NITROGEN UTILIZATION AND PERFORMANCE IN RUMINANTS FED OSCILLATING DIETARY PROTEIN LEVELS

by

Sarah Jordan Simpson

Thesis submitted to the Faculty of the

Virginia Polytechnic Institute and State University

in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

in

Animal and Poultry Sciences

APPROVED:

_____

J. P. Fontenot, Chair

_____ _____

D.E. Eversole C.E. Polan

_____

M.L. Wahlberg

August 2, 2000

Blacksburg, Virginia

NITROGEN UTILIZATION AND PERFORMANCE IN RUMINANTS FED OSCILLATING DIETARY PROTEIN LEVELS

by

Sarah Jordan Simpson

Committee Chair: Joseph P. Fontenot
Animal and Poultry Sciences

(ABSTRACT)

Nitrogen excreted by ruminants may negatively impact the environment, and N not retained is not utilized for growth and production. Experiments were conducted to examine the effect of 48 h oscillation of two levels of low ruminally degradable dietary CP on N metabolism in lambs and performance of steers. In Exp. 1, a metabolism trial was conducted with 28 lambs (31 kg), allotted to four different diets: 8% CP, 10% CP, 12% CP, and 8% and 12% CP diets oscillated every 48 h. After adaptation, transition, and preliminary periods, feces and urine were collected for 10 d. Ruminal fluid and blood samples were taken at the end of collection and again 2 d later. In Exp. 2, 24 crossbred steers (228 kg) were allotted to four diets: 1) 7.5% CP, 2) 9% CP, 3) 10.5% CP, and 4) 7.5% and 10.5% CP diets oscillated every 48 h. Feed intake was measured during the 112 d study, and ADG and gain to feed ratio were calculated. Cattle were weighed every 14 d and blood samples were taken every 28 d. In Exp. 1, N retention was lowest ($P < 0.05$) for the lambs fed the 8% CP diet, with no differences among lambs fed the other diets. Differences in urinary N excretion accounted for most of the differences in total N excretion. Ruminal NH_3-N and BUN levels were greater in animals fed higher amounts of CP. Ruminal pH and VFA concentrations were not affected by diet. In Exp. 2, feed intake did not differ among

steers fed different diets. Average daily gain was lowest for cattle fed the 7.5% CP diet. No significant difference was evident for ADG between steers fed the 7.5/10.5% CP oscillating diet and those fed the 9% or the 10.5% CP diet. Gain to feed ratio was lower ($P < 0.05$) for steers fed the 7.5% CP diet compared to steers fed all other diets. Blood urea N level was higher for cattle fed the 10.5% CP diet than those fed the two lower CP levels, and differences were usually significant ($P < 0.05$). No consistent significant difference in BUN levels existed between steers fed the 7.5/10.5% CP oscillating diet and those fed the 9% and 10.5% CP diets continuously. Oscillating two levels of low ruminally degradable dietary CP every 48 h had no significant effect on N retention in lambs nor on the performance of steers compared to animals fed the same level of CP daily in these experiments.

Key Words: N retention, Protein, Daily gain, Ruminants, Environment

Acknowledgements

I would like to thank God for the strength and ability to complete this degree, and for the blessing of a wonderful family and many true friends, all of whom have helped me immeasurably throughout this process.

To my major professor, Dr. Joseph Fontenot, I would like to express my gratitude for not only his guidance, encouragement, and wisdom, but also for his understanding and kindness throughout the past 2 yr. I would also like to thank the members of my committee, Dr. Dan Eversole, Dr. Carl Polan, and Dr. Mark Wahlberg, for their advice and support.

A special thanks is due to the university employees, graduate students, and friends who helped me complete the experiments and were a source of support and friendship: Jesse Austin, Gary Bradley, Judd Culver, Jessica Davis, Richard Dietz, Jeff Ford, Nancy Frank, Shane Horsley, Katie Poole, Tina Shanklin, Don Shaw, and Kenny White. It was a pleasure to work with all of these individuals and the many others whose kind words and encouragement made this endeavor easier and much more fun.

Finally, I would like to thank my family: my mother and father, Beverly and Edward Simpson, my sister and brother, Rachel and Ed, my aunt, uncle, and cousin, Diane, Richard, and Larkin Goff, my grandmother, Dorris Porter, and the memory of my grandfather, Hobart Porter. My family inspires me every day to do my best, work hard, be honest, be kind, and never to forget the things in life that are really important. I thank them for their unwavering support, inspiring example, and unconditional love.

Table of Contents

List of Tables

Introduction

Modern animal production and agricultural practices have the potential to negatively impact the environment. Nitrogen excreted in the urine of grazing animals is of particular concern in that it can be easily volatilized to ammonia (NH_3) or nitrous oxide (N_2O). These gaseous forms of N have been shown to influence pollutant deposition (ApSimon et al., 1987) as well as contribute to global warming (Rodhe, 1990) and ozone destruction (Crutzen, 1981).

Several studies have been conducted to evaluate the contribution of ruminants to N pollution. Sherlock and Goh (1983) demonstrated that the application of sheep urine to pasture resulted in immediate release of N_2O at a rate two times greater than applications of ammonium sulfate and urea, or a water control. A study by Jarvis et al. (1989) demonstrated that dietary N content could influence the amount, form, and distribution of N excreted. Pastures fertilized with high levels of N resulted in higher N content of the forage consumed, and greater NH_3-N loss from pasture swards per grazing steer than pastures fertilized with a medium level or no N fertilizer. Differences in N excretion between treatments were almost entirely attributable to differences in urinary N. Urea, which is degraded to NH_3, was the major N-containing component in the urine of all steers, regardless of treatment.

Another environmentally-oriented study was conducted by Flessa et al. (1996) to examine the effects of cattle excrements on the gas flux of N_2O and CH_4 in pastures. Data were collected and calculations were made on a global scale. Of the total amount of N excreted in the urine and feces, 3.2% became N_2O -N. When this was multiplied by the data collected on total N excretion by large, grazing ruminants world-wide, global N_2O emission from these animals was estimated at ~ 1.18 million metric tons per year, making it a significant source of N_2O in the atmosphere. Tomlinson et al. (1996) predicted N excretion for 635 kg Holstein dairy cows on a

diet formulated for 22.7 kg milk/d (15.3 % CP) to be 150 g urinary N and 175 g fecal N per day. Both urinary and fecal N presented a problem, due to volatilization as well as runoff concerns in this type of confinement operation. The runoff water from the dairy operations contains excess N, which can be converted to nitrates and potentially contaminate drinking water. These researchers stressed the need for the development of prediction equations to estimate the quantities of potentially harmful elements being excreted into the environment in this type of situation.

In addition to decreasing environmental pollution, decreasing N excretion in ruminants could result in positive effects on animal performance. If more N could be retained as a percentage of total N fed, more N would be available to maximize growth and production in the animals. Feeding ruminants low ruminally degradable protein sources may increase N retention. The method of feeding protein may also positively influence the retention of N in ruminants.

The ability to manipulate the excretion of N in ruminants has important environmental and nutritional applications, and this topic has been investigated in several studies using various approaches. The objectives of these studies were to determine the effect of oscillating dietary CP levels on N metabolism and performance in ruminants.

Review of Literature

Protein Metabolism in Ruminants

Ruminants can utilize both true protein and non-protein N in feed sources. Ruminal microbes break down the degradable fraction of true protein into NH_3 and then utilize it to synthesize microbial CP. The microbial CP then passes into the small intestine, where it may be digested and is absorbed. Non-protein N contains little if any ruminally undegradable nitrogen, and therefore is extensively broken down into NH_3 by ruminal microbes (Smith, 1989). True protein can contain a ruminally undegradable fraction, which bypasses the rumen-reticulum and passes directly to the small intestine for digestion and absorption. Because this ruminally undegradable protein does not undergo break down and resynthesis before absorption, it may represent a more efficient supply of protein to the animal, and therefore can be of particular benefit to animals functioning at high levels of production and growth. This type of protein is especially beneficial if it supplies the amino acids needed to complement those amino acids supplied by microbial CP (Owens and Zinn, 1988). Depending on ruminal pH and NH_3 concentrations in the blood and rumen, NH_3 in the rumen can diffuse into the blood stream and be carried to the liver, where it is used for urea synthesis. Nitrogen in the form of urea can then follow three routes in the body: 1) filtration at the kidney and excretion in the urine, 2) recycling to the rumen through saliva or the rumen wall, or 3) recycling to the intestinal tract, rendering less nutritional benefit to the animal (Smith, 1989).

The metabolism of protein is of particular importance as it relates to the retention of N in the animal. Nitrogen retention is defined as the difference between N intake and the combination of both fecal and urinary N output (Owens and Zinn, 1988). Increasing the amount of N retained

by ruminants, without feeding more N could potentially increase animal productivity as well as minimize wasteful and potentially harmful excretion of N into the environment.

Protein Degradability

Determination of Protein Degradability. Several methods exist to evaluate the ruminal degradability of intake protein. Although not without flaws, the *in situ* nylon bag technique (Mehrez and Orskov, 1977) is commonly used for this type of analysis (Broderick et al., 1988). In the *in situ* method, Dacron bags of a specific pore size, number, and distribution are washed, dried, and weighed. A measured amount of the feed in question is placed in the bag, and the bag is sealed securely. After rinsing with water, the Dacron bags are placed in the rumen of a ruminally-cannulated animal, and at the completion of each incubation interval, they are removed, washed thoroughly until the rinsed water is clear, dried to a constant weight, and nutrient disappearance is calculated (Mehrez and Orskov, 1977).

Several *in vitro* methods for determining protein degradability exist as well. Incubation in ruminal fluid, first described by Little et al. (1963), is one method of *in vitro* determination. This method measures the amount of NH_3 that accumulates after a particular feed is incubated with ruminal fluid at typical rumen environmental conditions. This method does not, however, account for the NH_3 taken up by ruminal microbes for protein synthesis. Procedures to correct for this fact have been investigated, and a method of using $^{15}NH_3$ has shown promising results (Hristov and Broderick, 1994). Different protein sources were mixed with a buffer solution, a reducing solution, citrus fruit pectin, and a soluble carbohydrate mixture before being combined with ruminal fluid. The $(^{15}NH_4)_2SO_4$ was added to the mixture just before incubation. After 6 h samples were taken, centrifuged, and supernatants were analyzed for non- NH_3-N, NH_3, AA, and

^{15}N-enrichment. Protein degradation was then calculated using several equations that corrected for the incorporation of ^{15}N into bacterial protein.

Incubation with proteolytic enzymes has also been examined as an *in vitro* technique to estimate ruminal protein degradability. *Streptomyces griseus*, ficin, and neutral protease with amylase are three proteolytic enzymes that have been studied (Roe et al., 1991). When results from the use of these methods were compared to those of an *in situ* trial, little correlation between the methods was found. However, the neutral protease with amylase method did relate to the degradation curve of the *in situ* method at certain time intervals, but not overall. Susmel et al. (1993), however, did find a correlation between the *Streptomyces griseus* method of proteolytic incubation and the *in situ* method in two areas. The rate of degradation of the potentially degradable protein fraction was correlated between these two methods. The *in situ* values for calculated effective degradability and the *Streptomyces griseus in vitro* values for percentage of N degraded were correlated, as well.

A study by Mehrez and Orskov (1977) investigated the effects of several factors on the consistency of results using the *in situ* method. They found that homogeneity of the physical form of the feed incubated and the size of the bags used could affect consistency of results. Variability was also found between animals used and between days of incubation. Microbial contamination has also been identified as potential source of error in the *in situ* method (Kennedy et al., 1984). With current technology, *in vitro* studies cannot exactly reproduce the environment of the animal body, and therefore are subject to many sources of error. Comparison of results from *in situ* and *in vitro* trials with those from *in vivo* trials, taking into account animal species and stage of productive life, may provide a more accurate understanding of ruminal protein degradability in ruminants (Erasmus et al., 1988).

Protein Degradability Values. Although researchers have reported a variety of protein degradability values for individual feeds, feeds can usually be classified as having high, intermediate, or low ruminal protein degradability. In a study examining DM, CP, and starch degradability, Batajoo and Shaver (1998) conducted an *in situ* trial on ruminally-fistulated Holstein cows. Cows received a basal diet of alfalfa silage (55%) and a concentrate mix (45%), which consisted mainly of dried shelled corn (90.5%). Degradabilities were calculated using values for the rapidly and slowly degraded fraction, degradation rate, and rate of passage. Wheat middlings and corn gluten feed exhibited the highest ruminal CP degradation studied (71.9 and 70.3%, respectively). Protein degradability of SBM, barley, and soybean hulls was intermediate, with values of 62.9, 60.0, and 58.2%, respectively. Brewers dried grains, corn, and distillers dried grains had the least amount of ruminally degradable protein, with values of 48.0, 40.0, and 39.6%, respectively. In other studies involving *in situ* trials with mature cows similar results as reported above were reported for soybean meal (53%, 52%, 66%) and brewers dried grains (43.5%) (Broderick et al., 1988; Erasmus et al., 1988; Susmel et al., 1993). However, Erasmus et al. (1988) did find brewers dried grains were much less ruminally degradable (23.0%) than Batajoo and Shaver (1998) (48.9%) and Susmel et al. (1993) (43.5%). Feedstuffs such as fish meal (Broderick et al., 1988; Susmel et al., 1993; Erasmus et al., 1988) , feather meal, meat and bone meal (Broderick et al., 1988), blood meal (Erasmus et al., 1988), and corn gluten meal (Susmel et al., 1993) have exhibited low ruminal degradability values (23 to 50%) in several *in situ* trials, while the ruminal degradability of SBM has been consistently high (> 50%) in several studies (Broderick et al., 1988; Erasmus et al., 1988; Susmel et al., 1993).

Effects of Protein Degradability

Nitrogen Metabolism. Stock et al. (1981) conducted two lamb metabolism trials to examine the effects of three slowly degraded protein sources (corn gluten meal, blood meal, and meat meal) and SBM on N utilization. Lambs were fed a basal diet of ground corn cobs and molasses. Eight treatments were used. All treatments were equalized in crude protein and TDN, varying only in type of supplemental protein employed. Supplements made up 10% of the total diet dry matter. The first treatment supplement was made up of 100% urea, while the second, third, fourth, and fifth treatments were 60% SBM, blood meal, meat meal, or corn gluten meal, respectively, and 40% urea. The sixth, seventh, and eighth treatment supplements were combinations of equal amounts of corn gluten meal and blood meal, corn gluten meal and meat meal, or corn gluten meal and SBM, with 40% urea in each.

In the first metabolism trial, the addition of slowly degraded protein increased N retention, expressed as g/d, compared to the urea and SBM combination treatment, or the 100% urea treatment. The meat meal-urea treatment lambs had the highest N retention values, and the blood meal-urea-fed lambs had the lowest N retention values among the more undegradable protein supplement treatments. Data also indicated the possibility of a complementary effect between the protein in corn gluten meal and that in SBM and blood meal. Lambs receiving those treatments exhibited N retention values only slightly lower than the meat meal-urea lambs.

In the second metabolism trial, comparing protein supplements of blood meal (60%)-urea (40%), commercial meat meal (60%)-urea (40%), two types of pork meat meal (60%)–urea (40%), and two types of beef meat meal(60%)-urea (40%) to SBM (60%)-urea (40%) and 100% urea, no significant differences for N retention were distinguished except between two sources of meat meal. The highest and lowest N retention values were exhibited by lambs fed the two beef

sources of meat meal (in comparison with pork or commercial sources). Protein degradability had no effect on N retention in this trial.

Gill and England (1984) compared fish meal (a less degradable protein) and ground nut meal (a more degradable protein source) as supplements to low quality perennial ryegrass silage. Twelve steers (119 kg) were randomly assigned to three treatments: 1) silage only, 2) silage and fish meal, or 3) silage and ground nut meal (diets 2 and 3 were isonitrogenous). These researchers found no effect on N retention between the two supplements of differing degradability, in agreement with the second metabolism trial of Stock et al. (1981). The researchers attributed the lack of significant results to possibly poor quality of the silage used, suggesting that degradability of protein supplement may have little influence when combined with low-quality silage.

Petersen et al. (1985) conducted two metabolism trials with 330 kg steers to compare the effects of rate of protein degradability. The steers were fed a ground mixture of mature, native forages in addition to one of four supplemental N sources: 1) 15% corn, 85% urea, 2) 100% SBM, 3) 10% corn, 40% SBM, 50% urea, or 4) 14% corn, 36% blood meal, 50% urea. The diets were formulated to supply 9.6% CP. Calculated percentage of escape (ruminally undegradable) N was: 0 (assumed for urea), 21.5, 16.5, and 54.2% for treatments 1, 2, 3, and 4, respectively.

Protein supplementation did not affect non-NH_3 N flow, the flow of feed N, the flow of bacterial N, or microbial protein synthesis efficiency. However, the researchers reported a positive correlation between amount of slowly degraded protein fed and non-NH_3 N and feed N flow. Although the data were not evident, the researchers stated that as the amount of slowly degraded protein in the diet increased, non-NH_3 N and feed N flow also increased. This led to

their suggestion that greater non-NH_3 N flow may indicate greater ruminal escape properties of a protein source.

In a second metabolism trial by Petersen et al. (1985), the urea supplement was replaced with a control (no supplement), and dietary CP was increased to 10.1%. Nitrogen retention was significantly higher in the 100% SBM and corn-blood meal-urea diets, compared to the corn-SBM-urea and no supplement diets. The trend in N retention data suggested a greater percentage of escape N may increase the growth of cattle fed this type of forage.

Hassan and Bryant (1986a) investigated the interaction of ruminally undegraded protein and forage: concentrate ratio by feeding 31.4 kg lambs four diets. The diets consisted of NaOH-treated barley straw, tapioca, extracted rapeseed meal, urea, and minerals formulated at either a 60:40 or 40:60 forage to concentrate ratio. Each forage to concentrate ratio was fed with either no fish meal supplementation, or 100 g/kg DM fish meal. The diets provided either 3 (without fish meal) or 9 (with fish meal) g ruminally undegradable N/kg DM and an equal amount of ruminally degradable N (1.4 g/kg DM). Inclusion of fish meal significantly increased N retention per kilogram digestible OM intake, but the N retention values were not significant as proportion of N intake or N digested.

In another experiment by Hassan and Bryant (1986b) the effects of feeding different levels of fish meal were examined. Lambs (31.4 kg) were given NaOH-treated barley straw, tapioca, extracted rapeseed meal, urea, and minerals in a 40:60 forage:concentrate ratio, and either 0 or 90 g fish meal supplemented to diets formulated for 100, 150, or 200 g daily gain. Diets contained either 2.2 g (without fish meal) or 6.7 g (with fish meal) of ruminally undegradable N/d. Nitrogen retention expressed as a proportion of N intake was increased by inclusion of fish meal in the diet, although only at the medium and high levels of feeding. The

researchers concluded that lambs gaining at 150 to 200 g/d would benefit from supplementation with a ruminally undegradable protein source.

Al Jassim et al. (1991) housed 10 lambs and 10 goats (21 kg) in metabolism cages and fed them two experimental diets. The basal diet was a mixture of NaOH-treated wheat straw, molasses, and varying amounts of barley, corn, and either SBM or formaldehyde-treated SBM (making it less ruminally degradable). The low undegradable protein diet contained 8.29 g undegradable N/kg DM, and the high undegradable protein diet contained 17.59 g undegradable N/kg DM. Nitrogen retention was increased significantly only for sheep on the more ruminally-undegradable diet. The researchers concluded that although there were differences in the digestion and utilization of protein between sheep and goats, more undegradable protein appeared to increase N retention, although not always significantly, in both animal species.

Dawson et al. (1988) performed a metabolism trial with four ruminally and duodenally fistulated steers. Animals were fed ryegrass silage hourly either alone or supplemented with 150 g fish meal/kg silage DM. The objective was to determine the effect of fish meal supplementation on rumen microbial metabolism. They found that total N flow, non-NH_3-N flow (largely as undegraded dietary protein), and AA flow to the duodenum were all increased by the addition of fish meal. Microbial N flow as well as efficiency of microbial protein synthesis were also increased in animals receiving fish meal. Although the researchers attempted to determine the source of N used by rumen microbes, unconfounded results could not be obtained. However, there was evidence to suggest that less ruminal NH_3 may have been utilized for microbial N synthesis in fish meal diets, perhaps indicating a greater uptake of peptides by microbes on the fish meal diet. The researchers also suggested that the improved efficiency of microbial synthesis with fish meal could be attributed to the hourly feeding, which allowed for a

more continuous N supply. This apparently improved synchronization of energy and N release could have reduced the loss of N before it reached the duodenum. It was concluded that the addition of fish meal to this type of silage and feeding regimen produced metabolic results favorable to growing steer performance.

Titgemeyer et al. (1989) used six Simmental steers (336 kg) with ruminal, duodenal, and ileal cannulas, to investigate the effects of SBM, corn gluten meal, blood meal, and fish meal on AA and N availability in the small intestine. Over eight periods, 13 diets were fed containing corn silage, pelleted wheat straw, ground corn, corn starch, urea, and casein. The basal diet contained 20.4% corn starch and 1.75% N. The four protein sources, all of relatively low ruminal degradability except for SBM, were each fed at three different levels: either 3, 6, and 9% CP (SBM and corn gluten meal) or 2, 4, and 6% CP (blood meal and fish meal). The protein was added at the expense of the corn starch in the diet.

When ruminal protein degradabilities were calculated, SBM had the lowest ruminal N escape value (21%), blood meal had the highest value (92%), followed by corn gluten meal (86%), then fish meal (68%). Higher ruminal N escape values resulted in more nonbacterial N absorbed in the small intestine. The greatest amount was absorbed from corn gluten meal and blood meal, followed by fish meal, and then SBM. Degradation of individual AA was also determined in this study, and the researchers found that each protein source was limiting in at least one essential AA. This led to the suggestion that combinations of protein sources may offer the best AA profile for ruminant animals.

Studies have also been done to examine the effects of protein degradability in dairy cattle. Zerbini et al. (1988) fitted six lactating Holstein cows with ruminal and duodenal cannulae to determine the effect of two protein sources differing in ruminal degradability on

protein digesta flow to the duodenum. The experiment was conducted during both early- and mid-lactation, and protein sources used were SBM (rapidly degradable) and fish meal (slowly degradable). Diets were formulated using corn silage, chopped orchardgrass hay, and ground corn, and supplied equal amounts of protein (15.5% CP) and fiber (20.7% ADF). Digesta flow was greater for the SBM diet, as was microbial N flow to the duodenum. However, residual N flow (total N – microbial N) was greater for the fish meal diet, resulting in total N flow being similar for both diets. Recovery of N at the duodenum was 9% greater for fish meal than SBM, indicating less ruminal N loss with the fish meal diet and less ruminal degradability of fish meal. True efficiency of microbial protein synthesis (g microbial N/kg OM truly digested in the rumen) was lower for the fish meal diet and in mid-lactation. Although total AA intake was greater for the SBM diet, AA flow to the duodenum showed no significant difference between diets. More total AA were lost in the stomach in the SBM diet. Individual AA flow varied between diets. Methionine, lysine, and histidine, being of particular importance to milk production, were less degraded in the fish meal diet; however, this was not reflected by an increase in milk production. The researchers concluded that diets for animals of this type should contain adequate degradable protein to maximize microbial synthesis.

Volden (1999) used six ruminally and duodenally cannulated, lactating cows (600 kg) in two experiments to examine the effects of ruminally undegraded protein on milk protein and various aspects of protein metabolism. The experiment was done both during early lactation, in which cows were fed at a high level (20 kg DM/d) and during late lactation, in which cows were fed at a low level (10 kg DM/d). The basal diet was a 60:40 mixture of concentrate (barley, oats, rapeseed meal, molasses) and grass silage, DM basis. Treatment diets were formulated to be either high protein, low ruminal degradability; high protein, high ruminal degradability; or low

protein, low ruminal degradability (diets contained 69, 53, and 48 g ruminally undegraded protein/kg DM, respectively). Fish meal was used as the low ruminally degradable protein source (24%), and SBM and a more degradable fish meal (65%) were used as the high ruminally degradable protein sources.

In early lactation, the high protein, low ruminal degradability diet had the greatest duodenal flow of non- NH_3 -N and total AA, while the low protein, low ruminal degradability diet had the lowest flow values. During late lactation, however, no significant differences among diets were found for non- NH_3 -N or total AA flow. More methionine, histidine, and arginine were found in the duodenal digesta of cows fed the high protein, low ruminal degradability diet than those fed the other diets at both early and late lactation. Using diaminopimelic acid as a bacterial marker, the high protein, high ruminal degradability diet was shown to have the greatest bacterial N synthesis during early lactation. Milk protein production measured in g/d and milk protein percentage were greatest for the high protein, low ruminal degradability diet during early and late lactation, respectively. He concluded that low ruminally degradable fish meal has the potential to increase total and individual AA supply to the small intestine, making AA more available for maximum milk protein production in dairy cattle.

Ruminal Characteristics, Intake, and Digestibility. Veen (1986) conducted five trials using Dutch Friesian or crossbred Holstein Friesian x Dutch Friesian cows to determine the effects of feeding slowly- or rapidly- degraded concentrate protein. Diets were formulated from a large variety of feedstuffs differing in ruminal degradation. Each trial involved feeding diets of either low or high protein ruminal degradability and sampling ruminal fluid pre- and post-feeding for pH, NH_3, and in some cases, VFA and lactate.

Ruminal pH and lactate concentration were not affected by protein degradability. However, ruminal NH_3 was greater when a more easily degraded concentrate protein was fed. The concentration of VFA increased more rapidly when a more rapidly degraded concentrate protein was fed, but the molar acetate:propionate ratio was greater with more slowly degraded protein diets. Veen (1986) concluded that concentrates with more slowly degraded protein allow for "stabilization" of rumen fermentation, and therefore, cellulolytic bacteria were less likely to be displaced by amylolytic bacteria, and a higher acetate:propionate ratio was maintained. Zerbini et al. (1988), however, found no significant effect of protein degradability on acetate:propionate ratio, but did find total VFA concentration and acetate concentration were greater for animals eating a more ruminally degradable protein source.

In agreement with the results of Veen (1986), other researchers have found ruminal NH_3 concentration to be greater in animals fed more degradable sources of protein compared to animals fed less degradable protein sources (Stock et al., 1981; Zerbini et al., 1988; Titgemeyer et al., 1989). However, data have also indicated the addition of fish meal, a low ruminally degradable protein source, to sheep diets tended to maintain a higher ruminal NH_3 concentration over time, compared to diets containing urea and rapeseed meal as protein sources (Hassan and Bryant, 1986a). Results from that study, however, may have been confounded by the fact that the fish meal diets also supplied a greater amount of total N than those diets not containing fish meal. Stock et al. (1981) suggested that low ruminal NH_3 levels were indicative of a slower rate of ruminal protein degradation, whereas high ruminal NH_3 levels implied greater ruminal degradation and release of NH_3 over time.

Varying results have been reported in studies investigating the effect of protein degradability on digestibility. No significant effect was found on OM, DM, or N digestibility in

two different studies using sheep fed groundnut meal compared to fish meal (Gill and England, 1984) and using cattle fed four different diets containing variable levels of ruminally degradable protein (Petersen et al., 1985). However, more ruminally undegradable protein in the diet of Holstein cows was reported to decrease fiber digestibility in these animals (Zerbini et al., 1988). The addition of fish meal to sheep diets formulated for both low and medium levels of gain resulted in increased apparent ADF digestibility. Digestibility of other fiber fractions was not affected, but this trend was not seen in animals fed diets formulated for high levels of gain (Hassan and Bryant, 1986b).

Performance. Stock et al. (1981) conducted two growth trials of 137 and 128 d to examine the effects of three slowly degradable protein sources on performance. Crossbred steer calves (200 kg) were fed either 50% corn silage, 40% chemically treated corn cobs, and 10% supplement (Trial 1), or 65% corn silage, 15% oat straw, 10% molasses, and 10% supplement (Trial 2). Both diets were formulated to provide 11.5% CP. Supplements consisted of 100% urea, SBM (60%)-urea (40%), blood meal (60%)-urea (40%), corn gluten meal (60%)-urea (40%), meat meal (60%)-urea (40%) and a combination of blood meal (30%)-corn gluten meal (30%) and urea (40%) or meat meal (30%)- corn gluten meal (30%) and urea (40%) (Trial 1). Trial 2 compared only the 100% urea, SBM-urea, blood meal-urea, and meat meal-urea supplements. Daily gain of steers was significantly increased with the addition of low ruminally degradable protein sources, compared to supplementation with urea alone. These protein supplements also numerically increased daily gain compared to SBM-urea supplementation. Results on feed conversion and conversion of protein to gain suggested a complementary effect of the blood meal-corn gluten meal combination, in that the combination of the two protein sources resulted in greater performance than either source alone. The researchers concluded that

while DM and N digestibilities were not greatly affected by the varying protein sources, blood meal, meat meal, and corn gluten meal fed with urea have the potential to increase animal performance over SBM-fed animals. In addition, using these high-quality, by-product feeds could reduce the cost of protein supplementation.

Gill et al. (1987) examined the effect of fish meal supplementation as well as oestradiol-17â implantation in growing calves (119 kg) fed well-fermented ryegrass silage. Animals were fed silage only, or 50, 100, or 150 g fish meal/kg silage DM/d, and one half of the animals on each diet were implanted with oestradiol-17â. The trial lasted 63 d. Results indicated a significant interaction between implantation and fish meal supplementation in that no response to implantation was apparent except at supplementation levels of 100 or 150 g/kg silage DM supplementation of fish meal. The 150 g/kg silage DM level of fish meal increased protein gain by 50 g/d over silage alone, and addition of implants to this diet increased protein gain by another 23 g/d. The researchers concluded that 100 g fish meal/kg silage DM is probably the optimum level for maximum response of cattle of this size, whether or not receiving growth implants.

Anderson et al. (1988) conducted two 75 d grazing trials to examine the effect of four levels of supplemental protein with low ruminal degradability on steers grazing smooth brome. Steers weighed approximately 250 kg and were fed corn starch and molasses (control), or 0.11, 0.23, or 0.34 kg/d of a mixture of corn gluten meal and blood meal with molasses. Soyhulls (0.23 kg/d) and additional molasses were added to the treatments in Trial 2, due to palatability problems. When data for both trials were combined, steer daily gain increased both linearly and quadratically as amount of supplemental low ruminally degradable protein increased. The maximum gains occurred at 0.23 kg/d (Trial 1) and 0.11 kg/d (Trial 2). Forage analysis

indicated the smooth brome protein was highly degraded in the rumen (80 to 90% of the potentially degradable protein was degraded in 12 h *in situ*). The improved performance of steers supplemented with escape protein over the energy controls steers and the extensive degradation of the forage used, led researchers to conclude that metabolizable protein was limiting steer performance while grazing smooth brome.

Gutierrez-Ornelas and Klopfenstein (1991) designed an experiment to investigate the effects of level and method of supplementation of escape protein on steers (230 kg) grazing irrigated and non-irrigated corn residue fields. Two trials of 63 and 66 d were used. In the first trial, 60 steers were fed 60, 88, 116, 144, 172 or 200 g escape protein per day, and all steers grazed non-irrigated fields. In the second trial, there were three variables: steers grazed either irrigated or non-irrigated fields, and were fed 60, 95, 130, 165, or 200 g escape protein daily, and were fed either their respective level of escape protein continuously, or they were fed the lowest escape protein level (60 g) for 21 d and then one of the other escape protein levels for the remainder of the trial. Escape protein was formulated from a combination of blood meal and corn gluten meal, and supplements were isonitrogenous and isocaloric.

In the first trial, no effect of escape protein supplementation was reported until d 20. Over the next 2 wk, however, feeding escape protein resulted in a dramatic increase in ADG as the amount of escape protein increased to 172 g/d. In the second trial, they reported increased gains with escape protein supplementation, but no effect from method of supplementation. Thus, the researchers concluded that escape protein was not critical for maximum growth of these animals in the first 3 wk.

Karges et al. (1992) fed steers (326 kg) one of eight diets: a negative control, energy control (cornstarch and molasses), either 0.15, 0.27, or 0.37 kg/d of degradable protein (corn

steep liquor and urea) or 0.07, 0.14, or 0.21 kg/d of escape protein (treated SBM, feather meal, and molasses). The pasture grazed was mostly little bluestem, switchgrass, prairie sand reed, sand bluestem, and hairy grama. To determine any carryover effect, one half of the steers were removed from the trial after 83 d and finished in a feedlot. The other half continued receiving supplementation for approximately 5 more weeks before also being finished in a feedlot.

Level of degradable protein supplementation had no effect on daily gain during the pasture study; however, there was a linear effect of level of escape protein supplementation, with the greatest daily gains at the highest supplementation level (0.21 kg/d). Feedlot performance of steers showed no carryover effects from protein supplementation treatments. It was concluded that supplementation with escape protein supplied the needed metabolizable protein to maximize gains of steers grazing this type of pasture, hence, microbial protein alone was insufficient.

Hafley et al. (1993) conducted two grazing studies (75 and 73 d) over 2 yr to determine the degradability of certain warm-season grasses and how cattle performed while grazing these grasses alone or with protein supplementation. The pasture consisted of mostly big bluestem, switchgrass, and some indiangrass. The first trial involved steers (286 kg) fed one of three supplements: energy control (cornstarch and molasses), energy control plus 0.1 kg/d escape protein, or energy control plus 0.2 kg/d escape protein. The escape protein was supplied by a mixture of blood meal and corn gluten meal. Performance, measured as ADG, was improved in steers supplemented with escape protein at the 0.2 kg/d level; however, this was only evident for the first 40 d of the trial. For the remaining 35 d, there was no significant difference in ADG for steers supplemented at the two levels of escape protein.

In the second trial, heifers (240 kg) were fed one of five supplements: negative control (no supplementation), energy control (corn starch and molasses), energy control plus 0.14 kg/d

escape protein, energy control plus 0.18 kg/d ruminally degradable protein, or energy control plus 0.36 kg/d of both escape and ruminally degradable protein. In this trial, the escape protein was supplied by non-enzymatically browned SBM and feather meal (estimated ruminal escape *in situ*, 12h was 75%), and the ruminally degradable protein was supplied by corn steep liquor and urea. Average daily gain was significantly improved over negative control, energy control, and escape protein only diets by the combination diet of both escape and degradable protein. The cattle fed the ruminally degradable protein diet had the next highest ADG, but it was not significantly different from those fed the energy control diet. Ruminal fluid collected from fistulated steers also receiving the specified diets indicated that ruminal NH_3-N concentrations were low for the forage only, energy control, and escape protein diets. Only the cattle fed the ruminally degradable protein diet and the combination protein diet achieved levels of NH_3-N considered adequate for microbial protein synthesis. It was concluded that warm-season grasses might require supplementation with ruminally degradable protein, and perhaps escape protein, to maximize cattle performance, due to the apparently lower ruminal protein degradability of warm-season forages when compared to cool-season forages.

Blasi et al. (1991) conducted four grazing trials of 75 d each to determine the effects of supplementing lactating beef cows with escape protein. Cows were grazing either smooth brome or big bluestem pastures. Animals were fed one of five supplements: a negative control, control (corn starch, molasses), or 0.11, 0.23, or 0.34 kg escape protein per head daily. The escape protein was a mixture of blood meal and corn gluten meal. Milk production, measured by the weigh-suckle-weigh technique, and calf daily gain exhibited a cubic response for animals grazing smooth brome. Calf gains and milk production decreased when escape protein was increased from 0 to 0.11 kg per head daily, increased for the 0.11 to 0.23 kg per head daily, then

decreased again for the 0.34 kg per head daily level. Cow weight gain was only significantly affected by level of escape protein for cows grazing brome in one trial, where a quadratic effect was evident. Cow weight gain was greatest at the 0.34 and 0 kg supplementation level. However, calf ADG and weaning weight exhibited a quadratic effect when cows grazed big bluestem, with peak gains and weights at the 0.23 kg supplementation level. The researchers suggested that the lack of response from cows supplemented with escape protein while grazing big bluestem was due to adequate ruminal escape protein already present in the forage.

Broderick (1992) compared the effects of supplementation with fish meal or solvent-extracted SBM on the efficiency of protein utilization of lactating dairy cows. Three trials were conducted. The first trial involved early lactation cows fed alfalfa silage as 70% of the diet, supplemented with either fish meal (60% estimated ruminal escape) or SBM (37% estimated ruminal escape) at 0.46 kg CP/d. Compared to cows on the SBM diet, fish meal supplemented cows had greater BW gain (kg/d), percent milk protein, milk yield (kg/d), 3.5% fat-corrected milk (kg/d), milk protein (kg/d), and rumen acetate:propionate ratio. Ruminal concentration of propionate was decreased in fish meal-supplemented cows compared to SBM-supplemented cows.

In the second trial, mid-lactation cows were fed a higher level of alfalfa silage (89% of diet), supplemented with fish meal or SBM at four different levels: 0, 1.5, 3.0, or 4.5% CP. In this trial, protein escapes were estimated at 67% for fish meal and 31% for SBM. Although supplemental protein of both types had beneficial effects on performance, there was no significant difference between the two protein sources. In the third trial, early lactation cows were used, but alfalfa silage was fed at 56% of the diet and cows received either no supplementation, or 0.55 kg CP/d from one of three sources: SBM, high solubles fish meal, or

low solubles fish meal (estimated rumen escapes: 27, 43, and 63%, respectively). Again, performance was improved by addition of all three protein sources. However, protein yield was increased most dramatically with supplementation of low solubles fish meal, compared with other diets. The researchers concluded that undegradable protein was advantageous in cows fed highly ruminally degradable alfalfa silage, in that it enhanced protein utilization.

Protein and Carbohydrate Interaction

Not only does protein degradability affect the nutrient metabolism, nutrient utilization, and performance of ruminants, but the carbohydrate source fed in conjunction with a protein of a specific degradability may also influence these factors.

Effect on N Metabolism. Lee et al. (1986) conducted an experiment to examine N digestion by four ruminally, duodenally, and ileally cannulated wether sheep given four isoenergetic and isonitrogenous diets in a 4 x 4 Latin square design. The diets contained either 1) barley and meat and bone meal, 2) barley and soybean meal, 3) corn and meat and bone meal, or 4) corn and soybean meal. These concentrates were fed in pelleted form, as was the roughage component (alkali-treated barley straw) of each diet. When barley was fed, non-NH_3-N entering the small intestine was increased compared to corn. Sheep fed the meat and bone meal diets had a greater quantity of total N and of amino acid N exiting the ileum and total N in the feces than sheep fed the SBM diets. This resulted in apparent N digestibility and percentage of total N and amino acid N entering the small intestine and apparently digested there being greater for SBM diets. Although efficiency of synthesis of microbial N was similar among diets, more microbial N entered the small intestine/d when barley was fed. Apparent feed N degradability was lower for meat and bone meal diets compared to SBM diets, in that larger amounts of feed N (including endogenous N) entered the small intestine when the meat and bone meal diets were fed.

Apparent efficiencies of rumen microbial N synthesis were lower than Agricultural Research Council (1980) values. The researchers attributed this in part to a possible low release rate of N in the rumen for sheep fed these diets. However, mean ruminal NH_3-N concentrations for the diets should have been adequate for ruminal microbial N synthesis. Meat and bone meal was less degradable in the rumen and also in the small intestine than SBM. This led to virtually no difference in the quantities of amino acid N being absorbed in the small intestine between the two protein sources. The overall N degradability of meat and bone meal diets was 44% compared to 51% for SBM diets in this experiment.

McCarthy et al. (1989) conducted an experiment to evaluate the effects of protein and carbohydrate source on fermentation in the rumen, supply of nutrients to the small intestine, and also performance in four early lactation Holstein cows. The experiment was designed as a 4 x 4 Latin square and the four treatments were: 1) ground shelled corn and fish meal, 2) ground shelled corn and SBM, 3) steam-rolled barley and fish meal, and 4) steam-rolled barley and SBM. The animals were fitted with both ruminal and duodenal cannulas and fed their respective diets in total mixed form, *ad libitum* quantities two times per day.

Even though cows fed the corn-based diets had a greater DMI and therefore a greater total N intake, total N flow to the duodenum was not affected by energy source. Flow of microbial N to the duodenum was greater for cows fed the barley-based diets than those fed the corn-based diets. However, cattle fed corn-based diets had higher non-NH_3 and non- NH_3 - nonmicrobial-N (NANMN) flow to the small intestine. The researchers hypothesized that the increased ruminal OM digestion in the barley-based diets allowed more energy for microbial growth, compared to corn-based diets. The researchers found a higher proportion of dietary N flow to the small intestine than previous research, which they attributed to the higher feed intake,

and therefore more rapid flow rate, and the low ruminal pH, which may have decreased protein degradation in the rumen. Although SBM is more ruminally degradable than fish meal, N intake, amount of total N, non- NH_3 - N, and non- NH_3 -non-microbial N flow to the duodenum were not affected by protein source. Total non- NH_3 - N flow to the duodenum was not affected by protein source due to more non- NH_3 -non-microbial-N flow with fish meal diets and more microbial N flow with SBM diets. More energy, NH_3, amino acids, or peptides from the greater ruminal degradability of SBM may have explained the increased microbial N flow in SBM diets. The researchers suggested that feed N:microbial N flow to the duodenum may be more easily manipulated than total N or non- NH_3 - N flow to the same place. In these types of diets, the carbohydrate source may play an even more important role in microbial N:non- NH_3 - nonmicrobial-N ratio flow than the protein source, due to the carbohydrate source making up such a large percentage of the diet as well as supplying some additional protein. Efficiency of microbial synthesis was greater than other researchers have reported, which could be attributed to the high OM intakes. Cattle fed corn-based diets exhibited less N digestion post-ruminally (as a percent of N flow to the duodenum and a percent of N apparently digested in the total tract), compared to barley-based diets. However, quantity of N digested post-ruminally was not affected by energy or protein source. This may have been due to more starch flow in corn-based diets, which would increase cecal fermentation, thereby increasing nonabsorbable microbial N-containing compounds.

Amino acid intake and passage were also examined in this trial. Cattle fed corn-based diets had a larger proportion of AA in total N flow to the duodenum than those fed barley-based diets, although energy did not affect total N passage. This could be attributed to the greater DMI of corn-based diets, or that more microbial N was present in the total non- NH_3 - N flow to the

small intestine with barley *versus* corn diets. Little difference in AA flow to the small intestine was attributable to protein source, demonstrating the ability of rumen microbes to equalize the AA composition of digesta.

Herrera-Saldana et al. (1990) designed an experiment to evaluate the effects of synchronization of starch and protein degradation. Their main areas of interest were nutrient digestion and microbial protein synthesis. Four mid-lactation Holstein cows were fitted with duodenal cannulae, two of which were also fitted with ruminal cannulae. The animals were fed one of four diets composed of starch and protein of varying degradabilities. A barley and CSM diet was used to synchronize rapid ruminal fermentation, and a milo and brewers dried grains diet provided the synchronized slowly fermented treatment. The remaining diets of barley with brewers dried grains and milo with CSM were the unsynchronized diets. All diets averaged similar CP and energy concentrations. Alfalfa hay and cottonseed hulls provided the 35% forage component of each diet. Protein degradability of barley, CSM, milo, and brewers dried grains was 65, 56.6, 45, and 38%, respectively. Starch degradability of barley (90.5%) and milo (70.5%) was also determined. Diets containing barley had greater microbial N synthesis than milo diets, with the synchronized rapidly fermented diet of barley and CSM having the greatest synthesis. Also, microbial protein flow was increased by protein and concentrate synchronized for rapid ruminal fermentation (barley and CSM diet).

In an attempt to examine the effects of type of carbohydrate and protein in the diet, Stokes et al. (1991) fed three ruminally and duodenally cannulated, lactating Holstein cows diets with varying percentages of nonstructural carbohydrates and degradable intake protein. The basal diet consisted of corn silage, grass hay, and ground corn. However, the amounts of these feedstuffs were varied depending on dietary treatment. The three treatments were calculated to

have either 38 and 13.2%, 31 and 11.8%, or 24 and 9% of nonstructural carbohydrate and degradable intake protein, respectively, as a percent of DM. Wheat straw, corn gluten meal, wheat middlings, canola meal, fish meal, and urea were all feedstuffs utilized among the three diets to achieve these particular nonstructural carbohydrate and degradable intake protein percentages.

Results showed ruminal OM digestion for the 24% nonstructural carbohydrate and 9% degradable intake protein diet was lower than the other diets, resulting in cattle fed this diet having a lower microbial N flow per day. A similar trend was found for N and nonstructural carbohydrate digestibilities, VFA production and ruminal NH_3 -N. These results led researchers to conclude that diets containing more nonstructural carbohydrate and degradable intake protein than 24% and 9%, respectively, were likely to provide greater microbial protein synthesis.

Giraldez et al. (1997) designed a study to evaluate the effects of digestible OM in the diet and N intake on N excretion in sheep. Twenty-five diets were formulated in which five levels of digestible OM and five levels of N were used in all possible combinations. Merino ewes (41.7 kg) were used as experimental animals. Diets consisted of varying amounts of low quality meadow hay, barley straw, caseinate, gluten meal, tapioca meal, and sunflower oil. Forage:concentrate ratio and DM intake were constant within each level of digestible OM. Nitrogen degradability of the diets was estimated using the nylon bag technique with one diet. The results from the degradation of the one diet were used to calculate the degradability of the remaining diets.

Results indicated that increasing digestible OM in the diet increased metabolic fecal N, but decreased endogenous urinary N. The researchers concluded several things. By increasing digestible OM, the amount of readily fermentable carbohydrate in the diet was also increased.

This allowed for greater fermentation in the hindgut, explaining the greater metabolic fecal N excreted. The decreasing levels of urinary-urea with increasing digestible OM suggested urea recycling into the digestive tract. They found this explanation logical in that as more digestible OM was fed, less degradable N per kg of digestible OM was present in the rumen, meaning less NH_3 was available. This series of events could have induced urea recycling to the rumen. Although the route of N excretion was affected by the amount of digestible OM and N in the diet, total endogenous N excretion was not significantly changed.

Hussein et al. (1991) examined the effects of feeding different dietary protein and carbohydrate sources on protein metabolism and carbohydrate fermentation in the rumen, as well as AA absorption in the intestine of sheep. Cannulas were fitted in the rumen, duodenum, and ileum of eight Hampshire wether lambs. The lambs were allotted to four treatments differing in their combination of carbohydrate and protein source: 1) corn-SBM, 2) corn- fish meal, 3) barley – SBM, 4) barley – fish meal Diets were formulated to supply 15.5% CP (urea was added to corn diets), diluted with solka floc, and fed as completely mixed diets with chopped hay and straw.

The ruminal degradation of dietary CP in each diet reflected the degradability of the ingredients of which they were composed; the lowest degradability was found in the corn-fish meal diet (51.9%), and the highest degradability was found in the barley-SBM diet (75.9%). Diets containing SBM had a higher efficiency of bacterial protein synthesis (g of N/kg OM truly digested in stomach) than fish meal diets. There was no effect of diet on total N (g/d) or non-NH_3 -N flow to the duodenum. Addition of fish meal did increase dietary N flow to the duodenum, but due to its lower bacterial N flow when compared to SBM, it did not increase the non- NH_3 -N available to the duodenum for digestion and absorption over SBM diets, but only

changed the compositional proportions of the non- NH_3 -N. Total N and non- NH_3 -N flow to the ileum was greater in SBM diets *versus* fish meal diets, suggesting that fish meal protein is digested and absorbed in the intestine to a greater extent than bacterial protein. No effect of carbohydrate or protein source was evident on total N and non- NH_3 -N absorption in the small intestine. However, a carbohydrate x protein source interaction occurred for absorption of non- NH_3 -N (expressed as a percent of that entering the small intestine), in that fish meal diets provided more absorbable AA than other diets, regardless of carbohydrate source.

Fecal N output for SBM diets was greater than fish meal diets, and apparent total tract N digestibility was greatest in the barley-fish meal diet. When expressed as a percentage of AA entering the small intestine, AA absorption was highest with the barley-fish meal diet, possibly due to the high quality of dietary protein found in fish meal. The researchers concluded that there was an advantage to feeding fish meal instead of SBM in combination with barley, but not in combination with corn. The barley-fish meal combination (highly degradable carbohydrate, with lowly degradable protein) improved fiber digestion, digestion and absorption of non- NH_3 -N and AA, OM digestion, and N digestion.

Effect on Ruminal Characteristics, Intake, and Digestibility. Lee et al. (1981) found no significant differences for ruminal pH, total VFA, individual VFA concentrations, molar VFA proportions, or ruminal NH_3 -N among diets varying in carbohydrate and protein source degradability. Sheep fed the corn and SBM diet in this study did have lower numerical values for ruminal NH_3 -N than the other diets (barley and SBM, barley and meat and bone meal, corn and meat and bone meal). Animals fed diets containing the less ruminally degradable meat and bone meal protein source demonstrated lower rumen volumes and higher rumen dilution rates than animals fed SBM diets. Since the barley diets were lower in energy compared to the corn

diets, more OM was fed and ingested by the sheep on these diets, and the amounts of OM entering the small intestine, leaving the ileum, and found in the feces were all significantly greater in barley diets. Therefore, rumen digestibility of barley diets was higher than corn diets, but apparent OM digestibility of corn diets was greater than that of barley diets.

McCarthy et al. (1989) conducted an experiment with Holstein cows comparing corn-SBM and corn-fish meal diets with barley-SBM and barley-fish meal diets. Intakes of DM, OM, and starch were greater with corn-based diets, as was flow of OM and starch to the upper small intestine, and amount of starch and OM apparently digested post-ruminally. However, average true ruminal digestibility of OM, apparent ruminal digestibility of starch, and apparent total tract digestibility coefficient for starch was greater in barley-based diets. Acid detergent fiber and NDF intake were not affected by energy source. However, NDF digested ruminally was greater for corn-based diets. The researchers concluded that their results agreed with others (Lee et al., 1986) in that ruminal fermentation of starch and OM was increased with barley as an energy source instead of corn, but barley may have caused the decrease in the ruminal degradation of fiber as opposed to corn-based diets (DePeters and Taylor, 1985), due to lower ruminal pH found in barley diets. There was no effect of protein source on DM, OM, ADF, or NDF intakes, and the only effect on ruminal, post-ruminal, or apparent total tract digestibility coefficients for OM, starch, ADF, or NDF was found when fish meal replaced SBM. In this instance, apparent total tract digestibility for NDF was increased. The researchers could not explain the very low values of NDF ruminal degradation on the barley-SBM diet.

The low digestibilities of ADF and NDF in the rumen and total tract for all diets was attributed to the low ruminal fluid pH (< 6.0) due to high VFA concentrations from the large amounts of OM, especially starch, fermented in the rumen (barley more so than corn). The low

pH decreased the functioning of cellulolytic bacteria, decreasing fiber degradation. A protein x carbohydrate source interaction was evident for total ruminal fluid VFA concentrations, molar percentage of acetate and propionate, and acetate to propionate molar ratios. Cattle fed the barley-fish meal diet had greater total VFA concentration and a smaller molar ratio of acetate to propionate, when compared with the corn-fish meal diet; however, when SBM was fed, similar changes did not occur. Increased fermentation of starch and decreased fermentation of fiber in the barley diets resulted in a higher molar percentage of propionate and lower percentage of acetate *versus* the corn-based diets.

Ruminal NH_3-N concentrations in this study were below that suggested for optimal growth of ruminal bacteria (< 5 mg/dL), possibly decreasing the fiber degradation for all diets. Fish meal increased ruminal NH_3-N compared to SBM, but did not increase fiber degradation. Cows fed corn-based diets had increased ruminal NH_3-N compared to barley-based diets, but more OM was ruminally digested in barley-based diets. The high DMI of the animals may have played a role in the decreased OM and fiber degradation (i.e. increased rate of passage) (McCarthy et al., 1989).

Results from a study done with cannulated Holstein cows indicated protein degradability did not affect digestibility of DM, OM, CP, or starch, but these digestibilities were affected by degradability of starch source (Herrera-Saldana et al., 1990). Rumen digestion coefficients for animals fed the more degradable barley diets were greater than for animals fed the less degradable milo diets. Animals fed barley and brewers dried grains, a diet supplying a rapidly degradable carbohydrate source with a slowly degraded protein source, exhibited higher digestibilities than the animals fed the barley and CSM, a diet with two more rapidly degradable feedstuffs. Postruminal starch disappearance was greater for milo-based diets than barley-based

diets, the highest value being for the milo-CSM diet. It was concluded that in this study, the degradability of starch had a greater effect on ruminal nutrient utilization than degradability of protein.

Hussein et al. (1991) fed wethers corn and SBM, corn and fish meal, barley and SBM, or barley and fish meal diets and reported their effects on ruminal characteristics and digestibility. They found no interaction of carbohydrate source x protein source for ruminal pH, NH_3-N, or VFA concentration; however, an interaction was evident for intake, apparent digestibility in the stomach, true digestibility in the stomach, apparent digestibility in the total tract (all as a percent of intake), and digestibility in the small intestine (percent of entering). The intake of the corn-fish meal diet was lowest, and OM digestion increased only with the barley-fish meal diet (highest NDF digestion in the rumen). This greater ruminal and total tract digestion of OM of the barley-fish meal diet could possibly be attributed to the slow release of N in the rumen, and to the presence of AA and peptides favorable to ruminal microbes and cellulose digestion being present in this combination of a lowly degradable protein source with a highly fermentable carbohydrate.

Further results suggested a more lowly degradable protein source might improve ruminal fiber digestion. Neutral detergent fiber, ADF, cellulose, and hemicellulose were all digested at a significantly higher percentage of intake in diets containing fish meal *versus* those containing SBM. This could be due to fish meal being digested more slowly in the rumen than SBM. Slower protein digestion would allow for a more continuous and gradual supply of N in the rumen and a slower, more gradual release of NH_3 -N, peptides, and branched chain volatile fatty acids. This would be beneficial to the cellulolytic bacteria, in that these factors, which are essential for bacterial growth, would be available for a longer time post-feeding.

Total nonstructural carbohydrate intake and digestibility in the small intestine were higher for diets containing corn *versus* barley, and SBM *versus* fish meal. However, the opposite was true concerning total nonstructural carbohydrate digestion in the rumen. Total tract and large intestine digestibility of total nonstructural carbohydrates showed a carbohydrate source x protein source interaction; however, almost all of the total nonstructural carbohydrate consumed was digested, regardless of diet.

Effect on Performance. Herrera-Saldana and Huber (1989) conducted an experiment with lactating cows to determine the effects of synchronized *versus* unsynchronized protein and carbohydrate degradability on the performance of lactating cows. Protein sources used were CSM (65% degradability) and brewers dried grains (56.6% degradability). Carbohydrate sources used were barley (45% degradability of protein) and milo (38.8% degradability of protein). The highest NH_3-N and total VFA concentrations were found in CSM diets. However, barley diets resulted in the highest blood urea N values. Organic matter digestibility of CSM diets was significantly greater than that of brewers dried grain diets, and the CP digestibility of CSM diets was numerically greater than that of brewers dried grains. Starch digestibility of barley diets was greater than milo diets; however, DM, ADF, NDF, and hemicellulose digestibilities were not affected by protein or carbohydrate source.

Milk production was significantly higher in the barley-CSM diet, compared to all other diets, which were not significantly different from each other. Milk fat percent was higher for milo diets compared to barley diets, but milk fat expressed as kg/d did not exhibit any significant differences. The synchronicity, or lack thereof, of protein degradability in diets varying in protein and starch sources had no effect on milk protein or the efficiency of protein, starch, and DM conversion to milk. It was concluded that in this study, the decrease in digestibility caused

by less ruminally degradable protein was overcome by supplying more energetically efficient nutrients to the small intestine. This could result in little difference in performance for animals on diets having either synchronized or unsynchronized rates of protein degradation in their protein and carbohydrate components.

Results from a similar trial by McCarthy et al. (1989) had contradictory results on milk production and composition. Milk yield was increased with corn-based diets (a less degradable carbohydrate source), but this was probably because of the increased DMI, as well as increased AA and starch to the small intestine in these animals. Neither energy nor protein source significantly affected milk fat percentage, milk fat yield, or 4% fat-corrected milk production, and total milk yield was not affected by protein source.

Oscillating Dietary Protein

The effects of oscillating dietary protein (feeding two different protein levels at intervals) have been examined in several studies. The protein status of the animal, the ruminal degradability of the protein source, and perhaps the pattern of absorption in the animal may play a role in the protein oscillation effect on N retention.

Effect on N Metabolism. Coleman and Wyatt (1982) examined whether CSM fed to steers daily, every-other-day, or every fourth day as a supplement to tall, low-quality, native range hay affected nutrient digestibility or N utilization. Cottonseed meal was fed at 0.45, 0.9, or 1.35 kg/d, respectively, and the hay contained 8% CP. They found no significant differences in nutrient digestibility or N utilization among supplementation intervals, but did find that any addition of CSM significantly increased CP digestibility (by 14%) and tended to increase N retention. In a second experiment, using the same animals and design, Coleman and Wyatt (1982) changed the protein supplement from CSM to small grain forage fed at 11.3, 22.7, 45.4

kg daily, every other day, or every fourth day, respectively. They also fed three different forages as the basal diet: March-cut range hay (which had only 3% CP), wheat forage, and spring-planted oat forage. Wheat was fed through the first two collection periods and oat forage for the last two collection periods. These forages were fed in chopped form (2.5 cm screen).

Small grain forage fed daily or every other day showed no effect on N balance; however, N consumed, excreted, and absorbed decreased significantly for steers fed small grain forage every fourth day compared to all other treatments except control. In this experiment, N balance was negative for all steers on all treatments. However, of the four periods (four consecutive 8 d collection periods, all feces and urine collected), N retention was highest for steers receiving small grain forage every fourth day in period 3. In every other period, N retention was less when the animals received small grain forage every fourth day than the other small grain forage treatment groups. They concluded that this infrequent protein supplementation could be used to meet winter protein requirements of grazing cattle while decreasing costs from other forms of supplementation as well as costs from increased labor involved in more frequent supplementation.

Another experiment examining the effects of oscillating protein supplements in the diets of ruminants was done by Collins and Pritchard (1992). Four experiments were conducted to determine the effects of corn gluten meal or SBM supplementation at 24 or 48 h intervals on several nutritional aspects in sheep and the feedlot performance of steers. The experimental animals, either sheep or cattle, were fed a basal diet of corn stalks.

Results from a N balance trial with rams (58 kg) showed that N intake and fecal N were not affected by treatment, but urinary N excretion was greater for SBM supplemented diets than corn gluten meal-supplemented diets on d 1 of the trial. Lower urinary N excretion was

exhibited when supplements were fed at 48 h intervals on d 2 as well as overall. Sheep fed corn gluten meal at 48 h intervals had the least amount of urinary N loss, possibly reflecting decreased ruminal NH_3-N loss or more efficient N recycling with this treatment. Unlike the results of Coleman and Wyatt (1982) or Hunt et al. (1989), the researchers reported an interaction between protein source and feeding interval for N retention and N retained per unit of N intake. The corn gluten meal fed at 48 h intervals increased N retention and N retained per unit of N intake up to two times that of the SBM fed at 48 h intervals (0.97 vs. 2.34 g/d for N retention and 7.38 vs. 19.32 % for N retained per unit of N intake). A similar, but much less dramatic, trend was evident at the 24 h interval (0.37 vs. 0.45 g/d for N retention and 2.96 vs. 3.62% for N retained per unit of N intake). They attributed this response to possibly the differences in ruminal escape CP between the diets, which could be increased by the 48 h feeding interval if ruminal escape is dependent on CP intake and CP source.

Plasma urea N levels obtained from steers on a performance trial also by Collins and Pritchard (1992), exhibited a protein source x supplement feeding interval interaction effect. Values increased from 8.5 to 17.9 mg/dL for animals fed SBM at 48 h intervals over the 2 d collection period, and decreased from 14.0 to 11.7 mg/dL for animals fed corn gluten meal fed at 48 h intervals over the same period. Changes in plasma urea N reflect net losses of ruminal NH_3-N across the rumen wall and nonessential N absorbed from the gut, and this interaction effect indicated to researchers that N metabolism was being altered by these treatments. A feeding interval effect was also evident on d 34 and 35 in that the 24 h feeding interval resulted in higher average PUN values than the 48 h feeding interval on both sampling days.

Collins and Pritchard (1992) also looked at the effects of dietary CP level in the diet (7.64%, 9.09%, 10.5%) of crossbred wethers (40 kg), again comparing the protein sources corn

gluten meal and SBM. Dry matter intake and DDMI increased but DM digestibility decreased as dietary CP increased. Nitrogen digestibility increased with increasing CP level in the diet, and N retention was greater in corn gluten meal diets, except when fed at the 10.5% level. The N retention data were somewhat misleading, however, in that corn gluten meal diets contained more N fed/d than SBM diets. Even so, urinary N excretion was lower in corn gluten meal diets *versus* SBM diets, except, again, when fed at the 10.5% CP level.

The changes in urinary N losses seen in the N balance trial followed a similar pattern as changes in ruminal NH_3–N and PUN concentrations from the ruminal characteristics and steer performance trials. This suggests that the loss of NH_3–N from the rumen may be the explanation for the decreased N utilization in the SBM diets and the 24 h supplementation diets. The researchers concluded that feeding supplemental protein at 48 h intervals to ruminants on a low CP, all forage diet, was more beneficial when the supplement was higher in ruminal escape properties (i.e. corn gluten meal = higher escape protein than SBM).

Krehbiel et al., (1998) examined the effects of frequency of SBM supplementation on intake and nutrient flow in ewes consuming low-quality forage (bromegrass hay). Ewes were fed once per day either 1) hay alone (control), 2) hay with SBM every 24 h, or 3) hay with SBM every 48 h (SBM = 80 g/d). The 21 d periods of the 3 x 3 Latin square design allowed for collection of DM and sampling of blood on d 3 of each period. Blood samples were taken through previously fitted hepatic, portal, and mesenteric vein and abdominal aorta catheters. It was analyzed for paraminohippurate, alpha-amino-N, urea N, NH_3-N, and glucose. Oxygen concentrations and flow rates were also calculated.

For the 48 h treatment, portal-arterial difference and net portal-drained viscera flow of alpha-amino-N peaked on the second d of sampling, suggesting a delay in SBM digestion and

flow to the small intestine for the 48 h *versus* the 24 h and control treatments which had alpha-amino-N peaks on the first d of sampling. This indicated a possible change in the pattern of alpha-amino-N absorption across the portal-drained viscera between the 24 h and 48 h supplementation times. Recycling of urea N was greatest when N intake was low (control = 28 to 52% of intake; 24 h = 12.6 to 23% of intake), due to the net transfer of urea N from the rumen to the portal drained viscera. The 48 h treatment exhibited 12.3% recycling of urea N on the day of supplementation, but an average of 74% (of N intake) on the day after supplementation. This indicated that the ewes supplemented every 48 h removed more urea N, which may have enabled them to maintain heightened NH_3-N concentrations in the rumen on the second day following supplementation. This increased removal of urea N by the liver, however, also increased liver energy expenditure.

Cole (1999) fed lambs a 90% concentrate diet to examine the effects of oscillating dietary CP concentration on retention of N, P, Na, and K. The experiment consisted of two trials that differed in type of supplemental protein used. In Trial 1, cottonseed meal (CSM), and in Trial 2 a 50:50 blend on a N basis of CSM and urea were used. Each trial consisted of five treatments: 1) animals fed a diet of 10% CP continuously (formulated for maintenance), 2) animals fed a 12.5% CP diet continuously (formulated for 50 g ADG), 3) animals fed a 15% CP diet continuously, 4) animals fed a 10% CP diet for 24 h, then a 15% CP diet for 24 h, 5) animals fed a 10% CP diet for 48 h, then a 15 % CP diet for 48 h.

Results from Trial 1 indicated that the 48 h oscillation (treatment 5) increased N retention by 38% compared to the 12.5% CP diet, mainly due to decreased urinary N excretion; nitrogen retention as a percentage of N apparently absorbed was also significantly increased with the 48 h oscillation treatment compared to the continual 12.5% treatment, which led Cole to suggest there

was improved N utilization of the absorbed N in this treatment. In Trial 2, however, treatments did not show any significant effects on N metabolism. The author attributed this to the possibility that Trial 1 lambs were protein deficient and digestible intake protein (DIP) deficient on the 12.5% CP diet, whereas Trial 2 lambs were not protein deficient and were above their DIP requirement on the 12.5% diet. Cole (1999) concluded that oscillating the level of CP in the diet could possibly cause an increase in N recycling, an improvement in the quality of protein made available to the small intestine, and/or an increase the metabolic use of those amino acids absorbed.

Effect on Ruminal Characteristics, Intake, and Digestibility. In a trial using four ruminally-cannulated Hereford steers, Hunt et al. (1989) examined the effect of CSM supplementation frequency on nutrient digestion. Cottonseed meal supplemented at 12, 24, or 48 h had no effect on feed intake, digestibility, or passage rate; however, CSM significantly increased DM and NDF intakes compared to control (no addition of CSM to diet). *In situ* disappearance of NDF and ADF indicated no difference in ruminal fiber digestion activity among CSM supplementation times, although fiber digestion was significantly increased in all CSM-containing diets compared to control. There were differences observed in ruminal NH_3-N concentration, however. Cottonseed meal fed at 12 h intervals and at 24 h intervals had similar patterns in ruminal NH_3-N concentration at 0, 4, 12, and 16 h post-feeding, disregarding one collection. Cottonseed meal fed at 24 h intervals had ruminal NH_3-N concentrations greater than CSM fed at 48 h intervals at 0 h post-feeding on d 1 and 4 h post-feeding on d 2. All ruminal NH_3-N concentrations, for all treatments, however, were below the level for optimal microbial fermentation. No differences among treatments were found for ruminal pH, but all CSM-containing diets had greater VFA concentrations. They concluded that the grass hay used

did not supply more than marginal amounts of ruminally degraded protein, and therefore the effects of CSM supplementation frequency could not be ascertained.

Coleman and Wyatt (1982) also fed steers a basal diet of range hay, wheat forage, and oat forage alone or supplemented with 11.3, 22.7, 45.4 kg small grain forage daily, every other day, or every fourth day, respectively, to measure effects on intake and digestibility. Results showed a decrease in DMI for steers fed small grain forage every fourth day compared to the other small grain forage treatments, but overall, addition of small grain forage increased DMI over the control. Dry matter and OM digestibility were also increased by addition of small grain forage to the diet compared to the basal diet alone, but frequency of supplementation had no effect on DM or OM digestibility, nor on rate of passage. However, there was a tendency for these factors to decrease as small grain forage supplementation interval increased. The researchers attributed this effect to a function of decreased intake of small grain forage rather than an effect of frequency of feeding.

Collins and Pritchard (1992) used 75 kg wethers to examine the effect of oscillating dietary protein on ruminal characterisitics. Results showed higher ruminal NH_3-N concentration with SBM fed at 48 h intervals than SBM fed at 24 h, or corn gluten meal fed at 24 or 48 h. This was attributed to the quantity and degradability of the protein in this treatment. Neither supplementation time nor protein source alone had any effect on total VFA concentrations or ruminal fluid pH, although a diet x hour of supplementation interaction for both and for some individual VFAs was evident. Molar proportions of acetate:propionate:butyrate were similar for all diets, which would indicate the pathways of rumen fermentation were similar among diets. Dry matter intake was increased with diets containing SBM supplementation compared to corn gluten meal; however, apparent dry matter disappearance (DMD) and digestible dry matter

intake (DDMI) were not significantly affected by either source or frequency of protein supplementation. The researchers did find an interaction between protein source and feeding interval concerning both DMD and DDMI, however. Dry matter disappearance and DDMI were increased with corn gluten meal supplementation every 48 h, whereas they were decreased when SBM was supplemented every 48 h.

Effect on Performance. Hunt et al. (1989) examined the effect of CSM supplementation frequency on performance, using beef steers fed low-quality (6.6% CP) fescue hay. Grass hay was fed alone or supplemented with 3 g CP/kg $BW^{.75}$ CSM every 12, 24, or 48 h in a trial using 24 Hereford x Simmental steers. Total DM intake and DM digestibility did not differ significantly among treatments, but were numerically higher for CSM-containing diets. This difference, as well as the higher fiber digestion and VFA concentrations shown in a previous trial using CSM-supplemented steers, probably contributed to the greater daily gains of the CSM-supplemented steers *versus* control steers. For reasons unknown to the researchers, steers fed CSM at 12 h intervals and at 48 h intervals exhibited significantly greater daily gains than when fed at 24 h intervals. The researchers concluded that CSM supplementation increased the utilization of low-quality grass hay by increasing ruminal fiber digestion and the overall supply of nutrients available post-ruminally. However, frequency of CSM supplementation had no dramatic effect on the performance of steers on a basal diet of grass hay at this low level of ruminally degraded protein.

In a feedlot steer experiment by Collins and Pritchard (1992), the effect of oscillating SBM and corn gluten meal at 24 and 48 h intervals was examined. No urea was fed in the treatments due to previous experiments having indicated that the diet supplied adequate ruminal NH_3-N concentration. However, treatments containing monensin were added to evaluate the

effect of this additive. The results for N source comparison in this study were somewhat confounded by the fact that dietary CP percent was higher in CGM diets. The DMI was higher for daily *versus* 48 h supplementation of both supplements, unlike results reported by Coleman and Wyatt (1982) and Hunt et al. (1989). Corn gluten meal supplementation increased ADG and gain/feed ratio (G/F) compared to supplementation with SBM. Monensin decreased ADG when combined with corn gluten meal, but increased ADG when combined with SBM (protein source x monensin interaction for G/F). No definite conclusions as to the effect of oscillating protein levels on performance could be drawn due to a confounding effect found between protein source and level of protein fed. However, the researchers did conclude that monensin may not have been beneficial when fed with corn gluten meal due to its already high protein escape properties.

Experiment 1 – Metabolism Trial with Lambs

Objectives

The objective of Exp. 1 was to determine the effect of oscillation of two levels of low ruminally degradable dietary protein at 48 h intervals on N metabolism in lambs. The effect of this feeding regimen on apparent digestibility, ruminal pH, VFA concentrations, and NH_3 concentration, and blood urea N levels was also investigated.

Experimental Procedure

Animals and Diets

Twenty-eight crossbred (1/8 Finnsheep, 1/8 Rambouliet, 1/4 Dorset, 1/2 Suffolk) wether lambs (31 kg) were used in a metabolism trial. The lambs were raised at the Southwest Virginia Agricultural Research and Extension Center, Glade Spring, VA. They were castrated at birth and received vaccinations for *Clostridium perfringes* C and D and tetanus at approximately 6 wk of age. Lambs were administered injectable Ivomec before being transported from Glade Spring to the Smithfield Unit in Blacksburg, VA, on August 4, 1999. At Smithfield, the lambs were fed hay for 2 d, and starting on the third day, they were gradually supplemented with a high roughage diet, consisting of ground corn (40.5%), grass hay (50.4%), soybean meal (3.5%), molasses (5.0%), and trace mineralized salt (0.6%). The level of feeding this diet was increased daily until 0.45 kg/head twice daily (0.9 kg/d) was reached, and hay was deleted. The lambs remained on this diet until the transition period of the metabolism trial.

All animals were administered 20 cc of Levasole (Schering-Plough Animal Health Corp., Union, NJ) 5 d before being placed in the metabolism stalls. On the afternoon of August 25, 1999, the lambs were placed in metabolism stalls similar to those of Briggs and Gallup (1949),

which allow separate collection of feces and urine. The lambs were administered 1,000,000 I.U. of Vitamin A and 150,000 I.V. of Vitamin D intramuscularly. On August 27, 1999, the lambs received 5 cc of Panacur (Hoechst Roussel Vet, Warren, NJ), due to the presence of worms in the fecal material of several lambs. Three lambs were also given 5 cc of Nuflor (Schering-Plough Animal Health Corp., Union, NJ) for respiratory distress on August 30, 1999.

The lambs were weighed and blocked by BW into seven blocks of four lambs each. The lambs within each block were allotted at random to the following four diets: 1) 8% CP, 2) 10% CP, 3) 12% CP, and 4) 8% and 12% CP diets oscillated every 48 h. The diets were composed of ground barley straw, barley grain (harvested at Virginia Tech farms), and Menhaden fish meal, corn sugar, dry molasses, limestone, dicalcium phosphate, and trace mineralized salt (TMS), purchased from Big Spring Mill, Elliston, VA. The TMS contained 35 ppm Zn, 28 ppm Mn, 17.5 ppm Fe, 3.5 ppm Cu, 0.7 ppm I, 0.7 ppm Co, as fed basis. Ingredient and chemical and mineral composition of the diets are presented in Tables 1 and 2, respectively. Diets were formulated to provide equal concentrations of Ca, P, and TDN. The concentrate portion of each diet was mixed for 15 min using a 226.8 kg capacity Marion (Rapids Machinery Co., Marion, Iowa) horizontal mixer with paddles. Limestone, dicalcium phosphate, and TMS were premixed and added to each batch of concentrate. Premixes were mixed for 10 min each in a 13.6 kg capacity Hobart (H600 model, The Hobart Manufacturing Co., Troy, Ohio) mixer. The analyzed CP content of the diets was 7.1, 9.1, 10.8, and 9.3% for the 8, 10, 12, and oscillating 8/12% CP diets, respectively.

The lambs were adapted to the metabolism stalls. During the adjustment period, the high-roughage diet was fed, followed by a 10 d transition to the experimental diets, an 11 d preliminary period, and a 10 d collection of urine and feces. During the preliminary and

Table 1. Ingredient Composition of Different Diets Fed to Lambs[a]

	Crude protein of diets, %[b]		
Feedstuff	8	10	12
	----------	%	----------
Barley straw	52.30	51.84	51.44
Fish meal	4.55	7.51	10.47
Barley grain	20.80	20.80	20.80
Corn sugar	15.02	13.17	11.29
Dry molasses	5.0	5.0	5.0
Limestone	0.43	0.22	0.0
Dicalcium Phosphate	0.9	0.46	0.0
Salt	1.0	1.0	1.0

[a]DM basis
[b]Calculated

Table 2. Chemical Composition of Different Diets Fed to Lambs

	Crude protein of diets, %[a]			
Component	8	10	12	8/12[b]
Dry matter, %	90.79	90.69	90.60	90.68
Composition of dry matter				
Crude protein, %	7.10	9.07	10.82	9.31
NDF, %	54.41	52.26	53.72	53.99
ADF, %	29.97	29.68	30.04	30.00
Cellulose, %	4.04	4.00	4.02	4.03
Lignin, %	3.90	3.85	3.83	3.85
Ash, %	8.93	8.10	8.12	8.44
Calcium, %	0.76	0.72	0.70	0.72
Phosphorus, %	0.44	0.45	0.45	0.45
Magnesium, %	0.14	0.14	0.14	0.14
Potassium, %	0.13	0.14	0.14	0.14
Copper, ppm	7.10	6.96	5.43	6.10
Zinc, ppm	55.10	60.80	51.82	53.15

[a]Calculated
[b]Oscillated at 48 h intervals

collection periods, the lambs were fed 350 g of their respective diets at 0700 and 1900 h each day, for a total of 700 g/d. This amount was determined from the maximum DMI of the lambs which consumed the lowest amount of feed during the adjustment period. The concentrate and straw were weighed separately, then mixed by hand before feeding. Animals were allowed 2 h to eat before feed was removed and refusals, if any, were weighed, recorded, and stored at room temperature. Water was available at all times except during the 2 h feeding intervals. During the 10 d collection period, animals on the oscillating diet were fed the 12% CP diet for 6 d and the 8% CP diet for 4 d. Therefore, chemical and mineral composition of the oscillating diet was calculated by adding 60% of the 12% CP diet values to 40% of the 8% CP diet values. Samples of the straw and each concentrate were taken at each feeding beginning 2 d before the start of the collection period and ending 2 d before the final d of the collection period. The samples for each 2 d were composited and subsampled at the end of the trial. Any refusals during the collection period were weighed and stored in plastic bags.

During the collection period, feces were collected in metal pans. Each day the total fecal collection was weighed, recorded, and a 20% subsample was dried at 60°C. After a 48 h drying period, the fecal samples were weighed and stored in plastic bags. Samples were composited and subsampled at the end of the trial. Urine was collected daily in 4 L plastic jugs that contained 15 mL of a 1:1 (w:w) mixture of H_2SO_4 and H_2O, which was diluted with 500 mL of H_2O. The collection from each day was diluted further with H_2O to 5000 g, from which a 100 mL subsample was taken, placed in a plastic bottle, and refrigerated. At the end of the collection period, each composited sample was mixed thoroughly, and a subsample was taken and frozen.

On the final day of the collection period and again 2 d later, ruminal fluid samples were taken 2 h post-feeding using a stomach tube, equipped with a strainer, and vacuum pump. Blood

samples were taken from the jugular vein on the final day of the collection period and again 2 d later at 2, 4, and 6 h post-feeding.

Chemical Analysis

Straw samples, feed refusals, and feces were ground in a Wiley mill (Thomas Wiley, Laboratory mill Model 4, Arthur H. Thomas Co., Philadelphia, PA) to pass a 1 mm-mesh screen. Due to melting of the concentrate samples when ground in the Wiley mill, these samples were ground using a household Osterizer (Sunbeam-Oster, Milwaukee, Wisconsin) blender. The straw, refusals, feces, and concentrate samples were analyzed for DM and ash (AOAC, 1990), NDF (Van Soest and Wine, 1967), ADF (Van Soest, 1963), cellulose (Van Soest and Wine, 1968), and lignin (Goering and Van Soest, 1970). The N content of these samples was determined by the Kjeldahl method (AOAC, 1990). Only straw and concentrate samples were analyzed for minerals. The samples were wet-ashed with a 2:1 (vol:vol) mixture of HNO_3:$HClO_4$ (Muchovej et al., 1986) and analyzed for Ca, Mg, K, Cu, and Zn using an atomic absorption spectrophotometer (Perkin Elmer 5100 PC, Norwalk CT). Analysis for P content was by a colorimetric method (Fiske and Subbarow, 1925) using a Spectronic® 21D (Milton Roy, Rochester, NY). Urine samples were thawed, mixed thoroughly, and analyzed for N content using the Kjeldahl method (AOAC, 1990).

Ruminal fluid samples were filtered through four layers of cheesecloth , and the pH of the fluid was immediately measured using an Accumet portable pH meter (Model AP 61, Fisher Scientific Co.). A 5 mL sample was added to 1 mL of 25% metaphosphoric acid in a plastic tube and frozen. Later the samples were analyzed for VFA concentrations using a gas chromatograph (Varian Vista 6000 gas Chromatograph, column from Supelco, Inc., packed with 10% SP – 1200/1% H3PO4 on 80/100 chromosorb W AW). The detector (170°C), column (115°C), and

inlet (180°C) were programmed for specific temperatures, and the VFA concentrations were determined by integration. The standard used contained 51.66 umol/mL acetic acid, 30.63 umol/mL propionic acid, 4.96 umol/mL isobutyric acid, 10.41 umol/mL butyric acid, 4.95 uol/mL isovaleric acid, and 5.18 umol/mL valeric acid. Another 5 mL sample of ruminal fluid was combined with 1 drop of H_2SO_4 for determination of NH_3-N (Beecher and Whitten, 1970).

Blood samples were centrifuged at 600 x g for 15 min, and the serum was separated and frozen. After thawing, urea N was determined using an autoanalyzer (Beckman SYNCHRON CX® SYSTEMS, Beckman Instruments, Inc., Brea, CA) and BUN reagent. The BUN concentration of samples was measured by an enzymatic rate method. The urea in the sample is first broken down by urease into NH_3 and CO_2. The enzyme, glutamate dehydrogenase, then acts on NH_3 and α-ketoglutarate to form glutamate. At the same time, β-nicotinamide adenine dinucleotide (NADH) is oxidized to NAD. The change in absorbance at 340 nanometers is monitored by the system. The concentration of urea N in the serum is directly proportional to the change in absorbance. Therefore, the SYNCHRON CX System calculates the BUN concentration of the sample.

Statistical Analysis

Data were statistically analyzed using JMP (JMP, 1996) for analysis of variance of a completely randomized block design. The Tukey-Kramer test was used unless tests for normality and unequal variances required the use of the Wilcoxon nonparametric test or the Welsh ANOVA. However, in instances where these were used, results did not differ from those of the Tukey-Kramer test. The blood urea N data from one lamb fed the 8% CP diet taken on the second day of blood sampling was eliminated from the analysis due to extreme outlying values. This was attributed to decreased feed intake after the first blood sampling (2 d prior).

Results and Discussion

Apparent Digestibility

Dry matter and OM digestibilities were not significantly different among different diets (Table 3). Dry matter digestibility values ranged from 56.47 to 57.04%. Values for OM digestibility ranged from 57.92 to 58.80%. Digestibilities of NDF and ADF were not significantly different among diets. Average values across diets were 43.77% for NDF and 37.07% for ADF. Sultan and Loerch (1992) demonstrated DM, OM, and NDF digestibilities were affected more by energy level than level of N fed.

Apparent digestibility of CP was affected ($P < 0.05$) by diet (Table 3). Digestibility increased ($P < 0.05$) with CP concentration in the diet. Although true digestibility of protein has been estimated at 90% (Owens and Zinn, 1988), apparent digestibility includes metabolic fecal N, which remains constant at a constant level of intake (Merchen, 1988). Therefore, at high N intakes, metabolic fecal N makes up a smaller portion of total fecal N. The digestibility of CP of the 8% CP diet was lower ($P < 0.05$) than for the other diets. The average value for the 12% CP diet was higher ($P < 0.05$) than for the 10% diet, but was not significantly higher than for the oscillating diet. The CP digestibilities of the 10% CP diet and the oscillating diet were similar ($P > 0.05$).

In a study by Hussein et al. (1991) lambs fed a diet of barley and fish meal in addition to grass hay and wheat straw exhibited greater OM digestibility than the values reported in the current study. Concerning the effects of oscillating dietary protein, other research results agree with digestibility results presented here. No effect on DM or N digestion was reported by oscillation of dietary protein at 24 or 48 h intervals in lambs fed a 90% concentrate diet (Cole,

Table 3. Apparent Digestibility of Diets Fed to Lambs

Component	Crude protein of diets, %[a]				
	8	10	12	8/12	SE
	----------	----------	%	----------	
Dry matter	56.49	57.04	56.86	56.47	0.56
Organic matter	57.92	58.80	58.69	58.13	0.56
Crude protein	48.11[b]	59.61[c]	63.54[d]	60.76[cd]	0.84
NDF	44.06	42.77	44.49	43.76	1.02
ADF	36.70	37.51	37.42	36.65	1.31

[a]Calculated
[b,c,d]Means in the same row with unlike superscripts are different ($P < 0.05$)

1999). Lambs fed a straw-based diet supplemented with protein every 24, 48, or 72 h showed no differences in DM, OM, N, NDF, or ADF digestibility (Brown et al., 1995).

Hunt et al. (1989) reported no difference in DM and NDF digestibility by supplementation of grass hay with CSM at 12, 24, and 48 h intervals. Digestibilities of DM, CP, ADF, and cellulose were not different among steers fed hay supplemented with CSM every day, every other day, or every fourth day (Coleman and Wyatt, 1982). However, in a second trial by Coleman and Wyatt (1982), in which steers were fed a lower-quality hay supplemented with small grain forage, DM digestibility of the diet supplemented every day was significantly higher than that of the diet supplemented every fourth day. Crude protein digestibility of the diet supplemented every fourth day was also significantly lower for diets supplemented every day or every other day, but OM, NDF, ADF, and cellulose digestibility were not affected by interval of supplementation.

Nitrogen Balance

Average N intake was 7.22, 9.22, 10.97, and 9.46 g/d for the 8, 10, 12, and 8/12% CP diets, respectively (Table 4). Fecal excretion of N was higher ($P < 0.05$) for lambs fed the 12% CP diet than for those fed the 10% or 8/12% CP oscillating diets, but only numerically greater than for lambs fed the 8% CP diet. No difference ($P > 0.05$) in fecal N excretion was shown between lambs fed the 8/12% CP oscillating diet and those fed the 8% and the 10% CP diets continuously.

Urinary N excretion was highest ($P < 0.05$) for lambs fed the 12% CP diet (5.4 g/d), followed by those fed the 8/12% CP oscillating diet and the 10% CP diet (4.28 g/d and 3.92g/d, respectively). Values were lowest ($P < 0.05$) for lambs fed the 8% CP diet (2.76 g/d). Although numerically higher, the urinary N excretion of lambs fed the 8/12% CP oscillating diet was not

Table 4. Nitrogen Balance by Lambs Fed Different Diets

Item	Crude protein of diets, %[a]				
	8	10	12	8/12	SE
Intake, g/d	7.22	9.22	10.97	9.46	
Excretion, g/d					
Fecal	3.74[bc]	3.72[c]	4.00[b]	3.70[c]	0.07
Urinary	2.76[b]	3.92[c]	5.40[d]	4.28[c]	0.15
Total	6.50[b]	7.64[c]	9.40[d]	7.98[c]	0.14
Apparent absorption					
g/d	3.48[b]	5.50[c]	6.97[d]	5.73[c]	0.07
% of intake	48.16[b]	59.68[c]	63.54[c]	60.77[c]	0.84
Retention					
g/d	0.72[b]	1.58[c]	1.57[c]	1.45[c]	0.14
% of intake	9.97[b]	17.12[c]	14.34[bc]	15.38[bc]	1.52
% of absorbed	20.37	28.70	22.59	25.31	2.54

[a]Calculated
[b,c,d]Means in the same row with unlike superscripts are different ($P < 0.05$)

different ($P > 0.05$) than that of lambs fed the 10% CP diet continuously. Total N excretion followed the same pattern as urinary N excretion. Lambs fed the 12% CP diet had the highest ($P < 0.05$) total N excretion (9.4 g/d), followed by those fed the 8/12% CP oscillating diet and the 10% CP diet (7.98 g/d and 7.64 g/d, respectively). Again, no significant difference was found between lambs fed the 8/12% CP oscillating diet and those fed the 10% CP diet continuously. Lambs fed the 8% CP diet had the lowest ($P < 0.05$) total N excretion.

Apparent absorption of N, expressed in g/d was highest ($P < 0.05$) for lambs fed the 12% CP diet and lowest ($P < 0.05$) for those fed the 8% CP diet. Values for lambs fed the 10% CP diet and the 8/12% oscillating diet were similar and intermediate between those for lambs fed the 8% and 12% CP diets. Expressed as a percentage of intake, values were not significantly different among the 10%, 12%, and oscillating diets. However, these three diets had a higher ($P < 0.05$) N absorption, expressed as a percentage of intake, than lambs on the 8% CP diet.

Nitrogen retention values, expressed as g/d, were higher ($P < 0.05$) for lambs fed the 10%, 12% and 8/12% oscillating CP diets than for those fed the 8% CP diet. Expressed as a percentage of intake, values were not different for lambs fed the 10%, 12%, and 8/12% oscillating CP diets. Retention of N was lower ($P < 0.05$) for the lambs fed the 8% CP diet, compared to the 10% CP diet. Although numerical differences existed, no significant differences were found among diets for N retention, expressed as percent of absorbed.

The findings concerning N balance agree in part with those of Cole (1999) and Collins and Pritchard (1992). In one trial, Cole (1999) found oscillating 10% and 15% CP diets every 48 h in lambs resulted in a significant increase in N retention as a percent of N intake and as a percent of N absorbed, compared to feeding a 12.5% CP diet continuously. The difference was attributable to less urinary excretion by lambs on the oscillating diet. Due to the significantly

higher N retention when expressed as a percentage of N absorbed, the author suggested this was due to improved utilization of the N absorbed. In a second trial by Cole (1999), using a more degradable protein source in the diet than the first trial, however, no difference in N retention was reported between the lambs fed the oscillating diet and those fed the 12.5% CP diet continuously. Several explanations for this discrepancy were offered. The N intake of lambs in the first trial may have been inadequate compared to that of lambs in the second trial. Differences in protein ruminal degradability were also suggested. The degradable intake protein supplied by the diet was calculated as marginally adequate for lambs in the first trial, but in excess of the requirement for lambs in the second trial. Ruminal protein degradability was not measured in the current experiment, nor was degradable intake protein calculated. However, based on NRC (1985) CP requirements for lambs of this age and size, N intake was at a critical level, as was that of Cole's first trial. In the current experiment a low ruminally degradable protein source (fish meal) was used also, but the basal diet differed from that of Cole. Cole (1999) fed lambs a 90% concentrate diet, the majority of which was composed of dry rolled corn, a carbohydrate source of lower ruminal degradability than that of barley, which was used in this experiment. This discrepancy in carbohydrate source used could have resulted in more degradable intake protein supplied in this experiment than that of Cole.

Collins and Pritchard (1992) reported a significant increase in N retention as a percentage of N intake when both SBM and corn gluten meal were fed at 48 h intervals to lambs fed a basal diet of corn stalks on an *ad libitum* basis. Protein degradability may have played a role in this effect. The corn gluten meal, a less ruminally degradable protein source, fed at 48 h intervals resulted in N retention values greater than those of SBM, a more ruminally degradable protein source, also fed at 48 h intervals. They found no evidence of inadequate ruminally degradable

intake protein in the diets fed, unlike the results reported by Cole (1999). Based on the N retention results, Collins and Pritchard (1992) attributed the effect to the use of a less ruminally degradable protein source.

Results reported from several other studies agree with those found for N retention in the current experiment. Brown et al. (1995) found that feeding SBM at 24, 48, or 72 h intervals had no effect on N retention or metabolism in lambs fed a basal diet of barley and wheat straw. Krehbiel et al. (1998) fed ewes a basal diet of bromegrass hay supplemented with SBM at 24 and 72 h and found no differences in net N absorption, but did find an indication of differences in the pattern of alpha-amino N absorption from the portal-drained viscera. No effect on N retention expressed as a percent of intake, or percent of absorbed was evident in steers fed CSM or small grains forage daily, every other day, or every fourth day as a supplement to a basal diet of hay fed on an *ad libitum* basis (Coleman and Wyatt, 1982).

Ruminal Fluid pH and Volatile Fatty Acids

When ruminal fluid was sampled on the final day of collection (d 10), lambs on the oscillating diet had been fed the 8% CP diet that morning after having been fed the 12% CP diet the two previous days. Two days later, at the second sampling (d 12), lambs on the oscillating diet had been fed the 12% CP diet that morning. Ruminal pH was not affected by diet; values ranged from 6.59 to 6.70 (d 10) and 6.58 to 6.70 (d 12) (Table 5), indicating ruminal pH was at a desirable level for normal rumen function (Owens and Goetsch, 1988). Interval of supplementation did not affect ruminal pH of steers supplemented with CSM at 12, 24 or 48 h intervals (Hunt et al., 1989). Similarly, lambs supplemented with either SBM or corn gluten meal at 24 and 48 h intervals exhibited no differences in ruminal pH (Collins and Pritchard, 1992).

Table 5. Ruminal Fluid pH of Lambs Fed Different Diets

	Crude protein of diets, %[a]				
Day	8	10	12	8/12	SE
10	6.63	6.59	6.67	6.70	0.09
12	6.70	6.58	6.70	6.64	0.10

[a]Calculated

Diet had no effect on total (µmol/mL) VFA concentrations on d 10 or 12 (Tables 6 and 7). Values were lower on d 12. On both sampling days, the ratio of acetic:propionic:butyric acid was approximately 60:30:10 and were not significantly different for lambs fed the different diets. The ratios were normal for these acids in the rumen of animals on high-roughage diets (65:25:10) (Owens and Goetsch, 1988). No differences in individual VFA concentrations suggested that similar paths of ruminal fermentation occurred, regardless of diet.

Lambs fed a diet of barley and fish meal but with a higher CP content (15.5%) exhibited a ruminal pH level of 6.32 (Hussein et al., 1991), somewhat lower than reported in this study, but still within the acceptable range for adequate rumen function. Total VFA concentration reported by Hussein et al. (1991) was lower than values found on d 10 of this experiment, but similar to those reported on d 12. Molar proportions of acetate, isobutyrate, and valerate found by Hussein et al. (1991) were slightly higher than values reported in this study, and molar proportions of propionate and isovalerate were somewhat lower than values in the current experiment. Values for the molar proportion of butyrate were similar between the two experiments. The acetate:propionate:butyrate ratio in lambs studied by Hussein et al. (1991) was approximately 66:21:9. The discrepancy with the present study could suggest differences in ruminal fermentation between the two studies. This could possibly be attributed to differences in the ingredients of the basal diet. Hussein et al. (1991) included animal fat and grass hay in the basal diet of the lambs used in that study, and the diet was also formulated at a higher CP content.

Concerning the effect of oscillating dietary protein on VFAs and ruminal pH, Collins and Pritchard (1992), found no effect on either of these ruminal characteristics when feeding SBM or corn gluten meal at 24 or 48 h intervals. Total VFA concentration was lower for lambs

Table 6. Molar Proportions of Volatile Fatty Acids in Ruminal Fluid of Lambs on Different Diets. Day 10

Item	Crude protein of diets, %[a]				SE
	8	10	12	8/12	
Total VFA, umol/mL	108.33	109.64	100.84	105.79	6.16
Individual VFA, Mol/100 mol					
Acetic	58.34	58.24	60.57	56.78	1.16
Propionic	27.78	28.58	25.05	30.10	1.62
Isobutyric	0.57	0.66	0.88	0.69	0.11
Butyric	10.45	9.64	11.29	10.31	0.91
Isovaleric	0.53	0.66	0.69	0.66	0.07
Valeric	0.94	0.98	1.19	0.98	0.09

[a]Calculated

Table 7. Molar Proportions of Volatile Fatty Acids in Ruminal Fluid of Lambs on Different Diets. Day 12

Item	Crude protein of diets, %[a]				SE
	8	10	12	8/12	
Total VFA, umol/mL	60.25	83.97	73.32	80.31	7.63
Individual VFA, Mol/100 mol					
Acetic	57.63	57.59	59.00	57.29	1.28
Propionic	26.69	30.09	26.69	31.22	2.17
Isobutyric	1.07	0.53	0.63	0.59	0.20
Butyric	9.51	8.98	11.44	10.28	0.68
Isovaleric	1.55	0.67	0.77	0.77	0.35
Valeric	0.88	0.74	0.93	0.92	0.06

[a]Calculated

in the study by Collins and Pritchard (1992) than in the current experiment, and molar proportions of acetate, propionate, and butyrate were 74, 18, and 8 for lambs fed the less ruminally degradable protein source (corn gluten meal) at 48 h intervals. In agreement with this study, however, no significant differences were found among treatments for VFA concentrations.

Ruminal Fluid Ammonia Nitrogen and Blood Urea Nitrogen

As CP content of the diet increased, both ruminal NH_3-N (Table 8) and blood urea N (BUN) concentrations (Table 9) increased on both d 10 and d 12, but differences were not always significant. Lambs on the oscillating diet were fed the 8% CP diet on d 10 and 12% CP diet on d 12, but the ruminal NH_3-N concentration for both sampling days was 17.0 mg/dL. On both days, the ruminal NH_3-N values for lambs on the oscillating diet were not significantly different from the values for the lambs fed the 10% or the 8% CP diet. A ruminal fluid NH_3 – N concentration of at least 5 mg/dL is generally accepted as the minimum value for adequate microbial protein production (NRC, 1985). Values above this level have not increased microbial protein production consistently. In the present study, ruminal fluid NH_3 – N concentrations for animals on all diets exceeded 5 mg/dL, indicating microbial protein production was not limited.

Lambs fed the 8% CP diet had lower ($P < 0.05$) BUN values than lambs fed the 10%, 12%, and oscillating diets at 2 and 4 h after feeding on d 10. At 6 h after feeding, the values were similar for lambs fed the 8%, 10%, and 8/12% CP oscillating diets. Lambs fed the 12% CP diet had higher ($P < 0.05$) values than those fed the 8% and 10% CP diets. On d 12, the oscillating lambs had been fed the 8% CP diet for 2 d prior to this sampling, but were fed the 12% CP diet the morning of the sampling. This might explain their BUN values on d 12 being significantly lower than those of the lambs fed the 12% CP diet continuously.

It has been reported that plasma urea N (PUN) levels are correlated with CP intake

Table 8. Ruminal Fluid Ammonia Nitrogen of Lambs Fed Different Diets

	Crude protein of diets, %[a]				
Day	8	10	12	8/12	SE
	----------	----------	mg/dL	----------	----------
Day 10	13.86[b]	18.93[bc]	21.29[c]	17.00[bc]	1.66
Day 12	15.07[b]	20.93[cd]	21.93[d]	17.00[bc]	1.23

[a]Calculated
[b,c,d]Means in the same row with unlike superscripts are different ($P < 0.05$)

Table 9. Blood Urea Nitrogen of Lambs Fed Different Diets

	Crude protein of diets, %[a]				
Item	8	10	12	8/12	SE
BUN, mg/dl					
D 10					
Hours after feeding					
2	8.27[b]	11.58[c]	13.19[c]	11.88[c]	0.51
4	8.30[b]	11.48[c]	13.54[c]	11.46[c]	0.62
6	8.18[b]	9.57[b]	13.05[c]	10.70[bc]	0.65
D 12					
Hours after feeding					
2	9.18[b]	11.40[c]	13.58[d]	9.16[b]	0.54
4	9.08[b]	11.45[bc]	13.43[c]	9.56[b]	0.59
6	8.56[b]	11.54[cd]	12.84[c]	9.37[bd]	0.59

[a]Calculated
[b,c,d]Means in the same row with unlike superscripts are different ($P < 0.05$)

(Pfander et al., 1975). In the present study, blood urea N values for the oscillating lambs continued to drop at each subsequent sampling interval on d 10, but were numerically higher than those of lambs on the 8% CP diet at the 6 h post-feeding sampling time. On d 12, the BUN level of the oscillating lambs increased between the 2 h to 4 h post-feeding sampling times, but then decreased slightly at the 6 h post-feeding sampling time. Samples taken at later time intervals may have shown a return to increasing values, as could be expected with a higher protein diet (Pfander et al., 1975). Since lambs fed the oscillating diet had been fed the 12% CP diet for 2 d prior to the sampling on d 10, but were fed the 8% CP diet the morning of the sampling, it could be expected that BUN levels for these lambs would be higher than lambs fed the 8% CP diet continuously.

Krehbiel et al. (1998) found that ewes supplemented with SBM every 72 h exhibited greater NH_3-N and alpha-amino N release by the portal-drained viscera on the day after the supplement was fed, rather than the day of or the second day after supplementation. These researchers also found less urea N was removed from the rumen to the portal-drained viscera on the day of supplementation compared to the 2 d following supplementation. This could provide an explanation for the equal ruminal NH_3-N concentrations when lambs were fed both the 8% and 12% CP diets in the present study.

Average plasma urea N (PUN) levels sampled before feeding in lambs fed cornstalks supplemented with SBM or corn gluten meal at 24 or 48 h intervals showed no effect of interval of supplementation (Collins and Pritchard, 1992). Values reflected the dietary CP content, with one exception. The lambs fed corn gluten meal every 24 h had numerically lower PUN levels compared to all other treatments. These data are somewhat misleading, however, in that it appears that the PUN values taken throughout the trial were averaged into one value. This does

not allow for actual comparison of possible changes in PUN levels when supplements were fed compared to when they were not fed. Collins and Pritchard (1992) also reported a tendency for interval of feeding to affect ruminal NH_3 -N concentrations. Lambs supplemented with either SBM or corn gluten meal at 48 h intervals exhibited numerically higher ruminal NH_3-N concentrations than lambs supplemented at 24 h intervals.

In a trial by Collins and Pritchard (1992) using similar treatments as in their study with sheep, but using steers as the experimental animal, PUN levels were significantly affected by time of supplementation. Supplementation every 48 h resulted in lower PUN values compared to supplementation every 24 h for both SBM and corn gluten meal protein sources. Steers fed corn gluten meal at 48 h intervals maintained more constant PUN levels across a 24 h sampling interval than steers fed SBM at 48 or 24 h intervals or steers fed corn gluten meal at 24 h intervals.

Average PUN concentrations were not affected by protein oscillation in lambs fed 90% concentrate diets with CSM as the protein source (Cole, 1999). Plasma urea N values of lambs fed diets of 10% CP and 15% CP oscillated every 24 or 48 h were similar to those of lambs fed a 12.5% CP diet continuously. However, lambs fed the 48 h – oscillating diet exhibited numerically higher PUN values and values closer to those of the lambs fed the continuously-fed 12.5% CP diet than those of lambs fed the 24 h – oscillating diet. When lambs were fed a 50:50 CSM and urea mix as the protein source, results differed. Plasma urea N concentrations were numerically higher for lambs fed the 24 h - and 48 h – oscillating diets than those of lambs fed the 12.5% CP diet continuously. Again, however, lambs fed the 48 h – oscillating diet had higher PUN values than those lambs fed the 24 h – oscillating diet. As in the lamb trial by Collins and Pritchard, PUN values in the trial by Cole (1999) were difficult to interpret due to

only average values being presented, not values at the specific time intervals sampled. However, the author did mention that the day after lambs fed the 24 and 48 h oscillating diets were fed the 10% CP diet, they exhibited PUN values significantly lower than lambs fed the 10% diet continuously, when the supplement was 100% CSM. This was not the case for oscillating lambs the day after they were fed the 15% CP diet,when the supplement was 100% CSM. In that instance, the PUN levels of the oscillating lambs fed the 15% CP diet that morning were very similar to those lambs fed the 15% CP diet continuously. When the lambs were supplemented with the CSM/urea mix in the second trial, average PUN values of the oscillating lambs the day after being fed the 10% CP diet were similar to those of lambs fed the 10% CP diet continuously, unlike in Trial 1, when the supplement was 100% CSM. The author suggested that differences in ruminal degradability of the protein supplement may have been the cause of the discrepancies between the trials (Cole, 1999).

Experiment 2 – Growth Trial with Steers

Objectives

The objectives of this experiment were to evaluate the effects of oscillating two levels of low ruminally degradable dietary protein on steer performance. Daily feed intake, average daily gain, gain to feed ratio, and blood urea nitrogen levels were compared.

Experimental Procedure

Animals and Diets

Twenty-four black, white-faced crossbred steers (228 kg) were used in a 112 d growth trial. Steers were purchased from a feeder cattle auction in Dublin, VA, and brought to the Smithfield Unit in Blacksburg, VA. The animals were blocked by BW and by using random numbers, were allotted at random within blocks to feeding stalls. Using random numbers, steers were allotted at random within blocks to four treatments: 1) 7.5% CP diet, 2) 9% CP diet, 3) 10.5% CP diet, and 4) oscillating 7.5% and 10.5% CP diet every 48 h.

The experimental diets were mixed in 226.8 kg batches for 15 min each in a Davis (H.C. Davis Sons MFG, Co., Inc., Bonner Springs, Kansas) mixer. Each ingredient was sampled before being added to the mixer, and each batch mixed was also sampled before being bagged. Samples were stored in plastic bags for analysis. Premixes of limestone, dicalcium phosphate, salt, and Vitamin A were mixed in a 13.6 kg capacity Hobart (H600 model, The Hobart Manufacturing Co., Troy, Ohio) mixer for 10 min and added to each 226.8 kg batch of experimental diet mixed. From December 1, 1999, to December 14, 1999, the steers were fed a combination of hay and a high roughage mix in their respective stalls. The high roughage mix

consisted of 40.5% ground corn, 50.4% grass hay, 3.5% soybean meal, 5.0% molasses, and 0.6% trace mineralized salt.

The steers were trained to enter their respective stalls where they were fed the experimental diets once per day. Steers were fed at 1500 h and remained in the stalls overnight without water. Refusals were weighed and recorded each morning after the animals were placed into an adjoining pen with access to water at 0700 h where they remained until the next feeding. Water was available continuously from a group waterer while steers were located in the pen. A 5-d transition period began on December 15, 1999. The animals were fed 0.91 kg of their respective experimental diets and 3.63 kg of the high-roughage diet on d 1. The amount of experimental diet was increased by 0.91 kg each day and the high-roughage diet was decreased by 0.91 kg each day until only the experimental diet was fed. Steers were started on 4.54 kg feed/d. This amount was increased by 0.45 kg if an individual animal left no refusals for 4 d. If refusals were greater than 0.91 kg during 2 d, the amount fed was decreased by 0.45 kg at the next feeding.

Ingredient and chemical compositions of the diets are presented in Tables 10 and 11, respectively. From the beginning of the trial on December 15, 1999, until February 1, 2000, diets consisted of barley straw, fish meal, barley grain, corn, wet molasses, limestone, dicalcium phosphate, salt, and 1,000 I.U. of Vitamin A per 0.45 kg of diet mixed. Due to a shortage of barley straw, on February 2, 2000, the barley straw in the diets was replaced by wheat straw, and the diets were re-formulated (Table 10). Upon analysis, diets contained 6.73, 7.99, 9.68, and 8.21% CP for the 7.5, 9, 10.5, and oscillating 7.5 and 10.5% CP diets, respectively. The diets will be referred to as 7.5, 9, 10.5% CP, and 7.5/10.5% CP oscillating diets.

Table 10. Ingredient Composition of Different Diets Fed to Steers[a]

Date	Feedstuff	Crude protein of diets, %[b]		
		7.5	9	10.5
(12/15/99 – 2/1/00)		----------	%	----------
	Barley straw	63.54	63.77	63.96
	Fish meal	2.75	5.23	7.73
	Barley grain	14.72	14.73	14.73
	Corn	13.29	11.11	8.98
	Sugarcane molasses	4.06	4.07	4.07
	Limestone	0.41	0.21	0.00
	Dicalcium phosphate	0.68	0.33	0.00
	Salt	0.55	0.55	0.55
(2/2/00 - 4/4/00)				
	Wheat straw	61.10	61.31	61.50
	Fish meal	3.26	5.77	8.25
	Barley grain	14.93	14.94	14.93
	Corn	14.92	12.75	10.64
	Sugarcane molasses	4.12	4.12	4.12
	Limestone	0.41	0.21	0.00
	Dicalcium phosphate	0.69	0.33	0.00
	Salt	0.56	0.56	0.56

[a]DM basis
[b]Calculated

Table 11. Chemical Composition of Different Diets Fed to Steers[a]

Date	Component	Crude protein of diets, %[b]			
		7.5	9	10.5	7.5/10.5
12/15/99-2/1/00		----------	----------	%	----------
	Dry matter	93.0	93.59	93.67	93.34
	Composition of dry matter				
	Crude protein	6.63	7.70	9.73	8.18
	NDF	58.06	56.41	57.69	57.88
	ADF	34.47	33.03	33.85	34.16
	Ash	7.51	7.27	7.30	7.41
2/2/00-4/4/00					
	Dry matter	93.30	94.04	93.69	93.50
	Composition of dry matter				
	Crude protein	6.91	8.39	9.60	8.26
	NDF	61.42	59.25	60.64	61.03
	ADF	35.80	34.63	35.51	35.66
	Ash	5.85	5.20	5.71	5.78

[a]DM basis
[b]Calculated

Beginning on December 15, 1999, the cattle were weighed every 14 d at 0715 h, and blood samples were taken every 28 d at 0830 h. Weights were recorded and used to calculate ADG for steers during the 112 d study. Blood samples were taken from the jugular vein, centrifuged at 600 x g for 15 min, and serum was separated into plastic tubes and frozen for later analysis.

Chemical Analysis

Feed samples were ground to pass a 1 mm-mesh screen of a Wiley mill (Thomas Wiley, Laboratory Mill Model 4, Arthur H. Thomas Co. Philadelphia, PA), and analyzed for DM and ash (AOAC, 1990), NDF (Van Soest and Wine, 1967), ADF (Van Soest, 1963), and N, using the Kjeldahl method (AOAC, 1990). Blood samples were analyzed for urea N concentration using an autoanalyzer (Beckman SYNCHRON CX® SYSTEMS, Beckman Instruments, Inc., Brea, CA) and BUN reagent. The BUN concentration of samples was measured by an enzymatic rate method. The urea in the sample is first broken down into NH_3 and CO_2. The enzyme glutamate dehydrogenase then acts on NH_3 and α-ketoglutarate to form glutamate. At the same time, β-nicotinamide adenine dinucleotide (NADH) is oxidized to NAD. The change in absorbance at 340 nanometers is monitored by the system. The concentration of urea N in the serum being directly proportional to the change in absorbance, the SYNCHRON CX System then calculates the BUN concentration of the sample.

Statistical Analysis

Data were analyzed using the JMP (JMP, 1996) procedure for analysis of variance of a completely randomized block design. When tests for normality or equal variances were significant, results from the Wilcoxon nonparametric test and the Welch ANOVA were used. In all other instances, the Tukey-Kramer test was used.

Results and Discussion

Steer Performance

The performance data are presented in Table 12. Initial weights were not different among animals allotted to different diets ($P > 0.05$). Final weights of steers on the 10.5% CP diet were higher ($P < 0.05$) than those of steers fed 7.5% CP diet, but not different from those steers fed the 9% or 7.5/10.5% CP oscillating diets. Average daily gain of steers fed the 10.5% CP diet was higher ($P < 0.05$) than that of steers fed the 9% CP diet or the 7.5% CP diet. Daily gain for cattle fed the 7.5/10.5% CP oscillating diet was higher, but not significantly different from that of cattle fed the 9% CP diet. The ADG of steers fed the 10.5% CP diet was not significantly different from those fed the oscillating diet. Oscillating the CP content of the diet did not significantly increase the ADG of steers compared to steers fed the 9% CP diet continuously, although the oscillating steers did exhibit numerically higher ADG values. This could be attributable to the oscillating diet supplying slightly more CP than that of the 9% diet (Table 11).

Average daily feed intake among steers fed different diets was not significantly different. Other research has demonstrated variable results concerning the effect of oscillating dietary crude protein on DMI. Collins and Pritchard (1992) reported a trend toward higher DMI in steers fed a basal diet of corn stalks supplemented with either SBM or corn gluten meal every 24 h versus every 48 h. However, Coleman and Wyatt (1982) and Hunt et al. (1989) found no effect of interval of supplementation on DMI when steers were fed a basal diet of grass hay supplemented with CSM at 12, 24, 48, or 96 h. A second experiment by Coleman and Wyatt (1982) using small grains forage instead of CSM as the supplemental protein source did result in differences in DMI. Steers supplemented every 96 h exhibited lower DMI than steers

Table 12. Performance of Steers

Item	Crude protein of diets, %[a]				
	7.5	9	10.5	7.5/10.5	SE
	---------- kg ----------				
Initial weight (12/15/99)	227.66	232.65	225.85	226.30	7.64
Final weight (4/5/00)	264.40[b]	284.81[bc]	296.60[c]	287.07[bc]	7.51
Daily gain, 112 d	0.33[b]	0.46[c]	0.63[d]	0.54[cd]	0.03
Daily feed intake	5.09	5.37	5.63	5.44	0.21
Gain/feed	0.06[b]	0.09[c]	0.11[d]	0.10[cd]	0.004

[a]Calculated
[bcd]Means in the same row with unlike superscripts are different ($P < 0.05$)

supplemented every 24 h or every 48 h.

Steers fed the 10.5% CP diet had a higher ($P < 0.05$) gain to feed ratio than steers fed the 9% CP or 7.5% CP diets, but no significant difference was detected between steers fed the 10.5% diet and those fed the oscillating diet. Steers fed the oscillating diet had numerically higher gain to feed ratios compared to steers fed the 9% CP diet continuously, but the difference was not significant ($P > 0.05$). Gain to feed values were lower ($P < 0.05$) for cattle fed the 7.5% CP diet, compared to those fed the other diets.

Several studies have examined the effect of supplemental low ruminally degradable protein on steer performance, and it has been generally concluded to have a positive effect (Stock et al., 1981; Anderson et al., 1988; Gutierrez-Ornelas and Klopfenstein, 1991; Karges et al., 1992; Hafley et al., 1993). Less research has been done, however, on the effect of oscillating mixed diets that vary in percent of low ruminally degradable crude protein compared to feeding a certain amount of this protein in the diet continuously. The majority of studies done, however, agree with performance results presented here. An early study done by McIlvain and Shoop (1962) reported that steers grazing winter forage of sagebrush and bunch grasses in Oklahoma exhibited no difference in ADG when supplemented with cottonseed pellets daily, every third day, or weekly. In agreement with the results of McIlvain and Shoop (1962), Hunt et al. (1989) found no significant effect of interval of supplementation on the ADG of steers fed CSM at 12, 24, or 48 h. Animals in the study by Hunt et al. (1989) were not grazing, but they were fed a basal diet of chopped grass hay on an *ad libitum* basis in individual stalls. Collins and Pritchard (1992) found no significant effect of interval of protein supplementation on ADG of steers fed a basal diet of cornstalks. Both low (corn gluten meal) and high (SBM) ruminally degradable protein sources were fed. Although the lower ruminally degradable protein source (corn gluten

meal) resulted in a significantly higher ADG and gain to feed ratio compared to SBM supplementation, the 24 h or 48 h supplementation intervals had no effect.

An experiment with pregnant beef cows has also demonstrated a lack of response to frequency of protein supplementation (Beaty et al., 1994). Soybean meal and sorghum grain were supplemented every day or three times per week to pregnant beef cows grazing dormant tallgrass prairie. Cow performance, measured as weight changes, body condition changes, pregnancy rate, calving interval, and calf BW, ADG, and WW were slightly negatively affected by increasing the time interval of supplementation, but the researchers concluded that these effects were outweighed by the potential reduction in labor costs resulting from less frequent supplementation.

Blood Urea Nitrogen

Data collected on blood urea N (BUN) levels of steers every 28 d are presented in Table 13. Initial BUN values were similar ($P > 0.05$). However, after the first 28 d, a trend began to develop, similar to that found in the performance data. Steers fed the 10.5% CP and the oscillating diet exhibited BUN concentrations which were higher ($P < 0.05$) than those of steers fed the 9% CP and 7.5% CP diets. This trend was evident on the third and fourth sampling date as well, but differences were not always significant. On the fourth sampling, no significant difference among BUN levels was detected for steers fed the 10.5% CP diet, the oscillating diet, and the 9% CP diet. Blood urea N levels of steers fed the 10.5% CP diet remained higher ($P < 0.05$) than those of the steers fed the 7.5% CP diet at the fourth sampling date, however. On the fifth and final sampling date, steers fed the 10.5% CP diet had higher ($P < 0.05$) BUN values than steers fed all other diets, and no difference ($P > 0.05$) was evident among the 7.5% CP, 9% CP, or oscillating diets. A difference ($P < 0.05$) was found between the oscillating diet and the

Table 13. Blood Urea Nitrogen of Steers Fed Different Diets

	Crude protein of diets, %[a]				
Date	7.5	9	10.5	7.5/10.5	SE
	mg/dL				
12/15/99	5.38	4.85	5.15	5.53	0.49
1/12/00	3.87[b]	4.62[b]	8.08[c]	7.12[c]	0.61
2/9/00	4.05[b]	4.26[b]	6.30[c]	5.07[bc]	0.44
3/8/00	3.77[b]	4.20[bc]	5.16[c]	4.51[bc]	0.28
4/5/00	3.29[b]	4.71[b]	6.70[c]	4.69[b]	0.38

[a]Calculated
[bc]Means in the same row with unlike superscripts are different ($P < 0.05$)

9% CP diet only on the second sampling date, although the steers on the oscillating diet usually exhibited numerically higher BUN concentrations. On each sampling date, the oscillating steers had been fed the 10.5% CP diet for the previous 2 d, therefore it would be conceivable that their BUN concentrations would be greater than the steers fed the 9% diet continuously. More meaningful data may have been provided by sampling blood at varying intervals in order to evaluate the BUN levels of steers when they had been or were currently being fed both the 10.5% CP diet as well as the 7.5% CP diet. Also, the percentage CP fed to the oscillating steers was slightly greater than that of the 9% CP steers (Table 11).

Other researchers have demonstrated variable results concerning the effect of oscillating dietary CP on plasma urea N (PUN) concentrations in ruminants. Plasma urea N values for steers fed a basal diet of corn stalks supplemented with SBM or corn gluten meal at 24 or 48 h intervals were significantly affected by interval of supplementation in a study by Collins and Pritchard (1992). Both SBM and corn gluten meal supplemented at 48 h intervals resulted in lower PUN values than supplementation at 24 h intervals. A protein source x interval of supplementation interaction was evident in that experiment as well. Steers fed the corn gluten meal every 48 h exhibited a less dramatic change in PUN concentration over a 24 h sampling interval compared to steers fed the SBM every 48 h. The researchers concluded that this effect suggested an alteration in N metabolism between treatments. The combination of a low ruminally degradable protein source and feeding this protein source at 48 h intervals may have a stabilizing effect on N metabolism.

Results from a study done with lambs also demonstrated interesting effects of oscillating dietary protein on PUN levels. Cole (1999) fed lambs a 90% concentrate diet formulated for either 10% CP, 12.5% CP, or 15% CP. Lambs fed a 10 and 15% CP diet oscillated every 48 h

exhibited PUN levels significantly lower than lambs fed the 10% diet continuously when oscillating lambs were sampled the day after eating the 10% CP diet. The day after oscillating lambs were fed the 15% CP diet, PUN levels were similar to those of lambs fed the 15% CP diet continuously. However, in a second trial, using a more ruminally degradable protein source, PUN levels of oscillating lambs the day after being fed the 10% CP diet were similar to those of lambs fed the 10% CP diet continuously. Like Collins and Pritchard (1992), the author suggested that this discrepancy between trials was an effect of protein source degradability.

Results concerning the effect of oscillating low ruminally degradable dietary CP on BUN levels in lambs have been presented previously in Exp 1. Lambs fed an 8% and 12% CP diet oscillated every 48 h did not exhibit BUN levels significantly different from those of lambs fed a 10% CP diet continuously, regardless of the percent CP the lambs on the oscillating diet were fed the morning of sampling. The BUN values of the lambs on the oscillating diet appeared to reflect the diet they had eaten the 2 d prior to the day of sampling. When these lambs had been fed the 8% CP diet the morning of sampling, but the 12% CP diet for the prior 2 d, BUN levels were similar to those fed the 12% CP diet continuously. However, when lambs on the oscillating diet were fed the 12% CP diet the morning of sampling, after having been fed the 8% CP diet for the 2 previous d, BUN levels were similar to those fed the 8% CP diet continuously.

General Discussion

The present experiments were conducted to evaluate the effects of 48 h oscillation of low ruminally degradable dietary protein on the metabolism of N and the performance of ruminants. It had been hypothesized, based on data from other studies (Collins and Pritchard, 1992; Cole, 1999), that this 48 h oscillation feeding regime would increase N retention compared to feeding the same amount of CP in the diet daily. It was further surmised that if N retention were indeed increased with this method of feeding, more N would be available to these animals for growth. Thus the ADG of animals fed the oscillating CP diet would exceed that of animals fed a low CP diet with no variation in CP content over time.

In the present Experiments 1 and 2, oscillating the CP content of the diet every 48 h had no significant effect on the N retention nor the ADG of ruminants compared to those fed the same level of CP daily. Fecal, urinary, and total N excretion were not significantly different between lambs fed the 8/12% CP oscillating diet and those fed the 10% CP diet continuously ($P > 0.05$). Apparent absorption of N expressed in g/d and as a percentage of N intake also did not differ between lambs fed the aforementioned diets ($P > 0.05$). Nitrogen retention, expressed in g/d, as a percentage of N intake, and as a percentage of N absorbed was also not affected by oscillating dietary CP. In Exp. 2, no significant differences in final weight, ADG, and gain to feed ratio were exhibited between cattle fed the 7.5/10.5% CP oscillating diet and cattle fed the 9% CP diet continuously ($P > 0.05$). However, trends favored the animals fed the oscillating diet.

In both Experiments 1 and 2, oscillating dietary CP had no significant effect on BUN levels. There was no significant difference between BUN levels of lambs on the 8/12% CP oscillating diet and those on the 10% CP diet continuously at any sampling time ($P > 0.05$). In

Exp. 2, for the first three sampling dates BUN levels of cattle fed the 7.5/10.5% CP oscillating diet were numerically higher ($P > 0.05$) than those of cattle fed the 9% CP diet continuously.

Although the results of both Experiments 1 and 2 indicated that oscillating the CP content of the diet every 48 h had no effect on N metabolism or animal performance, discrepancies between the two trials limit their comparison. In both experiments, BUN was measured. However, sampling times differed greatly, thus diminishing the relevance of a comparison between the two. In Exp. 1, blood samples were taken at 2, 4, and 6 h post-feeding on two different days. On the first sampling day, the lambs fed the oscillating diet had been fed the 8% CP diet that morning. On the second day of sampling, 48 h later, the lambs fed the oscillating diet had been fed the 12% CP diet the morning of the sampling. This allowed for a comparison of BUN values after lambs on the oscillating diet were fed both the 8 and 12% CP diets. In Exp. 2, however, blood samples were taken from cattle every 28 d at 17.5 h after feeding. Each time the cattle were sampled, those on the oscillating diet had been fed the 10.5% CP diet for the previous 2 d. No samples were taken while the cattle fed the oscillating diet were being fed the 7.5% CP diet. Thus, it is impossible to determine whether BUN levels of cattle in Exp. 2 followed the same pattern as BUN levels of lambs in Exp. 1.

It has been reported that BUN levels are highly correlated with CP intake (Pfander et al.,1975), and results from the current experiments support these results. Blood urea N levels of lambs fed the 8/12% CP oscillating diet were numerically higher on d 10, when they had been fed the 12% CP diet for the 2 previous days, and numerically lower on d 12, when they had been fed the 8% CP diet for the 2 previous days, than BUN levels of lambs fed the 10% CP diet continuously. In Exp. 2, cattle on the 7.5/10.5% CP oscillating diet had been fed the 10.5% CP

diet for 2 d before each blood sampling. As in Exp. 1, BUN levels of these animals reflected the CP intake of the past 2 d.

Further limitations exist for the comparison of Experiments 1 and 2. Although similar, the diets utilized between the two studies were not identical. In Exp. 2, corn was used in addition to barley as a carbohydrate source, whereas only barley was the carbohydrate source in Exp. 1. Corn, containing more ruminally undegradable protein than barley, may have exerted an additional effect on animals in Exp. 2, due to reports of higher levels of low ruminally degradable protein in the diet influencing N retention (Stock et al, 1981; Anderson et al., 1988; Gutierrez-Ornelas and Klopfenstein, 1991; Karges et al, 1992). The roughage to concentrate ratio was higher for Exp. 2 (62:37) than Exp. 1 (52:48). Forage to concentrate ratio can influence microbial population of the rumen through the alteration of ruminal pH (Owens and Goetsch, 1988). However, the difference in the ratios between these two experiments was probably not large enough to have created significant differences in ruminal pH. In the second half of Exp. 2 wheat straw was used, and barley straw during the first half, as the roughage component. The wheat straw used was lower in CP than the barley straw, causing a slight increase in the percent of fish meal added to each diet to maintain proper CP values. No effect was distinguished from this change, however. In Exp. 1, lambs were fed diets in which the CP content was more critical than that for cattle in Exp. 2. The CP requirement for lambs of this age and weight was 12.8% CP (NRC, 1985), whereas the CP requirement for cattle in Exp. 2 was from 8.6% to 9.8% CP, depending on desired rate of gain (NRC, 1996). The highest CP level fed to the steers in Exp. 2 was 9.73% CP, during the first half of the trial. This value could have been expected to have met their CP requirement, if estimates from the NRC (1996) are used. Steers fed this diet exhibited higher daily gains, compared to steers fed all other diets. In

contrast, all lambs in Exp. 1 were fed below their suggested CP requirement (NRC, 1985). However, N retention values in g/d were similar between lambs fed the 10% and 12% CP diets, suggesting that the excess N supplied by the 12% CP diet was not utilized. Possibly, available energy intake was limited.

Feeding frequency also differed between experiments. Lambs in Exp. 1 were fed two times per day at 12 h intervals. Cattle in Exp. 2 were fed once per day at 24 h intervals, although feed was available for 16 h/d. It has been reported that feeding frequency can affect N retention in cattle. Cattle fed 90% of their individual maximum intake four times per day exhibited higher DM and OM digestibility and N retention than cattle fed once per d (Ruiz and Mowat, 1987). However, no effect on N retention was reported when lambs were fed fescue hay at 2, 4, 8, or 16 times per day (Bunting et al., 1987). Feeding frequency may affect other metabolic functions of ruminants such as microbial protein and VFA synthesis (Michalowski, 1979; Bunting et al., 1987), which could thereby potentially affect whole body protein metabolism.

The fact that different species were used in the current experiments may also limit the comparison between the two. Lambs were used as the experimental animals in Exp. 1, and cattle were used in Exp. 2. Although in many instances lambs are used as a model for cattle, differences in the effect of oscillating dietary CP between species has not been investigated.

Despite the lack of effect demonstrated in the current experiments, the possibility exists that 48 h oscillation of low ruminally degradable protein may affect N metabolism under certain circumstances. Cole (1999) reported increased N retention for lambs fed a 90% concentrate diet containing 10 or 15% CP oscillated at 48 h intervals compared to lambs fed a 12.5% CP diet continuously. This effect was not exhibited when lambs were fed a more ruminally degradable protein source. The author suggested that this effect could be attributed to several factors.

Decreasing ruminal protein degradability, minimizing degradable intake protein, and synchronizing mean retention time of digesta in the gut with time of dietary protein changes could all contribute to increased N recycling. Synchrony of digesta retention time and that of dietary protein oscillation would vary depending on feed intake, particle size, and other factors affecting rate of passage. It has been suggested that a high-concentrate basal diet may result in greater recycling of N to the rumen *versus* the gut (Huntington, 1989). Therefore, considering the characteristics of the basal diet before determining the hours of protein oscillation may be of importance to achieve the maximum effect on N retention.

Implications

Oscillating two levels of low ruminally degradable dietary CP in the diets of steers tended to improve performance compared to the lower protein levels. Retention of N was similar for lambs fed the oscillating protein diet and those fed the two higher protein levels. These results agree with findings of some previous studies. Some evidence has been presented by others to indicate oscillating dietary CP may affect protein metabolism, and that this may be due, in part, to the effects of protein degradability. The ability to manipulate this effect to produce greater N retention and higher levels of performance in ruminants would be beneficial to both animal production and environmental protection. Discrepancies between trials of this nature warrant further research in this area.

Literature Cited

Agricultural Research Council. 1980. The Nutrient Requirements of Ruminant Livestock. Commonwealth Agricultural Bureaux, Slough, England.

Al Jassim, R.A.M., S.A. Hassan, A.N. Al-Ani, and T.K. Dana. 1991. Effects of rumen undegradable protein supplementation on digestion and nitrogen balance in sheep and goats. Small Ruminant Res. 5:57-63.

Anderson, S.J., T.J. Klopfenstein, and V.A. Wilkerson. 1988. Escape protein supplementation of yearling steers grazing smooth brome pastures. J. Anim. Sci. 66:237-242.

AOAC. 1990. Official Methods of Analysis (15th Ed.). Association of Official Analytical Chemists, Arlington, VA.

ApSimon, H.M., M. Kruse, and J.N.B. Bell. 1987. Ammonia emissions and their role in acid deposition. Atmosph. Environ. 21:1939-1946.

Batajoo, K.K. and R.K. Shaver. 1998. *In situ* dry matter, crude protein, and starch degradabilities of selected grains and by-product feeds. Anim. Feed Sci. Tech. 17:165-176.

Beaty, J.L., R.C. Cochran, B.A. Lintzenich, E.S. Vanzant, J.L. Morrill, R.T. Brandt, Jr., and D.E. Johnson. 1994. Effect of frequency of supplementation and protein concentration in supplements on performance and digestion characteristics of beef cattle consuming low-quality forages. J. Anim. Sci. 72:2475-2486.

Beecher, G.P. and B.K. Whitten. 1970. Ammonia determination: Reagent modification and interfering compounds. Anal. Biochem. 36:243-251.

Blasi, D.A., J.K. Ward, T.J. Klopfenstein, and R.A. Britton. 1991. Escape protein for beef cows: III. performance of lactating beef cows grazing smooth brome or big bluestem. J. Anim. Sci. 69:2294-2302.

Briggs, H.M. and W.D. Gallup. 1949. Metabolism stalls for wethers and steers. J. Anim. Sci. 8:479-482.

Broderick, G.A. 1992. Relative value of fish meal *versus* solvent soybean meal for lactating dairy cows fed alfalfa silage as sole forage. J. Dairy Sci. 75:174-183.

Broderick, G.A., R.J. Wallace, E.R. Orskov, and L. Hansen. 1988. Comparison of estimates of ruminal protein degradation by *in vitro* and *in situ* methods. J. Anim. Sci. 66:1739-1745.

Brown, D.R., F.C. Hinds, and R.M. Collins. 1995. Effect of supplementation frequency on diet digestion and nitrogen metabolism of growing lambs fed low-quality forage. Prof. Anim. Sci. 12:24-27.

Bunting, L.D., M.D. Howard, R.B. Muntifering, K.A. Dawson, and J.A. Boling. 1987. Effect of feeding frequency on forage fiber and nitrogen untilization in sheep. J. Anim. Sci. 64:1170-1177.

Cole, N.A. 1999. Nitrogen retention by lambs fed oscillating dietary protein concentrations. J. Anim. Sci. 77:215-222.

Coleman, S.W. and R.D. Wyatt. 1982. Cottonseed meal or small grains forages as protein supplements fed at different intervals. J. Anim. Sci. 55:11-17.

Collins, R.M. and R.H. Pritchard. 1992. Alternate day supplementation of corn stalk diets with soybean meal or corn gluten meal fed to ruminants. J. Anim. Sci. 70:3899-3908.

Crutzen, P.J. 1981. Atmospheric chemical processes of the oxides of nitrogen, including nitrous oxide. In: C.C. Delwiche, (ed.) Denitrification, Nitrification, and Atmospheric Nitrous Oxide. p.17. John Wiley & Sons, Inc., New York, NY.

Dawson, J.M., C.I. Bruce, P.J. Buttery, M. Gill, and D.E. Beever. 1988. Protein metabolism in the rumen of silage-fed steers: effect of fish meal supplementation. Br. J. Nutr. 60:339-353.

DePeters, E.J. and S.J. Taylor. 1985. Effects of feeding corn or barley on composition of milk and diet digestibility. J. Dairy Sci. 68:2027-2032.

Erasmus, L.J., J. Prinsloo, and H.H. Meissner. 1988. The establishment of a protein degradability data base for dairy cattle using the nylon bag technique. 1. Protein sources. S. Afr. J. Anim. Sci. 18:23-29.

Fiske, C.H. and Y. Subbarow. 1925. The colorimetric determination of phosphorus. J. Biol. Chem. 66:375-380.

Flessa, H., F. Dorsch, F. Beese, H. Konig, and A.F. Bouwman. 1996. Atmospheric pollutants and trace gases: influence of cattle wastes on nitrous oxide and methane fluxes in pasture land. J. Environ. Qual. 25:1366-1370.

Gill, M. and P. England. 1984. Effect of degradability of protein supplements on voluntary intake and nitrogen retention in young cattle fed grass silage. Anim. Prod. 39:31-36.

Gill, M., D.E. Beever, P.J. Buttery, P. England, M.J. Gibb, and R.D. Baker. 1987. The effect of oestradiol-17â implantation on the response in voluntary intake, live-weight gain and body composition, to fishmeal supplementation of silage offered to growing calves. J. Agric. Sci. (Camb). 108:9-16.

Giraldez, F.J., C. Valdes, R. Pelaez, P. Frutos, and A.R. Mantecon. 1997. The influence of digestible organic matter and nitrogen intake on faecal and urinary nitrogen losses in sheep. Livestock Prod. Sci. 51:183-190.

Goering, H.K. and P.J. Van Soest. 1970. Forage fiber analyses (apparatus, reagent, procedures, and some applications). Agric. Handbook No. 379. ARS, USDA, Washington, D.C.

Gutierrrez-Ornelas, E. and T.J. Klopfenstein. 1991. Diet composition and gains of escape protein-supplemented growing cattle grazing corn residues. J. Anim. Sci. 69:2187-2195.

Hafley, J.L., B.E. Anderson, and T.J. Klopfenstein. 1993. Supplementation of growing cattle grazing warm-season grass with proteins of various ruminal degradabilities. J. Anim. Sci. 71:522-529.

Hassan, S.A. and M.J. Bryant. 1986a. The response of store lambs to dietary supplements of fish meal. 1. Effects of forage-to-concentrate ratio. Anim. Prod. 42:223-232.

Hassan, S.A. and M.J. Bryant. 1986b. The response of store lambs to dietary supplements of fish meal. 2. Effects of level of feeding. Anim. Prod. 42:233-240.

Herrera-Saldana, R. and J.T. Huber. 1989. Influence of varying protein and starch degradabilities on performance of lactating cows. J. Dairy Sci. 72:1477-1483.

Herrera-Saldana, R., R. Gomez-Alarcon, M. Torabi, and J.T. Huber. 1990. Influence of synchronizing protein and starch degradation in the rumen on nutrient utilization and microbial protein synthesis. J. Dairy Sci. 73:142-148.

Hristov, A. and G.A. Broderick. 1994. *In vitro* determination of ruminal protein degradability using [^{15} N]ammonia to correct for microbial nitrogen uptake. J. Anim. Sci. 72:1344-1354.

Hunt, C.W., J.F. Parkinson, R.A. Roeder, and D.G. Falk. 1989. The delivery of cottonseed meal at three different time intervals to steers fed low-quality grass hay: effects on digestion and performance. J. Anim. Sci. 67:1360-1366.

Huntington, G.B. 1989. Hepatic urea synthesis and site and rate of urea removall from blood of beef steers fed alfalfa hay or a high concentrate diet. Can. J. Anim. Sci. 69:215-223.

Hussein, H.S., R.M. Jordan, and M.D. Stern. 1991. Ruminal protein metabolism and intestinal amino acid utilization as affected by dietary protein and carbohydrate sources in sheep. J. Anim. Sci. 69:2134-2146.

Jarvis, S.C., D.J. Hatch, and D.H. Roberts. 1989. The effects of grassland management on nitrogen losses from grazed swards through ammonia volitilization; the relationship to excretal N returns from cattle. J. Agric. Sci. (Camb). 112:205-216.

JMP. 1996. JMP Start Statistics: A guide to statistics and data analysis. SAS Institute, Inc., Cary, NC.

Karges, K.K., T.J. Klopfenstein, V.A. Wilkerson, and D.C. Clanton. 1992. Effects of ruminally degradable and escape protein supplements on steers grazing summer native range. J. Anim. Sci. 70:1957-1964.

Kennedy, P.M., G.P. Hazelwood, and L.P. Milligan. 1984. A comparison of methods for the estimation of the proportion of microbial nitrogen in duodenal digesta, and of correction for microbial contamination in nylon bags incubated in the rumen of sheep. Br. J. Nutr. 52:403-417.

Krehbiel, C.R., C.L. Ferrell, and H.C. Freetly. 1998. Effects of frequency of supplementation on dry matter intake and net portal and hepatic flux of nutrients in mature ewes that consume low-quality forage. J. Anim. Sci. 76:2464-2473.

Lee, N., J.A. Rooke, and D.G. Armstrong. 1986. The digestion by sheep of barley and maize-based diets containing either meat and bone meal or soya bean meal. Anim. Feed Sci. Tech. 15:301-310.

Little, C.O., W. Burroughs, and W. Woods. 1963. Nutritional significance of soluble nitrogen in dietary proteins for ruminants. J. Anim. Sci. 22:358-363.

McCarthy, R.D., Jr., T.H. Klusmeyer, J.F. Vicini, J.H. Clark, and D.R. Nelson. 1989. Effects of source of protein and carbohydrate on ruminal fermentation and passage of nutrients to the small intestine of lactating cows. J. Dairy Sci. 72:2002-2016.

McIlvain, E.H. and M.C. Shoop. 1962. Daily versus every-third-day versus weekly feeding of cottonseed cake to beef steers on winter range. J. Range Manage. 15:143-146.

Mehrez, A.Z. and E.R. Orskov. 1977. A study of the artificial fibre bag technique for determining the digestibility of feeds in the rumen. J. Agric. Sci. (Camb). 88:645-650.

Merchen, N.R. 1988. 1988. Digestion, absorption, and excretion in ruminants. In: D.C. Church, (ed.) The Ruminal Animal: Digestive Physiology and Nutrition, p.172. Waveland Press, Inc., Prospect Heights, Ill.

Michalowski, T. 1979. Effect of feeding frequency on the diurnal changes in microbial protein, volatile fatty acids and ammonia contents of the rumen of sheep. J. Agric. Sci. (Camb.) 93:67-70.

Muchovej, R.M.C., V.G. Allen, D.C. Martens, L.W. Zelazny, and D.R. Notter. 1986. Aluminum, citric acid, nitrilotriacetic acid, and soil moisture effects on aluminum and iron concentrations in ryegrass. Agron. J. 78:138-145.

NRC. 1985. Nutrient Requirements of Sheep (6th Ed.) National Academy Press, Washington, D.C.

NRC. 1996. Nutrient Requirements of Beef Cattle (7th Ed.) National Academy Press, Washington, D.C.

Owens, F.N. and A.L. Goetsch. 1988. Ruminal fermentation. In: D.C. Church, (ed.) The Ruminal Animal: Digestive Physiology and Nutrition, p.145. Waveland Press, Inc., Prospect Heights, Ill.

Owens, F. and R. Zinn. 1988. Ruminant nitrogen metabolism. In: D.C. Church, (ed.) The Ruminant Animal: Digestive Physiology and Nutrition, p.227. Waveland Press, Inc., Prospect Heights, Ill.

Petersen, M.K., D.C. Clanton, and R. Britton. 1985. Influence of protein degradability in range supplements on abomasal nitrogen flow, nitrogen balance, and nutrient digestibility. J. Anim. Sci. 60:1324-1329.

Pfander, W.H., S.E. Grebing, C.M. Price, O. Lewis, J.M. Asplund, and C.V. Ross. 1975. Use of plasma urea nitrogen to vary protein allowances of lambs. J. Anim. Sci. 41:647-653.

Rodhe, H. 1990. A comparison of the contribution of various gases to the greenhouse effect. Science 248: 1217-1219.

Roe, M.B., L.E. Chase, and C.J. Sniffen. 1991. Comparison of *in vitro* techniques to the *in situ* technique for estimation of ruminal degradation of protein. J. Dairy Sci. 74:1632-1640.

Ruiz, A. and D.N. Mowat. 1987. Effect of feeding frequency on the utilization of high-forage diets by cattle. Can. J. Anim. Sci. 67:1067-1074.

Sherlock, R.R. and K.M. Goh. 1983. Initial emission of nitrous oxide from sheep urine applied to pasture soil. Soil. Biol. Biochem. 15:615-617.

Smith, R.H. 1989. Nitrogen metabolism in the ruminant stomach. In: H.D. Block, B.O. Eggum, A.G. Low, O. Simon, and T. Zebrowska (ed.) Protein Metabolism in Farm Animals: Evaluation, Digestion, Absorption, and Metabolism. p.165. Oxford University Press, New York, NY.

Stock, R., N. Merchen, T. Klopfenstein, and M. Poos. 1981. Feeding value of slowly degraded proteins. J. Anim. Sci. 53:1109-1119.

Stokes, S.R., W.H. Hoover, T.K. Miller, and R. Blauweikel. 1991. Ruminal digestion and microbial utilization of diets varying in type of carbohydrate and protein. J. Dairy Sci. 74:871-881.

Sultan, J.I. and S.C. Loerch. 1992. Effects of protein and energy supplementation of wheat straw-based diets on site of nutrient digestion and nitrogen metabolism of lambs. J. Anim. Sci. 70:2228-2234.

Susmel, P., C.R. Mills, M. Colitti, and B. Stefanon. 1993. *In vitro* solubility and degradability of nitrogen in concentrate ruminant feeds. Anim. Feed Sci. Tech. 42:1-13.

Titgemeyer, E.C., N.R., Merchen, and L.L. Berger. 1989. Evaluation of soybean meal, corn gluten meal, blood meal and fish meal as sources of nitrogen and amino acids disappearing from the small intestine of steers. J. Anim. Sci. 67:262-275.

Tomlinson, A.P., W.J. Powers, H.H. Van Horn, R.A. Nordstedt, and C.J. Wilcox. 1996. Dietary protein effects on nitrogen excretion and manure characteristics of lactating cows. Trans. Am. Soc. Ag. Engin. 39:1441-1448.

Van Soest, P.J. 1963. Use of detergents in the analysis of fibrous feeds. II. A rapid method for the determination of fiber and lignin. J. Assoc. Off. Agri. Chem. 46:829-835.

Van Soest, P.J. and R.H. Wine. 1967. Use of detergents in the analysis of fibrous feeds. IV. The determination of plant cell wall constituents. J. Assoc. Off. Anal. Chem. 50:50-55.

Van Soest, P.J. and R.H. Wine. 1968. Determination of lignin and cellulose in acid detergent fiber with permanganate. J. Assoc. Off. Anal. Chem. 51:780-785.

Veen, W.A.G. 1986. The influence of slowly and rapidly degradable concentrate protein on a number of rumen parameters in dairy cattle. Neth. J. Agric. Sci. 34:199-216.

Volden, H. 1999. Effects of level of feeding and ruminally undegraded protein on ruminal bacterial protein synthesis, escape of dietary protein, intestinal amino acid profile, and performance of dairy cows. J. Anim. Sci. 77:1905-1918.

Zerbini, E., C. E. Polan, and J.H. Herbein. 1988. Effect of dietary soybean meal and fish meal on protein digesta flow in holstein cows during early and midlactation. J. Dairy Sci. 71:1248-1258.

Vita

Sarah Jordan Simpson was born in Blacksburg, Virginia, on June 3, 1975. In August of 1983, she moved with her family to Athens, Georgia, where she remained until graduating from Cedar Shoals High School in June of 1993. She attended Washington and Lee University (1993-94), then transferred to the University of Georgia and completed her Bachelor of Science degree in Animal Science in June of 1997. After working for the University of Georgia for one year, she entered the Master of Science program in Animal Science (Ruminant Nutrition) at Virginia Polytechnic Institute and State University.

Sarah J. Simpson