RUMEN MICROBIAL PROTEIN SYNTHESIS FROM UREA WHEN FED WITH LOW QUALITY ROUGHAGE RATIONS

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CHAPTER I

INTRODUCTION

Ruminants have as a part of their digestive system, a microbial fermentation stage in which plant materials such as cellulose are broken down to usable nutritional forms. This unique capacity enables ruminants to convert many plant materials and industrial by-products that would otherwise be wasted to meat, milk and other human foods.

Furthermore, ruminants have the additional capacity to harvest their own food. Approximately 1.2 billion acres, or 63%, of the total land area of the continguous United States are classified as grazing or range land (Heady, 1974). Most of this range land is unsuitable for production of crops; therefore, grazing is the only feasible way to harvest most of this renewable natural resource.

By far the majority of the 38 million beef cows in the United States subsist on this range land at or just above maintenance levels of nutrition for three to seven months of the year. With the marginal profit potential in the production of beef, supplementary feeding is provided only to insure survival or, at the most, maintenance until the next grazing season when the natural pasture will provide most of the nutritional requirements both in terms of quantity and quality. For maximum productive life of the cow and greatest economic advantage, beef cows can be maintained on sub-maintenance rations during the wintering period so long as adequate forage is available during spring and

summer to regain lost weight (Hughes, 1971).

The basic concept in the utilization of poor quality roughages is to supplement with adequate nutrients to nourish the rumen microorganisms in order to maintain an active population for the digestion of the fibrous feeds and for the synthesis of microbial protein to be used as a nitrogen source by the host animal. Nitrogen is the chief deficiency in poor quality forages. Because nitrogen is an essential element for rumen flora activities, nitrogen deficiency reduces digestibility of feeds. Feed energy is consequently not released and the animal suffers from an energy deficiency. If the energy required for normal body functions can not be obtained from the feed, body tissues are mobilized and the animal loses weight and condition. Non-protein nitrogen (NPN) compounds to supply ammonia for the rumen microbes are very appealing in terms of cost per unit of nitrogen as well as conservation of natural proteins for human food use.

Innumerable experiments on the utilization of NPN in rations providing maintenance nutritional levels have been conducted in the past. Inconclusive results have been presented often because the exact protein requirement at a maintenance level of nutrition is not well established. Traditional measurements of weight gain, nitrogen balance and nitrogen digestibility are inadequate indications of nitrogen utilization under these conditions.

New methods for the measurement of NPN utilization must evaluate the ability of the nitrogen source to support rumen fermentation and to promote the synthesis of microbial protein. The development of new research techniques in recent years should prove useful in accomplishing this task. The use of rumen and abomasal cannulae plus dietary markers

enables the quantitative measurement of food passage through the digestive tract. Since feed nucleic acids are rapidly degraded in the rumen (Smith, 1969) and ruminal microbes contain nucleic acids, the measurement of abomasal nucleic acid levels permits partitioning of abomasal protein into microbial or feed by-passed nitrogen. For simplifying sampling and interpretation, metabolite pool sizes must remain fairly constant; therefore, an automatic feeding system designed to feed animals hourly and reduce diurnal variation in digesta passage was used to assist in the maintenance of constant metabolite pools.

The purpose of this study was to evaluate the utilization of NPN in supporting rumen fermentation and microbial protein synthesis for steers fed maintenance energy rations when (1) varying levels of urea were substituted for soybean meal in isocaloric and isonitrogenous rations and (2) urea served as the main source of supplemental nitrogen at various nitrogen levels in isocaloric diets.

CHAPTER II

REVIEW OF LITERATURE

Introduction

Urea was discovered in 1773 by Rouelle and its composition established by Prout in 1818. Whereas most proteins contain 16 percent nitrogen, pure urea contains 47 percent. As early as 1891, Zuntz suggested that the rumen microflora were able to break down cellulose as a source of energy and convert non-protein nitrogen (NPN) into true protein. Early research of NPN utilization cast dim views on utilization of NPN. Due to critical shortages of vegetable protein during the war period of the early 1940's, research on the utilization of urea was much more widespread. Reid (1953) reviewed numerous studies which gave evidence that urea nitrogen fed to ruminants was indeed retained in the body and often increased the digestibility of cellulose and crude fiber in low protein diets.

The use of urea as a protein replacement has been one of the major developments in ruminant feeding during recent years and its utility has been well demonstrated as reviewed by various authors (Reid, 1953; Briggs, 1967; Chalupa, 1968; Loosli and McDonald, 1968; Oltjen, 1969; Helmer and Bartley, 1971). No attempt will be made to discuss all the research concerning NPN in this review. However, work involving nitrogen supplementation of maintenance and low productivity cattle rations

containing roughages or forages and various aspects of nitrogen metabolism in the ruminant will be reviewed.

Urea as a Protein Substitute in Submaintenance Rations

In the last thirty years, considerable research has been conducted concerning NPN supplementation on low quality forages in Oklahoma.

Briggs et al. (1947) used either crystalline urea or pelleted supplements containing various percentages of urea and cottonseed meal to study forage consumption and nitrogen retention. The supplements in which approximately 25, 50, 75 or 100% of the supplemental nitrogen was furnished by urea were fed with a basal ration of low grade prairie hay (crude protein = 3%) to steers in metabolism stalls. In general, the addition of protein to a low protein hay diet stimulated greater forage consumption. Greater apparent digestibility of urea nitrogen was noted as compared to cottonseed meal nitrogen; however, nitrogen storage was appreciably less indicating that the supplemental nitrogen supplied by urea was not converted to microbial protein in the rumen, but was absorbed as ammonia and eliminated in the urine.

In a second trial, pellets which supplied 25 and 50% of the supplemental nitrogen as urea induced about the same nitrogen storage as cottonseed meal. However, when further supplementation of urea was made, approximately the same nitrogen storage was noted as with prairie hay alone.

Dinning et al. (1949) employed steers receiving maintenance rations consisting of 10 pounds of prairie hay and one pound of supplement containing 25 or 50% of the supplemental nitrogen as urea or no additional

nitrogen to study nitrogen balance. The use of urea improved nitrogen retention, but as the nitrogen of urea increased from 25 to 50%, the percent of the urea-nitrogen retained decreased from 50 to 10% indicating loss by absorption.

Nelson and Waller (1962) summarized 16 trials over a seven year period in which urea furnished one-third to one-half of the nitrogen in protein supplements which were fed to wintering beef cows on dry range grass (crude protein = 2.5%). The addition of trace minerals tended to improve urea utilization, but the cows did not efficiently utilize supplements containing 50% of the nitrogen from urea. The results of feeding a 40% protein supplement with one-half of the nitrogen being supplied by urea was similar to that of a 20% cottonseed meal supplement in terms of cow weight loss.

Williams et al. (1969) noted that cows fed urea based supplements lost more weight and condition through the wintering period than cows fed cottonseed meal, but urea feeding had no adverse effects on weaning weights of their calves nor subsequent calving dates or weights of the cows at calving. When fed once daily, urea was rapidly hydrolyzed resulting in high rumen ammonia values within an hour after feeding. Ruminal ammonia levels peaked at 35 mg NH_3-N/100 ml rumen fluid within two hours post-feeding, then decreased to 10 mg NH_3-N/100 ml at six hours and tended to stabilize. A ratio of NFE:N of 28:1 provided rumen conditions which allowed greater protein synthesis than narrow ratios. The authors concluded that the combination of poor-quality roughages and urea supplements resulted in poor utilization.

Rush (1974) conducted seven trials to evaluate the supplemental value of various NPN sources for cattle consuming low quality forage.

In the first four trials, urea or feed grade biuret provided approximately 50 and 94% of the nitrogen in 30% dry and liquid supplements, respectively. Urea utilization as measured by cow weight loss and condition was poor; however, birth weight and weaning weight of calves were not significantly affected by treatment imposed on the cows. Spring and summer gain was greater for cows that lost more weight during the wintering period and fall weights of the cows were not significantly different.

Two winter trials involving 304 range cows were also conducted by Rush (1974). Urea provided one-half of the nitrogen in a 30% supplement and was compared to a negative (15% natural) and a positive (30% natural) control supplement. Urea appeared to be poorly utilized, since the performance of cows fed urea or fed the negative control supplement were similar.

An additional trial involving yearling heifers in drylot fed prairie hay or late winter harvested range grass suggested that urea could be utilized with prairie hay, but gains indicated that urea was poorly utilized when winter range grass was fed.

Therefore it would appear that results of Oklahoma work suggest that urea addition to low quality forages may increase forage consumption as a result of stimulating the rumen fermentation system, but the overall utilization of urea may be no better than 25%.

Most of the other research involving supplementing low quality roughage rations for maintenance have been aimed at improving the nutritive value of these roughages. In almost every instance, urea supplementation in combination with some energy source has resulted in improved consumption of forage and some reduction in body weight losses

when compared with the feeding of the forage alone. However, almost invariably, when low productivity rations contained less than 6% protein, response to supplementary preformed protein exceeded response to supplementary urea.

Nitrogen Metabolism in the Ruminant

Work with various aspects of nitrogen metabolism in the ruminant animal is extensive. Many excellent reviews (McDonald, 1958; McLaren, 1964; Phillipson, 1964; Hungate, 1966; Waldo, 1968; Smith, 1969; Allison, 1970; Chalupa, 1972; Chalupa, 1973) present full and in depth studies of nitrogen utilization. Only a general overview relating specific areas of digestion and absorption of nitrogenous compounds in the ruminant will be presented here.

Food Nitrogen

Most of the nitrogenous materials ingested by the ruminant is true protein. This protein is degraded to differing degrees by rumen microbes to yield free amino acids and subsequently ammonia. The rate of ammonia release is closely related to the solubility of the protein in rumen fluid (Blackburn, 1965). Initial work on protein solubility and digestion showed distinct patterns in solubility rates of different purified proteins as well as in rates at which different proteins were degraded to ammonia (Annison, 1956; McDonald, 1954; Lewis, 1962).

Amino acids produced by hydrolysis of dietary proteins are rapidly deaminated by ruminal bacteria. The rate of deamination is only slightly less than that of their production, so the level of free amino acids in the rumen is usually less than 1 mg amino-N/100 ml rumen fluid. Ex-

ceptions may occur, especially when high protein rations are ingested. During periods of high protein intakes, ruminal levels of free amino N may increase five to ten fold (Allison, 1970). The usual end products of amino acid catabolism are ammonia, carbon dioxide and volatile fatty acids. Hydrolysis of branched chain amino acids are of particular importance in that they furnish the keto acid carbon skeleton for the synthesis of branched chain microbial amino acids (Oltjen, 1969).

A considerable amount of NPN is ingested with some feeds. Amino acids, peptides, nucleic acids, nitrates and amines make up a majority of ingested NPN. These compounds are rapidly degraded in the rumen and, in some instances, form ammonia. However, without doubt, the NPN source most frequently fed to ruminants is urea. Pearson and Smith (1943a,b) demonstrated, by *in vitro* techniques, that rumen liquor had a high urease activity at all times of the day. They calculated that 100 g of rumen contents could convert 100 mg of urea to ammonia in one hour. The ability to hydrolyze urea appeared to reside in the rumen microbes. Numerous bacterial species undoubtedly contribute to the bulk of urease activity. Jones *et al.* (1964) reported that 35% of the viable bacteria in strained rumen fluid were associated with urease production.

Endogenous Nitrogen Entering the Rumen

Ruminants secrete large volumes of alkaline buffered saliva during food ingestion and rumination. Bailey (1961) computed the total volume of saliva secreted per day by cattle to range from 98 to 190 liters. Part of the variation in total secretion may be accounted for by the physical nature and moisture content of the feed being consumed.

The nitrogen content of saliva is quite variable, but generally is

in the order of 0.1 to 0.2%, of which 60 to 80% is urea nitrogen. The concentration of urea in saliva is closely related to dietary nitrogen intake or urea infusions (Somers, 1961); therefore, the blood urea concentration may have the greatest influence on nitrogen content of saliva (Houpt, 1970). Considering the estimated volumes secreted and the estimated nitrogen content, it is not unreasonable to assume that 8 to 12 g of nitrogen may enter the rumen in saliva of adult cattle.

Urea may also diffuse directly into the rumen from the blood. The amount of urea nitrogen entering the rumen directly is determined, primarily, by blood urea concentration (Houpt and Houpt, 1968) and could amount to 8 to 16% of the dietary nitrogen intake in sheep (Houpt, 1959). Vercoe (1969) reported a possible nitrogen transfer of 17 to 20 g to the rumen of cattle daily.

The concentration of ruminal ammonia is an important factor governing the uptake or loss of nitrogen from the rumen. Varady et al. (1967; cited by Church, 1971) reported a negative correlation between ruminal ammonia levels and passage of nitrogen into the rumen. Other factors, especially rumen pH, also influence ammonia absorption. Under most conditions, buffering action of rumen fluid converts over 99% of the ammonia (NH_3) to the ammonium ion (NH_4^+). Ammonia has a pKa of 8.8 at 40C (Bloomfield et al., 1963) and the absorption of NH_3 across the rumen wall is slow if the pH is below 7. As the pH of rumen fluid becomes more alkaline, NH_4^+ is converted to NH_3 which can penetrate the lipid layer of the rumen wall. By calculating the ratio of NH_3 to NH_4^+, Hogan (1961) concluded that free ammonia absorption was a function of concentration gradient.

The absorbed NH_3 is carried to the liver, where, unless the con-

centration is unusually high, it is reconverted to urea. The urea so formed may be either excreted in the urine, utilized as a nitrogen source by body tissues or recycled into the rumen via the saliva. Under more extreme conditions, when ammonia is being formed rapidly in the rumen, conversion of ammonia to urea in the liver may be incomplete and ammonia may enter the general circulation causing toxicity symptoms to develop (Word et al., 1969). Bypass of liver detoxication of ammonia by lymphatic uptake is another proposed cause of urea toxicity (Chalmers et al., 1971).

Nitrogen Requirement of Rumen Microbes

Ammonia is an essential nutrient for growth of many bacteria even when preformed organic nitrogen is present in the media (Hungate, 1966). Of 44 strains of rumen bacteria, 80% could be grown with ammonia as the sole source of nitrogen and 26% would not grow unless ammonia was present (Bryant, 1963).

Ammonia nitrogen is primarily incorporated into amonio acids and protein by bacterial cells and appears in protozoal cells as a consequence of ingestion of bacteria by protozoa. Amination and transamination reactions appear to be the major pathways of ammonia assimilation (Allison, 1969). Intermediates and end products of carbohydrate fermentation form the primary sources of carbon for bacterial amino acid biosynthesis. The key system for the initial fixation of ammonia to a carbon skeleton apparently is glutamate dehydrogenase.

The amount of ammonia that can be utilized by the rumen microbes depends largely on bacterial numbers and growth or turnover rate. The point at which ammonia becomes limiting for growth of rumen bacteria is

not clearly defined (Allison, 1970). In a continuous culture system, growth of Bacteroides amylophilus was limited at ammonia concentrations lower than 4.6×10^{-3} M (6.4 mg NH_3-N/100 ml).

In order to determine the rumen ammonia concentrations necessary to support maximum growth rates of rumen bacteria, Roffler and Satter (1973) and Satter and Slyter (1974) related ruminal ammonia concentration to crude protein and TDN content of the ration. Continuous culture fermentors were charged with ruminal digesta obtained from steers fed either a protein-free purified ration, a corn-based all concentrate ration or a 77% concentrate ration. Diets fed to the fermentor were similar to those fed steers supplying the ingesta except that the crude protein level of the culture diets ranged from 4 to 28%. Urea was the source of supplemental nitrogen and was continuously infused into the fermentors to maintain constant ammonia concentrations. Protein output measured as tungistic acid precipitable nitrogen increased as the level of urea supplementation was increased and then plateaued. Further increases in urea supplementation were without effect on protein output. The upper point of protein output coincided with the point at which ammonia accumulation began and was approximately 5 mg NH_3-N/100 ml of rumen fluid.

The in vivo influence of ration composition on mean ruminal ammonia concentrations was studied using 1038 ruminal ingesta samples from 207 cows in different stages of lactation and maintained under a variety of feeding conditions. Mean ruminal ammonia levels were found to be positively correlated to the percent crude protein in the ration dry matter. As the dietary crude protein was increased above 13% on a dry matter basis, rumen ammonia levels tended to increase sharply and were

in excess of 5 mg NH_3-N/100 ml rumen fluid.

Further evidence suggested that the efficiency with which NPN was converted to microbial protein was inversely proportional to ammonia concentration. With most high energy rations, approximately 40% of the protein escapes rumen degradation and becomes available to the animal post-ruminally. However, when NPN replaces some portion of the ration protein, the amount of rumen bypass of dietary nitrogen is reduced. The point of excessive ammonia accumulation in the rumen is affected by the amount of NPN added to the ration as well as the TDN and total protein content of the unsupplemented ration. As the amount of NPN in the ration increases, the point of zero utilization of NPN is reached at a correspondingly lower level of ration protein.

Microbial Protein Synthesis

McDonald (1948) suggested that quantitatively estimating microbial protein synthesis in the rumen was the outstanding problem in the study of the digestion of proteins by ruminants. Information on ruminal protein synthesis and the contribution of bacterial protein to the protein nutrition of the host was limited due to difficulties in measuring the extent of ruminal digestion, the quantities of metabolites produced during digestion and the composition and rate of flow of digesta from the rumen (Hogan and Weston, 1970). However the development of cannulation techniques for various portions of the digestive tract as well as modern laboratory procedures have made possible the measurement of digesta degradation and the partitioning of abomasal or duodenal nitrogen into microbial and non-microbial fractions. In recent years a considerable effort has been directed toward the measurement of microbial

protein synthesis in the rumen. Most such research has been conducted with sheep and information with cattle is very limited. Excellent reviews of microbial protein synthesis have been published (Purser, 1970; Hogan and Weston, 1970; Thomas, 1973). This review will outline various techniques for studying microbial protein synthesis as well as *in vitro* and *in vivo* production data.

Estimation Procedures

Quantitative estimation of microbial cell synthesis in rumen contents has long been a difficult problem. Essentially, the difficulty lies in differentiating plant material from microbial cell material.

Purified zein (McDonald, 1954) and purified casein (Blackburn and Hobson, 1960) have been used to study dietary protein degradation. However, the purified test proteins must be furnished in the diet; therefore, they cannot be applied under normal feeding conditions.

The use of radioisotopes and their incorporation into microbial protein has also been employed. Walker and Nader (1968) described a procedure for labeling the sulfide pool of rumen contents *in vitro* with radioactive $Na_2{}^{35}S$. Harrison *et al.* (1972) suggested infusion of $Na_2{}^{35}SO_4$ into the rumen of sheep would yield duodenal digesta containing labeled sulfur amino acids. Further work and procedures are outlined by Beever *et al.* (1974). Pilgrim *et al.* (1970) measured the extent to which ammonia-N served as a starting point for synthesis of microbial nitrogen when $(^{15}NH_4)_2\ SO_4$ was continuously infused into the rumen of sheep maintained in steady state conditions. Mathison and Milligan (1971) assessed incorporation of ^{15}N into microbial protein by infusing $^{15}NH_4Cl$.

Certain chemical compounds have been found to exist primarily in bacteria and protozoa. Weller et al. (1958) and el-Shazly and Hungate (1966) described procedures for the determination of α-ε-diaminopimelic acid (DAP). DAP is a constituent of the bacterial cell wall mucopeptide and exist almost exclusively in bacteria. By measuring the concentration of DAP in rumen or abomasal contents, the contribution of bacterial N to total duodenal N can be assessed. Hutton et al. (1971) determined a N:DAP ratio for rumen bacteria of 18 to 19:1.

Abou Akkada et al. (1968) suggested the possibility of using 2-aminoethylphosphonic acid (AEP) as a marker of rumen protozoal populations. Since no AEP is present in rumen bacteria and most rations are devoid of AEP, all AEP in rumen liquor was from protozoal cells.

Ellis and Pfander (1965) demonstrated that ribonucleic acid (RNA) and deoxyribonucleic acid (DNA) increased in samples of rumen fluid incubated in vitro and together accounted for about 15% of the microbial nitrogenous compounds formed. Research on the extraction of nucleic acids from animal tissues is extensive. McAllan and Smith (1969) determined that procedures of Schmidt and Thannhausen (1945) and Schneider (1945) proved the most useful to use for estimating nucleic acids in digesta. The procedure of McAllan and Smith (1969) involves repeated extractions with organic solvents, acidification with perchloric acid and neutralization with potassium hydroxide and a final removal of interference compounds by ion-exchange resins.

Smith (1969) suggested that DNA in pure cultures tended to reflect the number of organisms present but that the ratio of DNA to total nitrogen varied markedly between different organisms. RNA was much more closely associated with protein synthesis and the RNA-N to total nitro-

gen ratio was much more constant. Dietary nucleic acids appear to be rapidly degraded in the rumen and do not contribute appreciably to the nucleic acid content of rumen fluid (Smith and McAllan, 1970). McAllan and Smith (1972), in further investigations, concluded that nucleic acid, and particularly RNA, concentrations are suitable for estimating the contribution of total microbial nitrogen to rumen or duodenal digesta samples. Furthermore it appeared unnecessary to sample and analyze rumen bacteria in each experiment, but calibration was necessary to establish bacterial composition for the particular animals and diets used in a particular environment.

McAllan and Smith (1974) compared RNA and DAP as markers for determining microbial nitrogen of a Fresian calf fed several different diets and a cow fed dairy cubes. Since the calf was protozoa-free, measures of microbial nitrogen to total non-ammonia nitrogen in duodenal contents were almost identical between procedures. However, a marked discrepancy existed between the two techniques for the cow. DAP underestimated the microbial contribution to duodenal passage in the cow because it did not consider the protozoa present.

In Vitro Synthesis

Because of the anaerobic conditions which predominate in the rumen, protein synthesis is usually limited by available energy. Under some conditions it may be limited by available nitrogen (1 mg NH_3-N/100 ml rumen fluid) (Hogan and Weston, 1970). Reporting bacterial growth in batch culture where energy alone was limiting, Bauchop and Elsden (1960) calculated the yield of dry bacterial cells to be equivalent to 10 to 12 g per mole of ATP produced by substrate fermentation. Similar

results have been reported by Payne (1970) and Hobson and Summers (1972). However, even under optimum conditions, yield values show considerable variation between organisms and range between 8.5 and 13.1 g per mole of ATP. Since bacteria contain 10.5% nitrogen, this represents 0.9 to 1.4 g bacterial nitrogen per mole of ATP produced by substrate fermentation (Thomas, 1973).

Variations in rumen environment, such as dilution rate, proportions of different organisms, different dietary ingredients and level of feeding, may effect the growth of microorganisms in the rumen. Walker (1965) applied theoretical estimates of ATP yield during forage digestion to data on sheep consuming wheaten hay. Y(ATP) values were consistent with the range of 10 to 12. Later studies with mixed rumen microbes in vitro suggested values for Y(ATP) of 12.8 to 14.4 (Walker and Nader, 1968).

Walker and Nader (1970), from in vitro calculations, estimated that 5.9 g true protein was synthesized per mole of ATP produced. Protein synthesis per mole of VFA generated during fermentation was calculated to be 12.3 g crude protein or 10 g true protein. Data from Walker (1965) stated that 100 g carbohydrate would yield 1.24 moles of acetate and propionate or 1.18 moles of VFA, if 10% of the total VFA is present as butyrate formed from acetate. Using this information, Walker and Nader (1970) calculated a theoretical yield of 14.4 g microbial crude protein per 100 g OM digested in the rumen.

Satter and Slyter (1974) reported a yield of microbial dry matter per mole of estimated ATP production of 15.6 g when nitrogen was not limiting. Under nitrogen-limiting conditions, Y(ATP) values decreased substantially.

Isaacson et al. (1975) utilized mixed bacterial cultures derived from the rumen of a steer on an all hay diet to study the effects of dilution rate and levels of energy source (glucose) on fermentation product formation and the efficiency of microbial growth. Three dilution rates (2, 6 and 12% per hour) approximated 0.5, 1.5 and 3.0 volume turnovers per day and were chosen to represent the physiological range of fluid turnover rates encountered in the rumen. Y(ATP) values were 7.5, 11.6 and 16.7 g for increasing dilution rates, respectively. Bacterial protein synthesized per 100 g OM fermented also increased. Values for efficiency of production were 15.6, 22.3 and 31.1 for 2, 6 and 12% per hour, respectively. A calculated theoretical maximum of 33.1 g bacterial protein per 100 g fermented OM was noted.

In Vivo Synthesis

In recent years, much attention has been directed to the study of ruminal protein synthesis. As energy is usually the limiting factor for microbial growth, protein synthesis has been expressed as a function of either the organic matter (OM) apparently digested in the rumen (OM disappearance between food and duodenum) or the organic matter truly digested (OM apparently digested plus OM incorporated into the microbes). Most of the in vivo work has been conducted with sheep; therefore, emphasis in this review will center on efficiency of production and the effect of nitrogen intake on protein synthesis and nitrogen utilization.

Hogan and Weston (1967b) studied the utilization of a 7 and 17% crude protein diet with lambs in metabolism crates. A net gain of 2.6 g nitrogen or 47% of the dietary intake was noted on the low protein

diet, while 31% of the daily nitrogen intake was lost from the rumen on the high protein diet. The quantity of nitrogen leaving the stomach was similar for both diets as was the apparent digestibility of nitrogen in the intestines; therefore, the quantity of nitrogen apparently absorbed was similar regardless of nitrogen intake.

The organic matter digested in the rumen was considered equal to the grams of organic matter apparently digested in the rumen plus the quantity of microbial organic matter leaving the rumen. The quantity of microbial organic matter was estimated assuming 1) 1 g nitrogen per day was secreted into the abomasum, 2) the remaining non-ammonia nitrogen (NAN) leaving the abomasum consisted of microbial nitrogen, and 3) microbial organic matter contained 10.5% nitrogen. On this basis, 15.0 and 15.6 g microbial protein were synthesized per 100 g OM truly digested in the rumen on the 17 and 7% crude protein diets, respectively. No estimate of the contribution of dietary nitrogen bypassing rumen degradation to NAN leaving the abomasum was made; therefore, these estimates of efficiency are imprecise.

Twenty-three different diets prepared from seven species of pasture grasses and legumes were used by Hogan and Weston (1970) to estimate bacterial protein synthesis in sheep. Dietary nitrogen intakes ranged from 4.5 to 50 g nitrogen per day and the nitrogen content as a percent of digestible organic matter (DOM) ranged from 1.6 to 6.7%. When the dietary nitrogen/DOM ratio was less than 4%, the amount of NAN reaching the intestine exceeded nitrogen intake; however, net losses of nitrogen were noted on the higher protein diets. Within all diets, the ratio DAP:NAN in the digesta leaving the stomach tended to decrease as the crude protein level increased, suggesting more quantitative bypass of

dietary nitrogen on the higher protein diets. Bacterial protein synthesis was 23.1 g bacterial crude protein/100 g OM apparently digested in the rumen. This is somewhat higher than 19.4 g bacterial crude protein/100 g OM apparently digested as previously reported (Hogan and Weston, 1967b); however, higher dilution rates were noted in this study.

Hume et al. (1970) measured the effect of nitrogen intake on the daily flow of nitrogen through the omasum of sheep. Protein-free purified diets supplied either 2.6, 4.4, 9.2 or 16.0 g nitrogen per day. A constant nitrogen status was maintained while varying the ruminal nitrogen level by continuously infusing casein into the abomasum to give a total nitrogen input of 18 g per day. The concentration of ammonia increased in both the rumen and omasum with increasing levels of nitrogen intake. A similar pattern was noted in the total nitrogen contents of the rumen; however, omasal total nitrogen showed very little change. In terms of daily flow, a linear response was noted in the flow of total nitrogen out of the rumen with increasing nitrogen intake, but the flow of tungistic acid-precipitable nitrogen showed no increase between 9.2 and 16 g dietary nitrogen intake. No differences were noted in apparent organic matter digested in the rumen; therefore, g microbial protein synthesized per 100 g OM digested increased as nitrogen intake increased up to 9.2 g daily. Yields of protein per 100 g OM digested in the rumen were 9.1, 10.5, 12.8 and 13.3 g for nitrogen intakes of 2.6, 4.4, 9.2 and 16.0 g, respectively.

Considerable net influx of nitrogen into the rumen was noted on the two low nitrogen diets. The amount of recycled nitrogen entering the rumen was 157 and 71% of dietary intake when 2.6 and 4.4 g nitrogen were fed, respectively. Nitrogen equilibrium appeared to be met at

9.2 g nitrogen intake, but a loss of 5.8 g or 36% of intake was noted on the high nitrogen diet.

The author concluded that for maximum protein production in the rumen, approximately 4 mg digestible protein nitrogen per kcal maintenance digestible energy must be provided. With 4000 kcal DE/kg feed, this equals 10% digestible protein.

In further work, Hume (1970a) reported the addition of four- and five-carbon VFA's to a 11.5% protein diet increased rumen volume, flow of tungistic acid nitrogen and protein synthesized per 100 g OM digested in the rumen from 12.5 to 13.4 g. Hume (1970b) added casein, gelatin or zein to rations in which urea supplied 50% of the added nitrogen, the remainder being in the form of the test proteins. The concentrations of ammonia, total nitrogen and tungistic acid-precipitable nitrogen did not vary among urea, casein and gelatin; however, feeding zein reduced ammonia levels and increased passage of total nitrogen and tungistic acid-precipitable nitrogen. This is attributable to protein by-pass. Protein synthesis per 100 g OM digested in the rumen was 17.1, 19.8, 22.5 and 23.3 g for urea, gelatin, zein and casein supplemented diets, respectively. Ammonia loss or gain from the rumen was negligible on all diets except zein. Because of low ruminal ammonia levels, a net gain of 7.5 g nitrogen or 40% of dietary intake was noted with the zein supplemented diet.

Protein synthesis from gelatin and VFA/urea diets may have been limited by the rate of synthesis of sulfur-containing amino acids. In order to investigate sulfur supplementation, Hume and Bird (1970) fed diets containing no added sulfur, supplemented sodium sulfate, supplemented cystine or supplemented sodium sulfate plus cystine. Diets con-

tained 16.3% crude protein, half of which was gelatin and the remainder urea. Neither the level nor form of dietary sulfur had any effect on the concentration of nitrogen components in either the rumen or omasum. The amount of protein leaving the rumen was increased when sulfur was added to the basal diet. Protein synthesis per 100 g OM digested in the rumen was 18.5, 19.0, 17.7 and 20.2 for no added sulfur, 6.2 g sodium sulfate, 5.3 g cystine and 6.2 g sodium sulfate plus 5.3 g cystine, respectively. Added sulfur appeared to have little influence on efficiency of production.

Pilgrim et al. (1970) studied the extent of ^{15}N incorporation into microbial protein when $(^{15}NH_4)_2SO_4$ was continuously infused into the rumen of a sheep for periods of 78 to 98 hours. On a low nitrogen diet, nearly 80% of the bacterial nitrogen contained ^{15}N. Approximately 68 and 54% of the dietary nitrogen was converted into microbial nitrogen on the low and high nitrogen diets, respectively.

Mathison and Milligan (1971) estimated microbial protein synthesis in sheep fed either a rolled barley (1.8% N) or chopped brome grass-lucerne hay (2.5% N) by using $^{15}NH_4Cl$ and lignin as markers. Protein synthesis yields of 16.3 and 12.5 g per 100 g dry matter fermented in the rumen were calculated for the barley and hay diet, respectively. Rumen ammonia levels were much higher and considerably more nitrogen was lost from the rumen on the high protein hay diet.

Hogan and Weston (1971) fed semi-purified diets based on alkali-treated wheat straw to sheep and used urea as the source of supplemental nitrogen. The calculated rate of synthesis was 2.6 to 3.7 g nitrogen per 100 g OM fermented in the stomach. This is equivalent to 16.3 to 23.1 g protein per 100 g OM truly digested or 27.5 g protein per 100 g

OM apparently digested in the rumen. The authors suggested that these values are somewhat higher than previously reported and should be considered not as precise figures but only relative. Similar values on efficiency of production were reported by Lindsay and Hogan (1972). Defaunation resulted in more efficient production, increasing yields by 2 to 4 g bacterial protein per 100 g OM apparently digested in the rumen. The extent of the increase could not be assessed without data concerning the quantity of protozoal OM synthesized.

Beever et al. (1971) fed ground and pelleted barn-dried ryegrass (nitrogen = 1.1%) alone or together with continuous urea infusion into the rumen, bring the nitrogen content to 2.5%. Despite differences in nitrogen intake, 4.3 and 9.9 g per day, the flow of nitrogen was 11.5 and 12.2 g per day, including 54.7 and 57.0 g α-amino acid per day for the control and urea-supplemented diets, respectively. Available energy, rather than nitrogen, appeared to limit synthesis.

Allen and Miller (1972) investigated the effect of supplementing a 6% crude protein diet with 0.8, 1.6 and 2.4% urea on the flow of nitrogen into the abomasum of ewe lambs. Increasing the urea content resulted in a linear increase in nitrogen flow to the abomasum. Recycled urea appeared to be very important in increasing the effective conversion of urea nitrogen into microbial nitrogen. The net influx of nitrogen as a percent of dietary intake was 71, 38, 25 and 17% for diets supplemented with 0, 0.8, 1.6 and 2.4% urea, respectively.

Leibholz and Hartmann (1972) used sheep to study the effect of protein and energy intake on the flow of digesta. The sheep were fed diets hourly containing 1.4, 16 or 30 g nitrogen from barley roughage with or without a nitrogen supplement of lucerne, wheat gluten or casein.

Another diet containing 83% barley roughage also supplied 16 g nitrogen per day but only 1.34 Mcal ME per kg compared to 1.85 to 1.89 for the other diets. The total flow of nitrogen into the duodenum was similar in all sheep fed either 16 or 30 g nitrogen per day and all diets showed a loss of nitrogen in the stomachs. However, sheep fed only 1.4 g nitrogen per day had a significantly lower flow of nitrogen, but a net influx of 3.4 g nitrogen or 343% of intake was noted. Almost all the nitrogen at the duodenum was microbial with the low nitrogen ration, but considerable variability was noted with the other diets reflecting differences in protein solubility (Leibholz, 1972). Microbial protein per 100 g OM digested in the stomach was 9.5, 6.3, 10.5, 11.6, 6.9 and 6.2 for the lambs fed 1.4 g nitrogen, 15.5 g-lucerne supplemented, 16.1 g - casein supplemented, 19.1 g - gluten supplemented, 16.1 g - low energy and 30 g nitrogen, respectively. The percentage of microbial protein in the rumen and duodenum was inversely related to the dietary nitrogen intake and was dependent on both the source of nitrogen and intake of energy.

In review, Miller (1973) and Thomas (1973) concluded that energy-limited bacterial synthesis corresponded to 27 g bacterial nitrogen per kg OM truly digested or 36 g bacterial nitrogen per kg OM apparently digested in the rumen. These values, in terms of microbial crude protein, are 16.8 and 22.5 g microbial protein per 100 g OM truly and apparently digested in the rumen, respectively.

Al-Rabbat *et al.* (1971b) fed a Holstein cow either 13 g alfalfa pellets or 8 kg alfalfa-barley pellets to achieve nitrogen intakes of 370 and 186 g per day. An *in vitro* ^{15}N-tracer technique (Al-Rabbat *et al.*, 1971a) was used for measuring ruminal microbial growth derived

from ammonia nitrogen. Ammonia nitrogen pool sizes were considerably higher with the high nitrogen intake; however, more rapid turnovers were noted on the low nitrogen ration. Therefore, comparable rates of ammonia nitrogen incorporation were achieved with both rations. The rate of microbial growth via ammonia nitrogen was independent of ammonia concentration. Using two different methods for calculation, average microbial protein yields per day were 675 and 426 g microbial protein for the high and low nitrogen intakes, respectively. Despite large differences in gross energy and nitrogen intake between the diets fed, microbial growth via ammonia nitrogen per mole of ATP was 20.1 and 20.9 g for the high and low nitrogen diets, respectively. Under the conditions of this experiment, microbial growth via ammonia nitrogen was independent of nitrogen intake and dependent on energy intake.

Ibrahim and Ingalls (1972) used DAP and AEP as markers to estimate microbial protein synthesis in dairy cows. Total amounts of amino acids passing to the lower gut were 882 and 937 g daily with a starch-glucose straw diet without and with diethylstilbestrol (DES) and 1108 and 1271 g daily when an alfalfa-barley diet without and with DES was fed, respectively. Assuming 90% dry matter in the diet and 60% dry matter digestion in the rumen, 16.3 and 17.3 g amino acid synthesis occurred per 100 g dry matter digested in the rumen. No estimate can be made with the conventional diet due to rumen bypass.

Pitze n (1974) conducted three experiments with steer calves to measure protein synthesis in the bovine. In the first experiment, two 100 kg calves, fitted with re-entrant cannula, were fed starch and cellulose purified diets equal to 1.5 and 3.0% of their body weight. Various feeding intervals were employed with the use of an automatic feeding

system. Chromic oxide impregnated paper was administered through the rumen cannula every 12 hours. Chromic oxide recoveries were poor and resulted in considerable variation. Therefore, it was concluded that chromic oxide was an unsuitable marker when administered through the fistula. Microbial protein synthesis per 100 g OM digested was estimated to be 18.1 and 17.8 g for starch and cellulose, respectively. However, the amount of organic matter digested in the rumen was 1.25 times greater on the starch diet, so the amount of synthesis per 100 g dry matter intake was 1.4 times greater when starch served as the carbohydrate source.

In the second experiment, natural feeds were fed along with chromic oxide paper to four steers. Bacterial true protein was estimated using DAP as a marker. Bacterial synthesis per 100 g OM digested was 20.2, 16.2 and 15.1 for corn, corn cobs plus soybean meal and corn cobs, respectively. Much greater organic matter digestion occurred on the corn diet; therefore, synthesis per 100 g dry matter intake was also higher (11.9 vs. 7.9, 7.6).

Another trial, designed to study the effect of intake levels, was conducted using a diet containing 62.5% corn, 12% soybean meal, 20% ground corn cobs and various minerals and vitamins. The ration was fed at 1.5, 2.5 and 3.5% of body weight. No effect of feeding level on protein synthesis was noted. Bacterial protein synthesis per 100 g OM digested was 16.2, 15.4 and 16.8 g with increasing level of intake, respectively.

Cole et al. (1975) determined the influence of roughage level and corn processing method on microbial protein synthesis in beef steers fed high concentrate rations. Microbial nitrogen passing the abomasum

increased as the amount of roughage in the diet increased. Efficiency of production followed a similar trend. Microbial protein synthesis per 100 g dry matter fermented in the rumen was 7.5, 8.0, 11.8 and 12.7 g for 0, 7, 14 and 21% roughage in the diet, respectively. Protein yield per day on the 21% roughage ration was 360 g. Processing method had no significant influence on microbial nitrogen passage per day. Significantly more dry matter was digested in the rumen with the steam flaked corn as compared to dry rolled; therefore, efficiency of production was significantly reduced. Microbial protein synthesis per 100 g DM fermented was 7.0 and 10.5 g for steam flaked and dry rolled corn, respectively. Protein yield per day on the 21% roughage was 285 g.

Other work in our lab with steers fed high concentrate rations suggest no effect of corn processing method on daily microbial nitrogen production, but efficiency of production was significantly higher for propionic treated-high moisture corn (Galyean and Prigge, personal communication). Nitrogen intakes were approximately 80 g per day. Microbial nitrogen synthesis per day was 37, 35, 35 and 29 g for acid treated-high moisture corn (AHMC), steam flaked (SF), ground high moisture corn (GHMC) and dry rolled (DR), respectively. Efficiency of production (g/100 g DMD) was 14.8, 9.4, 9.5 and 7.5 g for AHMC, SF, GHMC and DR, respectively. Ruminal dry matter digestion was significantly lower on the AHMC treatment. Microbial protein synthesis per 100 g DM fermented appeared to be closely related to dilution rate in a positive manner.

Intestinal Utilization of Nitrogen

The nitrogenous compounds entering the small intestine consist mainly of microbial protein synthesized in the rumen, dietary protein

which has escaped rumen degradation and some endogenous secretions.

Much work has been done in determining the amino acid requirement of the ruminant. In a review, Purser (1970) concluded that protein quality of microbial protein is relatively constant across different rations or experimental conditions. Although the diet has some influence, the abomasal and duodenal digesta presented to the host animal's intestinal tract has an amino acid composition which is the average of microbial protein and residual feed protein present. Clarke et al. (1966) fed poor quality hay alone or with flaked maize, soya protein or both to sheep. Even though the diets had very different amino acid patterns, the individual duodenal amino acids, as a percent of total amino acids, varied only slightly. Similar results have been reported by Bergen et al. (1968). This change in amino acid composition may be a disadvantage if a high quality protein is ingested. Consequently, considerable research effort has examined ruminal protein bypass and the amino acid requirements of the ruminant.

The digestion of protein in the intestines of adult ruminants has been reviewed by Kay (1969). In the small intestine, digestion may differ from that in non-ruminants. The flow of digesta is almost continuous and fairly constant in consistency and composition. Furthermore, the acidity of the abomasal contents tends to persist much longer than in the non-ruminant and may effect protein digestion. The low pH of contents in the upper intestine apparently extends the time of action of abomasal pepsin and delays the onset of pancreatic enzyme activity. Nevertheless, substantial amounts of nitrogen are absorbed from each part of the intestine, suggesting that protein degradation continues throughout the length of the small intestine.

Fermentation of food residues begins anew in the large intestine with the production of considerable amounts of VFA's and ammonia (Faichney, 1968c). Urea may also diffuse into the large intestine to supply nitrogen. The net effect of secretion, microbial metabolism and absorption is the disappearance of 0.5 to 2.0 g nitrogen daily from the digesta passing through the large intestine of sheep (Clarke et al., 1966). Even though the bulk of the nitrogen is absorbed as ammonia, nitrogen recycling to the rumen when nitrogen intakes is low may be of benefit in nitrogen conservation.

Frequency of Feeding and Steady State Conditions

Increased frequency of feeding may increase utilization because of 1) increased total surface area open to attack by the rumen microbes, 2) decreased fermentation energy loss by increased rate of passage, 3) more even supply of substrate and production of metabolites giving rise to a more suitable microbial environment and 4) time spent ruminating and masticating food may result in differences in digestibility (Gordon and Tribe, 1952).

Feeding frequency of urea has been studied in hopes of increasing nitrogen utilization and decreasing urea toxicity. As early as 1949, Dinning et al. reported that the feeding of urea supplements on alternate days as compared to daily and twice daily had no effect on urea utilization by steers. Rush and Totusek (1973) reported no benefit from more frequent feeding of urea than three times per week.

Oltjen et al. (1973) suggested that daily feeding of urea was required for best performance. Campbell et al. (1963) found that feeding urea six times per day improved growth rate and feed efficiency as com-

pared to twice daily feeding. Bloomfield et al. (1961) reported that feeding 16 times per day appeared to increase urea utilization, but no improvement in nitrogen balance with frequent feeding of urea was noted. Deif et al. (1970) reported a significant increase in nitrogen balance when 10.58 g of urea nitrogen was given to lambs in three equal portions a day instead of once daily feeding. Knight and Owens (1973) infused equivalent amounts of urea directly in the rumen over a 1, 3 and 12 hour period post feeding. With twice daily feeding of high energy diets, the slow release of ammonia was not beneficial. With higher fiber diets, infusion of the urea over a three hour period resulted in a higher percentage of nitrogen retained. Sheep consuming the low energy diet and receiving urea infusions over a 1 and 3 hr. period had significantly higher nitrogen balance than control sheep receiving no supplemental urea and those receiving continuous infusion of urea.

Feeding frequency studies often are interpreted with respect to ammonia release alone, despite confounding influence of simultaneous energy intake (Knight and Owens, 1973). Frequent feeding of the carbohydrate may improve urea utilization through reduction in rumen pH, ruminal retention of ammonia or enhanced bacterial growth.

When using markers to measure digestion and digesta passage it is necessary that the concentration of the marker in rumen fluid remain relatively constant and not exhibit diurnal variations. A "steady state" condition indicates that the rate of flow of marker into the rumen is equal to the flow from the rumen with rumen volume and metabolite pools remaining relatively constant within and between days.

The concentration of ^{51}Cr-EDTA was relatively constant when continuously infused into the rumen of sheep (Weston and Hogan, 1967). It was

not possible to show that "steady state" conditions were met, but two different estimates of rumen volume were in good agreement and there was no reason to expect significant changes since a constant ration had been given for some time.

Faichney (1968b) fed animals a constant ration in equal quantities at three hour intervals. Marked changes in concentration of rumen metabolites which followed once daily feeding were largely eliminated. Hume et al. (1970) fed sheep at two hour intervals. Since the rate of disappearance of PEG injected into the rumen as single doses was relatively constant within and between days, it was assumed that "steady state" conditions were approached. Pilgrim et al. (1970) constantly infused $(^{15}NH_4)_2SO_4$ into the rumen of a sheep fed hourly. A close approach to "steady state" conditions was obtained after two days.

Thompson (1973) reported that feeding hourly resulted in less variability in daily flow of digesta. When sheep were fed in 24 equal feedings throughout the day, the pattern of flow was much more even and no distinct, diurnal rhythm was noted.

CHAPTER III

MICROBIAL PROTEIN SYNTHESIS WITH LOW QUALITY ROUGHAGE RATIONS: ISONITROGENOUS SUBSTITUTION OF UREA FOR SOYBEAN MEAL

Summary

Four 275 kg steers, fitted with permanent rumen and abomasal cannulae, were fed low quality roughage with soybean meal and urea supplements to study microbial protein production in the rumen. The total daily feed intake was 5 kg/day and consisted of a pellet of 75% ground weathered range grass (nitrogen < 0.5%) and 25% nitrogen supplement. Urea replaced 0, 25, 50 or 75% of the supplemental soybean meal nitrogen. Ground milo was added with urea to make all rations isocaloric and isonitrogenous. Steers were fed hourly with an automatic feeding system to maintain a constant flow of digesta.

Inclusion of urea depressed nitrogen retention ($P < .05$) and apparent digestibility of dry matter, organic matter and nitrogen ($P < .01$).

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Microbial nitrogen production was relatively constant among rations regardless of source of nitrogen. But the amount of protein nitrogen and feed protein which reached the abomasum decreased linearly ($P < .01$) as the percentage of soybean meal decreased in the supplement. Microbial protein synthesis per 100 g dry matter digested in the rumen was 9.9, 10.4, 10.9 and 11.6 g for rations supplemented with 0, 25, 50 and 75% urea, respectively. This suggests that protein synthesis was not limited by nitrogen availability, but was limited by available energy and rumen turnover time. Equal microbial protein production on diets ranging from 0 to 75% of the supplemental nitrogen as urea nitrogen illustrates that non-protein nitrogen can be utilized with low quality roughage rations for the synthesis of microbial protein if frequent ingestion can be facilitated. However, urea was inferior to soybean meal in the supporting digestion of dry matter and organic matter and retention of nitrogen.

Introduction

Supplements for low quality forages must contain adequate nutrients to nourish the rumen microorganisms in order to maintain an active population not only for ingestion of the fibrous feeds but also for the synthesis of microbial protein to be used as a nitrogen source by the host animal. Since nitrogen is one major limiting nutrient in certain low quality forages, the use of non-protein nitrogen (NPN) to supply ammonia for the rumen population is very appealing economically and ecologically (for conservation of natural proteins for human food use). Many experiments on NPN utilization with low quality roughage rations have been conducted. Traditional measurements of nitrogen utilization

are imprecise. New methods must evaluate the ability of the nitrogen source to support rumen fermentation and to promote the synthesis of microbial protein.

The objective of this study was to investigate the influence of isonitrogenous substitution of urea for soybean meal on microbial protein synthesis, ammonia release patterns, dry matter, organic matter and cellulose digestibility and nitrogen retention of steers fed isocaloric low quality roughage rations.

Experimental Procedures

Trial 1

Four 275 kg steers, fitted with permanent rumen cannulae, were housed in matabolism stalls equipped with an automatic feeding system designed for hourly feeding. This continuous feeding system was used to maintain a constant flow of digesta through the digestive tract and to reduce variations in sampling rumen contents.

The total daily feed intake was 5 kg/day and consisted of a pellet of 75% ground weathered range grass (nitrogen < 0.5%) and 25% nitrogen supplement (Table I). The four rations differed only in the amount of urea and milo substituted for soybean meal on an isocaloric, isonitrogenous basis. Urea supplied 0, 25, 50 and 75% of the supplemental nitrogen in the four rations which were fed in a 4 x 4 Latin square design. Each period of the Latin square consisted of a 10 day adjustment followed by a seven day collection period.

Apparent digestibility of dry matter, organic matter, cellulose and nitrogen was measured by total collection of feces on days 2

TABLE I

COMPOSITION OF RATIONS

Ingredient	IRN[a]	Ration (% DM Basis)[b,c] 0	25	50	75
Bluestem, aerial pt. fresh weathered, mature (2)	2-08-358	73.9	74.0	74.0	74.0
Alfalfa, aerial pt, dehy grnd (1)	1-00-025	1.4	1.4	1.4	1.4
Sugarcane, molasses dehy (4)	4-04-695	1.4	1.4	1.4	1.4
Sorghum, milo, grain, grnd (4)	4-04-444	9.5	12.8	16.3	19.6
Soybean, seed, solv-extd grnd, mx 7 fbr (5)	5-04-604	12.2	8.2	4.1	0.1
Urea, mn 45% nitrogen (5)	5-05-070	--	0.6	1.2	1.9
Sodium phosphate, monobasic (6)	6-04-287	0.7	0.7	0.7	0.7
Sodium sulfate (6)	6-04-292	0.1	0.1	0.1	0.1
Salt, T.M.		0.3	0.3	0.3	0.3
Chromium sesquioxide		0.5	0.5	0.5	0.5

[a] International Reference Number. Atlas of Nutritional Data on United States and Canadian Feeds. 1971. National Academy of Sciences, Washington, D.C.

[b] Rations identified by supplemental nitrogen equivalent supplied by urea.

[c] All rations contained added vitamin A palmitate, commercial, 7-05-143, 4950 IU/kg feed and vitamin D_3, commercial, 440 IU/kg feed.

through 6 of each collection period. Feces were weighed daily and a 10% aliquot stored at 4 C in a polyethylene bag. Daily aliquots were composited and a sample obtained for nitrogen determination. A second sample was dried in a forced air oven at 40 C, ground through a 2 mm screen in a Wiley mill and stored in polyethylene bags at room temperature. Urine volume was also measured daily and a 10% aliquot stored at 4 C. A 250 ml sample was obtained from the total composite for nitrogen determination.

To measure fluctuation in rumen pH and ammonia, rumen samples were taken by suction pump every 10 min for two hr in the morning and afternoon of day 7. The pH of the whole rumen contents was read immediately and the sample strained through two layers of cheesecloth. One ml of saturated mercuric chloride was added to inhibit bacterial action. A 10 ml aliquot of strained rumen fluid was used for ammonia nitrogen determination.

Trial 2

A second trial was conducted to measure microbial protein synthesis. Four 275 kg steers, fitted with permanent rumen and abomasal cannulae, were housed in individual pens with slotted floors. A continuous feeding system was used to simplify sampling of rumen and abomasal contents and to obtain more accurate estimates of digesta composition. The same 4 x 4 Latin square design with a 10 day adjustment and seven day rumen and abomasal sampling period was used in this trial. Rumen and abomasal samples were obtained twice daily approximately 30 min post-feeding on days 2, 4 and 6 of the sampling period. All samples were immediately frozen for later chemical analyses.

Laboratory Procedures and Calculations

Feed, rumen, abomasal, fecal and urine samples were analyzed for proximate composition (A.O.A.C., 1965) and cellulose and lignin (Van Soest, 1963). Ruminal and abomasal total nitrogen were fractionated by determining ammonia nitrogen by MgO distillation (A.O.A.C., 1965) and urea nitrogen as the increase of ammonia nitrogen after incubation of 10 ml of sample with 10 ml of urease solution (3490 units/liter) in a pH 7 phosphate buffer followed by MgO distillation.

Abomasal samples were also analyzed for ribonucleic acid (RNA) according to procedures outlined by McAllan and Smith (1969). Microbial nitrogen was estimated from abomasal RNA assuming a RNA nitrogen content of 13.2% and a microbial nitrogen to RNA nitrogen ratio of 10:1 (Males, unpublished data). True microbial protein was calculated as microbial nitrogen times 5.12, thereby correcting for the 19 to 20% of microbial nitrogen being in the form of microbial nucleic acids (Smith, 1969).

Non-ammonia nitrogen (NAN) was calculated as total nitrogen minus ammonia and urea nitrogen. Feed protein nitrogen bypassing rumen degradation was calculated as abomasal NAN minus microbial nitrogen. Nitrogen lost in the rumen was calculated as nitrogen intake minus total abomasal nitrogen. Lignin was used as an internal marker for determination of digesta passage through the abomasum. Ruminal dry matter digestion was calculated by the equation:

$$\% \text{ D.M. Digestion} = 100 - 100 \left[\frac{\% \text{ RL}}{\% \text{ AL}}\right]$$

where RL and AL are ration lignin and abomasal lignin, respectively.

The data were analyzed by analysis of variance as a 4 x 4 Latin square design. Treatment effects were tested for linear quadratic and

and cubic components (Snedecor and Cochran, 1967) when a significant treatment effect was noted in the analysis of variance.

Results and Discussion

The mean chemical composition of the rations and the intakes of various nutrients are presented in Table II. All rations were formulated to be as isocaloric and isonitrogenous as possible; however, the 50 and 75% urea supplemented rations were about 0.7 and 0.9% lower in crude protein content. Rations containing over 50% of the nitrogen from soybean meal had larger negative calculated UFP values (Burroughs et al., 1975), suggesting a surplus of ammonia nitrogen. A positive UFP value of 6.27 g urea/kg DM was noted on the high urea supplemented ration.

Little variation in rumen pH (range = 5.9 to 6.3) and only small fluctuations in rumen ammonia levels (range = 11 to 14.5 mg NH_3-N/100 ml) were noted with the hourly feeding system (Figure 1). Morning and afternoon values were rather constant and variation due to period was negligible. Similar results have been reported by Faichney (1968b) and Thompson (1973). Since all samples in Trial 2 were gathered on alternate days, day was analyzed as a source of variation. Day was a minor source of variation. Rumen pH and ammonia release patterns and the absence of day effects suggests that "steady state" ruminal conditions were approached.

Nitrogen retention data and apparent nutrient digestibilities are presented in Table III. Replacement of soybean meal nitrogen by urea depressed nitrogen retention linearly and quadratically ($P < .01$) and apparent digestibility of dry matter, organic matter and nitrogen

TABLE II

CHEMICAL COMPOSITION OF RATIONS AND NUTRIENT INTAKES

Item	Urea N/Supplemental N[a] 0	25	50	75
Rations (%)[b]				
Dry Matter	92.2	92.4	91.3	91.9
Organic Matter	92.0	92.1	91.8	92.0
Cellulose	29.5	30.3	33.7	33.5
Lignin	7.7	7.3	8.0	8.1
Crude Protein	10.1	10.0	9.4	9.2
DE (Mcal/kg)[c]	2.38	2.36	2.33	2.31
UFP (g/kg)[d]	-40.6	-25.5	-10.0	6.3
Intakes (g)				
Dry Matter	4609	4619	4567	4595
Organic Matter	4239	4252	4193	4227
Cellulose	1358	1398	1537	1540
Nitrogen	74.5	74.1	68.7	67.4

[a]Rations identified by supplemental nitrogen equivalent supplied by urea.

[b]Dry matter basis.

[c]Estimated digestible energy.

[d]Urea fermentation potential (Burroughs, Nelson and Mertens, 1975).

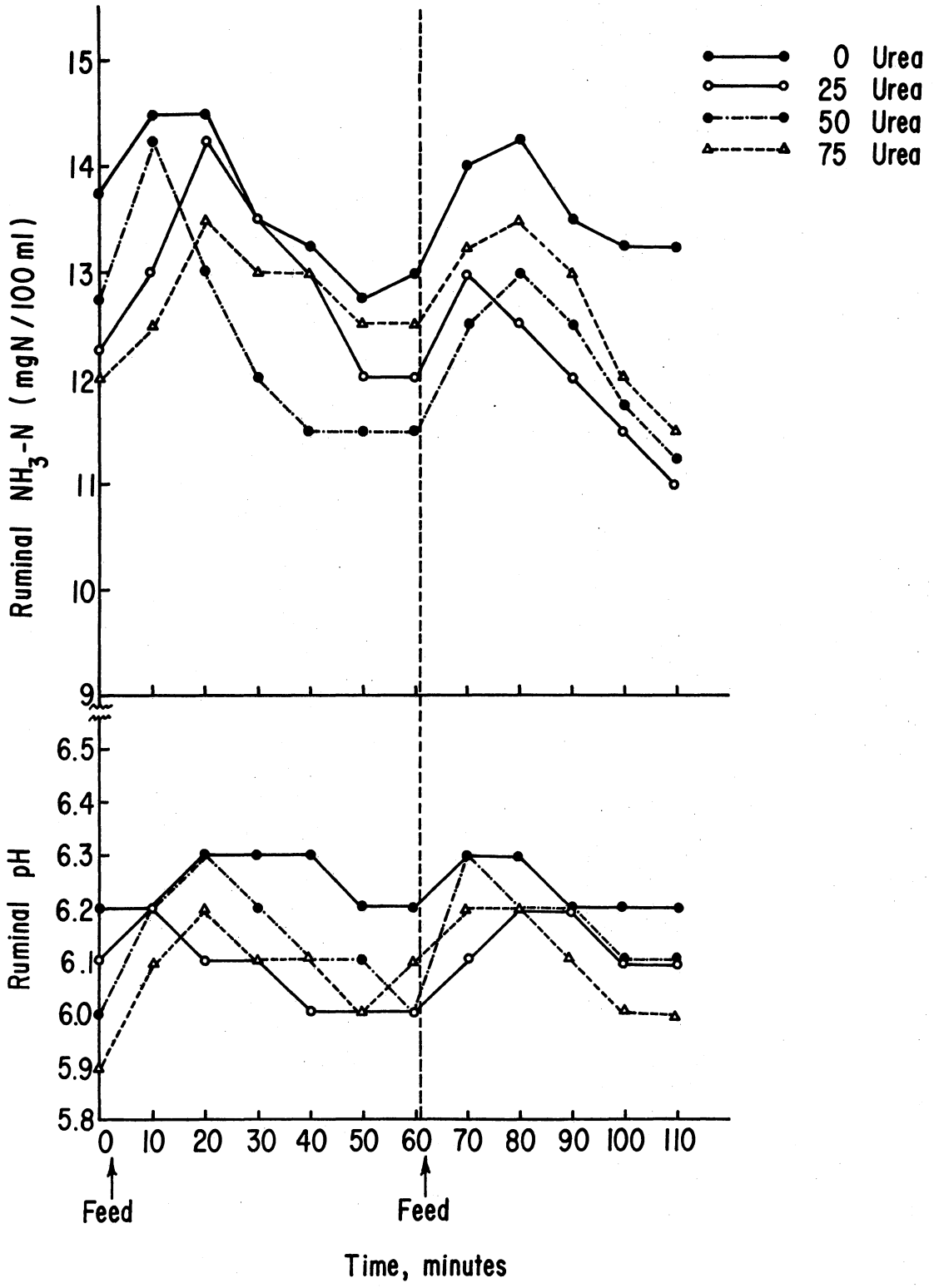

Figure 1. Rumen Ammonia and pH Constancy Curves

TABLE III

NITROGEN RETENTION AND APPARENT DIGESTIBILITY BY STEERS FED RATIONS CONTAINING VARIOUS UREA LEVELS

Item	Urea N/Supplemental N				
	0	25	50	75	SE
Nitrogen utilization, g/day					
Nitrogen intake	74.5	74.1	68.7	67.4	
Nitrogen excretion	67.8	75.7	75.2	70.9	
Fecal[b]	30.5	32.8	35.4	31.5	0.7
Urinary	37.3	42.9	39.8	39.4	2.4
Nitrogen retention[a,b]	6.7	-1.6	-6.5	-3.5	2.6
Apparent digestibility, %					
Dry matter[a,b,c]	55.2	51.9	46.1	49.9	0.7
Organic matter[a,b,c]	58.0	55.1	49.8	53.0	0.6
Cellulose	56.2	55.4	55.2	56.5	0.9
Nitrogen[a,b,c]	59.0	55.8	48.4	53.3	0.9

[a]Significant linear effect ($P < .01$).

[b]Significant quadratic effect ($P < .01$).

[c]Significant cubic effect ($P < .01$).

($P < .01$), however, the latter depression has a cubic component. All rations containing urea resulted in negative nitrogen balance. Nitrogen balance improved again slightly at the highest urea level. Oltjen and Putnam (1966) have reported significantly lower nitrogen retention for steers fed urea as compared to isolated soy protein. Freiteg et al. (1968) found no significant difference in nitrogen retention between 10% crude protein diets with urea supplying either 30 or 70% of the total nitrogen. Apparent digestibility of dry matter, organic matter and nitrogen decreased cubicly with urea replacement of soybean meal nitrogen. Whether this was a ruminal or postruminal effect is unclear. All digestibilities increased at the highest urea level, but remained inferior to soybean meal. Digestion coefficients were similar to those previously reported by Raleigh and Wallace (1963) with 9 and 12% crude protein hay diets supplemented with urea and/or cottonseed meal. However, their work found no significant differences between urea and cottonseed meal supplementation.

Differences in the apparent total digestion of nitrogen may be due to ruminal bypass of soybean meal. In trial 2, as the percentage of urea increased in the ration, the quantity of NAN or residual feed protein reaching the small intestine declined from 26.6 to 16.4 (Table VI). Feed proteins other than soybean meal may have a lower intestinal digestibility than soybean meal. Since only 5 g SBM was fed daily in 75% urea supplemented ration, the 16.4 g of NAN would be mostly non-degraded grass and milo nitrogen. Therefore, the quantity of soybean meal nitrogen reaching the small intestine would be 10.2, 5.1 and 4.2 g for 0, 25 and 50% urea supplemented rations, respectively. No significant differences were noted in quantity of microbial nitrogen reaching

the small intestine among rations. Thus, protein reaching the lower gut may have been more digestible with the 100% SMB supplemented ration since a higher proportion of the nitrogen reaching the small intestine would have been residual soybean meal nitrogen (19.8% vs. 10.6, 9.0%). Nitrogen digestibility is imprecise since it represents only a net feed-feces difference and generally increases when NPN is included in the ration. The decrease in nitrogen digestibility noted with the higher urea supplements may have been due to lower dietary nitrogen intakes. Reasons for the depressions in dry matter and organic matter digestion noted with the 50% urea supplement are not apparent.

Nitrogen fractionation of rumen and abomasal contents expressed as mg/g nitrogen are presented in Table IV. Ruminal total nitrogen decreased linearly ($P < .01$) as urea replaced soybean meal nitrogen in the diet. No urea nitrogen was detected in the rumen contents of steers fed the 100% SBM supplement. Also, no significant differences in rumen ammonia concentration were noted among rations. Soybean meal is fairly soluble and is partially degraded in the rumen. As a result, large quantities of ammonia nitrogen do pass through the ammonia pool before being utilized or absorbed. Mean ruminal ammonia concentrations were 13.6, 12.4, 12.3 and 12.7 mg NH_3-N/100 ml of rumen fluid for supplements containing 0, 25, 50 and 75% urea, respectively. The percentage of ammonia nitrogen present in the rumen tended to increase as the percentage of urea in the urea-containing supplements increased, but ruminal urea nitrogen levels were unchanged. Non-ammonia nitrogen (NAN) per g total nitrogen in the rumen was relatively constant regardless of the source of nitrogen in the ration.

Basically the same pattern was noted in the abomasum as found in

TABLE IV

NITROGEN FRACTIONATION OF RUMINAL AND ABOMASAL CONTENTS

Item	Urea N/Supplemental N				
	0	25	50	75	SE
Rumen					
Total nitrogen (mg/g DM)[a]	19.5	20.0	17.8	16.7	0.5
NH_3-N (mg/g N)	81.0	61.2	65.9	79.2	7.9
Urea-N (mg/g N)	0.0	10.0	10.8	10.9	1.1
NAN (mg/g N)	919.4	929.6	925.3	909.4	8.0
Abomasal					
Total nitrogen (mg/g DM)[a]	20.1	19.2	17.5	17.1	0.9
NH_3-N (mg/g N)	61.0	65.5	61.4	72.1	8.0
Urea-N (mg/g N)[a]	0.0	2.9	8.7	8.0	2.5
NAN (mg/g N)	939.0	931.7	929.9	919.8	7.9

[a]Significant linear effect ($P < .01$).

the rumen. Abomasal total nitrogen decreased ($P < .01$) and urea nitrogen increased ($P < .01$) as urea replaced soybean meal. No influence of urea replacement of soybean meal was noted among rations for abomasal NAN concentrations; however, abomasal NAN tended to decrease as the percentage of urea increased in the supplement.

Increasing the level of urea decreased ($P < .01$) the abomasal concentration of total nitrogen and non-ammonia nitrogen (NAN) when expressed per unit of dry matter (Table V). Microbial nitrogen concentration was rather constant regardless of the amount of urea present in the ration. Therefore, differences in abomasal NAN must reflect differences in feed protein escaping rumen degradation. As the percentage of soybean meal decreased, a decrease ($P < .01$) in bypassed nitrogen was noted.

Daily abomasal passage of nitrogen and digesta are presented in Table VI. More total nitrogen passed through the abomasum of steers fed the 100% SMB supplement and total nitrogen passage decreased ($P < .01$) as the percentage of soybean meal decreased. Previous reports (Potter et al., 1968; Potter et al., 1971; Young et al., 1975) also suggest more nitrogen reaches the abomasum of steers fed soybean meal rather than urea. Net losses of nitrogen from the rumen were calculated to occur with all rations. Similar ruminal nitrogen losses have been reported by Young et al. (1975) with ground ear corn diets of comparable nitrogen intakes. Considerable higher estimates of ruminal nitrogen loss were reported by Potter et al. (1968), while net influx of nitrogen into the rumen was reported by Potter et al. (1971) with similar rations. Nitrogen recovery in the abomasum relative to intake was high on all their rations and Potter et al. (1971) suggested that

TABLE V

FRACTIONATION OF NITROGEN IN ABOMASAL DRY MATTER

Item	Urea N/Supplemental N				
	0	25	50	75	SE
	Mg N/g DM				
Total Nitrogen[a]	20.11	19.18	17.46	17.13	.89
NH_3-N	1.23	1.24	1.08	1.24	.16
Urea-N[a]	.00	.06	.15	.13	.04
NAN[a]	18.88	17.90	16.24	15.78	.86
Microbial N	10.09	10.77	9.68	10.55	.87
Bypass N[a]	8.79	7.13	6.56	5.23	1.12

[a]Significant linear effect ($P < .01$).

TABLE VI

TOTAL DAILY ABOMASAL PASSAGE OF NITROGEN

Item	Urea N/Supplemental N				
	0	25	50	75	SE
Nitrogen intake (g)	74.5	74.1	68.7	67.4	
Nitrogen lost in rumen (g)	13.6	16.3	12.8	13.7	2.7
Total abomasal nitrogen (g)[a]	60.9	57.8	55.9	53.7	2.5
Microbial nitrogen (g)	30.5	32.5	31.0	33.0	2.2
Protein-N bypass (g)[a]	26.6	21.5	20.6	16.4	4.0
True protein reaching small intestine	322.4	300.8	290.0	271.5	14.8
Ruminal DM digestion (%)	34.3	34.7	31.2	31.8	2.1
Protein synthesis/100 g DM digested in rumen	9.9	10.4	10.9	11.6	1.5
Digesta passage (liters)	25.0	24.7	25.5	26.2	1.5
Dilution rate (%/hr)	2.08	2.06	2.12	2.18	.13
Rumen turnover time (days)	2.00	2.03	1.96	1.91	.14

[a]Significant linear effect ($P < .01$).

the high abomasal nitrogen levels might be due to indogenous nitrogen secretion or incomplete recovery of chromic oxide in the abomasal samples. The measurement of digesta passage through the abomasum by using marker techniques depends on obtaining representative samples of abomasal digesta (Hogan and Weston, 1967a). Chromic oxide was included at 0.5% of the diet to be used as one marker for measurement of digesta passage. But large abomasal nitrogen values suggested incomplete recovery and chromic oxide was determined to be an unsuitable marker. Drennan et al. (1970) and Pitzen (1974) also have obtained variable results using chromic oxide as an indicator to estimate ruminal digestion from abomasal samples. Faichney (1972) found chromic oxide completely unsuitable for quantitating abomasal flow. Young et al. (1975) suggested that when comparing rations of similar composition, little evidence exists to refute validity of marker techniques for estimating relative differences. Nevertheless, in view of the impossible absolute values observed based on chromic oxide, lignin was used as the reference marker in this study.

Microbial nitrogen production per day was relatively constant among rations regardless of level of nitrogen supplied as urea. Daily passage of protein nitrogen escaping ruminal degradation decreased ($P < .01$) as the percentage of urea increased in the supplement. No influence due to the addition of urea was noted among rations for true protein reaching the small intestine; however, the quantity of protein reaching the intestine tended to decline with the percentage of soybean meal in the diet. This decline was probably attributable to rumen bypass of soybean meal protein. As daily intake of soybean meal nitrogen decreased across rations from 39.6 to 0.3 g, protein nitrogen bypass decreased

from 26.6 to 16.4 g, suggesting that about 23, 17 and 28% of the soybean meal nitrogen escaped ruminal digestion for the 100, 75 and 50% soybean meal supplementation levels.

Microbial protein synthesis per 100 g DM digested in the rumen was in good agreement with estimates obtained in our laboratory with steers fed high concentrate diets hourly at the rate of 5 kg/day (Cole et al., 1975). Similar results have been reported with sheep (Hume et al., 1970; Leibholz and Hartmann, 1972). However, all these values are below the commonly accepted value of 22 to 23 g microbial protein synthesized per 100 g OM apparently digested in the rumen (Hogan and Weston, 1970; Miller, 1973; Thomas, 1973). The satisfactory conversion of ammonia nitrogen to microbial nitrogen depends upon an ample supply of alpha-keto acids for conversion to amino acids. When cattle are subsisting on low quality roughage, biochemical conditions in the rumen do not appear satisfactory for urea utilization. Cellulose is hydrolyzed and fermented too slowly to furnish adequate supplies of carbon skeletons to keep pace with the rapid release of ammonia following urea hydrolysis. Previous research has suggested that the rate of urea hydrolysis exceeds the rate of use of released ammonia for synthesis of amino acids by a factor of four (Bloomfield et al., 1960). Under most conditions, there is enough buffering action in rumen fluid to convert ammonia to the ammonium ion. Ammonia has a pka of 8.8 at 40 C (Bloomfield et al., 1963) and the absorption of ammonia across the rumen wall is slow if the rumen pH is below 7.

Increasing the frequency of urea feeding may increase urea utilization. Bloomfield et al. (1961) reported that the feeding of urea 16 times per day appeared to increase urea utilization, but frequent

feeding did not improve nitrogen balance. Campbell et al. (1963) reported improved growth rate and feed efficiency by feeding urea six times per day as compared to twice daily. Deif et al. (1970) reported a significant increase in nitrogen balance when lambs were fed urea in three equal portions per day instead of once daily feeding. Knight and Owens (1973) infused equivalent amounts of urea directly in the rumen over a 1-, 3- and 12-hour period post-feeding to investigate the sustained release of ammonia. Sheep consuming low energy diets twice daily and receiving urea infusions over a 1 and 3 hr period had significantly higher nitrogen balance than control sheep receiving no supplemental urea or those receiving continuous infusion of urea. Rush and Totusek (1973) reported no benefit from more frequent feeding of urea than three times per week under Oklahoma range conditions.

Frequent feeding studies often are interpreted with respect to ammonia release alone, despite confounding influence of simultaneous energy intake (Knight and Owens, 1973). Frequent feeding of the carbohydrate may improve urea utilization through reduction of rumen pH, ruminal retention of ammonia or enhanced bacterial growth. The frequent ingestion of the rations in this experiment resulted in a continuous supply of substrate and production of metabolites giving rise to a more suitable microbial environment. Ruminal ammonia fluctuations were reduced and rumen pH was rather constant and below 7. These factors should optimize urea utilization. Therefore, extrapolation of these results to infrequent feeding of urea on range grass must be made cautiously.

To calculate rumen turnover rates, the amount of digesta passage from the rumen was estimated from lignin intake, abomasal lignin and

abomasal DM. Assuming a constant rumen volume of 50 liters (Cole et al., 1975), the rumen dilution rate and turnover rate were estimated. The assumptions and estimates are based on a solid phase marker (lignin) and may not directly indicate the flow of liquid digesta.

No influence due to urea addition was noted among rations for digesta passage, dilution rate or rumen turnover time. Estimates of dilution rate and turnover time are lower than estimates obtained on high concentrate rations (Cole et al., 1975). Rumen turnover rate tended to increase as urea replaced soybean meal in the ration. This increased turnover may help explain the increased efficiency of production (g microbial protein synthesis/100 g DM digested) with the higher urea rations.

Many experiments designed to delineate rumen microbial growth requirements have been limited by the confounding of several variables, especially intake. Restriction of intake to 5 kg/day was chosen since it represented maintenance DE intake (N.R.C., 1970) and approximated winter grazing conditions. Because of the anaerobic nature of the rumen, protein synthesis is usually limited by available energy (Hogan and Weston, 1970). The energy density of these rations was low. Nitrogen balance values close to zero suggest that maintenance conditions were approximated. Maximum ruminal protein production appeared to be achieved for the energy level supplied and rate of rumen turnover, regardless of whether nitrogen was supplemented as urea or soybean meal. Equal microbial protein production on diets ranging from 0 to 75% of the supplemental nitrogen as urea nitrogen illustrates that non-protein nitrogen can be utilized on low quality roughage rations for the synthesis of microbial protein if frequent ingestion can be

facilitated. However, urea was inferior to soybean meal in promoting digestion of dry matter, organic matter and nitrogen and retention of nitrogen.

CHAPTER IV

MICROBIAL PROTEIN SYNTHESIS WITH LOW QUALITY ROUGHAGE RATIONS: LEVEL AND SOURCE OF NITROGEN

Summary

Four 350 kg steers, fitted with permanent rumen and abomasal cannulae, were fed cottonseed hulls with soybean or urea supplements to study the influence of level and source of nitrogen on microbial protein production. Protein levels on a dry matter basis were 11.1, 8.5, 10.8 and 12.6% for S-100, U-75, U-100 and U-115, respectively. Total daily feed intake was 5 kg/day. Steers were fed hourly to maintain relatively steady state ruminal conditions.

Mean ruminal ammonia concentrations were 8.1, 3.7, 12.4 and 22.2 mg NH_3-N/100 ml rumen fluid for S-100, U-75, U-100 and U-115, respectively ($P < .01$). Soybean meal was superior to any level of urea for total apparent dry matter, organic matter and cellulose digestion. Dry matter, organic matter and cellulose digestibilities were improved by

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[1]Journal article of the Agriculture Experiment Station, Oklahoma State University, Stillwater.

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the addition of urea above 8 g/kg DM consumed ($P < .01$). A negative nitrogen balance ($P < .01$) was noted with the U-75 ration. No significant differences among rations were noted for microbial protein synthesis regardless of level or source of nitrogen. Microbial protein synthesis per 100 g OM apparently digested in the rumen was 18.9, 17.9, 19.9 and 19.3 g for S-100, U-75, U-100 and U-115, respectively. An estimated 20% of the soybean meal nitrogen escaped ruminal degradation. No significant differences were noted among rations for digesta passage, dilution rate or rumen turnover time. Microbial protein production per 100 gm DM digested in the rumen appeared to plateau at dilution rates of 5.5 to 6% per hour.

Introduction

The satisfactory conversion of ammonia nitrogen to microbial protein depends upon an ample supply of alpha-keto acids for conversion to amino acids and of energy for bacterial growth. When cattle are subsisting on low quality roughage, biochemical conditions in the rumen do not appear satisfactory for high urea utilization. Cellulose is hydrolyzed and fermented too slowly to furnish adequate supply of alpha-keto acids or energy to keep pace with the rapid release of ammonia from urea hydrolysis. Providing urea and forage at hourly intervals, however, appears to produce a more suitable microbial environment for urea use (Kropp et al., 1975). Microbial protein synthesis per day was relatively constant on diets ranging from 0 to 75% replacement of the supplemental soybean meal nitrogen by urea nitrogen, suggesting that non-protein nitrogen can be adequately utilized with low quality roughage rations if frequent ingestion or attenuated ammonia release

can be facilitated.

Previous work by Hume et al. (1970) and Allen and Miller (1972) shows a linear increase in nitrogen flow to the abomasum as nitrogen intake increases at low nitrogen levels. At higher levels, Hogan and Weston (1967b), Beever et al. (1971), Al-Rabbat et al. (1971b) and Leibholz and Hartmann (1972) have reported that the flow of nitrogen from the stomachs was similar regardless of large differences in nitrogen intake. Recycled urea can meet some of the need for dietary nitrogen. A large net influx of nitrogen into the rumen is reported with low nitrogen diets, while nitrogen efflux due to absorption has been observed on high nitrogen diets (Hogan and Weston, 1970; Hume et al., 1970; Allen and Miller, 1972; Leibholz and Hartmann, 1972).

The objective of this study was to evaluate the relative utilization of urea fed at three different rates for supporting rumen fermentation and microbial protein synthesis in steers consuming isocaloric maintenance energy rations and to compare urea with soybean meal as a nitrogen supplement for such a ration.

Experimental Procedures

Four 350 kg steers, fitted with permanent rumen and abomasal cannulae, were housed in metabolism stalls equipped with an automatic feeding system designed for high roughage feeding. This hourly feeding system has been shown to maintain relatively steady state ruminal conditions (Kropp et al., 1975).

The total daily feed intake was 5000 g/day and consisted of 3750 g cottonseed hulls (nitrogen < 0.9%) and 1250 g of a nitrogen supplement. Four rations, differing only in source and level of nitrogen, were fed

in a 4 x 4 Latin square design (Table VII). The control ration supplied 100% of the suggested protein requirement (NRC, 1970) for zero weight gain by 350 kg steers in the form of soybean meal protein. Other diets consisted of urea-based nitrogen supplements calculated to meet 75, 100 or 115% of the NRC protein requirement. Urea provided 0, 26.5, 39.1 and 55.6% of the total nitrogen, respectively. Roughage was added to the soybean meal supplement (S-100) and corn starch to the high urea supplement (U-115) to keep the rations isocaloric. Sodium sulfate was added to all urea supplements to maintain N:S ratio of 12:1. Protein levels on a dry matter basis were 11.1, 8.5, 10.8 and 12.6% for S-100, U-75, U-100 and U-115, respectively.

Each period of the Latin square consisted of a nine day adjustment, five day fecal and urine collection and seven day rumen and abomasal sampling periods. Rumen and abomasal samples were obtained twice daily approximately 30 min post-feeding on days 2, 4 and 6 of the sampling period. The pH of the whole rumen contents was determined immediately after sampling and the samples were strained through two layers of cheesecloth. One ml of saturated mercuric chloride was added to inhibit bacterial action. A 100 ml aliquot of strained rumen fluid was retained for ammonia nitrogen determination.

Abomasal samples were taken by removing the abomasal cannula stopper and collecting approximately one liter of abomasal contents in the subsequent 15 to 30 min. The samples were thoroughly mixed and 500 ml of whole abomasal contents were immediately frozen for later chemical analyses.

Feed, fecal and urine sampling procedures, laboratory procedures and calculations have been previously described (Kropp et al., 1975).

TABLE VII

COMPOSITION OF RATIONS

Ingredient	IRN[a]	Ration (% DM Basis)[b] S 100	U 75	U 100	U 115
Cotton, seed hulls (1)	1-01-599	74.8	74.9	74.9	74.6
Wheat, straw, chop (1)	1-05-175	2.5	0.9	--	--
Alfalfa, aerial pt, dehy grnd (1)	1-00-025	2.6	--	--	--
Sugarcane, molasses, dehy (4)	4-04-695	2.7	2.7	2.7	2.7
Sorghum, milo, grain, grnd (4)	4-04-444	7.2	20.0	20.1	15.8
Soybean, seed, solv-extd, grnd, mx 7 fbr (5)	5-04-604	9.8	--	--	--
Urea, mn 45% nitrogen (5)	5-05-070	--	0.8	1.5	2.5
Sodium sulfate (6)	6-04-292	--	0.1	0.2	0.4
Sodium phosphate monobasic (6)	6-04-287	0.2	0.3	0.3	0.3
Limestone, grnd, mn 33 Ca (6)	6-02-632	--	0.1	0.1	0.1
Salt, T.M.		0.3	0.3	0.3	0.3
Starch, corn		--	--	--	3.4

[a]International Reference Number. Atlas of Nutritional Data on United States and Canadian Feeds. 1971. National Academy of Sciences, Washington, D.C.

[b]All rations contained added Vitamin A palmitate, commercial, 7-05-143, 4950 IU/kg feed and Vitamin D_3, commercial, 440 IU/kg feed.

The data were analyzed by analysis of variance as a 4 x 4 Latin square design. Treatment effects were tested by least significant difference (Snedecor and Cochran, 1967) when a significant treatment effect was noted in the analysis of variance. Day was considered a sub-plot source of variation.

Results and Discussion

The mean chemical composition of the rations and the intake of various nutrients are presented in Table VIII. Rations were formulated to maintain all dietary constituents except nitrogen as constant as possible. Maintenance DE intake (N.R.C., 1970) was achieved by restriction of dietary intake to 5 kg/day. Calculated ration UFP value (Burroughs et al., 1975) for the S-100 rations would suggest that ammonia was present at a surplus level. According to their definition of UFP, an additional 10.4 and 3.2 g of urea could be productively used for every kg of feed DM consumed in rations U-75 and U-100, respectively. A surplus of 2.6 g urea was provided in the U-115 ration.

Mean ruminal ammonia and pH concentrations for rations containing various sources and levels of nitrogen are presented in Table IX. Differences in ruminal ammonia levels reflect differences in nitrogen intake and solubility (Blackburn, 1965). Higher ($P < .01$) rumen ammonia level resulted from the feeding of the U-115 ration. Although no significant difference was noted between ammonia levels of S-100 and U-100, a slightly higher ammonia level for steers fed the U-100 diet might be expected if some of the soybean meal protein bypasses ruminal degradation to ammonia.

Ammonia is an essential nutrient for growth of many bacteria even

TABLE VIII

CHEMICAL COMPOSITION OF RATIONS AND NUTRIENT INTAKES

Item	Treatment S 100	U 75	U 100	U 115
Rations (%)[a]				
Dry matter	90.3	90.3	90.1	89.9
Organic matter	95.4	95.9	95.8	95.5
Cellulose	34.1	32.7	32.5	32.1
Lignin	17.3	17.2	17.1	17.0
Crude protein	11.2	8.5	10.8	12.8
DE (Mcal/kg)[b]	2.55	2.54	2.53	2.50
Basal UFP (g/kg)[c]	-37.6	18.4	18.2	22.4
Urea added (g/kg)	--	8.0	15.0	25.0
Ration UFP (g/kg)[c]	-37.6	10.4	3.2	- 2.6
Intakes (g)				
Dry matter	4515	4513	4503	4497
Organic matter	4307	4326	4312	4292
Cellulose	1541	1476	1463	1446
Nitrogen	80.7	61.7	78.0	91.8

[a] Dry matter basis.

[b] Estimated digestible energy.

[c] Urea fermentation potential (Burroughs et al., 1975).

TABLE IX

MEAN RUMINAL AMMONIA AND pH CONCENTRATIONS

Item	Treatment S 100	U 75	U 100	U 115	SE
Ruminal ammonia (mg NH_3-N/100 ml)	8.1[b]	3.7[c]	12.4[b]	22.2[a]	1.8
Ruminal pH	6.2	6.0	6.0	6.1	.1

[a,b,c]Means on the same line with differing superscripts are significantly different ($P < .01$).

when preformed organic nitrogen is present in the media (Hungate, 1966). Although the ammonia nitrogen requirement of rumen microbes has not been precisely quantitated (Allison, 1970), data suggest that 5 mg NH_3-N/100 ml rumen fluid is necessary to support maximum growth rates of rumen bacteria (Roffler and Satter, 1973; Satter and Slyter, 1974). The rather lower ($P < .01$) level of 3.7 mg NH_3-N/100 ml with the U-75 ration suggests a possible ruminal nitrogen deficiency.

The influence of various rations on the apparent digestion of dry matter is shown in Table X. Ruminal digestion of dry matter was about 2% lower ($P < .10$) for steers fed the U-100 supplement, resulting in a higher ($P < .10$) quantity of dry matter entering the small intestine. Of the dry matter entering the intestine, only 27.8% was digested on the low protein U-75 diet, less ($P < .05$) than other rations. Total apparent DM digestibility was 59.5% for the S-100 ration, being 2 to 9% higher ($P < .01$) than for urea supplemented rations. Overall, ruminal dry matter digestion was unchanged by the addition of urea above 8 g/kg DM consumed, but intestinal and total digestibility were significantly improved by added urea. Some previous research has reported no difference in apparent dry matter digestion or site of digestion by sheep due to level or source of nitrogen (Hume et al., 1970; Hume, 1970b; Leibholz and Hartmann, 1972). Owens et al. (1973) observed, with lambs, no ruminal effect of added urea but showed post-ruminal improvement in digestibility. In contrast, Raleigh and Wallace (1963) found that total DM digestibility by steers of a low quality roughage diet supplemented with cottonseed meal increased when crude protein level increased from 9 to 12%. But the partial substitution of urea for cottonseed meal did not improve digestibility as crude protein levels were increased. Of

TABLE X

DRY MATTER DIGESTION

Item	Treatment S 100	U 75	U 100	U 115	SE
Intake	4515	4513	4503	4497	--
Ruminal digestion (g)	1500[a]	1415[ab]	1271[c]	1378[b]	46
Entering intestine (g)	3015[c]	3098[bc]	3232[a]	3119[b]	46
Intestinal digestion (g)	1185[a]	864[b]	1194[a]	1227[a]	81
Total digestion (g)	2685[f]	2279[h]	2465[g]	2605[fg]	48
Disappearance of Apparently Digested Dry Matter					
Ruminal, % of intake	33.2[a]	31.4[ab]	28.2[c]	30.6[b]	1.0
Intestinal, % of entering	39.2[d]	27.8[e]	36.9[d]	39.2[d]	2.2
Total, % of intake	59.5[f]	50.5[h]	54.7[g]	57.9[fg]	1.1
Ruminal, % of total	56.0	62.4	51.7	53.0	2.7
Intestinal, % of total	44.0	37.6	48.3	47.0	2.7

[a,b,c]Means on the same line with differing superscripts are significantly different ($P < .10$).

[d,e]Means on the same line with differing superscripts are significantly different ($P < .05$).

[f,g,h]Means on the same line with differing superscripts are significantly different ($P < .01$).

the total g DM apparently digested in the whole alimentary tract, approximately 55% was digested in the rumen in this study.

Results relating to the digestion of organic matter (OM) are shown in Table XI. Ruminal digestion of OM tended to be lower on rations containing urea rather than soybean meal; however, differences were non-significant. As with dietary dry matter, a depression of intestinal ($P < .05$) and total digestion ($P < .01$) of OM was noted for steers fed the low protein U-75 diet. Kropp et al. (1975) previously reported a significantly lower OM digestion coefficient for isocaloric low quality roughage rations containing various levels of urea as compared to rations supplemented with SBM. Raleigh and Wallace (1963) fed a low quality hay supplemented with urea, cottonseed meal or urea and cottonseed to steers at dietary crude protein levels of 6, 9 and 12%. A significant improvement in OM digestion resulted from an increase in protein level from 6 to 12%, but changes between 6 to 9% and 9 to 12% were small. Increasing nitrogen intake did not change OM digestion in some studies (Hume et al., 1970; Hume, 1970b; Leibholz and Hartmann, 1972). OM apparently digested in the rumen was equivalent to 62% of the OM digested in the total tract. Similar results have been reported by Hogan and Weston (1967a) and Beever et al. (1972).

The dietary intake and site and extent of cellulose disappearance are presented in Table XII. Replacement of soybean meal with urea at any level depressed ($P < .05$) ruminal cellulose digestibility. No significant differences were noted among urea rations for ruminal digestion of cellulose. Campling et al. (1962) reported that the addition of urea to the rumen of cows fed oat straw diets improved both the rate and extent of ruminal digestion of the crude fiber portion of the straw dry

TABLE XI

ORGANIC MATTER DIGESTION

Item	Treatment S 100	U 75	U 100	U 115	SE
Intake (g)	4307	4326	4312	4292	--
Ruminal digestion (g)	1580	1541	1410	1488	47
Entering intestin (g)	2727	2785	2902	2804	47
Intestinal digestion (g)	1026[a]	693[b]	1006[a]	1030[a]	78
Total digestion (g)	2606[e]	2234[g]	2416[f]	2518[ef]	47
Disappearance of Apparently Digested Organic Matter					
Ruminal, % of intake	36.7	35.6	32.7	34.7	1.1
Intestinal, % of entering	37.6[c]	24.7[d]	34.5[c]	36.7[c]	2.4
Total, % of intake	60.5[e]	51.6[g]	56.0[f]	58.7[ef]	1.1
Ruminal, % of total	60.7	69.4	58.6	59.3	2.8
Intestinal, % of total	39.3	30.6	41.4	40.7	2.8

[a,b]Means on the same line with differing superscripts are significantly different ($P < .10$).

[c,d]Means on the same line with differing superscripts are significantly different ($P < .05$).

[e,f,g]Means on the same line with differing superscripts are significantly different ($P < .01$).

TABLE XII

CELLULOSE DIGESTION

Item	Treatment S 100	U 75	U 100	U 115	SE
Intake (g)	1541	1476	1463	1446	--
Ruminal digestion (g)	781^{c}	679^{d}	673^{d}	693^{d}	13
Entering intestine (g)	760	797	790	753	13
Intestinal digestion (g)	196^{c}	30^{e}	114^{d}	153^{cd}	18
Total digestion (g)	977^{c}	709^{e}	787de	846^{d}	28
Disappearance of Digested Cellulose					
Ruminal, % of intake	50.7^{a}	46.0^{b}	46.0^{b}	47.9^{b}	.8
Intestinal, % of entering	25.8^{c}	3.9^{e}	14.4^{d}	20.4^{cd}	2.6
Total, % of intake	63.4^{c}	48.1^{e}	53.8^{de}	58.5^{cd}	1.9
Ruminal, % of total	80.0^{d}	96.7^{c}	86.5^{d}	82.2^{d}	2.0
Intestinal, % of total	20.0^{c}	3.3^{d}	13.5^{c}	17.8^{c}	2.0

^{a,b}Means on the same line with different superscripts are significantly different ($P < .05$).

^{c,d,e}Means on the same line with different superscripts are significantly different ($P < .01$).

matter resulting in an increase in voluntary intake. The voluntary intake of the oat straw was stimulated by additions of urea above 25 g daily, but no difference was noted between 75 and 150 g urea daily. In the present study, 36, 68 and 113 g urea were fed daily in rations U-75, U-100 and U-115, respectively. The breakdown of cellulose in the ruminant is dependent on cellulolytic bacteria which obtain some or all of their nitrogen from ammonia. Possibly the hourly feeding system and restriction of intake prevented the expression of differences among the urea rations for ruminal cellulose digestion.

Of the cellulose entering the intestine, significantly less digestion occurred on the U-75 ration, resulting in the lowest ($P < .01$) total digestion of cellulose among rations. More ($P < .01$) cellulose was digested on the S-100 ration and total cellulose digestion increased as nitrogen level increased among urea rations. Raleigh and Wallace (1963) reported a significant effect of nitrogen level on total cellulose digestibility; however, no difference was noted between urea and cottonseed meal.

Ruminal digestion of cellulose accounted for 80 to 96.7% of total cellulose digestion. Similar results have been reported with sheep fed low quality roughages (Hogan and Weston, 1967a; Beever et al., 1972). Even though steers fed the U-75 ration digested a higher ($P < .01$) percentage of cellulose in the rumen than steers fed the other rations, the post-ruminal digestion of cellulose was minor.

Apparent nitrogen digestion and retention data are presented in Table XIII. Negative ruminal nitrogen digestibilities were noted for all rations, reflecting endogenous transfer of nitrogen into the rumen. The amount of nitrogen entering the intestine from the rumen was approx-

TABLE XIII

NITROGEN DIGESTION AND RETENTION

Item	Treatment S 100	U 75	U 100	U 115	SE
Intake (g)	80.7	61.7	78.0	91.8	---
Ruminal digestion (g)	- 24.2^{b}	- 24.9^{b}	- 23.4^{b}	- 4.2^{a}	3.6
Entering intestine (g)	104.9^{a}	86.6^{c}	101.4^{ab}	96.0^{b}	3.6
Intestinal digestion (g)	63.4^{a}	37.3^{c}	54.0^{b}	54.6^{ab}	3.8
Total digestion (g)	39.3^{e}	12.4^{g}	30.6^{f}	50.4^{d}	.8
Disappearance of Apparently Digested Nitrogen					
Ruminal, % of intake	- 30.0^{e}	- 40.3^{e}	- 30.0^{e}	- 4.6^{d}	4.4
Intestinal, % of entering	59.4^{d}	42.6^{e}	52.4^{d}	55.9^{d}	2.2
Total, % of intake	48.6^{d}	20.1^{f}	39.3^{e}	54.9^{d}	1.2
Ruminal, % of total	- 60.6^{ef}	-203.2^{d}	- 77.9^{e}	- 8.0^{f}	15.0
Intestinal, % of total	160.6^{ef}	303.2^{d}	177.9^{e}	108.0^{f}	15.0
Nitrogen retention (g)					
Intake	80.7	61.7	78.0	91.8	---
Fecal	41.5^{e}	49.3^{d}	47.4^{d}	41.4^{e}	.8
Urinary	20.6^{ef}	14.9^{f}	22.5^{e}	33.9^{d}	1.8
Nitrogen retained	18.6^{d}	- 2.5^{f}	8.2^{e}	16.5^{d}	2.1

^{a,b,c}Means on the same line with different superscripts are significantly different ($P < .05$).

^{d,e,f,g}Means on the same line with different superscripts are significantly different ($P < .01$).

imately 130, 140, 130 and 105% of the dietary intake for steers fed S-100, U-75, U-100 and U-115, respectively. Of the nitrogen entering the intestine, 43 to 60% was digested. Intestinal nitrogen digestion was depressed ($P < .01$) on the U-75 ration. As nitrogen level increased, the total apparent digestion of nitrogen also increased. Steers fed the S-100 ration also had a higher ($P < .01$) apparent nitrogen digestibility than steers fed the isonitrogenous urea ration. Nitrogen digestibility represents only a net feed-feces difference and generally increases when NPN is included in the rations if nitrogen is in surplus of needs. Raleigh and Wallace (1963) reported increased nitrogen digestion as the level of urea increased in the protein supplement. Kropp et al. (1975) noted significant decreases in total apparent nitrogen digestion as urea was substituted for SBM in the ration. The low ruminal pH and retention of ammonia nitrogen in the rumen due to the hourly feeding system probably reduced the absorption of ammonia from the rumen and increased its usefulness, thereby increasing total nitrogen passage and intestinal digestion. Total apparent nitrogen digestion coefficients were similar to those previously reported for steers fed low quality roughages (Raleigh and Wallace, 1963; Kropp et al., 1975). Decreased digestion of nitrogen by steers fed U-75 and U-100 resulted in greater ($P < .01$) fecal nitrogen losses. Urinary losses reflected ruminal ammonia levels and nitrogen intakes. Steers fed the U-75 ration were in negative nitrogen balance ($P < .01$). Retention of nitrogen increased with addition of urea above 8 g/kg of feed dry matter, but still was inferior to soybean meal supplementation.

Nitrogen fractionation of abomasal contents expressed as mg nitrogen/g dry matter are presented in Table XIV. Higher concentrations of

TABLE XIV

NITROGEN FRACTIONATION OF ABOMASAL DRY MATTER

Item	Treatment S 100	U 75	U 100	U 115	SE
			MgN/g DM		
Total nitrogen	34.66^{c}	27.93^{d}	31.30^{cd}	30.67^{cd}	1.40
NH_3-N	1.33^{c}	.85^{d}	1.17^{cd}	1.48^{c}	.10
NAN	33.33^{c}	27.08^{d}	30.13^{cd}	29.19^{cd}	1.33
Microbial N	18.99	17.31	16.86	17.89	1.01
Bypass N	14.34^{a}	9.77^{b}	13.27^{a}	11.30^{ab}	1.43

^{a,b}Means on the same line with differing superscripts are significantly different ($P < .05$).

^{c,d}Means on the same line with differing superscripts are significantly different ($P < .01$).

total nitrogen ($P < .01$) and non-ammonia nitrogen (NAN) ($P < .01$) were noted in the abomasal contents of steers fed S-100 as compared to U-75. Differences among rations for microbial nitrogen concentration were small and non-significant; therefore, differences in total nitrogen and NAN were largely a result of nitrogen intake and undegraded feed protein.

Daily abomasal passage of nitrogen and digesta are presented in Table XV. A net influx of nitrogen between ingestion and the abomasum was noted in all rations. Total abomasal nitrogen levels were 130, 140, 130 and 106% of dietary intake for S-100, U-75, U-100 and U-115, respectively. Hogan and Weston (1967b) reported a net gain of 2.6 g nitrogen or 47% of the dietary intake of lambs fed a 7% crude protein diet, but with a 17% CP diet, loss of 31% of their nitrogen intake from the rumen was observed. Similar trends were noted by Hume (1970b) and Hume et al. (1970). Allan and Miller (1972) investigated the effect of supplementing a 6% CP diet with 0, 0.8, 1.6 and 2.4% urea. Net influx of nitrogen as a percent of dietary intake was 71, 38, 25 and 17% for diets supplemented with 0. 0.8, 1.6 and 2.4% urea, respectively. Kropp et al. (1975) noted a loss of nitrogen from the rumen in steers fed 10% CP diets consisting of ground and pelleted bluestem grass and nitrogen supplements containing various levels of urea.

By explanation, little or no rumination and decreased rumen volume turnover with ground and pelleted diets have been reported (Pearce and Moir, 1964; Freer and Campling, 1965; Hogan and Weston, 1967a). The lower level of tactile stimulation would reduce rumination and salivation. Wilson and Tribe (1963) noted that saliva flow decreased with grinding of diets. Ruminants secrete large volumes of saliva during

TABLE XV

TOTAL DAILY PASSAGE OF NITROGEN

Item	Treatment S 100	U 75	U 100	U 115	SE
Nitrogen intake (g)	80.7	61.7	78.0	91.8	--
Nitrogen influx in rumen (g)	24.9^{c}	25.1^{c}	24.0^{c}	5.2^{d}	6.4
Total abomasal nitrogen (g)	105.6^{c}	86.8^{d}	102.0^{cd}	97.0^{cd}	6.4
Microbial N (g)	57.4	53.6	54.5	55.7	2.8
Protein-N Bypass (g)	43.7^{a}	30.4^{b}	43.4^{a}	35.8^{ab}	5.4
True protein reaching small intestine (g)	567^{c}	464^{d}	550^{cd}	509^{cd}	36.6
CP synthesis/100 g OM digested in rumen (g)	23.0	21.8	24.3	23.5	1.5
TP synthesis/100 g OM digested in rumen (g)	18.9	17.9	19.9	19.3	1.3
Digesta passage (liters)	44.2	46.1	48.5	47.4	2.0
Dilution rate (%/hr)	3.69	3.84	4.04	3.95	0.18
Rumen turnover time (days)	1.17	1.13	1.08	1.09	0.06

^{a,b}Means on the same line with differing superscripts are significantly different ($P < .10$).

^{c,d}Means on the same line with differing superscripts are significantly different ($P < .05$).

food ingestion and rumination. Bailey (1961) computed the total volume of saliva secreted per day by cattle to range from 98 to 190 liters. The nitrogen content of saliva is quite variable, but generally ranges from 0.1 to 0.2%, of which 60 to 80% is urea nitrogen. Therefore, it is not unreasonable to assume that 8 to 12 g of nitrogen may be recycled to the rumen in saliva of adult cattle with additional amounts passing directly through the rumen wall.

The concentration of ruminal ammonia is an important factor governing the uptake or loss of nitrogen from the rumen. Transfer of urea and ammonia through the rumen wall is a function of concentration gradient (Hogan, 1961). Vercoe (1969) suggested that 17 to 20 g of nitrogen may be transferred to the rumen daily in cattle. The net influx of 5.2 g nitrogen observed with the feeding of the U-115 ration may be totally accounted for by salivary secretion. Since a rather constant but moderately high level of 22.2 mg NH_3-N/100 ml rumen fluid was noted on the U-115 ration, influx of urea across the rumen wall may have been limited. However, a considerable net transfer of urea across the rumen wall may have resulted from the rather low ammonia level of 3.7 mg NH_3-N/100 ml rumen fluid on the U-75 ration. Probably a combination of salivary secretion and ruminal diffusion accounts for most of the influx of nitrogen observed on S-100 and U-100 rations. However, "net recycling" of NPN to the rumen is meaningless if nitrogen passing out of the rumen is not in a NPN form. Since only about 5% of the total abomasal nitrogen is NPN, the majority of the recycled nitrogen must have been utilized for microbial protein synthesis before it was termed as "recycled nitrogen." This "net recycling" may therefore simply reflect the rumen microbes' need for nitrogen.

No significant differences among rations were noted for microbial protein synthesis regardless of level or source of nitrogen. Significant differences between S-100 and U-75 for total nitrogen passage and true protein reaching the small intestine are a reflection of protein nitrogen bypass ($P < .10$). The low level of bypass nitrogen noted on the U-75 ration may suggest more extensive ruminal degradation of dietary protein. Since the bypass nitrogen on the urea rations largely represents undegraded feed proteins, an estimate of soybean meal nitrogen bypass may be derived. Approximately 36.5 g of nitrogen bypassed ruminal degradations on the urea rations, 7.2 g less than noted with soybean meal. This would suggest that about 20.2% of the soybean meal nitrogen escaped ruminal degradation. Kropp et al. (1975) previously reported a 25% ruminal bypass of soybean meal nitrogen.

In recent years, much attention has been directed to the study of ruminal protein synthesis. As energy is usually the limiting factor for microbial growth, protein synthesis has been expressed as a function of either the organic matter apparently digested in the rumen (OM disappearance between food and duodenum) or the organic matter truly digested (OM apparently digested plus OM incorporated into the microbes). Furthermore, some authors have reported true protein synthesis while others have reported crude protein synthesis. Smith (1969) suggested that 19 to 20% of the microbial nitrogen was in the form of microbial nucleic acids. About 40-50% of the microbial nucleic acid nitrogen produced in the rumen is not utilized by the ruminant animal. Therefore, calculation of true microbial protein by multiplying microbial nitrogen times 5.12 corrects for the limited values of microbial nucleic acid nitrogen to the host animal. No significant

differences were noted among rations for efficiency of protein production. Microbial crude protein synthesis per 100 g OM apparently digested in the rumen ranged from 21.8 to 24.3 g. These results are equivalent to 22-23 g commonly reported (Hogan and Weston, 1970; Thomas, 1973).

Digesta passage was estimated from lignin intake, abomasal lignin and abomasal dry matter. Dilution rate per hour and rumen turnover time were estimated assuming a constant rumen volume of 50 liters. No significant differences were noted among rations for digesta passage, dilution rate or rumen turnover time. Values were similar to those obtained using a 21% roughage ration (Cole et al., 1975). However, turnover rate was approximately two times faster than that observed with low quality bluestem (Kropp et al., 1975). Efficiency of microbial protein production, at about 22 g/100 g apparently digested OM, was also double that observed with the low quality bluestem.

In the present study, the linear regression of microbial protein synthesis on dilution rate yielded an equation:

$$\text{MPS/DDM} = 11.59 + 2.35\ \text{DR/Hr} \quad (r = .77)$$

in which MPS/DDM is g microbial protein synthesis per 100 g DM digested in the rumen and DR/Hr is the dilution rate in percent per hour (Figure 2). A similar plot of data presented by Kropp et al. (1975) yielded a linear regression equation of MPS/DDM = - 1.89 + 6.01 DR/Hr. Since the data were from two different trials conducted in different manners and no overlapping of data points were noted, the data were not pooled. Cole et al. (1975) reported a linear regression equation of MPS/DDM = - 0.56 + 2.414 (DR/Hr). Isaacson et al. (1975), using a continuous fer-

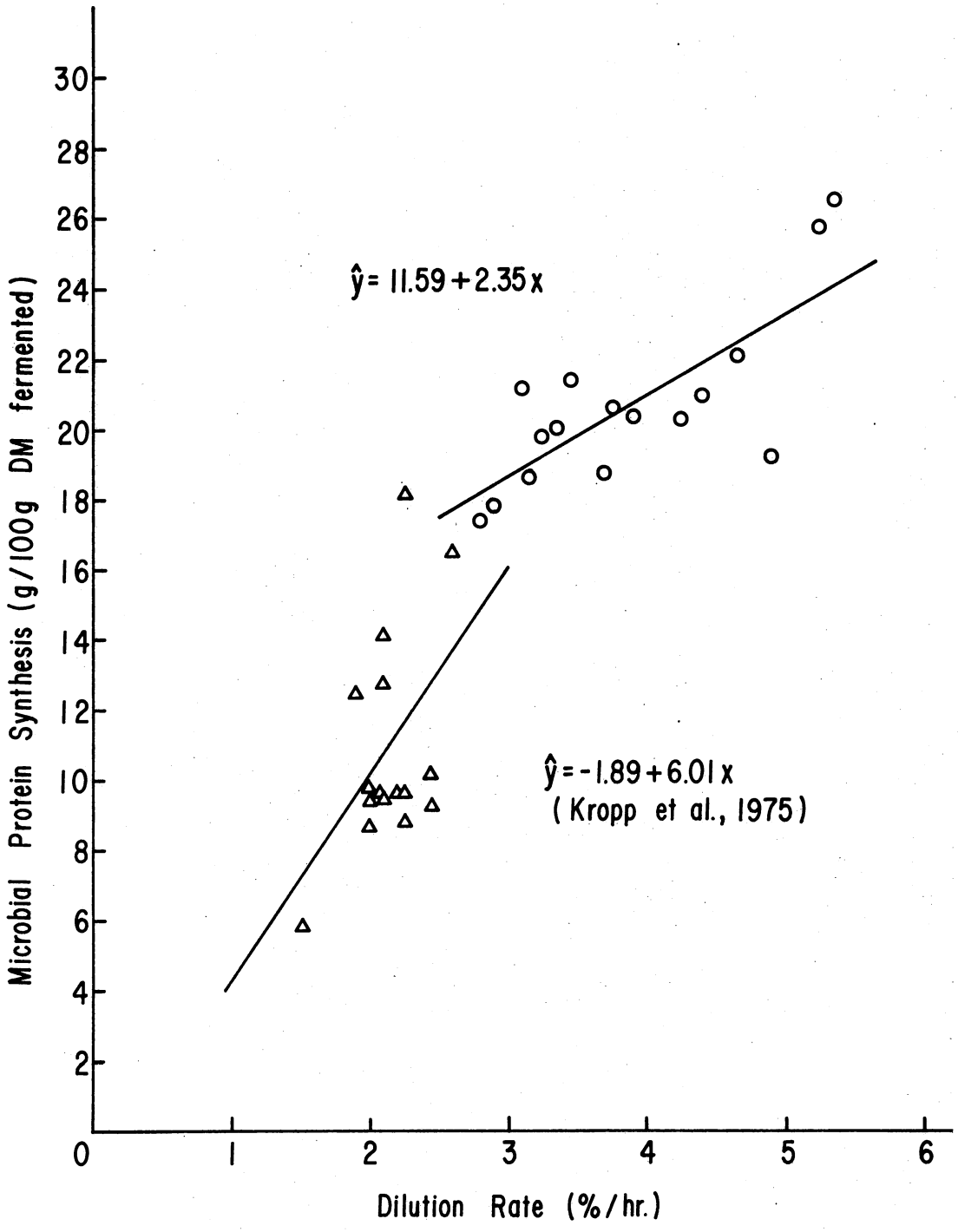

Figure 2. Relationship Between Microbial Protein Synthesis and Rumen Dilution Rate

mentation system and dilution rates of 2 to 12% per hour, noted a Michealis - Menten type saturation curve when microbial yield per mole of glucose was plotted against dilution rate per hour. Values approaching V_{max} were obtained only with dilution rates above approximately 6% per hour. Therefore at low dilution rates, ruminal microbial protein synthesis is highly dependent upon the rumen dilution rate. As the microbes grow faster, i.e., faster dilution rate, more of the energy from glucose fermentation would appear to be used for net bacterial growth. Isaacson et al. (1975) suggested that available energy could be divided into two fractions, one for maintenance, which varies as a function of time and population, and the second fraction, available for net growth. At low dilution rates, approximately 55% of the energy derived from glucose was used for maintenance, while at the high dilution rate, only 15% of the energy was used for maintenance.

In the present study, microbial protein production per 100 gm DM fermented in the rumen appeared to plateau at higher dilution rates. Apparently more of the energy derived from carbohydrate fermentation was available for net growth, while the microbial maintenance requirement utilized most of the available energy in the previous trial (Kropp et al., 1975).

The ability of the ruminant animal to utilize its food depends largely on the activity of the rumen microbial population and this activity depends on the supply of nutrients to support growth of the population. Nitrogen supplements added to a poor quality roughage diet, on which the maximum voluntary intake is insufficient to maintain the animal in energy balance, stimulates the rate of microbial digestion, thereby permitting greater feed intake and reduced loss of body weight

(Campling et al., 1962; Coombe and Tribe, 1962; Faichney, 1968b; Ammerman et al., 1972). Therefore, the influence of nitrogen supplementation on voluntary feed intake appears to be related to the rate of ruminal digestion. Steers fed the U-75 ration should have been in a sub-maintenance nitrogen condition. Thus, differences in total digestion should have been mediated by ruminal digestion. Lower apparent digestibilities of DM, OM, cellulose and nitrogen were noted in steers fed the U-75 ration as compared to steers fed the other diets. However, most of the effect of reduced digestion appeared to be post-ruminal. Owens et al. (1973) reported improved post-ruminal digestion of dry matter in lambs which received intraruminal urea infusions as compared to lambs fed a 1.39% nitrogen basal diet. Since total digestion was partitioned by lignin ratio technique into ruminal and post-ruminal digestion, marker problems could possibly account for partitioning differences. But due to the Latin square design and number of samples per estimate, marker problems seem improbable. Possibly the hourly feeding regime prevented the expression of ruminal digestive differences among rations. Under ad libitum feeding conditions, nitrogen supply has a definite bearing on ruminal digestion. However, explanations for the post ruminal influence noted in the present study are not readily apparent.

CHAPTER V

SUMMARY

Low quality roughage rations consumed by the majority of beef cows during the wintering period are primarily deficient in protein and minerals. Therefore, the provision of protein supplements is mandatory for the health and well-being of both the cow and her calf. Most of the protein supplements recommended to satisfy the deficiencies have consisted of natural proteins with such minerals as might be necessary for the specific geographic location. Previous research at Oklahoma State University has demonstrated rather poor results when high levels of non-protein nitrogen, especially urea, have been included in range protein supplements. New management systems are presently being tested to reduce the labor, effort and cost of providing the supplemental needs for animals grazing rangeland. Some research has suggested that the sustained release of ammonia from non-protein nitrogen compounds might improve nitrogen utilization from these compounds.

This study was conducted to evaluate the effect of source and level of nitrogen supplied by urea and/or soybean meal on microbial protein synthesis and site and extent of digestion of dry matter, organic matter and cellulose. The steers were fed hourly to eliminate the diurnal variation in digesta passing the abomasum as well as to impose a slow release of ammonia from the urea supplied in the diet.

Microbial protein production was relatively constant within experi-

ment regardless of level or source of nitrogen. However, protein synthesis was two times higher in experiment 2, apparently a function of faster rumen turnover rate. Efficiency of production appeared to plateau at dilution rates approaching 6% per hour. A similar observation was noted by Isaacson et al. (1975). More total nitrogen reached the small intestine in steers fed soybean meal rather than urea. Ruminal bypass of soybean meal was calcuated to be 20-25%.

Nitrogen retention data from experiment 1 suggested poor utilization of urea. Replacement of soybean meal nitrogen by urea depressed apparent digestion of dry matter, organic matter and cellulose ($P < .01$). In experiment 2, soybean meal was superior to any level of urea for total apparent dry matter, organic matter and cellulose digestion. Only the sub-maintenance nitrogen ration (U-75) produced negative nitrogen balance.

The satisfactory conversion of ammonia nitrogen to microbial nitrogen depends upon an ample supply of carbon skeletons for conversion to amino acids. Since cellulose is hydrolyzed too slowly to keep pace with the rapid release of ammonia following urea hydrolysis, frequent ingestion of both the nitrogen and carbohydrate sources should improve urea utilization. Apparently the frequent ingestion of the rations during this study resulted in a continuous supply of substrate and production of ammonia resulting in a more suitable environment for microbial protein synthesis. The simultaneous intake of energy and nitrogen may have improved urea utilization for protein synthesis through reduction in rumen pH, ruminal retention of ammonia or enhanced bacterial growth.

Results of this study demonstrates that ruminal protein synthesis was limited by available energy and rumen turnover rate and not by

nitrogen availability. Even though the U-75 ration in experiment 2 produced rather low rumen ammonia levels, microbial protein synthesis was not depressed. Soybean meal appeared superior to urea as a nitrogen source in conjunction with low quality roughage rations. However, results suggest that urea, fed at adequate levels, can be utilized on low quality roughage rations for the synthesis of microbial protein if frequent ingestion can be facilitated.

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APPENDIXES

TABLE XVI

RNA ANALYSIS FOR DETERMINATION OF MICROBIAL PROTEIN IN RUMEN FLUID OR ABOMASAL CONTENTS

Reference: McAllan, A. B. and R. H. Smith. 1969. Nucleic acid metabolism in the ruminant. Determination of nucleic acids in digesta. Brit. J. Nutr. 23:671.

Reagents: All aqueous reagents use deionized water

1. Refrigerator - 0 to 4°C

   a. 18% (w/v) TCA in ethanol
   b. 9% (w/v) aqueous TCA
   c. Ethanol saturated with sodium acetate
   d. 1 volume chloroform in 2 volumes ethanol
   e. 1 volume ethyl ether in 3 volumes ethanol
   f. 4N Perchloric Acid (287 ml of 70% $HClO_4$/500 ml glass distilled H_2O)
   g. 1N KOH (65.2 g/liter)
   h. 7N KOH (456.4 g/liter)

2. Room Temperature

   a. 1N KOH
   b. 0.025 m-tris buffer (2-Amino-2-[hydroxyl-methyl]-1,3-propanediol) at pH 7.8 (3.035 g/liter). Adjust pH with 3N HCl (251 ml conc. HCl/liter)
   c. 0.5 N HCl (41.8 ml conc. HCl/liter)

3. RNA Standard

   a. Digest 7.5 g RNA (Sigma Type VI torula yeast) with 150 ml 1N KOH for 18 hrs.
   b. Centrifuge at 35,000 xg for 15 min. Save supernatant and wash residue with 10 ml 1N KOH. Centrifuge again at 35,000 xg for 15 min. Combine supernatant.
   c. Cool and acidify with 64 ml cold 4N perchloric acid. Centrifuge at 35,000 xg for 15 min. Make supernatant fraction up to 250 ml with 4N perchloric acid. Store below 4°C and it is stable for 2 months.

4. Procedure

   a. Homogenize rumen samples (strained through double layer of cheesecloth) or abomasal samples.
   b. Weight out 25 g wet portion of homogenized sample.
   c. Following extractions should be carried out at 0 to 4°C. Centrifuge tubes should be in ice when not in the

TABLE XVI (Continued)

centrifuge.

1. Add to 25 g homogenized sample, 25 ml of 18% TCA in ethanol. Rehomogenize. Centrifuge two 25 ml portions at 35,000 xg for 15 min. Save residue, discard supernatant.

2. Wash residue successively with 25 ml portions of:

   a. 9% aqueous TCA
   b. 9% aqueous TCA
   c. Ethanol saturated with sodium acetate acetate
   d. Chloroform in ethanol
   e. Chloroform in ethanol
   f. Ether in ethanol
   g. Ether (room temperature)

   Separations all by centrifuging at 35,000 xg for 15 min. After solvent is added to residue, shake until pellet is loose. Save residue.

3. Residue from ether washing may be set out over night for evaporation. Dry under 17 lb. vacuum at 50°C for two hours. Then weighed and pulverized with mortar and pestle. This ground sample can be stored at 4°C.

d. Shake 100 mg of pulverized sample with 10 ml of 1N-KOH for 18 hr. at 37°. Centrifuge at 35,000 xg for 15 min.

   1. Save supernatant
   2. Wash residue with 1 ml 1N KOH
   3. Centrifuge at 35,000 xg for 15 min.
   4. Combine supernatants. Discard residue.

e. Reagents and operations at 0 to 4°C (ice).

   1. Add to combined supernatant, 4.4 ml of 4N perchloric acid.
   2. Leave for 30 min. in ice with occasional shaking.
   3. Centrifuge at 35,000 xg for 15 min. Save supernatant in 50 ml beaker.
   4. Neutralize supernatant with 7N KOH to pH 2. Use 1N KOH to bring to pH 7 on pH meter. If pH exceeds 7, use perchloric acid to bring pH down, then reneutralize.

f. Filter neutralized supernatant through Whatman No. 42 filter paper. Wash once with 10 ml of deionized H_2O. Wash out beaker. Get all residue. Make filtrate up to 50 ml

TABLE XVI (Continued)

with 0.025 M-tris buffer.

g. Neutralize 5 ml of RNA standard with KOH as above. Filter through Whatman No. 42 filter paper and wash with 10 ml of deionized H_2O. Dilute filtrate to 50 ml with 0.025 m-tris buffer.

5. Column Preparation

a. Weight out 3 g for small column or 4 g for large column of Bio Rad Ag 1 x 10 (50-100 mesh) Cl^- resin. Wash at least 3 times with deionized H_2O.
b. Place small piece of glass wool in bottom of column. Pour column in slurry and let resin settle on its own.
c. Wash column with 10 ml of 0.5 N HCl and then 30 ml of 0.025 n-tris buffer. Add tris buffer down the side and slowly.
d. Column should now be equilibrated and ready for use. Do not let column go dry.

6. Column Elutions

a. Use 0.5, 1 and 2 ml of the RNA standard filtrate solution as standards. Dilute each to 30 ml with 0.025 n tris buffer and place on column.
b. Place 50 ml of sample solution on resin column.
c. Wash all columns with 30 ml of 0.025 m tris buffer. Discard elutions.
d. Elute sample with 100 ml of 0.5N HCl.
e. Read HCl elutions on U.V. absorption at 260 nm.
f. Use 0.5 N HCl placed through steps 4d, 4e, 4f, 4g, 6b, 6c and 6d as the blank. Set slit width so that absorbance reads zero.
g. Read all samples at the same time.

Calculations:

1. 0.5, 1 and 2 ml of RNA standard filtrate solution are equivalent to 1.5, 3.0 and 6.0 mg of RNA, respectively.

2. $\frac{1.5 + 3.0 + 6.0}{\text{Reading Reading Reading}} = \text{mg RNA/unit absorption}$

3. Mg RNA/unit absorption x sample reading = mg RNA/sample.

TABLE XVI (Continued)

4. $\frac{\text{Mg RNA/Sample}}{\text{sample wgt}}$ = mg RNA/g sample

5. Mg RNA/g sample x pellet wgt = mg RNA/pellet

6. $\frac{\text{mg RNA/pellet}}{\text{Actual g DM of 25 g wet sample}}$ = mg RNA/g DM

7. mg RNA/g DM x .132 = mg RNA-N/g DM

8. $\frac{\text{mg RNA-N/g DM}}{.10}$ = mg Microbial N/g DM

TABLE XVII

DETERMINATION OF DIGESTA AMMONIA-N

Reagents:

1. Magnesium Oxide (MgO)
2. Pumice
3. 25% (w/v) $CaCl_2$ solution
4. Kjeldahl boric acid solution
5. 1/14 N HCl

Procedures:

1. To macro kjeldahl flask add 3 to 4 g MgO, approximately 1 g of pumice, 1 ml $CaCl_2$ solution and 0.5 ml octanol.
2. Add 10 ml of homogenized abomasal sample or strained rumen fluid and 250 ml distilled H_2O.
3. Distill approximately 200 ml into 50 ml of boric acid solution.
4. Tetrate with 1/14 N HCl to neutrality.

Calculations:

1. $\frac{\text{(ml of HCl - ml blank)}}{\text{10 ml sample}} = \text{mg } NH_3\text{-N/ml}$
2. mg NH_3-N/ml x 100 = mg NH_3-N/100 ml.

TABLE XVIII

DETERMINATION OF DIGEST UREA-N

Reagents:

1. Phosphate buffer - pH 7.0
   a. 4.36 g KH_2PO_4 + 10.54 g Na_2HPO_4
   b. Dilute to 1 liter with distilled H_2O
2. Jackbean Urease (Type III. 3490 units per gram; Sigma Chemical Co., St. Louis) Solution. 1 mg/ml phosphate buffer.
3. Abomasal ammonia-N determination reagents.

Procedures:

1. Incubate 10 ml of digesta sample with 10 ml of urease solution in a pH 7 phosphate buffer solution for one hour.
2. Run MgO distillation for ammonia-N determination.
3. Urea nitrogen is taken as the increase over ammonia-N.

VITA

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