from natural

protein sources. The reason for the low concentration of branched chain VFA in the rumen contents of sheep fed cottonseed meal is not clear.

Trial 5

Four lambs fed the basal purified diet which contained no nitrogen source and were administered biuret through the fistula showed irregular responses in feed consumption (Table XXIV). These results indicate that the limiting factor in their voluntary feed intake was not a palatability factor associated with the biuret. When two of the lambs were switched to urea supplementation the feed consumption improved slightly in one lamb but not in the other (Table XXV). These lambs were maintained in metabolism stalls where their physical activity was severely restricted. This may have had a restricting effect on feed intake. Previous adaptation to biuret did not have any effect on voluntary feed consumption.

TABLE XXIV

FEED CONSUMPTION OF LAMBS ADMINISTERED BIURET VIA FISTULA

Day	Animal Number			
	12	18	16	14
	Feed (gm)			
1	500	500	500	500
2	550	550	550	550
3	500	600	600	600
4	500	600	600	600
5	500	600	600	600
6	500	600	600	600
7	500	600	650	600
8	550	600	700	600
9	600	600	750	600
10	650	650	800	600
11	700	700	800	600
12	700	750	800	600
13	700	750	800	600
14	700	750	800	600
15	700	800	850	600
16	700	800	900	600
17	700	800	900	600
18	700	800	900	600
19	700	800	900	600

TABLE XXV

FEED CONSUMPTION BY LAMBS ADMINISTERED UREA VIA FISTULA

Day	Animal Number	
	12	16
	Feed (gm)	
1	500	500
2	500	500
3	600	600
4	700	700
5	800	800
6	800	800
7	850	850
8	900	900
9	900	900
10	950	900
11	950	850
12	1000	850
13	1050	850
14	1100	850
15	1100	850
16	1100	900
17	1100	850
18	1100	850
19	1100	850
20	1100	850
21	1100	850
22	1100	850
23	1100	850
24	1000	850
25	1000	850
26	1000	850
27	1000	850
28	1000	850
29	1000	850

CHAPTER V

SUMMARY AND CONCLUSIONS

This study was conducted to evaluate the effect of four levels of calcium (0.40, 0.48, 0.56, 0.64%) and four levels of phosphorus (0.30, 0.36, 0.42, and 0.48%) on growth performance of lambs fed purified diets. Evaluation of the effect of increasing the crude protein content of the diet from 11.0 to 13.7, 16.3 and 18.9% was also made. A wood hemicellulose extract was evaluated as an energy source in the purified diets when it was used to replace glucose, starch or wood fiber. Biuret and cyanuric acid were tested as possible nitrogen sources for lambs in a growth study. Biuret was further evaluated in a nitrogen balance trial. An in vitro digestion trial was conducted in which biuret disappearance from in vitro flasks, which had been innoculated with rumen fluid from lambs fed biuret for varying lengths of time, was measured. A trial was also conducted to test the influence of the palatability of biuret on feed consumption.

There was no significant effect of level of calcium and phosphorus or the use of a wood hemicellulose extract in the diet on feed intake, weight gain or feed conversion. Increasing the level of nitrogen in the ration had no significant effect on feed consumption or weight gain. A significant linear effect was found in feed conversion ($b = -22.9$). The efficiency of feed utilization tended to decrease with increased non-protein nitrogen level.

Weight gain, feed consumption and feed conversion were significantly depressed when either biuret or cyanuric acid was used to replace all of the urea in the purified diet. A 1:1 mixture of urea and biuret gave equivalent performance to urea alone. In general a 1:1 mixture of urea and cyanuric acid was inferior to urea as the sole nitrogen source but superior to cyanuric acid alone. A nitrogen balance study failed to show any difference between urea as the sole nitrogen source and a urea-biuret mixture or biuret alone. Attempts to measure the induction of biureolytic activity in the rumen ingesta of lambs by measuring biuret disappearance from *in vitro* flasks failed to show any adaptation to biuret. Palatability of biuret did not appear to influence feed intake.

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