

Supplemental protein sources for growing
cattle fed corn stover silage


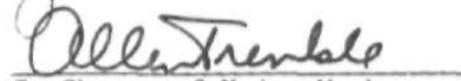

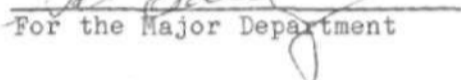
by

Ali Moses Adamu

A Dissertation Submitted to the
Graduate Faculty in Partial Fulfillment of the
Requirements for the Degree of
DOCTOR OF PHILOSOPHY

Department: Animal Science
Major: Animal Nutrition

Approved:


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In Charge of Major Work

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For the Graduate College



Iowa State University
Ames, Iowa

1985

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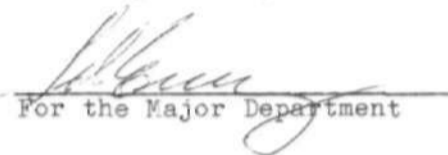
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Under the supervision of J. R. Russell and A. Trenkle
From the Department of Animal Science
Iowa State University

Four experiments were conducted to evaluate different sources of protein as supplements for growing cattle fed corn stover silage.

Isonitrogenous and isocaloric supplements containing soybean meal (SBM), dehydrated alfalfa (DEHY ALF), corn gluten meal (CGM), casein (C), urea (u) or a negative control diet (CNL) were compared in a digestion trial. Source of protein did not affect feed intake of the steers. Digestibilities of dry matter (DM) and organic matter (OM) were not affected by source of protein. Crude protein (CP) was most poorly digested by steers fed the DEHY ALF containing supplement. Digestion of CP by steers fed the CGM, SBM and U containing supplements was similar. Neutral detergent fiber (NDF) digestibility was not affected by source of protein. In a feedlot experiment involving 60 heifers and lasting 98 d, protein supplements containing U, SBM, formaldehyde treated SBM (TSBM), TSBM+U, TSBM+C or CNL supplement were studied. Crude protein and metabolizable protein content of the supplements were: 21.7, 8.0; 21.7, 10.5; 14.9, 10.8; 21.7, 10.8; 21.7, 10.9 and 14.9 and 9.0%, respectively. U, SBM, TSBM+U and TSBM+C supplementation resulted in higher rates of

growth and improved feed utilization over CNL and TSBM. Ruminant ammonia concentration and DM digestibility were higher for animals fed the U supplemented diet than animals fed all other supplements.

The effects of varying ruminal ammonia (NH_3) concentration on utilization of corn stover were also studied in a digestion and a feedlot trial. The protein supplements, SBM and 5 supplements containing TSBM along with 0.0, 1.43, 2.47 or 4.47% urea contained 20.9, -2.5; 14.4, 0.0; 18.4, -1.5; 21.3, -2.5; 24.1, -3.5; and 26.9 and -4.5% CP and urea fermentation potential, respectively. Ruminant NH_3 concentration increased linearly with increasing level, of urea in the diet. Digestion of OM and NDF increased with increased urea in the supplement and approached maximum when urea was 3.47% of the supplement. Microbial N synthesis increased with urea level and tended to plateau at 3.47% urea. Increasing urea level in the diet did not affect microbial N synthesized per kg OM digested in the rumen. Average daily gain and feed efficiency of heifers fed the 6 supplements increased linearly and quadratically with increasing urea in the supplement. It is estimated that ruminal NH_3 concentration for maximum microbial N synthesis and utilization of corn stover is about 9.32 mg/dl.

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LIST OF ABBREVIATIONS

AA	Amino acid/amino acids
AAP	Amino acid profile
ADF	Acid detergent fiber
AEP	Aminoethylphosphonic acid
AEP-N	Aminoethylphosphonic acid nitrogen
ATP	Adenosine triphosphate
BCFA	Branched chain fatty acids
C	Casein
CELL	Cellulose
CGM	Corn gluten meal
CHOH	Carbohydrates
CNL	Control
CP	Crude protein
d	day
DAP	Diaminopimelic acid
DEHY ALF	Dehydrated alfalfa
dl	deciliter
DM	Dry matter
DNA	Deoxyribonucleic acid
g	grams
GDH	Glutamate dehydrogenase
GS	Glutamine synthetase
h	hours

kg	kilograms
l	liters
LSD	Least significant difference
M	meters
MCP	Microbial crude protein
min	minutes
ml	milliliters
MP	Metabolizable protein
N	Nitrogen
NAN	Nonammonia nitrogen
NDF	Neutral detergent fiber
NH ₃	Ammonia
NH ₃ -N	Ammonia nitrogen
NPN	Nonprotein nitrogen
OM	Organic matter
RNA	Ribonucleic acid
SAS	Statistical analysis systems
SBM	Soybean meal
TDN	Total digestible nutrients
TSBM	Formaldehyde treated soybean meal
U	Urea
UFP	Urea fermentation potential
VFA	Volatile fatty acids

GENERAL INTRODUCTION AND REVIEW OF LITERATURE

Enormous quantities of crop residues, including those of corn and sorghum, are produced annually in the major cereal producing areas of the world. From 3.3 to 4.5 metric tons per hectare of cereal crop residues are produced annually in the United States and it is estimated that the utilization of half of this material would meet the winter (150 days) energy needs for 35 to 40 million beef cows (Ward, 1978). Thus, crop residues offer a large potential source of energy for ruminant farm animals.

Although corn stover contains considerable potential energy, it is classified as a low quality roughage because it may be deficient in nitrogen, minerals, vitamins, phosphorus and sulfur. Because of this, it has traditionally been used as maintenance feed for nonlactating and early gestating cows.

The nutritional value of a roughage to ruminants depends largely upon its intake and digestibility. Nitrogen intake is a major factor influencing the intake of low quality roughages by ruminants. Greater roughage intake and more efficient utilization of low quality roughages have resulted by increasing dietary nitrogen through supplementation with natural proteins (Ammerman et al., 1972; Burroughs et al., 1978; Horton and Nicholson, 1981) and with nonprotein nitrogen (NPN) (Ammerman et al., 1972; Falen et al., 1968; Holter and Kabuga, 1974). The rumen microbial population requires a source of nitrogen to ferment the fibrous constituents of low quality roughage and synthesize microbial protein.

Traditional measurements of nitrogen utilization in ruminants are imprecise because they do not differentiate between a protein shortage to the rumen bacteria or to the animal. With the present understanding of nitrogen metabolism in the rumen and the development of procedures for separating feed and bacterial nitrogen leaving the rumen, it has become possible to specifically evaluate the ability of a nitrogen source to support rumen fermentation and to promote the synthesis of microbial protein for the animal. Substitution of NPN for plant and animal sources of protein in a diet often lowers the cost of supplementation. This practice also relieves competition with humans and other nonruminant animals for plant and animal proteins that may be scarce or costly at certain periods of time or in various regions of the world. But, supplementation with NPN often results in lower animal performance. The optimal combination is likely to be a specific amount of ruminally available protein from NPN combined with a specific amount of a type of protein that will escape ruminal fermentation but be digested in the small intestine. Whether a slowly degraded supplemental protein together with a source of NPN is beneficial has been the subject of many research endeavors.

In order to improve corn stover utilization, while maintaining efficient use of protein, this study was conducted to:

1. Investigate the effect of varying the degradability of the protein supplement for growing beef animals fed corn stover silage.

2. Study the effect of varying the concentration of ruminal ammonia on the utilization of corn stover silage by growing beef animals.

This review of the literature concerns mainly nitrogen metabolism in the rumen and its effect on the utilization of energy from roughages by ruminant animals.

Rumen fermentation includes two major processes: microbial degradation of dietary compounds, mainly carbohydrates (CHOH) and proteins, and the synthesis of organic macromolecules in microbial biomass, mainly proteins, nucleic acids, CHOH and lipids.

It is not considered necessary to present an exhaustive discussion on CHOH and lipid metabolism because of the nature of the study but it must be emphasized that protein and energy metabolism are intricately interrelated, especially in ruminant animals. It has been well-documented that the rumen microbial populations degrade dietary CHOH and lipids to obtain energy and nutrients for the synthesis of their biomass and in the process produce by-products that serve as energy (volatile fatty acids) and protein (microbial protein) for the host animal. Eloquent reviews on this topic have been presented (Taminga, 1979, 1982; Baldwin and Allison, 1983).

Protein Degradation in the Rumen

Numerous studies investigating the extent of ruminal degradation of dietary proteins have been summarized (Chalupa, 1975; Hogan, 1975; Satter et al., 1977; Armstrong, 1976; Taminga and Van Hellemond, 1977; Taminga,

1979; Owens and Bergen, 1983; Stern and Satter, 1982). Feed protein may be extensively degraded in the rumen by both bacteria and protozoa into peptides and amino acids (AA), which are ultimately deaminated to form ammonia (NH_3) (Demeyer, 1976; Prins, 1977). Degradation of proteins is necessary to provide microbes with required precursors for their own protein synthesis, including NH_3 and presumably α -keto acids or intact AA. There is no one value for ruminal degradation of a particular protein as this varies with the feeding conditions and a host of bacterial factors and protein characteristics.

Measurement of Protein Degradation

Measurement of protein degradation in the reticulorumen has been accomplished by either in vitro or in vivo methods (Taminga, 1979; Owens, 1982; Broderick, 1982; Owens and Bergen, 1983). Generally, in vitro procedures have involved measurements of protein solubility in various solvents, loss of protein or accumulation of NH_3 or AA in vitro, and loss of protein upon incubation with various proteases. Buffer and enzyme systems frequently used to determine digestibility include: diluted sodium hydroxide (Lyman et al., 1953), artificial saliva (Tagari et al., 1962; Wohlt et al., 1973), autoclaved rumen fluid (Wohlt et al., 1973), diluted solution of pepsin in 0.1N hydrochloric acid (Beever et al., 1977) and water at various temperatures (Mertens, 1977). Of all these methods, incubation with artificial saliva at body temperature has been considered the most useful (Mertens, 1977; Waldo, 1978).

Solubility has received more attention as a simple predictor of degradation than any other factor. Generally, soluble compounds are attacked more rapidly and digested more completely in the rumen than are insoluble compounds due, in part, to differences in microbial access. However, certain proteins, though soluble, appear to resist proteases in vitro. Soluble proteins from soybean meal (SBM), rapeseed meal and casein were hydrolyzed at different rates when incubated with enzymes and rumen microorganisms (Mahadevan et al., 1980). Because the AA composition of the more soluble fraction usually differs from that of the insoluble fraction (feeds whose major fractions are albumens and globulins have a higher solubility than feeds containing mainly prolamins and glutelins in their protein) (Wohlt et al. 1976), bypass for some of the AA may be greater than others (Stern and Satter, 1982). Thirty-five to 50% of proteins insoluble in ruminal buffers are degraded in the rumen (Tamminga, 1979), indicating that solubility alone is a poor predictor for extent of ruminal degradation. Other factors, such as disulfide crosslinking (Mahadevan et al., 1980) and composition of the diet require consideration.

Measurement of protein degradability in vivo is usually performed with surgically modified animals, equipped with cannulae in the rumen, abomasum or duodenum. Microbial protein in the abomasal or duodenal ingesta is determined and undegraded dietary protein is estimated as the difference between total and microbial protein. The microbial protein can be estimated by the use of specific markers, such as nucleic acids,

diaminopimelic acid (DAP), aminoethyl phosphonic acid (AEP), D-alanine or one of the radioisotopes ^{35}S , ^{32}P or ^{15}N (Clarke, 1977). The use of regression calculations for estimating undegraded protein have also been employed (Jarrige et al., 1978; Hvelplund et al., 1976).

A technique for in situ determination of dietary protein degradation in the rumen has been developed by Mehrez and Orskov (1977). It involves placing a sample of feedstuff in a dacron bag which is incubated in the rumen for a given time. Estimates of both the rate and the extent of protein degradation can be obtained by suspending several bags containing samples of the same feedstuff in the rumen and withdrawing them at different time intervals (Mathers et al., 1977; Mehrez and Orskov, 1977). The rate of disappearance of protein from the dacron bag may not represent rate of degradation, because soluble protein may be washed out without actually being degraded (Mohamed and Smith, 1977).

Other factors that affect protein degradation in the rumen are: protein structure, type of diet, level of feed intake and pH. Structural differences caused by disulfide bridges and crosslinking of the protein may be important determinants of degradability (Nugent and Mangan, 1978). Increased feed intake has markedly reduced protein degradation in dairy cattle (Taminga, 1979) and steers (Zinn and Owens, 1983a), because of a decreased residence time and altered fermentation characteristics in the rumen (Bergen and Yokoyama, 1977). Cattle fed forage diets, as compared with those fed diets containing higher amounts of concentrate, have a greater rate and extent of ruminal protein degradation in vitro (Ganev et

al., 1979; Rode, 1981) and in vivo (Zinn and Owens, 1983b), because of a higher rumen pH (Taminga, 1979).

Quantitative determination of dietary protein which escapes rumen fermentation is central to newly proposed systems for calculating the nitrogen requirements of ruminants. The systems utilize values for protein degradability and efficiency of microbial growth to derive estimates of protein flow to the small intestine (Burroughs et al., 1975; Kaufmann, 1977; Roy et al., 1977; Satter and Roffler, 1975; Journet and Verite, 1979; Agricultural Research Council, 1980).

Protected Proteins

The quantity of protein entering the small intestine is the sum of the microbial protein produced in the rumen, endogenous proteins like enzyme fragments, mucins and cellular debris and the feed protein that has escaped ruminal degradation. In animals having high amino acid requirements, such as high producing dairy cows or growing cattle, microbial protein alone may be insufficient to meet the demands for production of animal protein (Satter et al., 1977; Huber and Kung, 1981). In such cases, a supplementary supply of high quality protein at the absorption sites may increase productivity. A variety of chemical and physical modifications to reduce degradation of dietary protein have been studied and reviewed (Broderick, 1975; Chalupa, 1975; Ferguson, 1975; Barry, 1976; Amos, 1980, Waldo, 1977; Hudson et al., 1970; Hatfield, 1973; Rodriguez et al., 1973; Thomas et al., 1979). These modifications

include treatment with formaldehyde, tannins, wood molasses, volatile fatty acids and heat.

The general idea of treatment of proteins with chemicals is to create a reversible, pH dependent, chemical modification that will inhibit breakdown of the protein at the pH usually found in the reticulorumen (very often close to neutral), but still enable proteolysis at the much lower pH found in the abomasum and proximal duodenum.

The formaldehyde treatment was developed in Australia and is now the most common treatment of protein having led to commercially available products. Substantial increases, 6 to 50%, in post-ruminal protein flow after treatment of dietary protein with formaldehyde seem possible (Kaufmann and Hagemester, 1976; Hagemester, 1977; Thomas et al., 1979). Taminga (1979) showed that at formaldehyde concentrations of over 10 g/kg protein, a negative rather than a positive response in milk yield was obtained. This may be the result of overprotection, causing not only reduced degradation of protein in the rumen, but also decreased susceptibility to proteolytic enzymes in the abomasum and small intestine. Suggested optimum levels for application of formaldehyde are 0.8 to 1.2% formaldehyde per protein (w/w) for the protection of casein, soybean meal (Broderick, 1975; Thomas et al., 1979) and 3% for legume grass silage (Broderick, 1975).

Grain processing methods, such as steam flaking and fermentation, appear to alter ruminal digestion of both protein and starch through heat or solubilization. Care must be taken using heat treatment because

excessive heating causes Maillard reactions, which result in proteins being bound to CHO, making them indigestible in the small intestine.

Some proteins are naturally protected because of their component amino acids or presence of certain compounds (e.g., tannins in tropical legumes). Example of such proteins are: bloodmeal, fishmeal, brewers or distillers dried grains, feathermeal, coconut meal, corn gluten meal, and palm kernel meal. The low degradation of fishmeal may be the result of fish being preserved with formaldehyde.

Synthesis of Microbial Nitrogen Compounds

Rumen microbial biomass is an important source of protein to the ruminant. Of the protein reaching the duodenum, 40 to 80% is microbial crude protein (MCP), depending on several dietary and animal factors (Owens and Bergen, 1983; Pilgrim et al., 1970; Smith and McAllan, 1970b).

Bacterial growth in the reticulorumen requires the provision of NH_3 , AA, essential minerals, notably sulfur and phosphorus, and OM to provide both an energy source and structural units. The relative importance of ammonia nitrogen ($\text{NH}_3\text{-N}$) and preformed AA in supplying microbes with N or other metabolites remains controversial. Specific preformed compounds including AA or peptides, branched chain carbon compounds and other growth factors are required by certain bacterial strains, although their importance for the mixed bacterial population in the rumen is ill-defined (Allison, 1969; Bryant, 1973; Smith, 1979). Additions of small amounts of AA to purified diets containing urea have increased MCP yields (Maeng and Baldwin, 1976). Appreciable amounts of

preformed AA are directly incorporated by mixed rumen bacteria if supplied by the diet (Al-Rabbat et al., 1971; Nolan et al., 1976; McMeniman et al., 1976b). The efficiency of microbial growth, however, may not be altered by supplying AA in vivo (Theurer, 1979). It is believed that certain peptides and AA may serve as sources of branched-chain fatty acids (BCFA) that are growth factors for a number of bacterial species including the cellulolytic bacteria (Bryant, 1973; Russell and Hespell, 1981). Fiber digestion is dependent on a supply of BCFA from the diet or from other microbes in the rumen (Russell and Hespell, 1981).

Mechanism of Ammonia-N Fixation by Rumen Bacteria

It is accepted that additions of preformed AA may stimulate growth of certain bacteria but the importance of NH_3 as a N source for most of the rumen bacterial population often has been observed. Over 80% of bacteria isolated from the rumen grew well in media containing NH_4 as the sole source of N (Bryant and Robinson, 1962). Fifty to 80% of microbial N in the rumen is from NH_3 (Mercer and Annison, 1976; Hespell and Bryant, 1979), though a recent estimate with lactating dairy cows has been even higher (Oldham et al., 1980).

The understanding of the mechanism of NH_3 fixation is vital for a proper understanding of N use by the ruminant. Bogg (1959) concluded that based on ^{15}N incorporation into AA hydrolysates of rumen contents, glutamic and aspartic acids played an important role in NH_3 fixation in the rumen. More information was obtained by Salter et al.

(1979), who fed rumen cannulated steers, isonitrogenous and isocenergetic diets consisting of straw, tapioca and supplemental N, provided by different proportions of decorticated ground nut meal or urea. Using ^{15}N -urea, it was concluded that much of the NH_3 entering the cell was initially captured in the form of amide groups of glutamine and (or) asparagine. These groups are used for subsequent amination of α -ketoglutarate to glutamate, either after the release of NH_3 or by direct incorporation (Erfle et al., 1977). Synthesis of alanine and aspartate follows through transamination, and these compounds, together with glutamate probably accumulate initially in the free forms. Amino groups are then transferred to other suitable carbon skeletons for the formation of AA which together with preformed compounds, are used in protein and nucleic acid synthesis. The enzymes required for the first stages of such a scheme have all been identified in mixed rumen bacteria (Palmquist and Baldwin, 1966; Chalupa et al., 1970; Erfle et al., 1977; Blake et al., 1983) and a variety of mechanisms, some following established pathways and others using less common routes, have been identified as providing the carbon skeletons for AA synthesis (Allison, 1969; Emmanuel and Millgan, 1972; Kristensen, 1974; Sauer et al., 1975).

The enzymes, glutamate dehydrogenase (EC 1.4.1.2 and 1.4.1.4) (GDH) and glutamine synthetase (EC 6.3.1.2) (GS) are particularly important in the assimilation of NH_3 into bacteria (Erfle et al., 1977; Wallace and Henderson, 1978; Jenkinson et al., 1979). Erfle et al. (1977) showed that enzymes of NH_3 assimilation by rumen micro-organisms in vitro

could be regulated by the concentration of NH_3 . The K_m of GDH for NH_3 was 56.1 mg/dl while that of GS was 3.06 mg/dl. It has been suggested that GDH plays the major role in NH_3 uptake (Allison, 1969; Smith et al., 1978), with GS providing an efficient means of glutamate synthesis at low rumen NH_3 concentrations (Erfle et al., 1977; Smith et al., 1978; Jenkinson et al., 1979). Other enzymes such as alanine dehydrogenase (EC 1.4.1.1) and asparagine synthetase (EC 6.3.1.1) have been identified in rumen bacteria (Burchall et al., 1964; Erfle et al., 1977; Wallace and Henderson, 1978), but their role remains unclear.

Source of Nonprotein Nitrogen

Substitution of NPN for plant and animal protein in diets for ruminants often lowers the cost of supplementation. Diets that contain over 98% of N as NPN have been fed to cattle and sheep (Virtanen, 1966; Oltjen, 1969). Dietary NPN generally is regarded to be useful only as a source of NH_3 for the ruminal bacteria, but it also serves as a base in the rumen to maintain pH in a desirable range for cellulose digestion. NPN may also improve the energetic efficiency of ruminal microbes as a result of the frequent feeding behavior of livestock fed it (Owens and Bergen, 1983). Post-ruminal administration of NPN may be useful because of cycling of N to the rumen or large intestine (Owens and Bergen, 1983).

Many sources of NPN have been tested for use in ruminant diets. These include: anhydrous NH_3 , applied as a liquid or gas to high or low quality forages (Huber and Kung, 1981), ammonium salts (chloride, phosphate, and lactate) and feed grade urea, containing 287% crude

protein equivalent (Briggs, 1967; Tillman and Sidhu, 1969). Other forms of NPN which have been used in ruminant diets include biuret, triuret, cyanuric acid and complexes of urea with formaldehyde, molasses and monosaccharides (Nikolic et al., 1980; Smith et al., 1976; Milligan et al., 1972). Sulfur coating of urea also has been used (Umunna and Woods, 1970).

Slow release NPN compounds have been developed to avoid NH_3 toxicity and improve the utilization of NH_3 . The first goal can be realized by the use of urea complexed to starch (STAREA), biuret, certain coating materials and complexes of urea with formaldehyde, molasses or sugar. These materials release NH_3 in the rumen at an attenuated rate that should more closely parallel energy availability for bacteria (Johnson, 1976). Slow release compounds, however, generally have not improved the utilization of N as measured by performance of cattle in laboratory or field trials (Forero et al., 1980; Mizwicki et al., 1980; Owens et al., 1980). Perhaps N recycling to the rumen compensates for the rapid NH_3 release, by maintaining an adequate supply of NH_3 in the rumen for several hours after peak ruminal NH_3 concentrations.

Rumen Ammonia Concentrations in Rumen Bacterial Synthesis

Although the maximum rate of fermentation and production of microbial protein per unit of substrate fermented depends largely on ruminal NH_3 concentrations, the optimal concentration of NH_3 for both may not coincide. Orskov et al. (1972) showed that, although microbial protein produced per unit of substrate fermented was not

altered as a result of urea supplementation of barley grain, the extent of rumen fermentation and digestibility was increased.

There is no general agreement on the ruminal NH_3 concentrations for maximum microbial protein production. Estimates of the optimal NH_3 concentration for microbial protein synthesis have ranged from 3.5 to 29 mg/dl (Satter and Slyter, 1974; Hespell and Bryant, 1979; Slyter et al., 1979). Most in vitro studies, however, have shown maximum microbial growth to occur when the NH_3 -N concentration was 5 to 8 mg/dl (Allison, 1970; Satter and Slyter, 1974; Annison, 1975; Nikolic et al., 1975; Okorie et al., 1977). Hume et al. (1970) observed in vivo that microbial growth attained a maximum level when rumen NH_3 -N concentration reached approximately 9 mg/dl. In contrast, Miller (1973) found a considerably higher value of approximately 29 mg/dl. More recent in vivo work by Okorie et al. (1977) indicated that maximal protein synthesis was achieved when the rumen NH_3 -N concentration reached 5 mg/dl, an observation consistent with the in vitro observations of Satter and Slyter (1974).

The rate of rumen fermentation has a great influence on both total and digestible feed intakes (Balch and Campling, 1962). Therefore, feed intake may be reduced if NH_3 concentration is limiting the rate of fermentation. Mehrez et al. (1977) used the dacron bag technique in situ to predict that the rumen NH_3 -N concentration for maximum rate of fermentation in animals fed a barley diet was 23.5 mg/dl, which does not agree with the in situ findings of Ortega et al. (1979) that

progressively increasing rumen $\text{NH}_3\text{-N}$ from 6.3 to 27.5 mg/dl did not result in any significant changes in the rate of fermentation. Some of the differences in ruminal NH_3 concentration obtained are due to different sampling techniques and variations in rumen NH_3 concentrations post feeding. The nitrogen requirements of bacteria associated with feed particles may be different from bacteria free in the rumen fluid. Cheng and Costerton (1980) showed that large numbers of bacteria adhered to feed particles by means of exopolysaccharide fibers and form microcolonies. The NH_3 concentrations in the environment of these microcolonies are likely to be different from those of rumen fluid. Allison (1980) postulated that the high NH_3 concentration for maximal bacterial synthesis and rates of fermentation in sheep on a barley diet reported by Mehrez et al. (1977) as compared with the low NH_3 concentration necessary for optimal microbial synthesis found by Satter and Slyter (1974) in a continuous culture study, could be due to formation of microcolonies on starch granules in the earlier case. Because of the high energy supply in the experiment reported by Mehrez et al., NH_3 assimilation rate was also high and the NH_3 concentration in the microcolonies was likely to be considerably lower than the concentration actually measured.

It has been suggested, from pure culture studies, that many of the predominant bacterial species produced GS, which has NH_3 saturation constants of <85.0 mg/dl, indicating that their maximum growth in continuous cultures would not be lowered more than 5% if NH_3 was 2.89 mg/dl (Schaefer et al., 1980).

Ruminal Ammonia Concentrations and Nitrogen Recycling

Ruminal distribution of NH_3 and postprandial changes complicate sampling and interpretation and may limit N availability in some locations in the rumen (Wohlt et al., 1976; Egan, 1980). Nitrogen recycling to the rumen is unique to ruminants and serves to augment the ruminal NH_3 concentration of ruminants fed low N diets. Nitrogen is conserved by decreasing urinary excretion of urea (Schmidt-Nielsen, 1977) for recycling to various portions of the digestive tract. Twenty-three to 92% of the plasma urea is recycled to the digestive tract, with higher values associated with lower N intakes (Kennedy and Milligan, 1980). The quantity of N recycled to the rumen appears to be negatively related to ruminal NH_3 concentrations and positively related to the plasma urea concentration and the OM digested in the rumen. Plasma urea enters the rumen via the saliva and by attenuated diffusion through the ruminal epithelium. High ruminal NH_3 concentrations reduce recycling either by: 1) inhibiting urease in the bacteria associated with the ruminal wall or 2) decreasing the NH_3 diffusion gradient. Incorporation of recycled N into MCP causes daily duodenal N flow to exceed N intake on animals fed low protein diets (Chamberlain and Thomas, 1979). In animals fed high protein diets, however, a net loss rather than a net gain of N in the rumen is more commonly seen. Besides N, other nutrients, notably P and S, are also recycled into the rumen.

Efficiency of Bacterial Growth in Terms of Energy Supply

Once the N need is recognized and quantified, it is relatively easy and cheap to ensure that the NH_3 supply does not limit bacterial growth in the rumen. A more difficult problem is to determine and maximize the yield of microbial protein in terms of energy ingested. The process of microbial protein synthesis is essentially a two stage one, involving the efficiencies with which ATP is first generated and then used. The process has been reviewed by Bergen and Yokoyama (1977) who described some of the ways in which these efficiencies may be expected to vary.

Individual values for the efficiency of bacterial growth varies widely (Smith, 1975). From a literature survey of such data, a mean value of 30 g microbial N incorporated/kg OM apparently digested in the rumen was derived which corresponded to a mean yield of microbial DM g/mole ATP (Y_{ATP}) of 14.5 (Smith, 1979). It has been shown in vitro that although Y_{ATP} is only about 10.5 for batch cultures (Forest and Walker, 1971), theoretically it can be as high as 25 for rapidly growing continuous cultures in which maintenance costs are minimal (Stouthamer and Bettenhausen, 1973; Isaacson et al., 1975).

A number of recent reviews have dealt with factors affecting microbial synthesis in the rumen (Smith, 1979; Stern and Hoover, 1979; Armstrong, 1980; Harrison and McAllan, 1980; Tamminga, 1980). These include N concentration, N source, level of intake and dilution rate. Other factors are dietary sulfur and frequency of feeding. Inadequate

N supply to the rumen bacteria affects rumen function in a number of ways. Digestion of starch, cellulose and other structural polysaccharides is depressed by a N deficiency to differing extents (McAllan and Smith, 1976). As a result of N deficiency, bacterial metabolism is diverted from protein synthesis to storage of polysaccharides (Walker and Nader, 1970; McAllan and Smith, 1977). The reduced growth rate means that a greater proportion of ATP is likely to be used for bacterial maintenance and less for protein synthesis (Isaacson et al., 1975) as evidenced by an increase in the proportion of microbial OM turning over (Smith and McAllan, 1971).

Determination of Rumen Microbial Protein Synthesis

The relative contribution of microbial and undegraded feed proteins to the total protein arriving at the duodenum is important in estimating protein requirements of ruminants (Kaufmann, 1977; Burroughs et al., 1975; Roy et al., 1977; Satter and Roffler, 1975; Journet and Verite, 1979).

Several natural markers and isotopic labels have been used to quantify the proportions of microbial protein in ruminant digesta. Once determined, the proportion of microbial protein can be used to estimate, by difference, the amount of dietary protein not degraded in the rumen. It would be desirable if markers of bacterial N occur only in bacteria at constant amounts and could be accurately and easily determined in a representative sample of rumen microbial population and digesta. The natural markers frequently used include RNA (McAllan and Smith, 1972;

Ling and Buttery, 1978; Zinn and Owens, 1982), DAP (Hutton et al., 1971), D-alanine (Garrett et al., 1982) and AEP, which has been used to measure the protozoal component in microbial protein (Hagemeister, 1975; Ling and Buttery, 1978).

The use of natural markers involves estimating the marker:N ratio in mixed rumen bacteria and the concentration of marker in digesta. From this, the concentration of the bacterial N in the digesta can be calculated (Hogan and Weston, 1970), and if digesta flow is estimated, total bacterial N production may be estimated.

DAP occurs in the cell wall of several bacteria, and is easily quantified with acid ninhydrin after the separation with ion exchange chromatography (Hutton et al., 1971). The main objection to the use of DAP is that its concentration varies from species to species (Work and Dewey, 1953). This means that the ratio, DAP:N of the bacterial fraction must be determined for each experimental situation. Another problem with DAP is that it has been detected in the hydrolysates of protozoa (Theurer, 1982; Weller et al., 1958; Hutton et al., 1971; Czerkawski, 1974; Ling and Buttery, 1978). DAP, however, present in protozoa could be attributed to engulfment of bacteria (Coleman and Hall, 1969; Thomas, 1973; Stern et al., 1977a,b). DAP has also been found in some feedstuffs (Theurer, 1982 and Czerkawski, 1974), although Ibrahim et al. (1970) and Ling and Buttery (1978) did not detect DAP in dietary constituents. When using DAP as a marker, it is important to remember that DAP is not a constituent of bacterial protein. Thus, any change in the ratio of

bacterial protein to bacterial cell wall, e.g., lysis of cells between the rumen and the digesta sampling site, may introduce bias.

The use of nucleic acids for microbial protein synthesis depends on the following factors: (1) the ability to acquire a satisfactory post-ruminal sample for analysis; (2) a reliable nucleic acid to protein ratio; (3) an insignificant contribution of non-microbial (dietary and endogenous mammalian) nucleic acids to the sample; and (4) a satisfactory analytical procedure for nucleic acids or their constituents. Analysis of rumen microorganisms taken from sheep fed diets devoid of nucleic acids, indicated that a relatively constant amount (14-18%) of the total microbial N could be attributed to nucleic acid N (Ellis and Pfander, 1965). Highly significant correlations were obtained between the concentration of total nucleic acid N ($r=0.80$) and RNA ($r=0.72$) and total microbial N. Smith et al. (1968) reported similar results in their work with rumen fluid from calves fed diets of various roughage to concentrate ratios. It was found that about 19% of the total microbial N was in the form of microbial nucleic acid-N, irrespective of the N content of the diet. Although literature cited previously indicate that nucleic acids are good indicators of microbial protein synthesis, some studies have noted variations in the nucleic acid concentrations related to rate and stage of microbial growth. In mature bacteria, nucleic acids account for 10 to 15% of the cell DM, while in rapidly growing bacteria they may account for as much as 21% of the cell DM (Adams et al., 1976). Another consideration in the use of nucleic acids as microbial markers

concerns the engulfment of bacteria by protozoa, making it difficult to characterize protozoa by their content of nucleic acids. Czerkawski (1975), in his study with relatively clean forms of protozoa and bacteria, however, found the nucleic acid to protein ratio of the protozoa to be 96% that of bacteria, suggesting a small difference between these organisms.

Samples for rumen bacterial analysis are usually taken at either the abomasum or proximal duodenum. It is felt that more uniform samples can be collected from the duodenum due to laminar flow as compared to abomasal sampling, which is subject to bias due to digesta stratification. It must be recognized that digestion of nucleic acid may occur in the duodenum. Extensive digestion of nucleic acid has been reported in the small intestine (Ellis and Bleichner, 1969; Smith and McAllan, 1971; Barnard, 1969) and duodenum (Jackson et al., 1977). Although secretion of nuclease prior to the pancreas is not reported, a backflow of pancreatic enzymes may result in some nucleic acid digestion in the duodenum.

A further consideration in the use of nucleic acids as a marker of bacteria concerns the extent of degradation of dietary nucleic acid in the rumen. This matter has been exhaustively investigated both with isolated and dietary nucleic acids (McAllan and Smith, 1968; Smith and McAllan, 1970a, 1970b; McAllan and Smith, 1973a and 1973b). It was reported that ruminal degradation of nucleic acids was essentially

complete in 1 hr, although substantial amounts of the purine and pyrimidine bases remained after 4 hrs of incubation.

Numerous methods of determining nucleic acid have been developed with varying accuracy. The method described by McAllan and Smith (1969) suffers from being very tedious, and accurate results are dependent upon using a satisfactory batch of chromatography resin. In order to overcome the analytical problems of the chromatographic method, Ling and Buttery (1976) adapted the much simpler method by Guinn (1966) so that it was suitable for rumen digesta samples. The method relies on extraction of the nucleic acid fraction and subsequent assay of the RNA using orcinol. Although the orcinol reaction is interfered with by the presence of DNA, this interference is minimal compared with the errors that would appear to be associated with chromatographic separation of the DNA from RNA. In addition, the effect of DNA could be corrected by determining the DNA content using the diphenylamine reaction. Efforts to develop a more satisfactory analytical method have yielded some hopeful results. Zinn and Owens (1982) reported a rapid assay for purine content of feeds and digesta by using silver nitrate. The procedure has the same biological basis as nucleic acids in polymer form. On the basis of quantitative recovery of the purine and pyrimidine bases following perchloric acid hydrolysis of nucleic acids (Marshak and Vogel, 1951) and the efficient separation of the bases using a strong cation exchange resin (Brown et al., 1974), Jackson et al. (1977) developed a procedure to quantify individual bases from ingesta by high performance liquid chromatography. This procedure has been modified by Koenig (1980) who reported that

although cytosine and adenine were found to hold most promise as markers of microbial protein synthesis, guanine, uracil and xanthine also could be utilized.

Abou Akkada et al. (1968) suggested the use of AEP as marker for protozoa. They detected no AEP in washed suspensions of mixed rumen bacteria or in pure strains of major rumen bacteria. The diet was also free of AEP. AEP in the rumen was mostly associated with solids, the small amount in the free state being probably due to ruptured protozoal cells. Similarly, Borhami et al. (1979) found that mean microbial N incorporation in the rumen contents using DAP and AEP as markers for bacterial and protozoa N was similar to the value obtained from ^{15}N incorporation in microbial cells. Ling and Buttery (1978), however, found AEP in samples of protozoa, bacteria and dietary constituents. Dufva et al. (1982) detecting no AEP in bacteria and different feeds and a high correlation ($r=.84$) between AEP-N:total protozoal N ratio, recommended that corrections for dietary AEP be made if AEP was used as a marker of protozoa. Both Theurer (1982) and Ling and Buttery (1978) found AEP unacceptable as a marker of protozoa.

D-alanine occurs in relatively constant amounts in the peptidoglycan component of most bacterial cell walls (Schleifer and Kandler, 1972). An enzymatic procedure for the analysis of D-alanine in the bacterial fraction of rumen contents and digesta has been proposed by Garrett et al. (1982). They observed high accuracy and precision of the procedure, but found a large variation in D-alanine and DAP concentrations of

bacteria isolated from cattle fed diets containing several different feeds. They, therefore, suggested that analysis of D-alanine in bacteria must be done in each experiment conducted.

Other markers which have been used for determining bacteria protein are: (1) amino acid profile (AAP), and (2) ATP (Forsberg and Lam, 1977). The use of AAP is based on the assumption that the profile of the digesta is the weighted sum of the various profiles contributing to it (Evans et al., 1975). The AAP is generated by computer, mixing in different proportions, the known profiles of the dietary and endogenous components arriving at the duodenum. The use of ATP is based on several assumptions: (a) ATP is ubiquitous in all living cells and absent in dead cells; (b) ATP concentration is similar in all microbes; and (c) the extraction and assay of ATP is relatively simple and inexpensive to perform. The concentration of ATP in rumen biomass, however, has been found to vary widely (Wolstrup and Jensen, 1978).

The most reliable methods for determining the microbial portion of the nonammonia nitrogen (NAN) flowing into the small intestine appear to be those using a nuclide label, normally ^{35}S , ^{15}N , or ^{32}P , which is incorporated into microbes after infusion of the label into the rumen (e.g., Mathison and Milligan, 1971; Leibholz, 1972; Beever et al., 1974; Mathers and Miller, 1980; Kennedy et al., 1984). The advantage of the ^{15}N isotope technique is that it deals directly with N.

The various methods used for separating microbial and feed N flowing into the small intestine have been extensively reviewed (Smith, 1975;

Buttery and Cole, 1977) and some of them have been compared (Smith et al., 1978; Ling and Buttery, 1978; Siddons et al., 1979; Harrison and McAllan, 1980; Mercer et al., 1980; Theurer, 1982; Kennedy and Milligan, 1984).

In comparisons between the AA profile and RNA techniques (Offer et al., 1979), the estimates of microbial protein production obtained with AA analysis were approximately 10% higher than those with RNA in two trials, but markedly lower in a third. In general, however, the results obtained with RNA have been higher than those obtained with other markers (Harrison and McAllan, 1980). In some instances (Smith et al., 1976), estimates of bacterial contribution to duodenal digesta using DAP were higher than estimates for the total microbial contribution obtained with ^{35}S and ^{15}N or ^{32}P . This may be partly due to DAP accumulation by bacteria (Harrison and McAllan, 1980). A comparison between the DAP, RNA or ^{35}S methods appeared to indicate a substantial contribution of protozoa to the duodenal microbial protein (Smith et al., 1976). Much of the variation observed in microbial protein production may be due to unrepresentative sampling of digesta, difficulties in isolating a pure bacterial fraction from the rumen or variation in the concentration of the marker in the cells.

The problem of assessing the bias of each technique is difficult, since no absolute standards are available. Following a comparison of the ^{35}S , DAP, RNA and AEP techniques, Ling and Buttery (1978) concluded that each technique had its own merits, but the most accurate

estimate of microbial protein synthesis was obtained by use of ^{35}S technique. The RNA method is very attractive since it requires no isotope, is much less complex to carry out and is more rapid than the ^{35}S method.

All techniques for microbial protein determination require the isolation of a representative sample of the microbial population which is entering the duodenum or abomasum for determination of marker concentration. Microbial samples are usually obtained by differential centrifugation of rumen liquor. Serious doubt must be cast on the assumption that representative samples of microbes are obtained by differential centrifugation. Many protozoa, large bacteria, aggregates of bacteria, bacteria closely associated with the rumen epithelium and bacteria firmly attached to feed particles (Weller et al., 1958) may be lost during this procedure. The bacterial fraction obtained after high speed centrifugation may represent only a small part of the total rumen bacterial population and may be less metabolically active than the greater number of bacteria that are associated with food particles (Smith, 1975). Although microbial markers present some problems, the variation in flow of the digesta marker may be more critical than the choice of the microbial marker (Theurer, 1979).

Effect of Source of Supplemental-N on the Utilization of Low Quality Forages

Various N sources have been used as supplements for animals fed roughages. The major differences between the sources are: (1) whether

they are of plant, animal or NPN origin, and (2) their ruminal degradability. Which protein supplement is used depends on availability, cost, storage and handling.

Microbial production and N flow to the duodenum

Several studies have indicated no differences in the amount and efficiency of microbial production between animals receiving supplements containing urea and protein N (Kropp et al., 1977a and b; McAllan and Smith, 1983, 1984; Sriskandarajah et al., 1982; Redman et al., 1980).

The influence of protein degradability on microbial protein production has been variable. Kellaway and Leibholz (1983) reported that increasing the concentration of urea in the diet of cattle fed sodium hydroxide-treated wheat straw did not improve amount of microbial protein production. Similar results have been obtained by Kropp et al. (1977a). Mahadevan et al. (1983), however, indicated that bacterial and protozoal protein synthesis in cows fed graded levels of formaldehyde-treated SBM decreased significantly with increasing concentrations of formaldehyde treatment because of decreased ruminal ammonia concentration.

Nitrogen flow to the duodenum increased linearly as N intake increases at low N levels (Hume et al., 1970; Allen and Miller, 1972), but at higher dietary N levels (Hogan and Weston, 1967; Beever et al., 1971; Al-Rabbat et al., 1971; Leibholz and Hartmann, 1972), the flow of N from the rumen is similar regardless of large differences in N intake.

Digestibility

Kropp et al. (1977a and b) showed that SBM was superior to urea for supporting digestion of DM and retention of N. Raleigh and Wallace (1963) fed a low quality hay supplemented with urea, cottonseed meal or urea plus cottonseed meal to steers at dietary crude protein levels of 6, 9 and 12%. They found that OM digestion increased when protein was increased from 6 to 12%, regardless of N source. The changes in digestibility between 6 to 9% and 9 to 12% were small. A similar result was obtained by Martin et al. (1981) using low quality pangola grass hay and graded levels of urea or biuret. Increasing N intake has not changed OM digestion in some other studies (Hume et al., 1970; Hume, 1970; Leibholz and Hartmann, 1972). Some previous research has reported no differences in apparent DM digestion by sheep due to source or level of N (Hume et al., 1970; Holter and Kabuga, 1974; Hume, 1970; Leibholz and Hartmann, 1972). However, Owens et al. (1973) with lambs, showed post-ruminal improvement in digestibility with urea supplementation.

Ammerman et al. (1972) fed sheep low quality pangola grass supplemented with different sources of N and found that source of protein did not affect fiber digestion, but the addition of N with an energy source increased apparent digestion of fiber and N. A similar result was obtained by Borhami et al. (1975) using rams fed corn stover hay supplemented with urea or cottonseed cake.

Horton and Nicholson (1981), compared soybean meal and alfalfa meal as protein supplements for steers fed barley grain and wheat straw and found that digestibilities of OM, CP and acid detergent fiber by the steers were increased by urea and SEM supplementation but not by alfalfa meal.

Intake

Intake of low quality forages is limited by inadequate N supply (Milford and Minson, 1965). Nitrogen supplementation has often increased voluntary intake of cereal straws (Campling et al., 1962; Horton, 1979). Several studies comparing sources of protein supplement for animals fed low quality forages indicate no differences between protein and NPN in improving feed intake (Holter and Kabuga, 1974; Kropp et al., 1977b; Ammerman et al., 1972). Some reports, however, indicate that animals fed protein containing supplements consumed more feed than do those fed urea containing supplements (Sriskandarajah et al., 1982; Clanton, 1978; NRC, 1976).

Increasing urea level in the diet has not improved intake in some experiments (Kropp et al., 1977b; deFaria and Huber, 1984; Martin et al., 1981), but other experiments have indicated improved feed intake with increasing urea in the diet (Church and Santos, 1981; Ernst et al., 1975; Jones et al., 1976).

Animal performance

Many reports dealing with N supplementation of beef animals wintering on low quality roughages have indicated that urea containing supplements were not as effective as natural protein supplements for

maintaining live weight (Nelson and Waller, 1962; Williams et al., 1969; Clanton, 1978; NRC, 1976). A similar result was obtained by Horton and Nicholson (1981) when comparing urea, soybean meal and alfalfa meal as supplements for steers fed barley and wheat straw. In contrast, similar daily gains have been obtained in growing cattle fed high straw diets supplemented with either plant protein or urea (Horton, 1979; Burris et al., 1975; Boling et al. (1972).

Burroughs et al. (1977) studied the effect of formaldehyde, wood molasses and heat treatments on the value of SBM supplements in high corn cob diets for calves. Protected SBM plus urea diets caused 20-26% improvement in live weight gains and feed utilization over similar diets containing untreated SBM and urea. In another experiment, Burroughs et al. (1978) found that corn gluten meal was superior to SBM as source of supplemental protein for calves fed a high corn cob diet containing urea.

Explanation of Dissertation Format

Sections I and II of the dissertation will be submitted for publication to the Journal of Animal Science. Section I will be submitted under the authorship of A. M. Adamu, J. R. Russell and A. Trenkle while Section II will be submitted under the authorship of A. M. Adamu, J. R. Russell, A. Trenkle and D. A. McGilliard. Information for the experiments discussed in Sections I and II but not included in the journal paper are presented in Appendices A, B and C.

SECTION I. UTILIZATION OF LOW QUALITY ROUGHAGES:
EFFECTS OF UREA AND PROTEIN SUPPLEMENTS
VARYING IN RUMINAL DEGRADABILITY ON
UTILIZATION OF CORN STOVER SILAGE BY
GROWING BEEF ANIMALS

UTILIZATION OF LOW QUALITY ROUGHAGES: EFFECTS OF
UREA AND PROTEIN SUPPLEMENTS VARYING IN RUMINAL
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GROWING BEEF ANIMALS

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SUMMARY

Two experiments were conducted to study the effect of varying ruminal degradability of the protein supplements on utilization of corn stover silage by growing beef cattle. In the metabolism trial (Experiment 1), 6 protein supplements were evaluated in a 6x6 Latin square using 6 Angus steers. The protein supplements, soybean meal, dehydrated alfalfa, corn gluten meal, casein, urea, or control, were formulated to be isonitrogenous at 17.25% CP and isocaloric at 56% total digestible nutrients except the control supplement which contains less CP (4.88%). The supplements were fed at 20% of diet dry matter with stover silage (8.19% CP). Protein supplementation improved ($P < .05$) feed consumption by the steers, but source of protein did not affect feed intake. Dry matter (DM) and organic matter (OM) digestibilities were not affected by protein supplementation. Crude protein was most poorly ($P < .05$) digested by steers fed dehydrated alfalfa and control supplements; the other supplements were not different. Neutral detergent fiber (NDF) digestibility was not different in steers fed the different protein sources and supplementation did not improve NDF digestion. Source of protein did not affect N-utilization but supplementation improved ($P < .05$) N-utilization. In experiment 2, feedlot performance of 60 Angus x Charolais heifers, initial weight 250 kg, given 6 protein supplements, was measured during a 98-d trial. Treatments included a negative control and treatments containing urea, soybean meal, formaldehyde treated soybean meal, formaldehyde treated soybean meal with

urea or formaldehyde treated soybean meal with casein. Supplements were isocaloric, fed at 30% of the ration DM with corn stover silage (6.13% CP) and contained 14.9, 9.0; 21.7, 8.0; 21.7, 10.5; 14.9, 10.8; 21.7, 10.8; and 21.7, 10.9% CP and metabolizable protein, respectively. Urea, soybean meal, formaldehyde treated soybean meal with urea, and formaldehyde treated soybean meal with casein supplementation resulted in similar and higher ($P < .05$) rates of growth and improved feed utilization over control and formaldehyde treated soybean meal supplementation. Ruminal ammonia concentration and DM digestibility, estimated by acid insoluble ash, were higher ($P < .05$) in heifers fed the urea supplement than heifers fed all other supplements. Heifers fed formaldehyde treated soybean meal with casein grew at the same rate, utilized feed similarly, and digested DM to the same extent as heifers receiving the formaldehyde treated soybean meal with urea or urea supplements. Supplementation with soybean meal did not improve growth and feed utilization over urea supplementation.

INTRODUCTION

The relative importance of protein and nonprotein N in the utilization of low quality forages has not been well-studied. Ruminal degradation and hydrolysis of dietary nitrogen sources are necessary to provide microbes with ammonia, α -keto acids and possibly intact amino acids for protein synthesis. Over 80% of bacteria isolated from the rumen grew well in media containing ammonia as the sole source of N (Bryant and Robinson, 1962). Fifty to 80% of microbial N in the rumen is produced from ammonia (Mercer and Annison, 1976; Hespell and Bryant, 1979), although a recent estimate with lactating dairy cows has been even higher (Oldham et al., 1980). Specific preformed compounds, including amino acids or peptides, branched-chain carbon compounds, and other growth factors are required by certain bacterial strains (Allison, 1969; Bryant, 1973). Small amounts of amino acids in purified diets containing urea have increased microbial crude protein synthesis (Maeng and Baldwin, 1976) and appreciable amounts of preformed AA are directly incorporated by mixed rumen bacteria if supplied by the diet (Al-Rabbat et al., 1971; Nolan et al., 1976; McMeniman et al., 1976). Peptides may serve as a source of branched-chain fatty acids (BCFA) that are growth factors for a number of bacterial species, including the cellulolytic bacteria (Bryant, 1973; Russell and Hespell, 1981).

Salter et al. (1979) reported that a deficiency of methionine and phenylalanine may limit bacterial growth on diets low in protein and high in NPN. Similarly, Blake et al. (1983) found that net bacterial

synthesis was significantly greater with a natural protein source than with urea diets.

Many reports have shown that urea-fed cattle utilized dietary N less efficiently than did cattle fed N supplements containing largely alpha-amino acids (Rock et al., 1983), which if not degraded in the rumen increase the amount of amino acids entering the small intestine (Horton and Nicholson, 1981; Williams et al., 1969; Oltjen and Putnam, 1966). Some reports, however, indicated no differences between animals fed either urea or natural proteins (e.g., Holter and Kabuga, 1974; Bolsen et al., 1968). Burroughs et al. (1977) found that soybean meal, protected from degradation by formaldehyde, molasses or heat, caused 20-26% improvements in live weight gains and feed utilization over similar diets containing untreated soybean meal and urea. Stock et al. (1983), used growing lambs consuming corn cob-corn based diets to evaluate soybean meal (highly degraded in the rumen) and blood meal (slowly degraded in the rumen), as protein supplements. They found that supplementation with blood meal resulted in significantly greater liveweight gains than soybean meal supplementation. They estimated that the relative value of blood meal compared with soybean meal was 251%.

The objective of this study was to investigate the effects of N sources having varying extent and products of ruminal degradability on utilization of corn stover silage by growing beef cattle.

MATERIALS AND METHODS

Digestion Trial

Six Angus steers, mean weight 250 kg, were restrained in individual metabolism crates and fed corn stover silage supplemented at 20% of the diet dry matter with one of six protein supplements in a 6x6 Latin square experimental design. The ingredient composition and nutrient concentrations of the protein supplements are shown in Table 1. The protein supplements: soybean meal, dehydrated alfalfa, corn gluten meal, casein, and urea were formulated to be isonitrogenous at 17.2% crude protein while the control supplement contained lower CP (4.88%). Except for the urea supplement which had lower digestible nutrients, the supplements were approximately isocaloric (56% TDN).

Corn stover silage was harvested in mid-November from a field of Pioneer hybrid 3541 corn and was stored in a 3x3.5x18.3 m trench silo. The corn stover silage contained 8.19% CP at feeding.

The diets were fed twice daily at 0800 h and 1600 h. Orts were taken once daily at the morning feeding. Steers were fed ad libitum during a 14 d adjustment period. This was followed by a 7 d period of total collection of feces and urine during which the intake was reduced to 90% of the ad libitum intake. During the collection period, feces were mixed thoroughly and 10% subsamples of the feeds and feces were frozen daily. Urine was collected in plastic buckets containing 1 ml of 50% sulfuric acid. Ten percent of the daily urine was added to a composite bottle and stored frozen. At the end of each period, aliquots

of feces, stover silage, and supplements were composited. There was a 1 wk rest between periods when steers were taken from the crates and fed together in a pen.

Heifer Growth Trial

Sixty Charolais X Angus heifers (mean weight 250 kg) were randomly allotted by weight to 12 pens and 2 pens assigned to each of six protein treatments (2 pens/treatment) in a 98-d growth trial. The composition of the protein supplements are shown in Table 2. The protein sources in the supplements included: urea, soybean meal, formaldehyde-treated soybean meal, formaldehyde treated soybean meal and urea and formaldehyde-treated soybean meal plus casein formulated to supply varying levels of CP, metabolizable protein (MP) and rumen degradable N and amino acids. Formaldehyde-treated soybean meal was prepared by treating soybean meal at 0.8% of the CP with formaldehyde (Thomas et al., 1979). The urea, soybean meal, formaldehyde treated soybean meal+urea and formaldehyde treated soybean meal+casein supplements were isonitrogenous (20.4% CP) and contained more CP than did the control and formaldehyde treated soybean meal supplements (Table 3). The calculated MP concentrations of the soybean meal, formaldehyde-treated soybean meal, formaldehyde-treated soybean meal+urea and formaldehyde treated soybean meal+casein supplements were equal and higher than those of the control and urea supplements. The supplements were fed at 30% of the diet dry matter with stover silage, containing 6.13% CP which was harvested when the grain contained 22% moisture and stored in a 6x18 m oxygen limiting silo.

After adding the supplements to the stover, the MP concentration of the total diet containing the soybean meal, formaldehyde treated soybean meal+urea and formaldehyde treated soybean meal+casein supplements were greater than those of diets containing the control, urea and formaldehyde treated soybean meal supplements.

Feed samples were taken biweekly. Animals were weighed on 2 consecutive days at the beginning and end of the experiment and once at 28-d intervals during the experiment. Weights were measured after a 12-h period without water and feed.

Rumen samples were collected using a stomach tube for the determination of the ruminal ammonia concentration and pH at 2h post-feeding on day 35 and 63 of the experiment. The rumen samples for analysis of ammonia were stored in 250 ml plastic bottles containing 1 ml of 50% sulfuric acid and frozen until analyzed.

After the 98 d trial, heifers were fed for an additional week at the end of which fecal grab samples were collected on 3 consecutive days for determination of diet digestibility using acid insoluble ash (AIA).

Chemical Analysis

Nitrogen was determined in freeze-ground feces and silage, oven-dried supplements and frozen urine samples by the Kjeldahl procedure (AOAC, 1980). Dried samples of the feed and feces were ground through 1 mm screen in preparation for further analysis. Neutral detergent fiber (NDF), acid detergent fiber (ADF) and cellulose (CELL) of the oven dried

feed and feces samples were determined according to Goering and Van Soest (1970). The concentrations of gross energy (GE) of the feed and feces were determined using a Parr adiabatic bomb calorimeter. Fecal and feed samples were analyzed for acid insoluble ash by the 2N hydrochloric acid procedure of Van Keulen and Young (1977). Ammonia in ruminal fluid was measured by the hypochlorite procedure of Van Slyke and Hiller (1933) adapted for automatic analysis (Technicon, 1960).

Statistical Analysis

The data from the digestion trial were analyzed as a 6x6 Latin square in which the rows represented the periods and the columns represented the animals and protein treatments (Steele and Torrie, 1960). Data were analyzed by using the General Linear Model (GLM) procedure of the Statistical Analysis System (SAS, 1979). In cases where significant F-values were obtained, significance between means were determined using the simple t-test. The data from the Heifer Growth Trial were analyzed as a complete randomized design using the Analysis of Variance (ANOVA) of the Statistical Analysis System (SAS, 1979). Means were separated by least significant difference (LSD) analysis.

RESULTS AND DISCUSSION

Digestion Trial

Feed consumption and apparent digestibilities of DM, OM, CP, gross energy and fiber are presented in Table 4. Feed consumption was lower ($P<.05$) for animals fed the control supplement than those fed the other supplements. Intake of low quality forages is limited by inadequate N supply (Milford and Minson, 1965). N supplementation has often increased voluntary intake and digestibility of cereal straws (Campling et al., 1962; Horton, 1979). Source of protein did not affect feed consumption.

Protein supplementation did not improve the digestibility of DM, OM and energy. Steers fed the casein supplement, however, tended ($P<.1$) to have higher DM, OM and energy digestibilities than did steers fed the other supplements. Cattle fed dehydrated alfalfa tended to have lower digestibilities of DM and OM than did those containing the urea, soybean meal, corn gluten meal and control supplements. Crude protein digestion was improved ($P<.05$) by protein supplementation. Steers fed the diet containing the CGM supplement had a higher ($P<.05$) CP digestibility than did those fed the soybean meal and dehydrated alfalfa supplementation but was not different from those fed urea or casein. Steers fed the diet containing the dehydrated alfalfa supplement had the lowest CP digestion among steers fed the other protein sources.

Protein supplementation tended ($P<.5$) to improve NDF digestion by the steers, but this effect was not significantly different from the control supplement (Table 4). Feeding the diet containing CGM resulted

in the highest NDF digestion and that containing dehydrated alfalfa resulted in the lowest NDF digestion. Acid detergent fiber digestion was significantly less ($P < .05$) in animals consuming the dehydrated alfalfa supplement than in those fed any other supplement. Supplementation with all other protein sources tended to increase ADF digestion, but the effect was not significant. Cellulose digestibility in the animals fed control supplement was not significantly different from those fed any other supplement. Animals fed the dehydrated alfalfa had lower ($P < .05$) cellulose digestibility than those consuming the soybean meal, urea and corn gluten meal supplements.

Nitrogen balance was improved ($P < .05$) by protein supplementation (Table 5), but was not influenced by the source of protein. These results agree with the results of Horton and Nicholson (1981) who found that digestibilities of OM, CP and ADF by steers were increased ($P < .05$) by supplementation of wheat straw and barley with urea and soybean meal. Similar to this study, they found that supplementation with alfalfa meal did not improve digestibilities of OM, CP and ADF. Similarly, Church and Santos (1981) showed that DM and ADF digestibilities were increased in animals fed wheat straw supplemented with either soybean meal or a liquid supplement containing urea and ammonium polyphosphate. These authors, however, reported that voluntary consumption of digestible energy was improved by feeding soybean meal, but not by feeding the liquid supplement. Kropp et al. (1977) also found that urea was inferior to soybean meal for supporting digestion of DM and OM and N retention in animals fed a pelleted ground weathered range.

The consistently poor digestibility of the diet containing the dehydrated alfalfa supplement may be due to heat damage during the dehydration process (Coering, 1976). Alternatively, the processing of alfalfa may have produced a feed of small particle size which resulted in a rapid rate of passage through the rumen and a subsequent reduction in digestibility (Horton and Nicholson, 1981).

Organic matter and energy digestion tended to be higher for the animals fed the diet containing the casein supplement than for the animals fed any other supplement. These effects of casein on OM or energy digestion, however, were not significantly different from those of urea, soybean meal, or corn gluten meal. The high digestibilities obtained when feeding casein may be due to the provision of amino acids in the rumen, which may be metabolized to branched-chain fatty acids needed for growth of the cellulolytic bacteria (Bryant, 1973 and Russell and Hespell, 1981).

Heifer Growth Trial

The data in Table 6 indicate that varying the ruminal degradability of the protein supplement to result in different concentrations of ammonia, and amino acids in the rumen and variable amounts of undegraded protein entering the small intestine did not significantly affect feed consumption by the heifers. Heifers receiving the formaldehyde-treated soybean meal supplement, however, tended to consume less feed and had a lower ($P < .05$) rate of gain than did the heifers receiving any of the

other supplements. Daily gains of heifers fed the control and formaldehyde-treated soybean meal supplements were lower ($P < .05$) than those of heifers fed the supplements containing urea, soybean meal, formaldehyde treated soybean meal with urea and formaldehyde-treated soybean meal with casein. Heifers fed the formaldehyde-treated soybean meal supplement had a higher ($P < .05$) feed:gain ratio than did those fed the other supplements. Feed efficiency was equal for heifers fed the urea, soybean meal, formaldehyde-treated soybean meal+urea and formaldehyde-treated soybean meal+casein supplements. Therefore, addition of rumen degradable N either as urea or casein to formaldehyde treated soybean meal improved feed utilization to approximately the same extent.

The effects of varying ruminal degradability of the nitrogen source on the digestibility of DM and the fiber constituents, ruminal pH and ammonia concentration in heifers are shown in Table 7. Heifers fed the soybean meal, formaldehyde treated soybean meal+casein and formaldehyde-treated soybean meal+urea supplements had similar ruminal ammonia concentrations 2h post-feeding. Heifers fed the urea supplement, however, had a significantly greater ($P < .05$) ruminal ammonia concentration than did those fed any other supplements. Ruminal ammonia concentrations of the heifers fed the formaldehyde treated soybean meal supplement were lower ($P < .05$) than other treatments. The low ruminal ammonia concentration in these animals may have caused the lower dry matter intake and daily gains of the heifers fed the formaldehyde treated

soybean meal supplement. Ruminal pH was not affected by protein treatments. As a result of low ruminal ammonia concentrations, diets containing the CNL and formaldehyde treated soybean meal supplements had lower ($P < .05$) digestibilities of DM, NDF, ADF and CELL than did any other treatment. The DM and CELL digestibilities by heifers fed the urea-supplemented diet were greater ($P < .05$) than those fed the diet supplemented with soybean meal. There was no difference in digestibilities of NDF or ADF between these diets. The digestibilities of DM, NDF, ADF and CELL for the diets containing the formaldehyde treated soybean meal+urea and formaldehyde treated soybean meal+casein were similar to those for the diet containing soybean meal.

The results of this experiment indicate that ruminal degradability of protein sources has an important influence on the utilization of low quality roughages. The products of protein degradation are used by bacteria for synthesis of bacterial protein. To supply the energy required for protein synthesis, bacteria degrade components of the feed including the fibrous constituents. Depriving the bacteria of adequate N resulted in low digestibilities of the DM and fiber fractions of the feed and in poor performance of cattle consuming the formaldehyde treated soybean meal and CNL supplements. Although supplementation with urea resulted in very high rumen ammonia as compared with soybean meal supplementation, the performance of the animals in these two groups was not different. Previous studies similarly found no differences in the performance of cattle fed diets supplemented with urea or soybean meal (Holter and Kabuga, 1974; Bolsen et al., 1968). In contrast, Oltjen and

Putnam (1966) and Braman et al. (1973) found that urea-fed cattle utilized dietary N less efficiently than did cattle fed N supplements containing mainly alpha-amino sources. Addition of urea or casein to formaldehyde treated soybean meal resulted in higher ($P < .05$) ruminal ammonia concentrations and subsequent increase ($P < .05$) in digestion of the fiber constituents. Since there was little difference in the digestibilities of steers fed the urea, soybean meal, corn gluten meal or casein, supplemented diets in the Digestion Trial, it can be inferred that little advantage was derived by supplying amino acids or keto acids through degradable true protein sources. Although animals receiving the urea supplement in the Heifer Growth Trial had the highest ammonia concentration and digestibilities of the fiber constituents, their performance was not different from animals consuming the formaldehyde-treated soybean meal+urea and formaldehyde treated soybean meal+casein supplements which resulted in a 50% less rumen ammonia concentration and lower digestibilities of the DM and fibrous constituents. This is related to the usefulness of urea in ruminant diets. The urea fermentation potential (UFP) is a measure of the amount of urea that can be used for microbial growth in a ruminant diet and depends on availability of energy and the amount of protein being degraded. Thomas et al. (1984) showed that during the initial 70 d of a growth trial with cattle consuming a corn grain-cotton seed hull diet supplemented with urea calculated to be either 25% deficient, equal to or 25% excess of the UFP, daily gains were improved ($P < .05$) by addition of urea up to the

level that fulfilled the UFP. After this point, energy became the limiting factor and addition of urea becomes useless.

This study showed that protected proteins by themselves are detrimental to performance of cattle fed high roughage diets. Urea addition to protected soybean meal resulted in improved performance of heifers over protected protein alone because of increased digestibility and possibly increased bacterial protein synthesis which contributed to the metabolizable protein needs of the animals. Since this response was similar whether the degradable N was supplied as casein or urea it appears that ruminal ammonia had a more important influence on utilization of corn stover silage than did the presence of amino acids in the rumen. But, it was also possible that slow ruminal degradation of the formaldehyde treated soybean meal protein provided the needed amino acids for bacterial growth in the supplements containing urea.

Table 1. Composition and nutrient concentrations of the protein supplements^a (Experiment 1)

Item	Soybean		Corn		Urea	Control
	Meal	Dehydrated Alfalfa	Meal	Gluten		
Ingredients	% of dry matter					
Ground corn (IFN 4-02-931)	---	---	---	---	---	29.80
Corn cobs (IFN 2-08-234)	59.60	---	59.6	59.60	59.60	59.60
Molasses (IFN 4-04-696)	5.35	5.35	12.71	18.90	30.05	5.35
Soybean meal (IFN 5-20-637)	29.80	---	---	---	---	---
Corn gluten meal (IFN 5-28-242)	---	---	22.44	---	---	---
Dehydrated alfalfa (IFN 1-00-023)	---	89.40	---	---	---	---
Casein (IFN-5-01-162)	---	---	---	16.25	---	---
Urea (IFN 5-05-070)	---	---	---	---	5.10	---
Dicalcium phosphate (IFN 6-01-080)	2.00	2.00	2.00	2.00	2.00	2.00
Salt (IFN 6-04-152)	2.50	2.50	2.50	2.50	2.50	2.50
Vitamin premix ^b	.50	.50	.50	.50	.50	.50
Trace mineral premix	.25	.25	.25	.25	.25	.25
Elemental sulfur	---	---	---	---	.2	---
Total	100	100	100	100	100	100
Nutrient Concentrations:						
Crude protein	17.25	17.23	17.25	17.24	17.29	4.88
Total digestible nutrients	55.98	59.30	56.00	56.90	49.60	59.60
Metabolizable protein	7.76	4.51	5.52	6.71	4.02	2.92
Urea fermentation potential	-2.38	-3.72	- .15	-2.70	-3.9	+1.06

^aSupplements were fed as 20% of the diet dry matter with corn stover silage.

^bVitamin A premix contains 4,409,245 units of vitamin A per kg.

Table 2. Composition of protein supplements fed with corn stover silage (Experiment 2)

Ingredients	Protein source of supplement						% of dry matter
	Control	Urea	Soybean Meal	Formaldehyde Treated Soybean Meal	Formaldehyde Treated Soybean Meal ^a + Urea	Formaldehyde Treated Soybean Meal ^a + casein	
Ground corn (IFN 4-02-931)	43.54	69.80	45.01	43.54	49.40	45.00	
Corn cobs (IFN 2-08-234)	20.00	10.00	15.00	20.00	17.40	25.00	
Molasses (IFN 4-04-696)	14.55	11.91	3.50	14.55	10.10	2.55	
Soybean meal (IFN 5-20-637)	18.46	---	33.04	---	---	---	
Treated soybean meal ^a	---	---	---	18.46	17.15	14.40	
Urea (IFN 5-05-070)	---	4.64	---	---	2.40	---	
Casein (IFN 5-01-162)	---	---	---	---	---	9.60	
Dicalcium phosphate (IFN 6-01-080)	1.50	1.50	1.50	1.50	1.50	1.50	
Salt	1.50	1.50	1.50	1.50	1.50	1.50	
Elemental Sulfur	---	0.20	---	---	0.10	---	
Trace mineral premix	0.15	0.15	0.15	0.15	0.15	0.15	
Vitamin A premix	0.30	0.30	0.30	0.30	0.30	0.30	
Total	100	100	100	100	100	100	100

^aSoybean meal treated with formaldehyde at 0.8% of the crude protein.

^bVitamin A premix contains 4,409,245 units of vitamin A per kg.

Table 3. Crude protein, metabolizable protein and urea fermentation potential concentrations of the protein supplements and complete diets (Experiment 2)

Supplement	Protein source of supplement						% of dry matter
	Control	Urea	Soybean Meal	Formaldehyde Treated Soybean Meal ^a	Formaldehyde Treated Soybean Meal ^a +Urea	Formaldehyde Treated Soybean Meal ^a +Casein	
Crude protein	14.9	21.7	21.7	14.9	21.7	21.7	21.7
MP	9.0	8.0	10.5	10.8	10.8	10.8	10.9
UP	-8.1	-34.8	-28.1	- .04	-24.7	-24.7	-24.7
Complete diet:							
Crude protein	8.0	10.0	10.0	8.0	10.0	10.0	10.0
MP ^b	5.9	6.1	6.8	6.0	6.9	6.9	6.9
UP ^c	---	---	---	---	---	---	---

^aSoybean treated with formaldehyde at 0.8% of the crude protein.

^bMP = Metabolizable protein.

^cUPP = Urea fermentation potential.

Table 4. Effect of protein source on intake and apparent digestibility of dry matter and nutrients (Experiment 1)

Item	Supplemental protein source						SE
	Soybean Meal	Dehydrated Alfalfa	Corn Gluten Meal	Casein	Urea	Control	
Dry matter intake, kg/d	4.7 ^a	4.6 ^a	4.4 ^a	4.4 ^a	4.5 ^a	3.7 ^b	.27
Digestion coefficient, %:							
Dry matter	53.9	49.7	55.8	57.4	55.0	53.5	1.46
Organic matter	55.3	51.7 ^b	57.9	59.5	57.5 ^{ab}	55.2	1.62
Crude protein	45.8 ^{ab}	41.4 ^b	49.6 ^a	41.5 ^b	48.0 ^{ab}	32.9 ^c	4.06
NDF	53.2	50.2 ^b	55.8	54.3	54.8	54.0	1.86
ADF	49.5 ^a	43.8 ^b	52.4 ^a	49.8 ^a	51.3 ^a	49.0 ^a	1.46
CELL	64.4 ^a	56.5 ^b	64.7 ^a	64.0 ^a	64.4 ^a	60.2 ^a	2.29
Energy	55.2	49.0	55.2	56.9	53.5	52.6	1.45
Energy, Mcal/kg	2.18	2.01	2.28	2.19	2.16	2.14	.059

^{a,b,c} Means in the same row with different superscripts differ (P<.05).

Table 5. Effect of protein source on nitrogen metabolism of the steers (Experiment 1)

Item	Supplemental protein source						Control	SE
	Soybean Meal	Dehydrated Alfalfa	Corn Gluten Meal	Casein	Urea			
Total N intake	72.1 ^{ab}	74.3 ^a	74.2 ^a	73.3 ^{ab}	72.2 ^{ab}	45.3 ^d	3.8	
Total N excreted (feces+urine)	49.2 ^{ab}	53.2 ^a	52.4 ^a	51.5 ^a	51.7 ^a	36.8 ^c	3.01	
N balance	23.0 ^a	21.1 ^a	21.8 ^a	21.8 ^a	20.5 ^a	8.5 ^b	4.72	

a, b, c, d Means on the same row with different superscripts differ (P<.05).

Table 6. Effect of varying ruminal degradability of the nitrogen source on the feedlot performance of the heifers (Experiment 2)

Item	Control		Urea		Soybean Meal		Formaldehyde Treated Soybean Meal + Urea		Formaldehyde Treated Soybean Meal + Casein		SE
	98	98	98	98	98	98	98	98	98	98	
Days on expt.	98										
Feed consumption, kg/d	5.1	5.4	5.4	5.8	4.8	4.8	4.8	4.8	4.8	4.8	0.32
Initial wt., kg	244	244	244	244	244	244	244	244	244	244	0.0
Final wt., kg	273	283 ^b	283 ^b	293 ^b	264	264	283	283	283	283	3.2
ADG, kg/d	.25 ^a	.40 ^b	.40 ^{ab}	.51 ^b	.20 ^a	.20 ^a	.44 ^b	.44 ^b	.40 ^b	.40 ^{ab}	.05
Feed/gain ratio	17.0 ^a	13.5 ^{ab}	13.5 ^{ab}	11.3 ^b	24.6 ^c	24.6 ^c	12.4 ^b	12.4 ^b	13.4 ^{ab}	13.4 ^{ab}	2.51

a, b, c Means on the same row with the same superscripts differ (P<.05).

Table 7. Effect of varying ruminal degradability of the nitrogen source on dry matter and fiber digestion, ruminal ammonia concentration and pH of the feedlot heifers (Experiment 2)

Item	Control		Urea		Soybean Meal		Formaldehyde Treated Soybean Meal		Formaldehyde Treated Soybean Meal + Casein		SE
	Control	Urea	Soybean Meal	Formaldehyde Treated Soybean Meal	Formaldehyde Treated Soybean Meal + Urea	Formaldehyde Treated Soybean Meal + Casein					
Digestion coefficient, %	48.2	58.1 ^c	52.7 ^d	48.1 ^b	54.0 ^d	56.4 ^c	0.98				
DM	48.2	58.1 ^c	52.7 ^d	48.1 ^b	54.0 ^d	56.4 ^c	0.98				
Fiber:											
NDF	45.7 ^{bc}	55.3 ^a	50.5 ^{abc}	45.2 ^c	52.8 ^{abc}	54.8 ^{ab}	2.71				
ADF	41.3 ^c	50.3 ^a	45.9 ^{abc}	39.9 ^c	47.5 ^{abc}	50.8 ^a	2.62				
CELL	54.7 ^{ab}	63.1 ^a	58.2 ^{ab}	53.2 ^b	60.6 ^{ab}	62.9 ^a	2.48				
Rumen NH ₃ -N, mg/dl	4.0 ^b	28.1 ^c	13.7 ^d	1.9 ^b	12.7 ^d	12.9 ^d	2.03				
Rumen pH	7.0	7.2	7.0	7.0	7.1	6.8	2.05				

a, b, c, d Means on the same row with different superscripts differ (P < .05).

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SECTION II. UTILIZATION OF LOW QUALITY ROUGHAGES: EFFECTS OF
RUMINAL AMMONIA CONCENTRATIONS ON UTILIZATION OF
CORN STOVER SILAGE BY GROWING CATTLE

UTILIZATION OF LOW QUALITY ROUGHAGES: EFFECTS OF RUMINAL AMMONIA
CONCENTRATIONS ON UTILIZATION OF CORN STOVER SILAGE BY GROWING
CATTLE

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SUMMARY

The effect of varying ruminal ammonia concentration on utilization of corn stover silage by growing cattle was studied in two experiments using 6 protein supplements. The protein supplements were: soybean meal, which served as a positive control and five supplements containing formaldehyde treated soybean meal and either 0.0, 1.43, 2.47, 3.47 or 4.47% urea. Crude protein (CP) and urea fermentation potential concentrations of these supplements were: 20.9, -2.5; 14.4, 0.0; 18.4, -1.5; 21.3, -2.5; 24.1, -3.5; and 26.9 and -4.5%, respectively. The protein supplements were isocaloric, contained similar metabolizable protein (MP) concentrations and were fed as 30% of the diet dry matter with corn stover silage containing 0.69% N. In the metabolism trial, the above supplements with the four highest levels of urea (1.43, 2.47, 3.47 and 4.47% urea) were fed to 4 Holstein steers fitted with ruminal and duodenal cannulae in a 4x4 Latin square. Ruminal ammonia concentrations were: 4.88, 7.70, 9.32 and 13.32 mg/dl for supplements containing 1.43, 2.47, 3.47 and 4.47% urea. DM intake increased with increased amounts of urea in the supplement. Ruminal DM and OM digestion tended to increase with urea level in the supplement up to a concentration of urea in the supplement of 3.47, and thereafter, a decline was observed. Similarly, NDF and ADF digestion and VFA concentration tended to increase with urea level and were highest when urea in the supplement was 3.47%. Bacterial N synthesis (g/d) tended to increase to a plateau when the supplement contained 3.47% urea, but the response was not significant. Increasing

the urea level in the supplement did not affect bacterial N synthesized per kg OM digested in the rumen. Sixty Charolais x Angus heifers (initial weight, 212 kg) were randomly allotted by weight to 12 pens and fed one of the 6 protein supplements (2 pens/treatment) for 84 days in a feedlot performance trial. Average daily gain and feed efficiency increased linearly ($P < .05$) and quadratically ($P < .05$) with increasing urea in the supplement. Both responses reached a plateau when the supplement contained 3.47% urea. The results of these experiments indicate that the ruminal-ammonia concentration for maximum bacterial N synthesis in animals fed corn stover silage was approximately 9.32 mg/dl. Although fiber digestion and VFA production tended to increase with urea level in the supplement up to 3.47% urea, bacterial-N synthesized per kg of OM digested in the rumen was not affected by urea level in the supplement, indicating that ruminal-ammonia concentration for optimal microbial-N synthesis was not the same as ruminal-NH₃ concentration necessary for maximum ruminal fermentation.

INTRODUCTION

Although it is known that some of the rumen microbes require preformed amino acids for their growth (McMeniman et al., 1976; Maeng and Baldwin, 1976; Nolan et al., 1976; Salter et al., 1979), the importance of ammonia as a precursor of microbial protein has always been stressed. Ammonia has been found to be essential for growth of certain rumen bacteria (Bryant and Robinson, 1962), but the amount required for maximal microbial growth and fermentative activity remains controversial. The yields of microbial biomass obtained in vitro were maximal with ammonia concentrations of 5.4-10.2 mg/dl (Allison, 1970; Annison, 1975; Satter and Slyter, 1974; Okorie et al., 1977) and these findings are supported by some in vivo data (Roffler et al., 1974) showing that ammonia concentrations of 6.8-8.5 resulted in maximal rumen microbial synthesis in sheep. In contrast, the ammonia concentration required for maximal microbial protein synthesis in sheep fed purified diets was found to be 10.71-16.15 mg/dl, by Hume et al. (1970) and 28.9 mg/dl by Miller (1973).

Maximum rate of fermentation and production of microbial protein per unit of substrate fermented depends largely on ruminal ammonia concentrations. Because of a partial 'uncoupling' of the fermentation, the optimal concentration of ammonia for both may not always be the same. Orskov et al. (1972) showed that when feeding barley, the microbial protein produced per unit substrate fermented was not altered

as a result of urea supplementation while the extent of rumen fermentation and in situ digestibility were increased. Similarly, Satter and Slyter (1974) found that ammonia concentrations of less than 6.12 mg/dl depressed microbial growth in the chemostat but had no significant effect upon VFA production, showing that the rate of fermentation is not dependent upon the rate of microbial growth. The large disparity in the previous data may be expected because variations in cell population make it improbable that one concentration of ammonia would give maximal microbial growth of the total population (Smith, 1979). To determine the rumen ammonia concentrations required for maximal microbial growth and digestion in animals fed a corn stover diet, two experiments were designed and performed evaluating the effect of varying ruminal ammonia concentrations on digestion by and performance of growing cattle fed corn stover silage.

MATERIALS AND METHODS

Two experiments were conducted using protein supplements shown in Table 1. The protein supplements were soybean meal, which served as a positive control, and five supplements containing formaldehyde treated soybean meal (Thomas et al., 1979) and 0.0, 1.43, 2.47, 3.47 or 4.47% urea. The protein supplements were isocaloric and contained approximately similar concentrations of metabolizable protein (Table 2). Crude protein (CP) of the supplements increased and urea fermentation potential (UFP) decreased with increased urea level. The soybean meal supplement was intermediate in both CP and UFP concentrations. The supplements were fed as 30% of the diet dry matter with corn stover silage containing 4.3% CP at feeding. The UFP concentrations of the total diet containing formaldehyde-treated soybean meal with 0.0 and 1.43% urea were calculated to be 0.4 and 0.0%, indicating that the ruminal ammonia concentrations of the animals fed these diets would be deficient and equal to the requirements of the microbes, respectively. Additional urea would result in excess ammonia in the rumen.

Experiment 1

Four Holstein steers (averaging 250 kg) were fitted with permanent ruminal cannulae and duodenal T-type cannulae. After a 3 wk post-operative recovery period, the steers were restrained in individual stalls with plywood on the sides and assigned to treatments in a 4x4 Latin square experimental design. The treatments used were the

supplements containing the four highest levels of urea (1.43, 2.47, 3.47 and 4.47%) shown in Table 1.

Each period of the Latin square consisted of a 10-d preliminary period during which mean voluntary intake for each steer was measured followed by a 6 d experimental period during which the animals were fed 90% of their mean voluntary dry matter intake and 42g of chromium-mordanted fiber (89.3 g chromium/kg) prepared according to Uden et al. (1980). During the preliminary period, animals were fed twice daily at approximately 12 h intervals. During the experimental period, steers were fed six times daily at 4 h intervals. Animals had access to water at all times.

Samples of feed and orts were taken daily and composited for chemical analysis. Sampling of the rumen and duodenal digesta and feces were done during d 4-6 of the experimental period. During the 3 d collection period, duodenal digesta was collected at three 6 h intervals and one 3 h interval daily to allow a shift in sampling time. At each sampling, 250 ml of duodenal digesta were collected from each steer. The sample was divided into two 125 ml portions. One portion was stored in a composite plastic jug and the other was stored in a plastic freezer container. Both samples were frozen until analyzed. Fecal samples were taken at each defecation, care being taken to exclude contaminants. On the last day of the experimental period, rumen liquor samples were taken through the cannulae hourly from 8 to 12 h and every 2 h from 14 to 18 h for determination of volatile fatty acids and ammonia concentration.

An aliquot of each sample was stored in 250 ml plastic bottles containing 1/2 ml of 50% sulfuric acid. pH was determined on another aliquot of the rumen liquor. A 2 l sample of rumen fluid was taken, 2 h post-feeding, from each steer for isolation of bacteria. Rumen fluid was filtered through 2 layers of cheesecloth and equal volumes of filtered rumen liquor and normal saline (0.9% NaCl) were centrifuged at 500 g for 5 min. The supernatant solution was decanted and recentrifuged at 20,000 g for 20 min. The resultant pellet was washed twice, once with saline and once with water, centrifuging after each washing for 20 min at 20,000 g. The pellet was dried at 60°C for 2 d.

Experiment 2

The 6 supplements in Table 1 were evaluated in 84 d growth trial. Sixty Charolais x Angus heifers (initial weight, 212 kg) were randomly allotted by weight to 12 pens and assigned to one of the 6 protein supplements (2 pens/treatment). Animals were weighed on 2 consecutive days at the beginning and end of the experiment and once at 28-d intervals during the experiment. Weights were measured after a 12 h period without water and feed.

Once during the experiment, rumen fluid samples were taken 2 h post-feeding for determination of pH and ruminal concentration of ammonia, volatile fatty acids. Fecal grab samples were collected at the end of the experiment for determination of digestibility using acid insoluble ash.

Chemical Analysis

Feces and protein supplements and stover silage were dried at 60°C for 3 d. All dried samples were ground in a Wiley mill through a 1 mm screen. Dry matter (DM) was determined on duplicate samples at 100°C overnight (AOAC, 1975). Ash was determined by heating in a muffle furnace at 550°C for 3 h (AOAC, 1975). The portion of duodenal samples which were stored in freezer containers were freeze-dried. Chromium was determined in the chromium-mordanted fiber and the dried duodenal and fecal samples by the method of Williams et al., (1962). Neutral detergent fiber (NDF), acid detergent fiber (ADF) and cellulose (CELL) in the feeds, feces and freeze-dried duodenal samples were determined by the methods of Goering and Van Soest (1970). RNA was determined in the dried mixed bacteria and duodenal samples according to Zinn and Owens (1982). Frozen duodenal composite samples duodenal contents were used to determine total nitrogen and ammonia nitrogen (NH₃-N). Total N was determined using the Kjeldahl procedure (AOAC, 1975). Ammonia-N was determined by the hypochlorite procedure (Van Slyke and Hiller, 1933), adapted for automated flow analysis (Technicon, 1960). Volatile fatty acids (VFA) in rumen fluid were determined using the Hewlett Packard 5830 gas liquid chromatograph packed with an SP 1200 H₃PO₄ column (Supelco, Inc., Bellefonte, Pa.). Fecal and feed samples in experiment 2 were analyzed for acid insoluble ash by the 2N hydrochloric acid procedure of Van Keulen and Young (1977). Equations for the calculation of flow of DM and nutrients, digestibilities and total bacterial synthesis are presented in Appendix B.

Statistical Analysis

The two experiments were analyzed using the General Linear Model (GLM) procedure of Statistical Analysis Systems (SAS, 1979). Regression analyses were performed to separate linear and quadratic responses to protein treatments. In experiment 1, two animals were withdrawn from the experiment during the fourth period because of health reasons. The use of the GLM procedure allowed numbers to be generated for the missing animals.

RESULTS AND DISCUSSION

Ruminal Ammonia Concentrations

In both trials increasing urea in the diet resulted in a linear increase ($P < .05$) in ruminal ammonia concentration (Table 3). The mean ruminal ammonia concentrations for the steers fed the four urea supplements in the metabolism trial were about 50% less than the mean ruminal ammonia concentrations for the heifers fed the same supplements in the feedlot trial. This difference may be attributed to frequency of feeding and sampling and method of sampling. In the metabolism trial, the animals were fed at 4 h intervals and rumen fluid was sampled 6 times daily through rumen cannulae. In the feedlot trial, the animals were fed once daily and rumen samples were taken once through the esophagus using a stomach tube. Frequent sampling through the rumen cannulae are likely to have given a more accurate estimate of the rumen ammonia concentrations than single sampling through the esophagus (Sriskandarajah et al., 1982).

Experiment 1

Although DM and OM intake tended (linear effect $P < .1$) to increase with increasing urea concentrations in the diet, the increase was not linear (Table 4). Ruminal DM and OM digestibilities also tended to increase with urea additions to 1.04% urea. Using the first derivative of the regression equation, it was found that the urea levels for the maximum ruminal DM and OM digestibilities were 1.02 and 1.035% urea, respectively. Feeding 1.34% urea, however, resulted in a decrease in

ruminal DM and OM digestibilities and, therefore, the increases were not linear. Total tract DM and OM digestibilities also tended (linear effect $P < .1$) to increase with increasing dietary urea concentration, but no statistically significant linear effects were observed. Similar results were obtained by de Faria and Huber (1984) who fed steers corn silage supplemented with levels of urea and found that increasing the level of urea increased intake and DM digestion.

Table 5 shows the effects of varying urea in the diet on digestion of the fiber fractions of the feed. Ruminal NDF (linear effect $P < .1$), ADF (linear effect $P < .1$) and cellulose (linear effect $P < .1$) digestion tended to increase with increased urea in the diet but further additions above 1.04% urea in the diet caused a decrease in the digestibility of the fibrous constituents. Computation of the first derivative of the regression equation shows that maximum ruminal NDF, ADF and cellulose digestibilities occurred when the urea levels in the diet were 1.06, 1.034, and 0.98%, respectively. The digestion coefficients for NDF, ADF and cellulose in the total tract follow the same trends as fiber digestion in the rumen. Total tract NDF and ADF digestion coefficients tended, however, to be lower than ruminal fiber digestion coefficient, possibly because of variations resulting from sampling procedures, marker flow or fiber digestion (Faichney, 1975).

Ruminal volatile fatty acid (VFA) concentrations and pH are shown in Table 6. Ruminal pH was not affected by increasing the urea concentration of the diet. Similar to the digestion coefficients for DM

and the fibrous constituents, the concentration of propionic acid in the rumen fluid increased until the diet contained 1.04% urea and then decreased (quadratic effect, $P < .05$). The concentration of acetic acid in the rumen fluid linearly increased ($P < .05$) and those of butyric, isovaleric and valeric acids tended to increase with increasing urea concentration in the diet.

Nitrogen flow to the duodenum of steers fed supplements containing varying levels of urea is presented in Table 7. Although total-N and feed-N flow was higher for steers receiving the diet containing 0.43% urea than for those receiving 0.74% urea, total and feed-N (linear effect $P < .1$ and $P < .01$, respectively) flow to the duodenum tended to increase with increasing urea in the diet. The high total N flow observed for the lowest urea level may be due to nitrogen recycling into the rumen. About 23 to 92% of the plasma urea is recycled to the digestive tract, with high values associated with lower N intake (Kennedy and Milligan, 1980). Incorporation of recycled N into bacterial crude protein will cause daily duodenal N flow to exceed N intake with lower protein diets (Chamberlain and Thomas, 1979). Bacterial protein synthesis tended to slightly increase with level of urea in the diet, however, the increase in daily bacterial-N synthesis was not linear. Bacterial-N synthesized per unit organic matter digested was not affected by increasing urea level in the diet. These results agree with the findings of Orskov et al. (1972) that the bacterial protein produced per unit substrate fermented was not altered as a result of urea supplementation.

Experiment 2

Feedlot performance of heifers fed corn stover silage with protein supplements containing varying concentrations of urea is shown in Table 8. Feed consumption increased linearly ($P < .05$) with urea additions to the diet. Feed consumption of heifers fed the soybean meal supplement was comparable to that of animals consuming the supplement containing the 3 highest urea levels. Average daily gain increased quadratically ($P < .05$) and feed efficiency, expressed as the feed to gain ratio, decreased linearly ($P < .05$) with increasing urea level in the diet. Feed was more efficiently utilized by animals on the soybean meal supplement than those fed any level of urea supplementation, but the differences were not significant.

Table 9 shows the effect of urea level in the diet on dry matter and fiber digestion. Unlike experiment 1, increasing urea level in the diet did not improve the digestibility of OM, NDF, ADF and cellulose. This lack of effect may be due to the greater intake observed when feeding increased urea levels in this experiment as rate of passage may have altered (Balch, 1950). Furthermore, sampling once at the end of the trial may not have been adequate to evaluate these diets. Table 10 shows ruminal VFA concentrations and pH of rumen samples taken from the heifers. Ruminal pH was not affected by urea level in the diet. Similar to the digestion coefficients, urea did not influence the ruminal concentration of any of the VFA.

In the metabolism trial, fiber digestion and VFA concentration tended to increase with increasing levels of urea up to a urea level of 1.04% in the diet which gave rise to a ruminal ammonia concentration of 9.43 mg/dl. This is in agreement with the findings of Satter & Slyter (1974), Okorie et al. (1977) and Roffler et al. (1974). Further additions of urea to the diet caused fiber digestion and VFA production to decline. Similarly, bacterial-N flow to the duodenum slightly increased up to when urea was 1.04% of the diet after which it plateaued. The results of the feedlot trial also indicate that average daily gain and feed efficiency increased with increasing urea in the diet and plateaued when urea level was 1.04%. Urea concentration for maximum rate of gain and feed utilization, as calculated from the regression, were 1.0 and 0.95%, respectively.

These data show that the ruminal ammonia concentration for maximum microbial-N synthesis and utilization of corn stover silage was 9.32 mg/dl.

Although fiber digestion and the VFA concentration increased with urea level in the diet up to 1.04% urea, bacterial-N synthesized per kg of OM digested was not affected by urea levels, indicating that the ruminal ammonia concentration for optimal bacterial-N synthesis did not coincide with ruminal ammonia concentrations for the maximal efficiency of microbial-N synthesis. Similar results have been reported (Satter and Slyter, 1974; Beever et al., 1974; Orskov et al., 1972; McAllan and Smith, 1983). This may be as a result of a partial 'uncoupling' of

fermentation in which energy from fermentation is not used for formation of high energy compounds needed for bacterial synthesis. It may also be due to bacterial maintenance energy requirement being high at low rumen turnover rate.

Table 1. Composition of protein supplements fed with corn stover silage

Ingredients	Soybean meal	Level of urea added to treated ^a soybean meal				
		0.0	1.43	2.47	3.47	4.47
		% of dry matter				
Ground corn (IFN 4-02-931)	45.01	43.54	43.54	43.54	43.54	43.53
Corn cobs (IFN 2-08-234)	15.00	20.00	17.56	15.95	14.42	12.38
Molasses (IFN 4-04-696)	3.50	14.55	15.50	16.00	16.50	17.50
Soybean meal (IFN 5-20-637)	33.04	---	---	---	---	---
Treated soybean meal ^a	---	18.46	18.46	18.46	18.46	18.46
Urea (IFN 5-05-070)	---	---	1.43	2.47	3.47	4.47
Dicalcium phosphate (IFN 6-01-080)	1.50	1.50	1.50	1.50	1.50	1.50
Salt (IFN 6-04-152)	1.50	1.50	1.50	1.50	1.50	1.50
Elemental Sulfur	---	---	0.06	0.13	0.16	0.20
Trace mineral premix	0.15	0.15	0.15	0.15	0.15	0.15
Vitamin A premix ^b	0.30	0.30	0.30	0.30	0.30	0.30
Total	100	100	100	100	100	100

^aSoybean meal treated with formaldehyde at 0.8% of the crude protein.

^bVitamin A premix contains 4,409,245 units of vitamin A per kg.

Table 2. Calculated crude protein, metabolizable protein and urea fermentation potential concentrations of the protein supplements and complete diets (Experiment 2)

Item	Soybean meal	Level of urea added to treated ^a soybean meal				
		0.0	1.43	2.47	3.47	4.47
% of dry matter						
Supplement						
Crude protein	20.9	14.4	18.4	21.3	24.1	26.9
MP	10.6	10.9	10.9	10.9	10.9	10.9
UFP	-2.5	0.0	-1.5	-2.5	-3.5	-4.5
Complete diet ^b :						
Crude protein	10.5	8.5	9.7	10.6	11.4	12.3
MP	6.8	6.0	6.9	6.9	6.9	6.9
UFP	-0.3	0.4	0.0	-0.3	-0.6	-0.9

^aSoybean meal treated with formaldehyde at 0.8% of the crude protein.

^bCP concentration of corn stover silage assumed to be 6% in formulating the diets but the analyzed CP was 4.3%, so that the total dietary CP concentration was lower than expected.

Table 3. Mean concentrations of ruminal ammonia in experiments 1 and 2

Experiment	Soybean meal	Supplemental protein source					SE
		% urea in the total diet dry matter					
		0.0	0.43	0.74	1.04	1.34	
							mg/dl
1 ^a	---	---	4.9	7.7	9.3	13.3	0.9
2 ^a	12.2	2.8	11.2	11.9	21.4	24.4	1.7

^aLinear effect of urea level (P<.05).

Table 4. Effect of varying the level of urea in the diet on dry and organic matter intake, dry and organic matter flow to the duodenum and dry matter and organic matter digestibility (Experiment 1)

Item	% urea in the diet dry matter				SE
	0.43	0.74	1.04	1.34	
Dry matter intake, kg/d ^a	5.8	6.2	6.2	6.6	0.39
Organic matter intake, kg/d ^a	5.2	5.6	5.7	6.0	0.27
Dry matter flow to the duodenum kg/d ^b	3.92	3.47	3.38	3.97	0.16
Organic matter flow to the duodenum kg/d ^a	3.18	2.89	2.84	3.23	0.14
Dry matter digestibility %:					
Rumen ^a	31.8	39.3	45.3	39.6	4.04
Total tract ^a	45.3	52.1	51.5	55.0	3.64
Organic matter digestibility; %					
Rumen ^a	39.01	44.1	49.2	44.9	3.74
Total tract ^a	49.4	55.9	54.7	59.0	3.54

^aNo linear or quadratic effect of urea level.

^bQuadratic effect of urea level (P<.01).

Table 5. Effect of varying the level of urea in the diet on fiber digestibility (Experiment 1)

Item	% urea in the diet dry matter				SE
	0.43	0.74	1.04	1.34	
Rumen:					
Neutral detergent, fiber, % ^a	41.5	44.2	52.2	47.2	4.44
Acid detergent fiber, % ^a	39.3	42.7	52.6	45.2	4.80
Cellulose, % ^a	64.2	64.5	73.6	65.9	4.06
Total tract:					
Neutral detergent, fiber, % ^a	34.7	42.7	43.1	46.1	4.81
Acid detergent fiber, % ^a	33.7	41.1	40.9	44.4	4.91
Cellulose, % ^a	63.1	67.1	66.6	67.8	2.82

^aNo linear and quadratic effect of urea level.

Table 6. Effect of varying the level of urea in the diet on ruminal volatile fatty acids and pH (Experiment 1)

Item	% urea in the total diet dry matter				SE
	0.43	0.74	1.04	1.34	
Volatile fatty acids, mmol/l:					
Acetic acid ^a	54.70	60.54	65.22	61.95	1.1
Propionic acid ^b	12.29	12.95	14.81	13.04	0.25
Butyric acid	8.02	8.12	8.96	8.40	0.70
Isovaleric acid	0.39	0.40	0.50	0.47	0.70
Valeric acid	0.47	0.57	0.66	0.55	0.025
pH	6.84	6.78	6.80	6.73	0.03

^aLinear effect of urea level ($P < .05$).

^bQuadratic effect of urea level ($P < .05$).

Table 7. Effect of varying the level of urea in the diet on nitrogen flow to the duodenum (Experiment 1)

Item	% urea in the diet dry matter				SE
	0.43	0.74	1.04	1.34	
Total N intake, g/d	76.97	82.61	96.79	105.82	5.09
N flow to the duodenum, g/d:					
Total N ^a	83.12	76.30	80.38	88.79	4.48
Microbial-N ^a	32.86	33.23	36.26	36.82	1.79
Feed-N ^b	50.23	43.04	44.09	52.85	3.85
Ammonia-N ^a	0.023	0.025	0.029	0.036	.005
Bacterial N synthesis, g/kg organic matter apparently digested ^a	22.02	22.41	22.18	20.95	2.6

^aNo linear or quadratic effect of urea level.

^bLinear effect of urea level (P<.01).

Table 8. Feedlot performance of heifers fed corn stover silage with supplements containing varying concentrations of urea (Experiment 2)

Item	Supplemental protein source						SE
	Soybean meal	% urea in the total dry matter					
		0.0	0.43	0.74	1.04	1.34	
Days on experiment	84	84	84	84	84	84	
Feed consumption ^a kg/d	5.4	4.5	5.3	5.5	5.5	5.6	0.19
Initial weight, kg	210	209	219	213	214	211	
Final weight, kg	259	232	261	258	259	257	
Average daily gain kg/d ^{a, b}	0.59	0.27	0.50	0.54	0.54	0.54	0.06
Feed/gain ratio ^a	9.2	16.6	10.6	10.2	10.2	10.4	0.64

^aLinear effect of urea level (P<.05).

^bQuadratic effect of urea level (P<.05).

Table 9. Effect of varying the level of urea in the diet on digestibility of nutrients (Experiment 2)

Item	Soybean meal	Supplemental protein source					SE
		% urea in the total diet dry matter					
		0.0	0.43	0.74	1.04	1.34	
		% digested					
Dry matter	49.5	57.3	55.0	50.8	42.3	48.4	2.6
Neutral detergent fiber ^a	44.5	51.3	50.9	44.0	34.9	43.4	2.5
Acid detergent fiber ^a	43.7	50.1	49.4	42.1	35.1	41.5	2.3
Cellulosa ^a	62.6	65.2	65.4	61.3	54.7	59.4	2.0

^aNo linear or quadratic effect of urea level.

Table 10. Effect of varying the level of urea in the diet on rumen volatile fatty acids and ammonia concentrations and pH (Experiment 2)

Item	Supplemental protein source						SE
	Soybean meal	% urea in the total diet dry matter					
		0.0	0.43	0.74	1.04	1.34	
Volatile fatty acids, mm/l:							
Acetic acid ^a	46.61	55.96	52.45	50.95	51.99	53.61	1.2
Propionic acid ^a	10.08	12.53	10.25	14.28	10.56	12.53	2.6
Butyric acid ^a	8.08	9.91	9.41	6.06	7.70	9.69	0.48
Isovaleric acid ^a	0.76	0.18	0.19	0.42	0.38	0.32	0.05
Valeric acid ^a	0.63	0.46	0.48	0.42	0.45	0.50	0.03
pH	6.8	6.7	6.8	6.7	6.8	6.9	0.04

^aNo linear and quadratic effect of urea level in the diet.

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SUMMARY AND DISCUSSION

To study the effect of differing supplemental protein sources for growing cattle fed corn stover silage, four experiments were conducted. Two trials were conducted to investigate the effect of varying ruminal degradability of the supplemental protein on the utilization of corn stover energy by growing cattle and two trials were performed to study the effect of ruminal NH_3 concentrations on digestion and performance of growing cattle consuming corn stover silage.

Supplementing steers consuming corn stover silage with protein from soybean meal, casein, corn gluten meal, urea or dehydrated alfalfa, improved feed consumption, digestion of nutrients and N-utilization over steers consuming a negative control supplement; but no differences between the protein sources were observed. Although differences in intake and digestion were not observed between steers fed the different proteins, the casein supplement resulted in the highest digestion of DM, indicating that protein sources which are degraded in the rumen to NH_3 and AA may be more useful in the utilization of roughage than urea (Salter et al., 1979). The poor digestibility of the diet containing the dehydrated alfalfa supplement may be attributed to heat damage of the protein or particle reduction during alfalfa processing, causing high rates of passage. The high digestibility by steers consuming the corn gluten meal cannot be explained by the low ruminal degradability associated with this product, but may be due to its being slowly degraded to supply AA, especially leucine, and NH_3 . Leucine

is catabolized to branched-chain fatty acids which are growth factors for some cellulolytic bacteria (Russell and Hespell, 1981).

The feedlot performance of heifers fed formaldehyde treated soybean meal was lower than the performance of heifers fed either urea or soybean meal, suggesting that rumen bacterial activity was impaired in heifers fed the highly "undegraded" protein (Mahadevan et al., 1983).

The hypothesis, that proteins which are highly degraded in the rumen, are important for microbial synthesis (Mahadevan et al., 1983; Pilgrim et al., 1970) is supported by the improved performance of heifers fed the TSBM+U or TSBM+C. The concept that AA are required for microbial synthesis (Salter et al., 1979; McManiman et al., 1976b; Maeng et al., 1976; Maeng and Baldwin, 1976a, b), however was not supported by the results of this experiment because performance of heifers consuming the TSBM+C supplement was not different from performance of heifers fed the TSBM+U. However, there are major differences between the present study and those just cited. In this study, free AA were not added to the diets of the animals and microbial synthesis was not measured. The ingredients of the diets consisted of soybean meal, formaldehyde-treated soybean meal, corn stover and molasses. Dietary proteins are slowly degraded in the rumen to peptides, AA and NH_3 . Although formaldehyde treated soybean meal protein is less degradable in the rumen (Thomas et al., 1979), it would be slowly degraded to AA, as would the proteins in corn stover and molasses, and therefore, could serve as sources of AA in the supplements containing urea. Addition of casein to formaldehyde treated

soybean meal may have resulted in the production of AA in excess of that necessary for maximum microbial synthesis. Most of the studies that have investigated the effect of AA on microbial synthesis have been done with washed rumen bacterial suspensions in vitro (Maeng et al., 1976; Maeng and Baldwin, 1976a, b). Maeng et al. (1976) examined the growth of washed rumen bacteria in batch cultures where urea was the sole N source and found that isonitrogenous replacement of 25% of the urea with a mixture of 18 AA increased the overall growth yields by approximately 100%. Despite this remarkable effect of AA on microbial growth, care must be taken in extrapolating in vitro results to what might happen in vivo. It is difficult to estimate the effects of AA additions to the diets of ruminants on microbial synthesis because AA are rapidly degraded in the rumen. The lack of transport systems for AA across bacterial cell walls (Allison, 1969), makes such a study less worthwhile. The use of dietary proteins, as examined in this study, seems more important and practical in investigating possible effects of AA on ruminal microbial synthesis because the slow degradation of dietary protein results in more AA being available. Moreover, dietary proteins are also degraded to peptides which are more efficiently utilized by rumen bacteria than free AA (Pittman and Bryant, 1964; Wright, 1967). More in vivo research is needed to confirm the increases in microbial synthesis and growth with AA additions as observed in in vitro studies. Similarly, animals fed the SBM supplement did not perform better than those consuming the urea diet. Among the factors examined in this experiment, ruminal ammonia

concentration was the most important in the utilization of corn stover silage.

Although increasing ruminal NH_3 concentration was accompanied by increased intake and digestion in steers fed corn stover silage, microbial protein synthesis per kg organic matter digested in the rumen was not changed with increased ruminal NH_3 concentration. Similar results have been obtained by others (Leibholz and Kellaway, 1979; Redman et al., 1980; McAllan and Smith, 1983; Orskov et al., 1972). Bacterial-N synthesized per day increased with higher ruminal NH_3 concentration up to an NH_3 concentration of 9.32 mg/dl, after which bacterial N synthesis plateaued. It is, therefore, estimated that the ruminal NH_3 concentration for optimal microbial N production in animals fed corn stover silage was 9.32 mg/dl. Ammonia concentrations in the rumen reflect the balance between production and utilization by bacteria and absorption, and as such vary with several factors including the extent of synchronism between energy and NH_3 release (Sriskandarajah et al., 1982). In low quality forages, energy would be a limiting factor in the utilization of urea-N by ruminants. Research is needed to estimate the amount of readily available energy which can be added to urea supplements for animals fed low quality forages.

To further improve utilization of low quality roughages, treatment with chemicals to increase fiber digestion should be combined with provision of rumen degradable protein.

A feeding scheme that maximizes ruminal microbial-N synthesis and at

the same time allows an increased flow of dietary protein to the duodenum should meet the metabolizable protein requirements of animals fed low quality forages.

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APPENDIX A. ADDITIONAL DATA FOR SECTION I

Table A1. Laboratory analysis of the stover silage (Experiment 1 and 2)

Item	Experiment	
	1	2
DM, %	37.16	65.00
	<u>% , DM basis</u>	
CP	8.16	4.95
NDF	63.00	74.34
ADF	40.66	50.56
Cellulose	31.78	40.31
Lignin	5.90	6.40
AIA	---	3.98
G.E. kcal/g	4.09	---

Table A2. Laboratory analysis of the protein supplements (Experiment 1)

Item	Supplemental protein source					
	SBM	DEHY ALF	CGM	C	U	CNL
DM, %	91.82	92.73	91.00	88.70	88.40	90.60
			% DM basis			
CP	15.72	18.00	15.75	13.25	17.63	5.38
NDF	56.41	49.14	52.74	46.16	48.93	51.69
ADF	31.79	34.03	29.65	27.00	27.82	30.45
Cellulose	25.66	22.74	22.84	21.40	21.77	23.58
Lignin	5.51	9.20	6.36	5.18	5.52	6.42
GE kcal/g	4.05	4.06	4.26	4.08	3.78	3.98

Table A3. Laboratory analysis of the protein supplements (Experiment 2)

Item	Supplemental protein source					
	CNL	U	SBM	TSBM	TSB+U	TSBM+C
DM, %	85.30	85.50	87.70	84.30	84.80	87.10
		% , DM basis				
CP	14.17	20.74	22.52	15.34	20.64	20.96
NDF	26.40	22.04	28.68	26.88	28.39	32.94
ADF	13.60	8.10	12.20	11.74	12.30	16.01
Cellulose	10.15	6.53	10.59	8.65	9.42	10.86
Acid insoluble ash	0.14	0.18	0.16	0.26	0.17	0.27

Table A4. Statistical model for least squares model of variance
(Experiment 1 and 2)

Source	Degree of Freedom	
	Experiment	
	1	2
Protein supplements	5	5
Row	5	--
Column	5	--
Error	17	6
Total	32	11

APPENDIX B. ADDITIONAL DATA FOR SECTION II

Table B1. Laboratory analysis of the stover silage

Item	
DM, %	71.18
	%, DM basis
CP	6.25
NDF	72.17
ADF	48.49
Cellulose	37.90
Lignin	5.00
AIA	5.95

Table B2. Laboratory analysis of the protein supplements

Item	Supplemental protein source % urea in the supplement					
	SBM	0.0	1.43	2.47	3.47	4.47
DM	91.18	87.15	85.38	84.47	83.68	84.75
	%, DM basis					
CP	19.11	14.72	17.00	20.13	21.68	23.71
NDF	16.00	17.55	19.70	17.65	15.30	14.30
ADF	12.19	11.95	13.07	12.46	11.65	10.44
Cellulose	10.69	10.58	11.38	10.88	9.95	9.19
Lignin	1.41	1.65	1.78	1.68	1.73	1.42
AIA	0.15	0.18	0.16	0.20	0.22	0.16

Table B3. Statistical model for the least square analysis of variance
(Experiment 1 and 2)

	Degrees of Freedom	
	Experiment	
	1	2
Protein supplements	3	5
Row	3	--
Column	3	--
Error	6	6
Total	15	11

Table B4. Isolation of mixed rumen bacteria

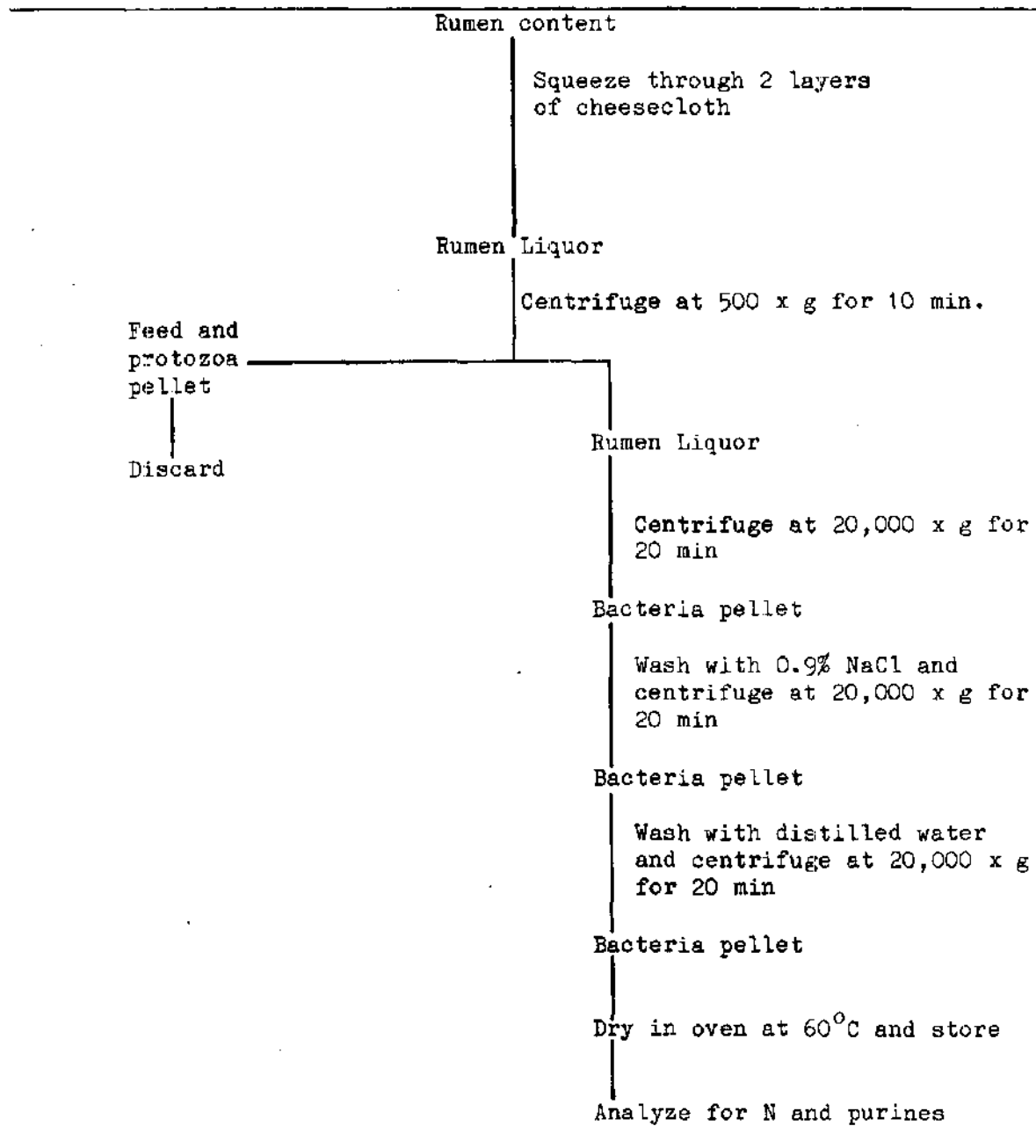


Table B5. Purine assay (Zinn and Owens, 1982)

1. Approximately 0.5 g duodenal samples, 0.2 g bacteria or RNA standard were weighed into 25 ml screw cap culture tubes provided with teflon lined caps.
2. 2.5 ml HClO₄ (70%) were added, tightly capped and vortexed until wet. The tubes were then incubated for 15 minutes in a water bath kept at 90-95°C after which they were removed, revortexed and replaced in the water bath for 45 minutes.
3. 17.5 ml 0.0285 M NH₄H₂PO₄ was added in two portions and vortexed to break up the charred mass and then filtered through Whatman #41 filter paper.
4. 0.5 ml filtrate were transferred to 15 ml centrifuge tube and 0.5 ml AgNO₃ (0.4 M) and 9 ml of 0.2 M NH₄H₂PO₄ were added and allowed to stand overnight.
5. The tubes were vortexed gently and centrifuged for 10 min using a bench centrifuge set at full blast. The supernatant was drawn off.
6. The pellets were washed with 6 ml of pH2 adjusted H₂SO₄ (adjusted with distilled water). Step 5 was then repeated, care being taken not to disturb the pellets.
7. 10 ml of 0.5 N HCl was added and vortexed.
8. The tubes were covered with marble and incubated in 90-95°C water bath for 30 minutes.
9. The tubes were vortexed and centrifuged.
10. Standards. These contained 0, 0.025, 0.100, 0.200 and 0.300 g yeast RNA. The standards were diluted to the range of the samples to be determined, usually 0.5 ml standard to 9.5 ml 0.5 N HCl.
11. The samples were read at 260 nm using a Beckman DB-G spectrophotometer.

Reagents

1. 0.2 N NH₄H₂PO₄ = 23 g/l distilled water.
2. 0.0285 M NH₄H₂PO₄ = 143 ml/l of 0.2 M NH₄H₂PO₄.

Table B5. (Continued)

-
3. 0.4 M AgNO₃ = 6.9 g/dl of pH 2 adjusted H₂SO₄ (adjusted with distilled water).
 4. 0.5 N HCl - 41.85 ml concentrated HCl/l.

Preparation of the standard curve and calculation of RNA content of duodenal samples.

The Y axis of the standard curve represented the dry weight of RNA in each tube corrected for dilution before absorbance was read.

The X axis represented the absorbance readings.

To obtain RNA concentrations of the duodenal samples, the absorbance readings of the duodenal samples were interpolated into the standard curve.

% RNA in the duodenal sample = (Predicted RNA concentration/duodenal dry sample weight) x 100.

Calculation of the ratio of bacterial nucleic acid N:total N from isolated ruminal bacteria.

This is done to facilitate comparison of values of the ratio of bacterial nucleic acid:total N obtained in different experiments and by different authors.

1. Average % N in ruminal bacterial RNA = 0.15292.
2. % RNA N = (% RNA in bacteria) x 0.15292.
3. RNAN:Total bacterial N = RNAN/total bacterial N.
4. In this trial, bacterial RNA averaged 7.06 %, bacterial N averaged 6.85 %.

% RNAN = 7.06 x .15292 = 1.08

RNAN:Total bacterial N = 1.08/6.85 = 0.157

The value of 0.157 compares with average of 0.16 obtained for animals fed low quality forages by Zinn and Owens (1982).

Table B6. Determination of chromium in chromium-mordanted fiber, duodenal and fecal samples (Williams et al., 1962)

Reagents:

1. Phosphoric acid-manganese sulphate solution:
 - a. Manganese sulphate solution - 3.6 g $\text{MnSO}_4 \cdot \text{H}_2\text{O}$ in 50 ml distilled water.
 - b. Add 7.5 ml manganese sulphate solution to 250 ml of 85% H_3PO_4 .
2. Potassium bromate solution: 11.25 g KBrO_3 in 250 ml distilled water.
3. Calcium chloride solution: 6.922 g CaCl_2 in 500 ml distilled water.
4. Chromium stock solution: 2.8285 g $\text{K}_2\text{Cr}_2\text{O}_7$ /l distilled water.

Procedure:

1. Ash 0.5 g sample in 30 ml crucible at 550°C for 2 hr.
2. Cool.
3. Add 1.5 ml phosphoric acid - manganese sulphate solution
4. Add 2 ml 4.5% potassium bromate solution.
5. Cover with watch glass and digest on a hot plate until effervescence ceases and purple color appears.
6. Cool, dilute with distilled water and wash into a 100 ml volumetric flask.
7. Add 10 ml calcium chloride solution and mix well.
8. Prepare standards - use feces from animals which were not fed chromium-mordanted fiber

Standard (ppm chromium)	mls stock
5	0.05
1	0.10
2	0.20
4	0.04
5	0.50
8	0.80
10	1.0

9. Allow to settle overnight and remove liquid off top for atomic absorption spectrometry. Read at 357.9 nm.
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Table B7. Calculation of flows and digestibilities

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- I. Flows of DM and its constituents to the duodenum. Dry matter flow to the duodenum was calculated from the amount of chromium marker ingested daily and the concentration of chromium in duodenal digesta (Faichney, 1975).

$$\text{DM flow (kg)} = \frac{\text{Total daily chromium ingested (g)}}{\text{concentration of chromium in duodenal digesta}}$$

The flow of any constituent of duodenal digesta was calculated by multiplying DM flow by the concentration of that constituent in duodenal digesta. For an example:

$$\text{Bacterial-N flow} = \text{DM flow} \times \% \text{ bacterial-N in duodenal digesta.}$$

Similar calculations were done for total N, fiber fractions and $\text{NH}_3\text{-N}$.

II. Digestibilities

DM digestibility was calculated from DM ingested and DM flows:

$$\text{Apparent DM digestion} = \frac{(\text{DM ingested} - \text{DM flow})}{(\text{DM ingested})}$$

Digestibilities of the constituents of DM were determined similarly.

Table B8. First derivatives of the regression equations (Experiment 1 and 2)

Item	First derivative
<u>Experiment 1</u>	
Feed intake	3.00
Dry matter, digestibility (total)	1.41
Dry matter digestibility (ruminal)	1.02
Fiber digestion:	
Ruminal NDF	1.06
Ruminal ADF	1.034
Ruminal cellulose	0.98
Total NDF	1.31
Total ADF	1.39
Total cellulose	1.17
Microbial-N, g/d	2.43
VFAs:	
Acetate	1.06
Propionate	1.00
Butyrate	1.40
Isovalerate	1.45
Valerate	0.95
<u>Experiment 2:</u>	
Feed intake	1.10
Average daily gain	1.0
Feed:gain	0.95

APPENDIX C. DETERMINATION OF ACID INSOLUBLE ASH

Table C1. Analysis of acid insoluble ash in feeds and feces (Van Keulen and Young, 1977)

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1. Use samples dried at 60°C for 3 d and ground to pass through a 1 mm screen.
 2. Weigh 2 g stover silage, 2 g feces or 5 g samples of protein supplement into tarred crucible.
 3. Heat at 100° for 3 hrs, cool and weigh.
 4. Ash at 550°C for 3 hrs, cool and weigh.
 5. Wash into 250 ml beaker with warm 2N hydrochloric (2N HCl) acid and add about 150 ml 2N HCl. Cover with watch glass and boil on hot plate, in a hood for 15 minutes.
 6. Filter with suction using Ashless Whatman 54 filter paper. Wash with hot water 3 times.
 7. Fold filter paper and return to respective crucible.
 8. Ash at 550°C for 3 hrs.
 9. Cool and weigh.
 10. Determine weight of ash and express at % of DM.
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