NITROGEN UTILIZATION BY STEERS FED HIGH CONCENTRATE OR LOW QUALITY ROUGHAGE RATIONS

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CHAPTER I

INTRODUCTION

Since the dawn of civilization, ruminants have played a decisive role in human welfare by providing food from plant materials and by-products. Ruminants, unlike monogastric animals, have as part of their digestive system a microbial fermentation stage in which cellulose and hemicellulose are digested to nutrients useable by the animal to form meat and milk.

The level of production of meat, milk, eggs or wool is a function of the animal's intake and digestibility of feed components. Microbial activity performs an important role in digestion. The concentration of microbes in ruminal digesta is a function of the ruminal environment (pH, quantity of microbial nutrients, etc.). When the rumen has reached a steady state condition on a given diet, the generation time of microbes is equal to the dilution rate or flow of material through the rumen. Thus, an increase in microbial output can arise from an increase in either: 1) microbial concentration in ruminal digesta, or 2) rate of flow of digesta from the rumen or both factors.

Protein enters the rumen from the diet, via saliva and diffusion through the rumen wall. Rumen fluid is always provided with ammonia from these sources. Ammonia is a substrate for bacterial growth in the rumen. The concentration of ammonia nitrogen in rumen fluid necessary to maximize bacterial growth remains uncertain. Ammonia may become

limiting only when below 2 mg/dl (Slyter et al., 1979) or whenever below 26 mg/dl (Allen and Miller, 1976). This point of limitation might change with ration type (all concentrate vs. all roughage). The amount of protein in the ration needed to attain an ammonia concentration may change as well as passage rate and quantity of ammonia recycled. There are academic and practical reasons to determine the ammonia requirement since ammonia level could serve as an indicator of response which might be expected with the addition of non-protein nitrogen (NPN) to the ration.

When NPN replaces intact protein in a ration and provides the nitrogen for microbial growth, other appropriate sources of energy and carbon must be supplied. These factors may be derived from carbohydrates, including cellulose, starches and sugars. Experimental data indicate that the amount of microbial protein produced per unit of organic matter digested in the rumen is variable. Estimates range from 8 to 20 g microbial protein synthesized per 100 g of organic matter fermented in the rumen. Typically, energy available to microbes for growth limits microbial protein synthesis in the rumen.

Ammonia limitation can also limit microbial growth. Although the optimal concentration of ruminal ammonia for maximal microbial growth and nutrient digestion remains in doubt, it has generally been concluded that coordinated release of the ammonia and energy would enhance nutrient utilization although no animal performance data are available to support this concept. The objectives of the present study were 1) to evaluate simulated slow release of ammonia with steers to test the theory that slow release is beneficial, 2) to observe the effects of added energy and nitrogen on intake of a low-quality, low-protein range

grass, and 3) to determine the influence of ruminal ammonia concentration on ruminal digestion and microbial synthesis and yield.

CHAPTER II

REVIEW OF LITERATURE

Introduction

Urea and other sources of non-protein nitrogen (NPN) materials were first used as protein substitutes in ruminant rations in 1891 when Zuntz showed that rumen microorganisms could utilize NPN to synthesize bacterial protein. But research in the early 1900's cast doubt on such utilization of NPN in ruminant rations. During World War II, due to critical shortages of vegetable protein, feeding of non-protein nitrogen to ruminants was necessary and became more prominent. Reid (1953) reviewed numerous studies which proved that urea nitrogen fed to ruminants was indeed retained by the body and often increased the digestibility of cellulose and crude fiber in low protein diets.

Urea utilization occurs as follows: Upon entering the rumen, urea is hydrolyzed by microbial urease to ammonia and carbon dioxide. Ruminal bacteria continually combine the released ammonia with α-keto acids derived from carbohydrates to form amino acids and subsequently protein. Ruminal bacteria are flushed to the intestines, digested and absorbed. The efficient utilization of urea is dependent upon the ability of the ruminal microbes to convert ammonia and energy sources to protein. Inefficiencies have been attributed to: 1) excessively rapid release of ammonia from urea by microbial urease, and

2) insufficient energy available for the bacteria to grow and use the liberated ammonia. Consequently, research has sought to attenuate ammonia release.

The extent to which urea or other NPN sources can replace natural protein in ruminant rations has received concentrated attention by researchers for the past 30 years (Reid, 1953; Briggs, 1967; Chalupa, 1968; Loosli and McDonald, 1968; Oltjen, 1969; Helmer and Bartly, 1971; FAO, 1975; NRC, 1976). The reader is referred to those reviews for general information. Specific areas of interest depicted in the first part of this review relate to: 1) attenuated release of ammonia nitrogen, 2) interaction of nitrogen utilization and carbohydrate availability, and 3) urea utilization with low quality forage.

A second area of concern relates to the protein level of the diet above which NPN supplementation ceases to improve microbial growth and animal performance. If the benefit from NPN addition to the diet is derived strictly from the incorporation of ammonia into the ruminal microbes, then the concentration below which ammonia nitrogen restricts the rumen microbial population is of grave concern. Studies indicate that the ammonia concentration necessary for maximal microbial growth and digestion may fall between 0.005 and 76 mg/dl of rumen contents (Bryant and Robinson, 1961; Allison, 1970; Hume et al., 1970; Miller, 1973; Satter and Slyter, 1974; Roffler and Satter, 1975; Allen and Miller, 1976; Merhrez et al., 1977; Slyter et al., 1979; Hespell, 1979). In part two of this review, research concerning the optimal ruminal ammonia concentration for maximal microbial growth and ruminal digestion is examined.

Nitrogen Metabolism

Rate of Urea Hydrolysis

Pearson and Smith (1943) demonstrated by in vitro techniques that ruminal fluid has high urease activity at all times. Because urease activity of ration ingredients was low, they concluded that rumen microorganism posses the ability to degrade urea. Hungate (1966) stated that a high concentration of urease is not necessary because of its extreme activity. Wegner et al. (1941) reported that when a 1 to 5% urea ration is ingested, urea disappeared from the rumen within 4 to 6 hours. Pearson and Smith (1943) found that 100 g of rumen contents could convert 100 mg of urea to ammonia in one hour. Increasing the concentration of the substrate from 30 to 414 mg urea N per 100 mg rumen fluid did not alter the rate of hydrolysis. Hydrolysis increased with temperature to 49 C while the optimal pH for hydrolysis was 7 to 9 with little activity below 3 or above 9.5. A similar study by Bloomfield et al. (1960) showed that urea was hydrolyzed at a rate of 80 mg urea N per hour per 100 ml of rumen. Pokop et al. (1971) observed that between 75 and 125 g of urea was hydrolyzed per minute in a steer and that the optimum pH for hydrolysis was between 8.17 and 8.35. Activity increased by 35% as temperature rose from 25 to 35 C.

The bacteria responsible for secreting urease have not been isolated although there is some evidence that Selenomonas ruminantium (John et al., 1974) and Streptococcus faecium (Cook, 1976) produce urease. Bacterial urease is an intracellular enzyme similar biochemically to jackbean urease (Jones, 1967; Brent et al., 1971; Mahadevan et al., 1976) and immunological cross-reactions occur. Sulfhydryl groups

are involved in the activity of both systems and both systems exhibit a high degree of substrate specificity. Electrophoretic studies suggest that there is only one type of urease present in mixed rumen populations of bacteria. The molecular weight of bacterial urease appears to be smaller than the active fractions of jackbean urease.

Ammonia Absorption

One major problem in efficient utilization of urea is the rapid release of ammonia. Bloomfield et al. (1960) indicated that urea hydrolysis occurred four times faster than bacterial uptake of the released ammonia. This may lead to absorption from the rumen and excretion of N which otherwise could be used by ruminal bacteria for protein synthesis. McDonald (1948) estimated that the amount of ammonia N absorbed from the rumen of sheep was between 4 and 5 g per day. However, Lewis et al. (1957) found that the rate of absorption from the rumen of sheep could be as high as 14 g of ammonia N per day. Hogan (1961) showed that rate of ammonia absorption depended upon the concentration gradient at pH 6.5 and that absorption stopped at pH 4.5. Smith (1975) stated that under normal conditions, rate of ammonia absorption from the rumen may not be particularly rapid and substantial amounts of ammonia remain available for microbial use for several hours. Rationale for this hypothesis are based on the concepts that: 1) at a rumen pH below 6.5, most of the ammonia exists as the ammonium ion for which the rumen mucosa has low permeability (Bloomfield et al., 1960); 2) peak blood ammonia concentrations occur several hours after corresponding peaks in rumen fluid (Hillis et al., 1971); and 3) studies with ^{15}N-labeled compounds suggest that much of the ammonia produced in

rumen of sheep fed alfalfa left with the digesta (Nolan and Leng, 1971).

Urea Recycling

Houpt (1959) observed that 48% of the urea-N injected intravenously into mature sheep receiving a low protein, carbohydrate supplemented ration was not recovered in the urine and did not remain as urea in body fluids. Therefore, it was presumed to be recycled to the rumen and used by the microorganisms for protein synthesis. Bailey (1961) computed, based upon the volume of saliva secreted per day of 98 to 190 liters for a cow and a nitrogen content of saliva of 0.1 to 0.2% of which 60 to 80% is urea nitrogen, that between 8 and 12 g of nitrogen may be recycled to the rumen via saliva. Packett and Groves (1965) reported that blood concentrations in the ruminal vein were high enough to directly transfer blood urea into the rumen. Vercoe (1969) suggested that between 17 and 20 g of nitrogen daily may be transferred directly into the rumen of cattle. Consequently, the combination of salivary secretion and ruminal diffusion permits a net influx of nitrogen between 25 and 32 g of nitrogen per day.

Urea Utilization by Rumen Microorganisms

Rumen microorganisms utilize ammonia-nitrogen for the synthesis of microbial amino acids and protein (Blackburn, 1965; Hungate, 1966). Even when the diet supplies nitrogen mainly in the form of protein, ruminal microbes derive 60 to 80% of their nitrogen from the rumen ammonia pool and only small percentages of carbon labelled amino acids are incorporated directly (Pilgrim et al., 1970; Mathison and Milligan, 1971; Nolan and Leng, 1972). Ammonia is an essential nutrient for the

growth of some strains of rumen bacteria and may stimulate growth of other strains.

Arthur et al. (1967) studied in vitro ammonia uptake by rumen microorganisms under various conditions. The pH value, if within the physiological range, had little effect on N uptake. Three different rates of uptake were reported during a nine hour incubation period. The rates were 1.15 mg N per dl per hour for the first three hours, 2.0 mg N per dl per hour for the second three hours, and 4.95 mg N per dl per hour for the third three hour period. Bloomfield et al. (1960) reported that ammonia released from urea by rumen microorganisms was utilized by the microorganisms at a rate of 20 mg ammonia-nitrogen per hour per 100 ml rumen fluid. The mechanisms for fixing of ammonia by rumen microorganisms utilize NAD and NADP-linked glutamic dehydrogenase, glutamine synthetase and carbamyl phophate synthetase. The concentration of ammonia needed for maximal microbial synthesis and ruminal digestion rate are discussed in Chapter V.

Interrelationships Between Ammonia Nitrogen Utilization and Carbohydrate Availability

Bacterial Energy Use

The feedstuffs consumed by ruminants are attacked by anaerobic microbes in the reticulo-rumen. Dietary polysaccharides and protein are degraded into VFA's and the microbes derive energy (ATP) for concomitant production of microbial cells.

The amount of microbial growth generally depends upon the amount of ATP generated. Bauchop and Elsden (1960) developed Y_{ATP} concept.

Y_{ATP} represents the grams of microorganisms produced per mole of ATP generated. This is one way to express efficiency of microbial growth and averaged 10 in many early studies. Based on a Y_{ATP} of 10, Hungate (1966) calculated that about 10 g of microbial protein would be produced from each 100 g carbohydrate fermented in the rumen. Later studies, using in vitro isotope incorporation (Bucholtz and Bergen, 1973), energy balance calculations from ruminal fermentations (Baldwin, 1970), and ingesta passage studies with sheep using NPN-containing diets or microbial cell markers (Hogan and Weston, 1971; Hume et al., 1970; Lindsay and Hogan, 1972) demonstrated that microbial protein synthesis ranged from 15 to 22 g per 100 g organic matter digested.

Strouthamer and Bettenhaussen (1973) proposed that Y_{ATP} was not constant. Instead, they suggested that efficiency of microbial growth depended upon the amount of energy remaining for growth after deducting that energy needed for maintenance. At a high growth rate or ruminal dilution rate, the ATP needs for maintenance are quite low, and much of the ATP is available for cell synthesis, while at low dilution rates much of the available ATP would be used for maintenance of microbial cells. The maximum Y_{ATP} possible (Y_{ATP}^{MAX}) was estimated to be about 25. Issacson et al. (1975) grew mixed ruminal cultures at various dilution rates in a continuous culture system and calculated a Y_{ATP}^{MAX} of 19.5. The Y_{ATP}^{MAX} was 7 to 8 at a 2% per hour dilution rate and 16 to 17 at a dilution rate of 12% per hour. They concluded that Y_{ATP} values depend upon the amount of energy used for maintenance which in turn is an inverse function of dilution rate.

For optimal rates and efficiencies of bacterial cell synthesis, other components are needed in addition to energy. These include NH_3-N,

carbon skeletons, sulfur, possibly free animo acids (Maeng et al., 1976) and other ill defined cofactors.

The processes for VFA production from carbohydrates and cellular constituents have been believed to be coupled so that production dropped with cell yield. Some limited experimental data (Satter and Slyter, 1974; Slyter et al., 1979) have led other workers (Hespell, 1979) to suggest that large amounts of energy may be released through uncoupling of the energy transfer chain. This would permit VFA production without concurrent bacterial growth. Conditions for and importance of uncoupling in vivo remain to be determined.

Nitrogen Utilization and Carbohydrate Availability

Numerous forms of carbohydrates exist in feedstuffs. They can be classified into three major classes based upon their relative rates of digestion in the rumen (Johnson, 1976). These are: 1) cell wall carbohydrates such as cellulose and hemicellulose, 2) readily fermented forms of glucose polymers such as starch and dextrins which are generally intracellular, and 3) various free simple sugars such as those present in molasses. These classification aid discussion of rate of energy release. The fermentation curves for these three classes of carbohydrates differ in applitude and time span. Cellulose is digested quite slowly, sugars are digested at a very rapid rate, and starches are intermediate. Many workers have suggested that starch is an ideal energy source to improve NPN utilization. Examination of the fermentative curves for starch relative to the ammonia release curve from urea suggest that starch may not be ideal but that some simple sugars may

be useful. Johnson (1976) concluded that the energy available from fibrous carbohydrates and simple sugars cannot be overlooked when considering feedstuff supplementation with NPN. He suggested that improved NPN utilization could be achieved by coordinating the release of ammonia and energy. This could be accomplished by: 1) altering the release rate of ammonia, or 2) supplementing the ration with energy sources which release energy at a rapid rate.

The "urea fermentation potential" concept (Burroughs et al., 1972) relates the amount of energy from a feed with the amount of ammonia available from that feed. Calculated deficiencies of ammonia are used to predict qualitative and quantitative urea usefulness. An alternative equation was developed by Roffler and Satter (1975) based upon ruminal ammonia concentrations with rations of various protein and TDN content. Both systems are based on the premise that nitrogen metabolism in the ruminant cannot be considered separately from carbohydrate digestion. Both systems also consider conditions to be "steady state" without the fluctuations in energy and ammonia supply inherent with meal feeding.

Synchronous Availability of Energy and Nitrogen

Simultaneous availability of energy and nitrogen may be one key to increase utilization of NPN compounds. To coordinate nitrogen and energy release, two options are possible: 1) create ruminal steady-state conditions by increasing the frequency at which feed is fed, or 2) with meal feeding, alter the ammonia or energy release rate.

Feeding frequency of urea has been studied in hopes of increasing nitrogen utilization and decreasing toxicity. Oltjen et al. (1973) suggested that daily feeding would improve urea utilization and animal performance. Campbell et al. (1963) fed dairy heifers a ration containing 50% corn, 30% urea, 10% molasses and 10% alfalfa twice or six times per day. More frequent feeding improved growth rate and feed efficiency. Bloomfield et al. (1961) measured nitrogen balance of whethers fed two times or sixteen times daily and noted increased nitrogen balance with the more frequent feeding regime. Deif et al. (1970) reported that nitrogen balance of lambs increased when a urea ration was fed in three equal portions than in one meal a day. In contrast to these benefits from frequent feeding, Dining et al. (1949) fed urea supplements to steers on alternate days, daily or twice daily. Feeding frequency had no effect on urea utilization by steers. Rush and Totusek (1976) also reported no benefit from more frequent feeding of urea to beef cattle.

Mizwicki et al. (1976) fed sheep an 80% concentrate 1% urea ration (12% protein) or this basal ration with 2% urea added. Sheep had ad libitum access to feed either 24 hours per day or for one hour each day. Urea addition to the ration fed ad libitum for 24 hours enhanced intake by 33% and increased gains by 14% as compared to the basal ration. But when the ration was fed once daily, the high level of urea depressed intake (46%) and decreased gain (30%). This suggests that reduced feed intake may explain some of the poor results with infrequent feeding of rations containing urea. Prior et al. (1976) conducted three experiments with frequency of feeding. In experiment 1, sheep were fed a purified ration which contained urea as the only nitrogen

source either twice or twelve times daily. Urinary citrate was decreased with more frequent feeding of urea which suggested that less ammonia was being detoxified by the liver (Prior and Visek, 1975a,b). Decreased citrate could indicate enhanced utilization of urea nitrogen. In experiment 2, sheep were fed either 2 or 12 times daily a natural ration containing 42% of the dietary N as urea. Urinary citrate excretion was not affected by more frequent feeding but intake, gain, and nitrogen retention were increased. Overall, the results were inconclusive and the authors suggested that further work was needed with higher fiber diets.

One explanation for decreased performance with urea is reduced bacterial protein synthesis in the rumen. To examine this, Kropp et al. (1977) studied the effects of urea (replacement of 0, 25, 50, and 75% of the supplemental soybean meal nitrogen) on microbial protein synthesis in the rumen of steers fed low quality roughage twelve times daily. They observed that microbial nitrogen production was relatively constant among rations regardless of the source of nitrogen. They concluded that non-protein nitrogen is satisfactorily utilized with low quality roughage rations for the synthesis of microbial protein when frequent feeding is practical. But they did not examine infrequent feeding of urea.

Feeding frequency studies often have been interpreted with respect to ammonia release alone, despite the confounding influence of simultaneous energy intake when the complete ration is meal-fed. Frequent feeding of the carbohydrate may improve urea utilization through maintenance of a higher ruminal pH, ruminal retention of ammonia, or enhanced bacterial growth.

When urea is provided at different intervals independent of the rest of the ration, benefits of frequent urea feeding have not been readily apparent. Knight and Owens (1973) infused equivalent amounts of urea directly into the rumen of sheep for periods of 1, 3, or 12 hours post feeding. Sustained infusion was intended to simulate an attenuated release rate of ammonia. Sustained infusion proved detrimental when high energy diets were fed twice daily. With higher fiber diets, infusion of the urea over a three hour period slightly improved nitrogen retention. Even sheep consuming a low energy diet which received urea infusion over a 1 and 3 hour period had higher nitrogen balances than sheep receiving a continuous infusion of urea. Streeter et al. (1973) infused urea continuously into the rumen of lambs or fed the urea with a basal 6% protein ration twice daily. They observed no difference in the utilization of nitrogen regardless of whether it was continuously infused or fed twice daily. Nitrogen reaching the abomasum and nitrogen balance were improved by either method of addition of urea above a zero urea basal. Romero et al. (1976) studied attenuated release of ammonia with cattle fed a low quality forage ad libitum. Slow release of ammonia did not improve digestibility, nitrogen balance, or bacterial protein synthesis in the rumen.

Huston et al. (1974) used a coated-urea product to examine a reduced rate of release of ammonia in the rumen. Slowed release slightly increased the value of urea as a supplemental nitrogen source when fed as a supplement for lambs fed a low quality ration. Pitzen et al. (1973) fed calves a high fiber ration containing 1.8% urea at two or twelve hour intervals. They observed that the amount of nitrogen leaving the abomasum was greater from the slow-release product or urea

fed at two hour intervals than from the twelve hour interval feeding of urea. Forero (1979) compared a new slow-release urea (SRU) compound with natural protein and prilled urea in winter supplements for dry, pregnant cows on range. He concluded that SRU improved palatability of supplements but did not consistently improve animal performance. In conclusion, benefits from an attenuated release rate of ammonia remain questionable and potentially attributable to ration acceptability.

Voluntary Intake of Low Quality Roughages

Factors Affecting Grass Intake

Voluntary intake of roughage by ruminants is determined primarily by the bulkiness of the digesta. Rate of disappearance of this bulk from the reticulo-rumen parallels intake (Balch and Campling, 1962).

Bulk limitation appears to be a function of ruminal rather than post-ruminal fill. This was determined from a clever trial by Grovum and Phillips (1978). They infused 93 to 534 g of the bulky material methyl cellulose into the abomasum of sheep fed a low quality forage. They observed no decrease in feed intake indicating that ruminal, not post-ruminal distention limits intake.

Disappearance of digesta from the reticulo-rumen can occur either by passage of residual food to the omasum or digestion in the rumen or both. According to Campling (1964), a number of factors can influence outflow. The small size of the reticulo-omasal orifice means that long or coarse particles of roughage must be reduced in size for passage to the abomasum. Particle size can be reduced by both mastication and by degradation by the microbial population. According to the review by

Balch and Campling (1962) some 60% of the organic matter of long hay rations must be digested before the digesta leaves the recticulo-rumen.

The Effect of Supplemental Concentrates on the Intake of Roughage

When supplemental high energy feeds are fed to animals receiving low-quality roughage, a decline in forage intake is generally noted. Lusby et al. (1976) noted that grass intake of cattle was depressed by 35% when 1.4 kg was added to a basal level of 1.2 kg of 30% protein supplement. Fick et al. (1973) similarly observed a depression of 14% in hay intake by sheep when 200 g of supplemental energy was provided. Campling (1964) showed that when concentrates were fed to cows receiving hay or silage, total feed intake increased but the intake of roughage declined. They hypothesized that the depression in hay intake may be due to a reduced rate of disappearance of digesta from the rumen. Head (1953) noted a depression in cellulose digestibility with additions of starch to rumen fluid. Reduced fiber digestion rate may be associated with suboptimal pH or insufficient concentrations of ammonia or other nutrients.

The Effect of Nitrogen on the Intake of Roughages

The effects of supplemental nitrogen on low protein roughage intake has been examined in many trials. Hemsley and Moir (1963) found that urea addition increased the intake of milled oaten hay by sheep. Urea addition increased rates of both cellulose digestion and passage of ingesta and increased the concentration of volatile fatty acids in

the rumen. Several authors have used a cotton thread technique to examine the rate of digestion of cellulose in the rumen (Combe and Tribe, 1962, 1963; Campling et al., 1962; Hemsley and Moir, 1963). All these studies reported that rate of digestion of cellulose increased with additional nitrogen. Such a change should permit increased feed intake. Loosli and McDonald (1968) reviewed a number of reports in which urea supplements increased the rate of digestion and food intake of poor-quality low-protein roughages. Coleman and Barth (1977) reported a 24% increase in intake with supplementation of nitrogen by steers fed low-quality low-protein forage. Forrero (1979) fed 1.2 kg of either a 15 or 40% protein supplement composed mainly of soybean meal to cows grazing winter range grass. Intake of grass was enhanced 32% by increasing the amount of supplemental protein from 180 to 480 g daily. In summary, it appears that a small amount of supplemental nitrogen improves the intake and rate of digestion of poor-quality low-protein roughages. The increased digestible energy intake improves the energy and nitrogen status of the animal. Although urea supplements may improve performance, when compared with soybean meal or other intact protein supplements, animal performance is generally disappointingly poor.

Microbial Synthesis in Relation to Ammonia Concentration

Nitrogen Needs of Rumen Bacteria

The dietary protein requirement of ruminant animals is partially met indirectly by bacterial protein synthesized in the rumen. Therefore, the needs of the rumen microorganisms for fermentable protein or

non-protein nitrogen become important. Supplying adequate ammonia to ensure normal rumen activity is necessary to maximize ruminal digestion and synthesis on microbial protein. The optimal ammonia concentration academically can be defined as that level which maximizes either: 1) rate of fermentation in the rumen, or 2) production of microbial protein per unit of substrate. These two definitions do not always coincide (Mehrez et al., 1977). On an experimental or practical basis, the definition of ammonia requirement is more complex as costs and feed intake effects become involved.

Ammonia-nitrogen levels in the rumen commonly range from 1 to 20 mg per dl of rumen contents. The optimal level of ammonia-nitrogen desired, however, has not been precisely determined. In vitro work by Bryant and Robinson (1961) indicated that pure cultures of cellulolytic bacteria may require ammonia-nitrogen in excess of 5.6 mg per dl of fluid. Utilizing continuous flow fermentors, Satter and Slyter (1974) suggested that a concentration of 2 to 5 mg ammonia-nitrogen per dl of rumen fluid was sufficient to support maximal microbial growth of rumen bacteria. They indicated that the more precise estimate of the limiting concentration may be 2 mg ammonia-nitrogen per dl. Levels far in excess of 5 mg per dl did not depress microbial growth. So for bacteria, excess ammonia may be harmless. In vivo studies (Satter and Roffler, 1975) indicated that bacterial protein concentration increased linearly with the level of crude protein supplied to a maximum at which point ammonia accumulates. As dietary crude protein was increased above this 13% crude protein break point, ruminal ammonia-nitrogen concentration increased from this baseline of one to five mg ammonia-nitrogen per dl of rumen fluid. Thus, when the ration contained above 13% crude protein,

more ammonia-nitrogen was present than was used efficiently for synthesis of microbial protein. It must be emphasized that the crude protein level needed to produce a ruminal ammonia-nitrogen concentration of five mg per dl of rumen fluid can be far below 13% for rations which contain less digestible energy. Added ammonia is supposedly useful only to the level at which microbial growth reaches the ceiling imposed by the amount of energy available for the bacteria. Recent in vivo studies by Slyter et al. (1979) infused urea into the rumen of steers to raise the ruminal NH_3-N concentration above 4.5 mg per dl. Added ammonia did not enhance ruminal protein concentration but increased nitrogen retention of steers slightly. Added ammonia did not increase in VFA concentration. Hume et al. (1970) indicated that microbial protein outflow from the rumen could be stimulated by increasing ammonia-nitrogen concentration to between 8.8 and 13.3 mg NH_3-N per dl rumen fluid. Maximum amounts of ruminal true protein were achieved with a diet containing 11.1% crude protein.

Meherez et al. (1977) continuously fed four cannulated sheep a barley ration with varying amounts of urea. Fermentation was maximized at an ammonia concentration of 23.5 mg NH_3-N per dl rumen fluid. Allen and Miller (1975) fed whethers 24 times per day barley rations containing 0, 0.8, 1.6, and 2.4% urea nitrogen. They observed that maximal microbial concentrations in the rumen occurred at 26 mg ammonia-nitrogen per dl of rumen fluid. Edwards and Bartley (1979) examined in vitro the hypothesis that microbial protein cannot be synthesized from urea when added to a ration containing more than 13% crude protein. They utilized 6 hour in vitro fermentations with rations containing sorghum grain plus soybean meal, or sorghum plus starea ranging in

protein from 17 to 30% protein. Maximum microbial protein production occurred at 76 mg per dl. In two preliminary studies with cows milk production was increased when protein was increased from 13% (all natural) to 16.5% (natural and starea). This indicated that cows can utilize additional nitrogen above the 13% limit suggested by Slyter et al. (1979). The above six trials indicate that considerable controversy exists about the optimal level of ruminal ammonia.

CHAPTER III

SLOW AMMONIA RELEASE FOR STEERS

Summary

Slow and rapid ruminal ammonia release rates were simulated by intermittently feeding urea with winter range grass to steers. Four 360 kg ruminally cannulated steers were fed 208 g prairie hay (2.56% crude protein) hourly with the following daily dietary supplements: urea continuously (C) as 3.5 g each hour; at a moderately high level for a shorter time (M) as 14.2 g hourly for 6 hours and no urea thereafter, pulsed or rapid (R) as 85 g urea in 1 hour with no urea at subsequent hours, and no supplemental urea (0). Ruminal ammonia remained stable with treatments C and 0 at 12.8 and 1.6 mg per dl, respectively. Treatments R and M peaked at 1.5 and 6.5 hours after urea feeding began at 41.8 and 37.1 mg per dl, respectively. Non-ammonia nitrogen concentrations in the rumen were increased by an average of 23% with urea supplementation. Dry matter digestibility was increased by 5% ($P < .05$) and nitrogen retention was increased (12.0 vs. 7.1 g per day, $P < .01$) with the addition of urea. Rate of urea administration did not influence dry matter digestibility or nitrogen retention. Metabolic use of ammonia in the rumen of steers fed low-quality forage was not improved by a simulated slow release rate of ammonia. (Key Words: Slow Release, Ammonia, Prairie Hay.)

Introduction

Cattle grazing low-quality forage utilize supplemental urea inefficiently. This has been attributed to rapid hydrolysis of urea by microbial urease (Bloomfield et al., 1960) with an insufficient supply of energy for bacteria to use the ammonia at this rate (Johnson, 1976). Excess ammonia is absorbed through the rumen wall (Lewis et al., 1957) and recycled or excreted. To balance ammonia and energy availability, attenuated ammonia release has been suggested. Several workers have reduced the rate of urea hydrolysis by various methods (Glimp and Tillman, 1965; Streeter et al., 1969; Ward and Cullison, 1970) but improvements in animal performance with attenuated ammonia release rates have been disappointing. Consequently, the theoretical advantage of attenuated ammonia release with forage diets needs reassessment. An ideal slow release ammonia compound would release ammonia in a pattern reflecting energy availability (Johnson, 1976). For animals grazing low quality forage, continuous ammonia release should prove most useful. Since no NPN source with specific, defined ammonia release characteristics is presently obtainable for experimental use, continuous intraruminal infusion or frequent feeding of urea have been used to produce various patterns of ruminal ammonia. Studies utilizing such techniques (Streeter et al., 1973; Pitzen et al., 1973; Knight and Owens, 1973) have used higher energy rations and show little advantage of attenuated release with infrequently fed rations. Frequent feeding of diets containing urea generally improves animal performance (Chalupa, 1972; Prior, 1976). Such results commonly have been attributed to slow release of ammonia, but may have an alternative explanation such as altered feed intake, metabolism, or nutrient bypass.

Although limited evidence suggests that moderately slow release of ammonia with medium-energy diets may prove slightly beneficial for sheep (Knight and Owens, 1973; Huston et al., 1974) and cattle (Romero et al., 1976), improvements have been slight. The objective of this study was to determine if a moderated release rate of ammonia would prove beneficial for digestion and nitrogen retention of steers fed winter range grass.

Experimental Procedure

Four crossbred steers weighing approximately 360 kg fitted with permanent rumen cannulas were utilized in a 4 x 4 latin square design. Steers were housed in metabolism stalls and fed hourly with timed automatic feeders.

The basal diet consisted predominantly of winter-harvested native prairie hay. The hay was fed at a fixed rate of 208 g per hour. The roughage contained 93% dry matter plus 2.56% crude protein, 52.9% ADF, 10.0% lignin, 39.4% cellulose and 12.0% ash on a dry matter basis. Daily nitrogen supplements provided urea continuously (C), 3.5 g of urea hourly for 24 hours, at a moderate rate (M), 14.2 g of urea hourly for 6 hours followed by no urea for 18 hours, rapidly (R), 85 g urea in the first hour with no urea the next 23 hours, or no supplemental urea (0). In addition, 606 g of an energy supplement (Table I) was provided the first hour of each 24 hour feeding cycle similar to commercial supplementation practices for cattle grazing winter range.

Each period of the latin square consisted of a 6-day adaptation period. On days 7 through 11 of each period, total urine and feces were collected. Fecal sub-samples were dried at 65 C for 48 hours and ground through a 1 mm screen in a Wiley mill. Rumen fluid samples were

TABLE I. DIET COMPOSITION

Ingredients	IRN	Energy supplement %	Urea supplement %
Corn, dent, yellow, grain, ground	4-02-931	74.7	---
Urea	---	---	62.1
Alfalfa, aerial part, dehy meal	1-00-023	18.4	---
Sodium sulfate	---	2.88	---
Monosodium phosphate	6-04-288	3.31	---
Sugarcane, molasses, dehy	4-04-695	---	37.9
Salt, trace mineralized	---	.61	---
Vitamin Premix[a]	---	.062	---

[a]To provide 2200 IU vitamin A/kg and 275 IU D_3/kg feed.

collected on day 12 from the ventral region of the rumen immediately below the cannula 0.5 hour prior to energy supplement feeding and 0.5, 1.5, 6.5, 7.5, and 15.5 hours later. Immediately following sampling, pH was determined and samples were acidified and frozen for later analysis.

Ration samples were obtained on days 6 through 11 of each period and handled in the same manner as feces. All samples were dried for 24 hours at 105 C to determine dry matter. Diet, feces, urine and rumen fluid were analyzed in the non-dried form for total nitrogen by the macro-Kjeldahl method (A.O.A.C., 1975). Ammonia-nitrogen in stained rumen fluid was determined by the phenol-alkaline hypochloride technique of Chaney and Marbach (1962) (Appendix A). Non-ammonia nitrogen was estimated as total nitrogen minus ammonia nitrogen concentration in the ruminal constituents. Cellulose was determined by the permanganate oxidation procedure of Van Soest and Wine (1968).

Apparent digestibilities of dry matter and crude protein were determined by difference between feed and feces. Ruminal dry matter digestibilities were calculated by the lignin ratio technique assuming that ruminal samples represented ruminal effluent. Continuous feeding should stabilize outflow to some degree.

Data were subjected to statistical analysis for a latin square design. Treatment means were compared by orthogonal contrasts (Steel and Torrie, 1960). First, rations providing urea (R, M and C) were compared with the ration without urea (O). Second, continuous feeding (C) was contrasted with more rapid ammonia release (R and M), and finally, the simulated 6 hour ammonia release pattern (M) was compared with 1-hour release (R).

Results and Discussion

Ruminal ammonia concentrations across time for each treatment are shown in Figure I. These patterns generally reflect infusion times. Consequently, these treatments would appear to simulate rapid, slow or continuous release compounds or no supplemental urea. Treatments C and O produced relatively stable ammonia-nitrogen concentrations of 12.8 and 1.6 mg per dl, respectively. For treatments R and M, ammonia-nitrogen peaked at 41.8 and 37.1 mg per dl at 1.5 and 6.5 hour postprandially.

Apparent dry matter (DMD) and cellulose digestibility were increased ($P < .05$) with urea feeding (Table II). The increase in DMD is attributable to an 8% increase in cellulose digestibility with urea addition. Similarly, Streeter et al. (1973) reported a 14% increase in cellulose digestibility with urea administered continuously or twice daily with sheep fed a wheat straw diet. Total tract lignin digestibility averaged 23% (range 16 to 30%) and was not affected ($P > .05$) by treatment. Lignin digestibility values question the validity of forage lignin as an internal marker for digestion or intake estimation.

Nitrogen digestibilities were increased ($P < .01$) from 13 to 60% with the addition of urea. This change may be due largely to the added urea. Protein digestibilities were recalculated to account for this urea based upon the assumption that urea was completely digestible. Adjusted values suggest that digestibility of non-urea nitrogen decreased ($P < .01$) from 13% with no additional urea to 4% for treatments with additional urea. This decrease in protein digestibility may be attributed to use of urea nitrogen for bacterial protein synthesis and incomplete digestion of such bacterial protein.

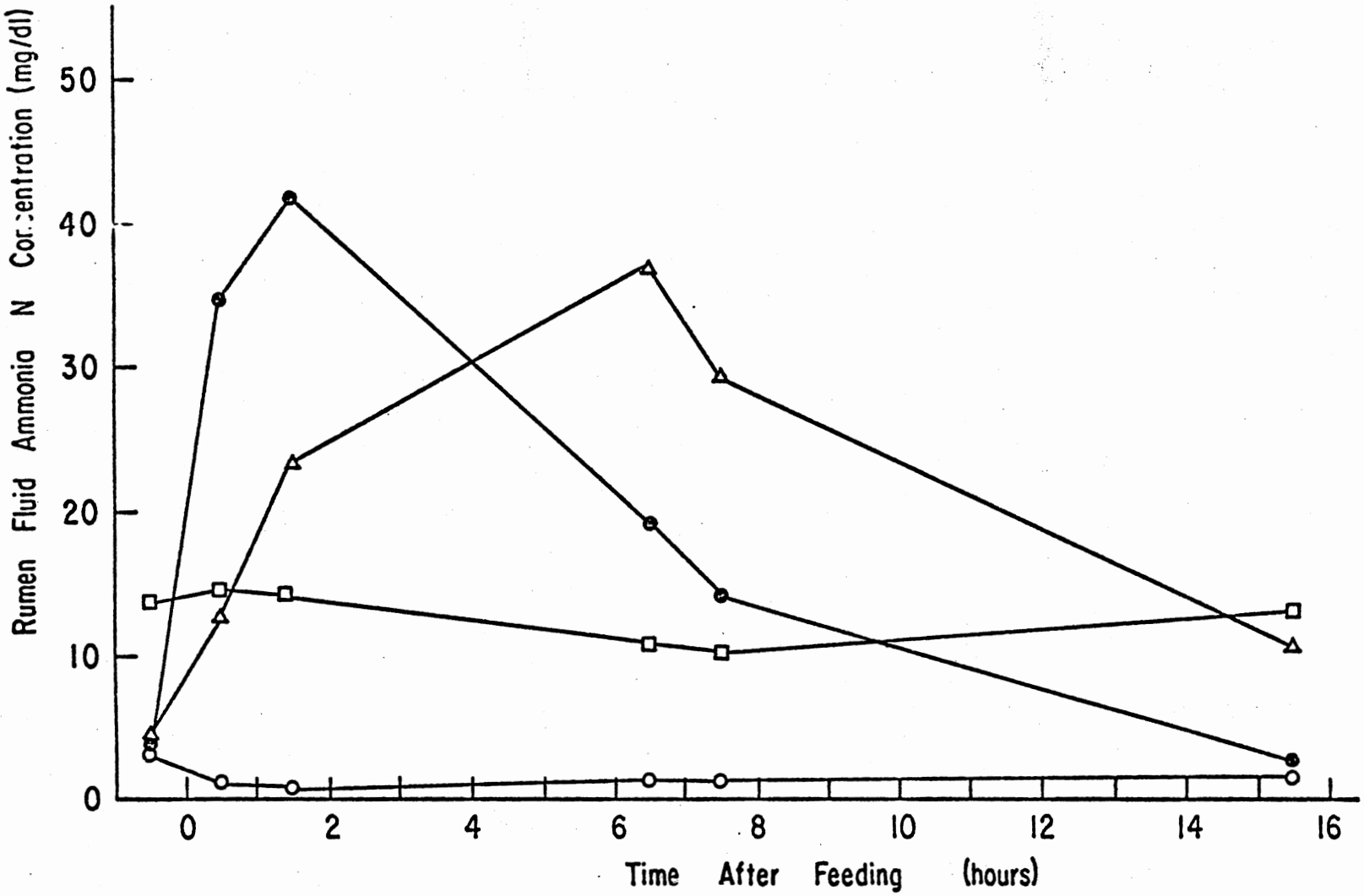

Figure I. Ruminal ammonia concentrations with interval urea supplementation. Treatments are no urea (O), rapid release (O), moderate release (Δ), and continuous release ([]).

TABLE II. DIGESTIBILITY OF NUTRIENTS BY STEERS RECEIVING UREA AT VARIOUS INTERVALS

Item	No urea	Urea administration interval: Rapid	Moderate	Continuous	SE[a]
	%				
Dry matter[b]	46.8	48.8	50.0	48.9	0.88
Cellulose[b]	50.8	54.3	55.4	55.4	1.39
Nitrogen[c]	13.4	60.2	60.3	60.3	1.44
Nitrogen, adjusted[c,d]	13.4	3.4	3.0	5.6	1.46

[a]Standard error of the mean.

[b]Mean of urea treatments differs from no urea ($P < .05$).

[c]Mean of urea treatments differs from no urea ($P < .01$).

[d]Calculated assuming complete digestion of urea.

Non-ammonia nitrogen (NAN) concentrations in rumen fluid (Table III) support the hypothesis that added urea enhanced bacterial protein synthesis. Addition of urea increased ($P < .01$) ruminal fluid NAN concentrations by a mean of 23%. Assuming that NAN values reflect bacterial protein concentrations, results support the suggestion that low rumen ammonia concentrations (under 2 mg/dl) reduce bacterial protein synthesis (Satter and Slyter, 1974). Rumen fluid pH versus time are presented in Figure II. The pH of rumen fluid remained virtually constant for treatments C and O. With treatments R and M, the pH increased following consumption of urea and gradually subsided. Added urea increased nitrogen retention ($P < .01$) expressed either as grams per day or percentage of nitrogen supplied (Table IV), which might be expected if protein flow to the intestine was enhanced. Rumen fluid dry matter percentage declined ($P < .05$) with urea feeding. This is partially attributed to increased ($P < .05$) ruminal dry matter digestibility (Table III) although enhanced salivary flow with urea may also be involved.

Results for nitrogen balance and dry matter digestibility agree closely with a recent intraruminal urea dosing study from Australia (Romero et al., 1976). With steers fed a low-quality roughage, organic matter digestibility, plasma urea nitrogen and nitrogen balance all increased when urea was added to the diet.

The time pattern of urea infusion caused no numerical improvement in digestibility, nitrogen balance or ruminal non-ammonia nitrogen in this experiment. In fact, except for the ruminal ammonia concentrations and ruminal pH, the interval over which urea was fed did not produce suggestive trends in any measured factor. Residual ammonia and/or

TABLE III. RUMINAL CHARACTERISTICS OF STEERS RECEIVING UREA AT VARIOUS INTERVALS

Ruminal measurement	Urea administration interval				SE[a]
	No urea	Rapid	Moderate	Continuous	
Non-ammonia nitrogen, mg/g DM[c]	8.40	11.12	11.00	11.00	0.28
Dry matter, %[b]	10.2	9.5	9.4	9.3	0.27
Dry matter[c] digestibility, %	28.4	33.8	32.9	33.4	1.52
pH	6.72	6.76	6.78	6.78	0.03

[a]Standard error of the mean.

[b]Mean of urea treatments differs from no urea ($P < .05$).

[c]Mean of urea treatments differs from no urea ($P < .01$).

TABLE IV. NITROGEN RETENTION BY STEERS RECEIVING UREA AT VARIOUS INTERVALS

Item	Urea administration interval				SE[a]
	No urea	Rapid	Moderate	Continuous	
Nitrogen intake, g/day[b]	23.2	60.4	60.2	60.4	0.80
Nitrogen excretion, g/day					
Fecal[c]	20.7	24.0	23.7	24.2	0.86
Urinary[b]	9.8	25.1	24.3	23.9	1.86
Nitrogen retention, g/day[b]	-7.1	11.4	12.2	12.4	1.58
Percentage of intake[b]	-34.6	18.8	20.4	20.5	4.21

[a]Standard error of the mean.

[b]Mean of urea treatments differs from no urea ($P < .01$).

[c]Mean of urea treatments differs from no urea ($P < .05$).

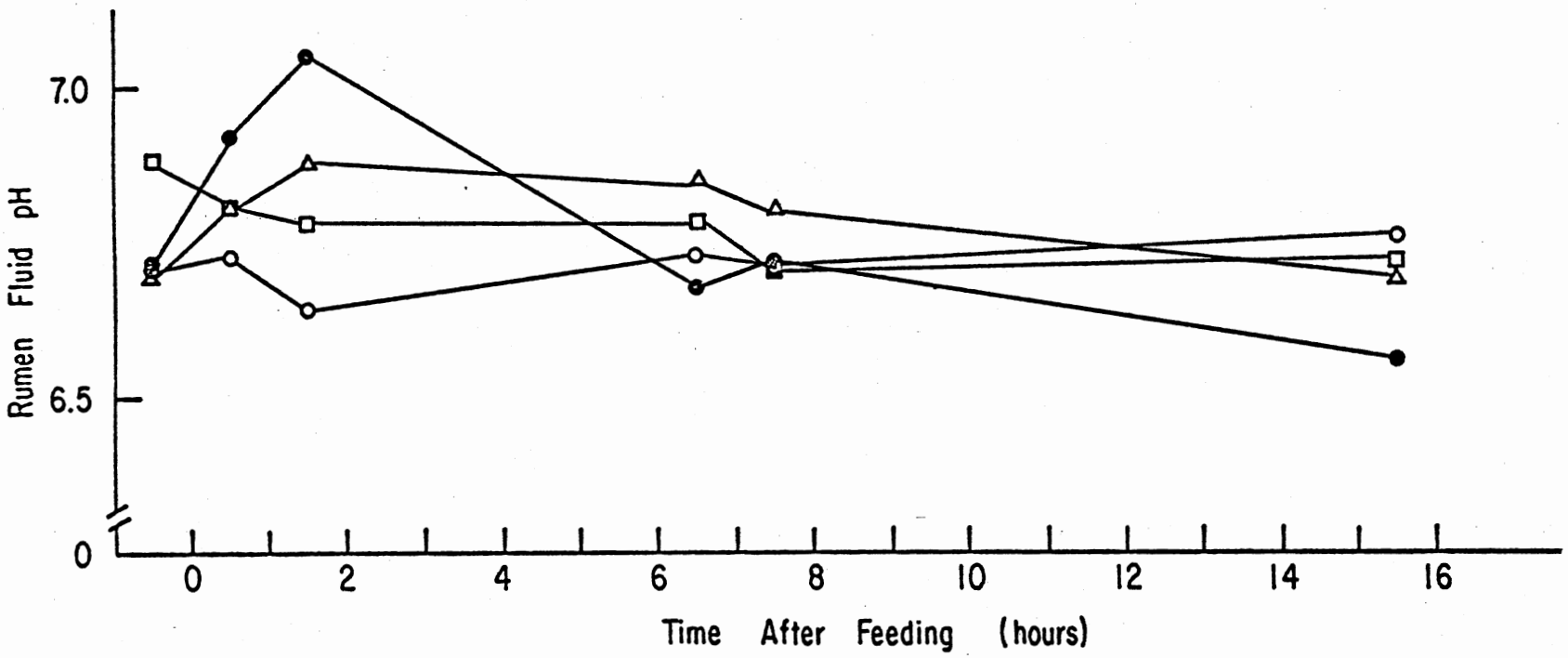

Figure II. pH curves with interval urea supplementation. Treatments are no urea (0), rapid release (0), moderate release (Δ), and continuous release (□).

recycled urea were apparently adequate to prevent ammonia deficiencies in the rumen and maintain conditions needed for fuminal digestion and microbial synthesis for the time periods with no infusion. Perhaps ad libitum feeding conditions would stress these homeostatic mechanisms and favor attenuated ammonia release. However, in the previously cited study with ad libitum intakes of low-quality forage, Romero et al. (1976) found that intake and dry matter digestibility were not influenced by attenuated release of ammonia. The theory that moderated release of ammonia is metabolically beneficial remains to be proven. Nevertheless, toxicity prevention remains as one definite advantage of compounds with an attenuated ammonia release pattern.

CHAPTER IV

SUPPLEMENTATION OF WINTER RANGE GRASS WITH ENERGY OR NITROGEN

Summary

Winter-harvested native range grass (2.6% crude protein) was supplemented with either energy or nitrogen and fed to four ruminally cannulated steers. Intake and digestibility were monitored. Supplements were composed of ground corn, soybean meal, alfalfa hay, minerals and vitamin A. The high protein (40%) supplement was fed at rates of 0.45 and 0.91 kg daily and the 20% protein supplement was fed at rates of 0.91 and 1.82 kg daily. Increasing protein intake from 182 to 362 g per day enhanced ($P < .05$) ad libitum hay intake (5.2 vs 5.9 kg) and ruminal digestibility of dry matter (28.2 vs 32.9%). Additional protein also enhanced ($P < .10$) turnover of ruminal polyethylene glycol (4.1 vs 5.8%/hr) and ruminal outflow of non-ammonia nitrogen (83 vs 122 g/day). Microbial protein synthesis averaged 9.8 g per 100 g dry matter digested in the rumen. Ruminal ammonia-nitrogen was higher ($P < .05$) with higher protein supplementation (4.4 vs 2.1 mg/dl). Added energy depressed ($P < .05$) ruminal ammonia by a mean of 42%. Ruminal outflow of non-ammonia nitrogen (NAN) was increased by added energy (18 g NAN/kg of supplemental dry matter) at the higher level of protein but added energy did not increase NAN outflow at the

lower protein level. Results suggest that supplementation of low-protein winter range grass with intact protein increases energy availability from forage and postruminal protein supply. In contrast, supplemental energy tended to reduce forage intake and increased postruminal protein supply only slightly. (Key Words: Protein, Energy, Ammonia, Non-ammonia Nitrogen Outflow.)

Introduction

The value of low-quality roughages for ruminant animals is a function of intake and digestibility of the roughage. Ruminal distention is thought to restrict intake of low quality forages (Balch and Campling, 1962; Campling, 1964). With low-protein, low-quality forages, nitrogen supplementation, either from non-protein nitrogen or intact protein often improves intake and utilization (Coombe and Tribe, 1963; Ammerman et. al., 1972; Coleman and Barth, 1977). Generally, differentiation between a nitrogen deficiency of ammonia for the bacteria and postruminal protein for the ruminant animal has been ignored (Johnson et al., 1978). Urea alone could meet the former need. Intact bypassed protein will help to meet the postruminal requirement. Postruminal nitrogen utilization typically is enhanced by adding energy to the ration (Chappel and Fontenot, 1968; Johnson, 1976). Such benefit of added energy with low-quality forages may be caused by an enhanced bacterial protein synthesis in the rumen and an increased postruminal supply of protein.

The objective of this study was to determine the effect of supplemental energy and/or nitrogen on forage intake and digestibility of low-quality forage by steers.

Experimental Procedure

Four 600 kg Holstein steers fitted with permanent rumen cannulas were housed individually in slatted floor pens and utilized in a 4 x 4 latin square experiment. The basal diet consisted predominantly of low-quality dry winter range grass harvested in late December. The grass contained 93.0% dry matter plus 2.56% crude protein, 52.9% ADF, 10.0% lignin, 39.4% cellulose and 12% ash on a dry matter basis. It was chopped to a maximum length of 18 cm and fed ad libitum with intake recorded daily. In addition to the grass, steers received daily either a) 0.91, or b) 1.82 kg of a supplement containing 20% protein, or c) 0.45, or d) 0.91 kg of supplement providing 40% protein (Table V). Steers were fed their respective supplements for a period of 13 days prior to measuring ruminal fluid composition. Fecal grab samples were obtained on days 13 and 14. Aliquots were dried at 65 C for 48 hours and ground through a 1 mm screen in a Wiley mill for later analysis.

To measure rumen volume and estimate liquid and solid outflow, 75 g of polyethylene glycol (PEG, molecular weight 3000-3500) and 30 g chromium sequioxide (Cr_2O_3) were dosed ruminally on day 13 of each period. Rumen samples were collected from the ventral region of the rumen at 2, 4, 8 and 24 hours post-dosing. Immediately following sampling, pH was determined and samples were acidified to stop microbial activity. Sub-samples were strained through 16 layers of cheesecloth to separate a fraction which shall be called the liquid fraction. This fraction contained much more particulate and suspended solids than the supernatant fraction which remains following slow speed centrifugation. Physically, it more closely resembles abomasal contents than a

TABLE V. SUPPLEMENT COMPOSITION

Ingredients	IRN	20 % 40	
Corn, dent, yellow, grain, grnd.	4-02-931	54.3	4.9
Soybean, seed, solv-extd, meal	5-04-604	25.2	74.6
Alfalfa aerial part dehy meal	1-00-023	15.0	15.0
Sugar cane, molasses, dehy	4-04-695	5.0	5.0
Trace mineral salt		0.5	0.5
Vitamin A[a]		0.015	0.015

[a]30,000 IU/g

supernatant sample does. The liquid fraction and the unfiltered (total) rumen fluid were analyzed for total nitrogen by the macro-Kjeldahl method (AOAC, 1975) and ammonia nitrogen as described by Chaney and Marbach (1962). Chromium and polyethylene glycol (PEG) concentrations were determined in the unfiltered and liquid phases of the rumen fluid, respectively. The unfiltered fraction was dried at 100 C for 48 hours and prepared for chromium analysis by the procedure of Williams et al. (1962). The liquid fraction was analyzed for PEG content as outlined by Smith (1959). Rumen volume and outflow rates were calculated by regression analysis of the natural logarithm of the marker concentration on time. Non-ammonia nitrogen was calculated as total nitrogen minus ammonia nitrogen. Since some filterable solids remained present in the unfiltered fraction and complete separation of a "solids" fraction was deemed infeasible, composition of the unfilterable fraction was calculated by the difference between the filterable and unfiltered materials. Calculations were based upon ratio of specific components to water in the two fractions and used the formulas presented in Appendix B. These formulas were used to subdivide the material passing from the rumen with the filterable (PEG) phase, and the amount passing with the non-filterable (Cr) phase. Outflow rates for the fractions were calculated from the dilution rate of these respective markers (Appendix C).

Ruminal fractions were also analyzed for ribonucleic acid (RNA) according to the procedure outlined by Ling and Buttery (1976) (Appendix D). Microbial RNA was calculated based upon a nitrogen content of RNA (13.2%) and an estimate that 10% of themicrobial nitrogen was RNA nitrogen (Smith, 1969; Cole et al., 1976). The total NAN in the unfilterable and filterable fractions reaching the abomasum was estimated

using the equation shown in Appendix B. Intact protein-nitrogen by-passing rumen degradation was estimated as non-ammonia nitrogen minus microbial nitrogen. Microbial protein was calculated as microbial nitrogen times 6.25.

Range grass and supplement samples were obtained on days 8 through 13 of each period and handled in the same manner as the fecal material. All samples were dried for 24 hours at 105 C to determine dry matter. Supplements and feces were analyzed in the non-dried form for total nitrogen. Cellulose was determined by the procedure of Van Soest (1963) and lignin was determined by the permanganate oxidation procedure of Van Soest and Wine (1968).

Ruminal dry matter digestibilities and total tract dry matter and nitrogen digestibilities were determined using lignin as a marker by calculating the ratio of lignin in feed to lignin in fecal material. Fecal lignin was the average of samples taken on days 13 and 14. Lignin disappearance of 23%, as determined previously (Mizwicki et al., Chapter III) was used to adjust nutrient digestibility values. Similar values were cited by Fahey (1979).

Data were subjected to statistical analysis for a 4 x 4 latin square design (Steel and Torrie, 1960). Single degree of freedom comparisons were used to examine the main effect of protein intake, the effect of energy intake within a protein level and the interaction (Table VI). The linear effects of protein level and energy level are partially confounded; thus treatment mean squares for single degree of freedom contrasts were adjusted by a tabular method (Appendix E). This provides a conservative estimate of the treatment effects.

TABLE VI. ORTHOGONAL COMPARISONS

Supplement Amount g/day	454	908	908	1816
Protein g/day	182	182	363	363
Level of Protein	-1	-1	+1	+1
Effect of Energy Level	-5	-1	-1	+7
Interaction	+2	-2	-1	+1

Results and Discussion

Forage Intake

Intake and digestibility of nutrients for steers fed weathered winter forage are presented in Table VII. Increasing the level of protein from 182 to 363 g per day enhanced ($P < .05$) intake of total ration forage ($P < .05$), and cellulose ($P < .05$). Loosli and McDonald (1968) have reviewed a number of reports in which urea supplements increased the rate of digestion and allowed increased food intake of poor-quality roughages. Coleman and Barth (1977) reported a 24% increase in intake with supplementation of nitrogen with steers fed low quality protein forage. Forero (1979) fed 1.2 kg of either a 15 or 40% protein supplement composed mainly of soybean meal to cows grazing winter range grass similar in composition to that used in this study. Intake of grass was enhanced 32% by increasing the amount of supplemental protein from 180 to 480 g daily. Lemenager et al. (1978) observed intakes of 9.6 kg per day for cows grazing range grass of similar composition. The greater intakes observed under grazing conditions when compared to stall confinement of this trial suggests the presence of intake stimulating factors such as exercise, pregnancy, lactation and grazing selectivity.

Adding energy tended to increase dry matter intake but supplemental energy reduced hay intake 1.3 and 9.1% at the low and high levels of protein, respectively. This suggests that additional energy from the supplement was directly reducing forage intake. Lusby et al. (1976) noted grass intake was depressed by 35% when cattle were fed 2.6 kg in place of 1.2 kg of 30% protein supplement. Fick et al. (1973)

TABLE VII. INTAKE AND DIGESTIBILITY OF NUTRIENTS BY STEERS FED LOW QUALITY ROUGHAGES

Supplement g/day	454	908	908	1816	
Protein g/day	182	182	363	363	SE_m
Intake, g DM/day					
Ration[c]	5612	5944	7008	7241	243.5
Hay[c]	5203	5136	6190	5626	261.3
Cellulose[c]	2076	2052	2472	2244	90.8
DM Digestibility, %					
Ration	44.7	44.8	47.8	48.0	1.18
Hay[e]	42.2	38.8	44.0	37.9	-
Cellulose	48.0	47.2	51.4	49.0	1.96
Digestible DM Intake, g					
Dry Matter[a]	2509	2663	3350	3476	108.2
Cellulose[ab]	996	968	1271	1100	46.0
Indigestible DM[d]	3103	3281	3658	3765	136.0
Nitrogen Digestibility, %					
Ration	42.4	35.4	55.9	58.0	7.13
Hay[e]	-0.47	-19.7	13.1	14.7	-

[a]Protein effect ($P < .01$).

[b]Energy effect ($P < .10$).

[c]Protein effect ($P < .05$).

[d]Protein effect ($P < .10$).

[e]Calculated assuming a digestibility of 73.1 and 78.1% for dry matter and 77.7 and 73.8% for nitrogen from the 40 and 20% supplement, respectively. Digestibilities were based on NRC (1976) values for component ingredients.

observed a depression of 14% in hay intake when 200 kg of supplemental energy was provided for sheep fed a low protein roughage.

Digestibility

Total tract digestibility (DMD) tended to increase with protein supplementation. The increase in DMD may be attributed partially to the 5% rise in cellulose digestibility with additional nitrogen. Streeter et al. (1973) and Mizwicki et al. (unpublished) similarly enhanced cellulose digestibility with the addition of nitrogen to low quality roughages. Bacteria may have been deficient in ammonia or other nutrients (amino acids, branched chained volatile fatty acids) at the low protein level to maximize the rate of digestion of cellulose (el-Shazly et al., 1961). Dry matter digestibility for the forage (38 to 44%) was low compared to the 49% observed by Williams et al. (1969) but more similar to the 39% reported by Lemenager et al. (1978).

Combining intake and digestibility together into values for digestible dry matter intake, added protein increased intake of digestible dry matter and cellulose ($P < .01$), and intake of indigestible dry matter ($P < .10$). The latter suggests increased bulk fill increased rate of passage or some other factor overriding the bulk fill limitation (Grovum and Phillips, 1978). Digestible dry matter intake appeared to increase with added energy probably due to an increased cellulose digestibility and to lower bulkiness of the supplement versus the forage.

Nitrogen digestibility was increased 46% by feeding additional protein. Enhanced nitrogen digestibility of the ration would be

expected as the supplemental protein should be more digestible than the protein from hay. But calculated by difference as well, added protein increased digestibility of the protein from hay.

Ruminal Conditions

Effects of nitrogen and energy supplementation on ruminal parameters are shown in Table VIII. Only small fluctuations were noted in dry matter (range 7.4 to 10.5%) and pH (range 6.4 to 6.7) of rumen contents. Ruminal digestion of dry matter increased ($P < .05$) from 28 to 33% with the addition of nitrogen. The increase with added protein may be attributed to increased cellulose digestibility.

Ruminal ammonia nitrogen was higher ($P < .01$) with the higher protein supplementation (4.4 vs 2.1 mg/dl) but added energy within each protein level decreased ($P < .05$) ruminal ammonia concentration. The decrease with added energy should be associated with enhanced microbial protein synthesis and outflow. This reduction in ammonia may explain the negative associative effect of supplement on grass intake. If less ammonia remains for cellulolytic microorganisms, reduced ammonia could reduce rate of digestion and, thereby, forage intake. If ammonia concentration had been maintained at the higher intake of supplement, grass consumption might not have declined. Although added protein enhanced rumen ammonia values, the mean ammonia concentration would be considered barely adequate for maximal microbial growth (Satter and Slyter, 1974).

Added protein tended to increase turnover of ruminal solids from 2.0 to 2.4% per hour. This may be associated with an enhanced rate of bacterial digestion, an increased rate of dry matter digestion and an increased feed intake. The increase in turnover rate of ruminal solids

TABLE VIII. RUMINAL CHARACTERISTICS OF STEERS FED LOW QUALITY ROUGHAGE

Supplement g/day Protein g/day	454 182	908 182	308 363	1816 363	SE_m
Dry matter %	10.2	9.1	10.3	10.0	0.53
pH	6.65	6.68	6.62	6.58	0.03
DM digestibility %[c]	28.1	28.3	33.5	32.3	1.18
Ammonia N gm/dl[ab]	2.3	1.8	5.8	2.9	0.47
Turnover rates %/hr					
Non filterable fraction	1.9	2.1	2.3	2.5	0.17
Filterable fraction[d]	4.8	4.1	5.4	6.1	0.36
Rumen volume ℓ	175.5	184.0	196.2	161.3	9.69

[a]Protein effect ($P < .01$).

[b]Energy effect ($P < .05$).

[c]Protein effect ($P < .05$).

[d]Protein effect ($P < .10$).

with supplemental nitrogen parallels those cited by Kropp et al. (1977a) for steers limit-fed winter range grass.

Ruminal liquid turnovers were 4.7 and 5.8% per hour ($P < .1$) for the low and high protein levels of supplementation. Increased saliva flow with enhanced intake may be responsible for this observation. No explanation is apparent for the 14% depression in turnover at the low protein level when energy was added although lower intake of forage and lower saliva flow may be involved.

Rumen volumes were unaffected by the addition of protein or energy. All volumes appeared excessively large. Animals ad libitum fed high roughage rations have previously produced unrealistic ruminal volume estimates (Lemenager et al., 1978). Incomplete mixing of the ruminal mass or binding of marker by digesta particles may be involved.

Ruminal Output

Ruminal outflow of non-ammonia nitrogen (NAN) in the solid and liquid phases (Table IX) calculated from ruminal turnover rates and ruminal NAN concentrations ranged from 130 to 155% of total nitrogen intake. Leme (1978) reported that flow of nitrogen to the abomasum averaged 138% of the dietary intake for prairie hay rations supplemented with low amounts of coated urea or of hydrolyzed feathermeal. Similar trends for greater outflow than intake were noted by Kropp et al. (1977b), Prigge et al. (1978), and Potter et al. (1972). Bacterial incorporation of recycled nitrogen is probably responsible for this increase. Yet the gain in ruminal nitrogen (25 to 43 g/day) appears large. Cattle secrete 98 to 190 liters of saliva per day (Bailey, 1961) with a nitrogen content of 0.1 to 0.2% for a total 12 g daily. Hogan

TABLE IX. NITROGEN FRACTIONATION OF RUMEN DIGESTA REACHING THE ABOMASUM

Supplement g/day Protein g/day	454 182	908 182	908 363	1816 363	SE_m
Nitrogen intake g/day	52.0	51.3	85.2	82.8	-
Non-ammonia nitrogen					
Total leaving the rumen g/day[a]	80.4	79.3	110.9	125.2	5.60
Non-filterable fraction g/day					
Total N[a]	33.6	35.8	53.7	64.4	4.20
Microbial N	33.0	36.0	38.6	49.1	6.07
Bypass N	0.6	-0.2	15.1	15.3	7.28
Filterable fraction g/day					
Total N[a]	46.8	43.4	57.2	60.8	4.05
Microbial N	11.5	8.8	8.8	11.2	1.80
Bypass N	35.3	34.6	48.4	49.6	5.23
Microbial protein g/100 g DM digested	8.3	8.3	10.8	10.6	1.54

[a]Protein effect ($P < .05$).

(1961) observed that the transfer of ammonia and urea through the rumen wall is a function of concentration gradients and transfers of 17 to 20 g of nitrogen a day at rumen ammonia concentrations below 12 mg per dl have been reported (Vercoe, 1969). In total salivary secretion and ruminal diffusion could explain the net influx of nitrogen. Nevertheless, imprecision in the solid and liquid phase marker procedures and separation may have introduced bias as well.

Total nitrogen reaching the abomasum was increased ($P < .05$) by 48% with the addition of protein. Outflow may be subdivided into bypassed feed protein and microbial protein based on RNA concentrations. Estimates of bypass suggest that 89% of the increase in NAN leaving the rumen when additional protein is fed was protein escaping degradation. For each 100 g supplemental protein fed, bypass increased by 43 grams. Added protein increased NAN outflow in both the solid and liquid fractions ($P < .05$), primarily by increasing bypass. Treatments with greater protein bypass also exhibited higher total protein digestibilities, supporting the hypothesis that bypassed protein was more digestible than either the hay or microbial protein. Microbial protein synthesis tended to be higher with the addition of protein (44.6 vs 53.8 g/day). Microbial protein synthesized per 100 g of dry matter digested in the rumen ranged from 8 to 11 g dry matter digested. These are in general agreement with those of Kropp et al. (1977a) and Leme (1978). A trend toward depressed microbial efficiency (18%) was noted at the lower protein level supporting suggestions of any of three hypotheses: 1) energetic uncoupling with ammonia deficiency (Hespell, 1979), 2) an increased microbial efficiency when amino acids are present (Maeng and Baldwin, 1976), 3) increased efficiency with an increased dilution rate (Isaacson et al., 1975).

Distribution of NAN in the solid and liquid phases in the rumen and that presented to the abomasum is presented in Table X. This is due to differential turnover of the solid and liquid phases from the rumen. In this study, about 60% of the protein in the rumen was associated with the ruminal solids, but due to faster outflow of liquids, only 45% of the protein outflow was with solids. Microbial protein was associated primarily with the solids. Therefore, care must be exercised in extrapolating from turnover of liquids or solids to turnover of ruminal bacteria. Less discrepancy may result with higher concentrate rations for which microbial distribution differs and solid and liquid outflow rates are quite similar.

In summary, forage intake and digestibility of dry matter and nitrogen were increased when supplemental protein was provided. Increased ammonia may have enhanced digestion rate and thereby permitted increased intake. Further, intake of indigestible dry matter increased when additional protein was added. Together with the increased turnover rates, this implies that rate of passage had increased with added protein. Added protein also increased ruminal solids turnover rate, and tended to increase bypass of intact protein. Added energy tended to reduce forage intake, possibly through reducing ruminal ammonia, and increased postruminal protein supply only slightly. If due to ammonia-nitrogen deficiency, perhaps supplement combinations of intact protein plus NPN or NPN with lower energy feeds (cottonseed hulls) might prove useful to prevent intake depression and enhance postruminal protein supply.

TABLE X. PERCENTAGE RUMINAL AND RUMINAL OUTFLOW OF NON-AMMONIA NITROGEN

Supplement g/day	454	908	908	1816
Protein g/day	182	182	363	363
Non-ammonia nitrogen				
Ruminal				
Solid fraction %	66.4	59.2	63.8	72.3
Liquid fraction %	33.6	40.8	36.2	27.7
Ruminal outflow				
Solid fraction %	41.8	45.4	48.4	51.4
Liquid fraction %	58.2	54.7	51.6	48.6

CHAPTER V

AMMONIA EFFECTS ON RUMINAL OUTPUT AND DIGESTIBILITY OF A HIGH CONCENTRATE RATION

Summary

Urea was added to a rolled corn steer ration at levels of 0, 0.14, 0.29, 0.56 and 1.12 g additional nitrogen per 100 g of feed. Ruminal dry matter, ammonia nitrogen and nitrogen digestibility tended to increase linearly ($P < .04$) as urea nitrogen was added to the basal ration. Rate of digestion of corn starch from nylon bags and ruminal non-ammonia nitrogen concentrations increased with ruminal ammonia concentrations from 11 to 24 mg per dl. But in vivo digestibility of starch and outflow of non-ammonia nitrogen were not altered. Ruminal volume appeared to decrease linearly ($P < .03$) with added urea. Microbial ammonia-nitrogen requirements appear to be less than 11 mg per dl, but higher concentrations may concentrate rumen contents and alter site and extent of digestion.

(Key Words: Ruminal Ammonia, Microbial Protein, Ruminal Dry Matter Digestibility.)

Introduction

The use of urea as a non-protein nitrogen source in ruminant

rations has been the subject of extensive research. Urea-nitrogen is optimally utilized when fed with low-protein, high-concentrate rations. What is not defined is the optimal level of dietary protein - that is the point above which addition of non-protein nitrogen will no longer enhance performance. The total amount of non-protein nitrogen that can be utilized should be primarily a function of microbial protein production which in turn is limited by the amount of substrate fermented in the rumen. The concentration of ammonia in the rumen, derived from dietary and salivary sources, can serve as an index of the nitrogen supply since non-protein nitrogen is utilized by the microbes in the form of ammonia.

Previous work suggests that some minimal concentration of ammonia (2.2 mg/dl) in the rumen is required for growth of bacteria and a slightly elevated concentration (4.5 mg/dl) will maximize nitrogen retention in steers without altering DMD (Slyter et al., 1979). The adequacy of these levels has been the subject of much debate. Hume et al. (1970) indicated that microbial protein synthesis would be stimulated by increasing ammonia concentration to a point somewhere between 8.8 and 13.3 mg per dl of rumen fluid. Orskov et al. (1972) showed with barley rations that the microbial protein produced per unit of substrate fermented was not altered by ammonia concentration, but the extent of rumen fermentation and digestibility was increased up to 22 mg per dl. Recent in vitro work with Starea (Edwards and Bartley, 1979) suggests that microbial protein synthesis is enhanced by levels up to 50 mg per dl. The optimal ammonia concentration in rumen fluid for maximum microbial protein production might differ from that needed to maximize the extent of rumen fermentation and digestibility.

The objective of the present trial was to examine the relationship between ruminal ammonia concentrations and, a) microbial growth, and b) digestibility of specific nutrients.

Experimental Procedure

Ten mature steers weighing approximately 550 kg were fitted with permanent rumen cannulae and utilized in two simultaneous 5 x 5 latin squares. Steers were housed individually in slatted floor pens and fed a diet of 92% rolled corn, 5% cottonseed hulls and 1% alfalfa. Urea replaced rolled corn at levels to provide 0, 0.14, 0.29, 0.56 and 1.12 g additional nitrogen per 100 g of feed (Table XI).

Five feeding periods of ten day duration were used. Steers were fed 3.2 kg of feed twice daily for the first six days. On days 6 through 9, 30 g of chromium sesquioxide (Cr_2O_3) and 75 g of polyethylene glycol (PEG) were incorporated into the feed as solid and liquid phase markers, respectively. On days 8 to 10, steers were fed 533 g in twelve equal portions in an attempt to maintain steady state ruminal conditions. In addition, on day 9 cotton strips of known weight and nylon bags containing either SBM or corn grain of 2 mm mean particle size were suspended in the rumen for eight hours. Upon completion of incubation, bags and strips were washed and dried for 24 hours in a 100 C forced air oven. Bags and strips were desiccated and weighed to estimate cellulose and dry matter digestion rate. Starch content of the corn grain before and after incubation was determined so that starch digestion rate could be calculated.

Fecal grab samples were attained on days 9 and 10 of each period and frozen. Sub-samples were dried at 65 C for 48 hours, and ground

TABLE XI. DIET COMPOSITION

Diet protein, % Ingredient	IRN	8.4	9.3	10.1	11.8	15.2
Corn, dent, yellow, dry rolled	4-02-935	91.96	91.64	91.32	90.70	89.45
Urea, 45% N	5-05-070	0	0.31	0.62	1.25	2.50
Cotton, seed hulls	1-05-599	5.00				
Alfalfa, aerial part, dehy grnd, mn 15% protein	1-00-022	1.00				
Calcium carbonate commercial, mn 38% ca	6-01-069	0.48				
Potassium chloride	6-03-656	0.35				
Sodium sulfate[a]	6-04-292	0.72				
Salt, trace mineralized		0.50				
Vitamin A, palmitate commercial	7-05-143	.0022				

[a] N:S (17:1)

[b] 30,000 IU/g

through a 1 mm screen in a Wiley mill. Five hundred ml of whole rumen fluid samples were collected on day 10 from the ventral region of the rumen immediately below the cannula approximately 15 minutes prior to the bi-hourly feeding at which markers were withdrawn from the ration and 2, 4, 8, and 24 hours later. The pH of the whole rumen fluid was determined immediately following sampling and microbial action was stopped by the addition of 5 ml of sulfuric acid per dl of rumen fluid. Equal aliquots from each sample were strained through sixteen layers of cheesecloth separating the whole rumen fluid into a liquid and solid fraction. In addition, a 20 ml aliquot of strained rumen fluid was centrifuged at 10,000 g for 30 minutes for ammonia nitrogen determination and PEG analysis. Ammonia nitrogen was determined by the phenol-alkaline hypochlorite technique of Chaney and Marbach (1962) and PEG concentration according to the procedure of Smith (1959). The solid ruminal portion was dried at 100 C for 48 hours, ground and prepared for chromium analysis by the procedure outlined by Williams et al. (1962). Rumen volume and outflow rates were calculated by regression analysis of the natural logarithm of the respective marker on time. The amount of marker present in the rumen at time zero which would have accumulated from the previous meals to an extent dependent on the dilution rate, was estimated from the determined dilution rate and the equation presented below:

$$N_w = \Sigma\, N_i e^{-\lambda t_i}$$

N_w = Amount in the rumen at time of marker withdrawal

N_i = Amount of marker dosed at time t_i.

e = 2.718

$-\lambda$ = Dilution rate determined following marker withdrawal.

t_i = Time prior to withdrawal of marker at which marker was fed.

Non-ammonia nitrogen was calculated as total nitrogen minus ammonia nitrogen. Non-ammonia nitrogen associated with the solid fraction in the rumen was estimated by formula presented in Appendix A. These formula were used to calculate the amount of material passing from the rumen with the liquid phase, and the amount passing with the solid phase. Total solid and liquid NAN reaching the abomasum was calculated from the dilution of the respective markers.

Ration samples were obtained on days 7 through 10 of each period, composited and ground. Dry matter, nitrogen, starch and chromium analyses were conducted on ration, rumen and fecal samples. All samples were dried for 24 hours at 105 C to determine dry matter content. Samples were analyzed in the non-dried form for total nitrogen by the macro-Kjeldahl method (AOAC, 1975). Starch was determined as total alpha-linked glucose polymers (MacRae and Armstrong, 1968). Chromium concentration in the ration and fecal samples was determined by atomic absorption spectrography. Total tract digestibilities of dry matter, protein and starch were calculated using the chromium ratio technique.

Data were subjected to statistical analysis for a latin square design. As no square by treatment effects proved statistically significant ($P > .05$), squares were combined. Effects of urea were statistically subdivided into linear, quadratic, cubic and quartic components. Simple correlation coefficients were calculated and are cited.

Results and Discussion

Steers receiving the basal ration in this experiment had concentrations of ammonia-nitrogen of about 11 mg per dl. This value is below

most values cited as being required for maximum production of microbial protein of 0.005 to 76 mg per dl (Hume et al., 1970; Allen and Miller, 1976; Mehrez et al., 1977; Slyter and Satter, 1979; Hespell, 1979; Edwards and Bartley, 1979). The high basal ammonia level may be a result of restricted feed intake (6400 g) which may enhance the potential for nitrogen recycling via saliva and depress turnover of digesta and reduce total protein synthesis by microorganisms. This study is therefore limited to discussion of ruminal ammonia concentrations above 11 mg per dl.

Ruminal ammonia-nitrogen concentrations and pH for steers fed graded levels of urea nitrogen are presented in Table XII. Ruminal ammonia concentration increased linearly ($P < .0001$) with urea addition. This indicates that the supply of ammonia to the rumen microbes was more than adequate for growth because a growth-limiting nutrient never accumulates in a biological system. Consequently, it appears that the concentration of ruminal ammonia required for microbial growth is below 11 mg per dl. Ruminal pH remained remarkably constant (range = 6.4 to 6.6) but appeared to decrease as ammonia concentration increased ($r = -0.48$, $P < 0.001$). Concentrations of dry matter in the rumen samples increased linearly with urea addition ($P < .05$) and with ammonia concentration ($r = 0.51$, $P < .001$).

Apparent digestibilities for dry matter (DMD), nitrogen and starch based upon chromium as a marker are shown in Table XIII. Digestibility of dry matter increased as additional nitrogen was provided from urea, although a drop was noted at the high urea level which produced an ammonia-nitrogen concentration of 24 mg per dl. Ruminal ammonia concentration was negatively correlated with dry matter digestibility

TABLE XII. MEAN RUMINAL AMMONIA NITROGEN CONCENTRATION AND pH

Item	Supplemental Urea N %					
	0	0.312	0.625	1.25	2.50	SE_m
Ruminal ammonia-nitrogen mg NH_3-N/dl[a]	11.9	12.3	14.5	18.6	23.9	1.31
Ruminal pH	6.50	6.45	6.55	6.47	6.42	0.186
Ruminal DM %[b]	4.36	4.06	4.37	4.74	4.82	0.238

[a]Linear effect ($P < .0001$).

[b]Linear effect ($P < .0381$).

TABLE XIII. DIGESTIBILITY OF NUTRIENTS

Item	Supplemental Urea N %					
	0	0.312	0.625	1.25	2.50	SE_m
Digestibility %						
Dry matter[a]	78.9	80.0	81.6	82.8	80.6	0.84
Nitrogen[b]	51.1	51.0	58.3	69.2	71.9	1.65
Starch	96.3	96.4	97.3	96.9	95.9	0.86
Fecal Starch %	10.7	8.6	8.3	8.5	10.7	1.30

[a]Quadratic ($P < .0035$).

[b]Linear ($P < .0001$), quadratic ($P < .0017$), cubic ($P < .0117$).

($r = -0.23$, $P < .11$) and starch digestibility ($r = -0.35$, $P < .01$). Hume et al. (1970) observed enhanced DMD up to 8.8 mg per dl and improved ruminal dry matter digestibility up to 13.3 mg per dl. Since the percent of dry matter of rumen contents had increased ($P < .0381$) with additional nitrogen (Table XII), part of the increase in DMD may be associated with an increased time for ruminal digestion. Digestibility of nitrogen appeared to be affected linearly ($P < .0001$), quadratically ($P < .0017$), and cubically ($P < .0117$) by urea intake. Because about six times as much of the variation due to regression could be accounted for by linear regression than by quadratic and cubic regression, the linear incease should receive the major emphasis. Digestibility of nitrogen would be expected to increase linearly with added urea since all of the addition increments of urea nitrogen (ammonia) should be absorbed. Neither starch digestibility nor fecal starch (Table XIII) were affected by additional nitrogen although correlations suggest that starch digestibility declined as ruminal ammonia increased ($r = -0.35$, $P < .02$).

Rate of starch, nitrogen and cellulose digestibility were estimated by the nylon bag digestion technique and are presented in Table XIV. Rate of starch degradation tended to increase with ruminal ammonia-nitrogen concentration (0.051% per hour per mg ammonia-nitrogen per deciliter). Digestion rates of soybean meal and cotton duck (a source of cellulose) were unaffected by the addition of nitrogen or ruminal ammonia concentration. Mehrez et al. (1977) found that rate of straw disappearance from nylon bags increased with ammonia concentration up to 23.5 mg per dl, similar to the highest ammonia level in this study. Unfortunately, neither study included levels of urea to

TABLE XIV. RATE OF RUMINAL DEGRADATION OF NUTRIENTS

Item	Supplemental Urea N %				
	0	0.312	0.625	1.25	2.50
% per hour					
Starch (in corn grain)	3.01	2.93	3.23	3.19	3.58
SBM	6.05	5.91	6.22	5.90	6.01
Cotton duck	1.06	1.04	1.04	1.02	1.02

produce ruminal ammonia concentrations above 24 mg per dl to deduce whether rate of fermentation would continue to increase. Penetration of ammonia into a nylon bag may limit extrapolation of results to in vivo conditions. Further, the amount of urea or concentration of ruminal ammonia-nitrogen required to maximize rate of digestion might differ from that level required to maximize yield of microbial protein. Finally, other in vitro effects of urea (on turnover rates and ruminal pH) complicate interpretation.

Turnover rates of liquid and solid digesta and ruminal volumes are presented in Table XV. Turnover rate of solid and liquid digesta responded cubically to additional nitrogen. A quadratic effect of urea on turnover would relate more readily to the DMD values discussed earlier, although dilution rate tended to decline at the higher nitrogen intake. Solid and liquid turnover rates were quite similar. This contrasts with the faster turnover of liquid than solids with higher roughage rations (Chapter III). Rumen volumes decreased linearly ($P < .05$) as more urea was fed. Dry matter concentration of ruminal contents was positively correlated with ruminal ammonia concentration ($r = +0.51$, $P < .001$) possibly because rumen volume decreased as ammonia increased ($r = -0.31$, $P < .01$, Figure III). A decrease in liquid volume might be attributed to a decrease in salivary flow. Such a depression in salivary flow could occur at eating or subsequently with reduced rumination, however rumination with this high concentrate diet was absent.

Decreased osmotic pressure might also decrease ruminal volume and outflow as fluid maybe absorbed through the ruminal wall. But added urea should increase, not decrease osmolarity of rumen contents. A

TABLE XV. DILUTION RATES AND RUMEN VOLUME

Item	Supplemental Urea N %					
	0	0.312	0.625	1.25	2.50	SE_m
Turnover rates %/hr						
Solid fraction[a]	4.42	4.47	5.08	3.58	4.25	0.35
Liquid fraction[a]	4.77	5.09	5.80	4.86	5.24	0.280
Rumen volume, L[b]	110.9	110.4	114.8	96.9	99.2	5.32
Outflow L/hr[c]	5.40	5.66	6.69	4.83	5.26	0.521

[a]Cubic effect of urea ($P < .027$).

[b]Linear effect of urea ($P < .034$).

[c]Cubic effect of urea ($P < .05$).

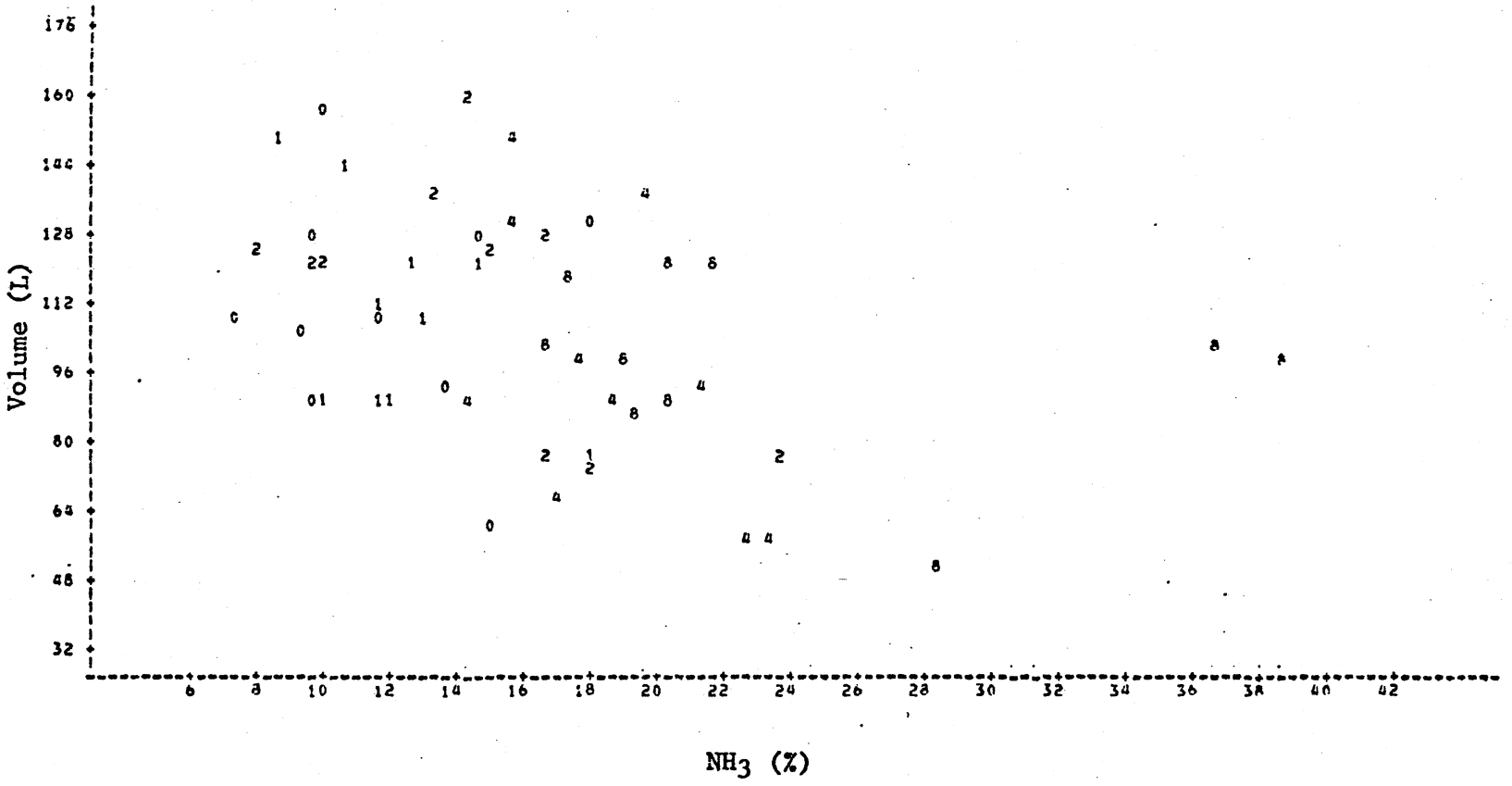

Figure III. Volume versus ammonia nitrogen

Legend: 0 = basal ration; 1 = basal ration + 0.312% urea; 2 = basal ration + 0.625% urea; 4 = basal ration + 1.25% urea; 8 = basal ration + 2.50% urea.

third possibility is decreased influx of fluid through the ruminal wall. If motility is decreased by ammonia, as occurs in ammonia toxicosis, the potential for fluid influx may be decreased since concentration gradients will be lower.

Non-ammonia nitrogen concentrations in ruminal fluid tended to increase ($r = 0.39$, $P < .01$) as ruminal ammonia concentration increased (Figure IV). Yet, total ruminal non-ammonia nitrogen leaving the rumen was unaffected by the addition of urea at any level (Table XVI). The relationship of total ruminal non-ammonia nitrogen in the rumen (bacterial plus feed nitrogen) and total non-ammonia nitrogen outflow to the abomasum is plotted against rumen ammonia concentration (Figures V and VI). Relationships were statistically nonsignificant ($r = 0.09$, $P < .55$; $r = 0.003$, $P < .98$). That concentrations of NAN increased while total ruminal NAN and NAN outflow were stable reflects the decreased ruminal volume with added ammonia discussed previously. The fact that ruminal protein yield was not enhanced by ruminal ammonia-nitrogen concentrations above 11 mg per dl supports the findings of Slyter and Satter (1979), Hume et al. (1970), and Hespell (1979), that ammonia-nitrogen concentrations above 11 mg per dl are sufficient to maximize yield of microbial protein. However, this contrasts with results of Edwards and Bartley (1979) who observed increases in ruminal microbial protein synthesis in vitro up to ammonia-nitrogen concentrations of 76 mg per dl. Buffering capacity of ammonia can easily prolong fermentation in vitro.

Starch digestibility effects appear complex. Although ruminal ammonia was negatively correlated with starch digestibility ($P < .02$), rate of digestion of corn starch increased with an ammonia concentrations

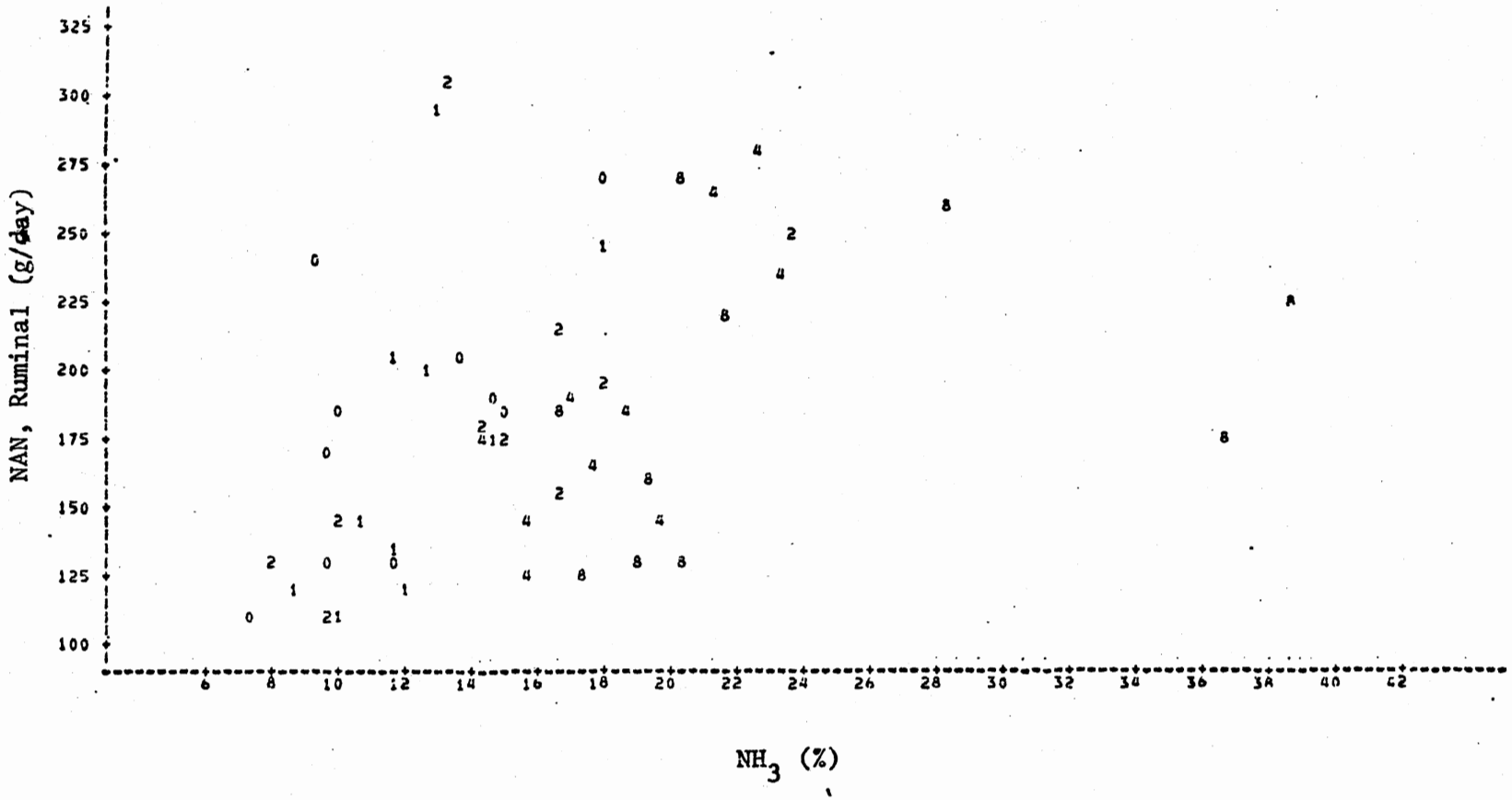

Figure IV. Non-ammonia nitrogen, whole rumen fluid versus ammonia nitrogen.

Legend: 0 = basal ration; 1 = basal ration + 0.312% urea; 2 = basal ration + 0.625% urea; 4 = basal ration + 1.25% urea; 8 = basal ration + 2.50% urea.

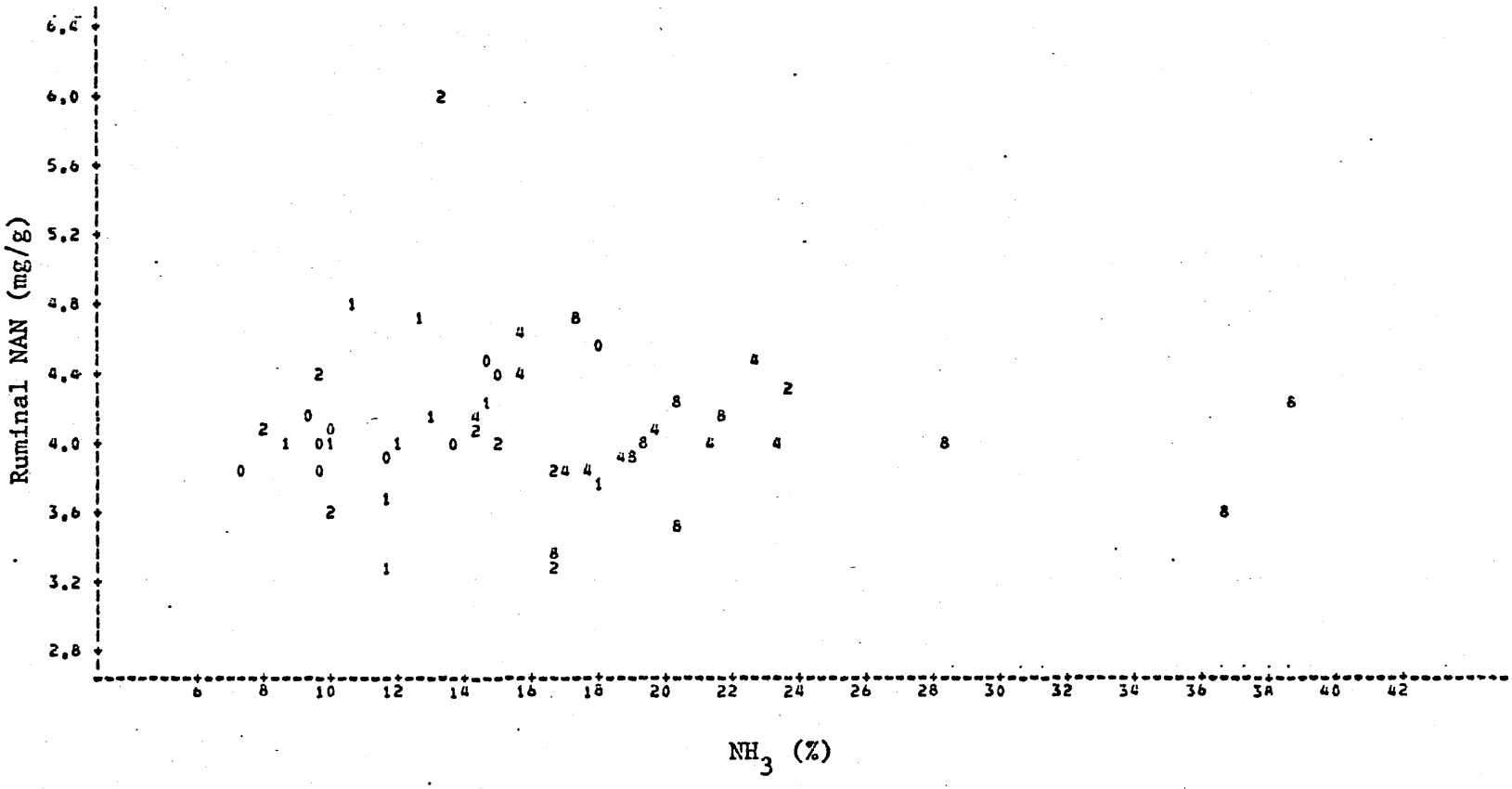

Figure V. Ruminal non-ammonia nitrogen versus ammonia nitrogen.

Legend: 0 = basal ration; 1 = basal ration + 0.312% urea; 2 = basal ration + 0.625% urea; 4 = basal ration + 1.25% urea; 8 = basal ration + 2.50% urea.

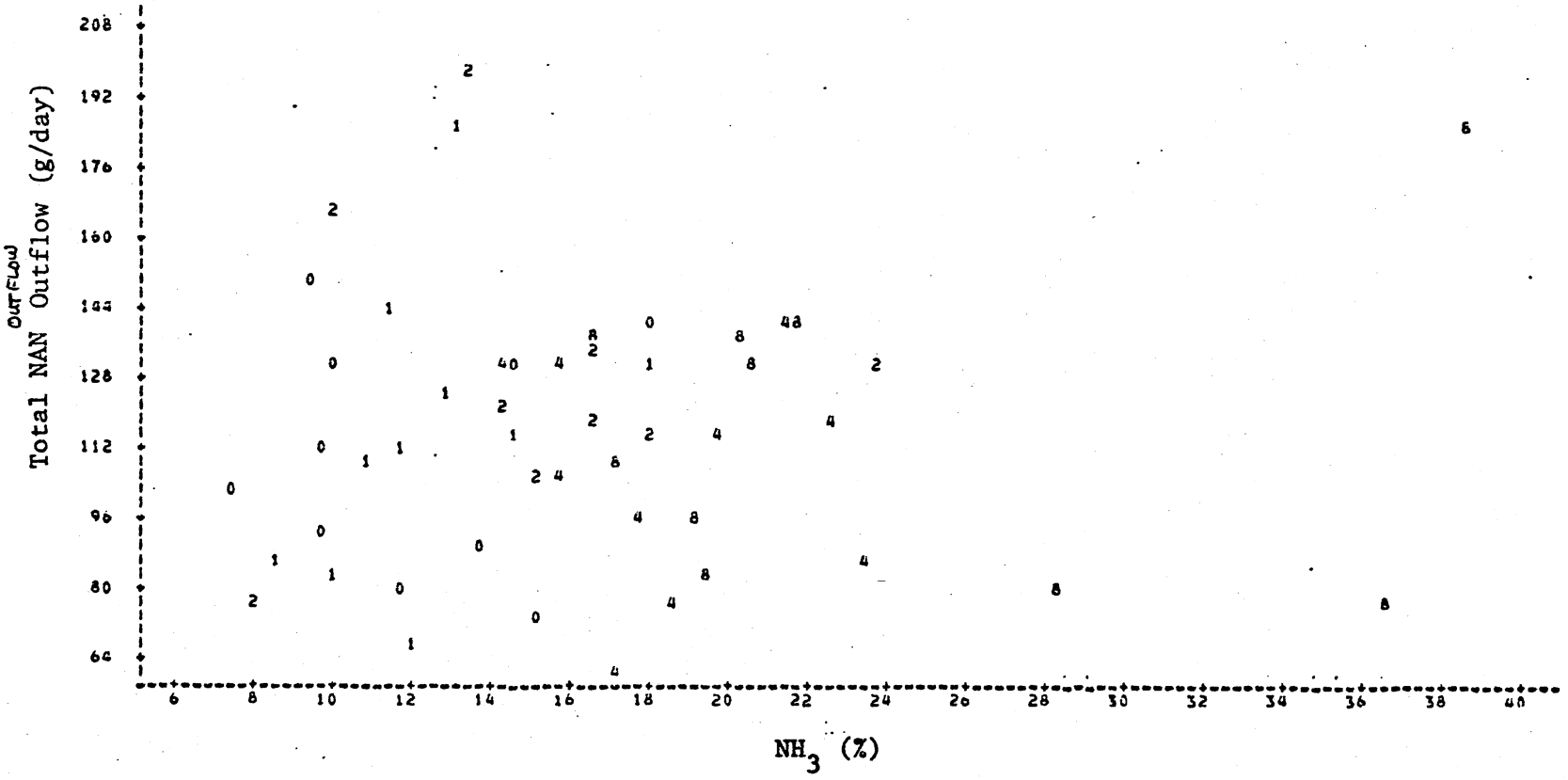

Figure VI. Total non-ammonia nitrogen versus ammonia nitrogen.

Legend: 0 = basal ration; 1 = basal ration + 0.312% urea; 2 = basal ration + 0.625% urea; 4 = basal ration + 1.25% urea; 8 = basal ration + 2.50% urea.

TABLE XVI. FRACTIONATION OF NON-AMMONIA NITROGEN

Item	Urea Supplementation					
	0	0.312	0.625	1.25	2.50	SE_m
Ruminal concentrations of NAN						
Liquid mg/ml	0.99	0.94	1.01	1.12	0.99	0.07
Solids mg/ml	19.04	19.69	19.46	16.64	18.24	1.40
Total mg/ml	1.82	1.74	1.86	1.91	1.87	0.10
NAN reaching the abomasum, g/day						
Solid fraction	46.6	48.3	52.5	34.0	50.3	6.8
Liquid fraction	63.6	67.2	73.5	73.0	67.6	6.5
Total	110.3	115.5	126.0	107.0	117.9	8.3

up to 23.9 mg per dl. This suggest an increased population of bacteria. The positive correlation of NAN concentration in rumen fluid ($P < .01$) supports this observation. Ruminal dry matter increased while ruminal volume and outflow rate decreased as urea was added. Consequently, *in situ* results are misleading when turnover rates change. Possibly, urea levels higher than that required to yield adequate ammonia for microbial growth may alter the site and extent of digestion. In summary urea supplementation of a high concentrate ration tended to increase both the ammonia-nitrogen concentration and nitrogen digestibility. Ammonia-nitrogen concentrations above 11 mg per dl did not increase ruminal non-ammonia nitrogen or outflow to the abomasum. However, ruminal volume appeared to be depressed linearly with added urea. In conclusion it appears that urea feeding may influence ruminal metabolism by altering, 1) availability of ammonia for microbial and protein production, and 2) site and extent of digestion through ruminal retention time.

Estimating ruminal microbial needs for ammonia by either ruminal non-ammonia nitrogen concentrations or nylon bag digestion rate appear futile. Measurement of total yield or of microbial protein reaching the abomasum is needed. Controversy remains over the ammonia concentration needed for maximal microbial production. Discrepancies between *in vitro* and *in vivo* effects may be attributed partially to the various physiological effects of urea or ammonia.

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APPENDIX A

MICRO-AMMONIA DETERMINATION

Micro-Ammonia Determination

Reagents: A. Phenol: 50 g Phenol (C_6H_5OH)
0.25 g sodium nitroferricyanide
distilled water to one liter volume

B. Sodium Hypochorite: 25 g Sodium Hydroxide (NaOH)
16.8 Sodium Hypochlorite distilled
water to one liter volume

Standards:
1. Dry Ammonium Chloride (NH_4Cl) in oven overnight
2. Weigh out 0.385 g/100 ml.
3. Add one drop acid to lower pH to about 3, then bring to volume with distilled water
4. Dilute to 1, 5, 10, 25 mg/100 ml

Procedure:
1. Turn on 37°C water bath
2. Pipet in duplicate, .02 ml, .1 ml, or .2 ml sample (depending on estimated ammonia concentration in sample), standards, and distilled water for blank.
3. Add 1 ml Phenol reagent to each tube.
4. Mix
5. Add 1 ml Sodium Hypochlorite reagent to each tube.
6. Mix
7. Incubate at room temperature 30 min., 37°C for 15 min. or 50°C for 7 min.
8. Add 8 ml distilled water.
9. Mix well. At this point color is stable and tubes can be read or stored for 4 hours.
10. Use blank to set slit width of the spectrophotometer at 630 nm and read tubes (Absorbance range = 500-660 nm).

APPENDIX B

FRACTIONATED RUMINAL NON-AMMONIA NITROGEN

Fractionated Ruminal Non-Ammonia Nitrogen

Solid NAN[a] (Rumen)

Total N_R[b] mg/100 g - (NH_3-N mg/100 ml x % H_2O_R) = mg NAN/100 g_R

Total N_L[c] mg/100 g - (NH_3-N mg/100 ml x % H_2O_L) = mg NAN/100 g_L

(mg NAN/100 g_L ÷ % H_2O_L) x % H_2O_R = mg NAN/100 g_{LC}[f]

mg NAN/100 g_R - mg NAN/100 g_{LC} = g NAN/100 g_S[d]

g NAN/100 g_S ÷ g DM/100 g_S = mg NAN/g DM_S

(mg NAN/g DM_S ÷ Cr mg/g DM) x g Cr Dosed = NAN_S

Liquid NAN (Rumen)

(mg NAN/100 g_{LC} ÷ PEG mg/ml) x g PEG Dosed = NAN_L[e]

Total G. NAN in Rumen

g NAN_S + g NAN_L = Total NAN_R

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[a]Non-ammonia nitrogen.

[b]Total nitrogen in raw rumen fluid.

[c]Total nitrogen in luquid fraction.

[d]Non-ammonia nitrogen associated with solid fraction.

[e]Non-ammonia nitrogen associated with liquid fraction.

[f]Liquid fraction corrected for solids.

APPENDIX C

FLOW OF NAN OUT OF RUMEN

Flow of NAN[a] Out of Rumen

(g NAN_S[b] x dilution rate %/hr) x 24 hr = g NAN_S/day

(g NAN_L[c] x dilution rate 5/hr) x 24 hr = g NAN_L/day

g NAN_S/day + g NAN_L/day = Total $NAN_{outflow}$

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[a]Non-ammonia nitrogen.

[b]Non-ammonia nitrogen associated with solid fraction.

[c]Non-ammonia nitrogen associated with liquid fraction.

APPENDIX D

RNA ANALYSIS

RNA Analysis

Sample Preparation:

A. Homogenize rumen samples (strained through double layer of cheescloth).

B. Weigh out .4-.6 g of very finely ground solid (or 25 ml. of the liquid portion of homogenized sample).

Purification Procedure:

A. Extract 10 min. @ 25°C in 20 mls ethanol-NaCl
Spin (15 min. 12,000 x g) and decant

B. Extract 10 min, @ 70°C in 20 mls ethanol
Spin (15 min., 12,000 x g) and decant.

C. Extract 10 min. @ 70°C in 20 mls ethanol-NaCl
Spin (15 min, 12,000 x g) and decant (repeat until the supernatant remains colorless.

Extraction:

A. Extract nucleic acid from moist residue with 10 ml. of 10% NaCl for 30-60 min. @ 100°C in plastic stoppered centrifuge tubes.

B. Centrifuge 5 min. @ 12,000 x g (decant supernatant and save).

C. Extract residue a second time for 30-60 min. at 100°C and filter the suspension while not through 4.25 cm discs of Whatman No. 1 filter paper with suction.

D. Wash centrifuge tubes with 5 ml of water then pour through the residue on filter paper. Add filtrate to supernatant from first extraction and chill in ice water.

Precipitate:

A. Precipitate nucleic acids by adding 5 mls. of cold 10% trichloroacetic acid.

B. Stir suspension and keep 1 hr. @ 0°C.

C. Centrifuge 10 min. @ 27,000 x g @ 0°C.

D. Discard supernatant

E. Wash pellet with .2 NPCA and centrifuge (10 ml PCA).

F. Solubilize in 7 mls .5 N perchloric acid for 46 min. in a 90°C water bath and mix periodically.

G. The supernatant resulting after centrifugation is decanted through glass wool into a 10 ml volumetric.

H. The ppt. in the tube is washed in 1 ml .5 N PCA and filtered into the 10 ml vol.

I. Glass wool is rinsed and the flask made to volume with .5 N PCA.

J. This solution is used for orcinol procedure.

Solutions:

ETOH-NaCl - 800 ml, 95% ETOH and 200 ml 10% NaCl

10% NaCl - 100 g NaCl per 1000 ml

10% TCA - 100 g TCA per 1000 ml

.5 N PCA - 4.25 ml PCA per 100 ml

Orcinol - .6 g orcinol per 10 ml 95% ETOH

HCl - .5 ml of 10% $FeCl_3$ .6 H_2O in 100 ml HCl

.1 N KOH - 5.6 g KOH per 100 ml

RNA - .0025 g per 25 ml of .1 N KOH

APPENDIX E

ADJUSTED TREATMENT SUMS OF SQUARES

Adjusted Treatment Sums of Squares

1) Trt. SS - IASS - ESS = SS corrected for protein

2) Trt. SS - IASS - PSS = SS corrected for energy

3) IASS

Trt. SS = Treatment sums of squares

IASS = Sums of squares associated with the interaction

PSS = Sums of squares associated with level of protein

ESS = Sums of squares associated with energy level

VITA

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