The influence of sodium sulfate, methionine and fishmeal on the protein quantity in the rumen of sheep
by David Justin Hoss

A thesis submitted to the Graduate Faculty in partial fulfillment of the requirements for the degree of
MASTER OF SCIENCE in Animal Science
Montana State University

Abstract:
Four mature wethers were fed five semipurified diets. The basal ration (Diet 1) consisting of chopped hay, straw, corn cobs, corn starch, and biuret supplemented with one of the following ingredients: sodium sulphate (Diet 2), methionine (Diet 3), fishmieal (Diet 4), and soybean meal (Diet 5) . The animals were fed each diet 17 days prior to sampling. Rumen samples were obtained via permanent rumen fistula and blood samples obtained by jugular venipuncture. The rumen samples were TCA precipitated and nitrogen was determined by a modification of the micro-Kjeldahl procedure. Methionine was assayed because of its suspected role as the limiting amino acid in NPN diets. Blood urea nitrogen means for Diets 1 and 5 were significantly lower ($P<.05$) .

The other rations did not differ significantly. The nitrogen content of the TCA precipitates from the rumen samples were not significantly different. The nitrogen content of the rumen fluid for Diets 1, 2 and 4 differed significantly ($P<.05$) from Diets 3 and 5. The methionine levels of the rumen precipitate, as determined by microbiological assay, showed a pronounced influence due to the diet. The rumen precipitate from Diet 4, basal + fishmeal, was significantly higher in methionine than that from the remaining diets. Total amino acid analysis of the feed protein and rumen precipitate showed a marked effect due to sulfur source. The nitrogen retained in the rumen was influenced by the sulfur source. The rumen fluid and nitrogen content of the rumen precipitate was higher for diets 2, 3 and 4. Total nitrogen of diet 1 was slightly higher and did not follow this pattern.

Diet 5 retained less nitrogen in the rumen which was associated with an increase in the BUN.

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Date May 26, 1970

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Approved:

R. W. Blackwell
Head, Major Department

C. W. Newman
Chairman, Examining Committee

K. Goering
Graduate Dean

MONTANA STATE UNIVERSITY
Bozeman, Montana

June, 1970

ACKNOWLEDGEMENTS

I wish to express my gratitude and appreciation to Dr. C. W. Newman whose assitance is in large part responsible for the completion of this project. I also wish to thank Dr. R. L. Blackwell for his encouragement and help, which made my coming to and remaining at Montana State University possible.

I wish to thank Drs. K. D. Hapner and D. G. Stuart for their technical skill and assistance in obtaining the amino acid data. I also wish to thank Dr. J. E. Catlin for performing the surgery on the sheep and help in maintaining their health.

I also wish to thank Drs. O. O. Thomas, E. L. Moody, P. J. Burfening, and S. J. Rogers for their help and assistance for the past two years.

A special thank you goes to my wife, Peg, whose understanding, love, and support makes the effort worthwhile.

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ABSTRACT

Four mature wethers were fed five semipurified diets. The basal ration (Diet 1) consisting of chopped hay, straw, corn cobs, corn starch, and biuret supplemented with one of the following ingredients: sodium sulphate (Diet 2), methionine (Diet 3), fishmeal (Diet 4), and soybean meal (Diet 5). The animals were fed each diet 17 days prior to sampling. Rumen samples were obtained *via* permanent rumen fistula and blood samples obtained by jugular venipuncture. The rumen samples were TCA precipitated and nitrogen was determined by a modification of the micro-Kjeldahl procedure. Methionine was assayed because of its suspected role as the limiting amino acid in NPN diets. Blood urea nitrogen means for Diets 1 and 5 were significantly lower ($P<.05$). The other rations did not differ significantly. The nitrogen content of the TCA precipitates from the rumen samples were not significantly different. The nitrogen content of the rumen fluid for Diets 1, 2 and 4 differed significantly ($P<.05$) from Diets 3 and 5. The methionine levels of the rumen precipitate, as determined by microbiological assay, showed a pronounced influence due to the diet. The rumen precipitate from Diet 4, basal + fishmeal, was significantly higher in methionine than that from the remaining diets. Total amino acid analysis of the feed protein and rumen precipitate showed a marked effect due to sulfur source. The nitrogen retained in the rumen was influenced by the sulfur source. The rumen fluid and nitrogen content of the rumen precipitate was higher for diets 2, 3 and 4. Total nitrogen of diet 1 was slightly higher and did not follow this pattern. Diet 5 retained less nitrogen in the rumen which was associated with an increase in the BUN.

INTRODUCTION

Protein is normally the most expensive item in an animal's diet. Ruminants have the ability to use a significant percentage of non-protein nitrogen (NPN) to substitute for the more expensive natural proteins. It has been shown, however, that ruminants which have only non-protein nitrogen in their diet consistently exhibit growth curves of 70% of those animals fed natural protein diets.

The sulfur containing amino acids, particularly methionine, have been implicated as possibly the limiting amino acids in non-protein nitrogen diets (Hungate, 1966). The purpose of this study was to determine the effect of the addition of inorganic sulfate, methionine, and a methionine-rich natural protein to a basically non-protein nitrogen diet upon the protein produced in the rumen.

To evaluate the effect of these additives on the basal diet, the protein fraction of the rumen was hydrolized and analysis was made for changes in total nitrogen content and the percent methionine of the protein. An attempt was made to relate this information with the protein free amino acid composition of the rumen and plasma and blood urea nitrogen. Changes in the dietary protein quality, as well as quantity, have been observed to be related to these parameters.

The free amino acid composition of the rumen contents may be of questionable value. Nearly all proteinaceous material entering the rumen is reduced to ammonia and short-chain carbon compounds. Ammonia may be used by the microorganisms present if sufficient energy is

present at time the ammonia is released. If a large amount of ammonia is present, it may pass through the rumen wall. When the ammonia level passing the rumen wall is high, as may occur when excessive amounts of urea are fed, it is toxic to the animal and may prove fatal. The ammonia, regardless of concentration, is picked up in the portal vein and transported to the liver, where it is converted to urea with a large percentage excreted through the kidneys. Urea may be recycled through the animal's body and arrive back at the rumen either as a constituent of saliva, or if the blood level is higher than the rumen concentration, it may diffuse back into the rumen, where it can be used in the synthesis of microbial protein.

Free amino acids in the rumen fluid may be the result of microbial synthesis or the result of partial hydrolysis of injested protein but would not necessarily reflect the quality of protein progressing to the lower digestive tract and nourishing the animal. Block et al. (1951) determined that the largest portion of amino acids was present in the TCA precipitated portion of the rumen contents. The literature to date has not indicated where the major nitrogen concentration of the rumen contents is located. It would appear that this would depend on the protein in the diet, time after eating, and adaptation to the protein or nitrogen source.

In order to obtain maximum utilization of protein, it is necessary to have a significant quantity of high quality protein reaching the

small intestine. Methods of evaluating and improving the quality of this protein in the ruminant are the objectives of this study.

REVIEW OF LITERATURE

Non-Protein Diets

Non-protein nitrogen has been utilized in the diet of ruminants for some time. The most widely used source has been urea. Under some conditions there is a problem in feeding urea because it is toxic when fed in high concentrations. Urea has proven to be valuable as a nitrogen source in high concentrate diets. Recent South African research (Clarck et al., 1963, and McKenzie and Altona, 1964) showed biuret to be superior to urea when fed in low quality roughage diets. Animals fed urea diets lost weight and in general did poorly when compared to those fed biuret. During the early studies with biuret, it was noted that those animals used in a succeeding trial with diets containing biuret showed improved roughage utilization. This led to the conclusion that it was necessary to adapt animals to biuret for thirty days or more before they can effectively utilize biuret.

Much of the research to date has compared non-protein nitrogen diets to diets of isolated plant proteins using purified and semi-purified diets. This technique would appear to be valid in determining the comparative value of one nitrogen source to another. These comparisons have shown that diets containing NPN perform from 50-80% as well as the diets containing natural protein.

Many reasons for this difference have been suggested. They range from lack of vitamins and minerals to type of carbohydrates present and suspected amino acid deficiencies. It would appear from the general

trend of the literature that there is a basic deficiency of an unknown factor(s) in NPN diets but these ingredients remain undiscovered at the present time. Nitrogen balance studies have been conducted and there has been some improvement shown by adding many of the suspected missing ingredients to basically NPN diets. However, none of these additions have improved animal performance to equal that of animals fed natural or isolated protein sources. It is suspected that there is not a single limiting factor missing from these diets, but a combination of factors.

Munro and Allison (1964) state, "The ideal ration for maximum use of nitrogen should contain protein of good digestibility with a low solubility in the rumen. Rumen ammonia should be low and a large amount of intact protein passing to the abomasum." From this we should be able to adapt the principle to include NPN. Urea is highly soluble and produces large amounts of ammonia. Hungate (1966) postulated that rapid nitrogen assimilation by rumen microorganisms is the chief factor limiting the performance of the host animal. In order to get the best advantage from urea, it would be necessary to have energy available as ammonia is produced in the rumen. In order to allow the microorganisms to most efficiently utilize ammonia from urea, it should be produced in quantities proportional to rapid energy release.

Biuret has a low solubility in the rumen, and the adaptation of the animal and its micro-flora has improved its utilization (Clarck et al.,

1963). Ammonia is released much slower than from urea or highly soluble proteins and as a result the energy requirements of microorganisms synthesizing protein from biuret is spread over a longer period of time, which is more consistent with cellulose digestion. Ideally for those animals on high NPN diets, the ammonia production curve and the volatile fatty acid curves should rise and fall at nearly the same rate.

Rumen Protein

The worth of a protein source can be shown in various ways. Factors which may be observed are weight gains or losses, improvement in wool growth, and increase or decrease in milk production. Since the discovery that ruminants can utilize NPN to form protein and the subsequent realization that NPN was not utilized as efficiently as natural proteins, attempts have been made to improve the performance of animals on NPN diets by the addition of various dietary factors.

McDonald (1954) determined that 40-50% of zein protein was converted to microbial protein. He also stated that this figure may be higher for other diets. Duncan (1953), in studying the amino acid content of the rumen from natural and purified diets, found higher amino acid values for the natural protein. The purified diet used in Duncan's study was amino acid free so that any amino acids found were presumed to be the result of bacterial synthesis. Loosli et al. (1949) also studied the amino acid balance in sheep and found that urine and

feces losses of amino acids exceeded the intake, yet the animals gained weight, further indicating amino acid synthesis.

The nutritional value of rumen protein is realized by the animal after the protein is degraded in the abomasum and intestinal tract and the amino acids are absorbed into the animal's amino acid pool for synthesis of body protein, McLaren (1964).

Annison (1956) and Leibholz (1965) indicated that all protein entering the rumen is degraded first to free amino acids and subsequently to ammonia. The reduction to ammonia is quite rapid, which accounts for the low concentrations of free amino acids found in the rumen liquor. Annison (1956) indicated that amino acids are not transported across the rumen wall and as a result the source of amino acids for building body protein is passed into the intestinal tract.

McLaren (1964) indicated the bacterial protein formed in the rumen of animals on NPN diets was comparable to plant protein. Bergen et al. (1967) indicated that the bacterial protein was equal that of casein. It would appear then that protein produced in the rumen from NPN diets could be equal in quality to protein from natural sources, if all the factors associated with synthesis were made available.

Inorganic Sulfur

One of the factors mentioned most often concerning NPN diets is the presence or absence of sulfur compounds. It is substantially accepted that microorganisms can utilize many sources of sulfur in

the synthesis of methionine and cystine. Block et al. (1951) fed radio-sulfur in the form of sodium sulfate to ewes. The rumen liquor was precipitated with trichloroacetic acid, and the radioactivity of both the precipitate and the supernate was measured. The greatest percentage of radioactive sulfur was present in the proteinaceous precipitate, indicating that the bacteria had incorporated the inorganic sulfur into the sulfur amino acids.

Starck et al. (1954) studied the response of lambs fed varied levels of elemental sulfur, sulfate sulfur, and methionine. This study showed an improvement in weight gains and wool growth from the diet containing sulfur over non-supplemented diets. They found no difference due to source or level of sulfur fed.

Sokolowski et al. (1969) fed sulfur to lambs on NPN diets and indicated that nitrogen retention was improved but no effect on weight gain was shown. Albert et al. (1956) calculated the sulfur requirement of growing fattening lambs to be 0.64% of the diet when fed as sulfate sulfur and 0.47% of the diet when supplied as elemental sulfur. Whanger (1969) suggested that the level of sulfur is not as critical as the nitrogen-sulfur ratio. He postulated that this ratio should not exceed 10:1. In this study a definite sulfur deficiency was produced on a urea diet, which indicated that a sulfur deficiency limits the value of NPN diets.

Organic Sulfur

The relationship of methionine as a possible limiting amino acid in NPN diets has been studied for some time. Loosli and Harris (1945) suggested that the addition of urea to the diet produced an inefficient protein of low quality. When they added isolated methionine to a urea diet for lambs, the average daily gain improved. They postulated that the protein formed in the rumen by animals fed diets containing urea was deficient in methionine. The addition of methionine to the diet made it equal to the linseed oil meal based diet fed the control lambs. Lofgreen et al. (1947) also showed a significant increase in nitrogen retention with sheep fed urea based diets by the addition of 0.2% methionine.

Oltjen et al. (1962) fed a urea diet supplemented with 0.4% methionine without effect. The lack of effect in this study may have been due to the level of methionine fed. Albert et al. (1956) determined the level of methionine required by sheep to be 0.64% of the diet. McLaren (1965) reported that the addition of methionine and/or tryptophan increased nitrogen retention in lambs fed high levels of NPN.

Leibholz (1965), in studying the free amino acids in plasma and rumen liquor of sheep, found that methionine increases in plasma due to the diet were low, which suggested that methionine may have been a limiting amino acid. The relationship between blood levels of amino

acids and needs of the animal are discussed by Longenecher and Hause (1961). In another study, Schelling et al. (1967) studied the effect of amino acid supplementation. Their data suggested that a lack of methionine synthesis in the rumen was the result of a methionine deficiency in the diet. However, Clifford and Tillman (1968) found the methionine content of the rumen liquor changed little from urea to soybean diets. Oltjen et al. (1968) found that methionine was one of six amino acids in the blood plasma which was affected by the diet. Starks et al. (1954) found a significant response in sheep to the addition of methionine to an NPN diet. McCarthy et al. (1968) found that the addition of methionine to a diet for dairy cows improved production. In association with this, animals on NPN diets had a higher incidence of ketosis, which was related to low levels of methionine in the diet and subsequently in the blood.

Protein Quality

Research indicates that the quality of protein fed to ruminants may be as important as the quantity (Loosli and Harris, 1945; Oltjen et al., 1962; Oltjen and Putnam, 1966; Matrone et al., 1964; and Bunn et al., 1968). Matrone et al. (1964) substituted urea for casein and depressed the performance of lambs. This depression was reduced when alfalfa was added to the urea diet. Bunn et al. (1968) used a basal diet of urea and added alfalfa and amino acids based on the amino acid composition of alfalfa. The alfalfa and amino acid supplemented

diets were superior to the basal diet.

Oltjen (1969) suggests several reasons for the inconsistency of the results in the studies where NPN diets were supplemented with amino acids. Most of the studies used the DL form of the amino acid rather than the active L form. The amino acids may have been too readily available in the rumen and were rapidly catabolized instead of being incorporated into microbial protein. The amino acid added was not limiting growth.

Evaluation Techniques

In deciding which analytical methods were to be used, it was necessary to relate recognized evaluation methods to the particular problem. The main objective was to determine the value of the feeds to the animal for protein synthesis. Determining the value of a protein source to ruminants is particularly difficult because of the action of proteolytic bacteria. In this study, four maintenance diets with biuret as the primary source of nitrogen were altered by the addition of various sulfur sources. The sources were sodium sulfate, feed grade DL methionine, and methionine rich fishmeal. These diets were compared to a basal diet which was not supplemented with sulfur and the basal supplemented with soybean meal.

The amount of protein which is reduced to ammonia in the rumen is dependent on the type and amount of protein in the diet. McDonnald (1954) contended that the main nitrogen component of NPN diets was

found as ammonia in the rumen. Chalupa et al. (1964) stated that as the microbial activity increased, rumen ammonia was higher. Munro (1964) stated that very little plant protein leaves the rumen intact and that most of the protein passed to the omasum was the protoplasmic protein of bacteria and protozoa. Microbial biosynthesis of protein is not comparable in rate to urea hydrolyses.

The above statements indicate it is necessary to measure the amount of ammonia produced in the rumen of animals fed a particular diet in order to evaluate nitrogen metabolism in ruminants. Lewis (1957) indicated two direct methods of determining rumen ammonia: measuring the actual concentration in the rumen and measuring the ammonia concentration in the portal blood system. However, the first method is subject to wide variation over a short period of time; and the latter method is difficult as the blood flow must be measured and this measurement requires cannulation of the portal vein. Indirect methods include a nitrogen balance and measurement of the blood urea nitrogen.

Lewis (1957) in his review states, "It is clear that there is a loss of ammonia from the rumen of sheep and the extent of that loss is proportional to the concentration of ammonia in the rumen." The ammonia lost from the rumen is picked up by the portal vein and carried to the liver where it is converted to urea. As the diet fed to sheep becomes more likely to produce rumen ammonia, so there is an increase in blood urea. The blood urea nitrogen (BUN) in sheep is fairly

constant but is dependent upon the diet. Changes in BUN concentration occur nearly as rapidly as rumen ammonia concentrations; however, BUN tends to reach a maximum four hours after feeding. Variation tends to be greater when sheep are fed a diet which produces high levels of rumen ammonia. It has been shown that the concentration of urea in the peripherial blood follows very closely the changes in rumen ammonia without the marked diurnal variation in the latter (Lewis, 1957). Fluctuations in the BUN concentrations probably arise following the absorption of NPN from the rumen and the intestine. As the BUN levels rise, there is an increased loss of nitrogen through the urine.

Lewis (1957) stated that when BUN levels exceeded 50 mg./100 ml., there was too much rumen ammonia present and resulted in inefficient utilization. Munro (1964) observed that high concentrations of alpha amino nitrogen were present when soluble carbohydrates were absent from the diet.

Lewis (1957) made the following conclusions concerning BUN concentrations:

a. For a given diet, the BUN concentration will remain constant.
b. Changes in the diet are indirectly reflected by BUN level changes which are in direct response to rumen ammonia levels.
c. Fluctuations in the BUN levels are not due to overall nitrogen intake.

It would appear from the above information that low blood urea levels

(20-40 mg./100 ml.) indicate nominal ammonia losses and consequently efficient utilization of ammonia by the rumen microorganisms.

Amino Acid Determinations

The importance of amino acids in the nutrition of monogastrics is well recognized. Because of the proteolytic action of rumen microorganisms, however, the amino acid requirements of the ruminant are less understood. In recent years, the amino acid requirements of ruminants have been studied, in an attempt to obtain maximum performance of ruminants through a greater understanding of their amino acid requirements.

McLaren (1964) stated the nutritional significance of injested protein was realized by the ruminant as it became digested and absorbed. This suggested that the amino acid composition of the protein was important. Loosli and Harris (1945) suggested that the protein formed in the rumen was deficient in methionine. Ellis et al. (1956) studied the nutritional value of bacterial protein from several sheep diets and found them to be unequal. Urea and gelatin diets had similar biological values but were lower in value than a casein diet which was lower than soybean and blood fibrin diets. Bergen et al. (1967) stated that rumen protein of bacterial origin was comparable to that of casein. McLaren (1964) indicated that the protein found in the rumen was comparable to plant protein. Of all the amino acid determinations made concerning the ruminant, the least amount of

information available concerns the amino acid content of ruminal protein.

Free amino acids were found in the rumen contents but their concentrations were generally low (Leibholz, 1965). The low concentrations may be partially responsible for the lack of absorption of these amino acids across the rumen wall, but it was the opinion of Duncan et al. (1953), Annison (1956) and Leibholz (1965) that no amino acids were absorbed across the rumen wall. The levels of free rumen amino acids are lower than the systemic levels which would also support this. Clifford and Tillman (1968) and Freitag et al. (1968) were able to change the concentrations of the amino acids present in the protein free portion of the rumen liquor but these changes had no effect on the nitrogen balances in the studies. Free amino acids were present in the rumen liquor of sheep fed purified amino acid-free diets by Duncan et al. (1953), which indicated that bacterially synthesized amino acids appeared in the rumen liquor. The concentrations of these acids were similar to those present in rations of natural protein. The fact that free rumen amino acid concentrations are small and that there has appeared to be no difference due to diet suggests that the parameter may be of questionable value in evaluating the effect of diets.

The amino acid levels most often observed in the literature are those of the plasma. Early work by Dent and Schilling (1948)

suggested that the levels of amino acids in the blood were not necessarily reflecting changes in the diet. Puchal et al. (1962) suggested that, for swine, the value of the protein was determined by its amino acid content. Longenecher and Hause (1961) proposed a method of determining the relationship between the dietary amino acid composition and the effect on plasma amino acid levels. They suggested that the free essential amino acids were removed from the plasma by the body tissues at rates proportional to the amino acid requirements of the animal.

Leibholz (1965) observed that the increase of methionine in the blood plasma of sheep supplemented on a NPN diet was low and suggested that this indicated methionine to be limiting. Oltjen et al. (1968) studied amino acid levels in the blood plasma of steers fed NPN diets. Amino acids that showed significant changes due to the diet were hydroxy proline, serine, alanine, methionine, isoleucine, tyrosine, and phenylalanine. The concentrations of threonine, serine, proline, glycine, valine, methionine, and lysine were all found to be lower four hours after feeding. Schelling et al. (1967) were able to influence only methionine in the plasma of sheep by supplementation of natural diets with NPN. They also noted that all of the amino acids were lower in plasma due to the purified diet when compared to the natural diet. Virtanen (1966) indicated that blood levels of amino acids were important in milk production.

The anatomic source of plasma does not seem to affect the interpretation of the effect due to diet. Dent and Schilling (1948) indicated the increases in plasma amino acid levels were larger in the portal system but the jugular showed similar qualitative changes. This finding was confirmed by Threurer et al. (1966), who also found the readings of plasma amino acids to be highest twenty-four hours after feeding.

This review suggests that, in the evaluation of protein or NPN for ruminants, blood urea nitrogen and amino acid concentrations of the protein and/or the plasma are necessary in evaluating different protein sources.

METHODS AND MATERIALS

Four mature white-face wethers were surgically prepared with permanent open rumen fistulas. After allowing for recovery, the sheep were nutritionally conditioned to a non-protein nitrogen diet. This adaptation was accomplished by feeding a 32 percent protein supplement pellet of which biuret furnished 8.4 percent of the equivalent crude protein for 7 weeks, followed by 30 grams of biuret daily with chopped straw ad libitum for 3 weeks. Five semi-purified diets were prepared as shown in Table I. The diets were fed to all animals at the same time in the order they are presented (1 through 5). It was felt that

TABLE I. COMPOSITION OF DIETS[1,2]

Ingredient: %	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5
Corn cobs	30.00	29.60	29.75	29.60	28.50
Corn starch	30.00	29.60	29.75	29.60	28.50
Molasses	7.50	7.50	7.50	7.50	7.50
Chopped hay	15.00	14.60	15.00	14.60	13.50
Chopped straw	15.00	14.60	15.00	14.60	13.50
Biuret	2.28	2.28	2.28	1.52	---
Salt	0.20	0.20	0.20	0.20	0.20
Sodium sulfate	---	1.60	---	---	---
Methionine	---	---	0.50	---	---
Fishmeal (70% Protein)	---	---	---	2.40	---
Soybean meal (48% Protein)	---	---	---	---	8.30
Protein	9.24	8.18	7.89	7.84	8.78

[1] Monosodium phosphate added to meet NRC requirements for 54 Kg. ram. Vitamin A palmitate and vitamin D as activated sterol to meet NRC requirements for 54 Kg. ram.

[2] Calculated to provide 60% by weight TDN and 113.5 grams of digestible protein per animal per day. Animals fed 600 grams twice daily.

each diet would be an improvement on the previous diet and consequently that changes in parameters would be due to diet. The animals were

weighed initially and at the end of each diet period. Weight changes of the animals by diet during the epxeriment are shown in Table II.

The diets were individually fed at a rate of 600 grams twice daily and made available 1 to 1½ hours at each feeding, which was sufficient for total consumption. Each diet was fed for a period of 17 days. On the 17th day, rumen fluid and blood samples were taken four hours after the morning feed was consumed. The animals were then weighed and placed on the next diet.

TABLE II. WEIGHTS IN KILOGRAMS OF SHEEP BY EXPERIMENTAL PERIOD.

Diet Treatment		1 Basal	2 Sodium Sulfate	3 Methio-nine	4 Fishmeal	5 Soybean Meal
Sheep #1	Begin	47.2	49.9	50.8	52.2	53.5
	End	49.9	50.8	52.2	53.5	54.9
Sheep #2	Begin	40.8	39.0	34.9	36.3	36.3
	End	39.0	34.9	36.3	36.3	38.1
Sheep #3	Begin	42.6	41.7	41.7	39.9	38.1
	End	41.7	41.7	39.9	38.1	38.1
Sheep #4	Begin	42.6	43.5	44.0	44.0	42.6
	End	43.5	44.0	44.0	42.6	46.3
Total Weight		174.3	171.6	172.5	170.7	177.5
Gain or Loss		---	-2.7	+0.9	-1.8	+6.8

Rumen samples were obtained at random depths and locations via the fistula with 10.0 mm tygon tubing. Approximately fifty milliliters were taken and placed in a glass bottle. The rumen samples were filtered through four layers of cheesecloth and an equal volume

of 10 percent Trichloroacetic acid (TCA) was added and mixed with the samples. After approximately one hour, the samples were centrifuged until clear. A sample of the supernatent and the precipitate were saved from each animal. Both portions were frozen at -23.3°C. for future analyses.

Blood samples were obtained by jugular venipuncture and collected in heparinized tubes. Blood urea nitrogen was measured by the procedure of Lavine et al. (1961). Determinations were made within two hours after collection. Five milliliters of blood for amino acid analyses were mixed with five milliliters of 10 percent TCA and filtered through Watman No. 40 filter paper. The filtrate was collected and stored at -23.3°C. for future methionine analysis.

The rumen precipitate was separated into three parts. One portion was ether extracted and hydrolized under standard procedure (Block and Weiss, 1956) with the following modifications. Prior to hydrolysis, the protein was boiled in 5 percent TCA to reduce the amount of methionine lost due to presence of carbohydrates (Block and Weiss, 1956). A one hundred-fold volume of six normal HCL was added to the protein precipitate to further reduce losses of methionine (Blackburn, 1968). A second portion of the rumen precipitate was divided into three sub-samples and the nitrogen content was determined by the improved micro-Kjeldahl method (Albanese, 1963). The moisture content was determined on the third portion by the AOAC (1960) method in order to convert the nitrogen content to a dry matter basis.

The nitrogen content of the protein-free portion of the rumen contents was determined by the improved micro-Kjeldahl method (Albanese, 1963).

Methionine analysis was accomplished by the microbiological assay of Shochman et al. (1954). The method of Lavine (1943) was initially employed to determine methionine levels. This method was found to be not sensitive enough for the size samples obtained and did not measure the oxidized forms of methionine. Methionine was oxidized by exposure to air for 48 hours and compared to unoxidized methionine in the method of Shochman et al. (1954) with no difference in standard curves. This method was found to be highly sensitive.

The samples which contained TCA, rumen fluid and protein-free plasma were prepared in the same manner for the microbiological assay. However, during autoclaving a pH change occurred which made the environment unsuitable for the organisms. It would appear desirable to separate the amino acids from the TCA prior to analysis in this type of assay. The best method for this could possibly be by ionic exchange.

Total amino acid analyses of feed and rumen precipitate were obtained by column chromatography on the Technicon Automatic Analyzer. The method of preparation for hydrolysis was the same as previously sited, however, the method of Hapner (1970), which included spinning the samples to evaluate the air on a Vortex centrifuge, was used, in

addition, on these samples. This procedure allowed for more complete air withdrawal and further reduced methionine losses. The chromatography was conducted on two polysulfonated Dowex cation exchange columns. A short column run of 0.5 ml. of sample was used for the basic residues. A long column run of 0.25 ml. of sample for the rumen portion and 1.0 ml. of the feed sample was used to separate the remaining residues. The higher protein concentration in the rumen protein allowed use of a smaller sample.

Statistical analysis was obtained by the least squares method of Harvey (1960). The diet X sheep interaction was used to test for significance as the error mean square contained sub-sample variance. Differences between diet means were determined by Duncan's new multiple range test (Duncan, 1955).

RESULTS AND DISCUSSION

The amino acid content of feed protein and rumen protein are shown in Tables III and IV, respectively.

TABLE III. AMINO ACID CONTENT IN MICROGRAMS/MILLIGRAM OF FEED PROTEIN ANALYSED.

Diet	1	2	3	4	5
Treatment	Basal	Sodium Sulfate	Methio-nine	Fishmeal	Soybean Meal
Aspartic acid	31.94	33.14	44.59	81.46	51.11
Threonine	21.68	16.56	22.63	36.45	33.00
Serine	18.39	16.18	20.81	32.16	14.19
Glutamic acid	58.26	49.58	59.29	87.68	*
Proline	21.07	26.13	26.25	43.75	*
Glycine	26.80	21.47	27.04	51.65	*
Alanine	32.43	26.10	33.23	54.52	*
Valine	28.93	30.11	33.86	80.60	*
Methionine	3.88	5.52	7.91	11.40	1.04
Isoleucine	21.25	19.28	22.95	40.14	89.59
Leucine	40.00	36.60	44.86	80.28	16.53
Tyrosine	*	7.97	8.33	27.72	4.89
Phenylalanine	23.46	18.17	23.79	37.99	9.42
Lysine	12.28	21.49	13.30	33.62	50.72
Histidine	*	12.57	7.14	11.79	22.65
Arganine	*	2.44	13.24	26.65	57.31
	341.37	343.31	409.25	737.86	350.27

*Residues not sufficiently separated to allow computation.

In comparing the amounts of amino acids per milligram of protein in the feed and rumen protein, the synthesis of animo acid by rumen microorganisms is obvious. The effect of added sulfur and natural protein can be seen on the total milligrams of amino acids produced in the rumen. Diet 4, which is one-third natural protein and two-thirds NPN,produced a protein which was very high in amino acid levels when compared to the other NPN diets.

TABLE IV. AMINO ACID CONTENT IN MICROGRAMS/MILLIGRAM OF RUMEN PROTEIN ANALYSED.

Diet	1	2	3	4	5
Treatment	Basal	Sodium Sulfate	Methio-nine	Fishmeal	Soybean Meal
Aspartic acid	153.86	92.24	100.09	117.79	121.12
Threonine	49.67	40.02	41.93	55.51	50.86
Serine	24.70	66.63	28.27	58.53	32.37
Glutamic acid	107.85	106.52	114.61	120.65	144.04
Proline	30.97	29.70	34.88	34.77	29.59
Glycine	45.94	48.12	53.83	72.82	61.26
Alanine	55.68	59.33	60.22	63.70	69.94
Valine	56.70	61.39	56.23	178.19	52.25
Methionine	27.01	28.03	26.71	28.35	19.56
Isoleucine	51.03	48.40	48.01	57.58	60.99
Leucine	70.42	72.14	62.44	85.13	86.44
Tyrosine	30.44	43.30	30.08	36.78	34.06
Phenylalanine	38.82	53.52	44.44	63.93	181.38
Lysine	60.82	69.00	138.15	86.25	83.47
Histidine	18.77	21.10	39.57	28.55	24.36
Arganine	40.94	32.75	82.92	45.64	48.08
	863.62	872.19	962.38	1134.17	1099.77

Tables V, VI, VII, VIII, show the means and analysis of variance for nitrogen percentage in the rumen precipitate, milligrams of nitrogen per 100 milliliters of rumen fluid, blood urea nitrogen per 100 milliliters of blood and micrograms of methionine per milligram of rumen precipitate, respectively. A summary of the means for these parameters is shown in Table IX.

The analysis of the percent nitrogen in the rumen precipitate (Table V) showed no difference in either the diet or the response of the individual sheep to diets.

TABLE V. LEAST SQUARES ANALYSIS AND DIET MEANS OF THE NITROGEN PERCENTAGE IN THE RUMEN PRECIPITATE.

Diet	1	2	3	4	5
Treatment	Basal	Sodium Sulfate	Methio-nine	Fishmeal	Soybean Meal
Mean	7.43	6.96	7.31	7.65	6.35

Source	D.F.	S.S.	M.S.	F.
Diet	4	10.41	2.60	0.77
Sheep	3	4.58	1.52	0.45
Diet X sheep	12	40.08	3.34	
Error	31	31.61	1.019	

The analysis of the milligrams of nitrogen found in the rumen fluid (Table VI) showed a significant difference ($P<.05$) due to diet, with no difference due to sheep. The analysis of the means showed that diets 1, 2, 3, and 4 were not different from each other but differed from diet 5. Diets 3 and 5 were lower than diet 4. The inference which can be drawn from this is that a larger portion of the nitrogen in the rumen was being lost from diets 3 and 5; which allowed for higher levels of blood urea nitrogen. This was indicated by BUN means from diets 3 and 5, shown in Table VII. More nitrogen was retained in the rumen fluid from diets 1, 2 and 4 and these values are reflected in the lower levels of blood urea nitrogen in the means from animals fed diets 2 and 4. Diet 1 does not follow this pattern which may in part be due to the fact that this diet had a slightly higher amount of nitrogen in it (Table I).

TABLE VI. LEAST SQUARES ANALYSIS AND DIET MEANS OF THE MILLIGRAMS OF NITROGEN/100 MILLILITERS OF RUMEN FLUID.

Diet	1	2	3	4	5
Treatment	Basal	Sodium Sulfate	Methio-nine	Fishmeal	Soybean Meal
Mean	22.68[a]	24.03[a]	20.16[ab]	27.18[a]	13.77[b]

Source	D.F.	S.S.	M.S.	F.
Diet	4	1024.31	256.07	3.55*
Sheep	3	163.92	54.64	0.76
Diet X sheep	12	865.12	72.09	
Error	35	582.44	16.64	

* $P<.05$

[a,b] Means on the same line bearing different superscript letters are significantly different ($P<.05$).

The analysis of the BUN (Table VII) showed no difference due to diet or sheep. This lack of significance was largely due to the amount of variation between the sheep while on the same diet (Appendix Table I). More observations of the blood urea nitrogen would help to evaluate these differences with observations taken at different times to determine if rates of response were different from animal to animal.

These three parameters, BUN, protein nitrogen in the rumen precipitate and the milligrams of nitrogen/100 milliliters of rumen fluid all reflect the effect of diet on production of protein in the rumen. Blood urea nitrogen levels reflect rumen ammonia levels. High BUN levels indicate a highly soluble protein in the rumen or a

TABLE VII. LEAST SQUARES ANALYSIS AND DIET MEANS OF BLOOD UREA NITROGEN IN MILLIGRAMS OF NITROGEN/100 MILLILITERS OF BLOOD.

Diet	1	2	3	4	5
Treatment	Basal	Sodium Sulfate	Methio-nine	Fishmeal	Soybean Meal
Mean	12.89	7.45	7.54	5.58	11.59

Source	D.F.	S.S.	M.S.	F.
Diet	4	308.99	77.25	1.93
Sheep	3	121.79	40.59	
Diet X sheep	12	481.05	40.08	
Error	20	70.84	3.54	

condition of inefficiency in protein production (Lewis, 1957). This supposition is supported by the BUN levels of diets 1 and 5. Biuret is insoluble but a diet composed largely of NPN would be an inefficient diet. Diet 5, which had biuret replaced by soybean meal, the nitrogen of which is more soluble, showed a higher level of BUN and a lower level of nitrogen, retained in the rumen. Nitrogen content of the rumen precipitate and of the rumen fluid have not been reported in the literature as yet. However, it would appear that the diets affected these parameters somewhat even though the analysis of the data showed no significant differences in the BUN and nitrogen content of the rumen precipitate.

The other parameter measured, methionine content of the rumen precipitate, showed quite clearly the effect of diet on rumen protein quality. This is illustrated in Table VIII.

TABLE VIII. LEAST SQUARES ANALYSIS AND DIET MEANS OF THE MICROGRAMS OF METHIONINE/MILLIGRAM RUMEN PROTEIN PRECIPITATE.

Diet	1	2	3	4	5
Treatment	Basal	Sodium Sulfate	Methio-nine	Fishmeal	Soybean Meal
Mean	0.99^{b}	1.18^{b}	1.99^{b}	3.74^{a}	2.27^{b}

Source	D.F.	S.S.	M.S.	F.
Diet	4	43.17	14.39	3.63*
Sheep	3	11.72	2.93	0.74
Diet X sheep	12	47.49	3.96	
Error	20	0.89	0.04	

*P<.05

a,b Means on the same line bearing different superscript letters are significantly different.

In comparing the means for the methionine content, diet 4 was significantly higher than the other diets. While no differences existed between diets 1, 2, 3 and 5 it is very obvious that the source of sulfur had an effect on methionine production in the rumen.

Table IX contains a summary of all means by diet. In comparing the parameters within the diet it is possible to see the effect of

TABLE IX. SUMMARY OF ALL MEANS BY DIET.

Diet	Bun Level	% Nitrogen In Rumen Precipitate	Milligrams of N/100 ml. of Rumen Fluid	Methionine Content of Rumen Precipitate in Micrograms/Milligrams
1	12.89	7.43	22.68^{a}	0.99^{b}
2	7.45	6.96	24.03^{a}	1.18^{b}
3	7.54	7.31	20.16^{ab}	1.99^{b}
4	5.58	7.65	27.18^{a}	3.73^{a}
5	11.69	6.35	13.77^{b}	2.89^{b}

a,b Means in the same column bearing different superscript letters are significantly different (P<.05).

the sulfur additions on the diet even though the differences were not significant. The addition of sulfate sulfur to the diet lowered the BUN level and retained more nitrogen in the rumen fluid. The addition of methionine had little effect on the BUN but tended to concentrate the nitrogen present in the rumen protein. The addition of fishmeal lowered the BUN level and brought about further retention of a larger amount of nitrogen in the rumen. Diet 5 tended to have more nitrogen reflected in the BUN, which was temporarily, at least, lost to rumen microorganisms.

This study tends to indicate an influence of sulfur on amounts of nitrogen retained in the rumen and a significant effect on protein quality.

SUMMARY

Four mature, rumen-fistulated wethers were fed five diets. The basal ration (Diet 1), consisting of chopped hay, straw, corn cobs, corn starch, and biuret, supplemented with one of the following ingredients: sodium sulphate (Diet 2), methionine (Diet 3), fishmeal (Diet 4), and soybean meal (Diet 5). The animals were fed each diet seventeen days prior to sampling. Rumen samples were obtained via permanent rumen fistula and blood samples obtained by jugular venipuncture. Blood urea nitrogen levels, which have been correlated to protein quality, were not significantly different. The percent nitrogen in the rumen precipitate was unaffected by diet. The amount of nitrogen present in the rumen fluid was lower for Diets 3 and 5.

A summary of means from all the parameters indicated the source of sulfur influenced the amount of nitrogen retained in the fluid. These differences were not significant in all cases but indicated a definite trend.

The results of the methionine analyses and the amino acid analyses indicated a more dramatic influence due to diet on the quality of protein produced in the rumen. Diet 4 (fishmeal) was particularly effective in producing amino acids from a largely NPN source.

APPENDIX

APPENDIX TABLE 1. Means of sample observations.

Percent nitrogen in rumen precipitate.

Diet		1	2	3	4	5	Total
SHEEP	1	7.43	7.93	7.67	8.46	5.41	36.90
	2	7.43	6.13	7.52	9.06	4.59	34.73
	3	7.43	8.08	7.50	6.75	7.66	37.42
	4	7.43	5.71	6.54	6.31	7.73	33.72
Total		29.72	27.85	29.23	30.58	25.39	142.77
Mean		7.43	6.96	7.31	7.65	6.35	

Milligrams of nitrogen in rumen fluid.

Diet		1	2	3	4	5	Total
SHEEP	1	22.68	25.56	20.52	18.36	22.32	109.44
	2	22.68	17.64	19.80	24.84	9.00	93.96
	3	22.68	28.08	18.00	25.56	15.12	109.44
	4	22.68	24.84	22.32	39.96	8.64	118.44
Total		90.72	96.12	80.64	108.72	55.08	431.28
Mean		22.68	24.03	20.16	27.18	13.77	

Blood urea nitrogen/100 milliliters of blood.

Diet		1	2	3	4	5	Total
SHEEP	1	10.22	3.15	5.33	10.68	14.09	43.47
	2	15.33	19.73	11.30	2.40	9.84	58.60
	3	11.53	3.78	4.93	2.57	11.36	34.17
	4	14.51	3.15	8.63	6.71	11.52	44.52
Total		51.59	29.81	30.19	22.36	46.81	180.76
Mean		12.89	7.45	7.55	5.59	11.70	

Micrograms of methionine/milligram of rumen protein.

Diet		1	2	3	4	5	Total
SHEEP	1	1.98	3.62	10.16	9.70	2.87	28.33
	2	1.98	.70	1.10	8.02	5.27	16.99
	3	1.98	.63	2.50	2.17	2.93	15.11
	4	1.98	4.50	2.18	10.00	7.08	25.74
Total		7.92	9.45	15.94	29.89	18.15	86.17
Mean		.99	1.18	1.99	3.74	2.27	

LITERATURE CITED

Albanese, A. A. (1963) Newer Methods of Nutritional Biochemistry. Academic Press, New York and London.

Albert, W. W., U. S. Garrigus, R. M. Forbes and H. W. Norton. (1956) The sulfur requirement of growing fatting lambs in terms of methionine, sodium sulfate and elemental sulfur. J. An. Sci. 15:559.

Annison, E. F. (1956) Nitrogen metabolism in the sheep - protein digestion in the rumen. Biochem. 64:705.

AOAC. (1960) Official Methods of Analysis, 9th Edition. Assoc. of Official Agr. Chemists, Washington, D. C.

Bergen, W. G., D. B. Purser, and J. H. Cline. (1967) Enzymatic determination of the protein quality of individual rumen bacteria. Journal of Nutrition 92:357.

Blackburn, S. (1968) Amino Acid Determination, Marcel Dekker, Inc., New York.

Block, R. J., J. A. Stehal, J. K. Loosli. (1951) Synthesis of sulfur amino acids from inorganic sulfate by ruminants. II. Synthesis of cystine and methionine from sodium sulfate by the goat and by the microorganisms of the rumen of the ewe. Archives of Biochem. and Biophy. 33:353.

Bunn, C. R., J. J. McNeill and G. Matrone. (1968) Comparison of amino acid and alfalfa supplementation of purified diets for ruminants. J. of Nutr. 94:47.

Chalupa, Wm., J. L. Evans, and M. C. Stillions. (1964) Metabolic aspects of urea utilization by ruminant animals. J. Nutr. 84:77.

Clarck, R., E. L. Barrett, J. H. Kellerman. (1963). A comparison between nitrogen retention from biuret and urea by sheep on a low protein roughage diet. J. S. Afr. Vet. Med. Ass. 34:419-423.

Clarck, R., E. L. Barrett and J. H. Kellerman. (1965) A comparison between nitrogen retention from urea, biuret, triuret and cyanuric acid by sheep on a low protein roughage diet. J. S. Afr. Vet. Med. Ass. 36:79.

Clifford, A. J. and A. D. Tillman. (1968) Urea and isolated soybean protein in sheep purified diets. J. An. Sci. 27:484.

Dent, C. E. and J. A. Schilling. (1948) Studies on the absorption of proteins: The amino acid pattern in the portal blood. Biochem. J. 44:318.

Duncan, C. W., J. P. Agrawala, C. F. Huffman and R. W. Lueche. (1953) A quantitative study of rumen synthesis in the bovine on natural and purified rations. II. Amino acid content of mixed rumen proteins. J. Nutr. 49:41.

Duncan, D. B. (1955) Multiple range and multiple F tests. Biometrics 11:1-42.

Ellis, W. C., G. B. Gurner, M. E. Muhrer and W. H. Pfander. (1956) Nitrogen utilization by lambs fed purified rations containing urea, gelatin, casein, blood fibrin and soybean protein. J. Nutr. 60:413.

Freitag, R. R., W. H. Smith and W. M. Beeson. (1968) Factors related to the utilization of urea vs protein nitrogen supplemented diets by the ruminant. J. An. Sci. 27:478.

Hapner, K. D. (1970) Personal communication. Montana State University, Bozeman, Montana.

Harvey, W. R. (1960) Least squares analysis of data with unequal subclass numbers. ARS Bull 20-8, USDA, Washington D. C.

Hungate, R. E. (1966) The Rumen and Its Microbes. Academic Press, New York.

Lavine, J. M., R. Leon, F. Steizman. (1961) A rapid method for the determination of urea in blood and urine. Clin. Chem. 7:488.

Lavine, T. F. (1943) An iodometric determination of methionine. J. Biol. Chem. 151:281.

Leibholz, J. (1965) The free amino acids occurring in the blood plasma and rumen liquor of the sheep. Aust. J. of Ag. Res. 16:973.

Lewis, D. (1957) Blood urea concentration in relation to protein utilization in the ruminant. J. Ag. Sci. 48:438.

Lofgreen, G. P., J. K. Loosli, L. A. Maynard. (1947) The influence of protein source upon nitrogen retention by sheep. J. An. Sci. 6:343.

Longenecker, J. B. and H. L. Hause. (1961) Relationship between plasma amino acids and composition of the injested protein. II. A shortened procedure to determine plasma amino acid ratios. Am. J. of Clin. Nutr. 9:356.

Loosli, J. K. and L. E. Harris. (1945) Methionine increases the value of urea for lambs. J. An. Sci. 4:435.

Loosli, J. K., H. H. Williams, W. E. Thomas. (1949) Synthesis of amino acids in the rumen. Science 110:144.

MacKenzie, H. I. and R. E. Altona. (1964) Biuret as a supplement to roughage rations for sheep and cattle. J. S. Afr. Vet. Med. Ass. 35:301.

Matrone, G., C. R. Bunn and J. J. McNeil. (1964) Investigation of dietary factors in purified rations for ruminants. J. Nutr. 84:251.

McCarthy, R. D., G. A. Porter and L. C. Greil, Jr. (1968) Bovine ketosis and depressed fat test in milk: A problem of methionine metabolism and serum lipo protein aberation. J. Dairy Sci. 51:459.

McDonald, I. W. (1948) The absorption of ammonia from the rumen of sheep. Biochem. J. 42:548.

McDonald, I. W. (1952) The role of ammonia in ruminal digestion of protein. Biochem. J. 51:86.

McDonald, I. W. (1954) The extent of conversion of food protein to microbial protein in the rumen of sheep. Biochem. J. 56:120.

McLaren, G. (1964) Symposium on microbial digestion in ruminants. Nitrogen metabolism in the rumen. J. An. Sci. 23:577.

McLaren, G. A., G. C. Anderson and K. M. Barth. (1965) Influence of methionine and tryptophon on nitrogen utilization by lambs fed high levels of non-protein nitrogen. J. An. Sci. 24:231.

Munro, H. H. and J. B. Allison, Eds. (1964) Mammalian Protein Metabolism. Vol. I. Academic Press, New York and London.

N.R.C. (1964) Nutrient Requirements of Domestic Animals, No. 5. Nutrient requirements of sheep. National Academy of Sciences, Washington D. C.

Oltjen, R. R. (1969) Effect of feeding ruminants non-protein nitrogen as the only nitrogen source. J. An. Sci. 28:673.

Oltjen, R. R. and P. A. Putnam. (1966) Plasma amino acids and nitrogen retention by steers fed purified diets containing urea or isolated soybean protein. J. Nutr. 89:385.

Oltjen, R. R., R. J. Sirny and A. D. Tillman. (1962) Purified diet studies with sheep. J. An. Sci. 21:277.

Oltjen, R. R., L. L. Slyter, A. S. Kozak and E. E. Williams, Jr. (1968) Evaluation of urea, biuret, urea phosphate and uric acid as non-protein nitrogen sources for cattle. J. Nutr. 94:193.

Preston, R. L., D. D. Schnakenberg and W. H. Pfander. (1965) Protein utilization in ruminants. I. Blood urea nitrogen as affected by protein intake. J. Nutr. 86:281.

Puchal, F., V. W. Hays, V. C. Spee, J. D. Jones and D. V. Catron. (1962) The free blood plasma amino acids of swine as related to the source of dietary proteins. J. Nutr. 76:11.

Purser, D. D., T. J. Klopfenstein and J. H. Cline. (1966) Dietary and defaunization effects upon plasma amino acid concentrations in sheep. J. Nutr. 89:226.

Schelling, G. T., F. C. Hinds, E. E. Halfield. (1967) Effect of dietary protein levels, amino acid supplementation and nitrogen source upon plasma free amino acids concentrations in growing lambs. J. Nutr. 92:339.

Schochman, G. D., J. J. Kolb, G. Toennies. (1954) Improved bacterimetric method for cystine. Anal. Chem. 26:1657.

Sokolowski, J. H., E. E. Hatfield and U. S. Gairigus. (1969) Effects of inorganic sulfur on potassium nitrate utilization by lambs. J. An. Sci. 28:391.

Starks, P. B., W. H. Hale, U. S. Gairigus, R. M. Forbes and M. F. Jones. (1954) Response of lambs fed varied levels of elemental sulfur, sulfate sulfur and methionine. J. An. Sci. 13:249.

Theurer, Brent, W. Woods and G. E. Poley. (1966) Comparison of portal and jugular blood plasma amino acids at various intervals. J. An. Sci. 25:175.

Toennies, G. and D. L. Gallant. (1948) Bacterimetric studies. I. Factors affecting the precission of bacterial growth responses and their measurements. J. Biol. Chem. 174:451.

Virtanen, A. I. (1966) Milk production of cows on protein-free feed. Science 153:1603.

Whanger, P. D. (1969) Sulfur in NPN rations. An. Nutr. and Health, p. 10, Sept. 1969.