A STUDY OF UREA UTILIZATION IN THE BOVINE USING THE RE-ENTRANT DUODENAL FISTULA TECHNIQUE

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INTRODUCTION

The importance of rumen microorganisms for the synthesis of amino acids which are utilized by the host is well established. The discovery that these microorganisms are able to utilize non-protein nitrogen sources for this synthesis led to many investigations into the use of such compounds as urea as a source of crude protein for the ruminant. The majority of early studies were concerned mainly with urea utilization as measured by such parameters as body weight changes and milk or wool production. More recently, however, attention has been centered on the qualitative and quantitative aspects of protein synthesis and nitrogen metabolism in the rumen. Studies of rumen contents have contributed greatly to the knowledge of the mechanisms of nitrogen metabolism in the rumen, but the dynamic state of the rumen makes quantitative measurements difficult. Food is periodically ingested; nitrogen is continuously entering the rumen in the saliva; ammonia may be absorbed from the rumen and urea may be secreted into the rumen directly from the blood stream; dietary protein is broken down by the rumen microorganisms and synthesized into microbial protein; and digesta is continuously passing out of the rumen and on down the digestive tract. The complexity of this system has led investigators to study the digesta leaving the rumen in order to determine the net effects of ruminal digestion. It was this method, namely the quantitative study of duodenal contents, which was used in this study to determine the efficiency of utilization of urea nitrogen as compared to soybean oil meal protein nitrogen when supplemented to a ration which was deficient in protein.

A REVIEW OF THE LITERATURE

The peculiar position of the ruminant animal in the field of nutrition is brought about by the fact that the food ingested by these animals is digested by microorganisms in the rumen. It is these microorganisms which enable the ruminant to utilize large amounts of cellulose-rich roughages such as hays, silage and pasture. The rumen bacteria are also known to eliminate certain vitamin requirements of the animal by synthesizing all vitamins except A, D and E. A third major function of the rumen bacteria, and one which has been widely studied in recent years, is that of synthesizing true microbial protein from non-protein nitrogen sources derived from either protein in the feedstuffs or supplements of non-protein nitrogen compounds. The significance of this microbial activity was suggested by Hastings (1944) who pointed out that the rumen contents of a sheep may comprise 20 per cent of the total body weight, and that 10 per cent of the wet volume may be living protozoa and bacteria.

Utilization of Urea for Protein Synthesis

The importance of the ability of rumen bacteria to incorporate non-protein nitrogen into true protein in their bodies was not realized until the last two decades. It was in 1891, however, that Zuntz (1891) outlined the utilization of amides and ammonium salts and their conversion to protein by rumen microorganisms and subsequent digestion and assimilation by the host. After a series of extensive investigations, Armsby (1911) reported that non-protein nitrogen sources could serve as a partial substitute for protein in a protein deficient diet. He also concluded that the non-protein nitrogen sources were inferior to true protein in nutritive value, but that protein so formed was not inferior to body protein.

Wegner et al. (1940) incubated rumen liquor from fistulated cows with synthetic buffered media containing urea and readily available carbohydrates and studied the conversion of inorganic nitrogen to protein by the microorganisms present. They found that while the total nitrogen remained constant the ammonia nitrogen decreased over a period of time indicating an increase in the protein nitrogen. Identical results with ammonium bicarbonate and urea containing an equal amount of nitrogen indicated hydrolysis of the urea to ammonia. It was also concluded that the protein formed must be microbial since it did not pass through a cell of fine silica powder which did not pass bacteria. These same workers (Wegner et al., 1941a,b) with in vivo studies demonstrated the importance of the level of protein in the ration on the utilization of urea. When a basal ration of 8.5 per cent protein was fed there were no indications of protein synthesis, but a basal ration of 4.0 per cent protein showed a decrease in rumen ammonia content eight hours after feeding. The protein content increased from 9.8 per cent on the basal ration to 12.5 per cent on the basal plus urea. Disappearance of urea which comprised up to five per cent of the concentrate was credited to microbial synthesis of protein. Evidence that other factors may be involved in this disappearance will be discussed later.

Harris and Mitchell (1941a,b) also studied the value of urea in protein synthesis and found that when urea was added to a low protein ration it improved the digestibility of cellulose. The urea itself was utilized up to 88 per cent. When sheep were maintained on 202 mg. urea nitrogen and 161 mg. casein nitrogen per day the biological value of urea and casein were calculated to be 62 and 79, respectively. That higher concentrations of urea were less completely utilized was

pointed out by the fact that the biological value for rations containing 8, 11 and 15 per cent of the protein as urea nitrogen were 74, 60 and 44, respectively. Other workers have suggested an upper limit to the amount of urea which may be utilized by ruminants. Johnson et al. (1942) found an improvement in nitrogen retention in lambs when urea supplied up to 12 per cent crude protein but no further improvement by additional urea. It was also noted that supplementation with sugars enhanced urea utilization but depressed the digestion of cellulose. The results of this study led these investigators to state that a considerable portion of the protein utilized by the ruminant is microbial protein regardless of the nature of nitrogenous compounds in the diet.

Pearson and Smith (1943a,b,c) criticized the value of conclusions of some previous investigators (Wegner et al., 1941a,b) who used an in vivo technique for estimations of protein synthesis in the rumen and developed an in vitro procedure for their own investigations. Among the findings of these workers are the following: (1) Urea is rapidly hydrolyzed to ammonia in the rumen. (2) Protein synthesis occurred on the order of 8 mg. nitrogen per 100 ml. rumen liquor. (3) This synthesis is microbial in nature. (4) The degree of protein synthesis is related to the amount and type of carbohydrate present, starch being the most effective. (5) Protein synthesis occurring during the incubation of rumen liquor is accompanied by protein breakdown, either process predominating according to the general conditions or substances present. Pearson and Smith (1943c) estimated that microbial protein synthesis equivalent to 450 g. protein may occur in the intact rumen over a period of 24 hours.

Later work (Johnson et al., 1944) on the mechanism of this non-

protein nitrogen utilization by ruminants showed that it was the bacteria and not the protozoa in the rumen which were responsible for the protein synthesis. Defaunating the rumen with $CuSO_4$ did not reduce the ability of the animal to utilize urea. The importance of the protozoa, however, was pointed out when the quantity of bacteria and protozoa were studied over a period of time. One hour after feeding the flora had reached a peak and began to decrease, while the fauna began to increase. By the 16th hour after feeding the protozoa had doubled, and the flora had decreased to 1/16 of what it was at the end of the first hour. It was concluded that protozoa consume the bacteria and in this way incorporate the bacterial protein into their own body protein. Smith and Baker (1944) also studied the relative significance of rumen bacteria and protozoa in protein synthesis and found the protozoa unable to utilize urea. These studies, carried out in vitro, involved separating the bacteria, protozoa and plant debris centrifugally. Disappearance of non-protein nitrogen, which was correlated with an increase in total nitrogen as well as polysaccharide in the bacteria, was considered to be synthesis of protein. Analysis of the bacteria for crude protein, carbohydrate, ether extract and ash were found to be comparable to linseed cake.

The first direct evidence that urea is utilized itself in the buildup of microbial protein and doesn't merely exert some type of sparing action was demonstrated by Watson et al. (1949). Ten to twelve grams of urea containing N^{15} were fed over a four day period. Samples of liver, blood and kidneys showed excess N^{15} in the protein components of these sheep when compared to controls fed regular urea.

A realization of the importance of protein synthesis by rumen

microorganisms led to studies of the nutritive value of protein so formed. Reed et al. (1949) reported that, except for a mild deficiency of methionine, the nutritive value of bacterial protein was high. Loosli and Harris (1945) had previously reported that urea plus methionine gave growth results in lambs equal to that of natural protein. Urea alone was inferior. Lofgreen et al. (1953), however, found no differences in efficiency of feed utilization, nitrogen retention, serum sulfate level and wool growth with sheep on a basal ration plus urea and those with added sodium sulfate. These workers suggest that the variable results noted by different investigators is a function of the sulfur content of the ration. When sulfur is inadequate in the diet, the addition of sulfate or methionine to the ration will produce the desired growth response. Johanson et al. (1949) found the cystine, cysteine and methionine content of rumen bacteria to be higher than for leguminous seeds and higher than reported for casein and muscle protein.

Agrawala et al. (1953) and Duncan et al. (1953) reported considerable synthesis of the ten dietary essential amino acids of the rat by the microorganisms in the rumen. The amino acid composition of true protein was similar for both the purified and natural diets. This confirmed the earlier work of Loosli et al. (1949). Weller (1957) studied the amino acid composition of hydrolysates of microbial preparations from the rumen of the sheep on pasture or fed wheat hay chaff, alfalfa hay chaff or wheat hay chaff with oats and urea. The whole proteins were hydrolyzed and the amino acids studied by ion-exchange chromatography. The composition of the bacterial hydrolysates were found to be remarkably uniform. The amino acid distribution resembled that reported for protein from pasture herbage and from rumen contents. Protozoal

protein differed in that it contained somewhat higher proportions of leucine, isoleucine, phenylalanine and lysine.

Knowledge that the nature of the carbohydrate present has a significant effect of the degree of urea utilization has led to many recent studies of this relationship. Pope et al. (1950) and Gallup et al. (1952) studied the effect of substituting roughage with cottonseed hulls on nitrogen retention in lambs fed a control ration containing 6.5 per cent crude protein and control plus urea containing 10.5 per cent crude protein. The nitrogen retention values where cottonseed hulls replaced 30, 50 and 85 per cent of the roughage were 1.22, 0.26 and -0.54 grams per day, respectively, on the control ration and 2.30, 0.55 and -0.33 grams per day on the ration containing urea. A comparison of a control ration with 40 per cent cottonseed hulls with 7.0 per cent crude protein was made with rations to which urea was added up to 8.5, 10.2 and 12.5 per cent protein equivalent. Nitrogen retention values of 0.56, 1.72, 2.44 and 2.30 grams per day, respectively, were noted. These results indicate a decreased utilization of urea as the cottonseed hull content of the ration was increased and also as the proportion of urea was increased above a maximum level. In all trials digestibility of the ration was improved by addition of the urea.

Burroughs et al. (1951), in in vitro studies, incubated urea and cellulose with varying amounts of protein and found the increased protein greatly increased the ammonia present. It was stated that this excess ammonia would be wasted in the intact animal. In a succeeding paper, Arias et al. (1951) demonstrated that increasing the amount of added carbohydrate, whether sucrose, cellulose, starch or corn cobs, increased the utilization of urea which had been added in excess of microbial

protein synthetic capacity. With in vitro studies, Hudman et al. (1953) found that when rumen liquor from sheep fed alfalfa hay was incubated with saline media containing glucose and powdered cellulose, protein synthesis was increased when urea or ammonium citrate was added. The omission of the cellulose, however, made very little difference in the results, indicating that cellulose in the presence of sugar is only slowly digested.

Williams et al. (1953), in studies with sheep, showed that increasing the nitrogen content of a diet containing added starch significantly increased the bacterial counts, whereas the diet lacking the starch showed no such effect. Starch at all levels of feeding showed no increase in digestibility of the dry matter. Protein, however, at all levels of starch feeding significantly increased dry matter digestibility. It was concluded that the proportion of the total protein in the ration converted to microbial protein, on the assumption that this is reflected by the bacterial counts, is not a constant but diminishes as the intake of protein increases. Bacterial counts increased from 27 to 58 million per cubic milliliter while protein intakes increased by a wider ratio, from 14.2 to 82.3 grams per day. Gallup et al. (1954) demonstrated more efficient utilization of soybean meal nitrogen than urea nitrogen by ruminants fed high molasses and sugar rations, but both nitrogen sources increased the digestibility of organic matter and crude fiber over that of a basal ration. Fontenot et al. (1955) found reduced nitrogen retention by steers fed increasing amounts of cerelose in a low protein diet, whereas on a high protein diet the nitrogen retention was increased. At all protein levels the biological value of the protein was increased with increasing cerelose. Belasco (1956) related

urea utilization to the type and amount of carbohydrate present. He showed that urea utilization was increased more by starch supplementation than by addition of cellulose, zylan or pectin. Lewis and McDonald (1958) found greater utilization of rumen ammonia in sheep when levan or starch was added to the ration than when cellulose was added. When disappearance of ammonia was measured following the addition of 400 ml. of ammonium acetate in water to the rumen, starch and levan showed the greatest increase in nitrogen retention. Glucose and, to a lesser extent, xylan were intermediate, and cellulose showed no effect. These authors suggested that the ideal situation for utilization of nitrogen sources is where both the protein and carbohydrate are fermented at equal rates.

An excellent review of the use of urea as a protein replacement for ruminants is that by Reid (1953).

Absorption of Ammonia from the Rumen

McDonald (1948) reported that ammonia could be directly absorbed into the blood stream from the rumen of the sheep. It was found that while the blood of the general circulation contained almost no ammonia, blood from the ruminal veins contained about 1.7 mg. % ammonia nitrogen. In order to establish that this ammonia was in fact derived from the rumen contents further experiments were conducted. Ruminal vein blood samples were taken when the washed rumen was filled with warm water and again when filled with four liters of a solution of ammonium acetate. The marked rise in the level of ammonia in the ruminal vein blood after addition of the ammonium acetate left no doubt that ammonia could be absorbed from the rumen. It was calculated that the absorption could amount to about 4-5 g. ammonia nitrogen per day when rumen ammonia was at a level of 22.6 mg. %. McDonald (1948, 1952) also suggests that

since urea is present in the saliva, it appears that a nitrogen circulation system occurs in the normal digestive process. Salivary urea is converted to ammonia which may be absorbed from the rumen, converted back to urea in the liver and once again become available for secretion in the saliva. Chalmers et al. (1954) confirmed the absorption of ammonia from the rumen of anaesthetized sheep fed rations supplemented with casein. Casein supplemented via duodenal fistula was retained better than that fed orally. Casein which had been heat treated to reduce it's digestibility in the rumen gave similar results.

Dinning et al. (1948) noted a rise in ammonia and urea levels in the portal and systemic blood when 40 g. urea and 40 g. sucrose in 100 ml. water was injected into the rumen of sheep anesthetized with pentothal solium. When administered to steers by stomach tube, 114-490 g. urea produced an abrupt rise in ammonia in jugular blood. Bouckaert and Oyaert (1952) suggested that absorption of ammonia was related to the buffering capacity of the rumen, the alkaling reaction converting urea to ammonia paralyzing the rumen and allowing ammonia to be absorbed through the epithelium. When sheep which had been on a straw ration for 14 days were given a ruminal injection of urea an increase in rumen pH and high mortality were observed. When sheep on an alfalfa ration, however, were given the same injection no toxic effects were observed. Addition of ammonium chloride caused little change in rumen pH. When made alkaline with NaOH or NH_4CO_3 the rumen returned to neutrality. Observations of NH_4 and Cl showed selective absorption of these ions in order to reach neutrality. In poorly fed animals with a poor rumen buffering capacity, ammonia would be absorbed in order to regain neutrality.

El-Shazly (1952a,b) demonstrated the importance of ammonia production from degradation of protein and amino acids in the rumen. The relation of this to ammonia absorption as well as protein synthesis is discussed very thoroughly in a review by Chalmers and Synge (1954).

Lewis (1957) made estimations of portal vein ammonia in sheep and found a considerable increase as rumen ammonia exceeded 25-30 mmol per liter. The level of blood urea was found to be correlated to the rumen ammonia level following a delay of 4-8 hours. It was proposed that when blood urea exceeds a level of 25-30 mmol per liter losses of nitrogen are considerable. After making several assumptions a figure of 15-30 per cent loss of ingested nitrogen was proposed for conditions under which rumen ammonia concentration was very high. Lewis et al. (1957) estimated the amount of ammonia nitrogen carried to the liver in 24 hours to be in the region of 14 g. in a 40 kg. sheep fed 800 g. hay and 50 g. casein daily. This estimate assumed a ruminal ammonia level of 40 mmol per liter, portal ammonia concentration of 0.65 mg. % and portal blood flow of 37 ml. per min. per kg. body weight in conscious sheep as reported by Schambye (1956). Houpt (1959) while studying the movement of intravenously injected urea into the isolated, saline filled rumen of the sheep noted simultaneous absorption of ammonia of 3.8 mmol ammonia nitrogen per hour at the end of the second hour. Absorption from the rumen of another sheep and a goat were estimated at 2.4 and 1.5 mmol ammonia nitrogen per hour, respectively.

Observations of nitrogen:lignin ratios by Gray et al. (1958) in sheep fed high quality alfalfa hay suggested a loss of nitrogen from the rumen. It was concluded that the decreased N:L ratio of the rumen contents over that of the feed indicated a loss of nitrogen through

absorption of ammonia rather than a faster rate of passage on nitrogen out of the rumen. Had the latter occurred, the N:L ratio of abomasal contents would be expected to be higher. This was not observed. The ruminal ammonia levels reported here were in the range of 40-50 mg. % for the six hours immediately after feeding. This was much in excess of the 22.6 mg. % for which McDonald (1948) reported considerable ammonia absorption.

The absorption of nitrogenous compounds other than ammonia from the rumen is not believed to occur to any great degree. Tsuda (1956) demonstrated that urea at levels normally found in the rumen was not absorbed from the "miniature rumen" of the goat. As he pointed out, however, urea is rapidly broken down to ammonia which may be absorbed. Tsuda (1956) also found no absorption of glycine at levels of 110 mg. %. This was the only amino acid studied, however. Dinning et al. (1949) found no ammonia loss from the rumen in the expired air.

Utilization of Blood Urea

Urea in the Saliva

Although the major functions of saliva in the bovine are to provide sufficient liquid for lubrication of foodstuffs and for the microbial fermentation vat and buffer action to reduce the acidity of the fatty acids, it also contains certain nutrients for use by the nicroorganisms. Nitrogen in the saliva may be utilized by the rumen microflora for protein synthesis in the same manner as dietary nitrogen. In order to quantitatively evaluate the nitrogen metabolism scheme in the rumen it becomes necessary to consider the nitrogen contributed by the saliva.

McDonald (1948) has estimated the salivary secretions of the sheep to contain about 0.5 g. nitrogen per day. A slightly lower estimate,

20-26 mmol nitrogen per day, was given by McDougall (1948). Somers (1958), also working with sheep, found that 2.4 liters of saliva containing 290 mg. nitrogen were secreted per day.

Studies of bovine saliva by Conner (1959) and McGilliard (1957a) have led to an estimation of 10 g. nitrogen per day secreted in the saliva (McGilliard, 1957b). Phillipson and Mangan (1959) found the protein nitrogen content of orally collected saliva to be much greater than the urea nitrogen content. These workers estimate that 15 g. nitrogen per day in the form of protein which is readily metabolized by rumen microorganisms may be secreted by the adult cow.

Direct Passage of Urea Through the Rumen Wall

Schmidt-Nielsen et al. (1957) demonstrated that large amounts (259 and 475 mmol) of urea given intravenously to camels on a low protein diet was almost completely retained. Further work (Schmidt-Nielsen, 1958; Schmidt-Nielsen and Osaki, 1958; and Schmidt-Nielsen et al. 1958) revealed that sheep also had the ability to retain nitrogen while on a low protein diet. It was demonstrated that the excretion of urea in such sheep remained at a low level despite the increased blood urea following urea injections. The exact mechanism of this renal regulation of urea excretion has not been determined. It is known, however, that the blood urea level is not the primary factor (Schmidt-Nielsen and Osaki, 1958).

Later work by Houpt (1959) with urea injection and rumen saline experiments showed that considerable urea could pass directly from the blood into the rumen. When urea was given intravenously to sheep receiving a carbohydrate-supplemented ration, 53 per cent of the injected urea was retained compared to 22 per cent for the ration containing hay

alone. Total transfer of urea to the rumen amounted to 8.3, 7.7 and 13.0 mmol per hour during the second and fourth hours for one sheep and the fourth hour for another. In experiments where the rumen of the sheep was isolated, emptied and refilled with warm 0.9 per cent saline solution, urea accumulated at the mean rate of 4.9 mmol of urea nitrogen per hour. Simultaneous collection of saliva revealed that about 16 times as much urea entered the rumen by direct passage through the rumen wall as was secreted in the saliva. The author points out the greater utilization of blood urea for protein synthesis when ruminants are on a low protein or starvation diet as compared to a well balanced high protein ration. On high protein diets urea may enter the rumen as a result of increased ammonia absorption and high blood urea levels. The greater portion of the blood urea, however, would be excreted by the kidneys.

Amount of Protein Synthesis in the Rumen

Ruminants may well be compared with non-ruminants from the standpoint of what nutrients must be absorbed from the alimentary tract and how material entering the abomasum and passing on down the intestinal tract is digested and absorbed (Chalmers and Synge, 1954). The value then of a given diet, from the standpoint of supplying protein needs, must be related to the total amount of true protein plus amino acids which leave the rumen to be digested and absorbed further down the digestive tract. The total protein (dietary plus microbial) leaving the rumen must be the sum of dietary protein and net gain in protein as a result of breakdown of dietary protein and synthesis of microbial protein. As have been described above, the factors affecting the amount of protein synthesis in the rumen are many and complex. The rumen is in the true sense of the word a dynamic system. The processes of protein

breakdown and synthesis, ruminal ammonia absorption, addition of urea in the saliva and passage of urea into the rumen through the rumen wall may be going on simultaneously. Studies of some of these processes and factors affecting them have, however, led to estimations of the amount of protein synthesis in the rumen.

Rumen Studies

As indicated previously, Pearson and Smith (1943c) estimated that the bacteria in the rumen are capable of synthesizing 450 g. protein from non-protein nitrogen sources in 24 hours. Duncan et al. (1952) found 19-190 per cent more protein in the rumen six hours after feeding a purified diet containing urea as the only source of nitrogen. The corresponding increase on a natural ration was 78 per cent, which was accounted for by ingested protein. In sheep fed wheat hay, Gray et al. (1952) found twice as much nitrogen per gram of lignin in fodder as in plant residues in the rumen, and more than half of the total ruminal nitrogen in the rumen microorganisms. Agrawala et al. (1953) showed a 33 to 109 g. increase in true protein in the rumen of six month old calves following feeding of a purified diet and a 252 g. increase when the calves were on a natural diet. With a partially purified diet where casein provided 87 per cent of the nitrogen, McDonald and Hall (1957) found 90 per cent of the casein degraded and utilized for protein synthesis. Moore and King (1958) found 65 to 78 per cent of the total nitrogen in feed residues and microbial cells in the rumen of steers fed a variety of diets.

Weller et al. (1958) estimated the conversion of plant nitrogen to microbial nitrogen by assaying for diaminopimelic acid. References are given to work which found this amino acid to be absent from all common

plant feedstuffs but present in most rumen bacteria. Weller then described an elaborate procedure for calculating the nitrogen in plant residues, bacteria, protozoa and in solution. Results indicate that throughout the whole day about 61-82 per cent of the nitrogen in the rumen is present as microbial protein. This worker pointed out that the extent of conversion of plant nitrogen to microbial nitrogen is more likely to approach the upper limit of these figures since food leaving the rumen generally consists of well-digested food rather than an average of the rumen contents (Gray et al., 1958).

Duodenal Investigations

In recent years many investigators have turned to a more direct method of assessing the net effect of rumen fermentation of nitrogenous substances. This has been the study, both qualitatively and quantitatively, of material passing from the stomach into the duodenum.

Studies of different forms of nitrogen in various portions of the bovine digestive tract led Raynaud (1955) to conclude that there is little digestion of polypeptides in the stomach such as there is in non-ruminants.

Boyne et al. (1956) studied changes in composition of digesta along the alimentary tract of sheep by killing the animals and separating the tract into rumen, omasum, abomasum, small intestine, cecum and colon. A tremendous increase in the concentration of nitrogen in relation to dry matter in the intestinal contents was noted when compared with that in the rumen. Badawy et al. (1957, 1958a) later found that considerable of this nitrogen could be attributed to epithelial shedding and loss of material from Brunner's glands in sheep which had been killed by use of the humane killer. Badawy et al. (1958a) also reported a great increase

in duodenal non-protein nitrogen. He pointed out that his colleague, Dr. Hywel Jones, had found that non-protein nitrogen represented 49.5 per cent of total nitrogen in upper intestinal contents removed immediately at death. After standing at room temperature for four hours, however, the figure had risen to 68.6 per cent. This indicated that hydrolysis of protein was occurring at a rapid rate and would affect any protein and non-protein nitrogen studies.

Gray et al. (1958b) estimated the total nitrogen reaching the duodenum when sheep were fed wheat hay and straw (0.7 % N), wheat hay (1.1 % N), wheat and alfalfa hays (1.8 % N) and alfalfa hay (2.9 % N). The percentages of dietary nitrogen reaching the duodenum, on the basis of nitrogen:lignin ratios in the feed and abomasal contents, were calculated to be 117, 100, 65 and 48, respectively, for the four rations.

The low percentage of dry matter in duodenal contents as compared to rumen contents reported by nearly all observers is explained by the fact that large volumes of digestive juices are secreted into the abomasum. Masson and Phillipson (1952), on the basis of chloride concentration in abomasal contents and gastric juice collected in a Hollander pouch, estimated the ratio of gastric juice to omasal contents to be 2:1. Since Gray et al. (1958b) noted indications that the gastric juice contains no more nitrogen than is absorbed from this organ, it is not expected that these secretions would seriously alter the total nitrogen entering the duodenum. The absorption of water from the omasum (Badawy et al., 1958b; Raynaud, 1957) although not affecting the total nitrogen, would tend to increase the nitrogen concentration. The extent to which water absorption occurs, however, is still a subject of controversy.

Using a method of collecting duodenal ingesta described by

Phillipson (1948), McDonald (1948b) estimated 40 per cent conversion of zein in the diet of sheep to microbial protein. These calculations were based on the fact that zein has no lysine and is alcohol soluble, characteristics which enable it to be distinguished from microbial protein. Phillipson (1952) later, however indicated that duodenal flow rates obtained by this procedure would be minimal, since a balloon was inserted into the duodenum to divert the flow into a cannula. Even a lightly inflated balloon in this position caused a reduction in duodenal flow.

Hogan (1957) exteriorized the duodenum of eight sheep and with twelve observations of twelve hours each noted an average flow of 4.3 liters. The duodenal contents contained 225 g. dry matter and 9.6 g. nitrogen compared to 415 g. and 12.8 g., respectively, in the diet. This loss in total nitrogen can not be evaluated since the composition of the diet was not given.

Conner et al. (1957) described a method of establishing a re-entrant duodenal fistula in cattle where the entire duodenal flow is diverted through an external plastic tube which may be removed for collection and sampling. In preliminary results of extensive studies using this fistula, McGilliard et al. (1957b) reported a considerable increase in total nitrogen passing the duodenum over that contained in the diet. The increase, however, decreased as the ration was changed from all corn to all alfalfa hay.

Kameoka and Morimoto (1959) reported a study in which a fistula was established between the omasum and abomasum of goats. These workers estimated that the true ruminal digestibility of the digestible protein of the feed varied from 52.6 to 87.0 per cent. These figures were determined by using a rough estimate of total salivary nitrogen.

EXPERIMENTAL PROCEDURE

Description of the Animal Used

A rumen fistula and a re-entrant duodenal fistula were established in a Holstein heifer weighing 585 pounds. The duodenal fistula was established according to the procedure of Conner et al. (1957). The materials used for the fistula were, with slight modifications, the same as described by Conner et al. (1957). The cannula was constructed of thin stainless steel tubing with an inside diameter of 3/4 inch. The slight increase in size was an attempt to reduce the possibility of plugging of the cannulae. A flexible plastic milking machine hose 10 inches long with 3/4 inch inside diameter was used to connect the two cannulae. The tube was bent in the shape of a "U" and held in place with radiator type clamps. Rubber bands connecting the ends of this tube served to reduce the tension exerted on the cannulae.

In order to establish the duodenal fistula the animal was anesthetized with sodium pentobarbitol and the body wall entered on the right side of the animal near the eleventh rib. The duodenum was transected about ten inches below the pylorus and the two ends sutured to form two blind pouches. A small incision was then made in each blind end and a cannula was inserted and sutured in place. Each cannula was then projected through a small hole in the body wall to the outside of the animal. The final position of the cannulae was such that the efferent cannula, connected to the upper digestive tract, was located 4½ inches below the afferent cannula which was connected to the lower digestive tract. The cannulae were held in place by a plastic collar which fitted over and was fastened to the cannula and rested against the outside of the body wall. The cannulae were than connected with the plastic tube. The entire operation lasted about three hours and the animal appeared to

suffer no ill effects. Within two hours digesta could be observed moving through the plastic tube, and at the end of 24 hours the animal was eating well. Within two weeks the animal had regained all weight lost during and following the operation.

The only problem encountered in maintaining the fistulated animal was making sure the fistula remained unplugged at all times. For the first few days the fistula plugged over night, but after this trouble was experienced only very occasionally. The possibility, however, that plugging could occur required that the fistula be checked several times each day. If the fistula remained plugged for more than a very few hours the increased pressure in the duodenum made the animal very uncomfortable and resulted in leaking of digesta around the fistula.

The animal remained in good health and gained about 100 pounds body weight during the six months following the establishment of the fistula. The weight of the fistula then caused the upper cannula to be gradually pulled through the body wall. Frequent plugging of the cannula with resulting loss of digesta led to loss of appetite and body weight and, finally, disposal of the animal 190 days after the establishment of the fistula.

Collection Procedure

A total of twelve 24-hour collections (trials) were made. Each trial began at 8:00 a.m. and was continued until 8:00 a.m. the following morning. The animal was placed in the collection stall 48 hours before the beginning of each trial and remained there until the end of the trial. An automatic drinking cup on the stall allowed the animal to have water ad libitum. The animal was fed twice a day, at 0 and 12 hours during the trial.

Duodenal Contents

The total flow of digesta from the efferent cannula was collected over the 24 hours of each trial. A composite sample of material collected during each 4-hour period was saved for analysis. The digesta was collected by attaching a tube to the efferent cannula and allowing it to drain into a 1500 ml. graduated cylinder. When the cylinder was filled it was thoroughly mixed and a 5 per cent aliquot saved for the composite sample. At the end of the 4 hours all the aliquots taken during the period were mixed and refrigerated as the composite sample for that period. After taking a 5 per cent aliquot sample of each 1500 ml. the remaining portion was poured into a holding container and gradually readministered back into the animal through the afferent cannula. A clamp on the tube connecting the holding container and the cannula allowed the operator to regulate the rate of flow of digesta into the animal. This was necessary as it was soon observed that the rate of digesta flowing into the animal greatly affected the rate at which digesta flowed out of the animal through the other cannula. If digesta was readministered too rapidly the flow stopped completely for periods of up to 45 minutes. The most satisfactory method was to readminister the digesta at a slow but constant rate.

The holding container was surrounded by a water jacket through which warm water was continuously pumped. This prevented the digesta from cooling prior to readmission to the animal. The water was maintained at 39 degrees C. in a thermostatically controlled water bath. A stirring device in the holding container prevented the larger particles of digesta from settling to the bottom and plugging the afferent tube.

The holding container was located slightly above the fistula so that flow of the digesta could be effected by the force of gravity. The equipment which was used for collecting and readmitting the digesta was fastened to a carriage on the collection stall which could be raised or lowered according to whether the animal was standing or lying.

Rumen Contents

The rumen contents were sampled at 2-hour intervals during each trial beginning before feeding at 0 hour. A composite of the rumen contents was squeezed through cheesecloth and the filtrate saved for analysis. The samples were acidified with two ml. of concentrated hydrochloric acid and refrigerated immediately.

Urine

Collection of the urine was complicated by the fact that the animal used was a heifer. Separation of urine and feces could have been accomplished in a metabolism stall, but the duodenal collection procedure required that the animal be located in the specially designed collection stall. This stall provided for separation of urine and feces of a steer but not for a heifer. An attempt was made to catheterize the bladder via the urethra, but this was discontinued when blood appeared in the urine, apparently from irritation of the bladder. It was decided that collection of the urine would be made only during the 24 hours of the trial, while the operator was present. The continuous presence of the operator allowed collection of the urine in a pail during the normal urinations. Feces were not collected since separation of urine and feces was available only during a 24-hour period. The author suggests that any further work be done with a steer, unless adequate means for separation of urine and feces are provided.

Rations Studied

As indicated previously, the purpose of the present study was to determine the extent to which urea nitrogen was converted to protein nitrogen in the rumen of the bovine. To accomplish this two levels of urea were fed and compared to a similar ration containing no urea. To compare the effeciency of urea in promoting protein synthesis to that of a source of true protein a fourth ration containing soybean oil meal was also studied. Each ration consisted of six pounds of timothy hay and six pounds of concentrate per day. The timothy hay was of medium quality, containing 5.56 and 5.79 per cent crude protein for that fed during trials I-X and XI-XII, respectively. The composition of the concentrates and the proximate analysis of the hay and concentrates are given in tables 1 and 2, respectively. Ration 1, which served as the control ration, supplied less than the N.R.C. (Loosli et al., 1950) recommended allowances for protein. The recommendations for a 600 lb. growing dairy heifer are 0.85 lbs. digestible protein per day. While the digestibility of the feedstuffs was not determined an estimate can be made on the basis of digestion coefficients by Morrison (1957). Such an estimate provides values of 0.60, 0.79, 1.09 and 0.91 lbs. of digestible protein per day for rations 1, 2, 3 and 4, respectively. Total intake of crude protein amounted to 0.88, 1.07, 1.27 and 1.23 lbs. per day for the four rations. Since the only variable in the four rations was the source of nitrogen supplementation it was assumed that the T.D.N. remained constant. This was considered advisable in the light of reports that the amount and source of carbohydrate greatly affects nitrogen utilization in the rumen (e.g. Lewis and McDonald, 1958).

Table 1

COMPOSITION OF CONCENTRATES FED

Ration	Corn*	Urea	Soybean Oil Meal
	(%)	(%)	(%)
1 & 1a	100.0	0.0	0.0
2	98.5	1.5	0.0
3	97.0	3.0	0.0
4	85.0	0.0	15.0

* Contained 1.0 % dicalcium phosphate and 0.5 % salt containing cobalt

Table 2

PROXIMATE ANALYSIS OF CONCENTRATES AND HAY FED

Ration	Dry matter	Crude protein	Crude fiber	Ether extract	N-free extract	Ash
	(%)	(%)	(%)	(%)	(%)	(%)
1	85.41	9.19	2.01	3.67	68.63	1.92
1a	86.76	8.75	2.38	3.89	68.83	2.91
2	84.67	12.31	1.96	3.27	64.67	2.46
3	87.26	15.31	2.15	3.96	63.34	2.49
4	87.28	15.00	2.63	3.20	63.04	3.41
Timothy hay						
I to IX	88.33	5.56	28.27	2.21	48.55	3.74
XI to XII	87.99	5.79	34.49	2.01	41.63	4.07

Table 3

RATION FED DURING EACH TRIAL
AND DATE CONDUCTED

Trial	Ration	Date (1959)
I	3	March 14
II	3	March 26
III	2	April 11
IV	1	May 1
V	4	May 23
VI	2	June 6
VII	1	June 27
VIII	4	July 11
IX	4	July 14
X	3	July 25
XI	3	August 14
XII	3	August 17

Allocation of Rations to Trials

The ration which was fed during each of the twelve trials and the date the trial was made are given in table 3. Each ration was fed a minimum of ten days before the trial was made. Since the establishment of the re-entrant duodenal fistula necessarily places a certain stress on the animal it was considered advisable to begin the study with the animal on a high quality diet. Ration 3 was fed during the first two trials. These two trials were conducted in order for both the animal and the operator to become acquainted with the equipment and procedure involved in the duodenal collection, and the results were not included

in the study. Two other trials, VI and X, were eliminated when the animal went off feed during the trial period. It should be noted that trials VIII and IX and trials XI and XII involve only a three day interval in order to complete more trials while the duodenal fistula was still intact. Since trial VI was eliminated there remains only one replication of ration 2, trial III. The necessary disposal of the animal on August 26 eliminated the possibility of repeating this ration

Chemical Analysis

Rumen Contents

Samples of rumen contents were centrifuged at 1000 r.p.m. (200 x G) for five minutes. Total nitrogen in the supernatant was determined by the micro-kjeldahl procedure (Hawk et al., 1949). Following digestion the samples were steam distilled in a Markham still and the ammonia collected in 2 per cent boric acid. Non-protein nitrogen was determined in the supernatant solution following treatment with 80 per cent ethanol.

Duodenal Contents

Samples of duodenal contents were also centrifuged at 1000 r.p.m. (200 x G) for five minutes. Total and non-protein nitrogen in the supernatant were determined as described above for the rumen samples. A composite of duodenal contents collected during each trial was dried and subjected to the proximate analysis of feedstuffs (A.O.A.C., 1956). Crude protein, crude fiber, ether extract, nitrogen-free extract, ash and water content were determined.

Urine

Total nitrogen was determined by the kjeldahl method in composite samples of urine collected during each 24-hour trial.

RESULTS

Duodenal Contents

The total amount of duodenal contents collected during each 4-hour period of each trial is indicated in table 4. The flow was found to be quite irregular during a given trial and also between different trials. The total flow for 24 hours varied from 31.69 liters during trial I to 58.39 liters during trial VII. There is some indication that the total flow was leveling off during the latter portion of the study, a range of 43.77 to 45.69 liters being noted for the last four trials.

From table 4 the variation in flow rates during a given trial may also be observed. Had each period been shortened to one hour or even two hours the variation would have been even more dramatic. At times the flow averaged 100 ml. per minute for periods of 15 or 20 minutes, and at other times the flow stopped completely for a half hour or longer.

With few exceptions the composition of the duodenal ingesta showed no great variation over a period of time. The changes in nitrogen concentration in the supernatant portion (1000 r.p.m., 5 min.) was more consistant (table 5) than that observed in the rumen samples. The concentration of non-protein nitrogen followed very closely that of the total nitrogen in the supernatant. It will be observed in table 6 that the non-protein nitrogen made up a very high percentage of the total nitrogen in the duodenal supernatant. This was, at first, very surprising, since it was expected that the largest portion of the microbial population would be present in the supernatant fraction. Reports by Badawy *et al.* (1958a), however, indicate that there may be considerable hydrolysis of the protein in duodenal contents which are

Table 4

DUODENAL FLOW BY 4-HOUR PERIODS

Trial	Period 1	2	3	4	5	6	Total
	(L.)	(L.)	(L.)	(L.)	(L.)	(L.)	(L.)
I	5.56	2.36	2.89	7.30	5.97	7.61	31.69
II	7.96	6.02	4.50	5.89	6.03	6.56	36.96
III	5.87	4.90	7.37	8.44	7.21	7.18	40.97
IV	5.13	4.68	5.76	5.25	6.28	7.40	34.50
V	8.20	8.94	9.59	9.70	8.42	10.22	55.03
VI	4.54	4.24	7.20	11.05	9.40	10.98	47.41
VII	9.15	10.13	9.27	10.61	9.42	9.81	58.39
VIII	7.07	7.50	7.03	7.40	8.02	7.84	44.86
IX	7.97	7.80	6.06	7.81	6.81	9.06	45.51
X	8.99	3.88	*				
XI	7.27	5.59	8.49	6.60	6.56	11.38	45.69
XII	8.14	7.51	6.61	7.26	5.56	8.67	43.77

* Trial discontinued, animal off feed

allowed to stand at room temperature for four hours without some means of stopping the action of proteolytic enzymes. In this study the duodenal samples were left at room temperature until the end of each four-hour period before being refrigerated. It would seem possible then that the non-protein nitrogen fraction obtained here contained a large portion of amino acids and short polypeptides in addition to non-amino nitrogen materials.

The proximate analysis of the duodenal contents as obtained from

Table 5

TOTAL NITROGEN AND NON-PROTEIN NITROGEN CONTENT OF SUPERNATANT PORTION OF DUODENAL CONTENTS

Trial & Ration		Period 1	2	3	4	5	6
		(Expressed as mg. N per 100 ml. duodenal contents)					
III 2	N	95	94	85	140	140	104
	NPN	63	65	56	107	69	70
IV 1	N	91	96	89	100	104	108
	NPN	68	63	62	76	76	72
V 4	N	94	93	85	91	93	93
	NPN	77	74	71	73	70	79
VII 1	N	57	59	62	64	76	68
	NPN	53	55	57	62	66	64
VIII 4	N	86	96	94	98	104	103
	NPN	75	85	87	88	85	87
IX 4	N	104	118	110	111	106	103
	NPN	96	106	100	100	96	89
XI 3	N	88	96	91	87	88	78
	NPN	79	87	81	82	78	72
XII 3	N	72	89	88	96	104	98
	NPN	64	69	77	74	85	79

Table 6

NON-PROTEIN NITROGEN AS A PER CENT OF TOTAL NITROGEN IN SUPERNATANT PORTION OF DUODENAL CONTENTS

Trial &	Ration	Period 1	2	3	4	5	6
III	2	66	69	66	76	66	67
IV	1	75	66	70	76	73	67
V	4	82	80	84	80	75	85
VII	1	93	93	92	97	87	94
VIII	4	87	89	93	90	82	84
IX	4	92	90	91	90	91	86
XI	3	90	91	89	94	89	92
XII	3	89	78	88	77	82	81

Table 7

PROXIMATE ANALYSIS OF DUODENAL CONTENTS

Trial &	Ration	Dry matter	Expressed as % of dry matter: Crude protein	Crude fiber	Ether extract	N-free extract	Ash
III	2	5.57	21.69	15.71	6.94	39.76	15.89
IV	1	5.43	18.13	17.15	6.47	44.44	13.81
V	4	4.62	21.25	18.15	6.78	37.70	16.12
VII	1	4.78	17.50	18.18	5.24	43.01	16.07
VIII	4	4.90	23.31	15.73	6.22	39.82	14.92
IX	4	5.31	24.19	15.22	6.36	40.06	14.17
XI	3	4.78	22.42	18.31	7.52	37.74	14.01
XII	3	5.45	19.72	19.50	6.73	40.89	13.16

a composite sample from each trial is presented in table 7. The digesta was quite fluid and had a dry matter content of about 5 per cent. The crude protein content, when expressed as a percentage of dry matter, was lowest for the two trials on the control ration (ration 1). The highest values were observed for trials VIII and IX where the ration was supplemented with soybean oil meal. The percentage of crude protein in the duodenal dry matter was 23.31 and 22.42, respectively, for these two trials. A third trial with the soybean meal ration gave a slightly lower value, 21.25 per cent crude protein in the duodenal dry matter. No consistant trends were observed in the crude fiber content of the duodenal dry matter with the four different rations, although the last four trials indicate some differences. Values of 15.73 and 15.22 per

Table 8

TOTAL 24-HOUR DUODENAL PASSAGE

Trial	Dry matter	Ash	Crude protein	Ether extract	Crude fiber	N-free extract	Organic matter	Total Carbohydrate
	(g.)	(g.)	(g.)	(g.)	(g.)	(g.)	(g.)	(g.)
III	2285	363	495	159	359	907	1919	1266
IV	1873	259	340	121	321	832	1614	1153
V	2542	410	540	172	475	945	2132	1420
VII	2791	449	488	146	507	1200	2341	1707
VIII	2198	328	512	137	351	870	1870	1221
IX	2417	342	585	151	368	968	2072	1336
XI	2184	306	490	164	400	824	1878	1224
XII	2385	314	470	161	465	975	2071	1440

Table 9

TOTAL DIETARY INTAKE PER DAY

Trial	Dry matter	Ash	Crude protein	Ether extract	Crude fiber	N-free extract	Organic matter	Total CHO
	(g.)	(g.)	(g.)	(g.)	(g.)	(g.)	(g.)	(g.)
III	4712	169	487	149	823	3084	4543	3907
IV	4732	154	402	160	825	3192	4579	4017
V	4784	195	560	147	824	3040	4589	3882
VII	4769	181	390	166	835	3197	4588	4032
VIII	4784	195	560	147	842	3040	4589	3882
IX	4784	195	560	147	842	3040	4589	3882
XI	4774	179	575	163	998	2859	4595	3857
XII	4774	179	575	163	998	2859	4595	3857

cent crude fiber were observed for trials VIII and IX with the soybean oil meal ration. The corresponding values for trials XI and XII with the 3 per cent urea ration were 18.31 and 19.50, respectively. It should be noted, however, that the timothy hay fed during the last two trials was higher in crude fiber content than that fed during the previous trials (table 2) which resulted in a higher crude fiber intake for these two trials (table 9). The ether extract content of the duodenal contents showed little variation between trials but was significantly higher than that contained in the dietary dry matter. This was expected since the gastric secretions contain large quantities of soluble ash.

The total 24-hour duodenal passage and the dietary intake of dry matter, organic matter, ash, crude protein, ether extract, crude fiber,

nitrogen-free extract and total carbohydrates are presented in tables 8 and 9, respectively. A great variation in duodenal passage of dry matter was observed. It cannot be stated how much of this variation was due to the collection procedure and how much was due to day to day variation which may occur in an intact animal. A definite correlation was noted between total duodenal flow and the total passage of dry matter. This is apparent in table 10. The trials which resulted in the greatest total duodenal flow (55.03 and 58.38 liters) also showed the greatest passage of dry matter (2542 and 2791 g., respectively). The trial which resulted in the smallest flow (34.50 liters) showed the least passage of dry matter (1873 g.). It is obvious that such differences in dry matter passage will greatly affect the total passage of all constituents of duodenal digesta. This is confirmed by inspection of table 8.

Table 10

TOTAL DUODENAL FLOW AND DRY MATTER PASSAGE

Trial	Ration	Flow	Dry matter
		(liters)	(g.)
III	2	40.97	2285
IV	1	34.50	1873
V	4	55.03	2542
VII	1	58.39	2791
VIII	4	44.86	2198
IX	4	45.51	2417
XI	3	45.69	2184
XII	3	43.77	2385

Table 11

RUMINAL DIGESTION COEFFICIENTS CALCULATED FROM DIETARY INTAKE AND DUODENAL PASSAGE DATA

Trial	Dry matter	Ash	Crude protein	Ether extract	Crude fiber	N-free extract	Organic matter	Total CHO
	(expressed as per cent digested before duodenum)							
III	51.6	-115.8	-1.6	-6.7	56.4	70.6	57.8	67.6
IV	60.4	-67.5	15.4	24.4	61.1	73.9	64.8	71.3
V	46.9	-109.7	3.6	-17.0	43.6	68.9	53.5	63.4
VII	41.5	-148.1	-25.1	12.0	39.3	62.5	49.0	57.7
VIII	54.1	-68.2	8.6	6.8	58.3	71.4	59.3	68.5
IX	49.5	-75.4	-4.5	-2.7	56.3	68.2	54.8	65.6
XI	54.3	-70.9	14.8	-0.6	50.3	71.2	59.1	68.3
XII	50.0	-75.4	18.3	1.2	53.4	65.9	54.9	62.7

With the exceptions of crude protein and ether extract, the passage of all constituents of duodenal digesta was highest during trial VII and lowest during trial IV. These differences, in turn, affect the apparent loss of material from the digestive tract prior to reaching the duodenum and thus the ruminal digestion coefficients presented in table 11. These coefficients are expressed as the per cent of each constituent of the feed which was not observed to pass the duodenum. It is tempting at this point to adjust the ruminal digestion coefficients to constant dry matter passage rates to eliminate the effect of different passage rates. This would, however, mask any effects of protein supplementation on dry matter digestibility. It cannot be stated, from the available data, that such an effect exists, but neither can it be stated that the

Table 12

TOTAL PASSAGE OF DUODENAL NITROGEN AND NON-PROTEIN NITROGEN AS PERCENTAGE OF ORGANIC MATTER

Trial	Ration	Organic matter	Total nitrogen	Non-protein nitrogen
		(g.)	(%)	(%)
IV	1	1614	3.37	1.47
VII	1	2341	3.34	1.49
III	2	1919	4.13	1.55
XI	3	1878	4.17	1.95
XII	3	2071	3.63	1.56
V	4	2132	4.05	1.91
VIII	4	1870	4.38	2.03
IX	4	2072	4.52	2.15

addition of urea or soybean oil meal did not affect the digestibility of the dry matter of the ration. Despite the great variability there are some differences which may be noted from table 11. The digestibility of ether extract for trials IV and VII (ration 1) was considerably higher than for the remainder of the trials. The two trials with ration 3 (3 % urea) resulted in digestion coefficients for crude protein of 14.8 and 18.3 per cent. The three trials with ration 4 (15 % soybean oil meal) resulted in corresponding values of 3.6, 8.6 and -4.5 per cent. These data indicate a greater loss of nitrogen from the digestive tract prior to the duodenum for ration 3 than for ration 4. It should be remembered that the total dietary intake of nitrogen was very similar for these two rations.

In table 12 the total passage of duodenal nitrogen and non-protein nitrogen are expressed as a percentage of the total organic matter. Expressed in this manner, the total nitrogen was found to be somewhat higher for ration 4 than for ration 3. Ration 2 was intermediate between these two, and the control ration (ration 1) was lowest. As indicated previously, the non-protein nitrogen fraction may contain a variable amount of hydrolyzed protein, hence the significance of these values remains doubtful.

A summary of duodenal nitrogen data is presented in table 13. This table presents values for total duodenal flow, dietary intake of total nitrogen, duodenal passage of total nitrogen and non-protein nitrogen and a value for nitrogen loss as calculated by the difference between

Table 13

SUMMARY OF DUODENAL NITROGEN

Trial	Ration	Total flow	Dietary nitrogen	Duodenal nitrogen	Duodenal NPN	Nitrogen loss*
		(L.)	(g.)	(g.)	(g.)	(g.)
IV	1	34.50	64.3	54.4	23.8	9.9
VII	1	58.39	62.4	78.1	34.8	-15.7
III	2	40.97	77.9	79.2	29.8	-1.3
XI	3	45.69	92.0	78.4	36.6	13.6
XII	3	43.77	92.0	75.2	32.4	16.8
V	4	55.03	89.6	86.4	40.8	3.2
VIII	4	44.86	89.6	81.9	37.9	7.7
IX	4	45.51	89.6	93.6	44.5	-4.0

total dietary and total duodenal nitrogen. Nitrogen loss with ration 3 was found to be 13.6 and 16.8 g. per day. This is considerably greater than the 3.2, 7.7 and -4.0 g. per day losses observed for ration 4. A net gain of 1.3 g. nitrogen per day was noted for ration 2. It is difficult to evaluate the net loss or gain of nitrogen with ration 1 owing to the wide range of flow rates observed here, but the data seem to indicate a very small net gain of nitrogen for this ration.

Rumen Contents

The total nitrogen and non-protein nitrogen content of the supernatant portion of the rumen contents are presented in table 14. As was true with the duodenal studies, a considerable difference was noted between trials IV and VII on the control ration. During either trial there was little net change in total nitrogen or non-protein nitrogen in the rumen supernatant over the period of 24 hours. There was, however, a considerable difference in the level of nitrogen between these two trials. During trial IV, for which the total duodenal flow was a comparatively low 34.5 liters, the total nitrogen in the supernatant ranted from 29 to 44 mg. % while the non-protein nitrogen ranged from 23 to 29 mg. %. During trial VII, on the other hand, for which the total duodenal flow was 58.39 liters, the total nitrogen ranged from 14 to 21 mg. % and the non-protein nitrogen from 12 to 19 mg. %. These data would seem to indicate that factors other than that of the collection procedure itself were at play in determining the amount of duodenal material collected during the 24-hour period. It is possible that a greatly increased water consumption by the animal during trial VII could have diluted the rumen contents and also increased the rate of passage of material out

Table 14

TOTAL NITROGEN AND NON-PROTEIN NITROGEN IN RUMEN SUPERNATANT*

Hours after feeding	Trial IV Ration 1		Trial VII Ration 1		Trial III Ration 2		Trial XI Ration 3	
	N	NPN	N	NPN	N	NPN	N	NPN
	(expressed as mg. nitrogen per 100 ml. rumen supernatant)							
12	38	28	18	16	44	30	18	14
2	35	24	16	15	55	38	47	42
4	39	28	20	19	56	39	31	27
6	33	27	18	16	39	22	17	15
8	34	29	20	18	50	24	19	16
10	38	27	17	15	28	19	20	15
12	29	25	21	19	36	21	20	16
2	39	23	20	18	39	27	38	35
4	38	27	20	18	48	30	42	35
6	34	22	21	18	49	32	30	24
8	42	29	17	13	46	31	24	20
10	44	28	14	12	47	31	22	18
12	42	27	19	16	36	35	22	15

* Centrifuged at 200 x G for 5 min.

(continued)

Table 14 (concluded)

TOTAL NITROGEN AND NON-PROTEIN NITROGEN IN RUMEN SUPERNATANT*

Hours after feeding	Trial XII Ration 3		Trial V Ration 4		Trial VIII Ration 4		Trial IX Ration 4	
	N	NPN	N	NPN	N	NPN	N	NPN
	(expressed as mg. nitrogen per 100 ml. rumen supernatant)							
12	23	21	39	35	24	21	28	25
2	45	42	28	24	30	26	21	20
4	31	27	28	25	18	15	17	16
6	24	20	18	16	21	16	16	13
8	22	16	20	19	22	19	17	14
10	23	18	28	25	20	18	24	20
12	27	23	22	22	22	19	27	24
2	48	45	25	21	28	23	23	19
4	33	27	17	15	20	17	19	14
6	27	17	25	21	21	17	18	14
8	25	20	25	21	24	20	19	16
10	25	20	27	22	22	19	23	19
12	33	27	24	23	27	23	26	22

* Centrifuged at 200 x G for 5 min.

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of the rumen. This possibility will be discussed more fully in the following section

During the only trial on ration 2 the total nitrogen and non-protein nitrogen concentrations were higher than on the control. Four hours after the morning feeding the values for ration 2 were 56 and 39 mg. %, respectively, and at six hours following the evening feeding were 49 and 32 mg. %, respectively. The difference between total and non-protein nitrogen during this trial was greater than during any of the other trials conducted. There seemed to be a general trend, though not clearly defined, of increasing ruminal supernatant nitrogen during the first 4-6 hours following feeding and a gradual decrease until the next feeding.

The change in ruminal nitrogen levels during the trials on ration 3 were very marked (appendix figure 1), and close agreement was noted between trials. Both total nitrogen and non-protein nitrogen concentrations rose very quickly after feeding and reached a peak at about two hours. The levels then decreased rapidly and at 6 hours after feeding had reached a minimum level. The total nitrogen reached peak concentrations of 45 and 48 mg. % and the non-protein nitrogen, levels of 42 to 45 mg. %. The minimum levels attained were 17 to 25 and 15 to 20 mg. %, respectively. It cannot be stated with certainty that the maximum levels noted in this study are the true peak levels, since it is entirely possible that a peak concentration had been reached prior to the two-hour sampling. It is reassuring, however, to note the close agreement between feedings and between trials and also the fact that the peak following the evening feeding on trial XI did not occur until sometime between the two- and four-hour samplings. These facts indicate

that the concentration noted at the end of two hours may well reflect the true maximum

The results of trials V, VIII and IX on ration 4 provide a very interesting comparison with those mentioned previously. It should be noted that the total dietary intake of nitrogen on this ration was essentially the same as on ration 3. With the exception of one sample, however, the concentration of total nitrogen did not exceed 26 mg. %. There seemed to be a very moderate peak at the time of feeding or two hours following feeding and a gradual decline until midway between feedings (appendix figure 2). This was most noticable during trial IX.

Urinary Nitrogen

Urinary excretion of nitrogen increased as the urea level in the ration increased (table 15). The highest total excretion of nitrogen occurred during the trials conducted on ration 3 (3% urea) where 36.8, 37.7, 38.6 and 40.3 g. nitrogen were excreted per day. Corresponding values for ration 4 were 35.5, 30.7 and 35.5 g. nitrogen excreted per day. The data presented in table 15 include trials I, II and VI which were not considered in the remainder of the study for reasons which, it was felt, did not affect the total nitrogen excretion. Indeed, the results of these trials agree very closely with the other trials conducted on the same rations. It should be pointed out that the values obtained are the result of urine collection only during the 24-hour collection period and are not averages of collection during a number of days.

Body Weight Changes

A consideration of some body weight changes noted during the course

Table 15

TOTAL URINARY EXCRETION OF NITROGEN

Trial	Ration	Total urine	Total nitrogen
		(L.)	(g.)
IV	1	1.69	22.7
VII	1	4.75	24.1
III	2	2.33	28.9
VI	2	1.79	32.1
I	3	2.46	37.7
II	3	2.62	38.6
XI	3	6.30	36.8
XII	3	8.00	40.3
V	4	2.61	35.5
VIII	4	2.58	30.7
IX	4	4.60	35.5

of this study may shed some light on the large differences in total passage of duodenal contents which occurred during this study. The animal was weighed immediately before being placed in the collection stall prior to each trial and again immediately after removal from the stall at the completion of the trial. Both weighings were made just prior to the regularly scheduled feeding. The animal invariably lost weight between these two weighings. During most trials this loss amounted to about 30 pounds. During trial VII, however, the weight loss amounted to only 10 pounds. This trial was conducted during a very hot and uncomfortable day, and the animal seemed to spend much more

time drinking than during the other trials. If the water consumption was, in fact, increased during this trial it may have affected the water balance of the animal and also the passage of digesta out of the rumen.

DISCUSSION

The total collection of duodenal contents has been shown to be quite variable between the 24-hour collection periods, especially for trials IV and VII on the control ration. Whether this is due to a true difference in passage in the intact animal or merely a reflection of the collection procedure cannot be clearly stated. The much lower rumen nitrogen level during trial VII as well as the suspected larger water intake of the animal during this trial suggest that the passage of water through the rumen may be a factor affecting the movement of digesta out of the rumen. Balch et al. (1953) found that limited water intake did not affect the rate of passage of hay from the rumen of the cow. These workers stated that an increased flow of saliva during periods of limited water intake tended to keep the fluid conditions in the rumen constant. The observations of these workers, however, were made after the animals had been on a restricted water intake for 14 days, and were not an attempt to demonstrate day to day variation. These workers gave no indication of the effect of increased rather than decreased water consumption, although Balch (1951) had previously indicated that the ratio of dry matter consumed by the cows to their water consumption was related to the passage of hay through the omasum, abomasum and intestines. It seems reasonable that an increased water consumption on a given day would have a "flushing-out" effect on the rumen contents resulting in an increased passage of dry matter out of the rumen. It is unfortunate that water intake data are not available to clarify this point.

Despite the variation in total passage of digesta the average passage of organic matter for each ration was approximately the same,

1978, 1919, 1974 and 2027 g. per day for rations 1, 2, 3 and 4, respectively. The following discussion then will refer to the observed passage of nutrients rather than making any adjustment to constant passage of any one constituent such as organic matter.

The total passage of duodenal nitrogen was found to average 66.2, 79.2, 76.8 and 87.3 g. per day for rations 1, 2, 3 and 4, respectively. These figures include true protein, both dietary and microbial, as well as non-protein nitrogen. To estimate the conversion of urea to microbial protein it is necessary to know how much of this duodenal nitrogen is true protein nitrogen and how much is non-protein nitrogen. By determining non-protein nitrogen a value for protein nitrogen can be obtained by difference. The total passage of protein nitrogen was found to be 37.0, 49.4, 42.3 and 46.5 g. per day, respectively, for the four rations. As has been indicated previously, the non-protein nitrogen of the duodenal contents may contain a sizable amount of true protein which has been hydrolyzed to amino acids and short polypeptides by proteolytic enzymes from the duodenal mucosa. Badawy (1958a) referred to work by Jones who found that the non-protein nitrogen content of duodenal digesta comprised 49.5 per cent of the total duodenal nitrogen when the digesta was collected immediately after death and treated with 5.0 per cent trichloroacetic acid, whereas the figure had risen to 68.6 per cent after the digesta stood at room temperature for four hours. Two observations indicate that the digesta collected in this study, however, did not suffer the rapid hydrolysis of protein indicated in the work reported by Badawy. The digesta collected here passed through only eight inches of the duodenum and thus was much less likely to contain a large amount of

proteolytic enzymes from the duodenal mucosa than was the digesta referred to by Badawy, which was taken from a large portion of the upper intestine after the animal had been killed. Secondly, during none of the trials reported here did the percentage of non-protein nitrogen in the total duodenal nitrogen exceed the minimal figure of 49.5 reported by Badawy. There still remains an unknown amount of amino nitrogen in the duodenal contents, but this will be assumed to be relatively constant for all rations.

The degree to which the nitrogen in the experimental rations was converted to protein nitrogen and recovered in the duodenum can be observed in table 16. This table shows the amount of total nitrogen and protein nitrogen which passed the duodenum in excess of that observed for the control ration as well as the nitrogen added to the diet in the form of urea (rations 2 and 3) or soybean oil meal (ration 4). The 14.5 g. urea nitrogen supplemented in the diet of ration 2 was apparently very efficiently converted to microbial protein, since 13.0 g. was recovered in the duodenum, and of this, 12.4 g. was determined to be protein nitrogen. The supplementary nitrogen in the high urea ration (ration 3) was utilized to a much lesser degree. Of the 28.6 g. urea nitrogen in the diet, only 10.6 g. was recovered in the duodenum, and only 5.3 g. of this was protein nitrogen. The indication here is that of an 18.0 g. loss of nitrogen from the digestive tract before the digesta reached the duodenum. This nitrogen loss is much greater than would be expected in comparison with the efficient utilization of the lower level of urea in the diet. It should be remembered that only one replication of ration 2 was available, and it is quite possible that

Table 16

INCREASE IN TOTAL PASSAGE OF TOTAL AND PROTEIN NITROGEN OF EXPERIMENTAL RATIONS OVER THAT OF THE CONTROL (RATION 1)

	Increase over control ration		
	Ration 2	Ration 3	Ration 4
	(g.)	(g.)	(g.)
Total nitrogen	13.0	10.6	21.1
Protein nitrogen	12.4	5.3	9.5
Dietary nitrogen	14.5	28.6	26.2

the nitrogen loss with this diet, as reported in this study, is less than that found in the intact animal. The close agreement, however, noted between the two trials on ration 3 indicated that the 18.0 g. loss of nitrogen is quite representative of this feeding regime. This loss is not the net disappearance of nitrogen between the diet and the duodenal passage, but is a net loss of supplementary nitrogen as compared to the control. The total passage of nitrogen with the control ration included any nitrogen which entered the rumen in the saliva and is expected to represent the simple total of dietary plus saliva nitrogen, since little if any loss of nitrogen is believed to occur through ruminal absorption on a low protein diet. The total passage of nitrogen on the experimental rations, however, represented dietary plus microbial nitrogen less any nitrogen lost through ruminal absorption. Therefore, the 18.0 g. loss on ration 3 was the total absorption of nitrogen from the rumen, or at any rate prior to the duodenum. The higher non-protein nitrogen levels (42-45 mg. %) in the rumen during the trials on ration 3 suggest that considerable ruminal absorption of ammonia was the cause of this

nitrogen loss.

From table 16 it can also be seen that there was little loss of dietary nitrogen prior to the duodenum with ration 4. Of the 26.2 g. nitrogen supplied as soybean oil meal protein 21.1 g. was recovered in the duodenum. Although this diet supplied nearly as much nitrogen as ration 3 (89.6 g. compared to 92.0 g.) the maximum rumen non-protein nitrogen levels were much lower (24-26 mg. %). Hence, much less ruminal absorption of ammonia occurred, and a loss of only 5.1 g. was noted. The soybean oil meal was much more slowly broken down in the rumen, and the decreased rate of release of ammonia allowed the rumen microorganisms to utilize this ammonia for protein synthesis before a high ammonia level could be built up in the rumen. This scheme should provide for a greater passage of protein nitrogen from the rumen on ration 4. Although only a slight increase in the total protein nitrogen passing the duodenum was noted on ration 4, a measurement of the total passage of free amino acids may prove to be greater with this ration. If the amount of protein synthesized in the rumen is accurately represented by the figures given, it may be due to a lack of carbohydrate for the rumen microorganisms. The total digestible nutrients (TDN) provided by each of the rations was approximately constant at 7.8 pounds per day despite the nitrogen supplementation of rations 2, 3 and 4. Although the constant TDN was necessary to prevent biased results for the experimental rations as compared to the control, the amount supplied was less than the 8.5 pounds per day recommended by the N.R.C. (Loosli et al., 1950). This supply of energy may well have been adequate for the microbial protein synthesis at the lower nitrogen levels, but it is likely that in order to

obtain maximum protein synthesis at the higher nitrogen levels more carbohydrate was necessary. The addition of starch to all of the rations may well have resulted in an additional passage of protein nitrogen, especially with rations 3 and 4.

The urinary excretion of nitrogen supported the increased loss of nitrogen with the high urea rations. During the trials conducted on the control ration 23.4 g. nitrogen was excreted in the urine per day. As more urea was added to the ration the urinary excretion increased to 30.5 and 38.4 g. per day for rations 2 and 3, respectively. As was expected the nitrogen excretion was less with ration 4 than ration 3 and amounted to 33.9 g. per day.

With the exception of ether extract, the ruminal digestibility of all the constituents of the proximate analysis showed no significant differences between rations. The ruminal digestion of ether extract, however, was considerably depressed by all experimental rations as compared to the control. The explanation for this phenomenon may be a decreased digestion of the triglycerides in the corn when nitrogen was added to the diet. This would imply that the increased passage of ether extract noted for the experimental rations was due to undigested triglycerides in the corn. It was assumed that the majority of the volatile fatty acids present in the duodenal contents were lost in the drying process prior to the ether extract determination and thus did not appear in the ether extract fraction.

SUMMARY AND CONCLUSIONS

A re-entrant duodenal fistula and a rumen fistula were established in a 600 pound Holstein heifer. The total passage of duodenal digesta was collected, measured and sampled and readministered at approximately the same rate at which it was collected. Each collection was for 24 hours, and composite samples during six 4-hour periods were obtained. A supernatant portion (200 x G for 5 min.) of each sample of duodenal digesta was analyzed for total and non-protein nitrogen. One composite sample of all digesta collected during the 24-hour trial was subjected to the proximate analysis of feedstuffs. A composite sample of rumen contents was taken every two hours and the supernatant (200 x G for 5 min.) was analyzed for total and non-protein nitrogen.

A total of eight trials were conducted with four rations. All rations consisted of 3 pounds of timothy hay and 3 pounds of a concentrate fed twice per day at 12-hour intervals. The concentrates for rations 1, 2, 3 and 4 consisted of corn alone and corn plus 1.5 % urea, 3.0 % urea and 15 % soybean oil meal, respectively.

Total passage of nitrogen in the duodenal digesta amounting to 66.2, 79.2, 76.8 and 87.3 g. per day indicated losses of nitrogen between dietary and duodenal of -2.9, -1.3, 15.2 and 2.3 g. per day, respectively, for rations 1, 2, 3 and 4. Ruminal non-protein nitrogen which reached 42-45 mg. % with ration 3 at two hours after feeding indicated that ruminal absorption of ammonia may have been considerable with this ration, and that that this could account for the 15.2 g. net loss of nitrogen. An increase in urinary excretion of nitrogen with this ration supported this nitrogen loss.

It may be concluded from this study that urea when fed at a level

of 1.5 per cent of the concentrate in a nitrogen deficient ration containing corn and timothy hay is very efficiently utilized and largely converted to protein nitrogen by the rumen microorganisms. When the level of urea was increased to 3.0 per cent, however, a significant amount of nitrogen disappeared before the digesta reached the duodenum. This loss was presumably due to ruminal absorption of ammonia, higher urea level in the blood and increased excretion of urea nitrogen in the urine. Nitrogen supplied in the form of soybean oil meal in amounts approximately equal to the higher level of urea was retained more efficiently than the urea.

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APPENDIX

Figure 1

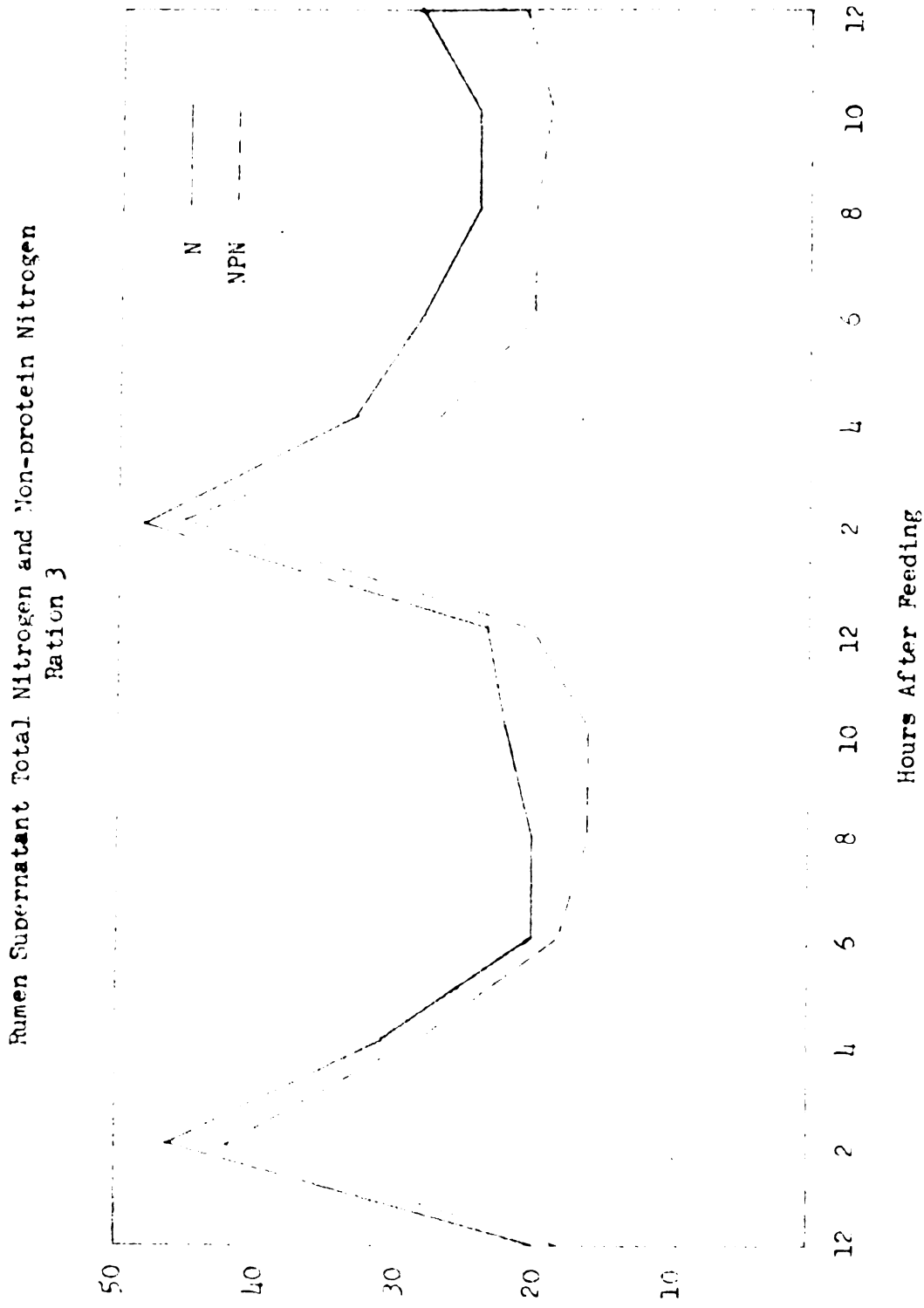
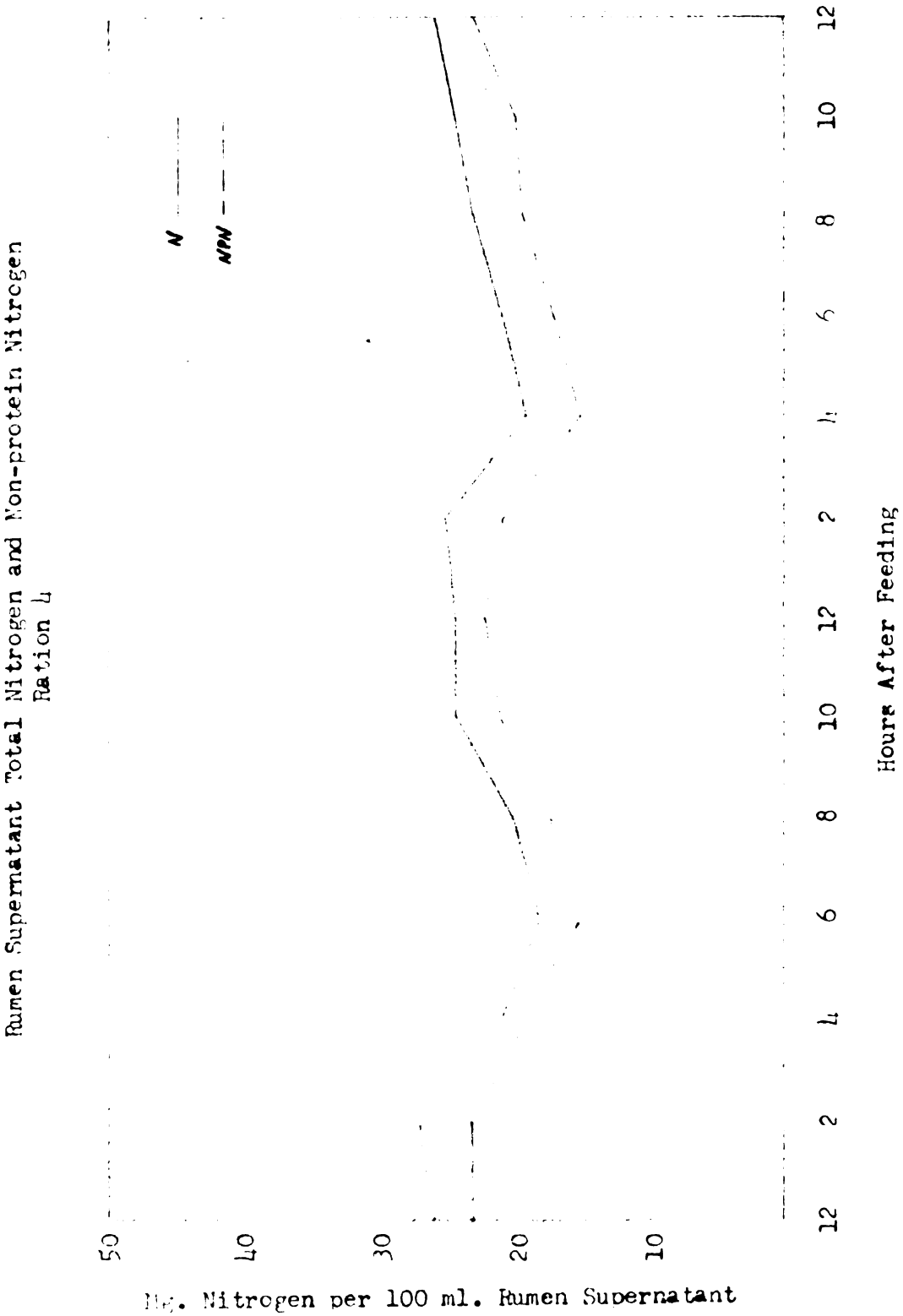

Rumen Supernatant Total Nitrogen and Non-protein Nitrogen
Ration 3
N
NPN
50
40
30
20
10
12
2
4
6
8
10
12
2
4
6
8
10
12
Hours After Feeding
Mg. Nitrogen per 100 ml. Rumen Supernatant

Figure 2


Rumen Supernatant Total Nitrogen and Non-protein Nitrogen
Ration 4
N
NPN
50
40
30
20
10
12
2
4
6
8
10
12
2
4
6
8
10
12
Hours After Feeding
Mg. Nitrogen per 100 ml. Rumen Supernatant