BLOOD, RUMEN LIQUOR, AND FECAL COMPONENTS AS AFFECTED BY DIETARY CRUDE PROTEIN

A Thesis

by

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Approved as to style and content by:

(Chairman of Committee)

(Member)

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(Head of Department)

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ABSTRACT

Blood, Rumen Liquor, and Fecal Components as Affected by Dietary Crude Protein. (December, 1979)

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Chairman of Advisory Committee: Dr. M. M. Kothmann

A technique was developed to predict deficiencies in crude protein (CP) intake of range cattle prior to production losses. A pen-feeding study was conducted using a 4 by 4 Latin square with four cows, and four steers. Four rations of varying CP contents (4.27%, 5.19%, 6.19%, and 7.14%) were fed during four, 14-day trials. Cottonseed hulls (3.82% CP) constituted the basal ration, sugarcane molasses (9.82% CP) was added to provide a maintenance level of digestible energy. Cottonseed pellets (35.74% CP) were added to vary levels of dietary CP from 4.27 to 7.14 (%). Blood (serum protein and blood urea nitrogen (BUN)), rumen fluid (total nitrogen, protein nitrogen, and microbial protein nitrogen), and fecal nitrogen were evaluated as predictors of dietary CP intake. Blood components were not suitable to predict maintenance, and sub-maintenance CP levels. Rumen fluid components were highly correlated ($r^2 > 0.97$) with dietary crude protein. Total nitrogen in rumen fluid was the easiest component to analyze, and the most effective predictor of the crude protein content of the diet. A total nitrogen level of less than 40 mg/100 ml of rumen fluid indicated a protein deficient diet for a steer or a dry cow. Fecal nitrogen was highly correlated ($r^2 = 0.92$) with dietary crude protein over the range studied. Fecal nitrogen levels less than 1.4% indicated a protein deficient diet. Fresh fecal

material collected by ranchers and routinely analyzed for total nitrogen would be a valuable aid to determining need for protein supplementation.

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INTRODUCTION[1]

Ruminants grazing rangelands are at times forced to consume forage which is nutritionally inadequate to meet their metabolic requirements. Protein is an important nutrient frequently found deficient in mature and dormant grasses (Kothmann and Hinnant, 1979).

Estimation of the crude protein (CP) content of the grazing ruminant's diet is a costly and laborious task. Three major methods have been used to estimate the CP content of diets selected by grazing cattle; total biomass clipping, hand plucking, and the use of esophageally fistulated animals. Total biomass and hand plucked samples generally contain lower levels of CP than samples from fistulated animals (Weir and Torell, 1959). Fistulated animals, however, may not be representative of the herd for which recommendations are to be made because of differences in sex, age, breed, and past environmental conditions. Another problem with this technique for practical use is that recommendations for supplementation could come too late to be of value by the time the samples are collected and processed.

A clinical test to estimate dietary CP which could be completed rapidly and relatively inexpensively would be invaluable to practical

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Literature citations and style of this thesis follow the style of the Journal of Animal Science.

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decision making for nutrition of range animals. The test should be able to predict quantitatively deficiencies in CP intake which could be corrected by supplementation. The method should be applicable to representative animals from the herd and should not have adverse effects on the test animals.

This study will evaluate indirect methods for predicting dietary CP intake. The objectives of the study were to monitor changes in certain constituents of blood, rumen liquor, and feces, as the CP content of the diet was changed. Specific objectives were to determine:

1. Blood urea nitrogen and serum protein levels as affected by dietary crude protein.
2. Total nitrogen, protein nitrogen, and microbial protein nitrogen levels in rumen liquor as affected by dietary crude protein.
3. Fecal nitrogen levels as affected by dietary crude protein.
4. Regression equations to predict dietary crude protein content from measurements of blood, rumen liquor, and/or fecal constituents.
5. The effects of various methods of collecting, storing, and processing on the total nitrogen content of rumen liquor samples from intact cows.

LITERATURE REVIEW

Crude protein is a basic and common indicator of the quality of a ruminant's diet. Protein requirements of ruminants varies with sex, age, and physiological development (NRC, 1976). Proteins in natural diets provide the major portion of the nitrogen used by rumen microbes and the host. The proteins are both of soluble and insoluble nature (Hungate, 1966). Ammonia is the chief end product of protein degradation in the rumen. Part of the ammonia is absorbed via the portal system and converted to urea in the liver, while some is used in the synthesis of microbial protein (Abou Akkada and Osman, 1967).

Urea in the blood system is not regulated by any mechanisms other than rate of formation in the urea cycle and excretion. Recycling occurs when urea is re-introduced into the rumen from saliva (Church, 1969), and directly into the rumen from peripheral blood vessels (Houpt, 1959). Houpt (1959) states that on low-protein diets, significant quantities of urea are reclaimed in the rumen and converted to microbial protein. Hogan (1973) found that blood urea nitrogen (BUN) movement into the rumen remains constant at BUN levels above 12 mg/100 ml in cattle. The body is indifferent to urea both chemically and physically. Urea is strictly an end product of protein metabolism and participates in no further chemical reactions. Urea concentrations in the body are determined not only by how much goes out via the kidneys, but also by how much goes to the kidneys from the liver (Abou Akkada and Osman, 1967). Differences in the rate of urea formation arise mainly from differences in the quantities of proteins the animals take

in as food (Addis et al., 1947). Thus, differences in the rate of urea formation are related to the nitrogen content of the feed (Blowey et al., 1973; Hewett, 1974; and Lewis, 1962). Abou Akkada and Osman (1967) concluded that changes of BUN are not a reflection of overall N intake but found BUN to predict N retention (a function of excretion and recycling).

BUN has been used primarily for diagnostic tests to follow BUN levels of dairy herds during the winter. Several researchers have found high correlations of BUN concentration with the protein content of the dairy cows diet. Prewitt et al. (1971) found a high correlation ($r = .986$) between dietary N and BUN concentrations. He also reasoned that this may be a measure of efficiency of protein utilization in ruminants. Hewett et al. (1975) concluded that BUN values appeared to be directly affected by protein level differences. BUN appeared to be a fairly distinct reflection of protein intake when energy and roughage remained constant. Blowey et al. (1973) monitored several dairy herds and found that changes in the mean BUN concentrations could be associated with changes in the diet, and with variations in production among the various farms. He suggested that BUN concentration changes could be useful in monitoring protein intake.

Manston et al. (1975) concluded that BUN concentrations were reflective of dietary protein intake and that concentrations of albumin, hemoglobin, and packed cell volume during lactation were affected by longterm protein status. Blood profiles of individual animals are of limited value, except where deviations are small. Herd means, when corrected for stage of lactation and age, are probably

reliable estimators for between herd comparisons (Hewett, 1974). Hewett (1974) also found that existence of a consistent seasonal effect is doubtful, and that differences in blood profiles are more related to current feedstuff quality and variations in sampling and analytical procedures. Large between herd differences were also found and results provided definite evidence that herd environment has a major effect on blood profile. Feeding was the most important environmental factor.

Similar results are reported from studies involving sheep. Muir et al. (1972) and Torell et al. (1974) found high correlations between BUN and dietary nitrogen. Preston et al. (1965) indicated that a BUN level in excess of 10 mg/100 ml in wethers would indicate adequate protein intake.

The use of BUN to predict the protein intake, and explain dietary-N differences have been demonstrated. BUN is a simple test to complete, but variability among individuals restricts its use (Torell et al., 1974). Lewis (1957), however, states that "such values are very difficult to determine...and impossible as a routine test". BUN can be a sensitive indicator of dietary protein levels, but reference samples must be taken when the herd is in good condition (Payne et al., 1970). Procedural problems also exist that cause high variability among samples, such as time of sampling in relation to feeding, analytical procedures, and storage. Other methods must be developed to facilitate the determination of the CP content of the ruminant's diet.

Plant proteins are the most common source of nitrogen used by rumen microbes and the host. Proteins are attacked by the microbes and some

assimilated directly into microbial protein. However, some amino acids are fermented to provide energy, and ammonia is formed. Ammonia may be reconverted to amino acids if adequate levels of carbohydrate are available. Ammonia passes through the rumen wall into the portal vein system. Rumen microbes and partially digested feed proteins are passed to the lower gastrointestinal tract (GIT) for absorption. Microbial protein constitutes the major part of the ruminant's nitrogenous food, and is important for synthesis of tissue proteins (Hungate, 1966).

Relationships of dietary-N content with regard to certain rumen content values have been investigated. As the ruminant digests feeds high in N, ammonia appears rapidly and many of the rumen bacteria assimilate it in preference to amino acids (Hungate, 1966). Thus, the rumen ammonia level may be an indicator of the protein content of the diet. Time of sampling is critcal due to ammonia fluctuations after feeding (Lewis, 1957, 1962; Roffler and Satter, 1975; and Davis and Stallcup, 1964). Satter and Slyter (1974) studied the effect of ammonia concentration on ruminal microbes in vitro and found 90% of the maximum output of tungstic acid precipitable nitrogen when ammonia concentrations were 5 mg ammonia-N/100 ml rumen liquor. This corresponds to about a 13% CP diet (steers) and N in excess was wasted.

Ammonia concentration in the rumen is variable as to time of collection after feeding. Ammonia levels are about twice as high four hr after feeding than at feeding (Lewis, 1957; Davis and Stallcup, 1964). Lewis (1957) found that mean ruminal ammonia concentration was positively related to dietary CP and negatively related to total digestible nutrients. Roffler and Satter (1975) reported that the

requirement of 5 mg ammonia-N/100 ml rumen liquor was adequate to support maximal microbial protein production in dairy cows. Mean ruminal ammonia concentrations, though seemingly an adequate indicator of the dietary-N content, is a laborious and time consuming procedure. Movement of ammonia to the blood and recycling of urea and hydrolysis back to ammonia in the rumen causes diurnal variation of ammonia concentrations in the rumen. Sampling at various times after feeding from the same animals is necessary to account for ammonia fluctuation. Therefore, mean ammonia concentration has limited value for predicting dietary protein intake.

The modes of action of digestion of feed proteins are complicated. As feed proteins are digested, microbial protein is synthesized and the rates of the two processes are not easy to measure separately. Rumen microbes contain a large portion of the N available for digestion and absorption in the rumen and lower tract. Weller et al. (1962) found that 80% of the feed N in the rumen was converted to microbial-N and only plant materials digested to this extent escaped into the omasum. Hogan and Weston (1972) found that when bacteria were considered 64 - 70% of the organic matter was digested and passed from the rumen. Hogan (1973) found that 80% of the digesta passing from the rumen was likely to be from microorganisms. Weller et al. (1962) also found rapid conversion of plant-N in the rumen to microbial-N. Six hr after feeding, 93% of the plant-N was converted to microbial protein. The digestion process consists of two major periods. During the first 6 hr, soluble proteins and carbohydrates are removed from cell contents, and cellulose breakdown occurs during the second 6-hr period. After 12 hr,

ruminal digestion practically ceases (Hale, 1947).

Davis and Stallcup (1964) measured total nitrogen, protein nitrogen, and ammonia concentration of the rumen liquor of steers fed a cottonseed hull basal ration. Ammonia nitrogen increased dramatically after feeding, and declined rapidly as ammonia was absorbed through the rumen wall. Total-N and protein-N 10 hr after feeding reached a slightly declining plateau, and by 12 hr after feeding, approximated the pre-feeding level. Differences in the total-N and protein-N levels were observed between the cottonseed hulls (3.74%) and cottonseed hulls plus soybean meal (9.26%) which were reflective of dietary crude protein intake. Elliot et al. (1965) and Elliot and Topps (1964) found seasonal fluctuation in the total-N content of rumen liquor with dietary CP content differences. Lower total-N values were observed during the winter months and highest values during spring green-up.

Fecal material excreted by ruminants is comprised of undigested feed particles, residues of digestive juices, sloughed material from the intestinal tract, and microorganisms (Church, 1969). The N fraction of fecal material is designated as undigested feed N and metabolic fecal nitrogen (MFN). Analytical separation of feed N and MFN cannot easily be determined (Thomas, 1909). Two major methods to determine MFN have been used. A direct approach which measures fecal-N from "nitrogen free" diets (Ellis et al., 1956) and the use of an indirect method which extrapolates to zero fecal-N losses from diets containing varying amounts of nitrogen (Blaxter and Mitchell, 1948). At a given level of feed intake, the higher the digestibility of its protein, a higher proportion of MFN will be present in the total fecal-N fraction.

Lowering the N content of the feed results in a decrease in the undigested component of the feces (Maynard and Loosli, 1969).

Fecal-N has been studied and used in the past 20 yr to predict and explain changes in intake and digestibilities of forage ingested by grazing ruminants. Several workers have found that intake is related in a highly significant manner to fecal-N (Hutchinson, 1958; Arnold and Dudzinski, 1963; and Fels et al., 1959). Relationships between organic matter intake (OM) and the N content of feces is then related to the amount of N in the organic matter.

Hutchinson (1958) found a strong influence of N intake with fecal N which could arise from either an increase in undigested N or increased MFN or "most likely both". Fecal-N, regardless of the source, is related to the amount of N ingested. Fels et al. (1959) working with sheep related the percentage N content of the feed (Y) to the percentage N of the feces (X) with the relationships $Y = .928X - .66$ ($r^2 = .86$). This regression equation was very similar to an equation by Raymond (1948), which was $Y = .795X + .14$. Raymond (1948) stated that "fecal nitrogen from grazing sheep gives a figure for nitrogen in feed grazed which is probably more accurate than that obtained by analysis of cut herbage".

MATERIALS AND METHODS

Pen-feeding Study

A pen-feeding study was conducted from December 18, 1978 to February 23, 1979 at the sheep and goat research facility on the campus of Texas A&M University. Eight partially covered pens were used for individual feeding of the cattle. Four steers averaging 386 kg and four pregnant cows averaging 425 kg were used in the experiment. All eight animals were assigned a number (1 to 4, steers and 5 to 8, cows) and randomly paired as 8 and 3, 7 and 4, 6 and 1, and 5 and 2.

Prior to initiation of this study the animals were grazed on a Coastal bermudagrass (Cynodon dactylon (L.) Pers.) pasture. The study began with a 12-day period of adjustment to the ration. Ration quantity was initially increased to determine intake potential, then decreased to ensure total intake of the ration (table 1). Visual examination of the rumens indicated that no Coastal bermudagrass remained by the 10th day of the adjustment period and the animals were adjusted to the ration. For the last 2 days of the adjustment period the animals were fed to meet maintenance requirements. The experimental design was a 4 X 4 latin square with two groups (cows and steers), four rations, and four 14-day trials (table 2).

Samples of cottonseed hulls, cottonseed pellets, and molasses were collected during each trial. Dry matter (DM) and organic matter (OM) contents were determined. Total nitrogen was analyzed by the Kjeldahl method (AOAC, 1975). In vitro digestible organic matter (IVDOM) was determined by a 48 hr fermentation of samples with rumen liquor (Tilley

TABLE 1. RATION COMPONENTS (KG AS FED) AND REFUSALS PER DAY DURING THE ADJUSTMENT PERIOD

Days	Ration components			Total refusal per day[a]
	Cottonseed hulls	Cottonseed pellets	Molasses	
1 - 2	4.9	.45	0.0	3.6
3 - 5	5.9	.45	1.3	1.7
6 - 7	7.0	.54	1.6	0.1
8 - 10	9.1	.54	1.6	1.7
11 - 12	5.9	.54	1.3	0.0

[a]Mean of all eight animals

TABLE 2. RATIONS FED TO PAIRS OF ANIMALS DURING EACH TRIAL IN A LATIN SQUARE DESIGN

Pairs of animals			Trial			
Cows		Steers	I	II	III	IV
5	and	2	1	4	3	2
7	and	4	2	1	4	3
6	and	1	3	2	1	4
8	and	3	4	3	2	1

and Terry, 1963) followed by a neutral-detergent fiber (NDF) extraction (Van Soest and Wine, 1967). Samples analyzed for IVDOM were corrected by a standard feed sample with a known in vivo digestibility. Digestible energy (DE) of the rations was estimated by multiplying the corrected IVDOM (g) times 4000 kcal/kg organic matter.

Cottonseed hulls were used for the basal ration to provide a low quality roughage, and for uniformity of particle size and chemical qualities. Cottonseed pellets were added to the basal ration to obtain four levels of crude protein intake, and molasses was added to provide equal digestible energy intake in all four rations. Chemical composition of ration components is given in table 3. Quantities fed and chemical composition and percent of maintenance requirement for CP and DE of each ration fed during the study are presented in table 4.

Ration components were combined to provide varying levels of CP, and to at least meet the maintenance energy requirement. Above maintenance energy levels were included in the rations to provide the rumen microflora an adequate energy source. Thus, levels of various blood and rumen liquor components would not be affected by inadequate energy in the diet. The IVDOM of ration components was converted to DE, and then to metabolizable energy by multiplying by .82 (NRC, 1976). These values when expressed as Mcal/kg ME agreed very closely with NRC (1976) tabulated values for the ration components.

In vivo DOM analyses of ration components were estimated during the fourth trial. In vivo DOM was analyzed by a 6-day in vitro fermentation of feed and feces (Tilley and Terry, 1963). Indigestible neutral detergent fiber (INDF) content of feed and feces was determined

TABLE 3. CHEMICAL AND PHYSICAL CONSTITUENTS (%) OF FEED STUFFS USED TO FORMULATE EXPERIMENTAL RATIONS

	Dry matter	Organic matter	*In vitro* digestible organic matter	Nitrogen	Crude protein
Cottonseed hulls	90.20	96.72	47.04	.61	3.82
Cottonseed pellets	90.77	95.54	70.25	5.72	35.74
Sugarcane molasses	59.92	50.88	91.00[a]	1.58	9.88

[a]Value was taken from NAS-NRC (1976).

TABLE 4. RATION COMPONENTS (KG DM) AND THE CP (%) AND ESTIMATED DE (MCAL/KG) CONTENT OF THE RATION

Components	Ration			
	1	2	3	4
Cottonseed hulls	6.79	6.79	6.79	6.79
Cottonseed pellets	0.00	0.25	0.40	0.78
Sugarcane molasses	0.54	0.41	0.25	0.08
Total DM fed	7.33	7.45	7.55	7.65
CP (% of maint.)	4.27 (71)	5.19 (87)	6.19 (103)	7.14 (119)
DE[a] (% of maint.[b])	2.53 (127)	2.63 (132)	2.80 (140)	2.79 (139)

[a]Estimated by IVDOM.

[b]Nutrient concentration in ration dry matter.

on the residue (Van Soest and Wine, 1967 and *in vivo* DOM calculated for apparent digestibility by the formula:

$$\text{In vivo DOM} = 100 - \left(100 \times \frac{\%\ \text{INDF in feed OM}}{\%\ \text{INDF in feces OM}}\right)$$

Digestibilities were also estimated by the use of radioactive material (^{141}Ce) and Cr-DTPA introduced into the rumen and collected in the feces. Rates of passage of ingesta, rumen volume, GIT volume, and fecal productions were also estimated (Loza, 1979).

Blood and rumen liquor samples were collected on the 1st, 10th, and 12th day of the adjustment period, and on the 10th, 12th, and 14th day of each trial. Blood samples were taken by jugular puncture into 10 ml evacuated tubes 20-hr after feeding during all four trials. Blood samples were also collected at 6-hr after feeding on the 9th, 11th, and 13th day, and 9-hr after feeding on the 13th day during the final two trials. Samples of rumen contents were collected at 20-hr after feeding via the rumen fistula. A one inch PVC pipe was inserted into the ventral anterior portion of the rumen, and samples withdrawn and placed in plastic containers.

Blood samples were taken to the laboratory immediately after collection and centrifuged. Blood serum was decanted and stored at 4° C until analyzed. Blood (serum) urea nitrogen (BUN) was determined by fearon condensation of urea with diacetyl monoxime in acid medium (Harleco, 1975). Serum protein was estimated with a hand refractometer (Barry *et al.*, 1960).

Rumen samples were taken to the laboratory immediately after collection and stored at 4° C until analyzed. Rumen samples were

squeezed through four layers of cheesecloth and the squeezed rumen liquor retained for analysis. Rumen liquor was separated into two aliquots. One was analyzed for total-N and protein nitrogen (protein-N). Total-N was analyzed by the Kjeldahl method. Protein-N was analyzed by a modification (R. E. Lichtenwalner, personal communication) of a method described by Folin and Wu (1919). Ten ml of the rumen liquor were pipetted into a pre-weighed centrifuge tube and 1 ml of a 10% (w/v) sulfosalycylic-acid solution was added. The tubes were centrifuged at 10,000 x g for 5 minutes. The supernatent was discarded, and the tubes and residue were oven dried (90° C) and weighed. The residue was expressed as mg protein-N/100 ml rumen liquor by dividing the mg of precipitated protein by 6.25. The second aliquot was further separated by low speed centrifugation (500 x g) to remove feed particles while retaining the microbial population in the supernatant. The supernatant was analyzed for protein-N by the method previously described and designated as mg microbial protein-N/100 ml rumen liquor.

Fresh fecal samples were collected at 10 am on the last day of each trial. Samples were frozen, freeze dried, and ground to pass a 1 mm screen. Fecal nitrogen (fecal-N) was analyzed by the Kjeldahl method.

The data were analyzed statistically using the model of Cochran et al. (1941). The analysis of variance table is presented in Appendix table 1. Rumen liquor and fecal components of cows and steers were analyzed separately where appropriate using the standard latin square design (Appendix table 2). Differences were said to be statistically

significant if the probability of a Type I error was less than or equal to five percent. Duncan's (1955) mean separation test was used to detect significant differences.

Sample Storage and Treatment Study

Rumen content samples were taken from two rumen fistulated lactating cows grazing on fresh oat pasture in May 1979. Experimental design was a 2 X 2 X 5 factorial experiment arranged as a randomized complete block with two replications. Treatments consisted of squeezed and non-squeezed rumen liquor, addition or absence of mercuric chloride ($HgCl_2$) and five time intervals (2-,4-,6-,8-, and 14-days) after collection until analysis. Collections were taken in the same manner as in the pen-feeding study. Samples were either placed in plastic containers or squeezed immediately through four layers of cheesecloth and decanted into vials. Five ml of $HgCl_2$ (10% w/v) were added to one-half of both squeezed and non-squeezed rumen liquor samples, and shaken to ensure mixture of the $HgCl_2$. Samples were then taken to the laboratory and stored at 4^{o} C until analyzed. Non-squeezed samples were squeezed through four layers of cheesecloth prior to analysis. Samples were analyzed for total-N by the Kjeldahl method. Data were tested by analysis of variance procedures (Appendix table 2) with blocks and squeezed treatment tested by Error A and time by Error B. Differences due to time of analysis after collection were said to be statistically significant if the probability of a Type IV error was less than or equal to five percent. Means were separated statistically by the use of Duncan's (1955) new multiple range test..

RESULTS AND DISCUSSION

Pen-feeding Study

Ration Digestibility

The in vivo digestibility analyses of ration components during the fourth trial resulted in lower energy values of the diets than anticipated. In vivo DOM for the 4.27, 5.19, 6.19, and 7.14% CP rations were 15.6, 32.0, 35.9, and 41.4% respectively. Digestible energy values expressed as a percent of maintenance for the 4.27, 5.19, 6.19, and 7.14% CP rations were 28, 58, 65, and 75% respectively (Appendix table 4) (Loza, 1979). The 4.27% CP ration contained approximately twice the DM volume in the GIT, and 20% greater fecal production than the other rations. Reduced DOM, and hence the high DM volume of the GIT, rumen, and feces reflected reduced microbial action upon the OM in the rumen, due primarily to the size and structure of the cottonseed hulls (Loza, 1979).

Elliot et al. (1965) found increased rumen activity on either protein-rich or protein and carbohydrate-rich concentrates. Carbohydrate-rich supplements alone did not increase rumen activity. This would explain the low DOM of the 4.27% CP ration fed in this study. The addition of N to the higher CP rations stimulated microbial activity resulting in a two-fold increase in OM digestibility.

Rations were totally consumed by all animals after four days on a new trial, except the 4.27% CP ration during trials II and III. Daily refusals of the low protein ration averaged .13 kg DM (steer #4), .75 kg

DM (cow #6), and .23 kg DM (steer #1) on trials II and III, respectively. Refusals only on the low protein ration can be explained by the low digestibility and high GIT volume as observed during trial IV.

Serum Protein

Serum protein levels in the blood reflect the long term protein status and health of the individual animal, and not current dietary CP (Camp, 1979). Serum protein was not significantly affected by the CP content of the diets in this study (figure 1). However, overall means of serum protein levels for cows (7.06 mg/100 ml serum) were significantly higher than for steers (6.71 mg/100 ml serum). Normal serum protein levels of cattle are approximately 7.6 mg/100 ml serum (Berrier, 1967). The lower values obtained in this study are probably due to the low protein content of diets from early winter grazing of the Coastal bermudagrass. The elevated levels of serum protein in the cows were a result of stage of pregnancy. Serum protein was lower for cows (6.64 mg/100 ml serum) during the final trial, which probably resulted from a cumulative effect of the low protein and low digestibility of the rations. Serum protein values of individual animals within sex also differed significantly.

Serum protein was monitored to be sure that no long-term protein deficiency was imposed on the animals. A trend was evident for higher serum protein levels on the 4.27, and 5.19% CP diets indicating that tissue proteins were possibly being mobilized into the blood system.

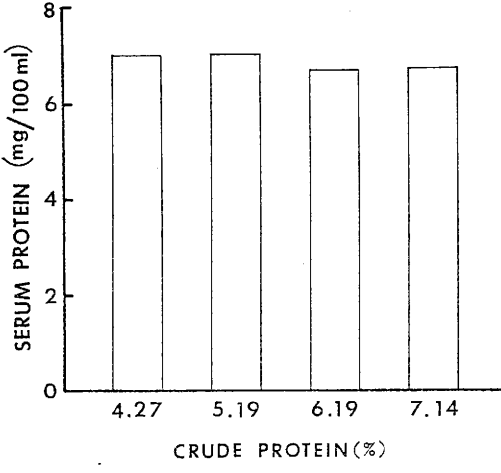

Figure 1. Mean blood serum protein was not affected by crude protein content of the diets fed.

Blood Urea Nitrogen

BUN concentrations in the serum produced minor treatment effects, but did not differ significantly as the CP content of the diet was increased (figure 2).

Strong linear relationships between dietary CP and BUN levels have been reported by Prewitt et al. (1971) with dairy cattle, and Muir et al. (1972) and Preston et al. (1965) with sheep. Linear relationships exist on diets with CP contents at maintenance and higher since almost all of the ammonia is converted to urea (Lewis, 1962). However, linear relationships do not appear to be as strong on sub-maintenance CP diets. An obvious "tailing effect" begins to appear below the maintenance requirement for CP, indicating N conservation, and possible mobilization from tissue. Low N intake results in decreased excretion and increased recycling via the saliva and transfer across the rumen wall independent of the urea nitrogen level (Schmidt-Neilson et al., 1957). N retention has been shown in both large and small ruminants by regulation of urea excretion in the kidneys (Schmidt-Neilson and Osaki, 1958; and Somers, 1961). N conservation by limiting urea excretion would retain higher levels of BUN in the blood system accounting for the non-linear response of BUN to sub-maintenance CP diets.

Blood samples analyzed following the first two trials produced levels far below the 10 to 12 mg/100 ml serum estimate by Prewitt et al. (1971) for diets containing maintenance CP contents (dairy cows). BUN values ranged between .6 and 8.8 mg/100 ml serum during this study. During the last two trials, blood samples were collected at 6- and 9-hr

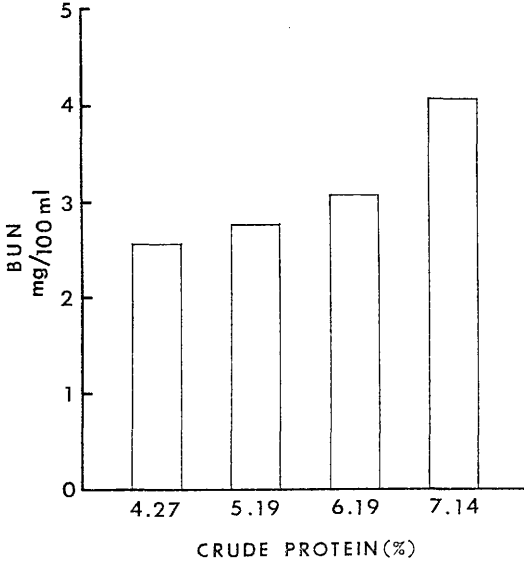

Figure 2. Blood urea nitrogen as affected by the crude protein content of four rations.

in addition to the 20-hr post-feeding collection. Samples collected at 6- and 9-hr after feeding were not different from the 20-hr collections. BUN levels expressed as a function of gms CP/$W^{.75}$, agreed with levels obtained by Preston et al. (1965) for sheep on low protein diets.

BUN was the only component analyzed which differed significantly among trials. Trial I was significantly higher than trials III and IV. BUN samples for trials I and II were analyzed within 3 weeks after collection, and trials III and IV were analyzed after the samples had been stored for 8 weeks due to delays of shipping reagents. The BUN levels could have decreased during storage due to bacterial degradation, thus resulting in lower values.

Protein Nitrogen

The protein-N content of squeezed rumen liquor was highly correlated (r^2 = .997) with the percent CP content of the ration (figure 3). Trials, sex X trials, and sex X treatment effects were not significant for the protein-N content of the rumen liquor. Sex and animal X sex were significantly different. Protein-N values ranged from 13.3 to 55.6 mg/100 ml of rumen liquor. Cows and steers were significantly different, and the treatment means and mean separations are presented in table 5. Mean separation of the protein-N values for steers produced significant differences between the 7.14 and 4.27% CP ration, and between the two highest and two lowest CP diets for cows. The treatment means for cows separated at approximately the maintenance requirement for crude protein. A protein-N value of less than 28 mg/100 ml of rumen liquor would represent a diet deficient in crude protein.

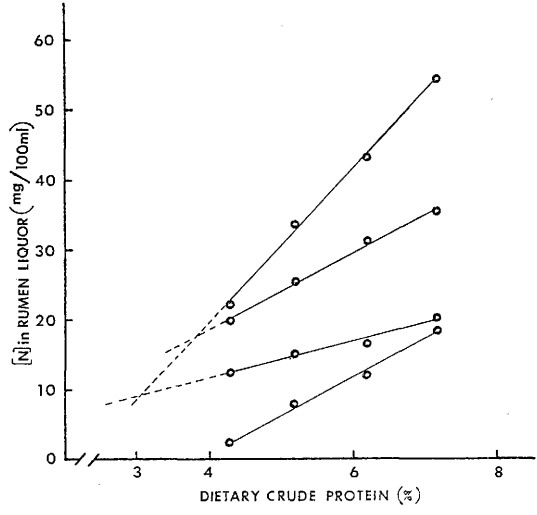

Figure 3. Mean content (mg/100 ml) of the total nitrogen, protein nitrogen, microbial protein nitrogen, and non-protein nitrogen fractions in the rumen liquor of cattle fed rations containing four different levels of crude protein.

TABLE 5. MEAN CONTENT (MG/100 ML) OF THE PROTEIN NITROGEN FRACTION IN THE RUMEN LIQUOR OF COWS AND STEERS FED RATIONS CONTAINING FOUR DIFFERENT LEVELS (%) OF CRUDE PROTEIN (CP)

	Rumen protein nitrogen	
Dietary CP	Cows	Steers
4.27	19.68[a]	20.17[a]
5.19	21.03[a]	30.08[ab]
6.19	28.89[b]	33.40[ab]
7.14	32.47[b]	38.63[b]

[ab]Means in the same column followed by the same letter do not differ significantly ($p < .05$).

Regression equations developed were Y = 0.249X + .15 for steers and Y = .0313X + .11 for cows, where Y = dietary N percent, and X = the protein-N content (mg/100 ml rumen liquor). Data from Davis and Stallcup (1964) were fitted into the regression equation for steers. Rations containing 3.14 and 10.85% CP were predicted for the 3.54 and 9.25% CP rations fed in their study respectively. The lower CP ration was slightly under estimated which is at approximately the range where the protein-N values would begin to become non-linear. The higher predictive value overestimated the actual CP percentage by only 1.5%.

Protein-N of the rumen liquor consists of partially digested feed particles and microbes (Hungate, 1966). The protein-N content of the squeezed rumen liquor was suitable for estimating the CP content of the diet between 3.5 and 10.0% crude protein.

Microbial Protein Nitrogen

Mean levels of microbial protein nitrogen of steers and cows across trials were highly correlated (r^2 = .976) with the CP content of the diet (figure 3, p. 25). The 7.14% CP diet was significantly different from all others, and the 6.19 and 4.27% CP diets were significantly different.

Significant differences were observed between sexes and treatments (table 6). Linear regressions of dietary CP content on the microbial protein-N in the rumen liquor of cows and steers were highly significant with r^2 = .945 and .944, respectively. Prediction equations computed for the means were Y = .075X - .20 for cows, and Y = 0.51X + .03 for steers (Y = % N in the diet, and X = microbial protein-N). Slopes of

TABLE 6. MEAN CONTENT (MG/100 ML) OF THE MICROBIAL PROTEIN NITROGEN FRACTION IN THE RUMEN LIQUOR OF COWS AND STEERS FED RATIONS CONTAINING FOUR DIFFERENT LEVELS (%) OF CRUDE PROTEIN (CP)

	Rumen microbial protein nitrogen	
Dietary CP	Cows	Steers
4.27	11.67^{a}	13.11^{a}
5.19	14.13^{ab}	15.87^{a}
6.19	15.33^{bc}	17.76^{ab}
7.14	17.96^{c}	22.19^{b}

^{abc}Means in the same column followed by the same letter do not differ significantly ($p < .05$).

the regression lines did not differ significantly. A microbial protein-N value of less than 15 mg/100 ml rumen liquor for cows and 17 mg/100 ml for steers would indicate inadequate levels of crude protein.

One hypothesis of this study was that the N content of the microorganisms would closely represent changes in dietary CP at 12 hr post feeding. Studies by Weller et al. (1962), and Hogan (1973) have found that 50 - 80% of the N indigesta passing from the rumen was from microorganisms. Microbial protein-N, which is in a constant state of production, and passage from the rumen into the lower GIT, is sensitive to the levels of N supplied to the rumen on a daily basis.

Microbial protein-N was found to be responsive to the CP content of the diet. The analysis procedure is more difficult to complete and, in view of other results in this study, not suitable for rapid determination to provide recommendations for supplementation.

Total Nitrogen

Total-N in rumen liquor was highly correlated with the CP content of the diet (figure 3, p. 25). The linear regression of total-N on diet CP gave the equation $Y = 10.97X - 24.24$, where Y = CP (%) of the diet and X = total-N content of the rumen liquor. The slope of this line was greater than the slope of protein-N, and microbial protein-N. Differences in slopes were due to reduction of NPN as the CP content of the diets was reduced.

Total-N was significantly affected by sex, sex X animals, and treatment. Treatment means for cows and steers were analyzed separately (table 7). Linear regressions of total-N (X) and percent N

TABLE 7. MEAN CONTENT (MG/100 ML) OF THE TOTAL NITROGEN FRACTION IN THE RUMEN LIQUOR OF COWS AND STEERS FED RATIONS CONTAINING FOUR DIFFERENT LEVELS (%) OF CRUDE PROTEIN (CP)

	Rumen liquor total nitrogen	
Dietary CP	Cows	Steers
4.27	21.41[a]	22.93[a]
5.19	28.02[b]	39.24[ab]
6.19	39.98[c]	46.26[b]
7.14	48.79[d]	59.57[b]

[abcd]Means in the same column followed by the same letter do not differ significantly ($p < .05$).

in the ration (Y) were calculated for steers (Y = .013X + .37) and cows (Y = 0.16X + .35). Treatment means of cows differed significantly. Total-N content of less than 36 mg/100 ml rumen liquor in cows would indicate an inadequate level of crude protein. A value of less than 45 mg/100 ml rumen liquor would indicate an inadequate level of CP for steers. Total-N was the rumen liquor most highly correlated with the percent CP in the diet. However, collecting rumen liquor samples from intact animals requires the equipment and expertise of trained individuals.

The total-N content of the rumen liquor was the most rapid rumen liquor component to analyze and produced significant differences among all treatment means. Squeezed rumen liquor contains N components such as ammoni-N, amino acids, partially digested feed residues, and micro-organisms. Prior to this study, total-N was expected to be the most variable and least defined component in the rumen liquor, mainly due to fluctuations of ammonia. The total-N values of total rumen contents have been used sucessfully to monitor the CP content of white-tailed deer (Odocoilus virginianus) diets (R. M. Robinson, personal communication). Actually, total-N should be the best rumen liquor component to relate to the CP content of the diet, because it combines all of the processes of rumination. As a feed is ingested, proteins are hydrolyzed, ammonia and partially digested proteins are produced, and a N source for micro-organisms made available. Total-N would therefore be related to digestibility, microorganism production, and rate of passage.

Rumen liquor components (total-N, protein-N, and microbial protein-N) increased linearly in their response to increased dietary CP

(figure 3). BUN and serum protein were not well correlated ($r^2 < .6$) with the dietary CP percentage; whereas, all rumen liquor components were highly correlated ($r^2 > .97$). The relationship between rumen liquor components and dietary CP was linear throughout the range of rations fed (4.27 to 7.14% CP). Rumen liquor components appeared more promising for predicting dietary CP than blood components for cattle receiving maintenance or sub-maintenance diets.

Digestibility of the ration did not affect the linear relationship and thus the values of the three rumen liquor components. The low digestibility of the 4.27% CP ration (15% in vivo DOM) did not adversly affect the rumen liquor components when compared to the other three rations (>30% in vivo DOM). The lower digestibility values were reflected in threefold reduction of non-protein nitrogen (NPN) (NPN = total-N - protein-N) (figure 3, p. 25). The rumen liquor of the 7.14% CP ration contained approximately 34% NPN compared to 11% NPN in the 4.27% CP ration. Moir and Harris (1962) found a decrease in bacteria, and ammonia nitrogen in sheep with decreased N in feed. They found that at their lowest CP level (2.0%) there was frequently no measurable ammonia nitrogen present in the rumen liquor. N available for assimilation or transport across the rumen wall was reduced as the CP content and digestibility of the ration decreased. Microbial protein-N was then reduced with corresponding reductions of precipitable protein-N in the rumen liquor.

A curvilinear relationship exists between the CP concentration and the apparent CP digestion coefficient. The digestion coefficient decreases to zero at approximately 3% CP as the CP content of the feed

decreased. The amount of apparently digestible protein in the feed is related as a linear function of the CP content of the feed (van Niekerk et al., 1967). At approximately 3% CP in the diet, digestibility of that CP reaches zero. The linear relationship of the rumen liquor components in this study could not be extended below 3.8% CP, since predicted total-N would be less than the protein-N values below 3.8% CP, and less than the microbial protein-N values below 3.1% crude protein. This supports the work by van Niekerk et al. (1967). At approximately 3.1% CP, the apparent digestibility would be near zero, resulting in a slight decrease in the N fraction of the rumen liquor. A slightly declining plateau would be reached as the microorganisms are introduced into the lower GIT until the death of the animal. The linear relationship is then bound to above 3.1% CP in the diet to at least approximately 10% crude protein. Total-N must begin to level out with protein-N and microbial protein-N following at some undefined point at CP levels below 3.8%.

Fecal Nitrogen

Fecal-N was correlated with the CP content of the diet in this study (figure 4). There were significant differences among treatments and between sexes. Animals within sex and trials were not significantly different. Since cows and steers differed significantly, they were also analyzed separately as two latin squares. There were no significant differences between animals and trials in either sex. Percent N in the feces of steers was significantly higher than cows (1.5 and 1.3%, respectively).

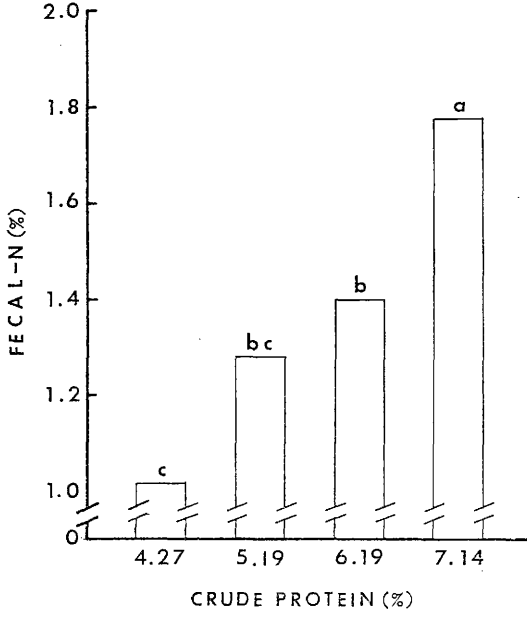

Figure 4. Mean content (%) of the fecal nitrogen component in the organic matter of feces of cattle fed rations containing four different levels of crude protein. Means with different superscripts are significantly different ($p < .05$).

Treatment means and mean separations for cows and steers are listed in table 8. Prediction equations for the N content of the diet (Y) from N content of the feces (X) were developed for steers ($Y = .662X - .09$, $r^2 = .91$) and for cows ($Y = .789X - .11$, $r^2 = .89$). The prediction equation for cows had a slope almost identical to the equation for sheep developed by Raymond (1948) of $Y = .795X - .14$. The points of intercept were different, and the prediction of dietary-N was higher for sheep than for cattle which reflects the higher N requirement of the sheep. The close agreement of equations developed in this study with earlier equations (Raymond, 1948; and Fels et al., 1958) indicated a good potential for using fecal-N to predict the CP content of a grazing ruminant.

The N content percentage of fecal material has been related directly (Raymond, 1948; and Fels et al., 1959) and indirectly (Hutchinson, 1958; and Arnold and Dudzinski, 1963) to the N content of the feed.

Fecal-N had several unique characteristics as a predictor of dietary crude protein. There were no significant differences in the percent fecal-N values of animals within sex in this study. Low variability between animals will reduce the number of samples needed to adequately predict the percentage of CP in diets of grazing ruminants. Samples can be collected by the rancher from fresh excreta in the pasture without disturbing the animals, and sent to a laboratory for rapid analysis. This would reduce costs as compared to having a veterinarian collect rumen liquor samples. Fecal samples could be collected during suspected periods of nutritional stress and

TABLE 8. MEAN CONTENT (%) OF THE NITROGEN IN THE FECES OF COWS AND STEERS FED RATIONS CONTAINING FOUR DIFFERENT LEVELS OF CRUDE PROTEIN (CP)

	Fecal nitrogen	
Dietary nitrogen	Cows	Steers
.68	1.07[a]	1.24[a]
.83	1.21[b]	1.36[b]
.99	1.28[bc]	1.51[b]
1.14	1.63[c]	1.90[b]

[abc]Means in the same column followed by the same letter do not differ significantly ($p < .05$).

supplements fed only when needed. This should reduce costs and increase effectiveness of supplementation.

Animal Variation

Variation among animals is an inherent factor in any biological system. Significant differences between animals within sex occured in values of serum protein, protein-N, and total-N. Significant differences between cows and steers existed in mean values of serum protein, all rumen liquor values (figure 5), and fecal-N. Mean values in all rumen components and percent fecal-N were significantly lower for cows than steers.

Cows had higher mean serum protein values than steers. The cows were in late pregnancy (third tri-mester) during the experiment, and were mobilizing body proteins into the blood system possibly for transport to the developing fetus (B. J. Camp, personal communication). Serum proteins, such as albumin which comprise 53 to 67% of the total serum proteins, and beta-globulins are elevated with total serum volume by various factors such as pregnancy and lactation (Berrier, 1967).

A decreased ability to digest the DM probably resulted in less N present in the rumen liquor of the cows. Cows were not able to digest DM in the rumen as well as steers, and thus rate of passage was decreased (Appendix table 5). Steers had a greater digestive ability, and more rapid rate of DM passage, resulting in higher N levels in the rumen liquor. GIT volume (DM) was approximately 14% greater for cows; and total-N, protein-N, and microbial protein-N content of the rumen liquor was approximately 14% less than in the rumen liquor of the

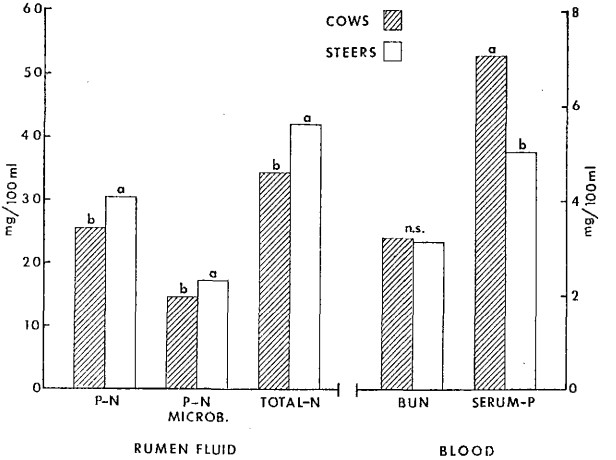

Figure 5. Mean content of rumen liquor and blood components of cows and steers averaged across trials and treatments. Means with different superscripts are significantly different ($p < .05$).

steers. DM digested seemed to be directly related to the total-N, protein-N, and microbial protein-N content of the rumen liquor. DM content of the rumen liquor was similar for cows (1.854 g/100 ml) and for steers (1.864 g/ml).

Variation of total-N and protein-N levels in the rumen liquor between animals was greatly reduced when the 4.27% CP ration was fed. Means and standard deviations of each treatment are listed in Appendix table 6. R. M. Robinson (personal communication) found a reduction in the variation of total-N (total rumen content) as the CP content of the diet of white-tailed deer decreased below their requirement. The CP content of the ration did not affect variations of microbial protein-N among animals as greatly as for total-N or protein-N.

Rumen Liquor Treatment and Storage Study

Total-N did not differ significantly in rumen liquor samples from the two animals. Neither squeezing nor the addition of $HgCl_2$ had any significant effect on the total-N content of the rumen liquor. Time delay prior to analysis significantly affected total-N, and the time X method interaction was significant. Means and mean separations of time delay and method for the corresponding total-N content are listed in table 9. The 2-, and 14-day analyses had the lowest total-N values. Samples analyzed the 2-day were not distilled as long during the Kjeldahl procedure as the other analyses, and since the total-N content was very high, complete conversion to ammonia might not have been accomplished. The total-N content was not decreased greatly during the 14-day analysis from the samples that were squeezed at collection.

TABLE 9. TOTAL NITROGEN (MG/100 ML) IN THE RUMEN LIQUOR OF TWO COWS AS AFFECTED BY METHOD OF SAMPLE PREPARATION AND TIME DELAY OF ANALYSIS AFTER COLLECTION

	Time delay (days)					
Method	2	4	6	8	14	Mean
Squeezed through cheesecloth immediately	132.1	154.0	143.6	142.2	141.6	142.4
Not squeezed through cheesecloth until day of analysis	133.2	131.5	147.6	152.2	126.2	138.1
Mean	132.6[a]	142.0[ab]	145.6[b]	146.8[b]	134.5[ab]	

[ab]Means in the same row followed by the same letter do not differ significantly ($p < .05$).

Samples that were not squeezed immediately decreased approximately 20% from the 8-day analysis to the 14-day analysis, however, the 14-day analysis was only approximately 5% less than the 2-day analysis.

Rumen liquor samples should be squeezed through cheesecloth immediately following collection and stored at 4^{o} C for up to 2 weeks. The addition of $HgCl_2$ is not necessary, and it is assumed that the microbial population was killed by the 4^{o} C refrigeration, or that changes in the microbial population did not adversely affect the total-N in the rumen liquor.

SUMMARY AND CONCLUSIONS

A pen-feeding study was conducted in 1979 to evaluate indirect methods for predicting the percentage of CP in diets of grazing animals to facilitate recommendations for supplementation. Serum protein and BUN in blood, total-N, protein-N, microbial protein-N in rumen liquor, and fecal-N levels were determined from four cows and four steers fed four different levels of CP in the diet. A 4 X 4 replicated latin square design with four trials and four different rations was used. Cottonseed hulls constituted the basal ration, with molasses and cottonseed pellets added to five 4.27, 5.19, 6.19 and 7.14% rations with approximately equal levels of digestible energy.

Significant differences between cows and steers existed for mean values of serum protein, all rumen liquor values, and fecal-N. The cows were in late pregnancy, resulting in higher serum protein values than the steers. Lower N values in the rumen liquor of the cows were probably due to a reduced ability to digest available OM which yielded higher DM fill, and a decrease in the rate of passage. An interesting relationship was found between the GIT volume (DM) (14% greater in the cows than steers), and all the rumen liquor components (14% less in the cows than steers). DM digested was thought to be directly related to the rumen liquor components.

Blood components were ineffective for predicting dietary crude protein. Serum protein levels declined to levels slightly below the average for cattle by the end of the fourth trial. BUN was responsive to CP in the diets, but not in a linear fashion. BUN levels decreased

only slightly at CP levels below the maintenance requirements due to increased N retention. BUN is not a sensitive indicator of the CP content of the ruminant's diet at sub-maintenance levels of crude protein, however BUN can be useful to predict maintenance and above levels of crude protein.

Total-N, protein-N, and microbial protein-N in the rumen liquor were correlated to the CP content of the diet. Close linear relationships were found for rumen liquor components and dietary crude protein. Linear regression equations were developed separately for cows and steers for each rumen liquor component. Rumen liquor components are sensitive indicators of the CP content of the diet of ruminants in the range from 3.5% to 10% crude protein. Linear relationships existed on sub-maintenance feeding levels of crude protein. Total-N has the highest correlation with the CP content of the diet and is easily determined. Rumen liquor samples can be collected in the field, squeezed through cheesecloth, stored at 4^{o} C, and analyzed within 14 days. The microbial population does not need to be killed with $HgCl_2$ during the collection process.

Total-N was the best rumen liquor component to predict dietary crude protein. However, there are several disadvantages for routine use of total-N: 1. A group of animals must be penned for at least 12 hr prior to collection. 2. A trained individual must take the samples via stomach tube. 3. The cost of sample collection may be high. Collection of rumen liquor and analysis of total-N may be a useful tool to predict dietary crude protein for animals used in grazing research.

Fecal-N has several unique characteristics for practical use to predict the CP content of a grazing animal's diet. Variation among animals was the lowest of any method used in this study. Thus, fewer samples are needed to obtain a representative sample from a herd. Samples may be collected by laymen (ranchers, etc.) and sent directly to a laboratory for rapid analysis at relatively low cost. Analyses can be completed in a short time to facilitate management planning for supplementation.

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APPENDIX TABLE 1. ANALYSIS OF VARIANCE MODEL USED TO ANALYZE BLOOD SERUM PROTEIN, BLOOD UREA NITROGEN, PROTEIN NITROGEN, MICROBIAL PROTEIN NITROGEN, AND TOTAL NITROGEN IN THE RUMEN LIQUOR IN A 4 X 4 LATIN SQUARE DESIGN WITH TWO GROUPS (COWS AND STEERS)

Source of variation	d.f.	Appropriate denominator for F test
Total	31	
Sex	1	Error
Animal within sex	6	Error
Trial	3	Error
Treatment	3	Error
Sex X treatment	3	Error
Sex X trial	3	Error
Error	12	

APPENDIX TABLE 2. ANALYSIS OF VARIANCE MODEL USED TO ANALYZE BLOOD SERUM PROTEIN, PROTEIN NITROGEN, MICROBIAL PROTEIN NITROGEN, TOTAL NITROGEN IN THE RUMEN LIQUOR, AND FECAL NITROGEN IN A 4 X 4 LATIN SQUARE DESIGN FOR COWS AND STEERS SEPARATELY

Source of variation	d.f.	Appropriate denominator for F test
Total	15	
Animal	3	Error
Trial	3	Error
Treatment	3	Error
Error	6	

APPENDIX TABLE 3. ANALYSIS OF VARIANCE MODEL FOR A 2 X 2 X 5 FACTORIAL EXPERIMENT FOR THE TOTAL NITROGEN CONTENT OF TWO COWS FOR THE SAMPLE STORAGE AND TREATMENT STUDY

Source of variation	d.f.	Appropriate denominator for F test
Total	76	
Block	1	Error a
Method	1	Error a
Additive	1	Error a
Method X additive	1	Error a
Error a	3	
Time	3	Error b
Method X time	4	Error b
Additive X time	4	Error b
Method X additive X time	4	Error b
Error b	16	
Residual	38	

APPENDIX TABLE 4. DIGESTIBILITY, RATE OF PASSAGE, DRY MATTER (DM) FILLS, AND FECAL OUTPUT DURING TRIAL IV[a]

	Ration			
	I	II	III	IV
In vivo DOM				
6-day IVDOM (%)[b]	15.6	32.0	35.9	41.1
Rate of passage[c]	0.018	0.064	0.038	0.033
Fecal output (kg)[c]	7.5	5.5	5.6	5.0
GIT volumes (kg)[c]	17.5	3.5	6.1	7.4
Rumen fill (kg)[c]	11.6	6.6	9.3	12.9

[a] Taken from Loza, 1979.

[b] Estimated from ^{141}Ce.

[c] Estimated by INDF analysis.

APPENDIX TABLE 5. DIGESTIBILITY, RATE OF PASSAGE, DRY MATTER (DM) FILLS, AND FECAL OUTPUT FOR COWS AND STEERS DURING TRIAL IV[a]

	Cows	Steers
In vivo DOM 6-day (%)[b]	30.5	31.8
GIT volumes (kg)[c]	9.3	8.0
Rumen fill (kg)[c]	8.8	11.4
Rate of passage [c]	.034	.044
Fecal output (kg)[c]	6.1	5.7

[a]Taken from Loza, 1979

[b]Estimated by INDF analysis.

[c]Estimated by ^{141}Ce.

APPENDIX TABLE 6. TREATMENT MEANS (MG/100 ML) AND STANDARD DEVIATION IN THE RUMEN LIQUOR COMPONENTS OF COWS AND STEERS IN A 4 X 4 LATIN SQUARE DESIGN

	Treatment (% CP)	Cows	Steers
Total-N	4.27	21.41 ± 2.13	22.93 ± 5.72
	5.19	28.02 ± 6.24	39.24 ± 18.55
	6.19	39.98 ± 11.59	46.26 ± 11.90
	7.14	48.79 ± 13.45	59.57 ± 10.48
Protein-N	4.27	19.68 ± 2.12	20.17 ± 2.73
	5.19	21.03 ± 5.00	30.08 ± 14.31
	6.19	28.89 ± 7.19	33.40 ± 5.72
	7.14	32.47 ± 6.17	38.63 ± 5.60
Microbial Protein-N	4.27	11.67 ± 2.61	13.11 ± 3.75
	5.19	14.13 ± .99	15.87 ± 4.43
	6.19	15.33 ± 1.55	17.76 ± 3.10
	7.14	17.96 ± 4.74	22.19 ± 4.18

VITA

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