THE EFFECT OF NITROGEN SOURCE ON RUMEN PH, AMMONIA, TOTAL AND PROTEIN NITROGEN

by

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INTRODUCTION

In non-ruminant animals the nitrogen requirements are met by the ingestion of proteins that are broken down in the stomach and small intestine, and absorbed as peptides or amino acids. The value of the ingested protein is determined by its digestibility and its amino acid composition and balance. The situation in ruminant animals differs markedly in that a large part of the ingested proteins are subjected to the activities of the rumen microbial population before passing on to the abomasum and small intestine. This function of the rumen is advantageous in that inferior proteins and non-protein nitrogenous compounds can be converted into presumably high quality microbial proteins. Yet research indicates that the fermentative process in the rumen may cause a loss in the value of proteins in that a part of the nitrogen therein may be lost as ammonia. There is considerable evidence showing ammonia to be a major end-product of protein degradation and the main component of non-protein nitrogen degradation in the rumen. Ammonia is utilized by certain rumen microorganisms for the synthesis of their body protein. The amount of ammonia found in the rumen depends on both the nature of the dietary protein and the proportion of carbohydrate present in the diet. In vitro studies have shown that as the ammonia level in the vessel decreases, the protein nitrogen content increases proportionately.

Considerable work has been conducted in comparing protein and non-protein nitrogen feeds for ruminant animals. As evidenced by its widespread use, urea by far has been the non-protein nitrogen feed of most interest. In general, urea as a source of supplemental nitrogen has been found to be inferior to plant protein supplements for ruminant animals.

In recent work by Akram (1964), nitrogen supplements for ruminant animals were compared by feeding isonitrogenous and isocaloric rations containing the various nitrogen supplements to a rumen fistulated animal and analyzing the total nitrogen and protein nitrogen content of the rumen six hours after feeding. The results obtained by this technique were in accordance with studies relevant to growth, production and nitrogen balance reported in the literature. It was found that soybean meal and cottonseed meal produced similar nitrogen values, while urea procuced lower protein synthesis that could be increased upon addition of grain to the ration. From this study, it was evident that the source of nitrogen did affect protein synthesis in the rumen. To further support the findings of Akram, this study was designed to compare the hourly changes in the percentage total and protein nitrogen that occur in the rumen of the fistulated steers fed rations supplemented with soybean meal, cottonseed meal or urea with and without added grain. In addition to the changes in total and protein nitrogen of rumen ingesta, rumen pH and rumen ammonia concentration were determined as additional criteria for comparisons. Determinations were made at a period immediately before the morning feeding and at hourly intervals from one to nine hours after feeding.

REVIEW OF LITERATURE

Sources of Nitrogen

Urea. As early as 1891 Zuntz suggested that rumen bacteria utilize non-protein nitrogen compounds.

Twenty years later, Armsby stated that non-protein nitrogen of vegetable substances appear to be converted to protein by the microorganisms of the digestive tract. By means of its conversion into bacterial protein, the non-protein nitrogen feed compounds may serve indirectly for maintenance and also as a source of protein for synthesis of milk, and probably for growth, in rations deficient in protein. He further stated that the limiting factor in the indirect utilization of the non-protein nitrogen feed compounds appears to be the extent to which it can be converted into protein in the digestive tract, rather than any inferior nutritive value of the protein thus formed as compared with that originally present in the feed.

Voltz (1919) showed the value of amides in ruminant nutrition and confirmed that urea was capable of replacing digestible protein in the metabolism of full grown ruminants. Voltz and Hansen (1922) stated that urea can be used as a protein carrier to some extent in the ration of lambs and milk cows without harmful results. Urea appeared to be beneficial when fed at a rate of 150 grams per day per cow in the presence of sufficient carbohydrate. In another report, Hansen (1922) stated that urea might be of practical value in feed rich in carbohydrates but poor in protein.

Klein, et al. (1936) found that when sheep were fed a ration of straw, molasses and starch, they were first in nitrogen equilibrium and later in positive nitrogen balance. When amide nitrogen was added to the ration, there was more true protein in the feces than in the feed. They concluded that the bacteria in the rumen were necessary for the conversion of this amide nitrogen to protein. Urea was found to be highly digestible and had no ill effects on either the digestibility of other nutrients of the ration or on the health of both cattle (Fingerling et al., 1937) and sheep (Wohlbier and Windheuser, 1937).

Hart et al. (1939) demonstrated that when urea nitrogen constituted 43% of the nitrogen of the ration, the growth rate of dairy heifers was but slightly less than that secured with a ration containing 66% of its nitrogen as casein. Schmidt and Kliesch (1939) found that substitution of urea for half the protein required for milk production did not affect production. Work and Henke (1939) demonstrated that growth of dairy heifers, on a ration in which 4% urea was substituted for a protein supplement, was inferior to that on a normal protein ration but was superior to that on a low-protein (8.4%) ration. They stated that urea appeared to be utilized, probably through bacterial synthesis as a substitute for protein.

With the use of an _in vitro_ technique, Wegner _et al_. (1940) presented evidence that conversion of inorganic nitrogen to protein nitrogen can occur through the use of bacteria from the cow's rumen. The decrease in ammonia content was accounted for by an increase in protein nitrogen and furthermore, the level of protein in the media had a negative influence on the decrease in ammonia nitrogen.

Harris and Mitchell (1941) conducted nitrogen balance studies with sheep and found considerable variation between individual sheep in the conversion of urea at the same level of intake; samll quantities were used more effectively than larger amounts.

By use of metabolism tests, Harris _et al_. (1943) further supported the theory that protein is synthesized in the paunch of ruminants. More true protein was found in the rumen of steers receiving urea (15.71%) than those subsisting on the same low protein ration without urea (9.62%).

Pearson and Smith (1943) used an _in vitro_ technique to demonstrate very high urease activity in bovine rumen liquor.

Mills _et al_. (1944) demonstrated that when fistulated heifers were fed Timothy hay as the sole ingredient of the basal ration, utilization of urea was low, whereas when corn molasses was added to the basal ration urea was fairly well utilized.

Loosli and Harris (1945) and Lofgreen et al. (1947) found that urea utilization by lambs was improved when the sulfur-containing amino acid, methione, was added to the ration.

McNaught and Smith (1948) stated that the deliberate use of non-protein nitrogen compounds in ruminant rations will be successful only when the available feed stuffs are rich in starch and poor in a slightly soluble protein.

Loosli et al. (1949) found that sheep and goats could maintain growth on a ration containing urea as the only source of nitrogen. The rumen material contained 9 to 20 times more of the amino acids than the diet fed. Watson et al. (1949) studied the formation of body protein from urea labelled with the isotope N^{15} and observed that the nitrogen of urea was utilized by sheep for the formation of body proteins.

From a urea tolerance study, Dinning et al. (1949) found that 2% urea in a cattle ration (dry matter basis) should be safe. Peirce (1951) indicated that feeding 15 grams of urea did not increase wool production of mature sheep when added to a low protein-high fiber ration. But addition of 15 grams of urea to a low protein-high starch ration increased wool production by 32% and fiber diameter by 9%.

McCall and Graham (1953) reported that urea was slightly less effective than protein when fed to fattening steers as a replacement of one-fifth and one-fourth of the supplemental protein. Steers fed the supplement containing urea had slightly lower feed conversion than the controls. There was little difference between the two groups in average carcass grade and dressing percentage.

Agrawala *et al*. (1953) found appreciable synthesis of protein (33 to 109 grams) from urea in the bovine rumen within six hours after feeding. Quantitative evidence obtained by Duncan *et al*. (1953) shows that rumen microorganisms can utilize urea nitrogen to synthesize amino acids. With the exception of histidine, the amino acid pattern of the mixed proteins in the ingesta of the calves on a purified diet containing urea as the only source of nitrogen was fundamentally similar to that found for calves on the natural ration.

Repp *et al*. (1955) reported that when urea replaced 50% of the protein nitrogen of the ration in lamb feeding experiments it did not support as high rates of gain as conventional protein. However, rations which had 15% and 30% of the protein replaced by urea were equal to those with conventional protein.

With an *in vitro* technique, Belasco (1956) showed urea utilization by rumen microorganisms to be dependent upon the amount and type of carbohydrate used as the energy source. The extent of urea utilization was slightly greater with starch than with cellulose. Xylan and pectin promoted urea utilization but not to the same extent as starch.

Gutowski *et al*. (1958) reported that when 30% of the protein in a fodder ration for heifers was replaced by urea, the total nitrogen in the liquid content of the rumen was slightly decreased but the protein nitrogen was increased.

Trials on addition of urea and molasses to the diet of sheep were conducted by Williams and Tribe (1958). They observed that the addition of 10 g. urea and 40 g. molasses per lb. to a diet of chopped oat straw for two months reduced loss in weight of sheep to about half that of sheep fed straw alone. In a similar study, Coombe (1959) showed that when sheep fed oat straw were supplemented with 240 g. molasses per day the animals lost weight at the rate of 2 lbs. weekly. However, when the molasses contained 15 g. urea, they maintained weight and if it contained 30 g. urea, the animals gained slightly. The urea supplementation increased feed intake markedly and wool growth was also increased.

Digestion and metabolism trials were conducted by Smith et al. (1960) to study the effect of adaptation of lambs to urea feeding with semipurified rations. Retention of absorbed nitrogen was significantly improved by approximately 2% with each consecutive 10 day feeding period up to 50 days with no measurable change in the digestibility of organic matter or crude fiber. Increasing the percentage of total nitrogen supplied as urea from 54 to 68%, significantly depressed the retention of absorbed nitrogen to the extent of approximately 12%.

Bloomfield et al. (1961) found that when sheep were fed a semipurified ration containing 3.2% urea, feeding sixteen times a day did not increase nitrogen retention but did decrease blood urea and increased whole blood albumin. They concluded from this evidence that feeding 16 times a day appeared to increase urea utilization compared to twice daily feeding.

The importance of a carbohydrate source in urea utilization was further investigated by Drori and Loosli (1961). They concluded that the performance of ruminants on urea-containing diets is not exclusively a result of the presence of urea in such diets. Carbohydrate may be an equally important factor in modifying the growth-promoting aspects of urea-containing diets.

According to Lizal et al. (1961), urea is well utilized by 7 to 9.5 month old heifers as long as it does not exceed 50 percent of the total digestible protein (or nitrogen) in the ration.

Compling et al. (1962) observed that when urea (with and without sucrose) was given by intraruminal infusion to non-lactating dairy cows, voluntary intake of oat straw was affected. Voluntary intake was increased 40% by infusion of 75 or 150 grams of urea per day. Increasing the sucrose level to 500 grams caused no further increase. Dry matter digestibility was increased from 41 to 50 percent by the 150 gram dose. The increased intake was thought to increase both digestibility of dry matter and its subsequent removal from the reticulo-rumen.

Soybean Meal versus Urea. Johnson et al. (1942) fed lambs a 6% protein ration in which urea nitrogen constituted 40-65% of the total nitrogen intake. The nitrogen was utilized by the host as well as nitrogen of soybean meal. In a comparative study of urea and soybean meal for steers (6-8 months of age), Harris et al. (1943) observed that the apparent digestion coefficient of nitrogen was 74 for urea and 78 for soybean meal. The biological value of urea when fed to the steers was 34 and that of the soybean meal was 60 when fed at 12 and 14% protein equivalent levels.

Klosterman et al. (1953) compared the value of various protein supplements for fattening cattle fed inferior hay. They found that urea was a satisfactory source of nitrogen. One lb. of urea and 7 to 8 lbs. of corn and cob meal were considered equal to 6 lbs. of soybean meal.

Using carbon-nitrogen balance studies on sheep, Tillman and Swift (1953) demonstrated that soybean meal promoted slightly greater storage of nitrogen in the body than did urea. The crude protein (N x 6.25) of the ration containing urea was practically equal to that of the ration containing soybean meal, however, the soybean meal ration contained the higher level of metabolizable energy. Urea was inferior to soybean meal in the storage of total carbon and body fat.

Nitrogen balance trials were conducted with beef steers by Gallup et al. (1954) to study the comparative value of urea and soybean meal as nitrogen supplements to a basal ration (5% protein) composed of prairie hay and molasses. Steers on the basal ration lost 60 lbs. over a 3 month period and were in negative nitrogen balance. Steers on basal plus urea or soybean meal (11% protein) maintained their weight and were in positive nitrogen balance. The estimated biological values for urea nitrogen was 57.6 and for soybean meal 65.5.

Albert (1955) conducted two comparative studies with lambs to study the nutritive value of nitrogen from urea and soybean meal. Supplements were formulated to be equal in energy and nitrogen to 0.25 pound of 44 percent soybean meal. One lot of lambs on a wintering ration received 0.25 pounds of soybean meal daily per lamb. A second lot received a supplement where urea replaced two-thirds of the protein with cerelose as an energy source. Average daily gains (lbs.) for 131 days were 0.137 and 0.115 for soybean meal and urea, respectively. Differences in gain were statistically significant. In a later study, urea supplements were formulated without soybean meal. It was observed that the lambs fed soybean meal made greater gains ($P < 0.01$) than the lambs fed urea supplement alone.

Ward et al. (1955) fed ten cows a protein depletion ration for 12 days. Two experimental rations consisting of basal + soybean meal and basal + urea on equal nitrogen bases were fed for 9 days. Under the conditions of this experiment, urea and soybean meal were considered to be of comparable value since maintenance of body weight and milk production did not differ significantly.

In a 196 day test, Rust et al. (1956) found that when soybean meal and urea supplied about 1/3 of the nitrogen in low protein concentrates, no significant differences in milk production were noted. During that time, however, the animals on the soybean meal rations gained an average of 3 lb. and those on urea lost 28 lb.

Light et al. (1956) reported that soybean meal was a better protein supplement than urea when the latter (42% nitrogen) was fed to sheep at the rate of 3% of a concentrate mixed with poor quality non-legume hay.

Smith et al. (1957) compared a self-fed urea molasses mixture to a molasses self-fed mixture plus 1.3 pounds of soybean meal in an attempt to determine if the urea-molasses mixture would serve as an adequate source of protein and energy for steer calves on dry grass. In 109 days, steers fed soybean meal and self-fed molasses gained 87 pounds more per head than steers on the self-fed urea-molasses mixture.

Anderson et al. (1959) showed that when lambs were fed semipurified, isocaloric and isonitrogenous rations with urea or soybean protein as the sole source of supplemental nitrogen, soybean protein significantly improved nutrient and nitrogen utilization over urea.

Theurer and Woods (1962) stated that soybean meal was superior to urea in promoting lamb growth when supplementing semipurified or natural rations. Blood plasma amino acid patterns differed considerably between the lambs fed soybean meal and urea.

Richardson and Tsien (1963) compared 19 amino acids in the rumen liquor of fistulated steers after feeding rations of alfalfa hay, prairie hay and corn supplemented with either 1 lb. of soybean meal or 60 gm. of urea plus 1 lb. corn. The rumen liquor of steers supplemented with soybean meal contained substantially more amino acids than rumen liquor from steers fed urea.

Akram (1964) compared the protein nitrogen content of rumen ingesta of fistulated steers fed isonitrogenous rations containing urea or natural proteins as added nitrogen, in two experiments (experiment I without added grain, and experiment II with added grain). It was found that the percent protein nitrogen in the rumen ingesta of steers fed rations supplemented with urea was significantly lower than that of steers fed soybean meal within both experiments.

Cottonseed Meal versus Urea. Weber and Hughes (1942) compared cottonseed meal and urea as sources of supplemental nitrogen for fattening cattle. The energy value of the ration was maintained at the same level by the addition of corn sugar. They found that the ration supplemented with urea was as palatable and produced equally large gains as the ration supplemented with cottonseed meal. Nitrogen retention value of urea nitrogen was fully comparable to that of cottonseed meal nitrogen.

Briggs et al. (1948) fed cottonseed meal and a pelleted feed containing urea to lambs as sources of supplemental nitrogen in digestion and nitrogen balance trials. They observed that the addition of urea to a basal ration of low protein prairie hay increased the apparent digestibility of hay nutrients and changed nitrogen balances to positive values. Pelleted feeds with 25% of their total nitrogen supplied by 4% urea permitted about the same storage of nitrogen as cottonseed meal. Nitrogen storage decreased as the proportion of total nitrogen supplied by urea was increased in pellets to 50-70%.

Bell et al. (1954) fed yearling steers a basal ration of grass hay, ground corn, cane molasses, cottonseed meal and minerals. They found that when urea and corn replaced 50% and 100% of the cottonseed meal in the basal ration, differences in average daily gains for 84 days were not statistically significant. In a study with dairy heifers, Frye et al. (1954) found that when urea replaced 30% of the nitrogen supplied by cottonseed meal in a concentrated mixture containing 12.5% crude protein, urea was comparable to cottonseed meal in feeding value.

Ratcliff and Stallcup (1954) stated that one lb. of urea and 7 lbs. of grain are equal to approximately 8 lbs of cottonseed meal in concentrate mixture for dairy heifers.

A series of digestion and balance trials by Bell et al. (1957) showed significantly greater digestibility of crude protein and significantly lower digestibility of ether extract for lambs fed rations containing urea as compared with lambs fed rations containing cottonseed meal.

Raleigh (1960) comparing protein sources with wether lambs, found that urea-cane-molasses and cottonseed meal were comparable with respect to weight gains, slaughter data, nitrogen digestibility and nitrogen balance, but digestibility of dry matter and energy was higher with urea-cane-molasses than with cottonseed meal rations.

In a comparative study of sources of supplemental nitrogen for fistulated beef steers, Akram (1964) found that the protein nitrogen content of rumen ingesta of animals fed rations supplemented with cottonseed meal was significantly higher than that of steers fed rations supplemented with urea (equivalent nitrogen basis) in the experiments with and without added grain.

Soybean Meal versus Cottonseed Meal. According to McCampbell (1924) soybean meal and cottonseed meal have the same value, pound for pound, as a protein supplement in feeding cattle and sheep.

Holdaway et al. (1925) stated that cottonseed meal and soybean meal protein are of approximately equal value for milk production, but soybean meal is slightly more efficient in maintaining body protein than cottonseed meal.

Henke et al. (1941) compared cottonseed meal and soybean meal as concentrate feeds in dairy cattle. Cows on cottonseed meal produced slightly less total weight of milk and butterfat than the soybean meal. From a similar study, Henke (1945) observed that both soybean meal and cottonseed meal satisfied the protein requirements for dairy cows. However, soybean meal resulted in slightly higher milk production than cottonseed meal though the differences were not statistically different.

In a nitrogen balance study, Gallup et al. (1952) found that cottonseed meal, soybean meal and corn gluten meal had similar nitrogen retention value when fed to sheep.

Akram (1964) in a comparative study of nitrogen sources for ruminants, confirmed the earlier reports that soybean meal and cottonseed meal are of equal value for cattle when fed on equivalent nitrogen bases. He found that the protein nitrogen content of rumen ingesta from fistulated steers fed rations supplemented with isonitrogenous amounts of soybean meal and cottonseed meal were very similar.

Rumen Ammonia. In a study using a mature rumen fistulated heifer, Mills et al. (1942) found that when the animal was fed 12 lbs. of Timothy hay alone, both the ammonia nitrogen and total protein in the rumen remained at constant low levels throughout the day. When Timothy hay (10 lbs.) was supplemented with 150 g. urea, hydrolysis of the urea to ammonia was delayed, being incomplete at one hour after feeding. Disappearance of the ammonia was very slow, about half remaining in the paunch 6 hours after feeding. The addition of 4 lbs. of starch to the Timothy hay + urea ration caused the urea to be completely hydrolyzed in less than one hour, and the ammonia thus formed disappeared in six hours. As the ammonia level fell, there was a concurrent rise in rumen protein. The rise in protein was approximately equivalent to the amount of ammonia disappearing from the paunch in the same period.

McDonald (1948) infused a solution of ammonium acetate into the rumen of fistulated sheep. Ammonia occurred only minutely in the circulatory system while the venous blood draining the rumen contained about 1.5 mg. ammonia/100 ml.; it was concluded that ammonia is absorbed from the rumen. In a similar experiment, Cuthbertson and Chalmers (1950) found that when casein was either fed or injected (into the rumen) there was an immediate rise in rumen ammonia concentration that reached a peak in 2 hours, then steadily declined. A rapid rise also occurred in the ammonia concentration of the venous blood draining the rumen.

Burroughs, et al. (1951) concluded from an in vitro study that the essential requirement for rumen microorganism was nitrogen in the form of ammonia that did not involve the more complex forms of nitrogen, such as amino acids.

Synge (1952) postulated that the overall utilization of protein is a balance between two conflicting tendencies, that of food protein of good amino acid composition being broken down to ammonia, part of which may be lost to the animal, and that of ingested non-protein nitrogen compounds low in "essential" amino acids, being built up into a soluble microbial protein.

El Shazly (1952) reported that the increase in rumen ammonia concentration could be correlated with increased concentrations of isobutyric acid and of the C_5 acids, and are therefore considered to arise from microbial attack on protein in the rumen.

According to Annison et al. (1954), the extent of ammonia production from a given amount of protein-rich material is decreased when the amount of starch or cereal meal being fed is increased. The presence of readily fermentable carbohydrates in the rumen encourage dominance of those types of microorganisms which obtain their energy therefrom. Amino acids arising by proteolysis will be assimilated by the growing microorganisms under these conditions, whereas in the absence of fermentable carbohydrate, amino acids will accumulate and undergo fermentation of the Stickland type, yielding products of little value for satisfying the nitrogen requirements of the host.

In a urea toxicity study, Coombe and Tribe (1958) observed toxicity symptoms in sheep when blood ammonia concentration rose above 80-120 mg. ammonia nitrogen/100 ml. However, the ammonia concentration in peripheral blood does not rise until the rumen ammonia level is raised above 80 mg./100 ml. They found that ammonia concentration in the rumen of sheep could be kept below 80 mg. ammonia nitrogen/100 ml. throughout the day be feeding 100 g. of urea incorporated into the ration as a solution mixed with hay.

Lewis (1960) stated that the microorganisms within the rumen rapidly reach a maximum level of adaptation (7 days) to handle large quantities of ammonia by a synthetic pathway.

Roberts (1961) collected rumen liquor from sheep at 0, ½, 1, 1½, 2, 2½, 3, 4, 5 and 6 hours after feeding rations containing urea and urea + lysine. The rumen liquor samples were analyzed for ammonia and non-protein nitrogen. He observed that the maximum concentration of ammonia and non-protein nitrogen in the rumen occurred one hour after feeding in both treatments.

Barnett and Reid (1961) suggested that where protein level is high, or where the protein is easily hydrolyzable, the rate of release of ammonia is too rapid for optimum conversion of the compound to microbial protein by the microorganisms of the rumen.

Rumen pH. The hydrogen ion concentration of rumen ingesta is subject to considerable variation. The extent of microbial activity in the rumen seems to be the major factor influencing rumen pH values.

Olson (1941) showed that rumen ingesta become more alkaline on exposure to atmosphere. Smith (1941) by use of an extended electrode of the Beckman pH meter made *in vivo* determinations of rumen pH through the rumen fistula of cows. He found that *in vivo* pH values of rumen ingesta were lower than *in vitro* values of rumen ingesta from the same animal. He stated that the more alkaline values for the *in vitro* determination was due to the loss of CO_2 to the atmosphere.

The effect of volatile fatty acid (VFA) concentrations in the rumen on the rumen pH values was demonstrated by Phillipson (1942). As the VFA accumulated to the extent of 57 to 162 mM/l., the rumen pH values ranged from 7.03 to 5.80.

In an abstract, Orth and Kaufmann (1958) reported that rumen pH values decreased with the increasing crude protein content of ingested fodder.

With *in vitro* techniques, Reis and Reid (1959) observed the accumulation of ammonia in rumen liquor during 4-hour incubation periods. The pH was controlled over the range 4.5 - 7.3 by the use of a buffer and casein hydrolyzate was added as substrate. They found that there was an optimum range of pH, between 6.0 and 6.7, for ammonia production. Ammonia production fell rapidly on the acid side of the optimum pH and usually less rapidly on the alkaline side. The effect of pH was considered to be an effect on enzymatic deamination rather than on proteolysis.

Purser and Moir (1959) infused 7, 35, and 70 g. of glucose into the rumen of sheep maintained on an adequate diet. It resulted in a fall of ruminal pH 2-4 hours after infusion from a mean of pH 5.66, without added glucose, to 5.40 at the 70 g. level. The decrease in pH values was related to the increase in VFA concentration in the rumen.

EXPERIMENTAL PROCEDURES

Experimental Animals. Three 5 year-old fistulated beef Shorthorn steers were used in this study. Two of the steers were a set of identical twins. The steers were housed in 12 x 6 ft. wooden stalls. Wood shavings were used as bedding.

Experimental Rations. The composition of the rations used in this study is shown in tables 1 and 2.

Table 1. Composition of rations in experiment I. Rations containing an additional source of nitrogen without added grain.

Rations	Total Nitrogen	Total Nitrogen	Added Nitrogen	Total Crude Protein
	lbs.	%	lbs.	lbs.
1 Prairie hay, 10 lbs. Soybean meal, 1 lb.	0.090 0.075 0.165	1.50	0.075	1.03
2 Prairie hay, 10 lbs. Cottonseed meal, 1.05 lbs.	0.090 0.075 0.165	1.49	0.075	1.03
3 Prairie hay, 10 lbs. Urea, 62 g. Corn, 1 lb.	0.090 0.058 0.017 0.165	1.48	0.075	1.03

The basal ration for experiment I was composed of 10 lbs. of prairie hay. The basal ration for experiment II was composed of 6 lbs. of prairie hay and 6 lbs. of cracked corn. Within each experiment, the 3 rations were formulated to be isonitrogenous and approximately isocaloric. Soybean meal,

Table 2. Composition of rations in experiment II. Rations containing an additional source of nitrogen with added grain.

Rations	Total Nitrogen	Total Nitrogen	Added Nitrogen	Total Crude Protein
	lbs.	%	lbs.	lbs.
4 Prairie hay, 6 lbs. Corn, 6 lbs. Soybean meal, 0.73 lbs.	0.054 0.101 0.055 0.210	1.65	0.055	1.31
5 Prairie hay, 6 lbs. Corn, 6 lbs. Cottonseed meal, 0.78 lb.	0.054 0.101 0.055 0.210	1.64	0.055	1.31
6 Prairie hay, 6 lbs. Corn, 7 lbs. Urea, 40 g.	0.054 0.118 0.038 0.210	1.60	0.055	1.31

cottonseed meal, and urea were used as additional sources of nitrogen. In the basal ration they were incorporated as a single source of nitrogen. To rations containing urea, one pound of cracked corn was added as a source of energy. Salt and steamed bone meal were added to each ration.

Feeding Schedule. The daily ration was divided equally between the 8:00 a.m. and 5:00 p.m. feedings. Fresh clean water was available to the animals at all times.

Sampling Schedule. Samples of rumen liquor and ingesta were collected by the same person from an arbitrarily selected site in the rumen of the fistulated steers. The steers were fed on these particular rations and on each fourteenth day, rumen samples were collected. Rumen liquor and rumen ingesta were collected from each steer just before the morning feeding (zero hour) and at hourly intervals (1, 2, 3, 4, 5, 6, 7, 8 and 9 hours) after feeding.

Sampling Techniques. Rumen liquor samples were collected by vacuum from the center of the rumen's ventral sac. The collection apparatus consisted of a Duo-Seal vacuum pump, a 500 ml. stemless vacuum flask, a 3½ x ½ in. polyethylene tube, and a 100 ml. plastic perforated (1/16") centrifuge tube. The perforated centrifuge tube attached to the polyethylene tube was inserted through the rumen fistula to the center of the rumen's ventral sac. Two hundred ml. of strained rumen liquor were drawn by vacuum into the vacuum flask that contained 50 ml. of mineral oil. The oil prevented contact of the liquor with air. Immediately after collection, the vacuum flask was stoppered and placed in a freezer.

Samples of rumen ingesta were collected by the technique described by Akram (1964). This involved the mixing and sampling of rumen ingesta in vivo. The ruminal contents of the fistulated steers were thoroughly mixed by inserting the hand and arm into the rumen and stirring for 5 to 7 minutes. After ascertaining homogeneity, a 250 ml. glass beaker was introduced into the rumen to collect the sample. Care was taken to prevent the entry of any material into the beaker by covering it with the palm of the hand until the contents were stirred again.

Treatment of Rumen Liquor

The samples of rumen liquor were kept in a freezer until all three steers were sampled and pH and ammonia determinations were ready to be performed.

pH Determination. Fifty ml. of rumen liquor were pipetted into a 100 ml. beaker and pH readings made with a Beckman pH meter.

Ammonia Determination. The microdiffusion method of Conway (1957) was used to determine rumen ammonia content. Determinations were made in triplicate and blanks were set up at every hour. The ammonia content was such that 0.5 ml. liquor was a satisfactory sample volume.

Treatment of Rumen Ingesta

Moisture Determination. The samples obtained from the rumen were placed in tared and appropriately labelled enamel pans, quickly weighed, promptly placed in a drying oven and dried at approximately 68°C. for 48 hours.

Grinding of Samples. The dried samples were scraped from the enamel pan and passed through a Wiley Mill fitted with a 1 mm. mesh screen. The samples were then thoroughly mixed and stored in air-tight glass jars until analyzed.

Determination of Total Nitrogen. A portion of the dry, ground sample was transferred to a clean and labelled 50 ml. glass beaker, and further dried in a vacuum oven for 12 hours to insure complete dryness of the sample. The sample was then transferred to a dessicator for cooling.

The cooled samples were thoroughly mixed with a spatula and 1 to 2 g. samples were quickly weighed in duplicate then analyzed for total nitrogen by the Macro-Kjeldahl Method (A.O.A.C., 1960).

Determination of Protein Nitrogen. The method used for this determination is fully described by Akram (1964).

Between 0.5 and 1 g. of the dried, ground and thoroughly mixed sample of rumen ingesta was weighed in duplicate and each placed in a 250 ml. Erlenmyer flask. Thirty ml. of 10% trichloroacetic acid was added to each flask and were shaken for 6 hours with an electric wrist-action shaker. The precipitated samples were then filtered through No. 42 ash free filter paper, using Buckner funnel and suction. The Erlenmyer flasks were flushed with distilled water to ensure complete transfer of the sample to the filter paper. The precipitate together with the filter paper was transferred into the Kjeldahl flask and analyzed for protein nitrogen by the A.O.A.C. Macro-Kjeldahl method.

Statistical Analysis

The combined data of experiments I and II were subjected to analysis of variance. The effect of treatments (soybean meal, cottonseed meal and urea as additional sources of nitrogen) were compared at all hours after feeding by the Least Significant Difference (LSD) method at the 5% level (Snedecor, 1962).

Preliminary Study

Before comparing the supplementary nitrogen sources, it was necessary to evaluate the variance criteria of response for only the basal diet of prairie hay. These preliminary data are summarized in table 3. Ruminal moisture, pH, ammonia, total nitrogen and protein nitrogen, as related to each animal, are shown in appendix tables 14, 15, 16, 17 and 18 respectively.

The data in table 3 show that variance of rumen moisture was equivocal throughout the 10 collection periods.

The pH values decreased during the first 2 hours after feeding and slowly increased in subsequent periods to values above that found at zero time.

Ruminal ammonia production was greatest one hour after feeding. By the fourth hour the level could not be detected. This suggests that there was little change in the organic nitrogen. This was confirmed by the analysis of total and protein nitrogen. These values were relatively constant.

Table 3. Average percentage moisture, pH, ammonia, total nitrogen and protein nitrogen in the rumen of fistulated steers fed paririe hay alone.

Hour after feeding	Moisture %	pH	Ammonia Mg/100 ml.	Total Nitrogen % of D.M.	Protein Nitrogen % of D.M.
0	89.59	6.85	0.146	1.37	1.23
1	89.09	6.77	0.697	1.35	1.20
2	89.12	6.73	0.089	1.31	1.19
3	88.99	6.84	0.081	1.30	1.14
4	88.99	6.85	-----	1.31	1.15
5	88.68	6.97	0.016	1.26	1.14
6	88.35	6.90	-----	1.27	1.12
7	88.44	6.98	-----	1.31	1.16
8	88.55	7.03	-----	1.29	1.15
9	88.37	7.03	-----	1.36	1.18

RESULTS AND DISCUSSION

Experiment I. Protein Supplementation Without Added Grain

Moisture. The average percentage moisture of rumen ingesta samples, collected at hourly intervals after feeding, from steers fed rations supplemented with soybean meal, cottonseed meal, or urea is shown in table 4. Individual animal results are shown in appendix table 19. The statistical comparisons of the treatments are shown in appendix table 30.

Marked differences in the moisture content of rumen ingesta were observed among steers. At almost all hours after feeding, the moisture content in the rumen of steer No. 3 was lower than that of steer No. 2 and steer No. 1 was lower than steer No. 2.

The moisture content changed in accordance with the time of sampling, with the highest value in all cases occurring at the collection period before feeding (0 hour). After feeding, the hourly moisture changes were not consistent, although relatively consistent in content.

The average moisture content in the rumen of steers fed soybean meal, was similar to that fed cottonseed meal at all hours of sampling except at hour 3 when the moisture content was significantly ($P < 0.05$) lower for steers on soybean meal.

The average moisture content of the steers fed urea was significantly ($P < 0.05$) lower than that of steers fed soybean meal at hours 4 and 5, and lower than steers fed cottonseed meal at hours 3 and 4.

Rumen pH. The average pH values of rumen liquor from steers fed rations supplemented with soybean meal, cottonseed meal, or urea are shown in table 5 and individual animal results are shown in appendix table 20. Statistical comparisons of the nitrogen supplements are shown in appendix table 32.

Table 4. Average percentage moisture of the rumen samples from three fistulated steers fed soybean meal (SBM), cottonseed meal (CSM), or urea without added grain.

Treatment	Time after feeding (Hour)									
	0	1	2	3	4	5	6	7	8	9
	%									
SBM	90.66	88.65	88.28	88.42	88.86	88.97	88.29	88.89	89.13	89.26
CSM	90.62	89.33	88.44	89.84	88.16	88.58	88.97	88.84	88.86	88.89
Urea	90.34	88.48	88.67	88.19	87.23	88.07	89.12	88.57	88.54	88.88

Table 5. Average pH values of the rumen liquor from three fistulated steers fed soybean meal (SBM), cottonseed meal (CSM), or urea without added grain.

Treatment	Time after feeding (Hour)									
	0	1	2	3	4	5	6	7	8	9
	pH									
SBM	7.17	6.93	6.93	6.93	6.85	6.97	7.09	7.10	7.10	7.11
CSM	7.15	6.98	6.93	6.93	6.88	7.01	6.99	7.01	7.06	7.05
Urea	6.93	6.91	7.03	6.84	6.91	6.89	6.92	6.97	7.01	6.05

The pH values of rumen liquor from fistulated steers on the three treatments showed only a slight trend in the direction of the hourly pH changes. The pH values of rumen liquor from steers fed rations supplemented with soybean meal or cottonseed meal were lowered after feeding and reached the lowest value at hour 4. Between hours 5 and 9, the values increased slightly.

The differences between the pH value in the rumen of the steers on the three treatments were statistically non-significant before and at all hours after feeding.

Rumen Ammonia. The average ammonia concentrations of rumen liquor from the steers fed rations supplemented with soybean meal, cottonseed meal or urea are shown in table 6. Individual animal results are shown in appendix table 21. Statistical comparisons - LSD, are shown in appendix table 34.

Table 6 shows that the average ammonia concentrations in rumen liquor from steers fed soybean meal or cottonseed meal reached peak levels 1 hour after feeding, then rapidly declined to the lowest level at approximately hour 5. After reaching the lowest level, ammonia concentrations steadily increased to hour 9. The average ammonia level of rumen liquor from steers fed urea reached a peak at hour 2, then very rapidly declined to hour 7 and slowly from hour 7 to 9. Although the peak of the average values was reached 2 hours after feeding, two of the three steers on this treatment showed peak level at 1 hour after feeding.

At all hours after feeding, steers on the ration supplemented with soybean meal had higher levels of rumen ammonia than that of steers supplemented with cottonseed meal, however the differences were not statistically significant.

The rumen ammonia levels of steers fed urea were significantly ($P < 0.05$) higher than that of steers fed soybean meal or cottonseed meal at 1 to 4 hours and 1 to 5 hours after feeding respectively.

Total Nitrogen. The average percentage of total nitrogen of the dried rumen samples from steers fed rations supplemented with soybean meal, cottonseed meal or urea are shown in table 7. Individual animal results are shown in appendix table 22. Multiple comparisons of the nitrogen supplements are shown in appendix table 36.

Steers on all three treatments showed an increase in total nitrogen in the rumen from the period before feeding to one hour after feeding but thereafter, inconsistent hourly changes were observed.

Similar values of total nitrogen were observed in the rumen of steers fed rations supplemented with soybean meal and cottonseed meal at all hours except the second hour after feeding, at which time the value for steers on the cottonseed meal supplement was significantly higher ($P < 0.05$).

At all hours after feeding, steers on the ration supplemented with urea had significantly ($P < 0.05$) lower total nitrogen values than that of steers supplemented with soybean meal or cottonseed meal.

Protein Nitrogen. The average percentage of protein nitrogen in the rumen of steers fed rations supplemented with soybean meal, cottonseed meal, or urea is shown in table 8. Individual animal results are shown in appendix table 23. Statistical comparisons of the treatments (LSD) are shown in appendix table 38.

The hourly changes of protein nitrogen values in the rumen of steers on all three treatments were not consistent.

The average protein nitrogen values in the rumen of steers fed soybean meal or cottonseed meal were very similar at all times.

Table 6. Average ammonia level of the rumen liquor from three fistulated steers fed soybean meal (SBM), cottonseed meal (CSM), or urea without added grain.

Treatment	Time after feeding (Hour)									
	0	1	2	3	4	5	6	7	8	9
	Mg. NH_3/100 ml.									
SBM	4.745	5.791	4.818	3.852	2.425	2.222	2.831	2.912	2.936	3.552
CSM	3.933	4.688	3.025	1.411	0.974	0.633	1.087	1.403	1.937	2.352
Urea	3.123	12.003	13.706	9.298	7.267	4.728	3.617	2.620	2.433	2.165

Table 7. Average percentage total nitrogen of the dried rumen samples from three steers fed soybean meal (SBM), cottonseed meal (CSM), or urea without added grain.

Treatment	Time after feeding (Hour)									
	0	1	2	3	4	5	6	7	8	9
	%									
SBM	1.71	1.87	1.78	1.96	1.83	1.83	1.80	1.74	1.74	1.75
CSM	1.78	1.88	1.91	1.90	1.82	1.79	1.81	1.79	1.83	1.71
Urea	1.58	1.63	1.58	1.50	1.52	1.38	1.52	1.45	1.43	1.51

The average protein nitrogen present in the rumen of steers fed urea was significantly ($P < 0.05$) lower all hours after feeding, than that of steers fed soybean meal or cottonseed meal.

Experiment II. Protein Supplementation With Added Grain

Moisture. The average percentage moisture of rumen samples from steers fed rations supplemented with soybean meal, cottonseed meal or urea is presented in table 9. Individual animal results are shown in appendix table 24. Statistical comparisons - LSD, are shown in appendix table 30.

As in experiment I, steer differences in regard to moisture content were observed. Under all treatments and at all hours of sampling, steer No. 3 had a lower moisture content than steers 1 and 2.

The average moisture content in the rumen of steers on all three treatments were very similar.

The moisture content of steers fed cottonseed meal was significantly ($P < 0.05$) higher than that of steers fed soybean meal at hour 7, and steers fed urea at hours 7 and 9.

Rumen pH. The average pH values of rumen liquor from steers fed soybean meal, cottonseed meal or urea are presented in table 10. Individual animal results are shown in appendix table 25. Statistical comparisons of the treatments - LSD, are shown in appendix table 32.

The average rumen pH values of the steers on all treatments decreased after feeding, for 2 to 4 hours, then unsystematically increased during the remaining periods.

Steers on the three treatments exhibited similar average pH values at all hours except at hour 5 where steers fed cottonseed meal had significantly ($P < 0.05$) higher values than that of steers fed urea.

Table 8. Average percentage protein nitrogen of the dried rumen samples from three fistulated steers fed soybean meal (SBM), cottonseed meal (CSM), or urea without added grain.

Treatment	Time after feeding (Hour)									
	0	1	2	3	4	5	6	7	8	9
	%									
SBM	1.62	1.75	1.65	1.73	1.64	1.67	1.59	1.57	1.60	1.59
CSM	1.61	1.77	1.72	1.66	1.65	1.72	1.64	1.65	1.65	1.59
Urea	1.46	1.51	1.46	1.36	1.39	1.29	1.38	1.30	1.34	1.38

Table 9. Average percentage moisture of the rumen samples from three fistulated steers fed soybean meal (SBM), cottonseed meal (CSM), or urea with added grain.

Treatment	Time after feeding (Hour)									
	0	1	2	3	4	5	6	7	8	9
	%									
SBM	89.75	87.35	87.19	87.62	88.15	88.32	88.68	89.38	88.58	89.34
CSM	89.02	86.99	86.97	87.13	87.39	87.85	88.34	88.13	88.36	88.56
Urea	89.56	87.61	87.20	86.88	87.48	87.53	88.10	88.01	88.20	88.12

Rumen Ammonia. The average ammonia concentration of the rumen liquor from steers fed soybean meal, cottonseed meal or urea are shown in table 11. Individual animal results are shown in appendix table 26. The statistical comparison of treatments - LSD, are presented in appendix table 34.

At all times average ammonia concentration in the rumen of steers fed cottonseed meal was higher than that of steers fed soybean meal; however, the differences were not statistically significant.

The first two hours after feeding, steers fed urea showed a significantly higher ($P < 0.05$) concentration of ammonia than steers fed soybean meal. Steers fed cottonseed meal displayed a similar high concentration ($P < 0.05$) before feeding and the first two hours after feeding.

Total Nitrogen. The average percentage total nitrogen of dried rumen samples from steers fed rations supplemented with soybean meal, cottonseed meal or urea are shown in table 12. Individual animal results are shown in appendix table 27. Statistical comparison of treatments - LSD, are presented in appendix table 36.

The hourly changes in total nitrogen showed no consistent pattern with steers on the three treatments.

No differences were observed among the average percentages of total nitrogen in the rumen ingesta of steers fed, at all times, soybean meal or cottonseed meal.

At the eighth hour after feeding, steers fed soybean meal had significantly ($P < 0.05$) higher total nitrogen in the rumen than steers fed urea. Only minor differences were observed during all other periods.

Steers fed cottonseed meal had significantly ($P < 0.05$) lower total nitrogen in the rumen at hour 7 and significantly higher at hour 8 than steers fed urea. At all other periods, only minor differences were observed.

Table 10. Average pH values of the rumen liquor from three fistulated steers fed soybean meal (SBM), cottonseed meal (CSM), or urea with added grain.

Treat-ment	Time after feeding (Hour)									
	0	1	2	3	4	5	6	7	8	9
	pH									
SBM	6.83	6.66	6.58	6.49	6.70	6.72	6.61	6.72	6.74	6.71
CSM	6.73	6.67	6.61	6.63	6.64	6.79	6.51	6.73	6.72	6.79
Urea	6.83	6.76	6.60	6.62	6.68	6.51	6.74	6.84	6.82	6.88

Table 11. Average ammonia level of the rumen liquor from three fistulated steers fed soybean meal (SBM), cottonseed meal (CSM), or urea with added grain.

Treat-ment	Time after feeding (Hour)									
	0	1	2	3	4	5	6	7	8	9
	Mg. NH_3/100 ml.									
SBM	5.734	6.894	4.436	2.084	1.760	1.736	1.997	2.117	2.831	3.317
CSM	7.713	9.067	5.546	3.025	2.028	1.703	1.935	2.774	2.993	3.236
Urea	4.387	15.904	9.740	4.566	1.817	1.590	1.517	1.914	2.044	2.182

Protein Nitrogen. The average percentage protein nitrogen of the dried rumen samples from steers fed soybean meal, cottonseed meal or urea are presented in table 13. Individual animal results are shown in appendix table 28. Statistical comparison of treatments are presented in appendix table 38.

The hourly changes in protein nitrogen showed no consistent pattern with steers on the three treatments.

The average percentage protein nitrogen in the rumen ingesta of steers fed soybean meal or cottonseed meal were very similar, only minor differences being observed.

At the seventh hour after feeding, the rumen ingesta of steers fed cottonseed meal was significantly ($P < 0.05$) lower in protein nitrogen than that of steers fed urea. However, the differences at all other periods were slight.

Experiment II Versus Experiment I

Moisture. The statistical comparisons of the treatments are shown in appendix table 30.

The steers fed soybean meal without added grain showed significantly ($P < 0.05$) higher moisture content in the rumen at 0, 1 and 2 hours after feeding than steers fed soybean meal with added grain.

Significantly ($P < 0.05$) higher moisture was found in the rumen of steers fed cottonseed meal without added grain before feeding and 1 to 3 hours after feeding, than steers fed cottonseed meal with added grain.

Moisture content in the rumen of steers fed urea without added grain was significantly ($P < 0.05$) higher than that of steers fed urea with added grain at 2, 3 and 6 hours after feeding.

Table 12. Average percentage total nitrogen of the dried rumen samples from three fistulated steers fed soybean meal (SBM), cottonseed meal (CSM), or urea with added grain.

Treatment	Time after feeding (Hour)									
	0	1	2	3	4	5	6	7	8	9
	%									
SBM	2.19	2.19	2.16	2.21	2.23	2.14	2.26	2.21	2.27	2.11
CSM	2.15	2.15	2.19	2.14	2.27	2.21	2.16	2.07	2.25	2.18
Urea	2.22	2.17	2.21	2.20	2.23	2.13	2.19	2.21	2.12	2.17

Table 13. Average percentage protein nitrogen of the dried rumen samples from three fistulated steers fed soybean meal (SBM), cottonseed meal (CSM), or urea with added grain.

Treatment	Time after feeding (Hour)									
	0	1	2	3	4	5	6	7	8	9
	%									
SBM	1.91	1.97	1.97	1.97	1.97	1.95	2.05	1.92	2.00	1.85
CSM	1.86	1.95	1.91	1.98	2.00	1.96	1.89	1.79	1.93	1.85
Urea	1.96	1.94	1.94	2.02	2.00	1.88	1.95	1.96	1.85	1.88

Rumen pH. The pH values of the rumen liquor from steers fed rations supplemented with soybean meal, cottonseed meal or urea, without added grain, where higher at all hours after feeding than that of steers fed the similar rations with added grain. The differences were significant at various times (appendix table 32).

Rumen Ammonia. The statistical comparisons of the treatments are shown in appendix table 34.

The ammonia content of rumen liquor from steers fed soybean meal with or without added grain were very similar.

Steers fed cottonseed meal with added grain had significantly ($P < 0.05$) higher rumen ammonia content than steers fed cottonseed meal without added grain before and one hour after feeding.

The steers fed urea without added grain had significantly ($P < 0.05$) higher rumen ammonia content than steers fed cottonseed meal without added grain before and one hour after feeding.

The steers fed urea without added grain had significantly ($P < 0.05$) lower rumen ammonia content at one hour after feeding and significantly ($P < 0.05$) higher at 2 to 5 hours after feeding, than steers fed urea with added grain.

Total Nitrogen. The statistical comparison of treatments are shown in appendix table 36.

The total nitrogen content of rumen samples from steers fed soybean meal, cottonseed meal, or urea with added grain were significantly ($P < 0.05$) higher than that of steers fed the similar rations without added grain at all hours of sampling.

Protein Nitrogen. The statistical comparison of treatments are shown in appendix table 38.

Steers fed soybean meal or urea with added grain had significantly ($P < 0.05$) higher protein nitrogen in the rumen than steers fed the same rations without added grain.

At all times the protein nitrogen content of the rumen of steers fed cottonseed meal with added grain was higher than steers fed cottonseed meal without added grain. The differences were significant ($P < 0.05$) at all hours except the seventh.

Statistical Analysis

The statistical analysis, the analysis of variance and multiple comparisons - LSD of rumen moisture content, pH, ammonia, total nitrogen, and protein nitrogen, are shown in appendix tables 29 through 38.

Since it would be difficult to explain the results of the variance analysis in the discussion of the individual experiments, it will be considered at this time.

Moisture. The analysis of variance of moisture content is shown in appendix table 29.

The data revealed that there were highly significant ($P < 0.005$) differences of moisture content. The variation of moisture content due to treatments and time of sampling were highly significant ($P < 0.005$). There were highly significant ($P < 0.005$) steers x treatment and treatment x time effects on moisture content. But steers x time effect was not significant.

Rumen pH. The analysis of rumen pH variance is shown in appendix table 31.

Variations of rumen pH, due to steers, treatments, time, and steers x treatments effects, were found to be highly significant ($P < 0.005$). The variation of rumen pH due to steers x time and treatment x time effects was non-significant.

Rumen Ammonia. The analysis of variance of ammonia content is shown in appendix table 33.

The variation in ammonia concentration of rumen liquor due to steers and steers x time effects, was non-significant. Variations due to treatments, time, steers x treatments and treatments x time effects were highly significant ($P < 0.005$).

Total Nitrogen. The variance analysis of total nitrogen is shown in appendix table 35.

It was found that all variation sources contributed highly significantly ($P < 0.005$) to variation in the total nitrogen content of the rumen samples.

Protein Nitrogen. The analysis of variance of protein nitrogen is shown in table 37 of the appendix.

The results indicate that the variation of protein nitrogen in the rumen contents were significantly ($P < 0.005$) affected by steers, treatments, and steers x treatments effects. The variation due to time was significant at the $P < 0.10$ level. The steers x time and treatments x time effects were non-significant.

GENERAL DISCUSSION

Experiment I

The moisture content of rumen ingesta did not seem to be influenced by the source of nitrogen supplements fed. The marked individual animal differences and the inconsistent hourly changes of moisture content could be due to the unmeasured water consumption of the steers, volume of salivary flow and the rate of removal of material from the rumen. The flow of saliva is of special interest since it was observed that the steer with the lowest percentage moisture (steer No. 3) consistently consumed the allotted ration very rapidly and the rumen ingesta was composed of materials of large particle size as compared to steers Nos. 1 and 2. The rapid consumption of the feed and, possibly, less frequent rumination, reduced salivary flow. Bailey (1961) reported that the mean rates of secretion from one parotid gland of a steer during eating, rumination, and periods of rest were about 20, 25 and 10 ml./min., respectively. In a similar study, Autrey (1964) found that the average salivary flow from two steers with parotid fistula while consuming coastal bermuda grass was 363 ml. per hour. Such data shows that salivary secretion may greatly affect rumen moisture content. Inconsistent hourly changes in percentage rumen moisture were also observed by Pearson and Smith (1943). They collected rumen ingesta from a fistulated steer 1 to 10 hours after feeding. They stated that the hourly changes in moisture content was due to the periodic passing of rumen contents from the rumen and reticulum into the other parts of the stomach, and that the material passing on at anytime may not be representative of the rumen contents as a whole.

The pH values of rumen liquor from the steers fed the different sources of supplemental nitrogen did not differ at all hourly determinations. Slight trends in the hourly changes were noted, but the values were too variable to arrive at any conclusion. The inconsistent changes in values between hourly measurements within each treatment could be due to the loss of carbon dioxide from the rumen liquor during the collection process. As stated in the experimental procedures, the rumen liquor was collected by vacuum. Earlier workers, Olson (1941) and Smith (1941), have shown that the pH of rumen ingesta becomes more alkaline when CO_2 is lost to the atmosphere.

The level of rumen ammonia for steers on soybean meal or cottonseed meal reached its maximum one hour after feeding, although at a much lower level than that of steers fed urea. Indications are that at least part of the protein therein was readily deaminated in the rumen. The high concentration of ammonia found in the rumen of steers fed urea demonstrated that urea was rapidly hydrolyzed in the rumen within 1 to 2 hours. This peak and the significantly higher concentrations of ammonia 1 to 5 hours after feeding, as compared to soybean meal or cottonseed meal treatments, may be explained by the statement made by Chalmers (1961). In a review article she stated that the high ammonia concentrations in the rumen indicated that the deamination of the protein had exceeded the synthetic capabilities of micro-organisms to use the degraded products for synthesizing their own protein. McDonald (1952) reported that the peak in the curve of ammonia concentration does not give an indication of the magnitude of total formation of ammonia since it is removed from the rumen in several ways: by passage in the ingesta from the rumen to the omasum and abomasum, and by direct absorption from the rumen. Bloomfield et al. (1960) reported that urea hydrolysis in the rumen was four times faster than the uptake of ammonia nitrogen by rumen organisms. Muhrer and Carroll (1964) stated that the capacity of rumen fluid to hydrolyze urea exceeds its capacity to utilize the ammonia formed. From their in vitro study, it was found that 246 mg. of urea is hydrolyzed in 100 ml. rumen contents per hour. The rate of ammonia release in the rumen of a steer supplemented with cottonseed meal, soybean meal or Morea was studied by Stallcup and Looper (1958). It was observed that rumen ammonia rose rapidly after a Morea feeding and reached its maximum 2 hours after feeding, afterwards declining steadily for 10 hours. Much smaller rises were noted with cottonseed meal and soybean meal with relatively constant rumen ammonia levels beyond a period six hours after feeding.

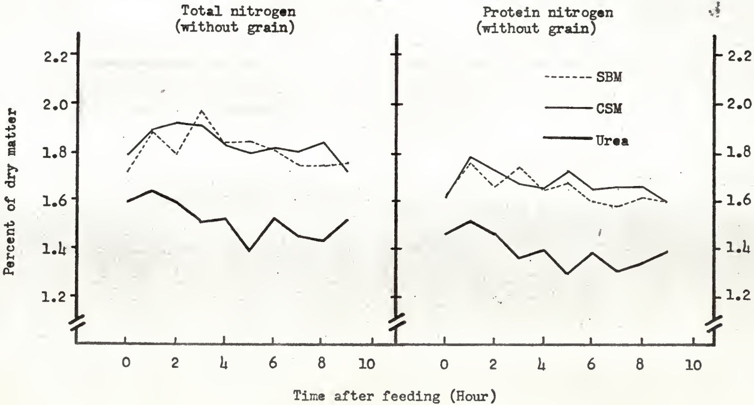
The rumen ammonia concentration and rumen pH values did not seem to be correlated. However, Reis and Reid (1959) observed that the optimum range of pH for ammonia production <u>in vitro</u> was between 6.0 and 6.7. They further found that production decreased rapidly on the acid side of the optimum pH and usually less rapidly on the alkaline side.

Within the respective treatments, the percentages of total nitrogen and protein nitrogen in the rumen ingesta of the steers fed soybean meal, cottonseed meal or urea showed similar patterns in hourly changes (figure 1). Protein nitrogen content was always lower than the total nitrogen. These results would be expected since the total nitrogen analysis includes the protein nitrogen (total nitrogen = non-protein nitrogen + protein nitrogen).

The increase in both total nitrogen and protein nitrogen in the rumen of steers on all three treatments one hour after feeding was due to the nitrogen (both non-protein nitrogen and protein nitrogen) supplied by the ration. But the explanations for the inconsistent hourly changes thereafter are difficult since no attempt was made to measure the rate of nutrient removal from the rumen. In a similar type of study, Pearson and Smith (1943) also observed variations in total nitrogen content of the rumen at different hours after feeding. They attributed the variations to the sampling techniques used and the rate of removal of nutrients from the rumen.

The total nitrogen and protein nitrogen values obtained for steers fed soybean meal or cottonseed meal were similar. These findings support the results obtained earlier (Akram 1964) using one sampling time (6 hours after feeding). This further supports the results of McCampbell (1924),

Figure 1. Average percentage total nitrogen and protein nitrogen of dried rumen samples from three fistulated steers.



Holdaway (1925), and Gallup et al. (1952), that soybean meal and cottonseed meal are of equal value for cattle and sheep.

The significantly lower percentage protein nitrogen in the rumen of steers fed urea at 1 to 9 hours after feeding ($P < 0.05$), may be explained by the extremely low level of total nitrogen present in the rumen before feeding (0 hour) and the subsequent high level of ammonia produced from 1 to 5 hours after feeding.

Experiment II

The moisture content of rumen ingesta at all collection periods did not seem to be influenced by the various nitrogen supplements fed with added grain. Similar animal differences were observed as in experiment I.

Rumen pH values of steers on all three treatments decreased after feeding, for 2 to 4 hours. This consistent decrease in pH would more likely be due to the added grain (corn) rather than the nitrogen supplements. Barnett and Reid (1961) stated that the addition of a readily available carbohydrate source to a diet produces a rapid fall in pH. Purser and Moir (1959) found that the infusion of glucose into the rumen of sheep maintained on an adequate diet will cause a fall in ruminal pH 2-4 hours after infusion.

For the first 2 hours after feeding, rumen ammonia concentrations of steers fed urea were significantly ($P < 0.05$) higher than steers fed soybean meal or cottonseed meal. Steers fed soybean meal or cottonseed meal showed similar rumen ammonia concentrations throughout the collection periods. As in experiment I, all three treatments produced peak levels of ammonia within 2 hours after feeding. The results indicate that urea is degraded to ammonia very rapidly even in the presence of large amounts of grain. The hourly changes in rumen ammonia concentrations did not correspond to the hourly changes of rumen pH values.

With added grain, the overall percentages of total nitrogen and protein nitrogen were quite similar for steers fed rations supplemented with soybean meal, cottonseed meal or urea (figure 2). The relatively constant content of total nitrogen and protein nitrogen at high levels, at all times, suggests that the nitrogen supplied by the added corn played a major role in this experiment. There seems to be some evidence that corn protein is quite insoluble in the rumen. Annison et al. (1954) stated that corn-gluten meal and other forms of corn yields very little ammonia.

Experiment II Versus Experiment I

The steers fed rations supplemented with soybean meal, cottonseed meal, or urea with added grain had a lower percentage of moisture in the rumen than steers fed the same rations without added grain.

The pH values of the rumen liquor from steers fed soybean meal, cottonseed meal, or urea with added grain were lower than that of steers fed the same rations without added grain. The lower values for the treatments with added grain, as discussed in the previous section, may largely be due to the greater microbial activities caused by the higher energy content of the rations.

The higher peak levels of rumen ammonia found for steers fed soybean meal, cottonseed meal or urea with added grain, as compared to those fed the same rations without added grain could be attributed to the higher level of nitrogen in the rations (figure 3). The rate at which the hourly concentrations of rumen ammonia decreased after the peak level, at one to two hours after feeding, for steers on the treatments with added grain, was much

Figure 2. Average percentage total nitrogen and protein nitrogen of dried rumen samples from three fistulated steers.

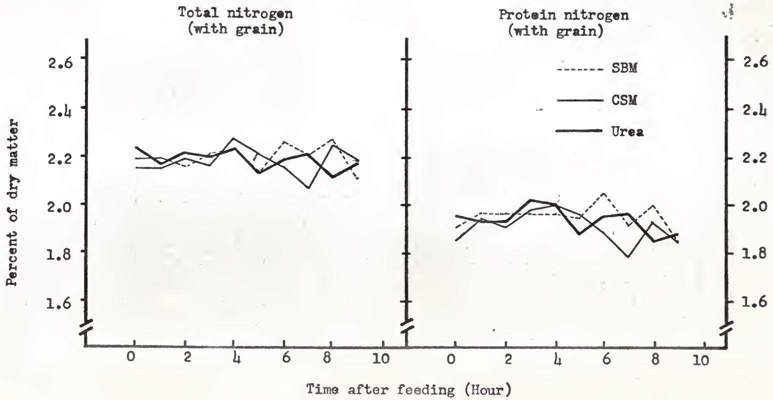
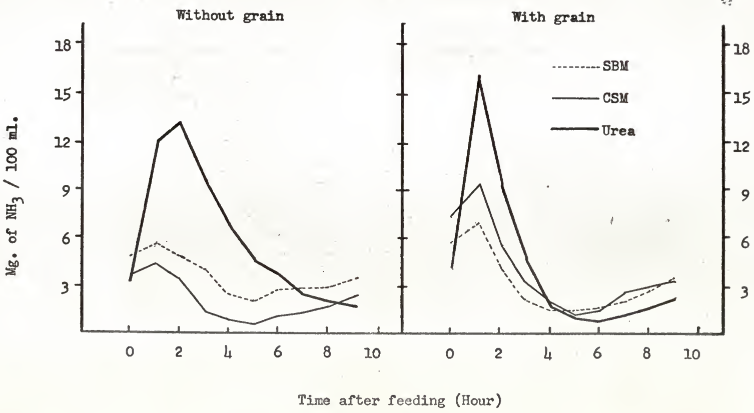


Figure 3. Average ammonia level of rumen liquor from three fistulated steers.



more rapid than that of steers on the same rations without added grain. This sharp decrease in ammonia level, and the lower pH values in the rumen of the steers fed grain, indicates that the added grain improved the rate of utilization of the ammonia by increasing the activities of the rumen micro-organisms. These findings further support the results obtained by Annison <u>et al</u>. (1954) and Phillipson <u>et al</u>. (1959), namely due to the increase in the numbers of bacteria that utilize ammonia nitrogen, the addition of starch to protein ration decreases the rumen ammonia level.

The percentages of total nitrogen and protein nitrogen in the rumen of steers fed soybean meal, cottonseed meal, or urea with added grain were higher at all hours of collection than those found in steers fed the same rations without added grain. The higher values for steers fed the rations with added grain may largely be due to the higher level of nitrogen in the rations. However, there was evidence indicating that the readily available energy from the added corn improved the utilization of the nitrogen supplements in that there were only slight differences in the protein nitrogen content in the rumen ingesta of steers on the three treatments in experiment II. The protein nitrogen content in the rumen of steers fed urea was very similar to that of steers fed soybean meal or cottonseed meal. With the similar comparisons in experiment I, steers fed urea had, at all hours after feeding, significantly ($P < 0.05$) lower protein nitrogen content in the rumen than that of steers fed soybean meal or cottonseed meal.

SUMMARY

Samples of rumen liquor and ingesta were collected from fistulated steers to study the comparative values of rumen pH, ammonia, total and protein nitrogen of the animals fed a basal ration supplemented with different

sources of additional nitrogen, soybean meal, cottonseed meal, and urea with and without added grain. The samples were collected at a period immediately before feeding and 1, 2, 3, 4, 5, 6, 7, 8 and 9 hours after feeding.

The moisture content of the rumen ingesta differed markedly both in experiment I and experiment II. In both experiments, the hourly moisture changes were not consistent, but relatively constant in content. The steers fed rations supplemented with soybean meal, cottonseed meal or urea with added grain had a lower percentage of moisture in the rumen than steers fed the same rations without added grain.

The pH values of rumen liquor from steers fed the various nitrogen supplement in both experiments showed only slight hourly pH changes. Generally, the rumen pH decreased after feeding for about 4 hours and thereafter increased until conclusion of the collection periods. Only minor differences in pH values were noted among treatments within experiments. However, lower pH values were found for steers on the treatments in experiment II than the same treatments in experiment I.

Within experiments, the rumen ammonia concentration of steers fed soybean meal or cottonseed meal was very similar. From 1 to 5 hours after feeding, steers fed the ration containing urea without added grain had significantly ($P < 0.05$) higher rumen ammonia content than steers on the rations supplemented with soybean meal or cottonseed meal without added grain. However, with added grain, (experiment II) steers fed urea had significantly ($P < 0.05$) higher rumen ammonia levels at only the first 2 hours after feeding. Steers on all treatments, in both experiments, showed peak ammonia levels within 2 hours after feeding. With added grain, the decline in rumen ammonia level after the peak was much faster than the treatments without added grain.

On all treatments and in both experiments, the hourly changes in total nitrogen and protein nitrogen in the rumen ingesta followed similar patterns. In experiment I, the total nitrogen and protein nitrogen contents in the rumen of steers fed the rations supplemented with soybean meal or cottonseed meal were very similar. Steers fed the ration supplemented with urea had significantly ($P < 0.05$) lower total nitrogen and protein nitrogen, at all hours after feeding, than the steers on the other two treatments. With added grain (experiment II), the total nitrogen and protein nitrogen contents in the rumen ingesta of steers fed rations supplemented with soybean meal, cottonseed meal, or urea were similar.

The percentages of total nitrogen and protein nitrogen in the rumen of steers fed soybean meal, cottonseed meal or urea with added grain were higher at all hours of collection than steers fed the same rations without added grain. The higher values for steers fed the rations with added grain was due to the higher level of nitrogen in the rations. However, there was evidence indicating that the readily available energy from the added corn did improve the utilization of the nitrogen supplements in that there were only slight differences in the protein nitrogen content of the rumen ingesta of steers on the three treatments in experiment II. The protein nitrogen content in the rumen of steers fed urea was very similar to that of steers fed soybean meal or cottonseed meal. With the similar comparisons in experiment I, steers fed urea had significantly ($P < 0.05$) lower protein nitrogen content in the rumen than that of steers fed soybean meal or cottonseed meal at all hours after feeding.

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APPENDIX

Table 14. Percentage moisture of the rumen samples from fistulated steers fed prairie hay alone.

	Steers			
Time after feeding	1	2	3	Average
Hour	%	%	%	%
0	89.52	92.01	87.23	89.59
1	88.31	91.52	87.45	89.09
2	89.07	90.51	87.78	89.12
3	88.45	90.90	87.63	88.99
4	88.56	90.97	87.43	88.99
5	87.91	90.92	87.22	88.68
6	88.01	90.25	86.79	88.35
7	88.28	90.50	86.54	88.44
8	88.09	90.29	87.26	88.55
9	87.47	90.60	87.04	88.37

Table 15. pH values of the rumen samples from fistulated steers fed prairie hay alone.

Time after feeding	Steers 1	2	3	Average
Hour	pH	pH	pH	pH
0	6.95	6.90	6.70	6.85
1	6.75	6.75	6.80	6.77
2	6.70	6.85	6.65	6.73
3	6.78	6.90	6.85	6.84
4	6.85	6.90	6.80	6.85
5	6.90	7.30	6.70	6.97
6	6.95	7.00	6.75	6.90
7	7.00	7.15	6.80	6.98
8	7.00	7.10	7.00	7.03
9	6.96	7.13	7.00	7.03

Table 16. Ammonia level of the rumen liquor from fistulated steers fed prairie hay alone.

Time after feeding	Steers 1	Steers 2	Steers 3	Average
Hour	Mg./100 ml.	Mg./100 ml.	Mg./100 ml.	Mg./100 ml.
0	-----	-----	0.438	0.146
1	0.389	0.292	1.411	0.697
2	0.122	0.097	0.049	0.089
3	-----	-----	0.243	0.081
4	-----	-----	-----	-----
5	0.049	-----	-----	0.016
6	-----	-----	-----	-----
7	-----	-----	-----	-----
8	-----	-----	-----	-----
9	-----	-----	-----	-----

Table 17. Percentage total nitrogen of the dried rumen samples from fistulated steers fed prairie hay alone.

	Steers			
Time after feeding	1	2	3	Average
Hour	%	%	%	%
0	1.43	1.40	1.29	1.37
1	1.43	1.32	1.31	1.35
2	1.41	1.20	1.31	1.31
3	1.35	1.30	1.24	1.30
4	1.38	1.30	1.25	1.31
5	1.28	1.30	1.21	1.26
6	1.34	1.30	1.16	1.27
7	1.36	1.22	1.36	1.31
8	1.44	1.22	1.20	1.29
9	1.35	1.32	1.41	1.36

Table 18. Percentage protein nitrogen of the dried rumen samples from fistulated steers fed prairie hay alone.

Time after feeding	Steers 1	2	3	Average
Hour	%	%	%	%
0	1.28	1.22	1.18	1.23
1	1.29	1.17	1.13	1.20
2	1.23	1.11	1.23	1.19
3	1.18	1.16	1.07	1.14
4	1.23	1.18	1.04	1.15
5	1.12	1.16	1.13	1.14
6	1.17	1.16	1.02	1.12
7	1.24	1.10	1.14	1.16
8	1.30	1.15	1.00	1.15
9	1.22	1.11	1.20	1.18

Table 19. Percentage moisture of the rumen samples from fistulated steers fed nitrogen supplements without added grain.

Time after feeding	Soybean meal Steers				Cottonseed meal Steers				Urea Steers			
	1	2	3	Average	1	2	3	Average	1	2	3	Average
Hour		%				%				%		
0	91.32	92.29	88.38	90.66	91.11	91.48	89.27	90.62	90.17	92.00	88.84	90.34
1	88.41	90.25	87.29	88.65	89.20	90.81	87.97	89.33	88.25	90.04	87.14	88.48
2	88.44	89.54	86.87	88.28	88.96	89.20	87.17	88.44	88.35	90.38	87.29	88.67
3	87.93	90.83	86.50	88.42	92.13	90.33	87.07	89.84	88.02	90.03	86.52	88.19
4	89.05	90.05	87.47	88.86	88.47	89.09	86.91	88.16	87.57	87.21	86.90	87.23
5	88.86	90.82	87.23	88.97	88.99	89.41	87.35	88.58	87.78	89.65	86.77	88.07
6	86.38	91.45	87.05	88.29	89.33	89.94	87.64	88.97	88.24	90.53	88.60	89.12
7	88.59	91.29	86.80	88.89	89.21	90.01	87.30	88.84	87.86	90.99	86.85	88.57
8	89.20	90	86.56	89.13	89.09	89.90	87.59	88.86	88.37	90.79	86.47	88.54
9	89.19	91.30	87.29	89.26	89.00	89.94	87.72	88.89	88.58	90.68	87.39	88.88

Table 20. pH values of the rumen liquor from fistulated steers fed nitrogen supplements without added grain.

Time after feeding	Soybean meal Steers			Average	Cottonseed meal Steers			Average	Urea Steers			Average
	1	2	3		1	2	3		1	2	3	
Hour		pH				pH				pH		
0	7.61	6.95	6.95	7.17	7.10	7.41	6.93	7.15	6.90	6.90	6.99	6.93
1	7.05	7.03	6.72	6.93	6.99	7.00	6.95	6.98	7.10	7.07	6.55	6.91
2	6.69	7.00	7.10	6.93	6.99	7.10	6.71	6.93	6.98	7.20	6.90	7.03
3	6.80	6.85	7.13	6.93	7.00	7.00	6.80	6.93	6.90	6.99	6.62	6.84
4	6.84	6.90	6.80	6.85	6.90	6.94	6.80	6.88	7.10	7.10	6.52	6.91
5	7.00	7.00	6.92	6.97	7.08	7.20	6.75	7.01	6.75	7.00	6.92	6.89
6	7.16	7.10	7.00	7.09	7.01	7.00	6.95	6.99	6.95	7.15	6.65	6.92
7	7.10	7.20	7.00	7.10	7.00	7.02	7.00	7.01	6.95	7.02	6.95	6.97
8	7.40	7.00	6.90	7.10	6.87	7.25	7.05	7.06	7.05	6.99	7.00	7.01
9	7.22	7.20	6.90	7.11	7.10	7.20	7.05	7.05	7.14	7.00	7.00	7.05

Table 21. Ammonia level of the rumen liquor from fistulated steers fed nitrogen supplements without added grain.

Time after feeding	Soybean meal Steers				Cottonseed meal Steers				Urea Steers			
	1	2	3	Average	1	2	3	Average	1	2	3	Average
Hour	Mg. NH_3/100 ml.				Mg. NH_3/100 ml.				Mg. NH_3/100 ml.			
0	4.623	4.428	5.183	4.745	4.355	4.379	3.066	3.933	2.409	3.553	3.406	3.123
1	4.136	5.304	7.932	5.791	5.815	3.406	4.842	4.688	5.401	18.686	11.922	12.003
2	1.946	3.699	8.808	4.818	3.942	2.433	2.701	3.025	19.756	12.116	9.245	13.706
3	0.973	2.725	7.859	3.852	1.800	1.217	1.217	1.411	14.900	5.207	7.786	9.298
4	0.729	1.071	5.474	2.425	1.460	0.536	0.925	0.974	10.414	7.007	4.379	7.267
5	0.584	1.752	4.331	2.222	0.755	0.657	0.487	0.633	6.691	4.379	3.115	4.728
6	0.487	2.532	5.475	2.831	1.679	0.730	0.852	1.087	5.353	2.920	2.579	3.617
7	1.266	2.968	4.501	2.912	2.141	1.217	0.852	1.403	3.625	2.433	1.801	2.620
8	1.363	3.285	4.161	2.936	2.433	1.947	1.431	1.937	2.798	2.555	1.947	2.433
9	1.703	3.796	5.158	3.552	2.409	2.676	1.971	2.352	2.019	2.384	2.093	2.165

Table 22. Percentage total nitrogen of the dried rumen samples from fistulated steers fed nitrogen supplements without added grain.

Time after feeding	Soybean meal Steers				Cottonseed meal Steers				Urea Steers			
	1	2	3	Average	1	2	3	Average	1	2	3	Average
Hour		%				%				%		
0	1.78	1.62	1.74	1.71	2.05	1.47	1.83	1.78	1.45	1.61	1.67	1.58
1	1.97	1.74	1.91	1.87	2.20	1.66	1.77	1.88	1.48	1.74	1.68	1.63
2	1.97	1.61	1.75	1.78	2.18	1.73	1.84	1.91	1.54	1.60	1.59	1.58
3	2.16	1.80	1.93	1.96	2.21	1.70	1.80	1.90	1.50	1.49	1.50	1.50
4	1.90	1.67	1.93	1.83	2.12	1.69	1.65	1.82	1.37	1.59	1.61	1.52
5	1.85	1.74	1.90	1.83	2.04	1.50	1.83	1.79	1.24	1.41	1.49	1.38
6	2.00	1.69	1.70	1.80	1.93	1.69	1.82	1.81	1.37	1.56	1.63	1.52
7	1.92	1.67	1.63	1.74	1.92	1.60	1.84	1.79	1.40	1.47	1.48	1.45
8	1.86	1.74	1.64	1.74	1.98	1.77	1.74	1.83	1.39	1.44	1.47	1.43
9	1.79	1.74	1.72	1.75	1.78	1.52	1.85	1.71	1.40	1.51	1.62	1.51

Table 23. Percentage protein nitrogen of the dried rumen samples from fistulated steers fed nitrogen supplements without added grain.

Time after feeding	Soybean meal Steers				Cottonseed meal Steers				Urea Steers			
	1	2	3	Average	1	2	3	Average	1	2	3	Average
Hour		%				%				%		
0	1.72	1.44	1.68	1.62	1.88	1.28	1.67	1.61	1.30	1.46	1.60	1.46
1	1.85	1.60	1.79	1.75	2.03	1.63	1.64	1.77	1.38	1.56	1.58	1.51
2	1.89	1.42	1.66	1.65	1.95	1.56	1.64	1.72	1.41	1.50	1.48	1.46
3	1.88	1.57	1.73	1.73	1.94	1.51	1.53	1.66	1.39	1.39	1.30	1.36
4	1.73	1.47	1.73	1.64	1.86	1.56	1.53	1.65	1.17	1.53	1.47	1.39
5	1.63	1.59	1.79	1.67	1.93	1.47	1.77	1.72	1.15	1.33	1.39	1.29
6	1.69	1.52	1.56	1.59	1.81	1.48	1.64	1.64	1.25	1.41	1.49	1.38
7	1.77	1.44	1.51	1.57	1.80	1.49	1.66	1.65	1.13	1.41	1.37	1.30
8	1.70	1.51	1.61	1.60	1.78	1.60	1.57	1.65	1.25	1.39	1.38	1.34
9	1.58	1.59	1.62	1.59	1.65	1.43	1.68	1.59	1.31	1.39	1.44	1.38

Table 24. Percentage moisture of the rumen samples from fistulated steers fed nitrogen supplements with added grain.

Time after feeding	Soybean meal Steers			Average	Cottonseed meal Steers			Average	Urea Steers			Average
	1	2	3		1	2	3		1	2	3	
Hour		%				%				%		
0	91.73	90.47	87.04	89.75	89.37	92.53	85.16	89.02	89.32	91.26	88.11	89.56
1	88.19	88.30	85.55	87.35	87.78	89.09	84.09	86.99	88.53	89.28	85.02	87.61
2	88.18	88.31	85.08	87.19	87.54	89.39	83.98	86.97	87.65	89.08	84.88	87.20
3	89.15	88.23	85.48	87.62	87.96	89.62	83.80	87.13	87.70	88.51	84.44	86.88
4	89.63	88.66	86.17	88.15	88.02	90.29	83.86	87.39	87.68	90.01	84.75	87.48
5	89.13	89.16	86.66	88.32	88.56	90.31	84.68	87.85	88.06	89.82	84.71	87.53
6	89.72	89.34	86.97	88.68	89.19	91.56	84.28	88.34	88.01	90.29	86.00	88.10
7	89.94	90.04	88.15	89.38	88.55	91.24	84.61	88.13	88.23	90.08	85.73	88.01
8	89.81	89.40	86.52	88.58	89.02	91.51	84.56	88.36	88.59	90.00	86.02	88.20
9	90.84	89.73	87.44	89.34	89.08	91.88	84.72	88.56	88.22	90.40	85.73	88.12

Table 25. pH values of the rumen liquor from fistulated steers fed nitrogen supplements with added grain.

Time after feeding	Soybean meal Steers				Cottonseed meal Steers				Urea Steers			
	1	2	3	Average	1	2	3	Average	1	2	3	Average
Hour		pH				pH				pH		
0	7.13	6.70	6.68	6.83	6.80	7.00	6.40	6.73	6.93	6.70	6.85	6.83
1	6.85	6.51	6.63	6.66	6.68	6.93	6.40	6.67	6.88	6.60	6.80	6.76
2	6.75	6.60	6.40	6.58	6.60	6.88	6.35	6.61	6.70	6.49	6.63	6.60
3	6.75	6.63	6.10	6.49	6.65	6.65	6.60	6.63	6.70	6.55	6.60	6.62
4	6.70	6.80	6.60	6.70	6.63	6.90	6.40	6.64	6.75	6.63	6.65	6.68
5	6.90	7.00	6.25	6.72	6.55	7.03	6.80	6.79	6.40	6.48	6.65	6.51
6	6.90	6.93	6.00	6.61	6.50	6.90	6.13	6.51	6.90	6.63	6.70	6.74
7	7.00	6.88	6.30	6.72	6.80	7.08	6.30	6.73	6.83	6.90	6.80	6.84
8	6.93	6.94	6.35	6.74	6.85	6.95	6.35	6.72	6.90	6.80	6.75	6.82
9	7.03	6.50	6.60	6.71	6.78	6.98	6.61	6.79	7.00	6.85	6.80	6.88

Table 26. Ammonia level of the rumen liquor from fistulated steers fed nitrogen supplements with added grain.

Time after feeding	Soybean meal Steers				Cottonseed meal Steers				Urea Steers			
	1	2	3	Average	1	2	3	Average	1	2	3	Average
Hour	Mg. NH_3/100 ml.				Mg. NH_3/100 ml.				Mg. NH_3/100 ml.			
0	6.132	5.864	5.207	5.734	5.767	6.496	10.876	7.713	4.452	4.306	4.404	4.387
1	6.545	7.080	7.056	6.894	7.226	9.124	10.852	9.067	17.932	14.549	15.231	15.904
2	4.355	4.817	4.136	4.436	3.844	5.961	6.834	5.546	9.829	8.199	11.192	9.740
3	1.752	2.798	1.703	2.084	2.262	2.871	3.942	3.025	4.623	4.915	4.160	4.566
4	1.411	3.504	0.365	1.760	1.898	2.506	1.679	2.028	2.531	1.898	1.022	1.817
5	1.825	2.896	0.487	1.736	1.630	3.042	0.438	1.703	2.287	2.239	0.243	1.590
6	2.336	3.188	0.438	1.997	1.995	3.129	0.681	1.935	1.801	2.458	0.292	1.517
7	2.847	3.017	0.487	2.117	3.163	4.087	1.071	2.774	2.190	2.969	0.584	1.914
8	2.774	3.698	2.020	2.831	3.893	3.893	1.193	2.993	2.433	3.212	0.487	2.044
9	2.968	4.306	2.676	3.317	3.820	4.185	1.703	3.236	2.312	3.260	0.973	2.182

Table 27. Percentage total nitrogen of the dried rumen samples from fistulated steers fed nitrogen supplements with added grain.

Time after feeding	Soybean meal Steers				Cottonseed meal Steers				Urea Steers			
	1	2	3	Average	1	2	3	Average	1	2	3	Average
Hour		%				%				%		
0	2.43	2.19	1.94	2.19	2.17	2.47	1.83	2.15	2.42	2.25	2.00	2.22
1	2.40	2.15	2.04	2.19	2.25	2.37	1.82	2.15	2.26	2.07	2.17	2.17
2	2.33	2.15	2.01	2.16	2.11	2.44	2.02	2.19	2.24	2.19	2.19	2.21
3	2.34	2.16	2.12	2.21	2.12	2.38	1.91	2.14	2.27	2.11	2.24	2.20
4	2.43	2.20	2.06	2.23	2.10	2.44	2.26	2.27	2.37	2.07	2.26	2.23
5	2.52	2.05	1.85	2.14	2.16	2.45	2.03	2.21	2.20	2.07	2.11	2.13
6	2.54	2.02	2.21	2.26	2.24	2.48	1.77	2.16	2.34	1.95	2.29	2.19
7	2.48	2.13	2.03	2.21	2.14	2.31	1.75	2.07	2.44	2.06	2.12	2.21
8	2.58	2.07	2.16	2.27	2.28	2.69	1.77	2.25	2.21	2.07	2.08	2.12
9	2.38	1.97	2.00	2.11	2.26	2.42	1.86	2.18	2.28	2.12	2.10	2.17

Table 28. Percentage protein nitrogen of the dried rumen samples from fistulated steers fed nitrogen supplements with added grain.

Time after	Soybean meal Steers				Cottonseed meal Steers				Urea Steers			
feeding	1	2	3	Average	1	2	3	Average	1	2	3	Average
Hour		%				%				%		
0	2.01	1.99	1.74	1.91	1.90	2.08	1.61	1.86	2.16	1.99	1.72	1.96
1	1.99	1.96	1.96	1.97	2.05	2.06	1.74	1.95	2.00	1.89	1.92	1.94
2	2.02	1.98	1.91	1.97	1.83	2.10	1.80	1.91	1.94	1.98	1.89	1.94
3	2.07	1.89	1.96	1.97	1.98	2.13	1.72	1.98	2.04	1.98	2.02	2.02
4	2.04	2.05	1.82	1.97	1.80	2.10	2.09	2.00	2.19	1.83	1.99	2.00
5	2.26	1.86	1.74	1.95	1.80	2.25	1.83	1.96	1.96	1.78	1.91	1.88
6	2.23	1.85	2.05	2.05	1.99	2.12	1.57	1.89	2.07	1.86	1.94	1.95
7	2.15	1.92	1.70	1.92	1.84	2.00	1.53	1.79	2.18	1.85	1.86	1.96
8	2.19	1.82	1.98	2.00	2.00	2.26	1.52	1.93	2.01	1.76	1.79	1.85
9	1.97	1.72	1.86	1.85	1.95	1.96	1.65	1.85	1.98	1.89	1.75	1.88

Table 29. Analysis of variance of moisture content.

Source of variation	Degree of freedom	Sum of square	Mean square	F value	Level of significance
Steers	2	434.488	217.244	749.117	P < 0.005
Treatments	5	38.912	7.782	26.835	P < 0.005
Time	9	67.610	7.512	25.904	P < 0.005
Steers x treatment	10	77.132	7.713	26.597	P < 0.005
Steers x time	18	7.621	0.423	1.459	N.S.[1]
Treatment x time	45	26.252	0.583	2.010	P < 0.005
Steers x treatment x time (error)	90	26.117	0.290		
Total	179	678.132			

[1]Nonsignificant.

Table 30. Comparison of treatments - LSD on moisture content.

Treatments	Time after feeding (hour)									
	0	1	2	3	4	5	6	7	8	9
SBM[1] vs. CSM[2]	N.S.[3]	N.S.	N.S.	S.[4]	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.
SBM vs. Urea	N.S.	N.S.	N.S.	N.S.	S.	S.	N.S.	N.S.	N.S.	N.S.
CSM vs. Urea	N.S.	N.S.	N.S.	S.	S.	N.S.	N.S.	N.S.	N.S.	N.S.
SBM vs. SBM & Corn	S.	S.	S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.
CSM vs. CSM & Corn	S.	S.	S.	S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.
Urea vs. Urea & Corn	N.S.	N.S.	S.	S.	N.S.	N.S.	S.	N.S.	N.S.	N.S.
SBM & Corn vs. CSM & Corn	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	S.	N.S.	N.S.
SBM & Corn vs. Urea & Corn	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	S.	N.S.	S.
CSM & Corn vs. Urea & Corn	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.

[1]Soybean meal.

[2]Cottonseed meal.

[3]Nonsignificant.

[4]Significant at 5% level.

Table 31. Analysis of variance of rumen pH.

Source of variation	Degree of freedom	Sum of square	Mean square	F value	Level of significance
Steers	2	1.831	0.916	38.167	$P < 0.005$
Treatments	5	4.019	0.804	33.500	$P < 0.005$
Time	9	0.858	0.095	3.958	$P < 0.005$
Steers x treatment	10	1.439	0.144	6.000	$P < 0.005$
Steers x time	18	0.388	0.022	0.917	N.S.[1]
Treatment x time	45	0.614	0.014	0.583	N.S.
Steers x treatment x time (error)	90	2.114	0.024		
Total	179	11.263			

[1]Nonsignificant.

Table 32. Comparison of treatments - LSD on rumen pH.

Treatments	Time after feeding (hour)									
	0	1	2	3	4	5	6	7	8	9
SBM[1] vs. CSM[2]	N.S.[3]	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.
SBM vs. Urea	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.
CSM vs. Urea	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.
SBM vs. SBM & Corn	S.[4]	S.	S.	S.	N.S.	N.S.	S.	S.	S.	S.
CSM vs. CSM & Corn	S.	S.	S.	S.	N.S.	N.S.	S.	S.	S.	S.
Urea vs. Urea & Corn	N.S.	N.S.	S.	N.S.	N.S.	S.	N.S.	N.S.	N.S.	N.S.
SBM & Corn vs. CSM & Corn	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.
SBM & Corn vs. Urea & Corn	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.
CSM & Corn vs. Urea & Corn	N.S.	N.S.	N.S.	N.S.	N.S.	S.	N.S.	N.S.	N.S.	N.S.

[1]Soybean meal.

[2]Cottonseed meal.

[3]Nonsignificant.

[4]Significant at 5% level.

Table 33. Analysis of variance of ammonia content.

Source of variation	Degree of freedom	Sum of square	Mean square	F value	Level of significance
Steers	2	5.559	2.776	0.910	N.S.[1]
Treatments	5	260.609	52.122	17.089	$P < 0.005$
Time	9	875.709	95.301	31.246	$P < 0.005$
Steers x treatment	10	141.042	14.104	4.624	$P < 0.005$
Steers x time	18	48.762	2.709	0.888	N.S.
Treatment x time	45	568.784	12.640	4.144	$P < 0.005$
Steers x treatment x time (error)	90	274.518	3.050		
Total	179	2174.983			

[1]Nonsignificant.

Table 34. Comparison of treatments - LSD on ammonia content.

Treatments	Time after feeding (hour)									
	0	1	2	3	4	5	6	7	8	9
SBM[1] vs. CSM[2]	N.S.[3]	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.
SBM vs. Urea	N.S.	S.[4]	S.	S.	S.	N.S.	N.S.	N.S.	N.S.	N.S.
CSM vs. Urea	N.S.	S.	S.	S.	S.	S.	N.S.	N.S.	N.S.	N.S.
SBM vs. SBM & Corn	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.
CSM vs. CSM & Corn	S.	S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.
Urea vs. Urea & Corn	N.S.	S.	S.	S.	S.	S.	N.S.	N.S.	N.S.	N.S.
SBM & Corn vs. CSM & Corn	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.
SBM & Corn vs. Urea & Corn	N.S.	S.	S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.
CSM & Corn vs. Urea & Corn	S.	S.	S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.

[1]Soybean meal.

[2]Cottonseed meal.

[3]Nonsignificant.

[4]Significant at 5% level.

Table 35. Analysis of variance of total nitrogen.

Source of variation	Degree of freedom	Sum of square	Mean square	F value	Level of significance
Steers	2	0.982	0.491	122.750	$P < 0.005$
Treatments	5	12.030	2.406	601.500	$P < 0.005$
Time	9	0.159	0.018	4.500	$P < 0.005$
Steers x treatment	10	2.867	0.287	71.750	$P < 0.005$
Steers x time	18	0.608	0.034	8.500	$P < 0.005$
Treatment x time	45	0.439	0.010	2.500	$P < 0.005$
Steers x treatment x time (error)	90	0.370	0.004		
Total	179	17.455			

Table 36. Comparison of treatments - LSD on total nitrogen.

Treatments	Time after feeding (hour)									
	0	1	2	3	4	5	6	7	8	9
SBM[1] vs. CSM[2]	N.S.[3]	N.S.	S.[4]	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.
SBM vs. Urea	S.	S.	S.	S.	S.	S.	S.	S.	S.	S.
CSM vs. Urea	S.	S.	S.	S.	S.	S.	S.	S.	S.	S.
SBM vs. SBM & Corn	S.	S.	S.	S.	S.	S.	S.	S.	S.	S.
CSM vs. CSM & Corn	S.	S.	S.	S.	S.	S.	S.	S.	S.	S.
Urea vs. Urea & Corn	S.	S.	S.	S.	S.	S.	S.	S.	S.	S.
SBM & Corn vs. CSM & Corn	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.
SBM & Corn vs. Urea & Corn	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	S.	N.S.
CSM & Corn vs. Urea & Corn	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	S.	S.	N.S.

[1]Soybean meal.

[2]Cottonseed meal.

[3]Nonsignificant.

[4]Significant at 5% level.

Table 37. Analysis of variance of protein nitrogen.

Source of variation	Degree of freedom	Sum of square	Mean square	F value	Level of significance
Steers	2	0.506	0.253	23.000	$P < 0.005$
Treatments	5	7.591	1.518	138.000	$P < 0.005$
Time	9	0.234	0.026	2.364	$P < 0.10$
Steers x treatment	10	1.927	0.193	17.546	$P < 0.005$
Steers x time	18	0.108	0.006	0.546	N.S.[1]
Treatment x time	45	0.325	0.007	0.636	N.S.
Steers x treatment x time (error)	90	0.997	0.011		
Total	179	11.688			

[1]Nonsignificant.

Table 38. Comparison of treatments - LSD on protein nitrogen.

Treatments	Time after feeding (hour)									
	0	1	2	3	4	5	6	7	8	9
SBM[1] vs. CSM[2]	N.S.[3]	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.
SBM vs. Urea	N.S.	S.[4]	S.	S.	S.	S.	S.	S.	S.	S.
CSM vs. Urea	N.S.	S.	S.	S.	S.	S.	S.	S.	S.	S.
SBM vs. SBM & Corn	S.	S.	S.	S.	S.	S.	S.	S.	S.	S.
CSM vs. CSM & Corn	S.	S.	S.	S.	S.	S.	S.	N.S.	S.	S.
Urea vs. Urea & Corn	S.	S.	S.	S.	S.	S.	S.	S.	S.	S.
SBM & Corn vs. CSM & Corn	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.
SBM & Corn vs. Urea & Corn	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.
CSM & Corn vs. Urea & Corn	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	S.	N.S.	N.S.

[1]Soybean meal.

[2]Cottonseed meal.

[3]Nonsignificant.

[4]Significant at 5% level.

THE EFFECT OF NITROGEN SOURCE ON RUMEN PH, AMMONIA, TOTAL AND PROTEIN NITROGEN

by

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Three rumen fistulated steers were used in two 3 x 3 factorial experiments designed to compare the hourly changes in the pH value, ammonia concentration and percentages total and protein nitrogen in the rumen of the steers fed a basal ration of prairie hay supplemented with soybean meal, cottonseed meal or urea without (experiment I) and with added grain (corn) (experiment II). Samples of rumen liquor and ingesta were collected at a period immediately before the morning feeding (0 hour) and 1, 2, 3, 4, 5, 6, 7, 8 and 9 hours after feeding.

In both experiments, rumen pH values generally decreased after feeding for about 4 hours and thereafter increased to the end of the collection period. Lower pH values were found for steers on the treatments in experiment II than the same treatments in experiment I.

Within experiments, the rumen ammonia concentration of steers fed soybean meal or cottonseed meal were very similar. In experiment I, the steers that were fed urea showed significantly ($P < 0.05$) higher rumen ammonia content than that of steers fed soybean meal or cottonseed meal from 1 to 5 hours after feeding. However, with added grain (experiment II) the steers fed urea showed significantly ($P < 0.05$) higher rumen ammonia levels for only the first 2 hours after feeding. Steers on all treatments in both experiments showed peak ammonia levels within 2 hours after feeding. With added grain, the decline in rumen ammonia concentration after the peak was much faster than the treatments without added grain.

In experiment I, the total and protein nitrogen content in the rumen of the steers fed soybean meal or cottonseed meal were very similar. Steers that were fed urea had significantly ($P < 0.05$) lower total and protein nitrogen, at all hours after feeding, than the steers on the other two treatments. With added grain (experiment II), the total and protein nitrogen content in the rumen of steers fed soybean meal, cottonseed meal, or urea were similar.

The percentages of total and protein nitrogen in the rumen of steers fed soybean meal, cottonseed meal or urea with added grain were higher at all hours of collection than steers fed the same rations without added grain. The higher values for steers fed the rations with added grain were due to the higher level of nitrogen in the rations. However, there was evidence indicating that the readily available energy from the added corn did improve the utilization of the nitrogen supplements in that there were only slight differences in the protein nitrogen content of the rumen ingesta of steers on the three treatments in experiment II. The protein nitrogen content in the rumen of steers fed urea was very similar to that of steers fed soybean meal or cottonseed meal. With the similar comparisons in experiment I, steers fed urea had significantly ($P < 0.05$) lower protein nitrogen content in the rumen than that of steers fed soybean meal or cottonseed meal at all hours after feeding.