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Protein Utilization by Cattle and Sheep as Affected by Feed Source, Processing Treatment and Level of Supplementation

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PROTEIN UTILIZATION BY CATTLE AND SHEEP AS AFFECTED BY FEED SOURCE, PROCESSING TREATMENT AND LEVEL OF SUPPLEMENTATION

BY

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This thesis is approved as a creditable and independent investigation by a candidate for the degree, Doctor of Philosophy, and is acceptable for meeting the thesis requirements for this degree. Acceptance of this thesis does not imply that the conclusions reached by the candidate are necessarily the conclusions of the major department.

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PROTEIN UTILIZATION BY CATTLE AND SHEEP AS AFFECTED BY FEED SOURCE, PROCESSING TREATMENT AND LEVEL OF SUPPLEMENTATION

Abstract

LUIS FERNANDO PALMER

Feeding experiments, digestion-nitrogen balance trials, rumen fermentation studies and blood parameter determinations were conducted to evaluate protein utilization by cattle and sheep as affected by feed source represented by ingredients varying in solubility or degradability of protein and amino acid profile, processing treatment and level of supplementation.

Growing and finishing cattle showed improved performance when fed ground ear corn or corn silage as basal diets supplemented with various sources of protein. Higher rates of gain and lower feed requirements were observed for cattle fed diets supplemented with urea, soybean meal, heat-treated soybean meal, heat-treated mix of 60% soybean meal and 40% soybeans, urea-dehydrated alfalfa meal and soybean meal-dehydrated alfalfa meal in comparison to cattle fed the control diets. No difference ($P>.05$) in animal performance was observed among the protein-supplemented diets.

Data obtained for these diets tested through nutrient digestibility and nitrogen balance trials with lambs showed that, generally, there were no improvements in organic matter apparent digestibility from any of the sources of protein supplementation. Crude protein

digestibility, in general, was higher for diets supplemented with protein than for control diets, with only slight differences among protein-supplemented diets. Protein supplementation resulted in elevated levels of nitrogen being retained as a percentage of the total nitrogen consumed for lambs fed the various sources of supplemental protein as compared to those which received only the control diets.

Rumen fermentation studies showed that lambs fed the various supplemental sources of protein had lower rumen pH and higher concentrations of rumen lactic acid, rumen ammonia-nitrogen and total volatile fatty acids than those which consumed control diets. Increased propionic acid and decreased acetic acid molar percentage concentrations were observed for lambs fed the protein-supplemented diets, resulting in significantly lower acetic to propionic acid ratios for these groups.

Hourly rumen fermentation studies showed that ruminal activity was at the maximum level between 2 to 4 h after feeding, since during this period lower rumen pH together with higher lactic acid and total volatile fatty acid concentrations were observed. There were some differences in the rate of fermentative activity in the rumen between dietary treatments at each sampling period with protein-supplemented lambs showing greater activity.

In studies conducted to evaluate growing-finishing lamb responses to soybean meal or urea at various levels of supplementation, it was observed that these sources appeared to be of similar value, since there were essentially no differences in average daily gain, feed intake or feed efficiency between the two supplements. Feeding lambs

diets containing increasing levels of protein (from 11.2 to 15.1%) resulted in improved average daily gain, increased feed intake and lower feed requirements. However, the rate of improvement in average daily gain decreased at the higher level of dietary protein, indicating that the highest level (15.1%) may have approached or exceeded protein requirements for the lambs. No indication of a period of adaptation was noticed during the feeding trial for lambs fed urea up to 1.08% of the dry diet. Under conditions of adaptational digestion-nitrogen balance studies, it was also observed that there appeared to be no evidence of a period of adaptation to diets with the various levels of protein from soybean meal or urea as measured by nutrient digestibility and nitrogen balance. Lambs receiving diets with urea had slightly higher digestibility for organic matter but similar digestibility for crude protein and for nitrogen balance as lambs fed soybean meal-supplemented diets. In general, feeding diets containing 12.5, 13.8 and 15.1% protein to lambs resulted in improved nutrient digestibility and nitrogen balance as compared to diets containing 11.2% protein.

During these lamb studies, nitrogen supplementation, particularly urea, resulted in increased rumen ammonia-nitrogen concentrations. Higher blood urea-nitrogen was observed for lambs fed increasing levels of protein, whereas protein supplementation at the levels fed during this research appeared to have no effect on plasma ammonia-nitrogen concentration.

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TABLE OF CONTENTS

LIST OF TABLES

Table | Page

LIST OF FIGURES

LIST OF APPENDIX TABLES

INTRODUCTION

Many experiments have been concerned with the amount of protein needed by cattle and sheep under various types and levels of production. Even though both animal and feed factors are known to affect requirements for dietary protein, recommended allowances have been established to provide rather efficient and economical production under practical feeding conditions. In these studies different sources of protein were also evaluated. Major emphasis was placed on the chemical and physical properties of proteins and how these properties affect nitrogen metabolism, digestibility and animal performance.

Research has shown that a major portion of the dietary protein ultimately available to the ruminant is converted to bacterial protein regardless of the dietary source. However, it has been well established that all sources of protein are not of equal value to the ruminant. While amino acid composition (quality) of the dietary protein is considered to be a factor of some importance, this is a matter of much debate in the case of cattle and sheep. Solubility of the nitrogen (particularly nonprotein nitrogen), rate of degradation of protein nitrogen in the rumen and type and availability of carbohydrates are important factors affecting the efficiency of synthesis of bacterial proteins. Rate of degradation of dietary proteins and availability of carbohydrates influence the amount of nitrogenous products that escape rumen digestion and which will be exposed to later enzymatic digestion and absorption. The amino acid composition of this escape protein and

its effect on animal performance has been an active area of research in recent years. Several methods for reducing protein degradation and increasing the amount of dietary protein and amino acids of higher quality leaving the rumen have been investigated and others are topics of current research. Studies with protein sources varying in protein solubility have suggested that the value of the protein which escapes degradation in the rumen depends on its ability to complement the amino acid composition of microbial proteins.

Processes used to reduce protein solubility and degradation in the rumen often add to the cost of protein supplementation. The added cost is likely to be only a small fraction of the total feeding costs. Thus, small improvements in performance can be of considerable economical importance. Therefore, potential processing methods and protein products should be examined thoroughly under a variety of dietary conditions, types of production and levels of production.

This study was conducted with the objective of determining the effects on performance of cattle and sheep fed commercially available sources of protein varying in amino acid composition and solubility, or degradability, as supplements to low protein diets for growing and finishing ruminants. In addition to feeding trials, the research included digestibility and nitrogen balance trials to evaluate the utilization of nitrogen and other nutrients, rumen fermentation studies to relate ruminal volatile fatty acids (VFA), ammonia and lactic acid concentrations to nitrogen metabolism and blood measurements of ammonia and urea to indicate how dietary proteins were utilized. The effect of

processing, special heat treatments or combining ingredients of varying protein solubility were investigated as ways of improving protein utilization by ruminants. The utilization of nutrients (nitrogen) in adaptation and finishing studies with lambs fed various levels of protein was also a part of this research.

REVIEW OF LITERATURE

The nutritional value of a protein depends upon the amount and proportion of amino acids (especially the essential ones) and their physiological availability in relation to the specific requirements of the animal (Friedman, 1978). Availability of amino acids varies and depends upon the feed source, interaction with other dietary components and the physiological state and age of the animal. The studies reported in this manuscript were concerned with the utilization of protein from several sources by ruminants and factors affecting their utilization. Research conducted for my Master of Science degree (Palmer, 1982) was concerned with a similar subject. However, the review of literature presented emphasized the principles of protein solubility and ways that protein sources could be treated to influence solubility. Animal performance, nitrogen metabolism and nitrogen utilization as affected by solubility were also reviewed to a lesser extent.

This literature review will be concerned, for the most part, with the effects of protein sources on nitrogen metabolism and utilization by the ruminant under various feeding conditions and the influence on the different metabolic nitrogen fractions. The two reviews should be considered complementary and together represent a more complete coverage of the subjects addressed during the research carried out during my graduate study.

Nutritional Quality of Protein Sources for Ruminants

Protein sources commonly used for ruminant feeding are those from nitrogen-bearing materials which include mainly the cereal grains, forages, oil-bearing seeds and by-products from these. However, there are several less used protein sources which include nonprotein nitrogen (NPN) sources, various products from the sea, microbial or single-cell proteins, meat and milk by-products and proteins recycled from animal waste (Oldfield, 1973). Measures of protein quality and nitrogen utilization may include yield of useful products such as meat, milk and wool. For more specific and usually more exact methods, nutritionists resort to evaluations based on digestibility, nitrogen retention in tissues of growing animals, biological value, protein efficiency ratio, net protein value or changes in rumen or blood components such as blood urea or amino acids (Church, 1979). In the following sections, the effect of protein source in relationship to nitrogen metabolism and utilization will be reviewed.

Rumen Nitrogen Sources

Nitrogen reaching the rumen originates mainly from the diet. However, salivary nitrogen, nitrogen infiltration through the rumen wall and endogenous nitrogen from abraded epithelial cells in the rumen wall make up a sizable portion (Waldo, 1968; Orskov, 1982).

Dietary Nitrogen

Feed protein is the input source of nitrogen into the animal and one of the most important sources of nitrogen for the rumen. The dietary nitrogen reaching the rumen can be classified into protein, NPN and some nitrogen in the form of nucleic acid (Van Soest, 1982). True protein generally makes up 60 to 80% of the total plant protein with soluble NPN, lignified nitrogen and small amounts of nucleic acid comprising the remainder (Van Soest, 1982). However, the relative amounts may be influenced by management procedures utilized to harvest the plants. Brady (1960) found that fresh forages contained 10 to 30% of the protein as NPN, whereas drying of forage for hay or haylage increased the NPN fraction to 25 to 50%. Unwilted silage had increased NPN concentration up to 60 to 75% of the protein during storage. In this NPN fraction was a volatile base or ammoniacal fraction amounting to 15 to 30% of the total nitrogen (Brady, 1960). On a related note, Sniffen (1974) reported that the ensiling process significantly increased protein solubility and as much as 90% of the soluble nitrogen was NPN. Further, Sniffen (1974) reported that under some conditions in the ensiling process as much as 50% of the protein can be tied up in the lignin fraction which has no digestibility. In earlier publications, Waldo (1968) stated that this association of indigestible nitrogen with lignin represented 1.2 to 1.6% of the lignin in grasses and 2.9 to 3.4% of the lignin in legumes. Data for corn grain and soybean seed showed a decrease from 30 to 40% of the total nitrogen as NPN in an immature stage to 4 to 5% in the dry seed stage (Waldo, 1968).

Seed proteins consist of the nitrogen reserves of the plants and include albumins, which are water-soluble but alcohol-insoluble; globulins, insoluble in water and alcohol but soluble in salt solutions of medium strength; prolamines, soluble in alcohol but insoluble in water and salt solutions; and glutelins, soluble only in dilute alkali (Van Soest, 1982). From the standpoint of the rumen environment, albumins and globulins are generally the protein fractions in the feedstuffs that represent the major soluble portion of the total protein. Solubility in this case is defined as that protein which will go into solution in the rumen fluid (Sniffen, 1974). The prolamines and glutelins represent the insoluble fraction in the rumen and have the potential of escaping degradation in the rumen. It is important to note that the quality of protein in albumins and globulins is generally higher than in prolamines and glutelins (Mosse, 1966).

Cereal seed proteins contain the various fractions previously indicated. Prolamines and glutelins are exclusively confined to the starchy endosperm, whereas albumins and globulins are also found in the aleurone layers of the seed and in the embryo (Bozzini and Silano, 1978). As shown in table 1, prolamines and glutelins generally make up 80 to 90% of the total grain proteins, with the exception of oat grain protein which contains about 80% globulins. Legume seed proteins, on the other hand, are composed of only two main fractions (albumins and globulins). Generally, these represent as much as 80 to 97% of the total legume seed proteins (Sniffen, 1974; Bozzini and Silano, 1978).

TABLE 1. PROTEIN CONTENT AND PROTEIN FRACTIONS OF VARIOUS CEREAL AND LEGUME PROTEIN SOURCES[a]

Name	Protein (% of DM)	Protein fractions % of total protein			
		Albumins	Globulins	Prolamines	Glutelins
Common wheat	10-15	3-5	6-10	40-50	30-40
Rye	9-14	5-10	5-10	30-50	30-50
Barley	10-16	3-4	10-20	35-45	35-45
Oats	8-14	1	80	10-15	5
Common millet	7-16	10-11		57	30
Soybean	30-50	trace	85-95	0	0
Peanut	42	trace	97		

[a] From Sniffen (1974).

Protein Quality for Ruminants. Church (1979) defined protein quality as the ability of a specific protein to provide essential amino acids in the required amounts to a given animal performing a specific function (growth, milk production). This quality (Knipfel et al., 1975) is dependent upon the total amount of amino acids present in the protein, their relative proportions (amino acid pattern) and the degree to which the animal can liberate and utilize the amino acids from the protein (amino acid availability). Nutritional availability of amino acids varies with protein source, processing treatment (especially heating) and interaction with other diet components (Friedman, 1975).

In the case of the ruminant, it frequently has been assumed that protein quality is of little or no importance and that proteins of equal digestibility are of equal value (Church, 1979). Church stated that this assumption has been based on the fact that ruminal micro-organisms synthesize the essential amino acids. However, a large

number of reports have indicated that proteins show different quality values when fed to ruminants. For example, early research indicated variations in biological values for soybean meal, casein and urea ranging from 63 to 73 (Turk et al., 1935; Johnson et al., 1942), 60 to 82 (Johnson et al., 1942; Lofgreen et al., 1947) and 51 to 71 (Lofgreen et al., 1947; McNaught et al., 1950), respectively, when these nitrogen sources were fed to ruminants. McDonald and Hall (1957) found that the conversion of zein to microbial protein (40%) was much lower than for casein (90%). More recent research conducted by Hembry et al. (1975) showed biological values of 84.8, 78.3, 84.3 and 76.4 for soybean meal, casein, zein and urea, respectively, when these sources were fed to mature sheep.

Other types of studies which measured the quality of protein were those where plasma amino acid patterns and pools were evaluated when feeding proteins from various sources. Elevated plasma level of glycine and serine and depressed levels of valine, isoleucine, leucine and phenylalanine were observed by Oltjen and Putnam (1966) in steers fed a purified diet containing urea as the sole nitrogen source as compared to steers fed the diets with isolated soy protein. Other workers (Frietag et al., 1968) noted a decrease in the concentration of several plasma amino acids in steers fed ground corn grain and cob diets when soybean meal was replaced by urea. Boling et al. (1972) observed that growth rate was less for steers fed urea than for those fed soybean meal with high grain diets of corn and cobs.

Bergen et al. (1973) showed that, when dietary corn protein escaped degradation in the rumen, the amino acid balance was somewhat reflected in the plasma and tissue pools. Improved plasma amino acid patterns and increased rate of gain observed with ruminants (Bergen, 1979a) are indicative of the importance of protein quality. However, data available suggest that protein quality in the ruminant is not only dependent on its amino acid composition but also in the rate of degradation and solubility in the rumen.

Protein Degradation. The quantity of protein presented to the small intestine for possible absorption is composed primarily of the microbial cell protein and feed protein that has escaped ruminal digestion (Owens and Bergen, 1983). During high production, microbial protein alone may not be sufficient to meet the demands of the animal for protein (Huber and Kung, 1981). Therefore, added protein of high quality may be needed to obtain optimum productivity. This added protein will undergo degradation and the extent of degradation in the rumen can vary from 0 to 100% of the dietary protein (Owens and Bergen, 1983).

Dietary protein is degraded by bacteria and protozoa and involves two steps. In one, proteolysis, the protein chain is broken by hydrolysis of peptide bonds yielding peptides and amino acids. The second is the amino acid degradation (Tamminga, 1979). The mechanism of protein degradation is somewhat different between bacteria and protozoa. Bacteria break down the protein chain into smaller parts by hydrolysis of some or all of the peptide bonds (Tamminga, 1979).

Rumen bacterial proteases are cell bound but located on the cell surface to provide free access to substrates and are composed of exopeptidase and endopeptidase (Chalupa, 1975; Tamminga, 1979). Protozoa, on the other hand, are capable of engulfing small feed particles and bacteria. Proteolysis of dietary protein takes place inside the protozoal cells. If the resulting amino acids are not incorporated into protozoal protein, they are often excreted into the surrounding medium rather than being degraded (Coleman, 1975).

Orskov (1982) indicated that it would be extremely useful if rumen bacteria degraded protein only to the extent to optimize their cell yield. However, the degradation of amino acids yields energy which can be used by the proteolytic organisms for their synthetic processes. Consequently, protein degradation is carried as far as possible (Tamminga, 1979; Orskov, 1982). Another reason for the degradation of amino acids by rumen bacteria may be due to the lack of mechanisms to transport amino acids from the cytoplasm across the cell wall into the surrounding medium. Therefore, in order to excrete excess amino acids, microbes need to degrade them first (Tamminga, 1979).

Tamminga (1979) indicated that the important biochemical reaction mechanisms in further degradation of amino acids by rumen microbes are deaminations, transaminations and decarboxylations. The most important degradative pathway for amino acids was reported by Prins (1977) to be deamination of the amino acid followed by decarboxylation of the resulting α-keto acid. The capacity for amino acid deamination generally exceeds the rate of amino acid release by proteolysis. This

is indicated by the fact that amino acids accumulate only briefly, if at all, even when large amounts of very soluble proteins are introduced into the rumen. Therefore, proteolysis is thought to be the step limiting ruminal degradation of proteins (Broderick, 1975; Tamminga, 1979).

The susceptibility of different feed proteins to degradation has been considered mainly to be a function of the solubility of its protein in ruminal liquor (Broderick, 1975; Chalupa, 1975; Tamminga, 1979). However, Mangan (1972) showed that ovalbumin, although quite soluble in the rumen fluid, was very resistant to degradation, which was due to the cyclic nature of the polypeptide chain. Mahadevan et al. (1980) showed that certain soluble proteins appeared to resist protease activity in vitro. The researchers indicated that soluble protein from soybean meal, rapeseed meal and casein were hydrolyzed at different rates. This suggested that not all soluble proteins undergo total degradation and also indicated that solubility is not the sole indicator of protein degradation. Structural differences caused by disulphide bridges and crosslinking of the protein together with ruminal environmental factors are also important components determining protein degradability in the rumen (Tamminga, 1979; Owens and Bergen, 1983). Among other factors influencing the extent of protein breakdown in the rumen are rate of proteolysis, rumen retention time, particle size of the dietary ingredients and level of feed intake (Hungate, 1966; Church, 1976, 1979).

Protein Solubility. Solubility of a protein refers to the amount of protein which will go into solution in the rumen fluid (Sniffen, 1974). It is considered an important factor in the rate of protein degradation and ammonia accumulation in the rumen (Waldo, 1968) and is partly determined by the relative amount of soluble albumins and globulins as compared to the relative amounts of less soluble prolamines and glutelins present in the feed (Tamminga, 1979). Feeds containing protein with albumins and globulins as the major fractions have a higher solubility in the rumen than feeds containing mainly prolamines in their protein (Wohlt et al., 1973). Waldo and Goering (1979) determined the insolubility of 15 feeds by four different methods and found that the mean insolubilities ranged from 49% of the total nitrogen for corn gluten feed to 94% for dried beet pulp. Wohlt et al. (1973) showed that there was a large range of protein solubilities from 3 to 93% of the total nitrogen.

Protein degradation in the rumen as indicated previously is determined to a great extent by protein solubility, but these characteristics are not similar as often indicated. Mertens (1977) denoted that generally in the rumen all the soluble protein is degraded, whereas only 40 to 50% of the insoluble protein is degraded. Tamminga (1979) reported that, based on multiple regression calculations in which intestinal flow of proteins was related to digestible organic matter and insoluble dietary protein, it was calculated that 65% of the insoluble dietary protein escaped microbial degradation.

The principle of protein solubility gave researchers the notion of feeding diets containing high quality protein that could escape rumen degradation and thus provide higher quality and be better balanced (than microbial protein) at the site of absorption in the small intestine. Since this principle was introduced by Chalmers et al. (1954), several methods to decrease protein solubility (heat, formaldehyde, tannic acid treatment, etc.) and to measure protein solubility (Burroughs salt solution, autoclaved rumen fluid, boiling water, etc.) have been reported. These were reviewed to a great extent previously (Palmer, 1982) and, since no new developments for either have been reported, these subjects will not be reviewed again in this manuscript.

Dietary Nonprotein Nitrogen Utilization. The ability of rumen microorganisms to utilize ammonia for protein synthesis permits the replacement of dietary protein with NPN sources which give rise to ammonia in the rumen (Mercer and Annison, 1976). Loosli et al. (1949) demonstrated that urea could serve as the sole dietary nitrogen source for lambs. Using the purified diet approach, they found that the 10 amino acids that are dietary essentials for the laboratory rat were synthesized within the rumen. Virtanen (1966) showed that lactating cows could be maintained indefinitely on protein-free diets but only modest levels of production were achieved. Data accumulated thereafter have been extensive and various important facets of NPN utilization have been elucidated.

Church (1979) indicated that utilization of NPN by ruminants has been less efficient than that of preformed protein, especially

under low-concentrate feeding conditions. Urea (most widely used NPN source) is readily hydrolyzed in the rumen (Bergen, 1979b), and the ammonia released may be used for microbial protein synthesis or it may be absorbed across the rumen wall, resynthesized into urea and excreted (Church, 1979). Satter and Roffler (1977) denoted that NPN may be utilized as well as true protein when ruminal ammonia-nitrogen concentrations are below 5 mg/100 ml. The researchers reported that supplementing NPN to animals whose ruminal ammonia concentration is in excess of 5 mg/100 ml would be without benefit. It was added that cattle may benefit from NPN added to high concentrate diets containing less than approximately 12 to 13% crude protein (DM basis) or to all-forage diets containing less than about 9 to 10% crude protein.

The amount of ammonia that microbes can utilize in the rumen is directly dependent upon the amount of energy which they can generate during the digestion of other dietary components (Satter and Roffler, 1977). Maximum utilization of ammonia for microbial synthesis in the rumen occurs at ruminal concentrations of 5 to 8 mg ammonia-nitrogen/100 ml (Mercer and Annison, 1976). Two enzymes involved in utilization of rumen ammonia are glutamate dehydrogenase and glutamine synthetase (Baldwin and Denham, 1979). The differences between these two enzymes are that glutamate dehydrogenase is constitutive and has a low affinity for ammonia, while glutamine synthetase is induced at low ammonia concentrations and has high affinity for ammonia (Baldwin and Denham, 1979).

Rumen ammonia concentrations below 3 to 5 mg/100 ml were found by Satter and Roffler (1975) to limit microbial function, whereas Mehrez et al. (1977) found that concentrations below 20 to 25 mg/100 ml were limiting. Baldwin and Denham (1979) stated that at 3 to 5 mg ammonia/100 ml glutamine synthetase is essentially saturated and velocity of this reaction approaches its maximum, while ammonia concentration in the 20 to 30 mg/100 ml range must be achieved before the velocity of glutamate dehydrogenase approaches maximum, indicating that both sets of observations (Satter and Roffler, 1975; Mehrez et al., 1977) may be correct.

Mercer and Annison (1976) indicated that, when conditions in the rumen are favorable for NPN feeding, a balance in the rate of release of ammonia against the rate in which energy for microbial growth is made available by microbial fermentation should be obtained in order to avoid wasteful and high rumen ammonia levels. A problem with this approach is that ammonium salts and urea generate ammonia rapidly in the rumen. However, this can be avoided by feeding small amounts at frequent intervals together with readily available sources of energy. This can also be avoided by reducing the rate of ammonia release into the rumen. Using biuret, uric acid, starea or processing urea together with a cereal (corn) have also been regarded as ways to improve NPN utilization (Mercer and Annison, 1976; Smith, 1979).

Under normal conditions, the Krebs-Henseleit pathway (urea cycle) copes with the temporary increases in the absorption of ammonia from the rumen. Urea, however, is not the only product of

detoxification. Hoshino et al. (1966) showed synthesis of glutamic acid in rumen mucosal homogenates and also reported that the ruminal mucosa splits and synthesizes glutamine. Intraruminal doses of urea in sheep resulted in rapid increases in plasma glutamine and asparagine concentrations followed by a more gradual increase in the concentration of plasma urea (Hoshino et al., 1966). It was also postulated that glutamine serves as a storage form of ammonia in the rumen mucosa.

Salivary Nitrogen

In addition to dietary nitrogen, a second source of rumen nitrogen is the saliva. The presence of salivary urea was demonstrated by McDonald (1948) and its concentration has been shown to increase when higher rumen ammonia levels occurred (Lewis, 1957). In cattle, Waldo (1968) reported that urea nitrogen represented an average of 77% of the total nitrogen in mixed saliva. Higher salivary concentrations of total nitrogen resulted in higher percentages of urea nitrogen, whereas in sheep Tillman and Sidhu (1969) reported that urea represented 60 to 70% of the total nitrogen in both mixed or parotid saliva and that there was a positive relationship between level of nitrogen intake and the amount of urea secreted in the saliva.

The extent to which urea is returned to the rumen via the saliva appeared to be directly proportional to blood urea concentration and to the amount of saliva secreted (Nolan and Leng, 1972), but Somers (1961) had found earlier that, while salivary urea concentrations were correlated with blood urea, they were consistently lower. An important factor affecting the amount of saliva secreted is the physical structure

of the diet, as the proportion of long fiber increased so did the amount of saliva secreted (Orskov, 1982).

Nolan and Leng (1972) reported that salivary urea in sheep accounted for most of the urea entering the rumen. However, in later studies, Kennedy and Milligan (1978) showed that up to 7.3 g of nitrogen daily was entering the rumen in sheep as urea and only 15% of that was accounted for by salivary urea, suggesting that normally the entry of urea from the blood is a more important route in terms of total endogenous nitrogen reaching the rumen.

Diffusion of Nitrogen Across the Rumen Wall

Endogenous urea enters the rumen as a third source of nitrogen by diffusion across the rumen wall and is hydrolyzed completely before reaching the rumen fluid (Houpt and Houpt, 1968). Tillman and Sidhu (1969) suggested that a possible explanation pertains to the penetration of ruminal urease into the rumen epithelial layers and the possibility that ammonia produced here diffuses more rapidly than urea through the remaining layers of rumen epithelium. The net result of this effect could greatly increase transfer of urea-nitrogen as ammonia into the rumen fluid. The rate of diffusion across the rumen membranes depends on the concentration gradient and the permeability of the membrane (Chalmers et al., 1976). Urea also diffuses into the abomasum and intestines and should not be regarded as completely lost to ruminant animals since urease activity is present in the intestinal fluids and ammonia can be absorbed from the intestines (Tillman and Sidhu, 1969).

Coccimano and Leng (1966) showed that the total endogenous urea influx (saliva and through the wall) into the rumen was a function of blood urea concentration. The total influx from the blood increased with increasing plasma urea concentrations. Weston and Hogan (1967) reported that the maximum amount of nitrogen from the blood transferred to the rumen was 4 to 5 g/d, the equivalent of 1.5 to 1.8 times the body urea nitrogen pool at blood urea nitrogen levels of 16 to 18 mg/100 ml. Orskov (1982) noted that on low-nitrogen diets the difference between nitrogen flow to the abomasum and that in the feed can be as great as 11 g/d in sheep, which is considerably greater than the return via saliva (4.3 g/d) reported by Nolan and Leng (1972).

Endogenous Nitrogen from Epithelial Cells Shed into the Rumen

Rumen epithelium is constantly sloughing off into the lumen of the rumen, and this can be considered as a source of nitrogen for the microbes. Orskov (1982) conducted epxeriments with lambs, steers and cows sustained entirely by intragastric nutrition to determine the quantities of nonammonia nitrogen leaving the rumen. Nonammonia nitrogen leaving the rumen was 1.4, 5.1 and 8.3 g/d, respectively, for lambs, steers and cows. Microscopic examination showed that the nitrogenous components appeared to be mainly in the form of abraded epithelial cells from the rumen wall. The researcher indicated that, while the quantity of nonammonia nitrogen from the rumen epithelial cells is not very large, it is greater than the amount of nitrogen contained in enzyme secretions in the abomasum. Orskov (1982) further

suggested that under normal feeding conditions the abraded epithelial cells could be partially degraded by rumen microorganisms.

Ruminal Nitrogen as Related to Microbial Protein Synthesis

Nitrogen Requirements for Microbial Growth

Microbial growth in the rumen requires the provision of ammonia, essential minerals, notably sulphur and phosphorus, and organic matter to provide both an energy source and structural units. Specific preformed compounds including amino acids or peptides, branched-chain carbon compounds and certain growth factors are required by ruminal bacteria, although their importance for the mixed bacterial population in the rumen have not been well defined (Smith, 1979). The rapid degradation of proteins in the rumen makes ammonia, free amino acids and peptides available for protein synthesis by the rumen microbes. Ammonia is quantitatively the most important source of nitrogen for rumen bacteria (Orskov, 1982; Steinhour and Clark, 1982). Some species of rumen bacteria require ammonia for growth and prefer ammonia to free amino acids as a nitrogen source (Bryant and Robinson, 1963). With the use of radioactive nitrogen (^{15}N), it was estimated that of the total microbial nitrogen in the rumen of sheep 50 to 80% was derived from ammonia (Mercer and Annison, 1976). The balance, 20 to 50% of microbial nitrogen, is presumably derived from preformed amino acids and peptides. Earlier work conducted by Bryant and Robinson (1962) showed that 82% of the rumen bacteria grown on a relatively non-selective medium could be grown with ammonia as the sole source of

nitrogen, 25% would not grow unless ammonia was present and 56% could use either ammonia or amino acids.

Rapid labeling of bacterial amide nitrogen from radioactive urea (^{15}N) as well as assays for enzymes in extracts from pure and mixed ruminal bacteria indicated that the enzyme glutamine synthetase has an important function in ammonia assimilation. Glutamine synthetase catalyzes the transfer of amide nitrogen from glutamine to α-ketoglutarate (Allison, 1982). Glutamate dehydrogenase is probably the other main mechanism for ammonia assimilation. The pathway involving glutamine synthetase appears to be especially important at low ammonia concentrations since (1) glutamine synthetase has greater affinity for ammonia than glutamate dehydrogenase and (2) glutamine synthetase levels increase when ammonia levels decrease. However, an additional mole of ATP is required for each mole of ammonia fixed via the glutamine synthetase pathway (Allison, 1982).

Peptides are probably the second most important nitrogen source for rumen bacteria (Steinhour and Clark, 1982). These researchers indicated that Bacteroides ruminicola can use ammonia or peptides but not free amino acids for growth. Additionally, labeled free amino acids were more rapidly converted to carbon dioxide and volatile fatty acid than labeled peptides. Steinhour and Clark (1982) further stated that the direct incorporation of free amino acids into microbial protein was inefficient since only 17% of the carbon (^{14}C) from glutamate and 8% of the carbon from aspartate appeared in bacterial protein after incubation of free amino acids with rumen fluid from sheep. However, Allison

(1982) observed that growth of mixed bacteria was much greater when various mixtures of amino acids supplemented or replaced urea. Teather et al. (1980) reported that corn silage diets supplemented with soybean meal supported ruminal bacteria populations that were 70% greater than an equivalent protein level from urea (12.5%). Using a similar amount of urea with corn silage which had gone through the silage forming process gave results comparable to those from the soybean meal supplemented diet. Owens and Bergen (1983) stated that certain amino acids and peptides may serve as sources of branched-chain fatty acids that are growth factors for a number of bacterial species including the cellulolytic bacteria.

A few of the bacteria require purines and pyrimidines for growth. However, measurements of disappearance rates of purines or pyrimidines in the rumen suggested that these may be assimilated by the microbes (Smith, 1975). Theoretical cell yields with glucose as substrate are increased about 10% when preformed nucleic acids and amino acids were supplied. With lactate or pyruvate rather than glucose, the increase was about 55% (Allison, 1982). In ruminants adapted to diets with high content of nitrate, bacterial populations are able to reduce nitrate to nitrite and then to ammonia. Some organisms are able to use nitrate as a substitute for ammonia (Allison, 1982).

Steinhour and Clark (1982) reported that protozoa, on the other hand, apparently do not utilize ammonia directly as a nitrogen source. It was estimated that, from labeled ammonia infusions in vivo, 31 to 64%

of the protozoal nitrogen in sheep rumen was derived from rumen ammonia. However, it was suggested that the uptake of labeled ammonia was probably a result of engulfment of labeled bacteria. Orskov (1982) denoted that some rumen microorganisms can capture and fix some gaseous nitrogen (N_2) and that it could possibly be about .4 mg/d in sheep, a rather insignificant amount in relation to the daily total nitrogen requirements.

Optimal ammonia nitrogen concentrations required for ruminal bacteria have been reported by Owens and Bergen (1983) to range between .35 to 29 mg/100 ml. The researchers indicated that pure culture in vitro generally showed that very low ammonia levels were adequate, whereas Mehrez et al. (1977) reported that digestion rate in situ plateaued only at much higher concentration of ammonia. Mehrez et al. (1977) denoted that the rumen ammonia concentration required for maximal microbial protein yield was less than required for optimal rate of digestion. It was also suggested that different substrates required different concentrations of ammonia to achieve maximum yield. When nitrogen availability is low in the rumen, bacteria shifted from the synthesis of microbial cell protein to synthesis of intracellular polysaccharides (Smith, 1975) and the chemical composition of bacteria changed. Bacterial storage of polysaccharides may be reduced with diets lower in starch and soluble sugar or with faster growth rates (Smith, 1975).

Microbial Protein Synthesis

The rumen environment is anaerobic and fermentation of organic matter produces heat, methane, volatile fatty acids and metabolically useful energy in the form of high-energy bonds, largely ATP. The ATP is used for a variety of purposes by the bacterial cell, but, in a rapidly growing culture, a great deal is used for the synthesis of oganic matter and in particular protein (Smith, 1979).

Much of the ammonia entering the microbial cell is initially captured in the form of amide groups of glutamine and(or) asparagine. These groups are used for subsequent amination of α-ketoglutarate to glutamate, either after the release of ammonia or by direct incorporation (Erfle et al., 1977). Synthesis of alanine and aspartate follows, and these compounds together with glutamate probably accumulate initially in their free forms. Amino groups are then transferred to other suitable carbon skeletons for the formation of amino acids which together with preformed compounds are used in protein and nucleic acid synthesis (Smith, 1979).

The fixation of ammonia, as previously indicated, is achieved via a number of systems, the most important of which is glutamine synthetase and glutamate dehydrogenase. Carbamyl phosphokinase, asparagine synthetase, aspartate and alanine amino tranferase activity have also been detected in extracts and mixed cultures of bacteria (Mercer and Annison, 1976). These researchers also mentioned the existence of a number of branched amino acid transferases involved in transamination reactions resulting in the formation of glutamic acid in

both protozoal and bacterial extracts. Mercer and Annison (1976) suggested that both, the ammonia fixing enzymes and the amino tranferase system, are responsible for the synthesis of amino acids by rumen bacteria. It was also indicated that some control of bacterial amino acid biosynthesis may occur later in the pathways than during the initial fixation of ammonia stage or during the operation of the amino transferase system. A possibility in this regard may occur in the sequence of reactions initiated by the enzyme aspartokinase during the synthesis by separate pathways of lysine, methionine, threonine and isoleucine (Mercer and Annison, 1976).

The quantity of microbial cells formed in the rumen is a nutritional function of supplies of nitrogen, energy and growth factors and is modulated by growth rates of rumen bacteria. Growth rate is an important determinant of yields and efficiency of microbial protein synthesis, because at high growth rates more of the energy derived from fermentation is used for cell growth rather than for maintenance of population (Chalupa, 1978). Stern and Hoover (1979) reported that results from numerous studies conducted under varied conditions showed that approximately 16.9 g microbial crude protein are synthesized per 100 g of apparent digested organic matter in the rumen. However, some variability between studies can be attributed to the microbial markers used or to the method for determining microbial protein. Kropp et al. (1977a) reported microbial protein production to be relatively constant among diets regardless of nitrogen source (urea vs soybean meal). However, the total nitrogen and feed protein reaching the

abomasum decreased significantly as urea replaced more soybean meal in the diet. Microbial protein synthesis per 100 g of dry matter digested in the rumen was 9.9, 10.4, 10.9 and 11.6 g for diets containing 0, 25, 50 and 75% of the supplemental nitrogen as urea. Chalupa (1978) concluded that 9.0 to 23.0 g microbial protein could be produced per 100 g organic matter digested.

Various methods have been used to estimate the quantity of microbial protein in digesta leaving the ruminant stomach. These techniques are based on determination of a single chemical marker believed to characterize the microbial components (Stern and Hoover, 1979). Diaminopimelic acid (DAP), aminoethylphosphonic acid (AEP), ribonucleic acid (RNA), adenosine triphosphate (ATP) and isotopes (^{35}S, ^{15}N, ^{32}P) incorporated into protein in the rumen have been used to measure the quantity of microbial protein synthesis (Stern and Hoover, 1979).

These techniques were reviewed recently by Stern and Hoover (1979). They stated that these procedures were based on the following facts. DAP is an advantageous technique since it is present in the cell membrance of many types of rumen bacteria but is absent from plant material. Traces of DAP can probably be detected in protozoa since these ingest bacteria. AEP is found in the lipid fraction of protozoa and is used to estimate protozoal protein synthesis. Both DAP and AEP can be used together to estimate total microbial synthesis in the rumen, whereas DAP alone only measures bacterial protein synthesis. The ratio of RNA to total nitrogen in rumen fluid and rumen microbes have been

used to estimate the extent of conversion of dietary nitrogen to bacterial and protozoal nitrogen. This technique relies on the assumption that nearly all dietary RNA is degraded in the rumen. However, it has been suggested that microbial protein synthesis may be amplified when large portions of the dietary protein and nucleic acid have been rendered insoluble by exposure to heat or chemical treatment (Stern and Hoover, 1979).

ATP was investigated as a rumen microbial marker because (1) ATP is present in all living cells and absent from dead cells, (2) ATP concentration is similar in all microbes and (3) extraction and assay of ATP is relatively simple to perform and inexpensive. Nevertheless, it was concluded that concentration of ATP reflected the level of activity of the biomass rather than the total amount of biomass and that it is also dependent upon the nitrogen source fed to the animal. Radioisotopes have been used as tracers to distinguish between microbial and dietary protein. The use of ^{15}N, ^{35}S and ^{32}P have been reported to be useful for this purpose in several studies (Stern and Hoover, 1979).

Factors Affecting Microbial Protein Synthesis

Stern and Hoover (1979) stated that during the measurement of rumen microbial protein synthesis some variability between studies can be attributed to the markers used. However, there are several factors that probably induced real differences and these factors include concentration and source of nitrogen and carbohydrates (energy), rumen dilution rate, dietary sulfur and frequency of feeding.

Carbohydrate (Energy) Source. Efficient conversion of dietary nitrogen to microbial nitrogen requires that the energy from the fermentation of organic matter must be available at a rate which matches the synthetic abilities of the microorganisms in the rumen (Oldham et al., 1977; Steinhour and Clark, 1982). There are several bacterial maintenance functions which require energy. Steinhour and Clark (1982) listed the maintenance requirements of microorganisms to include (1) energy for motility, (2) energy and nutrients needed for turnover of cell constituents, (3) energy and nutrients needed for production of extracellular proteins, carbohydrate polymers etc., (4) energy needed for active transport, (5) efficiency of phosphorylation, (6) energetic uncoupling, (7) lysis and resynthesis of cells and (8) unknown factors.

Readily available carbohydrates such as starches and sugars were reported by Stern and Hoover (1979) to be more effective than other carbohydrates in increasing utilization of degraded dietary nitrogen and(or) increasing microbial growth both "in vivo" and "in vitro." McAllan and Smith (1976) studied carbohydrate metabolism in young steers and found that of the sources studied starch provided the greatest amount of energy for rumen bacteria. Stern and Hoover (1979) observed that, when starch was added to high cellulose diets or replaced part of the cellulose, increased nitrogen utilization and decreased fiber digestion occurred. However, in previous reports Stern et al. (1978) noted that dietary energy level was not the only factor limiting microbial growth. An increased microbial growth in continuous cultures

from 15.0 to 19.5 g microbial crude protein per 100 g dry matter digested was found in response to increased dietary nonstructural carbohydrate levels, even though diets were isocaloric and VFA production and dry matter digestibilities did not differ markedly among diets. The researchers concluded that a major factor affecting utilization of degraded dietary nitrogen was the type and rate of availability of carbohydrates.

Nitrogen Concentration. An adequate supply of nitrogen is essential for microbial growth. If nitrogen level is not adequate, uncoupled fermentation may occur resulting in fermentation without useful ATP production, whereas, if the nitrogen level is excessive, energy may be the limiting factor for efficient utilization of nitrogen (Stern and Hoover, 1979). In studies conducted by Hume et al. (1970) with sheep fed semi-purified, protein-free diets containing urea as the dietary nitrogen source, increases in the intake of nitrogen from 2 to 9 g/d linearly increased the production of protein in the rumen from 32.5 to 50.0 g/d. It was concluded that microbial protein synthesis was depressed when the dietary crude protein concentration was below 11.0%. The "in vitro" experiments conducted by Satter and Roffler (1977) demonstrated that ammonia availability did not limit microbial growth until the ammonia-nitrogen level dropped below 5 mg/100 ml. Therefore, it was suggested that for dairy cattle the maximum microbial protein synthesis will occur with high concentrate diets which contain approximately 12 to 13% crude protein on a dry basis.

Several studies have been involved with levels of ruminal nitrogen concentration needed for a maximum microbial protein synthesis. Mercer and Annison (1976) suggested that peak microbial protein synthesis occurred at relatively low ruminal ammonia-nitrogen concentrations, indicating that the concentration was between 5 to 8 mg/100 ml. Hume et al. (1970) obtained maximum microbial protein growth when ruminal ammonia-nitrogen concentration was approximately 9 mg/100 ml. Miller (1973) and Mehrez et al. (1977) reported that maximum microbial protein synthesis occurred at much higher levels of rumen ammonia-nitrogen concentrations between 23.5 to 29.0 mg/100 ml. However, "in vivo" data presented by Kropp et al. (1977b) showed ruminal ammonia-nitrogen concentrations between 3.7 to 22.2 mg/100 ml, with a microbial protein synthesis per 100 g organic matter apparently digested varying only from 17.9 to 19.9 mg.

Nitrogen Source. The form of nitrogen available to bacteria is an important factor affecting microbial growth. Although ammonia is the major nitrogen source for rumen bacteria, it is not the only source used for protein synthesis (Steinhour and Clark, 1982). In studies conducted by Kropp et al. (1977b) where low quality roughage diets for steers were supplemented with urea or soybean meal, microbial protein synthesis showed relatively no changes. Stern and Hoover (1979) stated that, even though microbial protein synthesis can occur in the rumen on diets in which urea was the only nitrogen source, efficiency of microbial proteins may be limited by the lack of preformed amino acids. Hume (1970) reported that nitrogen provided to sheep by urea,

gelatin, casein and zein resulted in rumen microbial protein synthesis of 17.1, 19.8, 23.3 and 22.5 g/100 g organic matter digested, respectively. The researcher concluded that, since it was evident that degradation of both gelatin and casein in the rumen approached completion, microbial protein production from the gelatin and urea diets may have been limited by the rate of synthesis of one or more amino acids by the rumen bacteria with methionine appearing most limiting.

Salter et al. (1979) found that, when an adequate dietary supply of amino acids was available to steers, proline, arginine, histidine, methionine and phenylalanine were derived from the medium to a greater extent than other amino acids. While synthesis of proline, arginine and histidine increased on the urea containing diet, that of methionine and phenylalanine did not. It was suggested that methionine and phenylalanine may be limiting for bacterial growth on diets high in NPN and low in preformed protein. Stern and Hoover (1979) stated that, for ruminant diets which contain little or no preformed protein, a slowly degradable protein may be beneficial.

Dilution Rate. Rumen dilution rate is defined as the proportion of total volume leaving the rumen per hour (Stern and Hoover, 1979). Level of intake, the proportion of long fiber in the diet, intraruminal buffer infusions and environmental conditions have a marked effect upon dilution rate (Harrison and McAllan, 1980). High dilution rates are usually obtained with all forage diets and low dilution rates with diets containing a high proportion of grain. Increases in dilution rates observed by Cole et al. (1976) from .03 to .05/h resulted in increments

in the microbial protein synthesis from 7.5 to 11.8 g/100 g dry matter digested. To accomplish this increased dilution rate, the researchers switched steers from an all-concentrate diet to one containing about 14% roughage.

Harrison et al. (1976) infused artificial saliva into the rumen of sheep and increased the dilution rate from .03 to .08/h which resulted in an increase of total amino acid synthesized per mole of hexose fermented from 25.4 to 29.8 g. Hogan and Weston (1970) showed that increased dilution rates in sheep from .06 to .1/h were associated with enhanced microbial production rates of 31 to 37 g nitrogen per kg organic matter digested. Kennedy and Milligan (1978) raised the dilution rate from .07 to .12/h by maintaining sheep at colder temperatures and found an increment in the microbial synthesis from 35.9 to 50.9 g nitrogen per kg organic matter digested.

Sulfur and Frequency of Feeding. Sulfur is required by rumen microorganisms for the synthesis of methionine and cysteine, and intake of sulfur may limit the synthesis of protein when a large amount of NPN is fed in the diet (Stern and Hoover, 1979; Harrison and McAllan, 1980). Hume and Bird (1970) reported that, when sheep were fed diets with a nitrogen:sulfur ratio of 34.3:1, 82 g/d microbial protein were produced in the rumen; whereas, when the ratio was only 10.9:1, an increased production of microbial protein was observed (94 g/d).

Stern and Hoover (1979) observed that feeding sheep at 2-h intervals resulted in greater microbial protein synthesis as compared to feeding only once daily. The authors also reported that frequent

feeding resulted in a decrease of microbial metabolites in the rumen fluid and an increase in the microbial ATP pool, which is indicative of a greater microbial protein synthesis.

Microbial Protein Composition and Digestibility

Protein composition of rumen microorganisms is of great importance because, depending on dietary and animal factors, microbial protein reaching the duodenum could range between 40 to 80% of the total protein (Owens and Bergen, 1983). Earlier work conducted by Weller (1957) to determine the composition of rumen microorganisms showed that crude protein content of bacteria ranged from 58 to 77%, whereas protozoa varied between 24 and 49% when sheep were fed a variety of diets. Chalupa (1978) reported that isolated preparations of rumen bacteria and protozoa contained 35 to 80% and 17 to 55% crude protein, respectively. The researcher suggested that the wide range in crude protein contents was probably due to varying degrees of contaminations of microbial preparations with digesta. Smith (1979) denoted that the principal nitrogenous compounds of rumen bacteria are protein and nucleic acids comprising about 75 to 85 and 13 to 19%, respectively, of the total nitrogen.

Amino acid composition of bacteria have generally been shown to be fairly constant (Bergen et al., 1968a; Church, 1976), although Salter et al. (1979) reported that rumen bacteria from animals given NPN as the main nitrogen source had lower levels of methionine. Purser and Buechler (1966) found that amino acid nitrogen averaged 86% of the total nitrogen present in rumen microorganisms with a range between 56 and

100%. Church (1976) reviewed the amino acid composition of bacteria and protozoa reported by several researchers and concluded that protozoa appeared to have substantially higher lysine and glutamic acid contents in its protein than did bacteria. Bacteria, on the other hand, tended to be higher than protozoa in histidine, threonine, serine, cystine and methionine. Nucleic acids which are the other major fraction in ruminal microorganisms were reported by Smith (1979) to consist of about 60% RNA and 40% DNA for rumen bacteria, while the corresponding values for protozoa were 90 and 10%, respectively.

The nutritive value of rumen microbial protein has been determined by feeding isolated preparations of bacteria and protozoa to rats. Generally, rumen protozoa were only slightly higher in biological value than bacteria; but, because of higher true protein digestibility, net protein utilization of protozoal protein was much greater (Chalupa, 1978). Bergen et al. (1968a,b) found the biological value, true digestibility and net protein utilization of microbial protein to be 85, 75 and 63, respectively, for ruminal bacteria and 82, 87 and 71 for ruminal protozoa. Owens and Bergen (1983) reported that both digestibility and net protein utilization were generally higher for ruminal protozoa than for ruminal bacteria.

Orskov (1982) reported digestibility of bacteria from studies where isolated bacteria were infused as the only source of protein into the abomasum of sheep. Apparent digestibility measured between the abomasum and feces averaged 77.5%, whereas true digestibility determined by regression was approximately 79.5%. The researcher reported

that these digestibility values included nucleic acid nitrogen and that the true digestibility of only amino acids in the small intestine was about 84.7%. Smith (1979) reported that estimates of true digestibility in the small intestine of total microbial nitrogen compounds were between 75 to 85%. These estimates were made in steers by infusing predigested ^{15}N labeled rumen bacteria into the proximal duodenum and determining recovery of ^{15}N at the distal portion of the ileum.

Nitrogen Losses from the Ruminant

Feces and urine are the two major pathways for loss of nitrogen by the ruminant and are considered the excretory routes for unutilized nitrogen. Other nitrogen losses include those occurring through the hide, hoof and hair which amount to a small portion of the total nitrogen loss from the body. They do constitute a portion of the total requirements of nitrogen for the animal but are not generally considered in nitrogen balance studies.

Fecal Nitrogen

Fecal material excreted by ruminants contain nitrogen from undigested residues of feed material, residues of gastric juices, bile, pancreatic and enteric juices, cellular debris from the mucosa of the gut, excretory products discharged into the lumen of the gut, cellular debris and metabolites of microorganisms that grow in the large intestine and indigestible microbial protein originating from micro-organisms formed in the rumen (Church, 1976; Orskov, 1982).

To better understand the importance of fecal nitrogen, several researchers have attempted to fractionate this portion. Mason (1969) and Orskov (1982) have partitioned fecal nitrogen into different sources, which include (1) undigested dietary nitrogen, (2) bacterial plus endogenous nitrogen, (3) water soluble nitrogen and (4) nondietary fecal nitrogen. The undigestible dietary nitrogen varies directly with the content of insoluble nitrogen in the diet; and, unless the dietary protein has been heat damaged or chemically treated to a great extent, this fraction is usually small as the true digestibility of protein normally varies between 90 and 100% (Orskov, 1982). Another factor that may affect the undigestible dietary protein of the fecal nitrogen is that insoluble nitrogen associated with lignin (Waldo, 1968; Van Soest, 1982).

Mason (1969) indicated that the bacterial plus endogenous nitrogen fraction which could be centrifuged out amounted to about 70 to 80.1% of the nondietary fecal nitrogen. Later estimations by Mason and White (1971) with a wide range of diets showed that nondietary fecal nitrogen varied from 72 to 97% of the total fecal nitrogen. Diamino-pimelic acid analysis of the different fractions showed that endogenous nitrogen (intestinal cellular debris) ranged from 0 to 32.5% of the bacterial plus endogenous nitrogen fraction, indicating that bacterial residues constitute the major source of fecal nitrogen (Mason, 1969).

The water soluble nitrogen fraction was reported by Mason and White (1971) to be related with retention time in the large intestine and increased with increasing moisture content in the feces. These

researchers calculated this fraction and found that it varied from 16 to 58% of the total nitrogen in the feces.

Endogenous nitrogen lost by way of the feces is commonly called metabolic fecal nitrogen (Swanson, 1982). This nitrogen fraction is formed by nitrogen entering the gastrointestinal tract at all levels from the mouth to the rectum. It consists of urea, enzymes, bile, mucus, serum albumin, lymph, epithelial cells and other degradation products from the gastrointestinal lining and oral pharyngeal mucosa (Swanson, 1982). It was estimated by Swanson (1982) that 32.3 g of nitrogen passed into the gastrointestinal tract of a 60-kg sheep daily and for a 600-kg cow the amount was about 161.7 g daily.

Waldo (1968) indicated that assuming a constant metabolic nitrogen excretion and extrapolating fecal nitrogen to zero nitrogen intake, an estimate of metabolic fecal nitrogen which ranged from .545 to .576 g per 100 g of dry matter intake was obtained. However, Swanson (1982) suggested that this reference base (metabolic fecal nitrogen per amount of dry matter intake) was fairly constant when the low nitrogen diet is almost completely digestible; but, when the diet is high in fiber, metabolic fecal nitrogen varies with fecal dry matter closer than with dry matter intake. The researcher showed that fecal nitrogen and fecal dry matter increased together as fiber content in the diet increased. This resulted in a relatively constant ratio of metabolic fecal nitrogen per amount of fecal dry matter across a wide range of digestible dry matter.

Swanson (1977) reported that a summary of estimated metabolic fecal nitrogen from 57 experiments with low nitrogen yielded averages of 10.9 g metabolic fecal nitrogen/kg fecal dry matter and 4.7 g metabolic fecal nitrogen/kg of dry matter intake. The metabolic fecal nitrogen/kg of fecal dry matter was the preferred ratio.

The importance for determining fecal nitrogen is that it enables researchers to estimate the true digestibility of the dietary nitrogen. This is obtained by deducting metabolic fecal nitrogen from the total fecal nitrogen which accurately reflects the absorption of the feed nitrogen per se (Maynard et al., 1979). Estimates of the quantity of metabolic fecal nitrogen produced by cattle have been made by two methods, (1) directly from feeding a low nitrogen or "protein-free" diet and determining the quantity of nitrogen in the feces and (2) by feeding several diets of differing content of protein and then regressing the different digestible protein contents in dry matter against feed dry matter protein to zero nitrogen intake (Swanson, 1982). It was added that the two methods frequently disagree and those estimates from direct methods are usually lower. Swanson (1982) reported that both methods have basic defects. In the direct method a variable amount of undigested feed nitrogen appears in the feces. Also, the diet is so low in nitrogen that rumen fermentation cannot be normal. From the regression method, there is no evidence that can confirm a linear extrapolation to zero nitrogen intake (Swanson, 1982).

Recent reports by the NRC (1984) suggested that metabolic fecal nitrogen appeared to be a function of dry matter intake or fecal dry

matter excretion and constitute the major protein cost for mature ruminants. NRC (1984) denoted that the current estimates of metabolic fecal nitrogen loss are 4.8 g nitrogen/kg dry matter intake or 10.9 g nitrogen/kg of dry matter output in the feces. It was also reported that metabolic fecal nitrogen estimated from feed intake is greater than that estimated from fecal dry matter excretion for all diets with digestibilities over 55% (NRC, 1984).

Urinary Nitrogen

Urine represents the major route of excretion of nitrogenous metabolites of body tissues (Church, 1976). Creatinine in urine is used as an indicator of endogenous nitrogen metabolism because of the constant level irrespective of diet. Swanson (1982) observed that total urine nitrogen of cattle and sheep on low-nitrogen, energy-sufficient diets for 5 to 7 d reached a low plateau of which creatinine nitrogen represents 25 to 30%. The investigator stated that after a few days on low-nitrogen diets urinary excretion of ammonia plus urea will also plateau at a minimum level of 5 to 10% of the total urine nitrogen. Waldo (1968) and Church (1976) reported that, when diets contained near optimum amounts of protein (10%), urea was by far the largest fraction, constituting 70 to 71% of the total nitrogen in the urine. However, Swanson (1982) suggested that substantially larger proportions of urea in the urine indicate additions from exogenous nitrogen metabolism. The remainder of urinary endogenous nitrogen comes from the compounds bilirubin, allantoin, hippuric acid, uric acid and some amino acids (Church, 1976; Swanson, 1982). Only traces of amino acids and protein

are found in normal urine. The presence in quantity of proteins such as albumins and globulins are indicative mainly of renal disease in the adult ruminant (Church, 1976).

Endogenous urinary nitrogen can be estimated in simple stomached animals by determining daily urinary nitrogen excreted while they are consuming a diet which is adequate in energy but contains no protein or a minimum of protein of high utilization (Swanson, 1977). Ruminants cannot be maintained on such diets because of the nitrogen requirements of the microorganisms in the rumen. However, urinary endogenous nitrogen has been determined with preruminant calves fed low protein diets. Other estimates were also obtained by regressing daily urinary nitrogen from three or more liquid diets of graded protein concentrations to zero nitrogen intake (Swanson, 1977).

Swanson (1982) and NRC (1984) reported that a good reference base for computing endogenous urinary nitrogen is body weight. Swanson (1977) reviewed 21 published reports in which milk for preruminant calves or low-nitrogen diets for ruminating cattle were fed. This provided 82 estimates of endogenous urinary nitrogen associated with body weights ranging from 28 to 625 kg. The resulting regression was endogenous urinary nitrogen = .44 g per $kg^{.5}$, formula used by the NRC (1984) for obtaining the endogenous urinary nitrogen fraction when calculating protein requirements by the factorial method.

Urinary nitrogen loss is largely of endogenous origin with diets with proper level and quality of protein. At low levels of nitrogen intake, a large portion of nitrogen metabolized within the

animal is recycled, largely through the rumen, and very little appears in the urine. An increase in dietary nitrogen intake is associated with a larger urea entry rate, while a higher plasma level is associated with greater urinary nitrogen excretion rate above the threshold level in the blood. As dietary nitrogen is increased, the proportion of total urea that is degraded declines, with the balance being lost in the urine (Van Soest, 1982). Van Soest added that, at low dietary nitrogen intake, recycling is very efficient, with the source of urea being the endogenous metabolism of tissue and absorbed amino acids.

Protein and nitrogen sources which are rapidly hydrolyzed will cause higher levels of ruminal ammonia, plasma urea and urinary nitrogen with lower retention of dietary nitrogen (Lewis, 1957). Proteins with low solubility are usually related to decreased protein degradability in the rumen. Plasma urea is then decreased and urinary nitrogen losses are reduced, resulting in an increase in nitrogen retention (Sherrod and Tillman, 1962; Sniffen, 1974).

Other Endogenous Nitrogen Losses

Other sources of endogenous nitrogen losses include the integumental protein which include hair, scurf and scapes rubbed from the skin surface along with some loss of nitrogen compounds in skin secretions (Swanson, 1977). Also included in this fraction are losses occurring through hooves and horns that grow constantly at a slow rate which usually tend only to replace losses due to wear and tear but which result in some accretion that must be trimmed away, especially under confined conditions (Swanson, 1982).

Loss by way of hair and skin scrapings vary with season in that a thick coat of hair is grown in autumn and winter and it is mostly shed during spring and summer when hair growth is minimal. Some of the integumental nitrogen losses are recycled by cattle grooming themselves and by herd mates. The amount of protein ingested in this manner has been estimated to be as much as one-fourth of the daily losses (Swanson, 1977). Secretion loss is important only at high ambient temperatures. Jenkinson et al. (1974) reported that daily protein losses through Ayrshire calves skin secretions were less than .3 g at 15 C, but at 35 C the daily losses were 10 g.

Swanson (1977) reviewed data from 11 reports involving 61 individual animals and indicated that integumental nitrogen losses were .35 g per $kg^{.6}$ of body weight (W). This value ($W^{.6}$) was estimated by correlating surface area (which is related to skin losses) with animal body weight. Skin, hair and scurf nitrogen loss (S) in grams per day have been estimated by using the formula $S = .032\ W^{.6}$ (NRC, 1984). Swanson (1977) stated that, since endogenous urinary nitrogen varies with $W^{.5}$, then integumental nitrogen losses could be expressed according to the same power of W in order to have easier calculations when calculating nitrogen requirements by the factorial method. The formula then would be $S = .063\ W^{.5}$. Therefore, daily integumental protein losses could be calculated as $.39\ W^{.5}$. These formulas represent a daily protein loss from skin, hair and scurf losses of nearly 7 g for 300-kg cattle and about 10 g for cattle weighing 600 kg.

Dietary and Rumen Nitrogen in Relationship to Blood Nitrogen Changes

The protein entering the rumen is partly broken down to ammonia under the action of microorganisms. The amount of ammonia produced in the rumen is dependent upon the source of nitrogen (urea, soybean meal, heat-treated soybean meal) and the level of protein or NPN in the diet. The utilization of this nitrogen by rumen microorganisms will rely mainly upon the availability of highly soluble carbohydrate sources. Simultaneous destinations for ruminal ammonia include (1) uptake by microbes, (2) flushing to the omasum and (3) absorption through the rumen wall (Owens and Bergen, 1983).

Ammonia appears to be passively absorbed through the rumen wall in the nonionized form (Smith, 1975) and the absorption is influenced by rumen pH and concentration gradient (Tillman and Sidhu, 1969). Absorption is positively correlated to concentration in the rumen (Chalmers et al., 1976), being low at pH 7 and decreasing as pH decreases (Visek, 1968).

Absorbed ammonia is carried via the portal circulation to the liver where it is converted into urea. Generally, only small quantities of ammonia are found in the peripheral blood. Determinations reported by Bartley et al. (1976) indicated that in cattle peripheral blood ammonia-nitrogen concentration ranged between .10 to .15 mg/100 ml. Earlier work carried out by Lewis et al. (1957) in sheep showed normal portal blood ammmonia-nitrogen concentrations ranged between .51 to .85

mg/100 ml. These results suggest utilization or more correctly conversion of ammonia-nitrogen into urea by the liver.

Synthesis of absorbed ammonia into urea by the liver was reported by Lewis et al. (1957) to be complete. However, when ammonia in the peripheral blood reached a level of 1.36 mg/100 ml (obtained when ruminal fluid ammonia concentration was 93.5 to 102.0 mg/100 ml), leakage through the liver into the systemic circulation occurred. Lewis (1960) and Bartley et al. (1976) reported that, when circulating blood ammonia-nitrogen exceeded .8 mg/100 ml, the chance of toxicity was extremely high.

Under normal circumstances, as previously mentioned, the liver is able to detoxify all the absorbed ammonia by conversion into urea. This plasma urea can either be excreted via the urine when peripheral blood levels reach their threshold or recycled to the rumen via saliva or through the rumen wall (Owens and Bergen, 1983). Normal levels of blood urea were reported by Church (1979) to range between 6.3 and 25.5 mg/100 ml for lactating cows, while values for preruminant lambs were between 12 to 15 mg/100 ml. Somers (1961) fed growing lambs a series of diets containing variable levels of nitrogen and obtained blood urea concentrations ranging between 10.5 and 25.0 mg/100 ml. Lewis (1957) denoted that changes in the concentration of blood urea were a direct result of ammonia production in the rumen and not a direct reflection in the total dietary nitrogen intake.

Sniffen (1974) showed that increased intake of highly soluble protein sources or NPN resulted in increased levels of rumen

ammonia-nitrogen and thus increased plasma urea-nitrogen concentration. Similar results were reported in studies conducted by Preston et al. (1965), Coccimano and Leng (1966), McIntyre (1970) and Hammond (1983) where increased plasma urea concentration was obtained when increased levels of nitrogen were fed to ruminants. These studies also showed increased ammonia-nitrogen concentration in the rumen. Sources of protein with low solubility, on the other hand, such as heat-treated soybean meal have a lesser effect on nitrogen degradation in the rumen, thus showing lower levels of plasma urea nitrgoen (Wohlt et al., 1976). Chalmers et al. (1954) indicated that significant increases in blood urea levels were indicators of ruminal ammonia production and therefore good indicators of protein solubility.

The ruminant animal depends upon microbial cell protein plus dietary protein escaping degradation in the rumen for its supply of essential amino acids. This bacterial and undigested dietary protein arrives at the abomasum and small intestine at fairly constant rates (Smith, 1979). The pH in the abomasum is consistently low (2 to 3) and this low pH was reported to extend further into the small intestine than is generally true for monogastric animals. Smith (1979) added that apart from these modifying factors there appears to be no fundamental differences in the processes of digestion of proteins and nucleic acids in the small intestine of the ruminant and monogastric animal and, except for the fact that pancreatic ribonuclease is particularly abundant in the ruminant, the principal enzymes appear to be similar.

Smith (1979) denoted that the relatively low pH obtained in the duodenum may extend the activity of pepsin but causes a delay on the activity of pancreatic enzymes. This is consistent with work reported earlier by Ben-Ghedalia et al. (1974) which demonstrated that activities of trypsin, chymotrypsin and carboxypeptidase A reached a maximum at a distance of 7 m from the pylorus of the sheep. Ben-Ghedalia et al. (1974) also reported that the most intensive absorption of amino acids occurred between 7 and 15 m from the pylorus and that the net absorption of essential amino acids at this site exceeded that of nonessential amino acids.

Owens and Bergen (1983) summarized plasma amino acid responses to various physiological states as follows:

> "After a meal containing protein, venous plasma amino acid concentrations increase, but the amino acid profiles may or may not mirror the amino acid profile of ingested protein. Influx of exogenous amino acids from the gut will cause a 10% increase in plasma amino acids, but diets deficient in protein cause a reduction in essential amino acid levels in plasma and tissue pools, while nonessential amino acids concomitantly rise. Branched chain amino acids and methionine concentrations are reduced during protein deficiency but not during energy deficiency, while during fasting essential, branched and nonmetabolizable amino acids (N-methyl histidine, N-methyl lysine) concentrations increase."

Increasing available protein for digestion in the small intestine resulted in significant increases in plasma essential amino acid concentration, total nonessential amino acids remained unchanged but glycine levels decreased markedly (Bergen, 1979a). In earlier studies, Bergen and Potter (1975) investigated the effects of dietary protein level on plasma amino acid concentrations. In this study lambs

were bottle fed diets which contained 12, 24 and 35% protein as milk replacers. Plasma essential amino acids increased from 12.3 to 28.5 and 32.9 mg/100 ml for the three protein levels, respectively, whereas nonessential amino acid levels remained unchanged (11.1, 15.4 and 14.1 mg/100 ml plasma).

When NPN was the primary nitrogen source for growing or producing animals, plasma branched-chain amino acids and phenylalanine levels declined (Bergen et al., 1973). Elevated levels of glycine and serine with depressed levels of valine, isoleucine and phenylalanine were obtained by Oltjen and Putnam (1966) in steers fed a purified diet containing urea as the sole nitrogen source when compared with those fed isolated protein. The influence of dietary nitrogen levels and sources on plasma amino acids were summarized by Bergen (1979a). Bergen stated that in ruminants plasma amino acid concentrations reflected the amount of protein digested in and absorbed from the small intestine. Thus, a general increase in plasma amino acid concentrations with increased dietary protein content indicates that greater quantities of protein are passing to the small intestine for digestion and absorption. Increased passage of protein to the small intestine may be achieved by increasing microbial protein synthesis or by an increase of the preformed protein escaping degradation in the rumen.

MATERIALS AND METHODS

General Procedures

This research consisted of three separate experiments. General procedures as well as a general synopsis of the different studies involved in each experiment will be discussed in this section. More detailed information as to diets, analytical procedures, type of animals, number of animals, management practices, etc. will be covered separately further ahead in this manuscript for each experiment.

Experiment one evaluated four dietary treatments in a feeding trial using growing-finishing cattle, a digestion-nitrogen balance trial with growing-finishing lambs and rumen fermentation studies using lambs. In experiment two, six dietary treatments were evaluated when fed to growing cattle during a feeding trial and to growing lambs in a digestion-nitrogen balance trial. The third experiment was carried out utilizing growing-finishing lambs fed two sources of protein at four dietary levels. A series of trials was conducted to study adaptational responses of lambs to protein sources and levels of nitrogen supplementation as measured by nutrient digestibility, nitrogen balance and rumen fermentation.

All feeding trials during this study were conducted at the Beef Cattle and Sheep Nutrition Unit. The cattle feedlot facilities consisted of concrete-paved pens without shade or shelter. The pens were 7.01 x 7.92 m with fence-line concrete feed bunks. Each pen was also supplied with an automatic drinking fountain and allowed .88 m of feed

bunk space and 6.94 m^2 of total area per animal. The feeds for the cattle studies were mixed on a pen basis with a scale-mounted, batch mixer. Sheep feeding facilities at the same unit consisted of 4.9 x 4.9 m unpaved, outdoor pens. Each was equipped with a fence-line feed bunk and an automatic drinking fountain.

The digestion-nitrogen balance trials and rumen fermentation determinations were carried out in the Ruminant Metabolism Room at the Animal Science Complex. Studies at the Animal Science Complex were conducted in an air-conditioned room with temperatures maintained between 18 and 22 C.

Statistical analyses of the data were carried out based on the Statistical Analysis System (SAS). The analysis of variance procedure (ANOVA) was used to examine the main effects and interactions of the balanced data (Barr et al., 1976), while differences between treatment means of these data were compared by using Waller-Duncan K-ratio t test (Waller and Duncan, 1969) option from SAS. For those studies with unbalanced data, the General Linear Model (GLM) procedure from SAS was used to test the main effects and interactions, whereas the treatment means of the unbalanced data were compared by using the Duncan's multiple range test (Duncan, 1975), an option from SAS. Analysis of variance tables for the different parameters studied during this research are presented in appendix tables 1 through 72.

Experiment One

Feeding Trial

Two hundred eighty-eight Hereford, Angus or Hereford-Angus steers averaging about 291 kg in initial weight were used in this trial. They were purchased from a local auction market about 6 wk prior to the beginning of the trial. During the first 2 wk after arrival, steers were fed about 2.25 kg per head daily of high-moisture whole corn grain (approximately 73% DM) and a full feed of alfalfa-brome haylage (approximately 65% DM).

After the initial 2 wk, the diet was changed to a full feed of high-moisture ground ear corn over a period of 2 wk by gradually reducing the haylage and increasing ear corn. The cattle were full-fed high-moisture ear corn without any supplemental protein for an additional 2 wk prior to the beginning of the trial. The ground ear corn with approximately 64% DM used during the preliminary period was harvested during the 1979 season and stored in an oxygen-limiting silo. Processing of the cattle during the pre-experimental period included individual ear tagging, injection with Clostridium chauvoei-septicum-novyi-sordellii bacterin and implantation with 36 mg of zeranol.

The steers were weighed in early morning before feeding and again the following morning after withholding feed and water for about 16 h. The first weight was used to determine periodic performance during the course of the experiment, whereas the second weight was taken to determine overall performance on a shrunk basis at the end of the experiment. After the second weighing, steers were allotted to

36 pens on the basis of weight and breed group with five Hereford and three Hereford-Angus or Angus steers per pen.

The dry diets contained 92% ground ear corn and 8% of a supplement which was either a corn-based control, urea, soybean meal or heat-treated soybean meal. The soybean meals fed during this experiment were obtained from Farmland Industries, Inc., St. Joseph, Missouri. They were processed by conventional procedures through the step where the oil was extracted. The solvent was removed by a flash desolventizing method involving exposure to super-heated hexane vapor. The regular soybean meal had a protein dispersibility index (PDI) of about 40, while the heat-treated soybean meal was subjected to extensive heating and had a PDI of about 10. A urea supplement was fed at the same protein equivalent level as the soybean meal supplements for a comparison of a readily soluble source of nitrogen devoid of amino acids to one of preformed protein from soybean meal. The control supplement contained similar levels of calcium, phosphorus, vitamin A, vitamin E, monensin and trace mineral salt as the other supplements. No supplemental protein was included in order to measure the response to supplementation from the various sources of protein. Ingredient composition of the diets fed in this trial is shown in table 2.

The ground ear corn which contained about 20% cob and 80% grain (dry basis) was stored under three different methods. These methods were:

1. Air-dry (85% DM) stored in cribs and ground as needed in amounts for a 2- to 3-wk supply during the experiment.

TABLE 2. INGREDIENT COMPOSITION OF THE FEEDLOT DIETS FED DURING EXPERIMENT ONE[a]

Ingredients	Control	Urea	Soybean meal	Heat-treated soybean meal
		%		
Ground ear corn	92	92	92	92
		Supplement		
Ground ear corn	6.30	5.10	.40	.40
Soybean meal (53%)			5.90	
Urea (46% N)		.95		
Heat-treated soybean meal (57%)				5.90
Limestone	1.00	.85	1.10	1.10
Dicalcium phosphate	.30	.40	.20	.20
Trace mineral salt	.40	.40	.40	.40
Calcium sulphate		.30		
Avg protein content as analyzed (dry)	9.38	11.97	11.78	12.03

[a] Each diet contained 2200 IU of vitamin A, 33 IU of vitamin E and 33 mg of monensin per kg.

2. Ground with water added to give reconstituted high-moisture ear corn at 68% DM and stored in an oxygen-limiting silo.

3. Ground with water added as for 2 and stored in a concrete stave tower silo.

The corn storage methods were balanced as to supplemental treatments with three replicates for each storage method and a total of nine replications per supplement treatment. All the ear corn was ground in a tub grinder equipped with a 1.27-cm screen. Protein content obtained from periodic samples from the three sources of ground ear corn

varied from 9.0 to 9.7% and averaged 9.4% on a dry basis. The average protein content of the various supplements obtained from periodic samples during the experiment were control, 9.2; urea, 41.6; regular soybean meal, 39.3; and heat-treated soybean meal, 42.4%.

The initial rate of daily feeding was 5.5 kg of dry matter per head with daily increases of .7 kg until the steers were on full feed in about 1 wk. Thereafter, the rate of feeding was regulated to amounts that would be nearly consumed by the next feeding. Total feed offered each day on an as fed basis was determined by examining the feed remaining in the bunk for each pen and checking the amount fed the previous day. A feeding schedule was prepared for various levels of total feed to maintain the distribution as fed between ground ear corn and supplement for 92 and 8%, respectively, of the dry matter. The diets were batch mixed for each pen and fed once daily.

Intermediate weights were obtained in early morning before feeding. The trial was terminated after 124 d (June 10 to October 12, 1980) with a shrunk weight similar to that obtained at the beginning of the experiment. Weight of the cattle at this time averaged about 457 kg.

Digestion-Nitrogen Balance Trial

For this trial 30 crossbred wether lambs of similar weight (25 to 27 kg) were selected from a larger group purchased at a local livestock auction. The lambs were brought to the Animal Science Complex facilities and then ear tagged, sheared and implanted with 12 mg of zeranol. For 4 wk, these lambs were gradually adapted from a full feed

of alfalfa-brome haylage to a full feed of a diet with 10% alfalfa-brome haylage and 90% ground corn grain. During the first 2 wk, the lambs were group fed in groups of 7 to 8. They were penned and fed individually for another 2 wk. After this 4-wk period, ground ear corn was substituted at the same level for the ground corn plus haylage diet with 1 wk being allowed for the change.

Twenty-four of the lambs were selected based on weight and individual feed intake and allotted to four dietary treatments. The dietary treatments which consisted of control, urea, soybean meal and heat-treated soybean meal supplements to ground ear corn were similar to those fed during the cattle feeding trial (table 2). They were also fed during this trial at the same levels on a dry basis (92% ground ear corn and 8% supplement). For the next 5 wk, the lambs were adapted on slatted floor pens to the dietary treatments. During this period, dry feed intake was adjusted daily based on feed refusals.

Two wk prior to starting the collection period, lambs were weighed and placed in the metabolism crates. At this time the control group averaged 31.2 kg, while the urea, soybean meal and heat-treated soybean meal fed groups averaged 31.7, 33.0 and 33.9 kg, respectively. The lambs were weighed at the beginning of the 5-d digestion-nitrogen balance trial and again at the end of the collection period. Chemical analyses of feed samples taken daily gave an average protein content of 9.6% for the control, 12.6% for urea, 11.7% for soybean meal and 12.5% for the heat-treated soybean meal supplemented diets.

Urine from each lamb was collected in a plastic vessel containing 10 ml of a 6N HCl solution. A 10% aliquot of the total urine excreted was placed in glass bottles and stored under refrigeration until nitrogen analyses were conducted. Samples of the diet fed and the total quantity of feed refusals and feces excreted were collected daily, weighed and dried in a forced-air oven (Despatch) at 70 C for 36 h. Composites from the samples were ground through a Wiley mill equipped with a 1-mm screen. Samples from feed, orts and feces were analyzed for moisture, total nitrogen and ash (to determine organic matter) by using the AOAC (1975) procedures.

Rumen Fermentation and Blood Parameter Determinations

After termination of the digestion-nitrogen balance trial, all lambs were removed from their metabolism crates and placed in individual pens with slatted floors. During a 7-wk preliminary period, each lamb was fed the same diet as during the digestion trial (table 2) and the amount of feed was adjusted daily based on the amount consumed during the previous day. To insure good feed intake for all animals prior to rumen fluid collection, lambs were trained to consume the daily offering of feed in about 3 h. This was accomplished by removing the feed after offering it for 3 h every day. Several days were required before feed consumption was considered adequate for these studies. Additionally, just prior to starting these studies, lambs were weighed and their necks were sheared to facilitate the blood collection.

Rumen Fluid Determinations. Rumen samples were taken 3 h after feeding. Initially about 20 ml of rumen fluid were rapidly withdrawn by using the stomach tube and suction strainer procedure described by Raun and Burroughs (1962), examined for indication of saliva content and then discarded. Thereafter, 40 ml were taken and kept for analytical determinations. Immediately, the pH was determined by using a Sargent-Welch Model NX pH meter set at 36 C. Thereafter, the samples were placed in 50-ml polyethylene centrifuge tubes containing 1 ml of saturated 7% mercuric chloride ($HgCl_2$) to stop fermentation. After all samples were collected and the pH determined, the tubes were centrifuged at about 9,000 rpm for 20 min in a Sorvall SS34 centrifuge. Twenty ml of the supernatant were pipetted into plastic storage bottles containing 2 ml of metaphosphoric acid (25%, w/v) for deproteination, mixed and then frozen at -5 C.

After a few months (4 mo) of storage, the rumen fluid samples were thawed and centrifuged at 9,000 rpm for about 30 min to separate the precipitated protein. The fluid was decanted in clean storage bottles and frozen until chemical analyses were performed. When ready for analysis, the samples were thawed and 1-µl aliquots used to analyze individual volatile fatty acids (Baumgardt, 1964) with a Varian Model 1800 gas chromatograph. This instrument uses a hydrogen flame detector and is equipped with a 3.175 mm x 1.83 m stainless steel column packed with a 20% neopentyl glycol succinate with 2% phosphoric acid stationary phase on a support phase of Chromasorb PAW 60/80 mesh. The chromatograms were recorded with a Honeywell Electronic 16 Recorder equipped with a

disc integrator. The peaks were adjusted for baseline drifts and molar percentages for acetic, propionic, butyric, valeric and isovaleric acids were determined.

Total VFA concentrations were determined using a Markham steam distillation apparatus (Markham, 1942). Before distillation, 5 ml of the deproteinized rumen fluid sample were diluted to 50 ml with re-distilled water and three drops of concentrated sulfuric acid were added. Twenty ml of the diluted sample were inserted into the apparatus and approximately 150 ml of distillate were collected. Then, three drops of phenol red (.08%) were added and titration was accomplished with .09N potassium hydroxide. The calculations for total VFA concentration were carried out as follows:

$$\mu\text{mol/ml VFA} = \frac{(\text{ml KOH})\ (\text{N of KOH})}{(\text{ml rumen fluid distilled})} \times 1000$$

N = normality

In addition, rumen fluid samples were analyzed for lactic acid and rumen ammonia nitrogen. For the lactic acid determinations, 1 ml of deproteinized rumen fluid sample was mixed into 9 ml of glass distilled water. Then, the colorimetric procedure outlined by Barker and Summerson (1941) was used to determine the actual lactic acid concentration. Optical density readings were obtained by using the Beckman DU Quartz spectrophotometer. A standard curve obtained by using linear regression was utilized to convert the optical density readings into lactic acid concentrations.

Rumen ammonia was determined by using the ammonia specific ion electrode Model 95-10 from Orion Research connected to a Sargent-Welch

Model NX pH/mv meter. The direct measurement procedure outlined in the Orion Research (1979) ammonia electrode instruction manual was used to determine ammonia concentration.

Blood Ammonia-Nitrogen Determinations. Blood samples were drawn 8 h after feeding on the same days as sampling rumen fluid. About 10 ml of jugular blood were obtained in a vacutainer tube containing sodium heparin. These were placed immediately in an ice bath and kept here until centrifugation. After all samples were collected, they were centrifuged under refrigeration at 3,600 rpm for 10 min by using an International Model SBV centrifuge within 30 min after collection. The plasma was pipetted into plastic storage bottles and within 1 h the ammonia analysis was performed by using the Hyland (1979) blood ammonia (plasma) test kit procedure based on Miller and Rice (1963) procedures. This is a colorimetric procedure and a Beckman Model DU Quartz spectrophotometer was used to determine optical density.

The rumen fluid and blood sample collections were carried out in 3 d and consisted in obtaining specimens from eight different lambs daily (two from each dietary treatment). The procedure was repeated a week later to obtain a total of 12 samples per dietary treatment for each variable considered. Procedures and conditions during the second week of collection were similar as those employed during the first week.

Hourly Rumen and Blood Parameter Determinations

A week after the rumen fermentation and blood studies were finalized, two lambs from each dietary treatment were selected to conduct

a 1-d hourly study. Feed intake was the only criterion used to select the two lambs from each treatment group. Lambs consuming feed more uniformly from each group were utilized in this trial. For this study, rumen fluid samples were collected before feeding and at 1, 2, 3, 4 and 5 h after feeding. Blood samples were collected before feeding and at 6, 8 and 10 h after feeding. During the hourly rumen fermentation studies, a total of 12 observations were obtained for each dietary treatment, while for the blood determinations only eight observations per treatment group were obtained.

Rumen fluid and blood samples were collected and handled using similar procedures as those indicated in the previous section. Rumen fluid samples were analyzed for total VFA, individual VFA molar percent, lactic acid, pH and rumen ammonia, while blood samples were analyzed for plasma ammonia.

Experiment Two

Feeding Trial

This trial was conducted between January 9 and April 24, 1982, at the same location and utilizing the same facilities as those used for the feeding trial of experiment one.

The 192 steer calves used in this trial were selected from a larger group purchased at a local livestock auction market about 6 wk (preliminary period) prior to the beginning of the experiment. This group of steer calves averaged about 236 kg and consisted of Hereford, Angus and Hereford-Angus breeding. Upon arrival, the steers were fed a

mixture of low-quality grass and alfalfa hay at about 5.5 kg per head daily. Whole corn grain was started at .9 kg per head daily and increased by .5 kg daily to 2.4 kg. Alfalfa-bromegrass haylage (65% DM) replaced hay after 1 wk at a level to supply a similar amount of dry feed as the hay. A corn-based supplement with dicalcium phosphate, trace mineral salt, chlortetracycline, sulfamethazine and vitamin A was fed at .5 kg per head daily during the first 4 wk of the preliminary period.

Two wk prior to the beginning of the experiment, the alfalfa-brome haylage, whole corn grain and supplement were replaced with a full feed of corn silage (34% DM) without supplemental protein. This practice was carried out to adapt the cattle to the corn silage basal diet prior to starting the experiment. To achieve this, each animal received initially an average of 6.8 kg of corn silage and then intake was increased gradually to about 15.9 kg on an as fed basis. During the 2 wk, the cattle had access to dicalcium phosphate and trace mineral salt on a free-choice basis.

During the preliminary phase, the steers were processed and procedures included ear tagging, implantation with 36 mg zeranol, application of Warbex® (pour-on) for parasite control, vaccination against bovine virus diarrhea (BVD) and bovine rhinotracheitis (IBR) and injection of Clostridium chauvoei-septicum-novyi-sordellii bacterin.

The initial weight was a shrunk weight obtained after an overnight stand of about 18 h without water or feed. The steers were then allotted into 24 pens on basis of weight and breed group with six Hereford and two Hereford-Angus or Angus steers per pen. Dietary

treatments were replicated four times and consisted of a diet containing 90% corn silage and 10% supplement on a dry basis. Supplements used during this experiment included (1) control supplement, (2) urea supplement, (3) soybean meal supplement, (4) heat-treated soybean meal-whole soybeans supplement, (5) urea-dehydrated alfalfa meal supplement and (6) soybean meal-dehydrated alfalfa meal supplement.

Ingredient composition of dietary treatments is shown in table 3. The control supplement without a high protein ingredient served as a measure of response to the various sources of supplemental protein. The urea supplement was formulated to supply similar levels of protein as did the other protein-supplemented diets with .84% urea on a dry basis. This supplement was used to test the effects on animal performance of a protein source devoid of amino acids and totally solubilized in the rumen fluid.

The soybean meal supplement consisted of soybean meal flakes (50% protein on a dry basis) processed under conventional procedures by Triple "F" Feeds, Des Moines, Iowa. It is considered a high-quality protein as measured by amino acid composition but readily degraded in the rumen. The heat-treated soybean meal-whole soybean product (manufactured by the same company) consisted of a mixture of 40% whole soybeans and 60% solvent processed soybean meal. It will be referred to as heat-treated SBM-SB in this manuscript. The blend was dry extruded and heat-treated at 160 C under pressure by the Instapro method. The processing decreased the rate of ruminal degradation in comparison to soybean meal. This blend contained 6% ether extract and 7% crude fiber.

TABLE 3. INGREDIENT COMPOSITION OF DIETS FED DURING EXPERIMENT TWO[a]

Ingredient	Control	Urea	Soybean meal	Heat-treated SMB-SB[b]	Urea-dehydrated alfalfa	Soybean meal-dehydrated alfalfa
	% of dry matter					
Corn silage	90	90	90	90	90	90
	Supplement					
Ground corn grain	8.06	7.66	2.87	1.03		
Soybean meal (50%)			5.80			4.50
Urea		.84			.49	
Heat-treated SMB-SB (40%)				7.73		
Dehydrated alfalfa meal					8.22	4.36
Limestone	.22	.09	.20	.26		.07
Dicalcium phosphate	.68	.68	.63	.48	.68	.57
Calcium sulfate		.23			.11	
Trace mineral salt	.50	.50	.50	.50	.50	.50
Avg protein content as analyzed (dry)	9.19	11.64	11.22	11.29	11.20	11.23

[a] Each diet contained 2,200 IU vitamin A, 33 mg monensin and 9 mg tylosin per kg.
[b] SBM-SB was 60% soybean meal and 40% whole soybeans.

In the urea-dehydrated alfalfa meal supplement, each nitrogen source provided similar levels of protein or protein equivalent on a dry basis. Dehydrated alfalfa meal is considered to be solubilized at a slower rate than soybean meal, resulting in larger amounts of protein from alfalfa meal escaping degradation. In combination with urea, this protein source has been reported to improve utilization of the total dietary nitrogen. The soybean meal-dehydrated alfalfa meal supplement was formulated to determine benefits of alfalfa meal proteins combined with soybean meal. Each was used to measure the effect of combining ingredients of varying solubility as ways of improving protein utilization when fed as supplements to low protein diets for growing cattle.

The corn silage was stored in two tower silos, forage from one served as a control and the other silage was treated with a microbial silage additive (Lactobacillus plantarum). Protein supplements were balanced as to silage treatments. Protein contents on a dry basis for control, urea, soybean meal, heat-treated SBM-SB, urea-dehydrated alfalfa meal diets were 9.19, 11.64, 11.22, 11.29, 11.20 and 11.23%, respectively (table 3).

Upon initiation of the trial, supplements were fed for the first time on day 1. Total feed (corn silage plus supplement) was increased gradually from about 4.1 to a full feed of 5.9 kg (dry) per head daily in about 9 to 11 d. After this, procedures for daily feeding were similar to those employed in the feeding trial of experiment one.

Intermediate weights were taken at 4-wk intervals following an overnight stand of about 18 h without feed or water and the trial was terminated after 105 d when the steers averaged about 350 kg.

Digestion-Nitrogen Balance Trial

Thirty uniform, crossbred wether lambs were selected from a larger group kept at the Beef Cattle and Sheep Nutrition Unit for this trial. Upon arrival at the Animal Science Complex, lambs were ear tagged, injected with 1 ml of Clostridium perfringens type D bacterin-toxoid for prevention of enterotoxemia, implanted with 12 mg zeranol and placed in individual pens with slatted floors.

A preliminary period of 28 d was used for a uniformity period in which lambs were adapted to the corn silage basal diet, protein supplements and metabolism crates. During the first 15 d of this period, lambs were brought to a full feed of corn silage (37.3% DM) by daily increasing the amount of silage offered based upon appetite. The lambs were then weighed and allotted on the basis of weight into six groups for the dietary treatment (table 3) with five wether lambs per group. Diets (90% corn silage and 10% supplement on a dry basis) were similar as those fed during the feeding trial with the exception of the soybean meal group.

While mixing the soybean meal supplement, the amount of ground corn grain and soybean meal was switched inadvertently. Therefore, instead of formulating a 32% protein supplement a 21% protein supplement was devised. The soybean meal supplement fed during this trial contained 58.0% ground corn grain and 28.7% soybean meal in place of 58.0%

soybean meal and 28.7% ground corn grain as during the feeding trial. This, therefore, may not give a true measure of the diets fed during the feeding trial since the protein level for this group of lambs was much lower than that of the feeding trial. The crude protein levels for the control, urea, soybean meal, heat-treated SBM-SB, urea-dehydrated alfalfa meal and soybean meal-dehydrated alfalfa meal supplements fed during this trial were 8.1, 11.4, 9.3, 10.5, 10.3 and 9.9%, respectively.

After 1 wk of consuming the complete diet (90% silage, 10% supplement), four lambs were selected from each dietary treatment on the basis of feed intake, weighed and placed randomly into the 24 metabolism crates. Lambs were adapted to the metabolism crates in 7 d and then the collection period started. A 5-d total collection digestion-nitrogen balance trial was conducted by offering feed on an ad libitum basis to each lamb. The procedures for handling the feed, feed refusals, urine and feces were the same as described for the digestion-nitrogen balance trial in experiment one. Analytical procedures were also similar to those outlined in the digestion-nitrogen balance trial of experiment one. There were no missing values in the data obtained during this trial. Therefore, statistical analysis followed the same procedures as indicated in the general procedures section.

Experiment Three

Feeding Trial

Three hundred eighty-four crossbred ewe and wether lambs were selected from a larger group which had been sheared, implanted with 12 mg zeranol and vaccinated with Clostridium perfringens for prevention of overeating disease. Over a period of 3 wk, the diets were changed from .23 kg of ground corn grain and a full feed of alfalfa haylage to a full feed of the pelleted control diet (tables 4 and 5).

The lamb weights ranged from 24 to 39 kg and they were randomly allotted within weight groups into 48 pens. Experimental treatments were four levels of dietary protein using soybean meal or urea for eight treatment groups (table 6). Each treatment was replicated six times using four ewes and four wethers per pen. Allotment by sex groups was at random within weight groups.

Ingredient composition of the soybean meal and urea diets is presented in tables 4 and 5, respectively. Corn grain constituted the main portion of the diets ranging from about 90% in the control diets to about 82% at the higher level of supplementation with soybean meal. Sun-cured alfalfa was included at 8% of the diet to provide the roughage portion of the diets. Limestone, dicalcium phosphate and potassium chloride were used to supply levels of calcium, phosphorus and potassium of .50, .35 and .60%, respectively, of the dry diets. Each diet contained .5% trace mineral salt and 3,300 IU vitamin A per kg of dry diet. Calcium sulfate was used to furnish one part of sulfur to ten

TABLE 4. INGREDIENT COMPOSITION FOR SOYBEAN MEAL GROUPS DURING EXPERIMENT THREE

Ingredient	Protein level			
	11.2%	12.5%	13.8%	15.1%
	% of DM			
Corn grain	89.98	87.35	83.72	82.08
Soybean meal (44%)		2.80	5.60	8.42
Alfalfa, sun-cured	8.00	8.00	8.00	8.00
Limestone	.70	.71	.73	.75
Dicalcium phosphate	.47	.40	.32	.25
Potassium chloride	.35	.24	.13	
Trace mineral salt	.50	.50	.50	.50
Vitamin A[a]	+	+	+	+

[a] Each diet contained 3,300 IU of vitamin A per kg of dry diet.

TABLE 5. INGREDIENT COMPOSITION FOR UREA GROUPS DURING EXPERIMENT THREE

Ingredient	Protein level			
	11.2%	12.5%	13.8%	15.1%
	% of DM			
Corn grain	89.98	89.57	89.16	88.76
Urea (46% N)		.36	.72	1.08
Alfalfa, sun-cured	8.00	8.00	8.00	8.00
Calcium sulfate		.10	.20	.30
Limestone	.70	.64	.58	.52
Dicalcium phosphate	.47	.48	.49	.49
Potassium chloride	.35	.35	.35	.35
Trace mineral salt	.50	.50	.50	.50
Vitamin A[a]	+	+	+	+

[a] Each diet contained 3,300 IU of vitamin A per kg of dry diet.

parts of nitrogen from urea in the urea diets. Ingredients for each dietary treatment were mixed and pelleted (.635 cm diameter).

Diets were calculated to provide levels of 11.2 (control), 12.5, 13.8 and 15.1% protein from soybean meal and urea. Protein content obtained by analyzing semi-weekly samples of the diets were in good agreement with those calculated for treatment levels and protein source except for treatment group 3 (table 6), where there was some variation between protein sources. Average values for both sources were the same as those formulated which were considered appropriate to designate this level of protein.

Upon initiation of the test diets, the daily feed was increased gradually from an initial level of .9 kg to a full feed offered once daily over a period of about 12 d. Thereafter, lambs were fed once daily in amounts to be nearly consumed by the next feeding. Lambs were weighed at 17, 41, 61 and 81 d in early morning before feeding. The experiment was terminated at 81 d (August 7 to October 27, 1981) when the lambs averaged about 52.3 kg.

TABLE 6. PROTEIN LEVELS IN DIETS DURING EXPERIMENT THREE

Treatment group	Formulated level	Analyzed level		
		Soybean meal	Urea	Avg
	% of DM			
1	11.2[a]	11.1[a]	11.3[a]	11.2[a]
2	12.5	12.2	12.6	12.4
3	13.8	14.3	13.4	13.8
4	15.1	15.1	15.1	15.1

[a] No supplemental protein used at this level.

Total Digestion-Nitrogen Balance Trials (Adaptation Studies)

This study was conducted in two phases (I and II) with similar procedures in each phase. The only exception was that 2 mo elapsed between each phase. Therefore, procedures followed in phase I will be described entirely, while only those conditions or procedures of phase II that differed from phase I will be outlined.

In phase I of this study, 32 weaned, crossbred wether lambs were purchased from a local producer about 5 wk prior to the beginning of the trial. Upon arrival at the Animal Science Complex, the lambs averaged between 20.4 and 22.7 kg. Within a period of 2 wk, the lambs were adapted from a full feed of alfalfa haylage to a full feed of the unsupplemented control diet (tables 4 and 5). This was done by gradually increasing the amount of the control diet while decreasing the amount of haylage. The lambs were fed in four groups of eight lambs for the first week, but during the second week they were placed in individual pens with slatted floors to monitor individual daily feed intake. At the beginning of this period, lambs were ear tagged, implanted with 12 mg of zeranol and injected with Clostridium perfringens type D bacterin toxoid for the prevention of enterotoxemia.

The lambs were allowed 11 d to adapt to the control diet, then they were weighed and 24 lambs were selected based on weight and placed in the metabolism crates. Another 10 d were allowed for the lambs to adapt to the metabolism crates and individual feed intake was determined. The average daily feed intake for the last 3 d and the weight obtained prior to being placed in the crates were used to assign each

animal to its respective dietary treatment (tables 4 and 5). This allowed three lambs per dietary treatment.

Dietary treatments presented in tables 4 and 5 were fed for the first time on day 1 of the experiment based on appetite. Collection trials started 48 h after first feeding of dietary treatments. The protocol followed during this study is presented in table 7. Twenty-four h prior to collection, the urine, feed, water and feces containers were thoroughly cleaned and lambs were fed their diet based on appetite.

The first two collection periods consisted of 3 d with 3 d of no collection following each one. This was conducted to assess if there were any differences in digestibility and nitrogen balance between periods, therefore indicating if there is any adaptational period for nitrogen in lambs fed under the conditions of this experiment. The third collection period was a conventional 5-d total collection trial and served to further evaluate adaptational effects and also served to estimate digestibility and nitrogen balance coefficients for these diets once the lambs were adapted. As it can be observed in table 7, this third collection period was conducted 14 d after the lambs were consuming the experimental treatments.

During phase II of this study, 30 weaned, crossbred wether lambs obtained from the same source as for phase I were brought to the Animal Science Complex. Upon arrival the lambs were weighed, ear tagged, implanted and vaccinated for prevention of enterotoxemia. The lambs which averaged 23.3 to 29.5 kg were divided into four pens and group fed a full feed of alfalfa haylage. For the next 4 wk, the lambs (group fed)

TABLE 7. PROTOCOL FOLLOWED DURING THE DIGESTION-NITROGEN BALANCE ADAPTATIONAL TRIALS

Day number	Procedures
1	FEED DIETARY TREATMENTS
2	NO COLLECTION, FECES AND URINE CONTAINERS ARE CLEANED
3, 4, 5	FIRST COLLECTION PERIOD
6, 7	NO COLLECTION
8	ALL CONTAINERS ARE CLEANED
9, 10, 11	SECOND COLLECTION PERIOD
12, 13	NO COLLECTION
14	ALL CONTAINERS ARE CLEANED
15, 16, 17, 18, 19	THIRD COLLECTION PERIOD
20	LAMBS ARE REMOVED FROM DIGESTIVE CRATES AND WEIGHED

were switched from the alfalfa haylage diet to the unsupplemented control diet (tables 4 and 5) by slightly increasing the amount of the control diet and decreasing the daily amount of haylage offered. The lambs were placed in individual pens with slatted floors for 7 d. Then, they were weighed and 24 were selected and placed in metabolism crates. Lambs were allowed 3 d to adapt to the metabolism crates and then phase II of this study was started. As for phase I, lambs were allotted to the dietary treatments (tables 4 and 5) based on feed intake and the weight obtained prior to being placed in the crates. After this, the protocol (table 7) and procedures followed for collection of samples were similar as for phase I.

Procedures used for conducting these trials, handling and collecting feed, feed refusals, urine and feces samples during the three collection periods of phases I and II were similar to those described during the digestion-nitrogen balance trial outlined in experiment one. For statistical analyses of the data obtained, results from phases I and II were combined. This allowed a total of 48 observations for each collection period, thus giving six replications for each treatment group.

Rumen Fermentation and Blood Studies

After the conclusion of each phase of the adaptational studies (phases I and II), 16 lambs from each phase were selected and placed in individual pens with slatted floors. Selection was done by choosing the two lambs from each dietary treatment with the least variability in feed intake. Over a period of 21 d, lambs from phase I were trained to

consume their daily diets within a 3-h period. To accomplish this, feeders were removed 3 h after feeding. It was assumed that lambs were trained to eat the feed as indicated because at this time they were consuming similar levels of feed as during the last digestion-nitrogen balance trial. Additionally during this period, the neck of the lambs was sheared to simplify collection of jugular blood.

After this period, the collection of rumen fluid and blood samples was started. Collection was done twice for each lamb with 4 d separating each collection. Rumen fluid samples were obtained from eight lambs (one from each dietary treatment) on each collection day at 3 h after feeding, while the blood samples were taken at 8-h postprandial.

Rumen fluid samples were collected by using a stomach tube with a suction strainer connected to a .33 HP Klemm vacuum pump from General Electric for suction. About 200 ml of rumen fluid sample were quickly drawn from each lamb into a 250-ml Erlenmeyer flask. This procedure proved to be more efficient and successful in collecting rumen fluid samples than that described by Raun and Burroughs (1962) because of the characteristics of the rumen contents. The high concentrate diets formed a thick mash or slush in the rumen that made it impossible to draw rumen fluid by using the Raun and Burroughs (1962) procedure. The addition of the vacuum pump gave sufficient suction to draw rumen contents. It was also found that the contents were composed largely of feed. Therefore, the Erlenmeyer flask was added to the unit which allowed for collecting a larger quantity of material and thus yielding

the necessary amount of rumen fluid needed for the various chemical determinations.

Immediately after collection and after examining the rumen fluid samples to insure the lack of saliva, pH determinations were made by using an Orion Research Model 601A digital ion analyzer (pH meter) set at 25 C. Samples were then placed in 250-ml plastic centrifuge containers with 5 ml of 7% saturated mercuric chloride to stop fermentation. After all the samples were processed in the same manner, the containers were centrifuged at 6,000 rpm for 30 min in an International Model CS centrifuge. Then the supernatant was aspirated from the solids (feed particles) and an aliquot of 20 ml was placed in plastic storage bottles containing 2 ml of metaphosphoric acid (25%, w/v) for deproteinization, mixed and then frozen. The remainder of the supernatant was placed in plastic containers and frozen for ammonia-nitrogen determinations.

About 20 ml of jugular blood were collected from each lamb in two test tubes without any anticoagulant. When all daily blood samples were collected, they were left to coagulate for a period of 30 min. Then, each clot was carefully separated from the sides of the test tubes and centrifuged at 3,600 rpm for at least 30 min in an International Model SBV centrifuge. The serum was then carefully removed, placed in glass vessels and frozen. Soon after, these samples were transferred to plastic containers since some samples were lost due to breakage of the glass vessels during a power outage. Three samples of the first collection period were lost.

All rumen fluid and blood serum samples from the two collection periods of phase I were then stored frozen until all samples were ready for analysis, including those of phase II.

During phase II, 16 lambs were selected as for phase I and placed individually in pen with slatted floors. This group of lambs was trained to consume their total daily feed in a 3-h period. Conditioning required 14 d as compared to 21 d for lambs in phase I. Procedures for blood collection, rumen fluid collection and handling of the samples were similar as those described previously for phase I, including instrumentation used and period of time elapsed between the two collections (4 d) of phase II.

Once all the samples from phases I and II were in the same stage of preparation (frozen), the different analyses were conducted.

Rumen Fluid Determinations. The analyses of the rumen fluid samples were conducted immediately after all the samples from phase II were collected. Rumen fluid samples containing the metaphosphoric acid were thawed and centrifuged at 9,000 rpm for about 30 min for separation of the precipitated protein. The supernatant fluid was placed in storage bottles and stored frozen for chemical analyses. Equipment, handling and analytical procedures used during these analyses were similar as those described in the rumen fermentation studies of experiment one.

Rumen ammonia nitrogen analyses were carried out after thawing the frozen rumen fluid which was devoid of metaphosphoric acid. For this procedure, deproteination was not necessary. Orion Research (1979)

ammonia electrode direct measurement procedure as outlined in experiment one was used for these determinations.

Blood Urea-Nitrogen Measurements. For this analysis, frozen vessels containing the blood samples were thawed and allowed to reach room temperature. The American Monitor (1979) blood urea nitrogen methodology was followed during these determinations. The procedure utilizes a dialdehyde derivative of benzene in combination with a dihydroxylated naphthalene compound. The primary site of reaction occurs between the phthalaldehyde compound and urea (a dehydration-coupling reaction). A secondary condensation reaction between the product and 1,3-dihydroxynaphthalene results in an intensely colored chromophore which can be measured photometrically at 480 nm. The chromophore is linear and obeys Beer's law.

Standardization within runs and calibration curves were used to determine the blood sample urea concentrations. All water utilized was free of ammonia and optical density was measured by using the Beckman DU Quartz spectrophotometer. All samples were run in duplicate and optical density was read within 5 min after the chromophore was formed.

As for the digestion-nitrogen balance adaptational trials, rumen fluid and blood urea-nitrogen determinations were combined for statistical analyses following the procedures described.

RESULTS AND DISCUSSION

Experiment One

Feeding Trial

Data obtained from the feeding trial will be presented and discussed by weigh periods during the progress of the experiment. Animal response to levels and sources of dietary protein may be more pronounced during early stages of growing and finishing and become less evident if evaluated only over the total experiment. Therefore, examination of data at periodic intervals is important in studies of this nature.

Experimental diets were formulated with the various nitrogenous ingredients to provide variation in amino acid profile and rates of solubility or degradability in the rumen fluid. Studies were not conducted to determine the quality (amino acid content) of the protein sources fed during this research. However, assumptions were based upon known amino acid composition data of the feedstuffs and on solubility values reported by several researchers and from data provided by the companies manufacturing the ingredients.

Weight Gain. Accumulated average daily gain data by weigh periods are presented in table 8. The steers received a ground ear corn diet without supplemental protein for about 4 wk prior to supplementing with the various sources of protein. This allowed for a comparison between supplements under conditions in which the cattle were adapted to the basal diet prior to feeding the test supplements.

TABLE 8. WEIGHT GAIN DATA FOR FINISHING CATTLE FED EAR CORN DIETS SUPPLEMENTED WITH VARIOUS SOURCES OF PROTEIN (JUNE 10 TO OCTOBER 12, 1980--124 DAYS)

Item	Control	Urea	Soybean meal	Heat-treated soybean meal
No. of steers	71[a]	72	72	72
Avg initial wt, kg	292	291	292	291
Avg final wt, kg	452	457	460	459
Accumulated avg daily gain, kg				
29 d	1.50	1.48	1.70	1.67
57 d	1.27	1.37	1.42	1.41
86 d	1.38	1.45	1.46	1.48
114 d	1.37	1.39	1.42	1.43
124 d	1.36	1.38	1.40	1.41
124 d (shrunk)	1.30[c]	1.34[bc]	1.35[b]	1.35[b]

[a] Initially 72 steers but one died of acute polioencephalomalacia.
[b,c] Means within rows followed by unlike superscripts differ ($P<.05$).

After 29 d, weight gains were high for all treatment groups. The control (low protein) group gained an average of 1.5 kg daily during this period. The urea group which received .95% urea in the total dry diet gained at a similar rate. Steers fed diets which contained soybean meal showed faster rates of gain during this time. The advantages at 29 d amounted to about 13 and 11% over the control group, indicating a pronounced response to supplementation with soybean meal or heat-treated soybean meal but with almost no differences between these two sources.

During the second month, all treatment groups gained at lower rates, but the percentage reduction in comparison to the first month was considerably less for the urea-fed steers. The percentage reduction

for the urea group was 7.43% as compared to about 15.8% for the other groups. The advantages in total gain at 57 d for steers fed urea, soybean meal or heat-treated soybean meal supplements were 7.9, 11.8 and 11.1%, respectively, over steers fed the control diet. Protein-supplemented steers showed the maximum advantage in total gain over the control at this point (57 d). At this time all groups exceeded 360 kg in average weight.

There was a reduction in rate of gain during the remainder of the experiment and a narrowing of the differences between controls and protein-supplemented steers. There were only small differences in weight gain between sources of supplemental protein after the 57-d period. The results would indicate that the ear corn diet without supplemental protein (9.38%, dry) was adequate for steers weighing over 360 kg and the rates of gain obtained after this time. However, there were overall advantages on basis of shrunk weight upon termination of the experiment of 5.4, 6.8 and 6.8 kg per steer, respectively, for steers fed the urea, soybean meal and heat-treated soybean meal supplements. Weight gain data indicated no benefit from heat-treating soybean meal over the regular soybean meal, since rates of gain for these treatments were similar throughout the experiment.

Statistical analyses of the weight gain data on a shrunk basis for the 124-d experiment showed that soybean meal-fed steers gained at a faster rate ($P<.05$) than the unsupplemented controls. There were essentially no differences ($P>.05$) between sources of supplemental protein with cattle fed an ear corn diet.

Results obtained during this trial concerning average daily gain are in agreement with those reported by Swan and Embry (1973), Young et al. (1973) and Burris et al. (1974). Improvement in rate of gain for steers fed ear corn diets supplemented with urea or soybean meal over the unsupplemented controls was reported by these researchers.

Patterns as followed by the urea-fed steers early in and throughout the experiment have also been reported previously. Kirk et al. (1958), Meiske and Goodrich (1966) and Perry et al. (1967) stated that gains for cattle fed urea were lower during the initial phase of the feeding period but approached gains obtained with natural protein supplements later on. It was suggested that the response to urea was due to inadequate microbial protein synthesis from urea and the need for preformed protein early in the feeding period.

Published results of experiments where urea has been compared to supplements of preformed protein show considerable variability in animal performance. Reduced feedlot performance by cattle fed diets with urea in comparison to soybean meal has been reported by Swan and Lamming (1968), Braman et al. (1973), Schmidt et al. (1973) and Young et al. (1973). Researchers reporting no difference in performance of cattle fed supplements with urea or with preformed protein include Haskins et al. (1967), Bolsen et al. (1968) and Burris et al. (1974).

One important factor that may have contributed to these variable results is the type of basal diet fed. High-concentrate diets high in readily available energy are particularly suited for efficient urea utilization. Haskins et al. (1967) and Bolsen et al. (1968) demonstrated

that equal performance could be obtained with ruminants fed all-concentrate diets with urea or soybean meal as the supplemental sources of protein (nitrogen). In those studies in which the performance of steers fed soybean meal was better than that of urea-supplemented steers, levels of dietary energy were not as high as those fed during this study. Level of protein supplementation (Schelling et al., 1967; Veira et al., 1980a), total dietary protein (Boling et al., 1972) and percentage of urea in the diet (NRC, 1976b; Pendlum et al., 1976) could have been other factors contributing to the variability of the results reported in the literature.

One of the objectives of this trial was to evaluate the effects of protein solubility on animal performance. As previously indicated, no protein solubility tests were conducted. However, data available in the literature have shown differences in solubility between various sources of protein. Wohlt et al. (1973) and Broderick (1975) have considered urea as the most soluble (essentially 100%) source of nitrogen in the rumen fluid and it is devoid of amino acids. Soybean meal has been cited as a high quality source of protein on the basis of amino acid content but a readily degradable protein in the rumen (Bergen, 1979b). Heat treatment of soybean meal has been shown to render a product less degradable in the rumen liquor than the regular soybean meal (Tagari et al., 1962). The principles involved in protein solubility indicate that a source of protein of low solubility would be degraded less rapidly in rumen fluid and thus could escape the rumen with little or no change from the dietary form. If this highly soluble

protein is of high biological value and is protected from degradation, the result will be an increased amount of and a higher quality of protein reaching the small intestine which would be expected to improve animal performance.

Results reported by Thomas et al. (1979) indicated that heat-treating soybean meal improved rate of gain for steers over regular soybean meal-supplemented steers. In other studies conducted with lambs, heat-treated soybean meal showed improved average daily gain as compared to regular soybean meal (Glimp et al., 1967; Hudson et al., 1969; Peter et al., 1971). On the other hand, no differences in animal performance were observed by Wohlt et al. (1976) in lamb feeding trials where diets were varied in degree of protein solubility.

Under the conditions of the experiment reported herein, no differences in animal performance were shown between the three sources of supplemental nitrogen which varied fairly widely in solubility. However, it is important to keep in mind that these supplemental sources supplied only a small portion of the total dietary protein. Ground ear corn was fed at 92% of the total dry diet and provided a large percentage of the dietary protein.

Feed Intake. Accumulated average daily feed intake by weigh periods is presented in table 9. Feed consumption increased for all treatment groups with increasing weight and time on the experiment. Expressed as a percentage of body weight, daily feed intake on a dry basis for all groups decreased with increasing weight. This is in

TABLE 9. FEED INTAKE AND FEED EFFICIENCY FOR FINISHING CATTLE FED VARIOUS SOURCES OF PROTEIN WITH EAR CORN (JUNE 10 TO OCTOBER 12, 1980--124 DAYS)

Item	Control	Urea	Soybean meal	Heat-treated soybean meal
Accumulated avg daily feed, kg (dry)				
29 d	7.54	7.10	7.28	7.47
57 d	7.88	7.65	7.78	7.76
86 d	8.37	8.14	8.15	8.08
114 d	8.68	8.40	8.44	8.30
124 d	8.79	8.51	8.54	8.39
Accumulated avg feed/gain (dry)				
29 d	5.03	4.80	4.28	4.47
57 d	6.20	5.58	5.48	5.50
86 d	6.07	5.61	5.58	5.46
114 d	6.34	6.04	5.94	5.80
124 d	6.46[b]	6.17[a]	6.10[a]	5.95[a]
124 d (shrunk)	6.76[b]	6.35[a]	6.33[a]	6.21[a]

[a,b] Means with unlike superscripts differ ($P<.05$).

accord with dry matter consumption data listed in table of nutrient requirements (NRC, 1976a). Average feed intake was about 2.2% of body weight initially (29 d) and decreased to about 1.9% at the end of the experiment. Over the 124-d trial, feed consumption expressed as a percentage of body weight averaged approximately 2.0%.

At all periods, steers fed the control diet consumed the most feed. This was unexpected as research has shown that addition of supplemental protein to low protein diets generally results in an increase in feed intake, especially when associated with improvement in gain (Hatfield and Cantner, 1973; Swan and Embry, 1973).

There appeared to be no important or consistent differences in feed consumption between sources of supplemental protein with the exception that feed intake was slightly lower for steers fed the urea supplement during the first 57 d. These steers also had slightly lower rates of gain at 29 and 57 d in comparison to steers fed either form of soybean meal. Over the 124-d experiment, steers fed the diet with heat-treated soybean meal had the lowest level of intake ($P > .05$).

Results obtained during this study concerning feed consumption for supplemental sources of protein support the findings reported by several researchers. Studies conducted by Swan and Lamming (1968), Braman et al. (1973), Schmidt et al. (1973), Swan and Embry (1973) and Young et al. (1973) with diets similar to those fed in this trial also showed no differences in feed consumption with steers supplemented with soybean meal or urea. Thomas et al. (1979) stated that steers consuming diets containing heat-treated soybean meal as the supplemental source of protein consistently consumed higher amounts of dry feed as compared to steers fed untreated soybean meal ($P > .05$). It is also important to emphasize that in the majority of these studies steers consumed more total dry matter than did the steers in the trial reported herein.

Feed Efficiency. Feed efficiency data as affected by protein supplementation are presented in table 9. Good feed efficiency was observed for all treatment groups during this trial. Higher feed requirements may have resulted had the steers been fed to a more typical market weight of about 522 kg.

Steers fed the control diet consumed slightly more feed, gained at lower rates and had higher ($P<.05$) feed requirements than steers fed diets with supplemental protein. The improvement in feed efficiency for urea, soybean meal and heat-treated soybean meal fed steers over those consuming the control diet was about 6.1, 6.4 and 8.1%, respectively. Similar results were reported by Young et al. (1973) when all protein-supplemented diets (soybean meal or urea) showed more favorable feed conversions than unsupplemented controls.

The main difference among protein-supplemented steers was a higher feed requirement during the first 57 d for those fed the urea-supplemented diets. This was also the period when steers had lower feed intakes and made lower rates of gain than those supplemented with either source of soybean meal. Over the 124-d experiment, there were only small differences ($P>.05$) in feed to gain ratio among protein-supplemented steers but with lower feed requirements for the steers fed heat-treated soybean meal.

Several studies conducted with steers have shown that feed requirements usually are greater for steers fed diets with urea as compared to soybean meal. One such study was conducted by Braman et al. (1973) over a feeding period of 140 d. In this study, steers fed diets supplemented with soybean meal had more favorable feed conversions than steers fed urea supplemented diets. In similar studies, Swan and Lamming (1968), Schmidt et al. (1973) and Young et al. (1973) noted that steers fed diets with soybean meal exhibited lower feed requirements than steers fed the urea supplements. On the other hand, studies

conducted by Pendlum et al. (1976) showed no differences in feed conversion when either soybean meal or urea was fed. Results in comparisons between soybean meal and urea are often inconsistent, but a great majority of the data available in the literature indicate that steers fed soybean meal diets had lower feed requirements. Factors such as the type of basal diet fed (high-moisture corn, corn cobs, ground ear corn) may have contributed to the different responses observed between this study and those reported in the literature.

In studies where the solubility of the protein was evaluated, the data show that proteins of low solubility such as heat-treated soybean meal appeared to improve feed efficiency (Hudson et al., 1969). Steers fed heat-treated soybean meal supplements were reported by Thomas et al. (1979) to have consistently lower feed requirements than steers fed untreated soybean meal; but, similarly as during the present trial, differences observed were not statistically significant.

Digestion-Nitrogen Balance Trial

This study was conducted with lambs and using the same dietary treatments as for the cattle feeding trial (table 2). Sheep are frequently used as experimental animals in evaluating protein nutrition for ruminants because of economical considerations and the ease of collecting data as compared to cattle. Data obtained concerning the efficiency of nitrogen utilization with sheep are considered appropriate for cattle (Church, 1976; Maynard et al., 1979).

Prior to starting the experiment, the lambs were adapted to the basal diet and experimental protein supplements (92% ground ear corn and

8% supplement). When feed intake was considered adequate and consistent on a day-to-day basis, the collection period was started.

Digestibility of Nutrients. Feed consumption and digestibility data as affected by dietary treatments are shown in table 10. Daily feed was offered in amounts to result in some feed refusal. This was done to insure a full feed intake during the collection period. Dry matter intake data (table 10) showed some differences between dietary treatments. However, feed intake for all treatment groups was considered satisfactory to evaluate digestibility and nitrogen balance of the various diets.

TABLE 10. FEED CONSUMPTION AND NUTRIENT DIGESTIBILITY FOR LAMBS FED VARIOUS SOURCES OF SUPPLEMENTAL PROTEIN WITH EAR CORN

Item	Control	Urea	Soybean meal	Heat-treated soybean meal
No. of lambs	6	6	6	6
Avg initial wt, kg[a]	31.3	33.7	35.2	34.2
Avg final wt, kg	31.7	33.9	35.8	34.9
Dry matter consumed, g/d	547	729	898	754
Apparent digestibility, %				
Dry matter	74.6	73.4	71.3	71.5
Organic matter	75.6	74.8	72.0	72.0
Crude protein	68.9	72.4	69.0	69.4

[a] Lamb's weight when placed in metabolism crate.

Apparent dry matter, organic matter and crude protein digestibilities are presented in table 10. Digestibility of dry matter or organic matter is commonly used as a method for determining energy utilization and should be indicative of the overall fermentative

activity in the rumen. Each measures the same characteristics of the diet. Of the two, organic matter digestiblity should be preferred since it is not influenced by mineral content of the diet. Digestibility for dry matter and for organic matter was similar, however, with values for organic matter being slightly higher in all comparisons.

No differences ($P>.05$) in organic matter digestibility were observed between treatment groups but lambs fed the control diet had the highest value. The sources of supplemental protein did not appear ($P>.05$) to affect rumen activity as measured by organic matter digestibility during this collection period. Among the protein supplemented groups, lambs fed urea had slightly higher organic matter apparent digestibility. The results are in disagreement with data reported by Veira et al. (1980b). These researchers noted that protein supplementation (soybean meal) increased the organic and dry matter digestibilities of steers as compared to those fed the control diet. Also, differences in both dry matter and organic matter apparent digestibilities between urea and soybean meal ($P<.05$) as reported by Swan and Lamming (1968) were not observed in this study. However, data concerning the soybean meal treatments support the findings reported by Glimp et al. (1967) and Hudson et al. (1969, 1970) of no differences in apparent dry matter and organic matter digestibility between soybean meal and heat-treated soybean meal. The present study, nevertheless, does not support the findings of Wohlt et al. (1976) who concluded that dry matter and organic matter digestiblity of highly soluble protein diets tended to be lower than for diets of low solubility.

Protein supplementation appeared to offer no advantage in digestibility of the crude protein ($P > .05$). Urea-supplemented lambs, nonetheless, showed the highest crude protein apparent digestibility which was at least three percentage units greater than that obtained for other dietary treatments. Generally, protein supplementation has been shown to enhance crude protein digestibility (Gallup et al., 1952; Palmer, 1982), but this was not observed in this study. These results could have been affected by the extended length of the adaptation period to the diets (5 wk). Nicholson et al. (1956) indicated that steers tended to narrow initial differences in crude protein digestibility regardless of the diet when they were fed the diets for long periods of time. Differences may be due to an animal weight effect because requirements of the animals for protein as a percentage of body weight decrease as they increase in weight (NRC, 1975). At the time of the collection, protein requirements for the lambs could have been met by the control diet.

The results for crude protein apparent digestibility obtained during this trial were not supportive of the findings reported by Swan and Lamming (1968) and Veira et al. (1980b). Crude protein digestibility for soybean meal-fed animals was higher than for those fed the unsupplemented control diet (Veira et al., 1980b). Swan and Lamming (1968) also reported higher crude protein digestibility for lambs fed soybean meal diets as compared to those fed urea. Glimp et al. (1967), Hudson et al. (1969, 1970) and Wohlt et al. (1976) reported that heat-treated soybean meal-supplemented lambs did not appear to show any advantage in crude

protein apparent digestibility as compared to untreated soybean meal-supplemented lambs, which was in agreement with the results observed in this digestion-nitrogen balance trial.

Nitrogen Balance. The effect of protein sources on nitrogen utilization is shown in table 11. Lambs receiving protein-supplemented diets with about 12% protein consumed more feed and thus more nitrogen per day than lambs consuming the unsupplemented control diet with about 10% protein. The greater amount of nitrogen intake by the protein-supplemented lambs was significantly different ($P<.05$) than that of the unsupplemented controls but with no differences between sources of protein.

TABLE 11. EFFECT OF VARIOUS SOURCES OF PROTEIN SUPPLEMENTATION ON NITROGEN UTILIZATION FOR LAMBS

Item	Control	Urea	Soybean meal	Heat-treated soybean meal
Dry matter consumed, g/d	547	729	898	754
Nitrogen balance, g/d				
Intake	8.75^{a}	14.99^{b}	17.23^{b}	15.45^{b}
Fecal	2.73^{a}	4.09^{b}	5.30^{c}	4.73^{bc}
Urinary	3.15^{a}	5.44^{b}	5.30^{b}	4.98^{b}
Retained	2.87^{a}	5.46^{b}	6.63^{b}	5.74^{b}
Percent of intake				
Fecal	31.20	27.29	30.76	30.62
Urinary	36.00	36.29	30.76	32.23
Retained	32.80	36.42	38.48	37.15

^{a,b,c} Means within rows followed by unlike superscripts differ ($P<.05$).

Differences observed in the amount of fecal nitrogen excretion between the unsupplemented (control) lambs and those supplemented with urea or either source of soybean meal were significant ($P<.05$). Among protein-supplemented groups, lambs fed the diet containing soybean meal had higher ($P<.05$) amounts of fecal nitrogen than those fed the urea diet and slightly higher ($P>.05$) amounts than lambs consuming the heat-treated soybean meal diet. Fecal nitrogen excreted is related to the level of nitrogen intake and those groups which consumed higher levels of nitrogen had greater amounts of nitrogen excreted in the feces.

Protein supplementation increased ($P<.05$) urinary nitrogen excretion, with the urea group showing the highest nitrogen loss. Nitrogen retained was also affected by protein supplementation, with greater ($P<.05$) retention for protein-supplemented lambs as compared to those receiving the control diet.

Because lambs consumed variable amounts of feed and thus had different intakes of protein, it is probably more meaningful to discuss nitrogen utilization as a percentage of the total nitrogen consumed rather than as absolute amounts.

Small differences ($P>.05$) were observed for fecal nitrogen as a percentage of nitrogen intake between dietary treatments, with the urea group showing the lowest percentage (27.29%). The fecal fraction represents the undigested nitrogen portion and, therefore, is a reflection of the crude protein digestibility (table 10), which also showed no differences between dietary treatments.

Urinary nitrogen excretion as a percentage of the total nitrogen intake was somewhat higher for lambs receiving the control and urea supplements in contrast to the soybean meal-fed lambs, but differences were not significant ($P>.05$). Similar trends were reported by Palmer (1982). Lambs consuming the diets with higher levels of soluble protein (urea) showed greater urinary nitrogen losses than lambs fed protein sources of higher solubility (corn gluten meal).

Lambs supplemented with urea, soybean meal and heat-treated soybean meal retained more nitrogen than the unsupplemented control lambs. However, differences between treatment groups were not significant. Heat-treated soybean meal appeared to offer no benefit over regular soybean meal as measured by nitrogen retention.

Results found in the literature are inconsistent. For example, Palmer (1982) fed lambs ground ear corn diets supplemented with various sources of protein and found that protein supplementation improved ($P<.05$) nitrogen retention. However, in other studies, Veira et al. (1980b) did not show differences between control steers and those fed soybean meal diets. The trends reported in this manuscript tend to support the data published by Palmer (1982).

The results from heat-treated soybean meal as compared to untreated soybean meal are in agreement with earlier studies conducted by Hudson et al. (1969, 1970) but do not agree with results presented by Tagari et al. (1962) and Glimp et al. (1967). The objective of these studies was to determine the effect of protein solubility on nitrogen retention. Degradation of proteins in the rumen appears to

increase with increasing protein solubility. Increased losses of feed nitrogen as rumen ammonia prevail, thus decreasing the possibility of feed nitrogen escaping to the small intestine. The net result is to decrease the amount of nitrogen absorbed and also nitrogen retained (Wohlt et al., 1976).

Rumen Fermentation and Blood Parameter Determinations

This phase of the experiment was replicated over time with 1 wk separating each trial and the data collected were combined for the purpose of statistical analysis and discussion of the results. This study was conducted using the same dietary treatments (table 2) and the same lambs used in the digestion-nitrogen balance trial. These lambs were trained to consume most of the feed offered within 3 h after feeding. Rumen fluid samples were taken 3 h after feeding, while jugular blood samples were taken 8 h postprandial. Rumen samples were analyzed for pH, lactic acid, ammonia-nitrogen and total and individual VFA, while blood samples were analyzed for ammonia-nitrogen.

Rumen pH. Mean values for rumen pH as affected by dietary treatments are presented in table 12. Church (1976) indicated that rumen pH will generally reach a low from 2 to 6 h after feeding, depending on the nature of the diet and the rapidity in which the diet is consumed. Since diets were consumed within 3 h of feeding and rumen fluid samples taken at this time, the pH values obtained were assumed to indicate rumen pH at the peak of rumen fermentative activity.

TABLE 12. SOME RUMEN AND BLOOD CHARACTERISTICS AS AFFECTED BY DIETARY TREATMENTS

Item	Control	Urea	Soybean meal	Heat-treated soybean meal	SE
No. of observations	6	6	6	6	
Dry matter consumed, g/d	937	1049	1186	1137	
Nitrogen intake, g/d	14.42	21.06	22.24	22.69	
Rumen pH	6.15[a]	5.56[b]	5.60[b]	5.72	.09
Rumen lactic acid, µg/ml	19.56	221.41	37.79	1036.48	363.3
Rumen NH_3-N, mg/100 ml	.29[a]	4.21[b]	.73[a]	.74[a]	.05
Plasma NH_3-N, µg/100 ml	56	62	54	57	.71

[a,b] Means within rows followed by unlike superscripts differ ($P<.05$).

Rumen pH was higher ($P<.05$) for lambs consuming the unsupplemented control diet than for lambs receiving diets supplemented with protein. These results do not agree with the findings reported by Haaland et al. (1982) who showed higher pH values with protein supplementation. In the present study, the difference between the control group and protein supplemented groups was at least .43 unit values. Only small differences ($P>.05$) in rumen pH were observed between sources of protein supplementation.

These results fall in the range of 4.7 to 7.0 as reported by Hungate (1966) for ruminants fed a wide variety of diets. Animals consuming diets containing higher levels of concentrate tend to have a lower rumen pH (Briggs et al., 1957). Diets which resulted in the production of high levels of lactic acid are associated with low ruminal pH (Briggs et al., 1957; Fulton et al., 1979). Similar results were found in this study and the elevated rumen pH with the control diet could be explained in part by the lower lactic acid concentration in the rumen.

Results obtained for rumen pH during this study are in agreement with data reported by Oltjen and Davis (1965), Haskins et al. (1967) and Mackie and Gilchrist (1979). The findings obtained by Mackie and Gilchrist (1979) indicated that sheep fed diets containing 71% concentrate showed rumen pH levels averaging 5.78 ± .16 lb, which is a level similar to that found in this study for protein-supplemented lambs, whereas in studies conducted by Oltjen and Davis (1965) and Haskins et al. (1967) supplementation with urea or soybean meal showed no

differences in pH for steers fed high concentrate diets. Oltjen and Davis (1965) reported pH values of 5.6 for soybean meal-fed and 5.4 for urea-fed cattle. Differences in rumen pH reported by Loerch et al. (1983) between soybean meal and urea when fed to steers as supplements to ground corn grain and ground corn cobs were not observed during the present study.

Processing soybean meal with heat did not appear to have any effect on rumen pH, since values for lambs consuming the heat-treated or the untreated soybean meal diets were similar. These data also support the findings of Wohlt et al. (1976) which showed no differences in rumen pH between lambs fed diets of high and low protein solubility.

Rumen Lactic Acid. Ruminal lactic acid concentrations observed for lambs fed various dietary treatments are presented in table 12. Wide ranges in ruminal lactic acid values were also found during this study. The control group had 19.56 µg/ml, while the heat-treated soybean meal group showed 1036.48 µg/ml of rumen fluid. These values are within the ranges reported in the literature, except the low value for the control group. Rumen pH was observed to be higher for lambs on the control diet than for those on the protein-supplemented diets. Therefore, a greater concentration of lactic acid in the rumen fluid of protein-supplemented lambs was expected. Similar relationships between rumen pH and lactic acid was suggested by Briggs et al. (1957) and Reid et al. (1957).

The magnitude of the differences in ruminal lactic acid concentration observed between dietary treatments was large. The highest concentration observed, however, was lower than that reported by Mackenzie (1967) to cause lactic acidosis. Although the differences between dietary treatments were not significant ($P>.05$), lambs fed protein-supplemented diets showed higher levels of lactic acid in the rumen. Lactic acid concentrations between and within treatment groups varied widely, resulting in a large standard error and no difference ($P>.05$) between treatments.

Since most substances produced within the rumen are metabolic end-products or intermediates, their concentration can vary widely. Rumen lactic acid concentration is not an exception and Mackenzie (1967) has indicated that the rumen contents of animals fed low roughage diets may contain lactic acid values ranging from 90 µg/ml to 13500 µg/ml. Other studies using a variety of diets have also shown a wide range of ruminal lactic acid concentrations. Briggs et al. (1957) indicated that lambs fed a variety of diets containing different levels of concentrates and protein showed levels of ruminal lactic acid varying between 63 and 15210 µg/ml. Hungate (1966) reported pools of ruminal lactic acid ranging from 1500 to 3960 µg/ml.

There are several factors affecting lactic acid values. For example, Fulton et al. (1979), when feeding a 90% concentrate diet to steers, found that lactic acid concentration in the rumen ranged between 55.5 and 158.4 µg/ml. The measurements were taken between 2 to 4 h after feeding and average ruminal pH was 5.39. In studies

conducted by Mackie and Gilchrist (1979), the peak concentration of rumen lactic acid occurred between 30 to 60 min after feeding, with a concentration of 108.9 μg/ml of fluid when feeding a 71% concentrate to sheep. From their studies it is evident that factors such as percentage concentrate in the diet and time of sampling after feeding are factors influencing the rumen lactic acid production.

All diets fed during the present experiment contained similar levels of concentrates. However, those supplemented with protein showed a greater ruminal lactic acid concentration than the unsupplemented controls. No explanation was found in this regard, but perhaps the elevated nitrogen intake might have caused a proliferation of lactic acid producing microorganisms, thus increasing the level of lactic acid in the rumen.

Rumen Ammonia-Nitrogen. Average rumen ammonia-nitrogen concentrations as influenced by dietary treatments are presented in table 12. Results showed that urea supplementation increased ($P<.05$) the levels of ammonia-nitrogen in the rumen as compared to the other dietary treatments. Lambs receiving diets containing either source of soybean meal showed greater levels of ammonia-nitrogen in the rumen fluid than control lambs, but these differences were not significant. There were also no differences in the levels of rumen ammonia-nitrogen in animals fed the soybean meal or heat-treated soybean meal diets.

Rumen ammonia-nitrogen levels have been reported by Hungate (1966) to range from 0 to 130 mg/100 ml of fluid. A comparison of values obtained in this study with those reported by Hungate (1966) for

diets of this type indicate that the concentrations obtained herein would be considered low. Procedures and determinations used to obtain rumen ammonia-nitrogen concentrations during this study were double checked. This was done in order to insure that the low concentrations observed were not due to errors in the analytical procedures or in the chemical mixtures involved in these determinations.

Lewis et al. (1957) reported levels of 34 to 68 mg/100 ml of rumen fluid as normal ammonia levels for lambs fed a variety of diets. In later reports, Lewis (1961) noted that under relative normal conditions ruminal ammonia-nitrogen may be between 10 and 60 mg/100 ml of fluid. More recent reports have suggested a range between 10 to 45 mg of ammonia-nitrogen per 100 ml of rumen fluid as normal (Church, 1976). Ruminal ammonia-nitrogen levels being lower than normal might lead to concern for a nitrogen insufficiency in the rumen which would restrict microbial mass. Since measurements of this type were not made, the discussion of the rumen ammonia-nitrogen data will be based on the relative differences between dietary treatments.

As indicated previously, rumen ammonia-nitrogen levels increased with higher levels of nitrogen in the diet. Briggs et al. (1957), Hudson et al. (1969) and Veira et al. (1980a) indicated that ammonia-nitrogen levels in the rumen fluid were, in general, correlated with the protein content of the diet. Higher levels of protein in the diet gave greater ammonia-nitrogen levels in the rumen fluid. Veira et al. (1980a) showed that steers fed a 10.2% protein diet had 1.89 mg ammonia-nitrogen

per 100 ml of rumen fluid, while diets containing 12.2% protein resulted in rumen ammonia levels of 3.2 mg/100 ml.

The differences in rumen ammonia observed between the urea-supplemented lambs and those fed soybean meal were also found by Schmidt et al. (1973) and Pendlum et al. (1976). The researchers found that urea-supplemented steers fed high concentrate diets showed higher ($P<.01$) levels of ammonia nitrogen in the rumen than steers fed soybean meal-supplemented diets.

While there were no significant differences observed between soybean meal- and heat-treated soybean meal-fed lambs in this experiment, studies conducted by Chalmers et al. (1954), Sherrod and Tillman (1962) and Hudson et al. (1969) showed that rumen fluid ammonia-nitrogen was higher for animals consuming diets with regular soybean meal as compared to those fed diets with heat-treated soybean meal.

Blood Ammonia-Nitrogen. Average concentration of plasma ammonia-nitrogen for the different dietary treatments are presented in table 12. Protein supplementation did not appear ($P>.05$) to have any effect on the concentration of plasma ammonia-nitrogen. Lambs fed the unsupplemented control diet had similar ammonia-nitrogen concentration in the plasma as lambs consuming diets supplemented with protein. Lambs fed diets with urea (.95% of the total dry matter) had slightly higher plasma ammonia-nitrogen concentrations than lambs from other dietary treatments. This might be due to the nature of urea which is readily hydrolyzed to ammonia in the rumen (Bergen, 1979b), thus allowing more ammonia to be absorbed through the rumen wall and reaching the liver

via the portal vein. If the rate in which ammonia-nitrogen reaches the liver is greater than the rate that it is being converted into urea, peripheral blood ammonia levels increase (Lewis et al., 1957).

Under circumstances where energy and protein are normal, only small quantities of ammonia are found in the peripheral blood. Lewis et al. (1957) reported that portal blood ammonia-nitrogen ranges between .51 to .85 mg/100 ml. On the other hand, Bartley et al. (1976) stated that the concentration of ammonia-nitrogen in peripheral blood was only .10 to .15 mg/100 ml. Lewis et al. (1957) reported that the liver was able to convert all of the absorbed ammonia into urea until the level of ammonia reached a level of 1.36 mg/100 ml, which was observed with ruminal fluid ammonia-nitrogen concentration of 93.5 to 102.0 mg/100 ml.

In the study presented in this manuscript, rumen ammonia-nitrogen levels were lower than those reported by Lewis et al. (1957). Blood ammonia-nitrogen concentrations presented in this manuscript were much lower than those found by Bartley et al. (1976), but the values were similar to those reported by Schmidt et al. (1973). These researchers (Schmidt et al., 1973) indicated that blood ammonia-nitrogen levels were only slightly higher when urea was used as a nitrogen source than when soybean meal or formaldehyde-treated soybean meal were used in diets for steers.

In a symposium paper, Waldo (1968) stated that Holzschuh and Wetterau (1965) found that mg/100 ml of ammonia-nitrogen in jugular blood was equal to .06 + .00231 x (mg/100 ml ammonia-nitrogen in the rumen). By using this equation and the rumen ammonia-nitrogen data

(table 12), results for blood ammonia-nitrogen obtained in this trial would be considered within normal ranges for these lambs.

Rumen Volatile Fatty Acids. Total and individual VFA concentrations in the rumen fluid as affected by dietary treatments are presented in table 13. Values shown were from samples taken 3 h after the feeding of diets containing 92% ground ear corn and 8% supplement. Since the ear corn contained about 80% grain and 20% cob, these diets would be considered as being fairly high in concentrates.

TABLE 13. TOTAL AND INDIVIDUAL VFA DATA AS AFFECTED BY DIETARY TREATMENTS

		Moles/100 moles					
Treatment[a]	Total VFA µmol/ml	Acetic	Propionic	Butyric	Valeric	Isovaleric	C2/C3 ratio
Control	60.1[b]	28.19[b]	49.61	13.15	5.19	3.86	.57
Urea	79.3[cd]	24.04[bc]	56.07	11.98	5.92	1.99	.43
Soybean meal	89.3[d]	23.21[c]	52.40	14.73	7.90	1.77	.44
Heat-treated soybean meal	75.4[c]	23.57[c]	54.05	14.80	5.87	1.71	.44
SE	4.4	1.37	2.51	1.43	.84	1.21	.04

[a] There were six observations per value.
[b,c,d] Means within lines followed by unlike superscripts differ (P<.05).

Total VFA data obtained during this trial (table 13) showed differences (P<.05) between dietary treatments. Lambs receiving diets with supplemental protein had higher levels (P<.05) of total VFA in the rumen as compared to those fed the unsupplemented control diet. Among protein-supplemented diets, lambs receiving the diet with soybean meal

had higher total VFA levels as compared to lambs fed diets with urea or heat-treated soybean meal. Lambs fed heat-treated soybean meal-supplemented diets had the lowest concentration of total VFA in the rumen among protein supplemented groups.

Total VFA concentrations varied between 60.1 and 80.3 μmol/ml. These levels tend to be lower than many average values reported in the literature but are within the normal range given by Hungate (1966). In studies conducted by Briggs et al. (1957) when feeding diets containing about 80% concentrates, the total VFA concentration varied between 90 and 218 μmol/ml. Mackie and Gilchrist (1979) showed an average of 134 ± 14 μmol of total VFA per ml of rumen fluid when feeding sheep diets containing about 71% concentrates.

Discrepancies in the total VFA concentrations between data reported herein and those found in the literature may be due to factors such as analytical methods utilized, sampling methods and dietary treatments which are known to affect rumen VFA values (Church, 1976). Level of feed intake, physical form, level of concentrate and type of fiber could also be other factors involved. Rumen pH is another factor which has a major influence in rumen fermentation. Briggs et al. (1957) carried out extensive investigations with sheep fed several diets with various levels of concentrates and roughages. Their results as to pH and total VFA compare favorably with those presented in this manuscript and indicated that, as rumen pH declined, the total VFA production increased.

The response to protein supplementation observed during this study as measured by total VFA concentration in the rumen fluid has been reported by several researchers. Slyter et al. (1979) and Haaland et al. (1982) showed an increased level of total VFA production in the rumen with protein supplementation. On the other hand, no differences in total rumen VFA concentration were observed between steers fed the control diet and those receiving soybean meal-supplemented diets (Veira et al., 1980a). Davis and Stallcup (1967) reported greater total VFA concentrations in the rumen of soybean meal-supplemented animals than in those consuming a urea-supplemented diet. Similar trends were also reported by Cross et al. (1974) while feeding soybean meal- or urea-supplemented diets to steers. Ahrar and Schingoethe (1979) did not find any differences in total VFA concentration in the rumen due to heat treating of soybean meal, which was contrary to the results obtained in the present study.

Average molar percentages of the individual VFA are shown in table 13. Evaluation of these molar concentrations expressed as a percentage of the total rumen VFA indicated that, for all diets (about 80% concentrates), propionic acid was the most abundant VFA produced in the rumen of these lambs followed by acetic, butyric, valeric and iso-valeric acids. The concentration of propionic acid was at least two times greater than that of acetic acid for all treatments with the exception of the control group which was about 1.8 times greater. In most studies found in the literature in which ruminants were fed diets containing similar levels of concentrate, acetic acid concentrations

were higher than propionic acid concentrations. In cattle studies conducted by Ghorban et al. (1966), Cross et al. (1974) and Fulton et al. (1979), molar percentage acetic acid was greater than propionic acid in the rumen of animals fed high concentrate diets. Similar results were observed by Hudson et al. (1969) and Mackie and Gilchrist (1979) in studies conducted with sheep.

In other studies, Oltjen and Davis (1965) reported similar molar percentage concentrations for acetic and propionic acid, while Haskins et al. (1967) when feeding all-concentrate diets to steers observed greater molar percentages of propionic acid than acetic acid. However, the differences in molar percentage concentration between acetic and propionic acids were not as great as those observed during the study presented here. Data presented by Reid et al. (1957), Raun et al. (1962) and Woods and Luther (1962) indicated an increased molar percentage propionic acid with increasing levels of concentrate in the diet, but their levels were not as high as those used in this study.

The higher levels of propionic acid obtained here as compared to those found in the literature when feeding diets with similar levels of concentrate may be due in part to the supplementation of monensin sodium. Each diet contained about 33 mg of monensin sodium per kilogram of feed. These results agree with those of Dinius et al. (1976), which indicated a substantial decrease in the proportion of acetic acid and an increase in molar percentage of propionic acid when supplementing steers with monensin.

During this study, only acetic acid showed significant differences ($P<.05$) between dietary treatments. Protein-supplemented lambs had lower molar percentage concentrations of acetic acid in the rumen fluid than lambs fed the unsupplemented control diet, with lowest values ($P<.05$) for either source of soybean meal. Molar percentage propionic acid was greater for all protein-supplemented groups than the control group, but differences were not significant ($P>.05$). Butyric, valeric and isolvaleric acids showed slight differences between treatment groups with no apparent important tendencies during this study. However, it is important to note that valeric and isovaleric acids combined comprised between 7.58 and 9.67% of the total molar percentage. Head (1961) reported that valeric and isovaleric acids are derived from deamination of the amino acids valine, leucine and isoleucine during increased rumen fermentative activity. Lambs receiving the control diet had a slightly wider acetic-propionic acid ratio than those on the supplemented diets, but these differences were not statistically significant.

Data available in the literature show variable response to protein supplementation and heat treatment of soybean meal in terms of individual VFA molar concentrations. For example, Hudson et al. (1969) and Veira et al. (1980a) noted that rumen fluid VFA molar proportions were not affected by protein supplementation, while Slyter et al. (1979) showed a significant increase in butyric acid molar percentage concentration with protein supplementation. However, other findings such as the lack of significant differences in individual VFA molar proportions

between urea and soybean meal fed diets were in accordance with results reported by Oltjen and Davis (1965).

On the other hand, Glimp et al. (1967) stated that heat treating soybean meal contributed to a reduction in the levels of isovaleric and valeric acids in the rumen 3 h after feeding as compared to regular soybean meal fed diets. In similar studies, Hudson et al. (1969) reported that heat treatment of soybean meal did not affect the molar percentages of any of the individual VFA when fed to lambs in diets containing about 80% concentrates. Contrary to these results, Wohlt et al. (1976) found that highly soluble sources of protein such as soybean meal in the rumen decreased the molar percentage of propionate and increased butyrate concentrations when evaluating low soluble versus high soluble protein sources fed to lambs.

No data were found showing the effect of protein supplementation on acetic acid. However, as indicated by Church (1976), changes in chemical composition of the diet usually result in marked changes in protozoal and bacterial population, thus influencing rumen fermentation. Since conditions such as time of sampling, rumen pH, frequency of feeding, physical form of the diet, level of feeding, ionophores and feed processing methods among others used in the experiments reported in the literature usually are not constant, therefore, contradictory and different results are frequently obtained regarding individual rumen VFA molar proportions.

Hourly Rumen and Blood Parameter Determinations

The effect of sources of supplemental protein and time after feeding on rumen fermentation and blood ammonia-nitrogen were studied during this trial. Two lambs from each dietary treatment group were used in this study. The objective was to determine if the sources of supplemental protein were fermented at different rates, thus causing some differences in the utilization of nitrogen.

Ample time was allowed for the lambs to adapt to the test diets before starting sample collections. Early in the morning, blood and rumen samples were taken before feeding. These samples were taken at about 24 h after the last feeding. Thereafter, lambs were fed and sampled for rumen fluid at 1-h intervals up to 5 h. Blood samples were taken at 6, 8 and 10 h postfeeding.

On the days in which this study was conducted, lambs fed the control, urea, soybean meal and heat-treated soybean meal-supplemented diets were consuming an average of 1218, 1082, 1221 and 1340 g/d of dry matter, respectively, which gave an average daily nitrogen intake of 18.75, 21.73, 22.90 and 26.74 g, respectively. Data obtained for the rumen fluid parameters were analyzed statistically as a 4 x 6 factorial, while blood ammonia-nitrogen data were analyzed as a 4 x 4 with time and dietary treatments as the main effects.

Rumen pH. Rumen pH values for each dietary treatment determined at hourly intervals are presented in table 14 and graphically in figure 1. The data (table 14) show that rumen pH when averaged across dietary treatments decreased ($P<.05$) up to the 2-h sampling period

TABLE 14. RUMEN FLUID PARAMETERS AS AFFECTED BY SOURCES OF PROTEIN SUPPLEMENTATION AND TIME AFTER FEEDING[a]

Item	Treatment	Sampling time, h 0	1	2	3	4	5	Avg
pH	1	6.85	6.35	6.18	6.02	5.72	5.75	6.14
	2	7.26	5.86	5.09	5.36	5.62	5.59	5.80
	3	7.30	6.27	5.71	5.58	5.70	5.87	6.07
	4	6.69	5.64	5.47	5.59	5.80	5.89	5.84
	Avg	7.02[b]	6.03[c]	5.61[d]	5.64[cd]	5.71[cd]	5.77[dc]	
Ammonia-nitrogen, mg/100 ml	1	.28	.60	.56	.20	.60	.56	.46[b]
	2	3.21	6.86	3.56	8.99	12.36	5.33	6.72[c]
	3	2.18	1.23	.64	1.35	2.40	2.06	1.64[b]
	4	2.43	.87	1.43	1.11	.53	.63	1.16[b]
	Avg	2.02	2.39	1.55	2.91	3.97	2.14	
Lactic acid, µg/ml	1	6.63	224.24	66.83	57.44	145.26	120.41	103.46[b]
	2	4.97	871.20	3672.98	5.41	383.31	9.39	824.54[bc]
	3	10.50	1526.01	3185.20	4578.13	4431.88	2520.52	2708.71[c]
	4	662.24	3152.22	3589.80	1643.61	36.45	22.09	1517.74[bc]
	Avg	171.08	1443.42	2628.70	1571.15	1249.22	668.10	
Total VFA, µmol/ml	1	37.81	55.92	61.80	78.10	95.53	97.35	71.08[b]
	2	21.51	59.76	68.37	80.59	84.67	91.46	67.72[b]
	3	17.66	52.07	55.65	54.56	54.54	69.27	50.29[c]
	4	41.43	70.86	74.48	86.70	80.82	80.36	72.44[b]
	Avg	29.60[b]	59.65[c]	64.57[cd]	74.99[de]	78.89[e]	84.61[e]	

TABLE 14 CONTINUED

Item	Treatment	Sampling time, h 0	1	2	3	4	5	Avg
Acetic acid, moles/100 moles	1	28.00	26.44	27.07	25.50	26.08	26.62	26.58^{bc}
	2	26.68	25.32	24.82	24.13	23.05	23.99	24.66^{b}
	3	37.07	34.14	29.22	29.76	27.58	24.58	30.39^{c}
	4	26.17	21.66	23.60	23.85	20.62	24.60	23.41^{b}
	Avg	29.48	26.84	26.17	25.81	24.33	24.95	
Propionic acid, moles/100 moles	1	44.06	46.17	47.08	46.31	47.51	44.10	45.87^{b}
	2	49.77	56.07	56.31	56.09	59.87	58.86	56.16^{d}
	3	40.23	45.75	47.07	51.53	52.81	54.40	48.63^{b}
	4	45.21	55.27	56.06	53.84	51.67	48.38	51.74^{c}
	Avg	44.82^{b}	50.81^{c}	51.63^{c}	51.94^{c}	52.96^{c}	51.43^{c}	
Butyric acid, moles/100 moles	1	14.91	17.33	16.88	15.94	16.46	17.98	16.58^{b}
	2	11.76	9.97	12.23	13.19	10.54	10.24	11.32^{d}
	3	9.40	12.54	15.82	11.97	12.75	12.99	12.58^{cd}
	4	14.93	12.52	12.36	12.74	14.50	15.11	13.69^{c}
	Avg	12.75	13.09	14.32	13.46	13.56	14.08	
Valeric acid, moles/100 moles	1	6.85	7.94	7.41	8.48	9.96	9.36	8.33^{bc}
	2	4.81	5.27	6.64	6.60	6.56	6.91	6.13^{d}
	3	5.35	7.57	6.11	6.75	6.87	8.05	6.78^{cd}
	4	6.75	8.71	8.00	9.59	13.22	11.92	9.70^{b}
	Avg	5.49^{b}	7.37^{bc}	7.04^{bc}	7.85^{bc}	9.15^{c}	9.06^{c}	
Isovaleric acid, moles/100 moles	1	6.19	2.32	1.57	3.78		1.95	2.63
	2	6.99	3.38					1.73
	3	7.95		1.80				1.62
	4	6.94	1.86					1.47
	Avg	7.02^{b}	1.89^{c}	.84^{cd}	.94^{cd}	d	.49^{d}	

TABLE 14 CONTINUED

Item	Treatment	Sampling time, h 0	1	2	3	4	5	Avg
Acetic/propionic	1	.68	.58	.58	.55	.56	.61	.59[b]
	2	.54	.45	.44	.43	.39	.41	.44[c]
	3	.92	.75	.62	.58	.52	.45	.64[b]
	4	.58	.39	.42	.44	.40	.51	.46[c]
	Avg	.68[b]	.55[bc]	.52[bc]	.50[c]	.47[c]	.49[c]	

[a] There were four observations for each value shown.
[b,c,d,e] Means followed by unlike superscripts differ ($P<.05$).

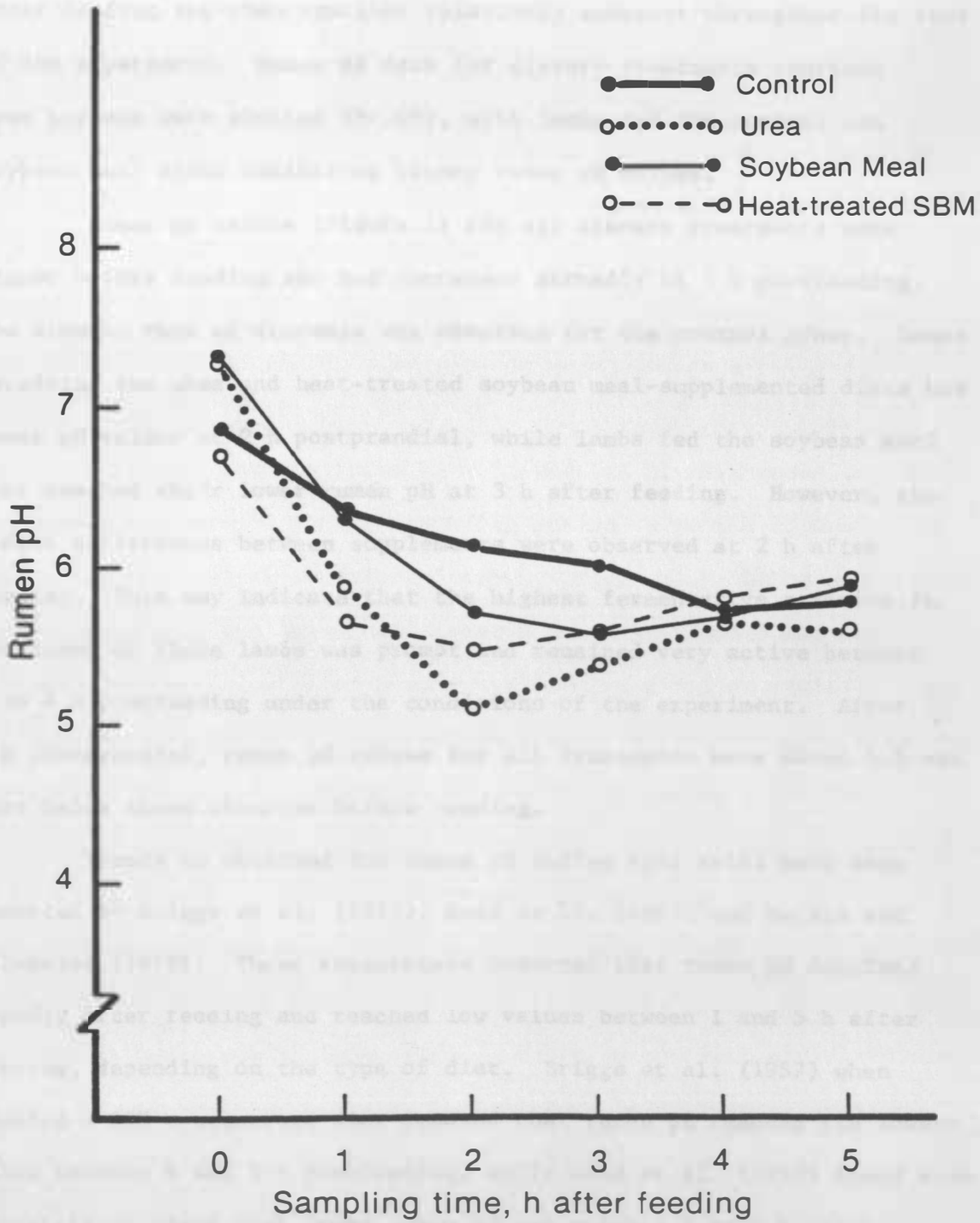

Figure 1. Rumen pH for lambs fed various sources of supplemental protein with ear corn diets.

after feeding and then remained relatively constant throughout the rest of the experiment. Rumen pH data for dietary treatments averaged over periods were similar ($P > .05$), with lambs fed the control and soybean meal diets exhibiting higher rumen pH values.

Rumen pH values (figure 1) for all dietary treatments were higher before feeding and had decreased markedly at 1 h postfeeding. The slowest rate of decrease was observed for the control group. Lambs receiving the urea and heat-treated soybean meal-supplemented diets had lower pH values at 2 h postprandial, while lambs fed the soybean meal diet reached their lower rumen pH at 3 h after feeding. However, the widest differences between supplements were observed at 2 h after feeding. This may indicate that the highest fermentative activity in the rumen of these lambs was prompt and remained very active between 2 to 4 h postfeeding under the conditions of the experiment. After 5 h postprandial, rumen pH values for all treatments were about 5.5 and were below those observed before feeding.

Trends as obtained for rumen pH during this trial have been reported by Briggs et al. (1957), Reid et al. (1957) and Mackie and Gilchrist (1979). These researchers observed that rumen pH declined rapidly after feeding and reached low values between 1 and 5 h after feeding, depending on the type of diet. Briggs et al. (1957) when feeding a 65% concentrate diet denoted that rumen pH reached its lowest value between 4 and 5 h postfeeding, while Reid et al. (1957) found with a variety of diets that lower rumen pH was reached 1 to 4 h after feeding. Mackie and Gilchrist (1979), on the other hand, reported the

lowest rumen pH level was reached in 2 h following feeding of a 71% concentrate diet to sheep.

Rumen Ammonia-Nitrogen. Mean rumen ammonia-nitrogen concentrations for the dietary treatments (table 14 and figure 2) showed that lambs consuming the urea diet had a higher ($P < .05$) level of ammonia in the rumen than lambs consuming the other diets. Averages over dietary treatments for time periods were similar ($P > .05$) at the various intervals.

Lambs fed the diet with urea showed the highest rumen ammonia-nitrogen concentration at each sampling period, with the highest concentration obtained at 4 h after feeding (figure 2). The unsupplemented control group, on the other hand, showed the lowest rumen ammonia-nitrogen concentration throughout the different sampling periods. The two soybean meal diets followed similar patterns with no particular trend observed for these diets at the different sampling periods.

The concentration of ammonia-nitrogen in the rumen over a period of time has been reported to vary, depending upon the diet fed. Higher levels of ammonia-nitrogen in the rumen of lambs fed diets with urea such as those observed during the study presented herein have also been reported by Davis and Stallcup (1967), McIntyre and Williams (1970) and Schmidt et al. (1973). However, these researchers reported peak concentrations from 30 min postprandial (Schmidt et al., 1973) to about 4 h after feeding (McIntyre and Williams, 1970) for cattle and sheep, respectively, fed a variety of protein supplements. Higher concentrations of rumen ammonia-nitrogen due to protein supplementation were

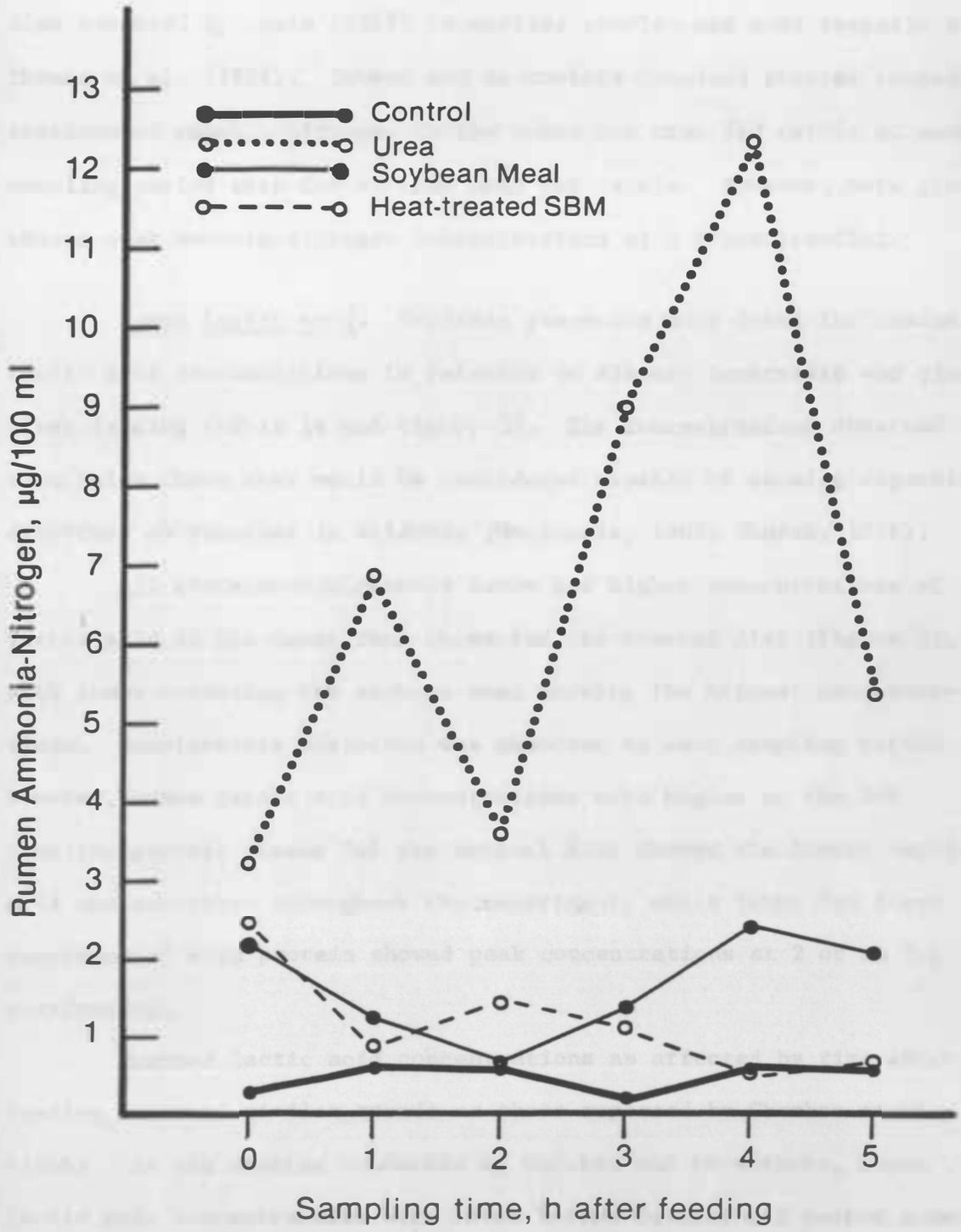

Figure 2. Rumen ammonia-nitrogen for lambs fed various sources of supplemental protein with ear corn diets.

also reported by Lewis (1957) in earlier studies and most recently by Thomas et al. (1984). Thomas and co-workers obtained greater concentrations of ammonia-nitrogen in the rumen for urea fed cattle at each sampling period than for soybean meal fed cattle. However, both groups showed peak ammonia-nitrogen concentrations at 3 h postprandial.

Rumen Lactic Acid. Variable responses were found for ruminal lactic acid concentrations in relation to dietary treatments and time after feeding (table 14 and figure 3). The concentrations observed were below those that would be considered capable of causing digestive disorders as reported in acidosis (Mackenzie, 1967; Church, 1976).

All protein-supplemented lambs had higher concentrations of lactic acid in the rumen than those fed the control diet (figure 3), with lambs receiving the soybean meal showing the highest concentrations. Considerable variation was observed at each sampling period. However, rumen lactic acid concentrations were higher at the 2-h sampling period. Lambs fed the control diet showed the lowest lactic acid concentration throughout the experiment, while lambs fed diets supplemented with protein showed peak concentrations at 2 or at 3 h postfeeding.

Ruminal lactic acid concentrations as affected by time after feeding followed similar trends as those reported by Ghorban et al. (1966). In the studies conducted by Ghorban and co-workers, rumen lactic acid concentrations were lower before feeding and peaked somewhere between 30 and 100 min postfeeding depending on the diet. Animals receiving diets with higher levels of concentrate showed peak

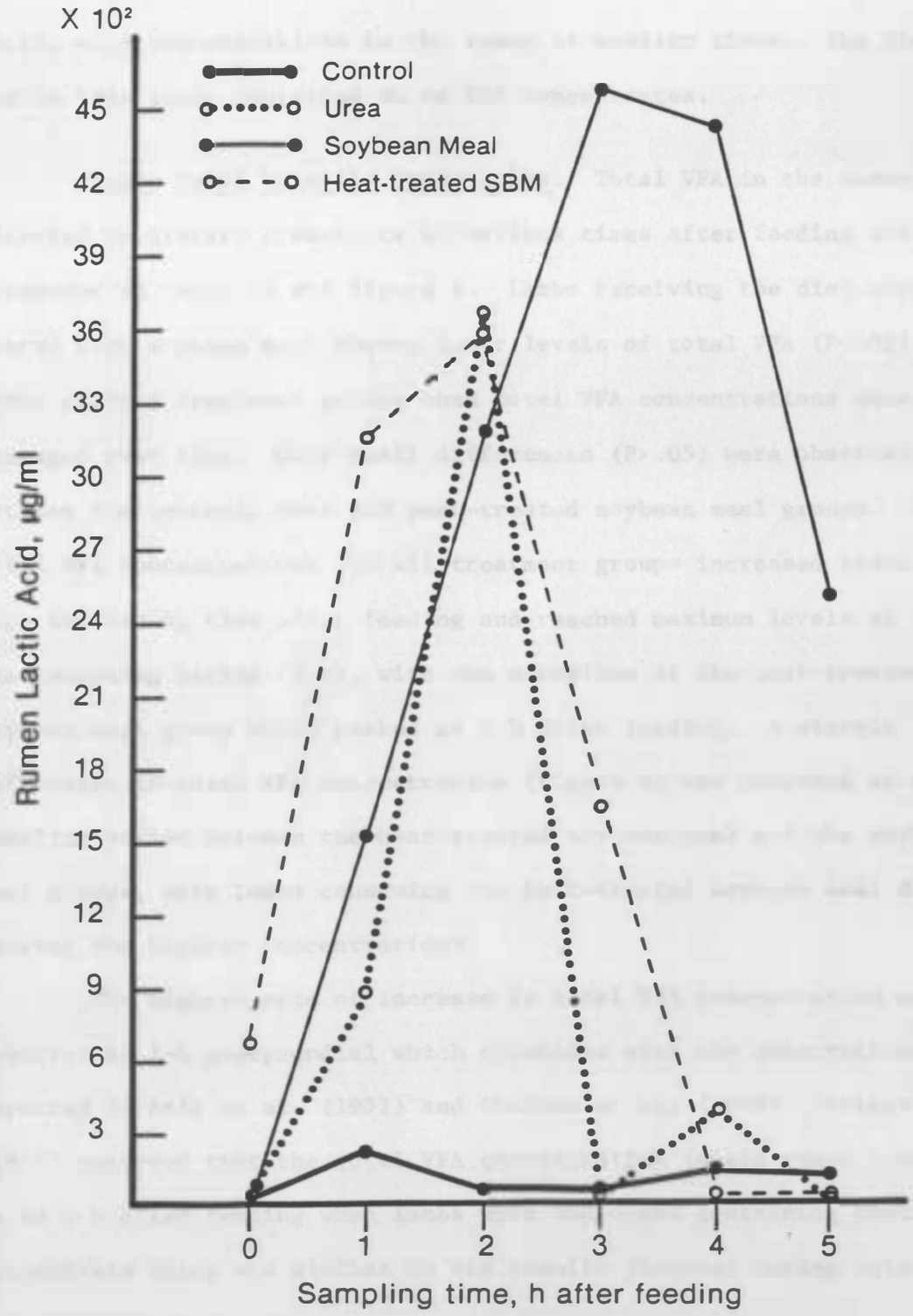

Figure 3. Rumen lactic acid for lambs fed various sources of supplemental protein with ear corn diets.

lactic acid concentrations in the rumen at earlier times. The diets fed in this study contained 40 to 80% concentrates.

Rumen Total Volatile Fatty Acids. Total VFA in the rumen as affected by dietary treatments at various times after feeding are presented in table 14 and figure 4. Lambs receiving the diet supplemented with soybean meal showed lower levels of total VFA ($P<.05$) than other dietary treatment groups when total VFA concentrations were averaged over time. Only small differences ($P>.05$) were observed between the control, urea and heat-treated soybean meal groups. Rumen total VFA concentrations for all treatment groups increased steadily with increasing time after feeding and reached maximum levels at the last sampling period (5 h), with the exception of the heat-treated soybean meal group which peaked at 3 h after feeding. A sizable difference in total VFA concentration (figure 4) was observed at each sampling period between the heat-treated soybean meal and the soybean meal groups, with lambs consuming the heat-treated soybean meal diet showing the highest concentrations.

The highest rate of increase in total VFA concentration was observed at 1-h postprandial which coincided with the observations reported by Reid et al. (1957) and Ghorban et al. (1966). Briggs et al. (1957) observed that the total VFA concentration in the rumen increased up to 5 h after feeding when lambs were fed diets containing about 65% concentrate which was similar to the results observed during this study.

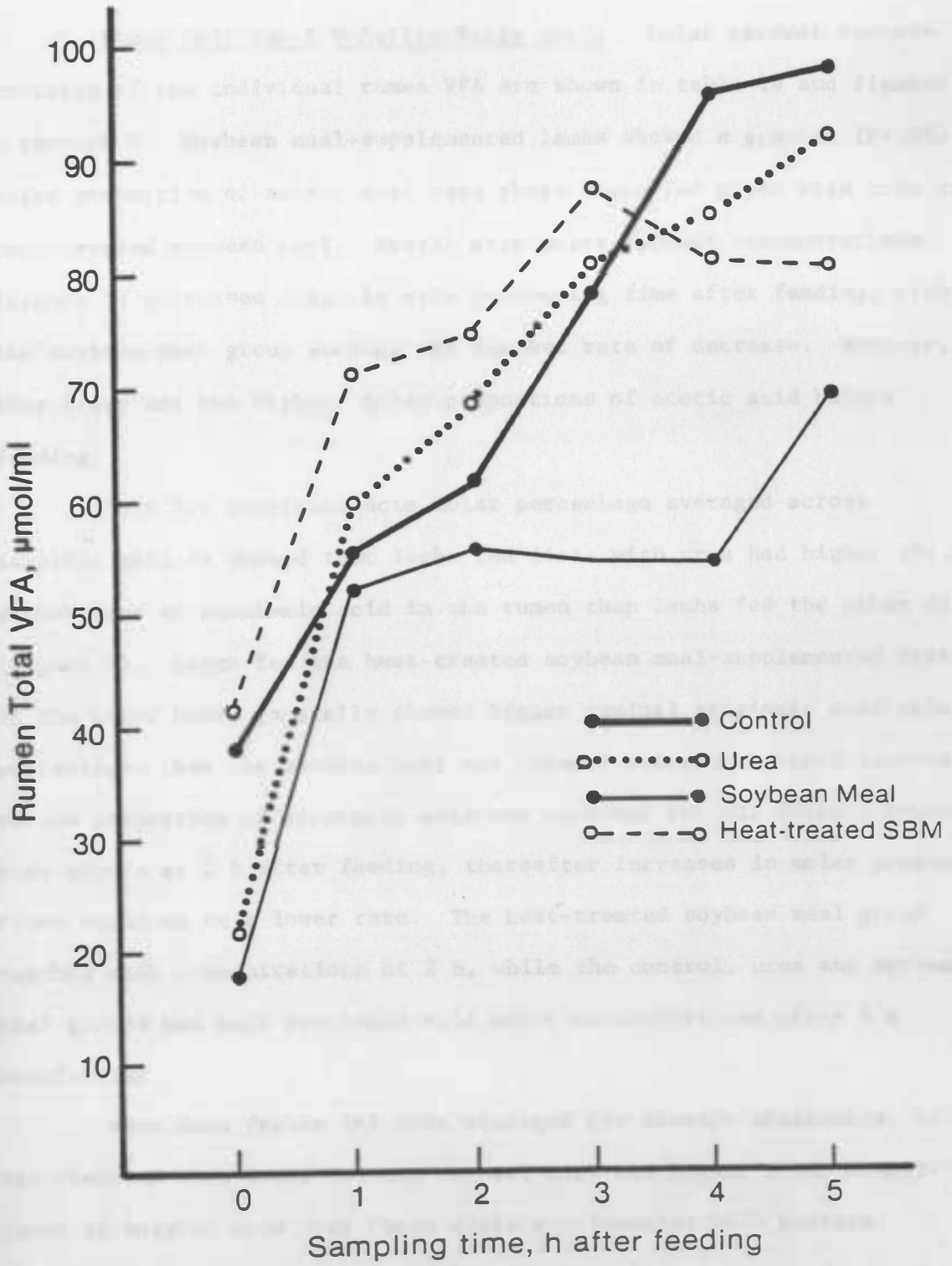

Figure 4. Rumen total VFA for lambs fed various sources of supplemental protein with earn corn diets.

Rumen Individual Volatile Fatty Acids. Molar percent concentrations of the individual rumen VFA are shown in table 14 and figures 5 through 9. Soybean meal-supplemented lambs showed a greater ($P<.05$) molar proportion of acetic acid than those lambs fed diets with urea or heat-treated soybean meal. Acetic acid molar percent concentrations (figure 5) decreased slightly with increasing time after feeding, with the soybean meal group showing the fastest rate of decrease. However, this group had the highest molar proportions of acetic acid before feeding.

Data for propionic acid molar percentage averaged across sampling periods showed that lambs fed diets with urea had higher ($P<.05$) proportions of propionic acid in the rumen than lambs fed the other diets (figure 6). Lambs fed the heat-treated soybean meal-supplemented diet, on the other hand, generally showed higher ruminal propionic acid molar percentages than the soybean meal and control groups. A rapid increase in the proportion of propionic acid was observed for all dietary treatment groups at 1 h after feeding, thereafter increases in molar proportions occurred at a lower rate. The heat-treated soybean meal group reached peak concentrations at 2 h, while the control, urea and soybean meal groups had peak propionic acid molar concentrations after 4 h postfeeding.

When data (table 14) were averaged for dietary treatments, it was observed that lambs fed the control diet had higher molar proportions of butyric acid than those diets supplemented with protein. Variable responses were observed for the protein-supplemented groups

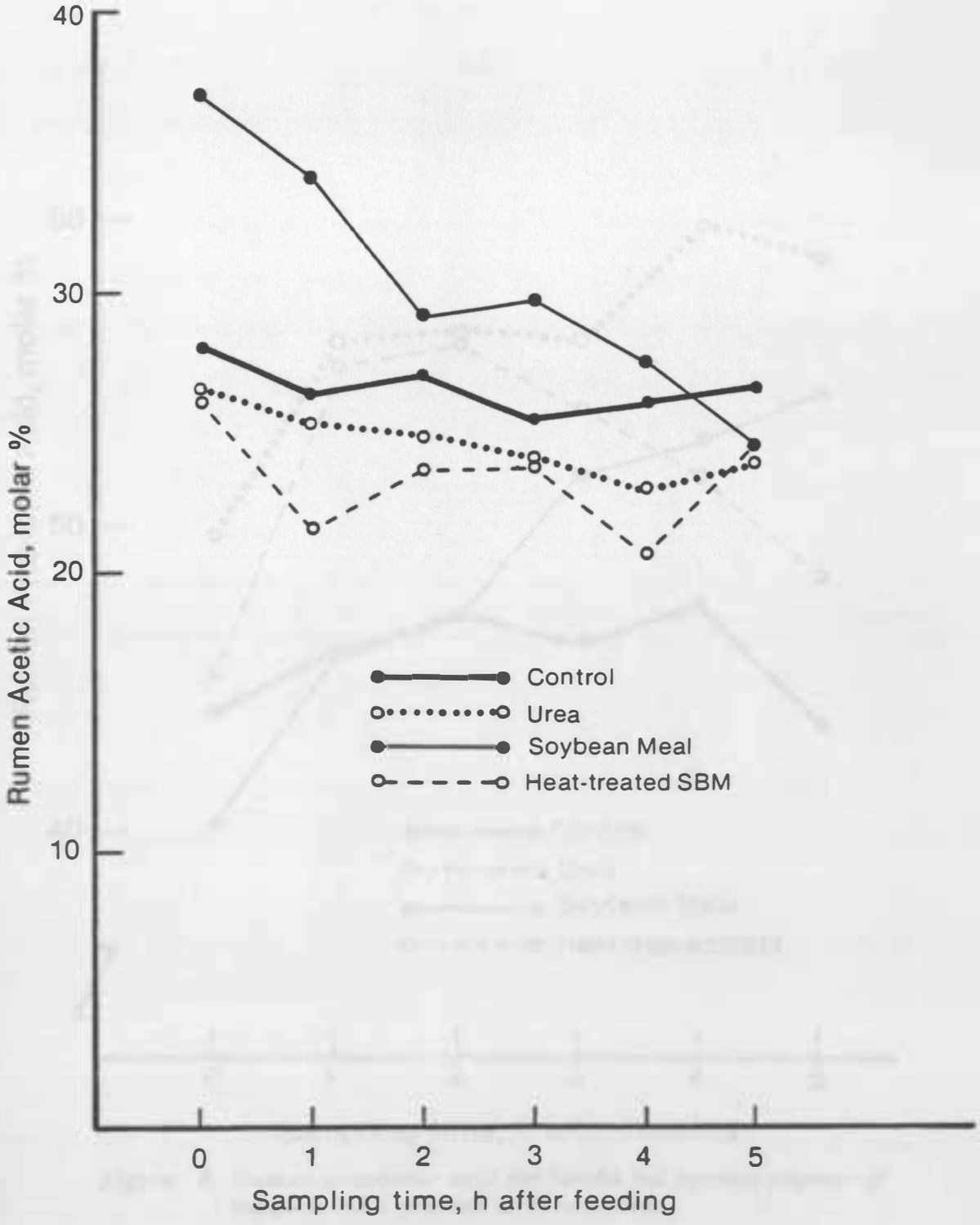

Figure 5. Rumen acetic acid for lambs fed various sources of supplemental protein with ear corn diets.

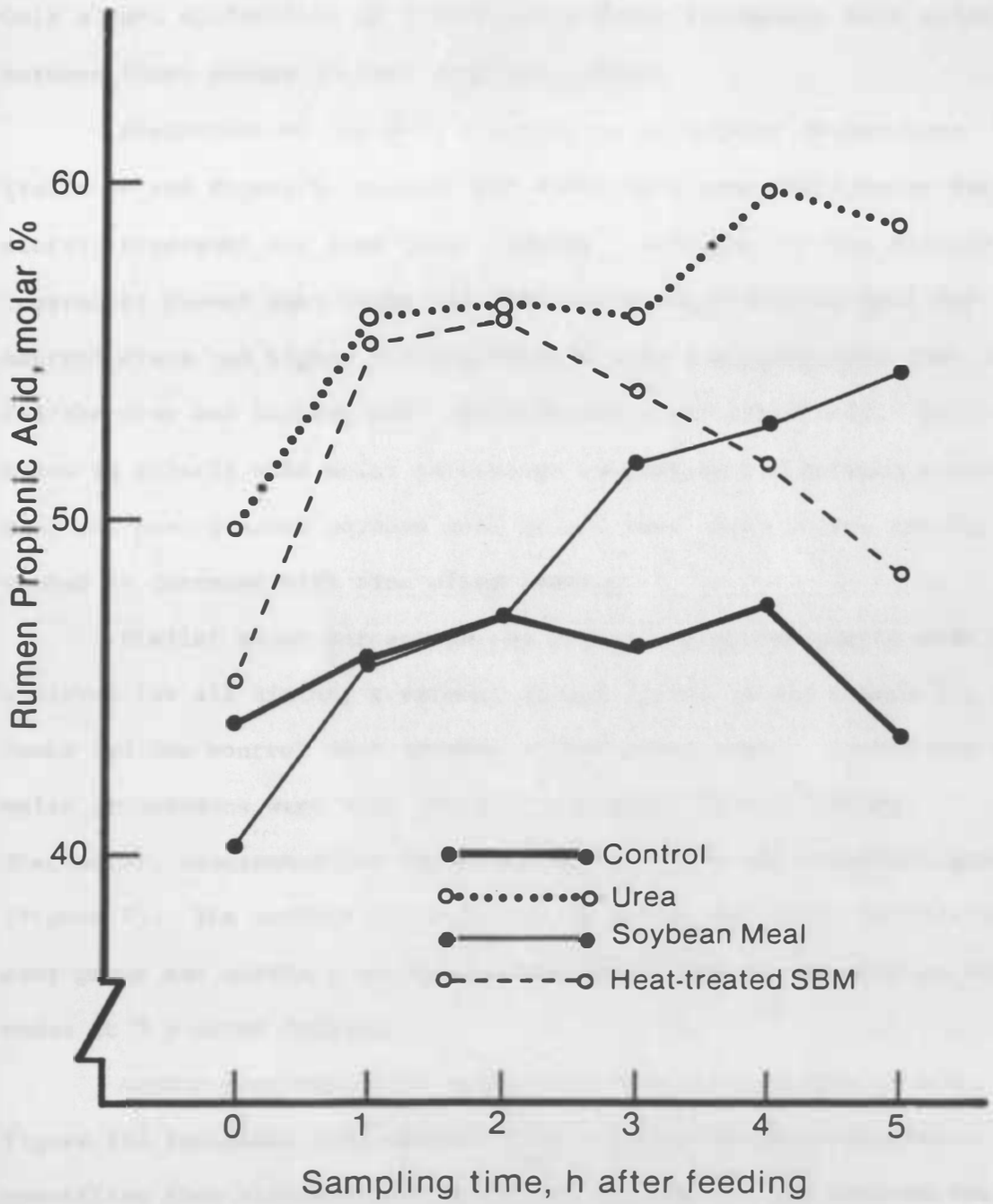

Figure 6. Rumen propionic acid for lambs fed various sources of supplemental protein with ear corn.

(figure 7) with no consistent trends observed as time periods advanced. Only slight differences in butyric acid molar percentage were observed between these groups at each sampling period.

Evaluation of the data for valeric acid molar proportions (table 14 and figure 8) showed that there were some differences due to dietary treatment and time after feeding. Averages for the dietary treatments showed that lambs fed the heat-treated soybean meal and control diets had higher ruminal valeric acid concentrations than lambs fed the urea and soybean meal-supplemented diets (figure 8). Differences in valeric acid molar percentage concentrations between soybean meal and heat-treated soybean meal groups were small before feeding but tended to increase with time after feeding.

Similar molar percentage concentrations of isovaleric acid were observed for all dietary treatment groups (table 14 and figure 9), with lambs fed the control diet showing higher proportions. Isovaleric acid molar proportions were high for all treatments before feeding. Thereafter, concentrations decreased markedly for all treatment groups (figure 9). The control group lagged more than any other dietary treatment group and showed a greater proportion of isovaleric acid in the rumen at 5 h after feeding.

Acetic-propionic acid ratios for this trial (table 14 and figure 10) indicated that propionic acid was produced in greater quantities than acetic acid. Lambs fed the control and soybean meal diets showed higher propionic-acetic acid ratios than lambs fed the urea or heat-treated soybean meal diets. A prompt decline in the

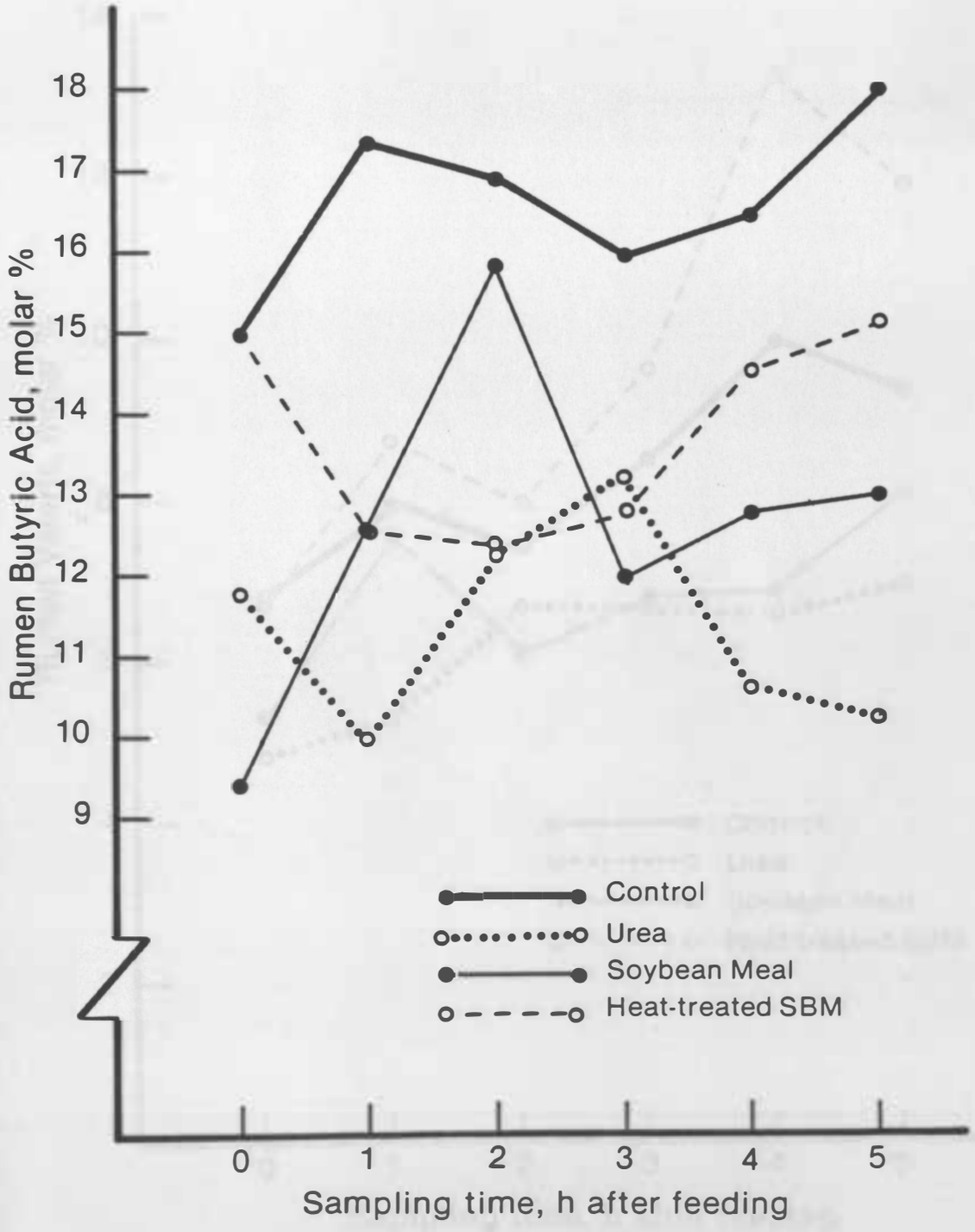

Figure 7. Rumen butyric acid for lambs fed various sources of supplemental protein with ear corn diets.

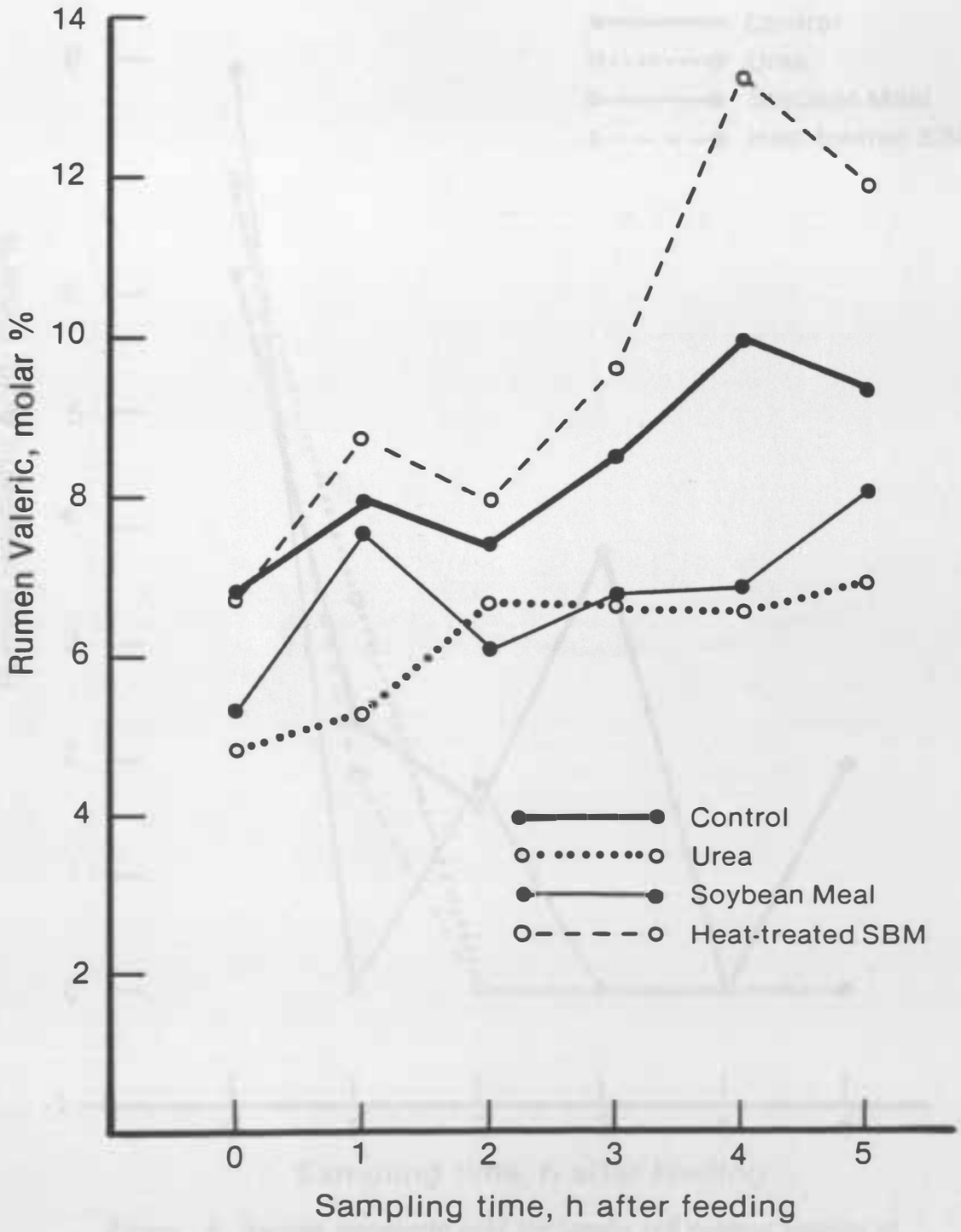

Figure 8. Rumen valeric acid for lambs fed various sources of supplemental protein with ear corn diets.

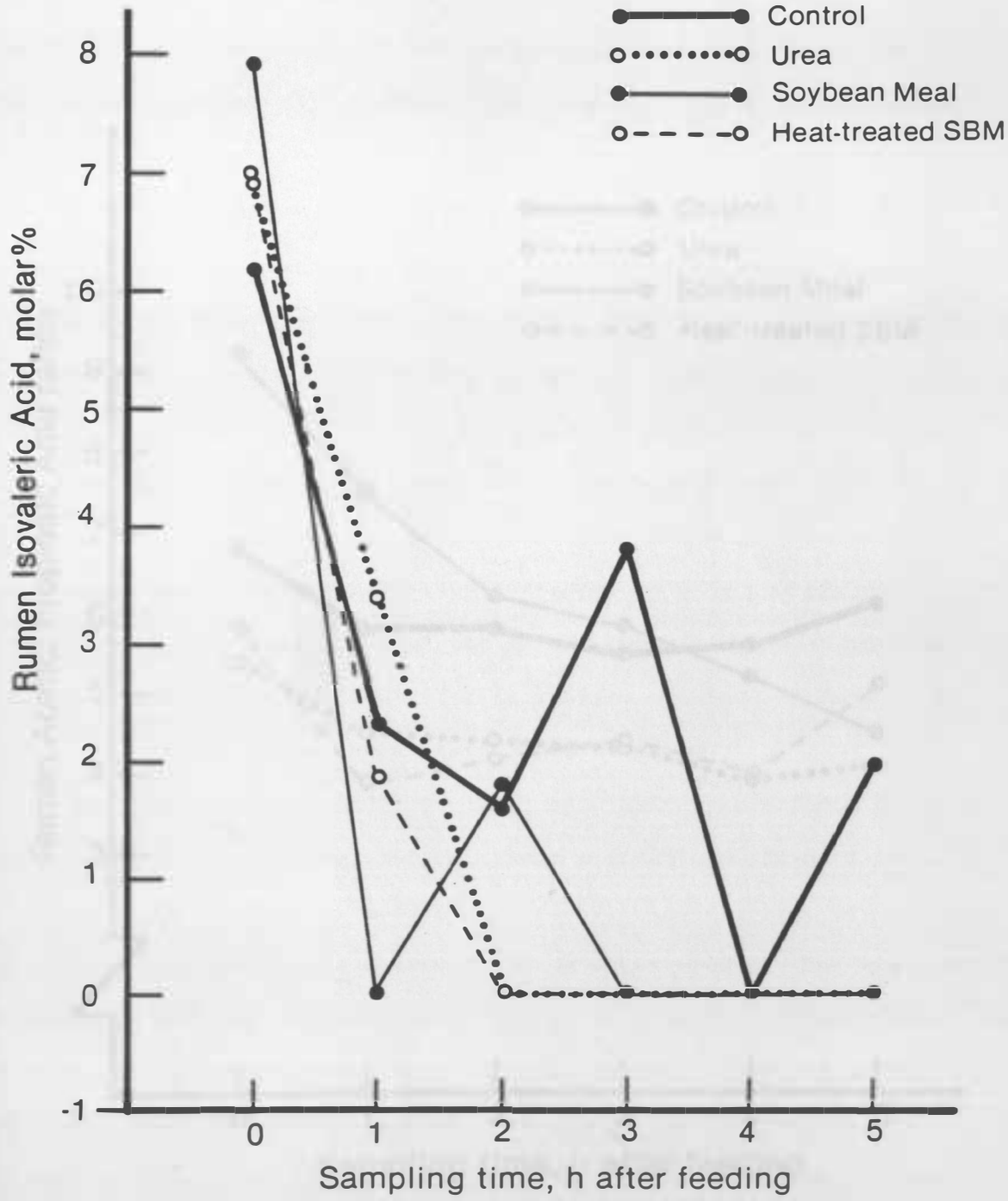

Figure 9. Rumen isovaleric acid for lambs fed various sources of supplemental protein with ear corn diets.

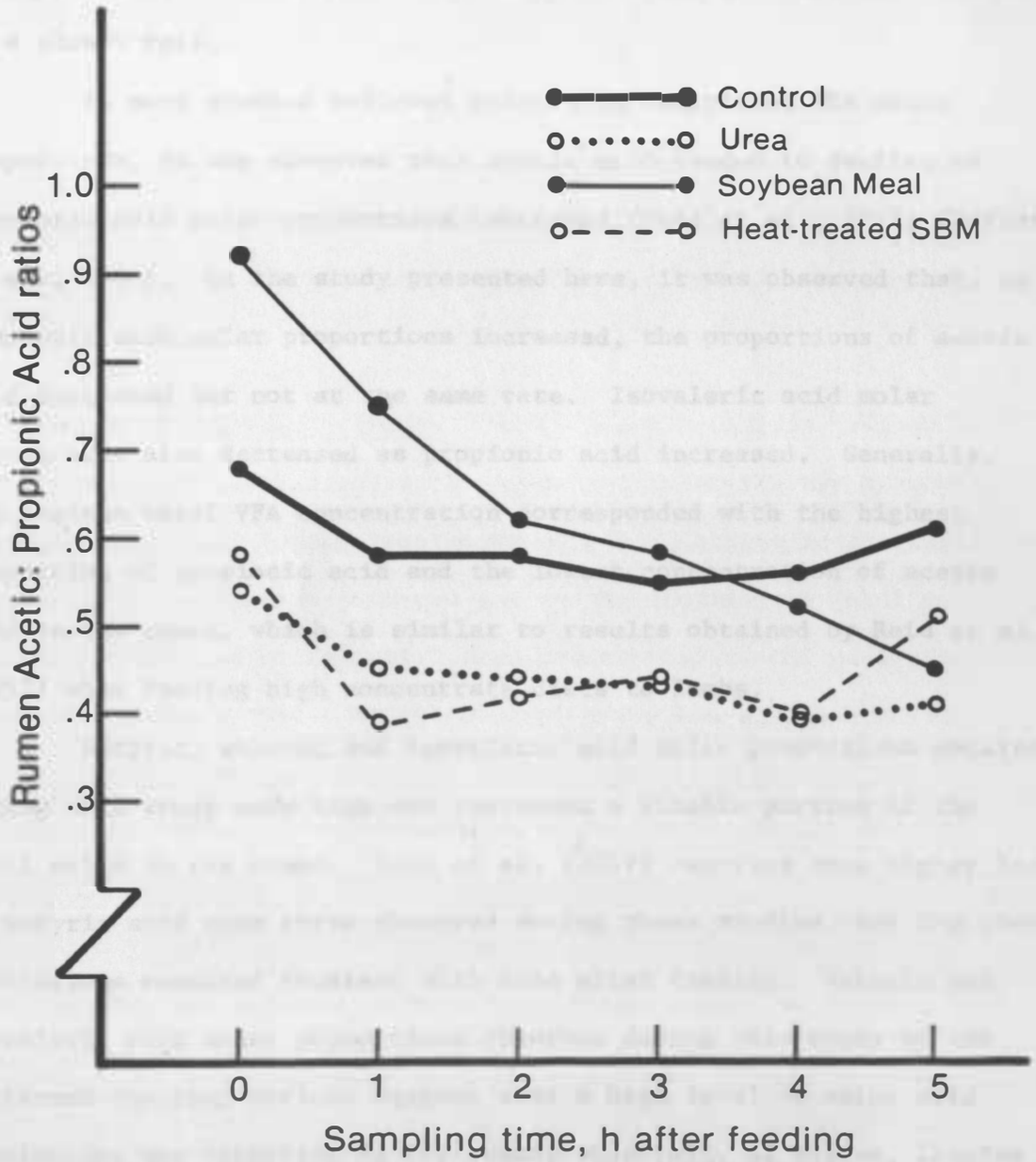

Figure 10. Rumen acetic: propionic acid ratios for lambs fed various sources of supplemental protein with ear corn diets.

acetic-propionic acid ratios was observed at 1 h after feeding, with lambs fed the soybean meal or heat-treated soybean meal diets showing the sharpest decline. Thereafter, the rate of decrease continued but at a slower rate.

In most studies reviewed concerning individual VFA molar proportions, it was observed that acetic acid tended to decline as propionic acid molar proportions increased (Reid et al., 1957; Ghorban et al., 1966). In the study presented here, it was observed that, as propionic acid molar proportions increased, the proportions of acetic acid decreased but not at the same rate. Isovaleric acid molar percentages also decreased as propionic acid increased. Generally, the maximum total VFA concentration corresponded with the highest proportion of propionic acid and the lowest concentration of acetic acid in the rumen, which is similar to results obtained by Reid et al. (1957) when feeding high concentrate diets to lambs.

Butyric, valeric and isovaleric acid molar proportions obtained during this study were high and represent a sizable portion of the total acids in the rumen. Reid et al. (1957) reported even higher levels of butyric acid than those observed during these studies, but the concentrations remained constant with time after feeding. Valeric and isovaleric acid molar proportions observed during this study at the different sampling periods suggest that a high level of amino acid deamination was occurring in the rumen, especially of valine, leucine and isoleucine (Head, 1961), resulting in an increase in the levels of these acids in the rumen. In other studies conducted by Ghorban et al.

(1966), no production of valeric and isovaleric was detected in the rumen fluid when feeding high levels of concentrates.

Data reported by Reid et al. (1957) and Ghorban et al. (1966) indicated that acetic-propionic acid ratios declined as time after feeding advanced. However, the ratios reported by these researchers were higher than those observed during the present study. Factors such as level of carbohydrates in the diet as well as the addition of monensin may have contributed to the higher propionic acid molar percentage concentrations observed during this study.

Blood Ammonia-Nitrogen. Plasma ammonia-nitrogen values as influenced by dietary treatments at various intervals are presented in table 15. All dietary treatments showed a decline in the level of plasma ammonia-nitrogen from the prefeeding sampling to the first sampling at 6 h after feeding. Mean concentrations for each dietary treatment group showed that the control group had a higher level of plasma ammonia-nitrogen than all other dietary treatments. No consistent trends were observed with time after feeding. However, concentrations were lower at 10 h after feeding.

The plasma ammonia-nitrogen data obtained at the various sampling periods indicate that perhaps blood samples were taken long after plasma ammonia-nitrogen changes occurred and that levels of nitrogen fed during this trial were not conducive to affect significantly blood concentrations. These suggestions are supported by the fact that the results obtained at the various postfeeding sampling periods were lower than those observed during the prefeeding collection.

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TABLE 15. PLASMA AMMONIA-NITROGEN AS AFFECTED BY SOURCES OF SUPPLEMENTAL PROTEIN AND TIME AFTER FEEDING[a]

Item	Treatment[b]	Sampling time, h 0	6	8	10	Avg
Plasma NH_3-N, μg/100 ml	1	146	91	182	92	128[c]
	2	106	99	82	46	83[d]
	3	104	80	94	92	93[cd]
	4	133	88	102	80	101[cd]
Avg		122[c]	90[cd]	115[cd]	78[c]	

[a] There were four observations for each value shown.
[b] 1 = control, 2 = urea, 3 = soybean meal and 4 = heat-treated soybean meal.
[c,d] Means followed by unlike superscripts differ ($P<.05$).

Rumen ammonia-nitrogen levels observed during this study were lower than those reported as normal by Lewis et al. (1957), which may also result in slightly lower blood ammonia-nitrogen concentrations. Work conducted by Webb et al. (1972) and Crickenberger et al. (1977) showed peak plasma ammonia-nitrogen concentrations at 1 h after urea was ruminally infused to cattle, supporting the suggestion that perhaps peak plasma ammonia-nitrogen levels were reached before postfeeding sampling periods.

Experiment Two

This experiment included a feeding trial and a digestion-nitrogen balance trial. The feeding trial was conducted over 105 d with growing cattle (236.7 kg) fed a diet consisting of 90% corn silage and 10% supplement (dry basis). The corn silage was stored in two

concrete stave tower silos. Forage from one silo served as a control and the other was treated with a microbial silage additive. Control, urea, soybean meal, heat-treated SBM-SB, urea-dehydrated alfalfa meal and soybean meal-dehydrated alfalfa meal supplements were tested with the corn silage. The digestion-nitrogen balance trial consisted of a 5-d total collection study conducted with growing lambs (27.3 kg) fed diets similar to those fed in the cattle feeding trial.

Feeding Trial

There were no significant interactions between silage treatments and sources of supplemental protein during this trial. Therefore, feedlot performance data are presented averaged across silage storage treatments. Performance obtained during the feeding trial is shown in table 16. Results are presented cumulatively at approximately 4-wk intervals with average daily gain taken on a shrunk basis. Statistical analysis of the data was for the 105 d of the experiment.

Weight Gain. Daily rate of gain was increased with increasing time and weight during the experiment. Steers fed each source of supplemental protein with corn silage gained at a faster rate ($P<.01$) than the unsupplemented control group. A good and relatively uniform response to protein supplementation was observed at all weigh periods. At 105 d, there was an advantage of 28.6 to 32.7 kg in average gain per head for protein-supplemented steers over the controls. These results are in agreement with work reported by Garret et al. (1982) which showed improved average daily gain for steers fed corn silage

TABLE 16. FEEDLOT PERFORMANCE DATA FOR GROWING CATTLE FED VARIOUS SOURCES OF PROTEIN WITH CORN SILAGE (JANUARY 9 TO APRIL 24, 1982--105 DAYS)

Item	Control	Urea	Soybean meal	Heat-treated SBM-SB[a]	Urea-dehydrated alfalfa meal	Soybean meal-dehydrated alfalfa meal
No. of steers	32	32	32	32	32	32
Avg initial wt, kg	237	236	237	237	237	236
Avg final wt, kg	320	349	352	354	349	352
Accumulated avg daily gain, kg						
26 d	.64	.80	.85	.90	.86	.87
54 d	.64	.93	.95	.99	.95	.98
82 d	.72	1.01	1.06	1.07	1.03	1.05
105 d	.80[b]	1.07[c]	1.10[c]	1.11[c]	1.07[c]	1.10[c]
Accumulated avg daily feed, kg (dry)						
26 d	5.06	5.54	5.58	5.68	5.93	5.79
54 d	5.38	6.09	6.00	6.11	6.23	6.15
82 d	5.59	6.34	6.36	6.44	6.55	6.45
105 d	5.77[d]	6.57[e]	6.61[e]	6.66[e]	6.78[e]	6.71[e]
Accumulated feed/gain (dry)						
26 d	7.91	6.93	6.56	6.31	6.90	6.66
54 d	8.41	6.55	6.32	6.17	6.56	6.28
82 d	7.76	6.28	6.30	6.02	6.36	6.14
105 d	7.21[d]	6.14[ef]	6.01[e]	6.00[e]	6.34[f]	6.10[ef]

[a] SBM-SB was 60% soybean meal and 40% whole soybeans.
[b,c] Means within rows followed by unlike superscripts differ (P<.01).
[d,e,f] Means within rows followed by unlike superscripts differ (P<.05).

supplemented with urea, soybean meal, dehydrated alfalfa meal or urea-dehydrated alfalfa meal as compared to steers fed corn silage without supplemental protein. While there was a good response to protein supplementation under the conditions of the experiment, only slight differences ($P > .05$) in daily gain were observed between the sources of supplemental protein over the 105-d feeding trial.

An improvement in average daily gain was observed at the first weigh period (26 d) for steers fed diets supplemented with protein over the controls. The differences in total gain between the control and the protein-supplemented groups increased throughout the experiment.

After 26 d, steers fed the urea supplement had the lowest daily gain among those fed supplemental protein. However, this urea group gained about 20% more than the controls during this time. Substituting dehydrated alfalfa meal (8.22% of diet dry matter) for about one-half of the protein provided by urea resulted in similar weight gains at 26 d as for steers fed diets supplemented with soybean meal. At the end of the 105-d experiment, average daily gain for both groups fed supplements with urea was the same and only slightly less ($P > .05$) than for those fed diets with soybean meal.

The substitution of about one-half of the protein from soybean meal with protein from dehydrated alfalfa meal (4.36% of diet dry matter) appeared to offer no advantage in comparison to the soybean meal alone, since daily gain for each group was similar throughout the experiment. Steers fed the heat-treated SBM-SB supplement initially gained at a slightly higher rate than those fed regular soybean meal

supplemented diets, but at the termination of the experiment average daily gains for both groups showed no difference ($P > .05$).

The treatments evaluated here included urea and soybean meal fed alone and combined with dehydrated alfalfa meal and soybean meal combined with whole soybeans and subjected to a heat treatment process. These ingredients, combinations and processing treatment gave diets with variations in level of protein solubility in the rumen and amino acid composition. Under the conditions of this experiment, these differences in protein supplementation did not reflect changes in average daily gain over the 105-d trial. This lack of response under such conditions of protein supplementation has also been reported by Boling et al. (1971), Cross et al. (1974) and Garret et al. (1982). These researchers also reported that steers fed corn grain-corn silage diets supplemented with urea for an entire growing-finishing period performed similarly as steers supplemented with preformed protein.

Differences between sources of protein supplementation have been reported by Boling et al. (1972) and Loerch and Berger (1981). Boling and co-workers reported higher rates of gain for steers fed a corn silage diet supplemented with soybean meal than for steers fed diets supplemented with urea. Loerch and Berger (1981) fed steers corn silage diets supplemented with urea, soybean meal or dehydrated alfalfa meal and found that the steers fed soybean meal or dehydrated alfalfa meal gained at faster rates.

The potential advantages of feeding protein sources that are slowly degraded in the rumen were discussed by Broderick (1975) and

Chalupa (1975). Processing procedures for commercially available protein sources such as soybean meal and dehydrated alfalfa meal involve heating, a procedure which has been shown to decrease solubility of protein in the rumen (Sherrod and Tillman, 1962). Dehydrated alfalfa meal and heat-treated soybean meal have been reported to be slowly degraded in the rumen (Krause and Klopfenstein, 1974; Thomas et al., 1979). Heat treating of soybean meal has also been shown to improve average daily gain in young ruminants by Glimp et al. (1967) and Hudson et al. (1969). Recent data obtained at this experiment station using corn silage supplemented with heat-treated soybean meal support the results found during the trial presented in this manuscript (Palmer et al., 1984).

Many common growing and finishing diets for cattle and sheep often furnish much of the protein needed to meet the requirements of the animal. Under such conditions, it may be difficult to demonstrate differences between supplemental sources which provide only a small fraction of the total dietary protein.

Feed Intake. Feed intake for all treatment groups increased with increasing weight and time on experiment. Steers fed protein-supplemented diets with corn silage consumed more ($P<.05$) feed than unsupplemented controls, but there were only small differences ($P>.05$) in feed intake between the various protein-supplemented treatments.

At each weigh period of the experiment, steers fed the control diet had lower feed intake than those fed protein-supplemented diets. This was in agreement with research reported by Hatfield and Cantner

(1973) and Swan and Embry (1973) when supplemental protein to low protein diets resulted in increased dry matter consumption. Similar average daily feed intake was observed for all protein-supplemented groups at the periodic intervals during the experiment.

Data obtained for feed intake during this experiment showed that there was essentially no reduction inf feed intake initially or throughout the experiment when urea was fed at .84% of the dry diet. Steers fed supplements with dehydrated alfalfa meal at 8.22% in the diet with urea and at 4.36% with soybean meal had similar feed intake as for these treatment groups without the dehydrated alfalfa meal. These levels of dehydrated alfalfa meal would have resulted in some reduction in energy concentration of the diets, but weight gains did not appear to be affected. Steers fed diets supplemented with heat-treated SBM-SB consumed similar amounts of feed throughout the experiment as did steers fed regular soybean meal.

Levels of dry matter intake for steers fed during this trial was about 2.36% of their body weight initially and decreased to about 1.88% by the end of the 105-d experiment. Dry matter intake for steers consuming diets composed largely of corn silage is generally expected to be less than for grain and hay diets containing similar energy levels. Loerch and Berger (1981) fed steers corn silage supplemented with urea, soybean meal or dehydrated alfalfa meal and obtained essentially similar levels of intake as those obtained in this study.

Feed Efficiency. Good feed efficiency was observed for all treatment groups during the experiment. Generally, all dietary

treatments showed lower feed requirements per unit of gain with increasing weight and time on experiment. Feed requirements would be expected to increase had steers been fed to a typical market weight and finish.

Steers fed the protein-supplemented diets consumed more feed and gained at a faster rate and, therefore, had lower feed requirements than steers fed the unsupplemented control diet. The improvement ($P<.05$) in feed efficiency ranged from 12 to 17% for steers fed the protein-supplemented diets over the controls. Weight gain and feed consumption for steers fed diets with urea at .49 and .84% in comparison to diets with soybean meal resulted in similar feed requirements for these groups ($P>.05$). Addition of dehydrated alfalfa meal at 8.22 and 4.96% of the dry diet in substitution for about one-half of the supplemental protein from urea and soybean meal, respectively, had only small effects on weight gain and feed intake, resulting in about the same feed requirements ($P>.05$) as compared to feeding urea or soybean meal alone. Depending on the price of alfalfa products in comparison to the other ingredients replaced, alfalfa products may be an economical source of protein. Steers fed the heat-treated SBM-SM-supplemented diet gained and consumed feed at similar levels as those fed soybean meal and, therefore, had similar feed requirements.

The effect of protein supplementation on feed efficiency observed during this study has been reported by Young et al. (1973) when feeding steers a basal diet of ground ear corn and recently by Palmer et al. (1984) with corn silage diets. On the other hand, results

reported by Loerch and Berger (1981) indicated that steers fed soybean meal diets were more efficient than those fed diets with urea or dehydrated alfalfa meal. A more recent study (Palmer et al., 1984) has also indicated a slight improvement in feed efficiency for steers fed corn silage supplemented with soybean meal (regular and heat-treated) in comparison to urea.

Digestion-Nitrogen Balance Trial

A group of 24 lambs was selected to evaluate digestibility and nitrogen balance of diets with corn silage and the various sources of supplemental protein. Diets were formulated to be of the same ingredient composition and protein content as those fed during the cattle feeding trial (table 3). However, as noted in the Materials and Methods section, the soybean meal supplement was formulated erroneously and contained only 21% protein instead of the expected 32%. The urea supplement was higher in protein (41.5%) than expected (32%) from the formulation. These samples were reanalyzed to check for possible analytical errors but with similar results. The protein contents consequently for the control, urea, soybean meal, heat-treated SBM-SM, urea-dehydrated alfalfa meal and soybean meal-dehydrated alfalfa meal diets were 8.1, 11.4, 9.3, 10.5, 10.3 and 9.9%, respectively.

Nutrient Digestibility. Daily dry matter consumption and digestibility data for lambs fed the various sources of supplemental protein are shown in table 17. Each lamb was offered an amount of feed daily to result in some feed refusal to insure full feeding of all

TABLE 17. FEED CONSUMPTION AND NUTRIENT DIGESTIBILITY FOR LAMBS FED VARIOUS SOURCES OF SUPPLEMENTAL PROTEIN WITH CORN SILAGE

Item	Control	Urea	Soybean meal	Heat-treated SBM-SM	Urea-dehydrated alfalfa meal	Soybean meal-dehydrated alfalfa meal
No. of lambs	4	4	4	4	4	4
Avg initial wt, kg[a]	27.0	27.4	27.3	27.3	27.3	27.3
Avg final wt, kg	27.4	28.5	27.9	29.8	26.8	28.3
Dry matter consumed, g/d	840	944	875	1089	857	968
Apparent digestibility, %						
Dry matter	64.1	65.1	65.9	64.0	64.9	63.7
Organic matter	65.9[b]	67.1[d]	67.4[c]	65.9[c]	66.8[c]	65.4[c]
Crude protein	55.0[b]	66.9[d]	60.1[c]	61.0[c]	61.0[c]	59.0[c]

[a] Lamb's weight when placed in metabolism crate.
[b,c,d] Means within rows followed by unlike superscripts differ ($P<.05$).

diets. Considerable variation in dry matter intake was observed between treatment groups during the 5-d collection period. The differences in feed intake were not expected to affect digestibility of diets, since intakes were at adequate levels for studies on digestibility. While dry matter and organic matter digestibilities are shown, organic matter was the better indicator of fermentative activity and the discussion will be focused on this parameter.

Organic matter digestibility ranged from 65.4 to 67.4%. The data showed high rates of digestiblity for these corn silage diets with only slight ($P>.05$) differences between treatment groups. Apparently, the protein level provided in the control diet (8.1% of the dry matter) was adequate for maximum digestibility of the organic matter and additional protein from any of the various sources of supplementation resulted in no improvement.

A lack of response in digestibility of the organic matter with as much protein as in the diets fed here has been reported by Nomani and Evans (1971) and Griffiths et al. (1973) when steers and heifers were fed corn silage diets supplemented with urea or soybean meal. Other workers (Poos et al., 1979; Loerch and Berger, 1981) have reported improvement in dry matter digestibility when evaluating corn silage diets supplemented with urea, soybean meal or dehydrated alfalfa meal.

Lambs fed diets supplemented with protein showed improved ($P<.05$) digestibility of crude protein (table 17) when compared to those fed the unsupplemented control diet. The highest digestibility of crude protein was observed for lambs supplemented with urea. The

protein content in the urea diet was higher (11.4%), but the daily nitrogen intake was similar to that of the heat-treated SBM-SM group (table 18). Urea being totally hydrolyzed and rapidly absorbed from the rumen is likely to result in higher apparent crude protein digestibility than protein sources less readily soluble in the rumen fluid. Other protein-supplemented groups--soybean meal, heat-treated SBM-SM, urea-dehydrated alfalfa meal and soybean meal-dehydrated alfalfa meal--had very similar crude protein digestibility coefficients.

The improvement in crude protein digestibility from protein supplementation observed during this study was also reported by Griffiths et al. (1973) and Poos et al. (1979). However, the improved digestibility of the crude protein reported by Loerch et al. (1983) for lambs consuming soybean meal as compared to those fed dehydrated alfalfa meal or the relative increase of about 10% in protein digestibility obtained by Tagari et al. (1962) when feeding lambs diets containing heated vs unheated soybean meal were not observed during the present study.

Nitrogen Balance. Nitrogen utilization data obtained for the various dietary treatments during this experiment are presented in table 18. Nitrogen intake which is a reflection of feed intake and the protein content of the diet varied from 10.90 to 17.73 g/d. Lambs fed the control diet consumed the least amount of dry matter and had the lowest intake of nitrogen. All protein-supplemented groups consumed more feed with higher amounts of nitrogen being consumed than the control group.

TABLE 18. EFFECT OF VARIOUS SOURCES OF PROTEIN SUPPLEMENTATION ON NITROGEN UTILIZATION FOR LAMBS

Item	Control	Urea	Soybean meal	Heat-treated SBM-SM	Urea-dehydrated alfalfa meal	Soybean meal-dehydrated alfalfa meal
Dry matter consumed, g/d	840	944	875	1089	857	968
Nitrogen balance, g/d						
Intake	10.90	17.31	12.92	17.73	13.95	15.11
Fecal	4.94^{a}	5.71^{abc}	5.15^{ab}	6.91^{c}	5.42^{ab}	6.20^{bc}
Urinary	3.78^{a}	6.24^{c}	4.66^{ab}	6.62^{c}	5.35^{bc}	5.38^{bc}
Retained	2.18^{a}	5.36^{b}	3.11^{a}	4.20^{ab}	3.18^{a}	3.53^{ab}
Percent of intake						
Fecal	45.32^{a}	32.99^{c}	39.86^{b}	38.97^{b}	38.85^{b}	41.03^{b}
Urinary	34.68	36.05	36.07	37.34	38.35	35.61
Retained	20.00	30.96	24.07	23.69	22.80	23.36

^{a,b,c,d} Means within rows followed by unlike superscripts differ ($P<.05$).

Since considerable variation was obtained for nitrogen intake which could have affected amounts of nitrogen absorbed from the diet and the amount excreted through the urine and feces, nitrogen balance data will be discussed as percentage of the total nitrogen consumed. This will allow a better interpretation of the effects of nitrogen utilization of the different dietary treatments.

Fecal nitrogen as a percentage of the total nitrogen consumed represents digestibility. Reduced fecal nitrogen ($P<.05$) was observed for all groups fed protein-supplemented diets, with the urea group showing the least nitrogen losses in the feces. The other protein-supplemented groups--soybean meal, heat-treated SBM-SB, urea-dehydrated alfalfa meal and soybean meal-dehydrated alfalfa meal--had similar fecal nitrogen losses ($P>.05$). Results reported by Poos et al. (1979) in two experiments where dairy cows were fed corn silage diets supplemented with urea or soybean meal also showed lower fecal nitrogen losses with diets containing supplemental protein as compared to the controls. The lower fecal nitrogen losses by the urea group could be explained in part by the nature of urea which is totally hydrolyzed to ammonia in the rumen (Bergen, 1979b), resulting in increased absorption through the rumen wall and showing lower amounts of nitrogen in the feces (Lewis, 1961).

Losses in urinary nitrogen (percent of nitrogen intake) for protein-supplemented diets were only slightly higher ($P>.05$) than for the controls, with only small differences between sources of supplemental protein. Increases in urinary nitrogen are indicative of excess

nitrogen absorption, protein content in relation to energy and amino acid profile (protein quality) inadequate for needs under conditions measured. The similarity in urinary nitrogen as percent of nitrogen intake for the protein-supplemented lambs would indicate no important differences between diets in regard to these conditions. Higher urinary nitrogen losses, however, have been reported by Sniffen (1974), Wohlt et al. (1976) and Poos et al. (1979) for ruminants fed sources of protein of high solubility (urea, soybean meal) in comparison to those containing low soluble proteins (heat-treated soybean meal).

Nitrogen retained was increased when lambs were fed supplemental protein as compared to the controls. Similar results were also reported by Griffiths et al. (1973) and Poos et al. (1979) when feeding corn silage diets supplemented with various sources of protein. Under the conditions of this experiment, the urea group had the highest nitrogen retention. This indicates a more efficient utilization of the nitrogen by the urea group as compared to the other protein-supplemented groups which had only small differences in nitrogen retention.

Poos et al. (1979) conducted two experiments with dairy cows fed corn silage diets supplemented with urea or soybean meal. The researchers obtained higher nitrogen retention for the urea group in one experiment but for the soybean meal group in the other experiment. However, in many studies such as those conducted by Nomani and Evans (1971) and Sniffen (1974), nitrogen retained was lower for ruminants consuming the more soluble nitrogen source.

The lack of improvement in nitrogen retention due to feeding diets containing heat-treated SBM-SB to lambs as compared to feeding regular soybean meal observed during this study are supported by earlier results reported by Hudson et al. (1969, 1970). Glimp et al. (1967), however, have reported better nitrogen retention from heat-treated soybean meal.

Experiment Three

This experiment consisted of a feeding trial, a series of digestion-nitrogen balance studies, a rumen fermentation trial and blood urea-nitrogen determinations. These studies were to evaluate soybean meal and urea as supplements to diets with various levels of protein (11.2, 12.5, 13.8 and 15.1%) for growing and finishing lambs. The pelleted diets consisted of 92% concentrate (mostly corn) and 8% sun-cured alfalfa.

Feeding Trial

Results obtained for the lamb feeding trial are presented in tables 19 through 22. Treatment effects on weight gain, feed intake and feed efficiency are presented cumulatively in table 19. Treatment effects for each protein source and level over the 81-d experiment for weight gain, feed intake and feed efficiency are presented in tables 20 through 22, respectively. The initial average weight ranged from 33.0 to 34.0 kg (table 20) and was considered to be a typical weight range to study the effect of protein supplementation for growing and finishing lambs.

TABLE 19. PERIODIC FEEDLOT PERFORMANCE FOR GROWING-FINISHING LAMBS FED SOYBEAN MEAL OR UREA AT VARIOUS PROTEIN LEVELS (AUGUST 7 TO OCTOBER 27, 1981--81 DAYS)

Protein level, %	11.2[a]		12.5		13.8		15.1	
Item	SBM	Urea	SBM	Urea	SBM	Urea	SBM	Urea
No. of lambs[b]	48	47	46	48	44	45	45	48
Avg initial wt, kg	33.0	34.0	33.5	33.4	33.5	33.3	33.3	33.7
Avg final wt, kg	50.5	50.8	51.1	51.9	53.2	53.0	53.9	54.0
Accumulated avg daily gain, g								
17 d	250	174	227	255	278	290	285	301
41 d	233	208	248	234	273	285	271	281
61 d	220	210	240	241	256	260	254	271
81 d	216	209	217	231	243	244	254	251
Accumulated avg daily feed, kg (dry)								
17 d	.94	.87	.90	.95	.92	.98	.91	.97
41 d	1.05	1.02	1.05	1.07	1.12	1.16	1.06	1.13
61 d	1.06	1.06	1.08	1.12	1.13	1.15	1.10	1.19
81 d	1.10	1.10	1.12	1.17	1.18	1.19	1.18	1.22
Feed/gain (dry)								
17 d	3.76	5.00	3.96	3.73	3.31	3.38	3.19	3.22
41 d	4.51	4.90	4.23	4.57	4.10	4.07	3.91	4.02
61 d	4.82	5.05	4.50	4.65	4.41	4.42	4.33	4.39
81 d	5.09	5.26	5.16	5.06	4.86	4.88	4.65	4.86

[a] Nonsupplemented controls.
[b] Initially 48 lambs per group, but some died of acute polioencephalomalacia.

Weight Gain. The overall data for the 81-d experiment were summarized by level and source of protein, analyzed statistically as a 2 x 4 factorial with six replications and results obtained are shown in table 20. A good rate of gain was observed from the lowest level of protein supplementation. Increasing levels of supplementation resulted in increased rates of gain up to the highest level fed (15.1%). Several researchers (Hinds et al., 1965; Hudson et al., 1967; Braman et al., 1973) have also observed increased rates of gain when feeding lambs diets which had protein levels from 10.0 to 19.4% of the dry diet.

TABLE 20. AVERAGE DAILY GAIN COMPARISONS FOR SOYBEAN MEAL AND UREA AT VARIOUS LEVELS OVER THE 81-DAY EXPERIMENT

Protein level, %	Soybean meal	Urea	Avg
	g		
11.2	216	209	213[a]
12.5	217	231	224[a]
13.8	243	244	244[b]
15.1	254	251	253[b]
Avg	233	234	

[a,b] Means followed by unlike superscripts differ ($P<.01$).

The advantages over the nonsupplemented control (11.2% protein) amounted to about 5, 15 and 19%, respectively, for the 12.5, 13.8 and 15.1% levels of protein. The reduction in the rate of response from 13.8 to 15.1% protein in the diet suggests that total protein requirements for lambs of this weight may lie between these two levels. NRC (1975) suggested protein requirements of 11.0% of the dry diet for

growing-finishing lambs averaging 30 to 55 kg with daily gains of 200 to 250 g. However, protein levels for lambs in excess of those recommended by the NRC (1975) have been reported by numerous researchers. Preston et al. (1965), Hudson et al. (1969), Bergen et al. (1973) and Insley et al. (1983) reported improved average daily gains for lambs fed diets containing protein levels above 11.0%. In other studies, Schelling et al. (1967) obtained optimum performance for lambs fed diets containing 13.5% protein, while Braman et al. (1973) reported maximum gains for lambs fed diets with about 15.0% protein. Hinds et al. (1964) and Glimp et al. (1967) fed protein levels up to 17% to lambs and observed maximum rates of gain at the highest level. It is important to note that, when protein needs are expressed as a percentage of the total diet, the amount of roughage and(or) concentrates in the diet, total feed intake, digestibility and type and level of production will have an effect on the level of protein.

No differences ($P > .05$) were observed in average daily gain between lambs fed diets supplemented with soybean meal or urea over the 81-d trial. Urea fed at .36, .72 and 1.08% of the total dry diet to provide the various levels of protein resulted in a similar response as for soybean meal at each level of supplementation. The results are in agreement with work reported by Jordan and Hanke (1969), Huston and Shelton (1971) and Braman et al. (1973). These researchers reported only slight ($P > .05$) differences in average daily gain for lambs fed high-concentrate diets supplemented with various sources of nitrogen

(urea, soybean meal, dehydrated alfalfa meal, guar meal, cottonseed meal or feather meal).

Weight gain data at the various periodic intervals (table 19) were generally quite variable. Of major concern was the response shown initially and at periodic intervals to increasing levels of protein provided by either soybean meal or urea. The preliminary period provided adequate time for adaptation to the basal diet without supplemental protein. This preliminary period treatment and the manner of initiating experimental diets were expected to provide a sensitive measure of response to source and to level of dietary protein.

Diets for lambs in both the soybean meal and urea groups at the 11.2% level of protein were the same without added soybean meal or urea. There was a sizable difference in daily gain between these two groups at 17 d, but the differences were rather small at subsequent periods of the experiment. These differences were due largely to poor performance of lambs in two of the six pens from the urea group. In contrast, the soybean meal group showed a very satisfactory rate of gain during this 17-d period. The average for the two groups (212 g) might represent a more typical performance when feeding the 11.2% protein diets during this period.

Average daily gain (table 19) at 17 d for lambs fed urea exceeded the gain of lambs fed soybean meal in the three comparisons with urea at .36, .72 and 1.08% of the dry diets. The results indicate no evidence that the lambs required a period of adaptation to urea in comparison to soybean meal under the conditions of this experiment.

The lambs were adapted to the high-grain diets without soybean meal or urea prior to including the supplemental sources of protein. There also was a gradual increase in the amount of feed offered from an initial level of approximately .85 kg per head daily to a full feed in about 12 d. This gradual increase in the level of feed intake would also result in a gradual increase in the amount of urea which likely would be beneficial for nonadapted animals.

Comparisons between soybean meal and urea at subsequent periods showed similar performance from feeding the two sources at the various levels of dietary protein. Haskins et al. (1967), Bolsen et al. (1968) and NRC (1975) have suggested that the most favorable responses from urea supplementation have occurred in diets containing relatively high concentrations of readily fermentable carbohydrates. Under these conditions, performance equal to that of urea could be expected with soybean meal-supplemented diets.

Feed Intake. Lambs fed diets supplemented with increasing levels of protein showed some slight increases in feed intake up to the 13.8% level over the 81-d experiment (table 21). Feeding diets with increasing levels of protein to lambs have resulted in variable responses concerning feed consumption. Preston et al. (1965) fed a 50% concentrate diet to lambs supplemented with increasing levels of protein (6.2 to 13.5%) and found that feed consumption increased with increasing the level of protein supplementation. Similar results were reported by Hudson et al. (1969) when feeding lambs diets containing 10 to 14%

TABLE 21. AVERAGE DAILY FEED INTAKE COMPARISONS FOR SOYBEAN MEAL OR UREA AT VARIOUS LEVELS OVER THE 81-DAY EXPERIMENT[a]

Protein level, %	Soybean meal	Urea	Avg
	kg		
11.2	1.10	1.10	1.10[b]
12.5	1.12	1.17	1.14[bc]
13.8	1.18	1.19	1.19[c]
15.1	1.18	1.22	1.20[c]
Avg	1.15	1.17	

[a] Feed values presented are on a dry basis.
[b,c] Means followed by unlike superscripts differ (P<.05).

protein. However, in studies conducted by Glimp et al. (1967), Schelling et al. (1967) and Braman et al. (1973), no differences in feed intake were observed for lambs fed diets containing different levels of protein (11.0 to 17.0%).

Lambs fed diets with urea consumed slightly more (P>.05) feed than lambs fed the soybean meal at each level of protein compared. This similarity in levels of dietary feed consumption for lambs fed different sources of nitrogen supplementation was also obtained by Jordan and Hanke (1969), Huston and Shelton (1971) and Braman et al. (1973).

Feed intake for the first period after 17 d (table 19) was influenced to some extent by feeding practices followed during this period. Upon initiation of the test diets, the daily feed was increased gradually from an initial level of .85 kg, as fed, to a full feed over a period of 12 d. In general, there was a small increase in feed consumption with increasing time on experiment. Lambs consuming urea

diets at all levels of supplementation showed slightly higher amounts of feed intake over the soybean meal groups at each period during the trial.

Feed Efficiency. Over the 81-d experiment, improved feed efficiency was observed due to protein supplementation (table 22). As the levels of protein supplementation increased, feed requirements declined with evidence of a slowing in the rate between the two higher levels. Improved feed to gain ratios were also obtained by Preston et al. (1965), Hudson et al. (1969), Braman et al. (1973) and Insley et al. (1983) when feeding lambs diets with increasing levels of protein which ranged from 6.5 to 17.0%. Hudson et al. (1969) and Insley et al. (1983) reported that a level of 14% dietary protein gave the most efficient gains. On the other hand, Hinds et al. (1964) showed that protein levels of 19.1% resulted in lower feed requirements for lambs as compared to diets with lower levels of protein.

TABLE 22. AVERAGE FEED/GAIN COMPARISONS FOR SOYBEAN MEAL OR UREA AT VARIOUS LEVELS OVER THE 81-DAY EXPERIMENT[a]

Protein level, %	Soybean meal	Urea	Avg
11.2	509	526	518[b]
12.5	516	506	511[bc]
13.8	486	488	487[cd]
15.1	465	486	476[d]
Avg	494	502	

[a] Feed values used were on a dry basis.
[b,c,d] Means followed by unlike superscripts differ ($P<.05$).

Differences in feed efficiency between soybean meal and urea groups were slight ($P>.05$). Only small and inconsistent differences in feed requirements between soybean meal and urea-fed lambs were observed at the various levels of protein supplementation. These results were similar to those reported previously by Jordan and Hanke (1969), Huston and Shelton (1971) and Braman et al. (1973) when feeding lambs various sources of protein (urea, soybean meal, cottonseed meal, blood meal, and hydrolyzed feather meal).

In general, better feed efficiency was observed during the first period at 17 d for all groups as compared to the subsequent periods (table 19). As the experiment advanced, greater amounts of feed were required for all groups. Differences in feed requirements between the two protein sources were small with no apparent difference in feed efficiency between urea and soybean meal at the various levels of protein. At each period it was also observed that protein supplementation improved feed efficiency, since lambs fed diets with higher levels of nitrogen showed better feed to gain ratios than those fed lower levels of protein.

Nutrient Digestibility

Diets for the digestion-nitrogen balance trials were formulated similarly as for the feeding trials (tables 4 and 5). During the pre-experimental period, lambs were fed the unsupplemented control diet and upon initiation of the experiment (day 1) all protein sources and level treatments were offered for the first time. Lambs for each dietary

treatment were allotted as to weight and feed consumption. Thus, initially each experimental group had similar feed intake.

The protocol (table 7) shows that this experiment was to be divided into three periods for collection. The first one was from day 2 through 4 after the protein supplemented diets were fed for the first time, the second period included day 8 through 10, while the third period was from day 14 through 18. This procedure was followed to assess the effects of time (adaptation) upon digestibility and nitrogen utilization of the diets with soybean meal or urea at various levels of protein supplementation. The first collection period was intended to evaluate the early response to the diets fed. During the last collection period, adequate time was considered to have been allowed for the lambs to be adapted to the diets fed.

The experiment was repeated a second time with different lambs in order to have a greater number of animals and thus more adequate data for measuring treatment effects. Data between phases did not show any statistical differences. Therefore, phases were combined and analyzed as a 3 x 4 x 2 factorial for period of collection, protein levels and sources of protein. Data for main effects are shown in table 23 and in table 24 for observation of interactions, even though none were significant ($P > .05$).

Feed consumption data showed an adequate level of dry matter intake and represents an average consumption of about 3% of the body weight. The intakes represent an adequate amount of feed consumption for measuring nutrient digestibility and nitrogen balance. Digestion

crates are not a good place to measure feed intake, and levels of intake could be affected by factors other than dietary treatments.

Dry Matter and Organic Matter Digestibility. Apparent digestibility data for dry matter and organic matter are presented in tables 23 and 24. As indicated previously, the dicussion will emphasize organic matter digestibility. Because of the two coefficients, this one is a better indicator of energy utilization.

Relatively high digestibility of organic matter was observed for these diets during the experiment with only small differences between periods of collection. There was a slight reduction ($P<.05$) in the digestibility of the organic matter at the second period during day 8 through 10. The reason for this was not apparent and the magnitude of the difference was considered to be of minor practical importance. Palmer (1982) reported similar initial responses when supplemental protein was added to a low protein basal diet. Initially at day 1 through 3, Palmer observed increased digestibility of the organic matter. Thereafter (day 4 to 6), the digestibility declined slightly and tended to stabilize at about day 10. Initial levels of organic matter digestibility in comparison to the subsequent periods indicated that a period of adaptation to the diets was not evident as measured by organic matter digestibility.

Lambs fed urea diets showed slightly higher ($P<.05$) digestibility of organic matter than those fed soybean meal diets. The advantage amounted to only 1.7 percentage units and digestibility for each group indicated good utilization of the energy fraction of these

TABLE 23. MAIN EFFECTS FOR FEED CONSUMPTION AND NUTRIENT DIGESTIBILITY FOR LAMBS FED UREA OR SOYBEAN MEAL AT VARIOUS LEVELS DURING ADAPTATIONAL STUDIES

Item	Period			Treatment		Protein level, %			
	1	2	3	SBM	Urea	11.2	12.5	13.8	15.1
Dry matter consumed, g/d	1017	1130	1184	1139	1082	1144	1125	1091	1082
Apparent digestibility, %									
Dry matter	79.3^{a}	76.9^{b}	78.2^{ab}	77.2^{a}	79.1^{b}	75.7^{a}	80.3^{c}	77.8^{b}	78.9^{bc}
Organic matter	80.7^{a}	78.2^{b}	79.4^{ab}	78.6^{a}	80.3^{b}	76.9^{a}	81.6^{c}	79.1^{b}	80.1^{bc}
Crude protein	67.8^{ab}	66.6^{b}	68.6^{a}	67.3	68.0	63.6^{a}	67.7^{b}	69.8^{c}	69.5^{c}

^{a,b,c} Means within each group followed by unlike superscripts differ ($P<.05$).

TABLE 24. FEED CONSUMPTION AND NUTRIENT DIGESTIBILITY FOR LAMBS FED UREA OR SOYBEAN MEAL AT VARIOUS LEVELS DURING ADAPTATIONAL STUDIES

Item	Treatment	Period	Protein level, % 11.2	12.5	13.8	15.1	Avg
No. of lambs	Soybean meal		6	6	6	6	
	Urea		6	6	6	6	
Avg initial wt, kg	Soybean meal		28.3	28.4	28.7	28.2	
	Urea		28.3	28.3	28.5	28.2	
Avg final wt, kg	Soybean meal		33.0	35.2	35.2	34.5	
	Urea		33.5	34.2	33.2	33.7	
Dry matter consumed, g/d	Soybean meal	1	1021	1049	1066	1004	1035
	Urea		1085	966	983	965	1000
Average			1053	1007	1025	984	
	Soybean meal	2	1166	1255	1195	1049	1166
	Urea		1209	1034	1050	1084	1095
Average			1188	1144	1123	1067	
	Soybean meal	3	1165	1289	1225	1188	1217
	Urea		1217	1157	1023	1203	1151
Average			1191	1223	1124	1196	

TABLE 24 CONTINUED

Item	Treatment	Period	Protein level, % 11.2	12.5	13.8	15.1	Avg
Apparent digestibility, %							
Dry matter	Soybean meal	1	76.7	82.2	77.3	78.5	78.7
	Urea		77.5	81.0	79.8	81.6	80.0
Average			77.1	81.6	78.5	80.0	
	Soybean meal	2	74.4	78.8	75.4	75.1	75.9
	Urea		73.1	79.5	80.4	78.7	77.9
Average			73.7	79.2	77.9	76.9	
	Soybean meal	3	76.0	78.3	75.1	78.9	77.1
	Urea		76.3	82.1	78.7	80.5	79.4
Average			76.2	80.2	76.9	79.7	
Organic matter	Soybean meal	1	78.1	83.5	78.8	79.8	80.1
	Urea		78.8	82.2	81.4	82.9	81.3
Average			78.5	82.8	80.1	81.3	
	Soybean meal	2	75.6	80.3	76.6	76.8	77.3
	Urea		74.4	80.8	81.6	79.8	79.1
Average			75.0	80.5	79.1	78.3	
	Soybean meal	3	77.2	79.6	76.1	80.2	78.3
	Urea		77.3	83.2	79.8	81.4	80.4
Average			77.3	81.4	78.0	80.8	

TABLE 24 CONTINUED

Item	Treatment	Period	Protein level, % 11.2	12.5	13.8	15.1	Avg
Crude protein	Soybean meal	1	62.1	70.4	69.8	69.3	67.9
	Urea		62.3	65.9	69.7	72.8	67.7
Average			62.2	68.2	69.7	71.0	
	Soybean meal	2	63.0	67.9	70.3	64.1	66.3
	Urea		63.0	64.5	70.4	69.6	66.9
Average			63.0	66.2	70.4	66.9	
	Soybean meal	3	64.7	68.0	69.6	68.2	67.6
	Urea		66.6	69.6	68.7	73.4	69.6
Average			65.6	68.8	69.2	70.8	

diets. These results were similar to those reported earlier by Palmer (1982), where only slight differences in organic matter digestibility were observed between lambs fed diets supplemented with soybean meal or urea.

Protein supplementation improved ($P<.05$) organic matter digestibility in comparison to the nonsupplemental control (11.2%). Only 2.5 percentage units in digestibility separated the three levels of supplementation, with maximum organic matter digestibility being obtained at the 12.5% level. The slightly lower organic matter digestibility at the higher levels (13.8 and 15.1%) was not readily apparent and probably was not of much concern, since improved rate of gain was observed for these groups during the feeding trial. Higher feed intake was also observed for these two groups in the feeding trial. Increased digestibility of organic matter with increased protein concentration in the diet was also reported earlier by Preston et al. (1965) and Glimp et al. (1967) when feeding lambs diets with soybean meal as the supplemental protein. Preston et al. (1965) fed diets with protein levels ranging from 6.5 to 13.5%, while Glimp et al. (1967) fed diets containing 12.1 to 17.2% protein.

Data for the interactions presented in table 24 show higher organic matter digestibility for urea-supplemented lambs during each of the three periods of collection and generally at each level of protein supplementation. Therefore, on the basis of organic matter digestibility, there was no apparent period of adaptation required for

lambs fed urea at levels up to 1.08% of the dry diet when previously adapted to high-concentrate diets.

Crude Protein Digestibility. Differences in crude protein digestibility between the three collection periods were small, with only slightly higher coefficients observed during the third collection period as compared to earlier periods. The similarity in crude protein digestibility initially and at the end of the 18-d trial would indicate no evidence of a period of adaptation to the diets fed under the conditions of the experiment. In earlier studies conducted by Palmer (1982), similar responses were observed when protein supplementation was evaluated over time.

Digestibility of the crude protein was not affected by source of supplementation since lambs consuming urea or soybean meal diets showed similar digestibility values ($P > .05$). Protein supplementation resulted in improved ($P < .05$) digestibility of the crude protein with the maximum response observed at the 13.8% level. These results were similar to those reported by Preston et al. (1965), Glimp et al. (1967), Waldo (1968), Hudson et al. (1969) and Palmer (1982) when feeding lambs diets with levels of protein ranging from 6.5 to about 17.0%. The data reported by Preston and co-workers suggest a maximum crude protein digestibility at 13.5% protein in the diet. Hudson and associates reported maximum digestibility at about 14% protein in the diet.

An increased crude protein digestiblity with protein supplementation was observed during each of three collection periods, with only slight differences between sources of supplementation. Early

detrimental effects often reported in the literature (Church, 1976) when urea was fed were not observed during this experiment. Factors such as prior adaptation to the control diet, level of soluble carbohydrates in the diet and rumen fill at the beginning of the experiment might have contributed to the results obtained.

In general, it was observed that there was no apparent evidence of a period of adaptation to the sources and levels of supplemental protein as measured by digestibility of organic matter and crude protein. Digestibility values obtained initially and during the third collection period 14 to 18 d later were similar. Nutrient digestibility for urea- and soybean meal-fed lambs was also similar throughout the experiment. There was an improvement in nutrient digestibility (organic matter and crude protein) with protein supplementation. Maximum digestibility of the organic matter appeared to be obtained at the 12.5% protein level, while maximum digestibility of crude protein was obtained at the 13.8% level.

Nitrogen Balance

Nitrogen balance data are shown in tables 25 and 26. These studies were conducted along with the nutrient digestibility studies. Therefore, procedures described for the digestibility studies are applicable to the nitrogen balance studies.

Nitrogen intake data showed some variation between period of collection, source of nitrogen and level of supplementation. There was, however, an adequate level of nitrogen intake and a positive nitrogen balance for all treatment groups. As for previous experiments,

nitrogen balance data will be discussed as a percentage of the total nitrogen intake, since this value is considered to more accurately measure treatment effects under conditions where feed intake was not controlled and with the variable levels of dietary protein.

Fecal Nitrogen. Fecal nitrogen (tables 25 and 26) is the undigested feed protein plus a metabolic fraction and thus represents apparent digestibility of protein. Digestibility of protein has been discussed in the previous section.

Urinary Nitrogen. Urinary nitrogen losses as a percentage of the total nitrogen intake (table 25) increased at each collection period, with losses at day 14 through 18 (third period) being highest ($P<.05$). Lambs fed diets containing urea had higher ($P<.05$) urinary nitrogen losses than those fed the soybean meal diets. Little et al. (1963), Egan and Kellaway (1971) and Sniffen (1974) have also reported higher urinary nitrogen losses when NPN sources were fed as compared to when preformed sources of protein were fed to ruminants.

The urinary nitrogen fraction was increased with protein supplementation. Lambs consuming higher levels of protein in the diet showed increased urinary nitrogen losses up to the 13.8% level of dietary protein. Church (1976) also reported higher urinary nitrogen levels when feeding ruminants increased amounts of protein in the diet and indicated this was due to increased levels of plasma urea which resulted in higher nitrogen losses in the urine.

TABLE 25. MAIN EFFECTS FOR NITROGEN UTILIZATION OF LAMBS FED UREA OR SOYBEAN MEAL AT VARIOUS LEVELS DURING ADAPTATIONAL STUDIES

Item	Period			Treatment		Protein level, %			
	1	2	3	SBM	Urea	11.2	12.5	13.8	15.1
Nitrogen balance, g/d									
Intake	19.98	22.49	23.97	23.12	21.17	19.97	21.48	23.02	24.13
Fecal	6.40^{a}	7.46^{b}	7.51^{b}	7.56^{a}	6.68^{b}	7.24	6.90	7.01	7.36
Urinary	5.00^{a}	5.95^{b}	7.02^{c}	5.88	6.10	4.79^{a}	5.47^{b}	6.68^{c}	7.02^{c}
Retained	8.58	9.08	9.44	9.68^{a}	8.39^{b}	7.94^{a}	9.11^{ab}	9.33^{ab}	9.75^{b}
Percent of intake									
Fecal	32.03^{ab}	33.17^{b}	31.33^{a}	32.70	31.55	36.25^{a}	32.12^{b}	30.45^{bc}	30.50^{bc}
Urinary	25.03^{a}	26.46^{a}	29.29^{b}	25.43^{a}	28.81^{b}	23.99^{a}	25.47^{ab}	29.02^{bc}	29.09^{bc}
Retained	42.94	40.37	39.38	41.87^{a}	39.64^{b}	39.76	42.41	40.53	40.41

^{a,b,c} Means within each group followed by unlike superscripts differ ($P<.05$).

TABLE 26. NITROGEN UTILIZATION OF LAMBS AS AFFECTED BY UREA OR SOYBEAN MEAL FED AT VARIOUS LEVELS DURING ADAPTATIONAL STUDIES

Item	Treatment	Period	Protein level, % 11.2	12.5	13.8	15.1	Avg
Nitrogen balance, g/d							
Intake	Soybean meal	1	17.19	20.56	22.68	21.67	20.52
	Urea		18.52	17.32	20.23	21.70	19.44
Average			17.85	18.94	21.45	21.69	
	Soybean meal	2	20.14	25.38	25.33	23.58	23.61
	Urea		21.26	18.48	21.86	23.88	21.37
Average			20.70	21.93	23.60	23.73	
	Soybean meal	3	21.29	25.76	26.74	27.15	25.24
	Urea		21.42	21.35	21.26	26.79	22.70
Average			21.35	23.56	24.00	26.97	
Fecal	Soybean meal	1	6.53	6.06	7.00	6.82	6.60
	Urea		7.00	5.92	6.02	5.87	6.20
Average			6.76	5.99	6.51	6.35	
	Soybean meal	2	7.45	8.08	7.62	8.44	7.90
	Urea		7.87	6.51	6.51	7.20	7.02
Average			7.66	7.30	7.06	7.82	
	Soybean meal	3	7.50	8.31	8.26	8.71	8.20
	Urea		7.10	6.50	6.64	7.08	6.83
Average			7.30	7.41	7.45	7.90	

TABLE 26 CONTINUED

Item	Treatment	Period	Protein level, % 11.2	12.5	13.8	15.1	Avg
Urinary	Soybean meal	1	4.26	4.99	5.45	5.09	4.95
	Urea		4.46	4.10	5.57	6.11	5.06
Average			4.36	4.55	5.51	5.60	
	Soybean meal	2	4.54	5.87	6.49	6.17	5.77
	Urea		4.87	4.37	7.02	8.25	6.13
Average			4.71	5.12	6.76	7.21	
	Soybean meal	3	4.96	7.71	7.47	7.55	6.92
	Urea		5.62	5.79	8.09	8.94	7.11
Average			5.29	6.75	7.78	8.24	
Retained	Soybean meal	1	6.40	9.51	10.23	9.75	8.97
	Urea		7.06	7.31	8.64	9.72	8.18
Average			6.73	8.41	9.43	9.74	
	Soybean meal	2	8.15	11.43	11.22	8.97	9.94
	Urea		8.53	7.59	8.33	8.42	8.22
Average			8.34	9.51	9.78	8.70	
	Soybean meal	3	8.83	9.75	11.01	10.89	10.12
	Urea		8.69	9.06	6.53	10.77	8.76
Average			8.76	9.40	8.77	10.83	

TABLE 26 CONTINUED

Item	Treatment	Period	Protein level, % 11.2	12.5	13.8	15.1	Avg
Percent of intake							
Fecal	Soybean meal	1	37.89	29.56	30.24	20.75	32.11
	Urea		37.71	34.08	30.35	27.24	32.34
Average			37.80	31.82	30.30	29.00	
	Soybean meal	2	37.00	32.13	29.66	35.93	33.68
	Urea		36.99	35.49	29.58	30.36	33.11
Average			36.99	33.81	29.62	33.15	
	Soybean meal	3	35.33	31.99	30.36	31.81	32.37
	Urea		33.39	30.38	31.30	26.63	30.43
Average			34.36	31.19	30.83	29.22	
Urinary	Soybean meal	1	25.20	25.79	24.02	25.14	25.04
	Urea		24.05	24.08	29.19	28.48	26.45
Average			24.62	24.93	26.61	26.81	
	Soybean meal	2	22.72	23.53	25.49	26.18	24.48
	Urea		22.85	24.40	32.47	34.19	28.48
Average			22.78	23.97	28.98	30.19	
	Soybean meal	3	23.25	29.75	27.90	27.77	27.17
	Urea		26.51	27.36	45.32	33.34	33.13
Average			24.88	28.56	36.61	30.56	

TABLE 26 CONTINUED

Item	Treatment	Period	Protein level, % 11.2	12.5	13.8	15.1	Avg
Retained	Soybean meal	1	36.92	44.65	45.74	44.11	42.85
	Urea		38.24	41.85	40.46	44.27	41.84
Average			37.58	43.25	43.10	44.19	
	Soybean meal	2	40.29	44.34	44.85	37.89	40.46
	Urea		40.16	40.10	37.94	35.44	41.20
Average			40.22	42.22	41.39	36.66	
	Soybean meal	3	41.22	38.26	41.74	40.41	38.41
	Urea		40.10	42.25	23.38	40.03	36.44
Average			40.76	40.26	32.56	40.22	

Nitrogen Retained. Nitrogen retention data for periods of collection, source of protein and level of protein supplementation are shown in table 25, while data for the interactions are presented in table 26. Only small differences ($P > .05$) in percent nitrogen retained were observed between periods of collection, with a tendency to retain slightly less nitrogen at each collection period. These data suggest no evidence of a period of adaptation to source and level of protein in diets fed during this experiment as measured by percent nitrogen intake that was retained. A possible explanation of the increase in excretion at each collection involved in the study is that of an increase in body stores of nitrogen.

A higher percentage of nitrogen was retained ($P < .05$) by lambs consuming diets with soybean meal as compared to those fed diets supplemented with urea. The soybean meal groups generally retained greater proportions of nitrogen than the urea groups at each period of collection and at each level of protein supplementation (table 26). The greater retention of nitrogen by the soybean meal group resulted mainly from lower urinary losses as compared to the urea group, since fecal nitrogen losses were slightly higher for the soybean meal groups. Data reported by Little et al. (1963) and Egan and Kellaway (1971) showed greater urinary nitrogen losses for ruminants fed diets containing urea as compared to those fed diets containing preformed protein, data which support the findings obtained during this experiment.

Only small differences ($P > .05$) in nitrogen retention were observed between lambs fed the different levels of protein supplementation. Lambs

fed the 12.5% protein diets retained a higher percentage of the nitrogen consumed than did those fed the other levels of dietary protein. These results were due to a combination of lower fecal nitrogen losses but higher urinary nitrogen losses with increasing levels of protein in the diet. Improved nitrogen utilization with increasing protein concentration in the diet such as that reported by Hudson et al. (1969) and Palmer (1982) was not observed during the present study.

These digestion and nitrogen balance trials show optimum digestibility of organic matter and percentage of dietary nitrogen retained at the 12.5% level of protein. A higher level of dietary protein was indicated in the feeding trial where rate of gain increased with increasing levels of protein up to the highest level fed (15.1%). Feed consumption and rate of gain were higher in the feeding trial and these tend to affect the need for protein. Digestion and nitrogen balance data are best used along with feeding trials to determine feed requirements.

A major objective of the digestion and nitrogen balance trials was to compare efficiency of nitrogen utilization of protein from soybean meal and urea at the various levels of dietary protein and the need for a period of adaptation to urea at the various levels fed. While there was a slight advantage ($P < .05$) for soybean meal on basis of nitrogen retention, there was no evidence of a need for a period of adaptation to urea up to the highest level fed (1.08% of the diet) under the conditions of the experiment. Results from the feeding trial were in agreement with this observation. For lambs adapted to a full feed

of a high-grain diet, there would appear to be no need for a period of gradual adaptation to urea when used as the supplemental protein at levels normally needed to meet requirements for growing and finishing lambs.

Rumen Fermentation and Blood Determinations

After each phase of the digestion-nitrogen balance adaptational studies, two lambs from each experimental group were selected on basis of uniformity in feed intake for the rumen fermentation and blood parameter studies. Rumen fluid and blood samples were collected during each phase twice with a 5-d period separating each collection. Experimental diets (tables 4 and 5) were fed once daily with collection of rumen fluid and blood samples at 3 and 8 h, respectively, after feeding. Data collected during each phase were combined and analyzed statistically.

Average weight, feed consumption and nitrogen intake for lambs fed soybean meal or urea at the various levels are shown in table 27. Average weight of lambs ranged from 41.0 to 46.8 kg and average daily feed consumption varied from 1176 to 1374 g. Differences in dry matter intake for lambs fed diets with soybean meal resulted in similar levels of nitrogen intake at the three higher levels of protein supplementation. In the urea groups, increased daily nitrogen intake was observed at each increase in level of protein supplementation.

TABLE 27. AVERAGE ANIMAL WEIGHT, FEED CONSUMPTION AND NITROGEN INTAKE FOR LAMBS FED SOYBEAN MEAL OR UREA AT VARIOUS LEVELS DURING THE RUMEN FERMENTATION STUDIES

Item	Treatment	Protein level, %			
		11.2	12.5	13.8	15.1
No. of animals	Soybean meal	4	4	4	4
	Urea	4	4	4	4
Animal weight, kg	Soybean meal	43.5	44.5	43.2	46.8
	Urea	41.1	41.0	43.4	43.9
Dry matter intake, g/d	Soybean meal	1239	1374	1264	1222
	Urea	1243	1277	1176	1251
Nitrogen intake, g/d	Soybean meal	22.70	27.57	27.58	27.86
	Urea	21.83	23.68	24.42	28.74

Rumen pH. Rumen pH was relatively constant for all dietary treatments throughout the experiment (table 28). Rumen pH ranged from 4.96 to 5.16 for lambs fed diets with soybean meal and from 5.00 to 5.13 with urea. These differences were small and indicated no important trends as to source of nitrogen or level of protein supplementation.

Rumen pH values determined at 3 h after feeding were within the range suggested by Church (1976) as that time after feeding at which rumen pH reaches its lowest value. The slight increase in rumen pH generally observed with protein supplementation followed trends reported by Haaland et al. (1982). Rumen pH values were generally within the range of 5.0 to 7.0 reported by Hungate (1966) when a variety of diets were fed. A rumen pH of about 5.0 appears typical for pelleted, high-concentrate diets.

TABLE 28. RUMEN FLUID AND BLOOD PARAMETERS FOR LAMBS FED SOYBEAN MEAL OR UREA AT VARIOUS LEVELS OF SUPPLEMENTATION

Item	Treatment	Protein level, % 11.2	12.5	13.8	15.1	Avg[Θ]
Rumen pH	Soybean meal	5.00	5.16	4.96	5.01	5.03
	Urea	5.00	5.04	5.12	5.13	5.07
Average		5.00	5.10	5.04	5.07	
Rumen lactic acid, μg/ml	Soybean meal	706.0	555.7	1360.6	822.6	861.2
	Urea	2030.4	387.3	271.0	91.3	695.0
Average[†]		1368.2[a]	471.5[b]	815.8[ab]	457.0[b]	
Rumen ammonia, mg/100 ml	Soybean meal	1.3	2.1	2.6	6.0	3.0[a]
	Urea	1.8	2.9	7.8	15.7	7.0[b]
Average[†]		1.6[a]	2.5[a]	5.2[b]	10.9[c]	
Blood-urea nitrogen, mg/100 ml	Soybean meal	11.3	12.7	15.0	15.5	13.6
	Urea	10.7	12.7	17.6	18.5	14.8
Average[†]		11.0[a]	12.7[ab]	16.3[b]	17.0[b]	

[Θ] Means within groups followed by unlike superscripts differ (P<.01).
[†] Means within rows followed by unlike superscripts differ (P<.05).

Rumen Lactic Acid. Lactic acid concentration in the rumen 3 h after feeding showed great variability between dietary treatments (table 28). Mackenzie (1967) reported that rapid production with accumulation of lactic acid often occurs following consumption of a large quantity of soluble carbohydrates. The conditions during this study as to diet composition and level of feed intake were favorable for lactic acid accumulation.

No particular trend as to lactic acid concentration was observed for the soybean meal-fed lambs at the various levels of protein supplementation during these studies, with lambs receiving the 13.8% protein diet showing the highest concentration among treatment groups. Lambs fed diets with urea generally had lower lactic acid concentrations than those fed diets with soybean meal and also showed a decline in the lactic acid concentration as level of protein supplementation increased. The wide range in lactic acid concentrations (91.3 to 2030.4 μg/ml) obtained during this study were within the range of 90 to 13500 μg/ml reported by Mackenzie (1967) as normal when feeding a variety of diets and were not indicative of problems with lactic acidosis.

When data were averaged for level of protein supplementation, it was observed in most instances that lambs fed protein-supplemented diets had lower ($P<.05$) concentrations of lactic acid in the rumen as compared to unsupplemented controls, with higher values for soybean meal ($P>.05$) than for urea at each level of protein.

Rumen Ammonia-Nitrogen. Rumen ammomia-nitrogen concentrations (table 28) increased with increasing levels of protein, with values for lambs fed urea being higher at all comparisons than those fed soybean meal. The spread between the urea and soybean meal groups increased as the level of protein supplementation increased.

Rumen ammonia-nitrogen concentrations were within the limits of 0 to 130 mg/100 ml reported by Hungate (1966) as being normal. However, only those lambs receiving urea in the 15.1% protein diet showed concentrations of rumen ammonia-nitrogen (15.7 mg/100 ml) within the ranges of 10 to 60 and 10 to 45 mg/100 ml reported by Lewis (1961) and Church (1976), respectively, as normal concentrations for ruminants.

Although there were some increases in rumen ammonia-nitrogen ($P>.05$) for lambs fed 12.5% protein diets over the controls, it was only at the 13.8% level of dietary protein that pronounced increases ($P<.05$) were observed. Tagari et al. (1964), Hudson et al. (1969) and McIntyre (1970) in studies with lambs and Veira et al. (1980a) with cattle also observed significant increases in rumen ammonia-nitrogen with each increment in the level of protein in the diet. Veira et al. (1980a) fed diets ranging from 10 to 14.1% protein and obtained rumen ammonia-nitrogen varying between 1.89 to 8.94 mg/100 ml, levels which were similar to those observed in the present study.

High concentrations ($P<.05$) of ammonia-nitrogen in the rumen obtained for the urea group as compared to the soybean meal group during this study were also reported by Schmidt et al. (1973) and Pendlum et al. (1976) in studies carried out with steers. There is no evidence

which would suggest that these trends would not hold true for lambs. Also, an insufficiency of nitrogen might have occurred for lambs showing rumen ammonia-nitrogen levels below 5 mg/100 ml. In this regard, Satter and Roffler (1977) reported that concentrations of ammonia-nitrogen in the rumen lower than 5 mg/100 ml limited microbial growth.

Blood Urea-Nitrogen. Jugular blood was utilized for these determinations and samples were collected 8 h after feeding. McIntyre and Williams (1970) conducted studies with lambs fed a variety of diets and found that blood urea-nitrogen generally peaked at 8 h after feeding.

Increased levels of urea-nitrogen in the blood were obtained when higher levels of nitrogen were supplied in the diet (table 28), with lambs fed the 13.8 and 15.1% protein diets showing the highest ($P<.05$) concentration. This increased blood urea-nitrogen concentration resulted with each protein source (soybean meal and urea) with larger values for urea at the two higher levels of supplementation.

Blood urea-nitrogen concentrations were within the range (10.5 to 25.0 mg/100 ml) reported by Somers (1961) for growing lambs fed diets with variable levels of nitrogen. The results are also in accordance with data reported by Church (1979) for a number of ruminants. Church reported a normal range of blood urea-nitrogen between 6.3 and 25.5 mg/100 ml (avg, 13.4 mg/100 ml) for lactating cows. For preruminant lambs, a range of 12 to 15 mg/100 ml was observed with an average of about 14.5 mg/100 ml.

A number of research papers have shown similar findings for blood urea-nitrogen as affected by levels of nitrogen supplementation. Among these were studies conducted by Lewis (1957), Preston et al. (1965), Coccimano and Leng (1966), McIntyre (1970) and Hammond (1983). In all these studies as during the one presented here, blood urea-nitrogen increased with increased nitrogen supplementation in the diet. It is important to note, however, that Lewis (1957) stated that changes in blood urea-nitrogen concentration were a direct result of ammonia production in the rumen and not a direct reflection of changes in the total nitrogen intake. The researcher indicated that the change in blood urea-nitrogen concentration followed increases or decreases in rumen ammonia-nitrogen after a delay of 4 to 8 h.

Similar concentrations of blood urea-nitrogen for lambs fed diets with soybean meal or urea as those observed during this study were also reported by Boling et al. (1972) in cattle experiments and by Prior et al. (1972) in studies conducted with lambs.

Rumen Volatile Fatty Acids. Total and individual concentrations of volatile fatty acids (VFA) in rumen fluid of lambs fed during this study are shown in table 29. Averages for protein supplementation across sources of nitrogen indicated slight increases ($P > .05$) in total VFA with increasing levels of nitrogen supplementation in the diet. Lambs fed the soybean meal diets had slightly higher total VFA concentrations ($P > .05$) than lambs fed urea. Differences observed between urea and soybean meal groups at 11.2% protein were not expected and are probably due to individual variations rather than dietary effects, since

TABLE 29. RUMEN VFA RESULTS FOR LAMBS FED SOYBEAN MEAL OR UREA AT VARIOUS LEVELS OF SUPPLEMENTATION

Item	Treatment	Protein level, % 11.2	12.5	13.8	15.1	Avg
Total VFA,	Soybean meal	99.27	90.27	95.77	103.94	97.31
µmol/ml	Urea	84.43	96.09	97.37	98.76	94.17
Average		91.85	93.18	96.57	101.35	
Acetic acid,	Soybean meal	34.96	28.10	25.34	26.87	28.82
moles/100 moles	Urea	30.88	26.50	29.46	27.23	28.52
Average		32.92[a]	27.30[b]	27.40[b]	27.05[b]	
Propionic acid,	Soybean meal	33.33	40.91	48.26	48.05	42.64
moles/100 moles	Urea	47.09	44.75	42.53	41.51	43.97
Average		40.21	42.83	45.39	44.78	
Butyric acid,	Soybean meal	23.03	21.86	18.89	17.98	20.44
moles/100 moles	Urea	16.27	20.10	20.05	24.52	20.23
Average		19.65	20.98	19.47	21.25	
Valeric acid	Soybean meal	8.69	9.13	7.51	7.10	8.11
moles/100 moles	Urea	5.75	8.65	7.95	6.72	7.27
Average		7.22	8.89	7.73	6.91	
Acetic/propionic	Soybean meal	1.05	.69	.53	.56	.71
	Urea	.66	.59	.69	.66	.65
Average		.85[a]	.64[b]	.61[b]	.61[b]	

[a,b] Means within rows followed by unlike superscripts differ ($P<.05$).

both groups were consuming the same diet. Total VFA concentration increased thereafter at each level of protein supplementation for soybean meal and urea groups.

Data obtained during this study for total VFA followed trends as reported by Davis et al. (1957) who observed higher total VFA production which resulted from feeding increasing levels of protein to dairy cows. On the other hand, Cross et al. (1974) fed corn silage diets to steers and reported that total VFA production was unaffected by dietary protein source (urea and soybean meal) and level of protein supplementation. In other studies, Davis and Stallcup (1967) showed higher ($P<.05$) molar concentrations of total VFA for steers fed soybean meal than for those fed urea. The trends observed during the study presented herein were in the same direction as those reported by Davis and Stallcup (1967), but the magnitude of the difference was much less.

Acetic acid molar percent concentration (table 29) was lower ($P<.05$) for protein-supplemented lambs than for controls with no apparent differences between levels of supplementation. Source of nitrogen supplementation appeared to result in no difference as to acetic acid concentration in the rumen. Davis et al. (1957) also observed decreased levels of acetic acid at higher levels of protein supplementation for lactating cows. However, Glimp et al. (1967) reported increased acetic acid concentration when lambs were fed higher levels of protein.

Some variability was observed for propionic acid molar percentage concentrations (table 29). While propionic acid

concentrations generally tended to increase with increasing protein supplementation for the soybean meal-fed lambs, concentrations tended to decrease with increasing levels of protein supplementation for the urea group. However, when the data were averaged for levels of protein supplementation, a slight increase ($P > .05$) in molar percentage propionic acid was observed up to the 13.8% level of protein. Propionic acid concentrations were similar ($P > .05$) for the soybean meal and urea groups when averaged for source of protein supplementation.

Level of protein supplementation and source of nitrogen appeared to have no effect on butyric acid molar percentage concentration ($P < .05$). However, lambs fed soybean meal diets showed a slight decrease in butyric acid concentration at each increment in the level of protein supplementation, whereas lambs fed the urea diets generally showed increased butyric acid concentrations with increasing protein supplementation.

Valeric acid molar percentage data (table 29) showed no consistent trend as to level of protein and source of nitrogen supplementation. The small differences observed between the main effects were not significant ($P > .05$). No branched-chain fatty acids (isovaleric acid) were obtained during this trial.

There appeared to be almost no response regarding individual VFA (moles/100 moles) to protein sources and level of protein supplementation during this study, with the exception of acetic acid concentrations which decreased with protein supplementation. These results were consistent with data presented in the literature which generally

indicated that the individual VFA molar proportions were not significantly affected by level of dietary protein (Davis et al., 1957; Hudson et al., 1969; Cross et al., 1974; Veira et al., 1980a). Under the conditions of this experiment, only slight differences in individual molar proportions should be expected because the fermentative potential of the different diets fed were quite similar, since all diets contained 92% concentrate and 8% sun-cured alfalfa.

A wide range in the acetic:propionic acid ratios was observed during this study (table 29). This was especially true for lambs consuming the soybean meal diets (1.05 to .53). Acetic:propionic acid ratios for the urea groups ranged between .69 and .59. When data were averaged for levels of supplementation, it was observed that lambs showed narrowed ($P<.05$) acetic:propionic acid ratios when protein was supplemented in the diet. Only slight differences were observed between sources of protein supplementation ($P>.05$) with the soybean meal group showing the wider ratios. Data presented by Glimp et al. (1967) and Hudson et al. (1969) showed acetic:propionic ratios to be higher than 1. However, diets fed did not contain as high levels of concentrates as those fed during this study. As reported by Woods and Luther (1962), pelleting may have also contributed to the low acetic:propionic acid ratios observed during the present study.

SUMMARY

Three experiments involving feeding trials, digestion-nitrogen balance trials, rumen fermentation studies and blood parameter determinations were conducted to evaluate protein utilization by cattle and sheep as influenced by feed source (represented by ingredients varying in protein solubility or degradability and amino acid profile), processing treatment and level of supplementation. For the feeding trial of experiment one, 288 Hereford, Angus and Hereford-Angus steers (291 kg) were fed for 124 d to a final weight of about 455 kg. The experimental diets consisted of 92% ground ear corn and 8% supplement. The supplements fed were control, urea, regular soybean meal and a special heat-treated soybean meal and diets contained 9.38, 11.97, 11.78 and 12.03% protein (dry), respectively.

Average daily gain, feed intake and feed conversion over the 124-d experiment for the control, urea, soybean meal and heat-treated soybean meal groups were (kg) 1.30, 8.79, 6.76; 1.34, 8.51, 6.35; 1.35, 8.54, 6.33 and 1.35, 8.39, 6.21, respectively. These data indicated good performance under the conditions of the experiment as to cattle and diets. A weight-gain response ($P<.05$) over the control group was observed for each source of soybean meal but was limited mostly to the first 2 mo of the experiment. Steers fed the urea-supplemented diet gained at essentially the same rate as the control during the first month. Thereafter, these steers gained at about the same rate as those fed either soybean meal-supplemented diet, resulting in similar performance upon termination of the experiment. There were only small

differences in feed intake between dietary treatment groups. Each source of supplemental protein improved feed efficiency over the control with greater improvements ($P<.05$) for soybean meal treatment groups. Feeding steers diets containing heat-treated soybean meal resulted in slightly lower feed requirements than for those fed regular soybean meal.

For further evaluation of these diets, a digestion-nitrogen balance trial was conducted using wether lambs as the experimental animals (25 to 27 kg). They were adapted to the ground ear corn diet over a period of 4 wk and allotted into groups of six lambs (32.5 kg) each for the four supplemental treatments. Crude protein contents for the diets (92% ground ear corn and 8% supplement) during this phase of the experiment were 9.6, 12.6, 11.7 and 12.5%, respectively, for the control, urea, soybean meal and heat-treated soybean meal treatments.

Similar apparent digestibilities for organic matter and crude protein were observed for all dietary treatments. Digestibilities of organic matter and crude protein for control, urea, soybean meal and heat-treated soybean meal diets were (%) 75.6, 74.8, 72.0 and 72.0; 68.9, 72.4, 69.0 and 69.4, respectively. Supplemental sources of protein did not result in any improvement in digestibility of organic matter or of crude protein over the control diet, indicating that it contained sufficient protein for optimum nutrient digestibility. Nitrogen retained (percent of intake) for the dietary treatments in the order listed were 32.80, 36.42, 38.48 and 37.15%, respectively. These values showed some increase ($P>.05$) in percent nitrogen retained over

the controls for protein-supplemented groups with similar values for each source of supplemental protein.

Following the digestion-nitrogen balance trial, rumen fermentation and blood parameter determinations were carried out after allowing a suitable period for the lambs to adjust to the new experimental conditions and training to consume the allotted feed within a 3-h period. Rumen samples were taken at 3 h after feeding, while blood samples were drawn from the jugular vein at 8 h postprandial. Two lambs from each dietary treatment were sampled each day with the procedure repeated using the same lambs a week later.

Rumen pH was higher ($P<.05$) with the control diet as compared to protein-supplemented diets. Great variability was observed for rumen lactic acid concentrations between dietary treatments ($P>.05$) with higher concentrations from protein-supplemented diets. All protein-supplemented diets resulted in greater ammonia-nitrogen concentrations in the rumen fluid than for the control, with the urea diet having the highest concentration ($P<.05$). Plasma ammonia-nitrogen concentrations were similar for all dietary treatments.

Total VFA concentrations in the rumen from the control, urea, soybean meal and heat-treated soybean meal were 60.1, 79.3, 89.3 and 75.0 μmol/ml of fluid, respectively. Protein-supplemented diets resulted in higher ($P<.05$) total VFA concentrations than did the control diet. Among protein-supplemented diets, soybean meal resulted in higher total VFA concentrations than did the heat-treated soybean meal ($P<.05$).

Percent molar concentrations of acetic acid obtained during these studies were greater for the control diet than for protein-supplemented diets, with some differences observed between the control and the soybean meals ($P<.05$). Differences between control and protein-supplemented diets for propionic acid (moles/100 moles) varied between 2.79 and 6.46 percentage units with the protein-supplemented diets giving higher concentrations ($P>.05$). Molar percentages for butyric, valeric and isovaleric acid varied only slightly ($P>.05$) between dietary treatments. Acetic to propionic acid ratio was slightly higher ($P>.05$) for the control diet than for diets supplemented with protein (.57 vs .43 to .44).

Hourly rumen fluid and plasma parameter determinations were obtained during a 1-d study by selecting two lambs from each dietary treatment. For this study rumen fluid samples were collected before feeding and at 1, 2, 3, 4 and 5 h after feeding, while blood samples were drawn before feeding and at 6, 8 and 10 h postprandial.

Results obtained during this hourly study indicated that rumen fluid and blood parameters showed some variability between dietary treatments. Rumen pH showed a steady decline to reach a low between 2 to 3 h after feeding, with protein-supplemented diets having the lowest pH values. Rumen ammonia-nitrogen concentrations were higher at each sampling period for diets with urea than the other dietary treatment groups with the control group showing the lowest concentrations. While total VFA concentrations in the rumen fluid increased with time, molar proportions of propionic acid increased readily only up to 1 h

postprandial. Valeric acid molar percentage concentrations, on the other hand, showed increased levels with increasing time after feeding, whereas isovaleric acid concentrations decreased. Acetic to propionic acid ratio was high before feeding and declined steadily up to 4 h after feeding with lambs fed protein-supplemented diets showing smaller ratios at this time. Blood ammonia-nitrogen and rumen lactic, acetic and butyric acids showed no apparent trends during this study. The results from this study indicated that, even though there were some differences between dietary treatments, it appeared that maximum activity for all diets occurred between 2 to 4 h.

These results showed that animal performance improved with protein supplementation, with only slight differences between the sources of supplemental protein. In terms of nutrient digestibility, nitrogen balance and rumen fermentation, it was observed that there were only small differences between the four dietary treatment groups. The data suggest that under these conditions the protein content of the control diet was sufficient for maximum rumen microbial activity.

During experiment two, various sources of supplemental protein were evaluated in a 105-d feeding trial with 192 steers (236 kg) of mixed breeding. Protein sources used during this experiment were fed alone and(or) in some combinations to give diets with a greater range in protein solubility or degradability and amino acid profile as compared to those diets fed during the previous experiment. The dry diet consisted of 10% supplement and 90% corn silage (34% DM). Dietary treatments were control, urea, soybean meal, heat-treated (160 C) blend

of 60% soybean meal and 40% ground whole soybeans, urea-dehydrated alfalfa meal and soybean meal-dehydrated alfalfa meal. Dehydrated alfalfa meal comprised 8.2 and 4.4%, respectively, of the dry diets. Each diet contained 9.19, 11.64, 11.22, 11.29, 11.20 and 11.23% protein, respectively.

Average daily gain, feed intake and feed conversion (kg) for these diets over the 105-d experiment were .80, 5.77, 7.21; 1.07, 6.57, 6.14; 1.10, 6.61, 6.01; 1.11, 6.66, 6.00; 1.07, 6.78, 6.34 and 1.10, 6.71, 6.10, respectively. Faster rates of gain ($P<.01$), higher feed intake ($P<.05$) and lower feed requirements ($P<.05$) were obtained for steers fed diets with supplemental protein as compared to those fed the control. Performance was similar for steers fed all diets which contained soybean meal. However, there was a slight reduction in average daily gain from steers fed urea due primarily from a lower initial response to supplementation. The combination of dehydrated alfalfa meal with urea or soybean meal did not appear to offer any improvement in feedlot performance as compared to these supplements without the dehydrated alfalfa.

For further evaluation of these diets through nutrient digestibility and nitrogen balance, wether lambs (27.3 kg) were adapted during a period of 28 d to a basal diet of corn silage (37.3% DM). Thereafter, lambs were allotted into six groups and fed a dry diet consisting of 90% corn silage and 10% supplement. The dietary treatments were formulated to be similar in ingredient composition as those fed during the cattle feeding trial and were control, urea, soybean meal, heat-treated soybean

meal-whole soybeans, urea-dehydrated alfalfa meal and a soybean meal-dehydrated alfalfa meal supplement. Dry diets contained 8.1, 11.4, 9.3, 10.5, 10.3 and 9.9% crude protein, respectively.

Nutrient digestibility data showed that there were only slight differences ($P>.05$) in dry matter (63.7 to 65.9%) and organic matter (65.4 to 67.4%) apparent digestibilities between dietary treatments. However, crude protein digestibility for the different dietary treatments was 55.0, 66.9, 60.1, 61.0 and 59.0%, respectively. Digestibility of crude protein was higher ($P<.05$) for protein-supplemented diets than for the control, with the urea diet having the highest digestibility ($P<.05$).

Fecal nitrogen as a percentage of the total nitrogen intake was higher ($P<.05$) for the control diet than for protein-supplemented diets. Among protein-supplemented diets, it was observed that the urea diet had the lowest ($P<.05$) amount of fecal nitrogen excreted. Urinary nitrogen losses (percent of intake) varied only slightly among treatments, while nitrogen retained ranged from 20% for the control diet to about 31% for the urea diet ($P>.05$).

These results with corn silage diets showed a response in animal performance to protein supplementation similarly as for experiment one with ear corn diets. There were only slight differences in animal performance among the sources of supplemental protein and generally no differences among sources as measured by nutrient digestibility and nitrogen balance.

Experiment three was conducted to evaluate lamb responses to soybean meal and urea at various levels of supplementation. For the 81-d feeding trial, 384 ewe and wether lambs averaging 33.4 kg were allotted on the basis of weight into a 2 x 4 factorial with six replications per treatment. The approximate protein averages for each source of supplementation were 11.2, 12.5, 13.8 and 15.1% of the total dry diet consumed. The pelleted basal diet consisted of ground corn and sun-cured alfalfa meal (8% dry basis) with mineral and vitamin supplements. Prior to feeding the experimental diets, all lambs were adapted to the unsupplemented control diet over a period of 3 wk. Thereafter, all dietary treatments were offered for the first time on day 1 of the experiment.

Average daily gain, daily dry feed intake and feed conversion for soybean meal at 11.2, 12.5, 13.8 and 15.1% protein were (kg) .216, 1.10, 5.09; .217, 1.12, 5.16; .243, 1.18, 4.86 and .254, 1.18, 4.65, respectively. Comparable values for the urea groups at 11.2, 12.5, 13.8 and 15.1% protein were (kg) .209, 1.10, 5.26; .231, 1.17, 5.06; .244, 1.19, 4.88 and .251, 1.22, 4.86, respectively. A slight increase in feed intake ($P<.06$) was obtained with increasing levels of protein supplementation. Average daily gain and feed conversions were improved ($P<.01$) with protein supplementation at the various levels fed up to the highest level but decreasing in degree with increasing levels of protein. There were no differences in average daily gain, feed intake or feed efficiency ($P>.05$) between soybean meal and urea at the various levels as fed during this experiment.

Digestion-nitrogen balance trials were conducted with lambs (20.3 to 29.5 kg) to study adaptational responses to the various diets fed during the feeding trial. These studies were conducted in two phases with 2 mo elapsing between phases. During each phase 24 lambs were placed in metabolism crates and adapted to the pelleted control diet. On day 1, all dietary treatments were fed for the first time. Feces, urine and orts were collected during three separate periods. The first two periods of each phase consisted of 3-d total collection studies and were carried out during day 2 through 4 and day 8 through 10. The third collection period was a 5-d total collection study and was conducted during day 14 through 18. Data obtained during both phases were pooled and analyzed as a 2 x 3 x 4 factorial (protein source x collection period x protein level).

Lambs showed only slight differences in dry matter and organic matter apparent digestibilities during each collection period, exhibiting slightly higher digestibility coefficients during the first collection period. Urea-fed lambs digested the dry matter and organic matter at a higher rate ($P<.05$) than lambs fed soybean meal. Lambs supplemented with protein showed improved ($P<.05$) dry matter and organic matter digestibility coefficients when compared to those fed the control diet. Lambs fed the 12.5% protein diets had the highest digestibility of dry matter and organic matter. Crude protein digestibility varied slightly between collection periods. There were no differences in digestibility of crude protein due to source of protein supplementation, but digestibility increased with level of protein

supplementation up to 13.8% protein. The nutrient digestibility data suggested that there appeared to be no evidence of a period of adaptation to urea under the conditions of the experiment.

Fecal nitrogen as a percentage of nitrogen intake appeared to be unaffected by the type of dietary nitrogen and only small differences were observed between collection periods. Lambs supplemented with protein (12.5, 13.8 and 15.1%) showed lower losses of nitrogen ($P<.05$) in the feces than those fed the unsupplemented controls. Urinary nitrogen losses increased at each collection period. Lambs fed urea diets had greater ($P<.05$) levels of nitrogen excretion in the urine than those fed the soybean meal diets. Higher levels of nitrogen were lost in the urine as protein supplementation increased, with the two highest levels showing the greatest losses.

Nitrogen retained as a percentage of the nitrogen intake decreased slightly at each period of collection ($P<.05$), but those lambs consuming soybean meal diets retained higher ($P<.05$) levels of nitrogen than those fed diets with urea. Nitrogen retention coefficients were higher for protein-supplemented groups than for the controls ($P>.05$), with lambs fed the 12.5% protein diets showing the highest percentage of nitrogen being retained. These data also suggest that no apparent period of adaptation to urea was needed during these studies and on the basis of nitrogen balance the 12.5% protein diet appeared to be the optimum diet.

After the digestion-nitrogen balance trial of each phase, rumen fermentation and blood parameter studies were conducted by using two

lambs from each dietary treatment for a total of 16 lambs per phase. Rumen fluid samples were drawn from eight lambs (one from each dietary treatment) on each collection day at 3 h after feeding, while blood samples were taken 8 h postprandial. Collections from each lamb were repeated 4 d later.

Rumen pH, lactic acid and blood urea-nitrogen were not affected by source of nitrogen supplementation, but rumen ammonia-nitrogen was higher ($P<.05$) for lambs fed the urea diets than for those fed the soybean meal diets. Only rumen pH was not affected by level of protein supplementation. Lactic acid concentration in the rumen was greater for the control group than for the protein-supplemented groups. Rumen ammonia-nitrogen and blood urea-nitrogen increased with increasing level of supplementation, especially for those lambs fed the 13.8 and 15.1% protein diets.

Total VFA concentrations and acetic, propionic, butyric and valeric acids molar percentage concentrations were not affected by source of protein supplementation. Only acetic molar percentage concentrations were affected by level of protein supplementation, showing higher concentrations ($P<.05$) for the control group than for lambs supplemented with protein. This resulted in a greater acetic to propionic acid ratio ($P<.05$) for lambs fed the control diets in comparison to those fed the protein-supplemented diets. In general, rumen fermentative activity was more adequate for those lambs receiving larger proportions of protein in the diet with no apparent difference between sources of protein supplementation.

Results of the lamb feeding trial showed that animal performance was improved up to the highest level of supplementation (15.1%), but a decline in the rate of increase was observed at the 15.1% protein level. This level of supplementation may have approached or exceeded the protein requirements of these lambs under the conditions of the experiment. As for nutrient digestibility, nitrogen balance and rumen fermentation studies, it was observed that generally lambs fed diets containing 12.5% protein showed optimum digestibility of organic matter and nitrogen balance. Digestibility of crude protein, total volatile fatty acid concentration and rumen ammonia-nitrogen appeared optimum at the 13.8% level of protein. It was also observed during these studies that for the most part similar performance was obtained with lambs fed diets with urea as for those fed diets containing soybean meal.

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APPENDIX

TABLE 1. ANALYSIS OF VARIANCE FOR AVERAGE DAILY GAIN DURING THE FEEDING TRIAL (EXPERIMENT ONE)

Source	df	Mean squares
Total	286	
Rep	8	1.013332
Trt	3	.289576
Rep x Trt	24	.240899
Error	251	.134097

TABLE 2. ANALYSIS OF VARIANCE FOR AVERAGE DAILY FEED INTAKE DURING THE FEEDING TRIAL (EXPERIMENT ONE)

Source	df	Mean squares
Total	35	
Rep	8	.928619
Trt	3	1.223477
Rep x Trt	24	.719019

TABLE 3. ANALYSIS OF VARIANCE FOR FEED TO GAIN RATIO DURING THE FEEDING TRIAL (EXPERIMENT ONE)

Source	df	Mean squares
Total	35	
Rep	8	7096.757
Trt	3	6442.444
Rep x Trt	24	1282.174

TABLE 4. ANALYSIS OF VARIANCE FOR PERCENT DRY MATTER DIGESTIBILTY DURING DIGESTION-NITROGEN BALANCE TRIAL (EXPERIMENT ONE)

Source	df	Mean squares
Total	23	
Trt	3	15.26181
Error	20	10.94502

TABLE 5. ANALYSIS OF VARIANCE FOR PERCENT ORGANIC MATTER DIGESTIBILITY DURING DIGESTION-NITROGEN BALANCE TRIAL (EXPERIMENT ONE)

Source	df	Mean squares
Total	23	
Trt	3	21.19845
Error	20	11.49368

TABLE 6. ANALYSIS OF VARIANCE FOR PERCENT CRUDE PROTEIN DIGESTIBILITY DURING DIGESTION-NITROGEN BALANCE TRIAL (EXPERIMENT ONE)

Source	df	Mean squares
Total	23	
Trt	3	16.02292
Error	20	8.62524

TABLE 7. ANALYSIS OF VARIANCE FOR GRAMS OF NITROGEN CONSUMED DURING DIGESTION-NITROGEN BALANCE TRIAL (EXPERIMENT ONE)

Source	df	Mean squares
Total	23	
Trt	3	2052.6069
Error	20	125.1271

TABLE 8. ANALYSIS OF VARIANCE FOR GRAMS OF FECAL NITROGEN/LAMB DURING DIGESTION-NITROGEN BALANCE TRIAL (EXPERIMENT ONE)

Source	df	Mean squares
Total	23	
Trt	3	7.338385
Error	20	.442017

TABLE 9. ANALYSIS OF VARIANCE FOR GRAMS OF URINARY NITROGEN/LAMB DURING DIGESTION-NITROGEN BALANCE TRIAL (EXPERIMENT ONE)

Source	df	Mean squares
Total	23	
Trt	3	6.782292
Error	20	.827205

TABLE 10. ANALYSIS OF VARIANCE FOR GRAMS OF NITROGEN RETAINED/LAMB DURING DIGESTION-NITROGEN BALANCE TRIAL (EXPERIMENT ONE)

Source	df	Mean squares
Total	23	
Trt	3	15.625685
Error	20	2.813724

TABLE 11. ANALYSIS OF VARIANCE FOR PERCENT FECAL NITROGEN OF TOTAL NITROGEN CONSUMED/ LAMB DURING DIGESTION-NITROGEN BALANCE TRIAL (EXPERIMENT ONE)

Source	df	Mean squares
Total	23	
Trt	3	16.022916
Error	20	8.625238

TABLE 12. ANALYSIS OF VARIANCE FOR PERCENT URINARY NITROGEN OF TOTAL NITROGEN CONSUMED/ LAMB DURING DIGESTION-NITROGEN BALANCE TRIAL (EXPERIMENT ONE)

Source	df	Mean squares
Total	23	
Trt	3	54.28296
Error	20	45.13564

TABLE 13. ANALYSIS OF VARIANCE FOR PERCENT NITROGEN RETAINED OF TOTAL NITROGEN CONSUMED/LAMB DURING DIGESTION-NITROGEN BALANCE TRIAL (EXPERIMENT ONE)

Source	df	Mean squares
Total	23	
Trt	3	37.27131
Error	20	58.05814

TABLE 14. ANALYSIS OF VARIANCE FOR BLOOD AMMONIA-NITROGEN DURING BLOOD PARAMETER STUDIES (EXPERIMENT ONE)

Source	df	Mean squares
Total	23	
Trt	3	.521250
Error	20	3.018542

TABLE 15. ANALYSIS OF VARIANCE FOR RUMEN pH DURING RUMEN FERMENTATION STUDIES (EXPERIMENT ONE)

Source	df	Mean squares
Total	23	
Trt	3	.543451
Error	20	.048862

TABLE 16. ANALYSIS OF VARIANCE FOR RUMEN LACTIC ACID DURING RUMEN FERMENTATION STUDIES (EXPERIMENT ONE)

Source	df	Mean squares
Total	23	
Trt	3	12124093752
Error	20	7918197384

TABLE 17. ANALYSIS OF VARIANCE FOR RUMEN AMMONIA-NITROGEN DURING RUMEN FERMENTATION STUDIES (EXPERIMENT ONE)

Source	df	Mean squares
Total	23	
Trt	3	.00198907
Error	20	.00015862

TABLE 18. ANALYSIS OF VARIANCE FOR TOTAL VFA DURING RUMEN FERMENTATION STUDIES (EXPERIMENT ONE)

Source	df	Mean squares
Total	23	
Trt	3	881.0572
Error	20	115.0497

TABLE 19. ANALYSIS OF VARIANCE FOR ACETIC ACID MOLAR PERCENT DURING RUMEN FERMENTATION STUDIES (EXPERIMENT ONE)

Source	df	Mean squares
Total	23	
Trt	3	32.17776
Error	20	11.24779

TABLE 20. ANALYSIS OF VARIANCE FOR PROPIONIC ACID MOLAR PERCENT DURING RUMEN FERMENTATION STUDIES (EXPERIMENT ONE)

Source	df	Mean squares
Total	23	
Trt	3	44.74943
Error	20	37.80725

TABLE 21. ANALYSIS OF VARIANCE FOR BUTYRIC ACID MOLAR PERCENT DURING RUMEN FERMENTATION STUDIES (EXPERIMENT ONE)

Source	df	Mean squares
Total	23	
Trt	3	11.00693
Error	20	12.31951

TABLE 22. ANALYSIS OF VARIANCE FOR ISOVALERIC ACID MOLAR PERCENT DURING RUMEN FERMENTATION STUDIES (EXPERIMENT ONE)

Source	df	Mean squares
Total	23	
Trt	3	6.342262
Error	20	8.756026

TABLE 23. ANALYSIS OF VARIANCE FOR VALERIC ACID MOLAR PERCENT DURING RUMEN FERMENTATION STUDIES (EXPERIMENT ONE)

Source	df	Mean squares
Total	23	
Trt	3	8.143925
Error	20	4.207703

TABLE 24. ANALYSIS OF VARIANCE FOR ACETIC TO PROPIONIC ACID RATIO DURING RUMEN FERMENTATION STUDIES (EXPERIMENT ONE)

Source	df	Mean squares
Total	23	
Trt	3	.02451238
Error	20	.01113505

TABLE 25. ANALYSIS OF VARIANCE FOR RUMEN pH DURING HOURLY RUMEN FERMENTATION STUDIES (EXPERIMENT ONE)

Source	df	Mean squares
Total	47	
Trt	3	.343430
Hour	5	2.344707
Trt x Hour	15	.017868
Error	24	.171527

TABLE 26. ANALYSIS OF VARIANCE FOR RUMEN AMMONIA-NITROGEN DURING HOURLY RUMEN FERMENTATION STUDIES (EXPERIMENT ONE)

Source	df	Mean squares
Total	47	
Trt	3	.0000978
Hour	5	.0000058
Trt x Hour	15	.0000069
Error	24	.0000065

TABLE 27. ANALYSIS OF VARIANCE FOR RUMEN LACTIC ACID DURING HOURLY RUMEN FERMENTATION STUDIES (EXPERIMENT ONE)

Source	df	Mean squares
Total	47	
Trt	3	147566385990
Hour	5	56571913118
Trt x Hour	15	31676943234
Error	24	49305926959

TABLE 28. ANALYSIS OF VARIANCE FOR TOTAL VFA DURING HOURLY RUMEN FERMENTATION STUDIES (EXPERIMENT ONE)

Source	df	Mean squares
Total	47	
Trt	3	1262.2990
Hour	5	3133.1466
Trt x Hour	15	123.1435
Error	24	210.3890

TABLE 29. ANALYSIS OF VARIANCE FOR ACETIC ACID MOLAR PERCENT DURING HOURLY RUMEN FERMENTATION STUDIES (EXPERIMENT ONE)

Source	df	Mean squares
Total	47	
Trt	3	111.29742
Hour	5	26.16604
Trt x Hour	15	9.13871
Error	24	22.03190

TABLE 30. ANALYSIS OF VARIANCE FOR PROPIONIC ACID MOLAR PERCENT DURING HOURLY RUMEN FERMENTATION STUDIES (EXPERIMENT ONE)

Source	df	Mean squares
Total	47	
Trt	3	233.86945
Hour	5	68.21416
Trt x Hour	15	17.60887
Error	24	15.17640

TABLE 31. ANALYSIS OF VARIANCE FOR BUTYRIC ACID MOLAR PERCENT DURING HOURLY RUMEN FERMENTATION STUDIES (EXPERIMENT ONE)

Source	df	Mean squares
Total	47	
Trt	3	60.50480
Hour	5	2.77555
Trt x Hour	15	4.85783
Error	24	6.67505

TABLE 32. ANALYSIS OF VARIANCE FOR VALERIC ACID MOLAR PERCENT DURING HOURLY RUMEN FERMENTATION STUDIES (EXPERIMENT ONE)

Source	df	Mean squares
Total	47	
Trt	3	30.756441
Hour	5	12.177847
Trt x Hour	15	1.987119
Error	24	5.558010

TABLE 33. ANALYSIS OF VARIANCE FOR ISOVALERIC ACID MOLAR PERCENT DURING HOURLY RUMEN FERMENTATION STUDIES (EXPERIMENT ONE)

Source	df	Mean squares
Total	47	
Trt	3	3.300941
Hour	5	54.140198
Trt x Hour	15	2.527118
Error	24	1.814769

TABLE 34. ANALYSIS OF VARIANCE FOR ACETIC TO PROPIONIC ACID RATIO DURING HOURLY RUMEN FERMENTATION STUDIES (EXPERIMENT ONE)

Source	df	Mean squares
Total	47	
Trt	3	.1159154
Hour	5	.0469892
Trt x Hour	15	.0107320
Error	24	.0192105

TABLE 35. ANALYSIS OF VARIANCE FOR BLOOD AMMONIA-NITROGEN DURING HOURLY BLOOD PARAMETER STUDIES (EXPERIMENT ONE)

Source	df	Mean squares
Total	31	
Trt	3	2946.7083
Hour	3	3569.2083
Trt x Hour	9	1035.3472
Error	16	1261.6250

TABLE 36. ANALYSIS OF VARIANCE FOR AVERAGE DAILY GAIN DURING THE FEEDING TRIAL (EXPERIMENT TWO)

Source	df	Mean squares
Total	191	
Rep	3	.046258
Trt	5	2.274488
Rep x Trt	15	.071299
Error	168	.101058

TABLE 37. ANALYSIS OF VARIANCE FOR AVERAGE DAILY FEED INTAKE DURING THE FEEDING TRIAL (EXPERIMENT TWO)

Source	df	Mean squares
Total	23	
Rep	3	.08097
Trt	5	2.69960
Rep x Trt	15	.14869

TABLE 38. ANALYSIS OF VARIANCE FOR FEED TO GAIN RATIO DURING THE FEEDING TRIAL (EXPERIMENT TWO)

Source	df	Mean squares
Total	23	
Rep	3	54.1111
Trt	5	9352.8666
Rep x Trt	15	352.1778

TABLE 39. ANALYSIS OF VARIANCE FOR PERCENT DRY MATTER DIGESTIBILITY DURING DIGESTION-NITROGEN BALANCE TRIAL (EXPERIMENT TWO)

Source	df	Mean squares
Total	23	
Trt	5	2.770124
Error	18	6.320601

TABLE 40. ANALYSIS OF VARIANCE FOR PERCENT ORGANIC MATTER DIGESTIBILITY DURING DIGESTION-NITROGEN BALANCE TRIAL (EXPERIMENT TWO)

Source	df	Mean squares
Total	23	
Trt	5	2.671816
Error	18	6.675370

TABLE 41. ANALYSIS OF VARIANCE FOR PERCENT CRUDE PROTEIN DIGESTIBILITY DURING DIGESTION-NITROGEN BALANCE TRIAL (EXPERIMENT TWO)

Source	df	Mean squares
Total	23	
Trt	5	59.682493
Error	18	6.933209

TABLE 42. ANALYSIS OF VARIANCE FOR GRAMS OF NITROGEN CONSUMED DURING DIGESTION-NITROGEN BALANCE TRIAL (EXPERIMENT TWO)

Source	df	Mean squares
Total	23	
Trt	5	686.87353
Error	18	72.29958

TABLE 43. ANALYSIS OF VARIANCE FOR GRAMS OF FECAL NITROGEN/LAMB DURING DIGESTION-NITROGEN BALANCE TRIAL (EXPERIMENT TWO)

Source	df	Mean squares
Total	23	
Trt	5	2.146405
Error	18	.562453

TABLE 44. ANALYSIS OF VARIANCE FOR GRAMS OF URINARY NITROGEN/LAMB DURING DIGESTION-NITROGEN BALANCE TRIAL (EXPERIMENT TWO)

Source	df	Mean squares
Total	23	
Trt	5	4.279526
Error	18	.727821

TABLE 45. ANALYSIS OF VARIANCE FOR GRAMS OF NITROGEN RETAINED/LAMB DUIRNG DIGESTION-NITROGEN BALANCE TRIAL (EXPERIMENT TWO)

Source	df	Mean squares
Total	23	
Trt	5	4.688873
Error	18	1.535859

TABLE 46. ANALYSIS OF VARIANCE FOR PERCENT FECAL NITROGEN OF TOTAL NITROGEN CONSUMED/LAMB DURING DIGESTION-NITROGEN BALANCE TRIAL (EXPERIMENT TWO)

Source	df	Mean squares
Total	23	
Trt	5	59.682493
Error	18	6.933209

TABLE 47. ANALYSIS OF VARIANCE FOR PERCENT URINARY NITROGEN OF TOTAL NITROGEN CONSUMED/LAMB DURING DIGESTION-NITROGEN BALANCE TRIAL (EXPERIMENT TWO)

Source	df	Mean squares
Total	23	
Trt	5	5.349103
Error	18	46.617993

TABLE 48. ANALYSIS OF VARIANCE FOR PERCENT NITROGEN RETAINED OF TOTAL NITROGEN CONSUMED/LAMB DURING DIGESTION-NITROGEN BALANCE TRIAL (EXPERIMENT TWO)

Source	df	Mean squares
Total	23	
Trt	5	54.090896
Error	18	41.838111

TABLE 49. ANALYSIS OF VARIANCE FOR AVERAGE DAILY GAIN DURING THE FEEDING TRIAL (EXPERIMENT THREE)

Source	df	Mean squares
Total	370	
Rep	5	.028245
Source	1	.000005
Rep x Source	5	.021429
Level	3	.152503
Rep x Level	15	.020265
Source x Level	3	.008392
Rep x Source x Level	15	.017264
Error	323	.014019

TABLE 50. ANALYSIS OF VARIANCE FOR AVERAGE DAILY FEED INTAKE DURING THE FEEDING TRIAL (EXPERIMENT THREE)

Source	df	Mean squares
Total	47	
Rep	5	.021132
Source	1	.048769
Rep x Source	5	.037189
Level	3	.117819
Rep x Level	15	.040062
Source x Level	3	.007397
Rep x Source x Level	15	.028110

TABLE 51. ANALYSIS OF VARIANCE FOR FEED TO GAIN RATIO DURING THE FEEDING TRIAL (EXPERIMENT THREE)

Source	df	Mean squares
Total	47	
Rep	5	1573.4375
Source	1	1271.0208
Rep x Source	5	1623.1708
Level	3	5333.8540
Rep x Level	15	595.0375
Source x Level	3	479.1319
Rep x Source x Level	15	752.6819

TABLE 52. ANALYSIS OF VARIANCE FOR DRY MATTER CONSUMED DURING DIGESTION-NITROGEN BALANCE ADAPTATIONAL STUDIES (EXPERIMENT THREE)

Item	df	Mean squares
Total	143	
Source	1	119418.15
Level	3	30222.54
Source x Level	3	92718.20
Period	2	346929.78
Source x Period	2	4730.43
Level x Period	6	15667.96
Source x Level x Period	6	8443.28
Error	120	54473.34

TABLE 53. ANALYSIS OF VARIANCE FOR PERCENT DRY MATTER DIGESTIBILITY DURING DIGESTION-NITROGEN BALANCE ADAPTATIONAL STUDIES (EXPERIMENT THREE)

Source	df	Mean squares
Total	143	
Source	1	125.0552
Level	3	139.1342
Source x Level	3	26.0997
Period	2	69.2361
Source x Period	2	2.9198
Level x Period	6	9.5923
Source x Level x Period	6	9.3062
Error	120	14.9558

TABLE 54. ANALYSIS OF VARIANCE FOR PERCENT ORGANIC MATTER DIGESTIBILITY DURING DIGESTION-NITROGEN BALANCE ADAPTATIONAL STUDIES (EXPERIMENT THREE)

Source	df	Mean squares
Total	143	
Source	1	111.5327
Level	3	141.8387
Source x Level	3	26.9903
Period	2	73.6317
Source x Period	2	2.2115
Level x Period	6	8.9171
Source x Level x Period	6	9.0311
Error	120	15.2478

TABLE 55. ANALYSIS OF VARIANCE FOR PERCENT CRUDE PROTEIN DIGESTIBILITY DURING DIGESTION-NITROGEN BALANCE ADAPTATIONAL STUDIES (EXPERIMENT THREE)

Source	df	Mean squares
Total	143	
Source	1	20.9003
Level	3	291.2273
Source x Level	3	75.7003
Period	2	48.1819
Source x Period	2	14.6229
Level x Period	6	27.6681
Source x Level x Period	6	8.5500
Error	120	18.2941

TABLE 56. ANALYSIS OF VARIANCE FOR GRAMS OF NITROGEN CONSUMED/LAMB DURING DIGESTION-NITROGEN BALANCE ADAPTATIONAL STUDIES (EXPERIMENT THREE)

Source	df	Mean squares
Total	143	
Source	1	136.9245
Level	3	118.5878
Source x Level	3	70.4483
Period	2	194.9345
Source x Period	2	7.0423
Level x Period	6	6.6966
Source x Level x Period	6	4.0572
Error	120	23.5562

TABLE 57. ANALYSIS OF VARIANCE FOR GRAMS OF FECAL NITROGEN/LAMB DURING DIGESTION-NITROGEN BALANCE ADAPTATIONAL STUDIES (EXPERIMENT THREE)

Source	df	Mean squares
Total	143	
Source	1	27.83709
Level	3	1.58996
Source x Level	3	4.37820
Period	2	18.80865
Source x Period	2	2.79356
Level x Period	6	.96002
Source x Level x Period	6	.34656
Error	120	3.44255

TABLE 58. ANALYSIS OF VARIANCE FOR GRAMS OF URINARY NITROGEN/LAMB DURING DIGESTION-NITROGEN BALANCE ADAPTATIONAL STUDIES (EXPERIMENT THREE)

Source	df	Mean squares
Total	143	
Source	1	1.74959
Level	3	39.07262
Source x Level	3	13.37836
Period	2	48.66965
Source x Period	2	.19316
Level x Period	6	2.18013
Source x Level x Period	6	.61807
Error	120	2.50548

TABLE 59. ANALYSIS OF VARIANCE FOR GRAMS OF NITROGEN RETAINED/LAMB DURING DIGESTION-NITROGEN BALANCE ADAPTATIONAL STUDIES (EXPERIMENT THREE)

Source	df	Mean squares
Total	143	
Source	1	60.03318
Level	3	21.66628
Source x Level	3	22.25814
Period	2	9.04069
Source x Period	2	2.63693
Level x Period	6	8.66019
Source x Level x Period	6	3.93424
Error	120	7.36893

TABLE 60. ANALYSIS OF VARIANCE FOR PERCENT FECAL NITROGEN OF TOTAL NITROGEN CONSUMED/LAMB DURING DIGESTION-NITROGEN BALANCE ADAPTATIONAL STUDIES (EXPERIMENT THREE)

Source	df	Mean squares
Total	143	
Source	1	20.90027
Level	3	291.22727
Source x Level	3	75.70029
Period	2	48.18189
Source x Period	2	14.62283
Level x Period	6	27.66805
Source x Level x Period	6	8.35501
Error	120	18.29412

TABLE 61. ANALYSIS OF VARIANCE FOR PERCENT URINARY NITROGEN OF TOTAL NITROGEN CONSUMED/LAMB DURING DIGESTION-NITROGEN BALANCE ADAPTATIONAL STUDIES (EXPERIMENT THREE)

Source	df	Mean squares
Total	143	
Source	1	518.0234
Level	3	332.3821
Source x Level	3	219.4936
Period	2	267.5656
Source x Period	2	62.4496
Level x Period	6	65.7833
Source x Level x Period	6	36.4343
Error	120	63.4813

TABLE 62. ANALYSIS OF VARIANCE FOR PERCENT NITROGEN RETAINED OF TOTAL NITROGEN CONSUMED/LAMB DURING DIGESTION-NITROGEN BALANCE ADAPTATIONAL STUDIES (EXPERIMENT THREE)

Source	df	Mean squares
Total	143	
Source	1	330.8196
Level	3	57.6417
Source x Level	3	206.1801
Period	2	153.9930
Source x Period	2	18.2221
Level x Period	6	154.3075
Source x Level x Period	6	67.7182
Error	120	75.1324

TABLE 63. ANALYSIS OF VARIANCE FOR BLOOD UREA-NITROGEN DURING BLOOD PARAMETER STUDIES (EXPERIMENT THREE)

Source	df	Mean squares
Total	31	
Source	1	11.8828
Level	3	66.4578
Source x Level	3	6.4111
Error	24	20.1468

TABLE 64. ANALYSIS OF VARIANCE FOR RUMEN pH DURING RUMEN FERMENTATION STUDIES (EXPERIMENT THREE)

Source	df	Mean squares
Total	31	
Source	1	.012207
Level	3	.015513
Source x Level	3	.032453
Error	24	.028649

TABLE 65. ANALYSIS OF VARIANCE FOR RUMEN LACTIC ACID DURING RUMEN FERMENTATION STUDIES (EXPERIMENT THREE)

Source	df	Mean squares
Total	31	
Source	1	2210189111
Level	3	14581314059
Source x Level	3	22627053596
Error	24	4946444054

TABLE 66. ANALYSIS OF VARIANCE FOR RUMEN AMMONIA-NITROGEN DURING RUMEN FERMENTATION STUDIES (EXPERIMENT THREE)

Source	df	Mean squares
Total	31	
Source	1	.013031
Level	3	.013716
Source x Level	3	.003786
Error	24	.000661

TABLE 67. ANALYSIS OF VARIANCE FOR TOTAL VFA DURING RUMEN FERMENTATION STUDIES (EXPERIMENT THREE)

Source	df	Mean squares
Total	31	
Source	1	79.0182
Level	3	143.5053
Source x Level	3	162.4870
Error	24	188.7994

TABLE 68. ANALYSIS OF VARIANCE FOR ACETIC ACID MOLAR PERCENT DURING RUMEN FERMENTATION STUDIES (EXPERIMENT THREE)

Source	df	Mean squares
Total	31	
Source	1	.69178
Level	3	64.42974
Source x Level	3	24.00709
Error	24	16.25845

TABLE 69. ANALYSIS OF VARIANCE FOR PROPIONIC ACID MOLAR PERCENT DURING RUMEN FERMENTATION STUDIES (EXPERIMENT THREE)

Source	df	Mean squares
Total	31	
Source	1	18.4300
Level	3	46.7518
Source x Level	3	174.1846
Error	24	80.7810

TABLE 70. ANALYSIS OF VARIANCE FOR BUTYRIC ACID MOLAR PERCENT DURING RUMEN FERMENTATION STUDIES (EXPERIMENT THREE)

Source	df	Mean squares
Total	31	
Source	1	.351751
Level	3	6.599028
Source x Level	3	61.941293
Error	24	24.314584

TABLE 71. ANALYSIS OF VARIANCE FOR VALERIC ACID MOLAR PERCENT DURING RUMEN FERMENTATION STUDIES (EXPERIMENT THREE)

Source	df	Mean squares
Total	31	
Source	1	5.56528
Level	3	6.02618
Source x Level	3	4.24043
Error	24	10.72134

TABLE 72. ANALYSIS OF VARIANCE FOR ACETIC TO PROPIONIC ACID RATIO DURING RUMEN FERMENTATION STUDIES (EXPERIMENT THREE)

Source	df	Mean squares
Total	31	
Source	1	.041946
Level	3	.105918
Source x Level	3	.123320
Error	24	.043384