Nitrogen Concentration in Blood and Rumen Liquor of Cattle Fed Low Protein Diets

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Abstract

Crude protein determination of a grazing animal's diet is difficult and expensive. Traditional methods include forage sampling (usually not representative of the diet selection process) and the use of fistulated animals for direct diet collections. Indirect methods were tested to provide a rapid estimate of diet protein at less cost. Concentration of blood serum urea N (BUN) and the concentration of total nitrogen (N), protein N, microbial protein N, and non-protein N (NPN) in rumen liquor were determined in 4 cows and 4 steers fed diets at maintenance (7.1%) and 3 sub-maintenance levels of crude protein (CP) (4.3, 5.2, and 6.2%). Cottonseed hulls constituted the basal diet, with cottonseed cubes added to vary the CP content and molasses added to provide isocaloric diets. However, diet CP affected the in vivo digestibility of the diets and hence their caloric values. Concentrations of BUN did not differ ($P<.05$) with changes in dietary CP. The concentration of total N, protein N, microbial protein N, and nonprotein N (NPN) in the rumen liquor ($P<.05$) increased as diet CP increased. The percentage of NPN in the total N was reduced when diet CP was below 5.2%, but it did not differ significantly when diet CP was between 5.2 and 7.1%. The ratio of microbial nitrogen to total protein nitrogen was not affected by level of dietary crude protein. Total N was a sensitive indicator of the CP content of the diet and was the easiest and quickest method tested.

Key Words: dietary crude protein, protein requirement, rumen nitrogen, blood urea nitrogen

Ruminants grazing rangelands are at times forced to consume forage that is nutritionally inadequate to meet their metabolic requirements. Crude protein (CP) intake of grazing ruminants is difficult to determine because they usually select their diets from heterogeneous vegetation. Blood profiles have been used in Europe to monitor and predict dietary CP levels for high producing dairy cattle (Hewitt et al. 1975, Manston et al. 1975). High correlations ($r^2>.90$) have been established between dietary N and blood urea N (BUN) concentrations in cattle and sheep (Muir et al. 1972, Torell et al. 1974).

Levels of ammonia in the rumen liquor reflect levels of dietary CP but are subject to extreme diurnal fluctuations (Lewis 1957, Davis and Stallcup 1964). Concentrations of other rumen liquor components such as total N and protein N have been shown to be more stable between 12 to 24 hr post-feeding and responsive to changes in dietary CP (Davis and Stallcup 1964, Elliot et al. 1965).

Objectives were to evaluate the quantitative relationship between dietary CP level and the concentrations of blood and rumen liquor components, and to determine their suitability as indicators of dietary nitrogen status.

Materials and Methods

Four Brahman × Jersey steers averaging 386 kg and four 4-year-old Brahman × Jersey pregnant cows, averaging 425 kg were used in 2 Latin square pen-feeding experiments. All animals were ruminally cannulated. The cattle were fed individually in 8 partially covered pens.

A cottonseed hull based diet was fed ad libitum once daily at 1000 hours. Experimental design was two 4 × 4 Latin squares, 1 with steers and 1 with cows. Each Latin square consisted of 4 animals, 4 CP treatments, and four 14-day trials.

Cottonseed hulls were used as the diet base to provide a low quality roughage and for uniformity of particle size and chemical qualities. Cottonseed cubes were added to the hulls to provide 4 CP diets, and molasses was added to make the diets isocaloric. Quantities fed, chemical composition, and percentage of maintenance requirements for CP and DE of each diet are presented in Table 2. Mineral blocks were provided for each animal. Ration refusals were minimal and occurred only infrequently with the 4.3% CP diet.

Samples of diet constituents were collected during each trial for chemical analysis. Dry matter (DM), organic matter (OM), CP content, and in vitro organic matter digestibility (OMD) of the diet constituents were determined. Total N was analyzed by the micro-Kjeldahl method (AOAC 1975), and in vitro OMD was determined by a 48-hr fermentation in rumen liquor followed by a neutral detergent fiber extraction (Goering and Van Soest 1970). Samples analyzed for in vitro OMD were corrected for batch variation by a standard feed sample of known in vivo digestibility (Engdahl 1976) (Table 1).

Table 1. Chemical and physical constituents (%) of feed stuffs used to formulate experimental rations.

	Dry matter	Organic matter	In vitro organic matter digestibility	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]This value was taken from NRC (1976).

Diets were formulated to provide approximately the same levels of digestible energy. Digestibilities were similar when estimated in vitro; however, Loza (1979), in an associated study, determined that in vivo OMDs of all rations were lower than those estimated in vitro (Table 2). Loza (1979) determined in vivo OMD by the ratio of the indigestible neutral detergent fiber (INDF) content of feed and feces (Jacobs 1975).

Blood and rumen liquor samples were collected on the 10th, 12th, and 14th day of each trial at 0800 hr, approximately 20 hr post feeding. Blood samples were collected by jugular puncture into 10-ml evacuated tubes. Blood samples were taken to the laboratory immediately after collection and centrifuged to retain the serum. BUN was determined by fearon condensation of urea with diacetyl monoxime in acide medium.[1,2]

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[1]Technique used was that described in the manual accompanying Blood Urea Nitrogen Reagent and Standard Set 64667, produced by Harleco, a Division of American Hospital Supply, Gibbstown, N.J.

[2]Mention of a trademark name does not constitute a guarantee or warranty of the product by the Texas Agricultural Experiment Station and does not imply its approval to the exclusion of other products that also may be suitable.

Table 2. Diet components (kg DM) and the contents of crude protein (%) and digestible energy estimated by in vitro OMD and in vivo OMD (%), and the estimated percent of maintenance these components contributed to the diet.

Components	Diet			
	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	7.44	7.65
Crude protein	4.3	5.2	6.2	7.1
Percent of maintenance	71.	87.	103.	119.
Digestible energy Estimated by:				
in vitro OMD	63.3	65.8	70.0	69.8
Percent of maintenance	127.	132.	140.	139.
in vivo OMD	15.3	32.0	36.0	41.5
Percent of maintenance	31.	64.	72.	83.

[a]Maintenance requirements taken from NRC (1976) for cattle of comparable weights.

Samples of rumen contents were collected via rumen fistula. Samples were collected by inserting a 2.54-cm PVC pipe into the ventral anterior portion of the rumen. Rumen samples were filtered through 4 layers of cheesecloth and separated into 2 aliquots. One aliquot was analyzed for total N by the micro-Kjeldahl procedure and the other sample aliquot was for N fractionization.

Protein N was analzyed by a gravimetric modification of a method described by Folin and Wu (1919). Rumen liquor (10 ml) was pipetted into a tared centrifuge tube and 1 ml of a 10% (w/v) sulfosalicylic-acid solution was added. The tubes were centrifuged at 10,000 × for 5 minutes. The supernatant was discarded, and the tubes and residue were oven-dried (105° C) and weighed. The amount of residue was expressed in milligrams protein N/100 ml rumen liquor by dividing the quantity of precipitated protein (mg) by 6.25. The second aliquot was separated by low-speed centrifugation (500 × g), which removed the remaining fine feed supernatant. The supernatant was analyzed for protein N by the method previously described, and the quantity was expressed as milligrams microbial protein N/100 ml rumen liquor. NPN was determined as the difference between total N and protein N.

The data were analyzed statistically by standard latin square procedures (SAS 1982). Differences were considered statistically significant if the probability of a Type I error was less than or equal to 5%. Duncan's (1955) mean separation test was used to rank significant differences.

Results

Blood

BUN did not differ ($P<.05$) for either cows or steers; however, there was a trend for BUN to increase with increased diet protein. BUN averaged 2.6, 3.2, 2.7 and 4.3 mg/100ml at the 4.3, 5.2, 6.2 and 7.1% diets, respectively, but the variation among animals was too great to detect a significant difference at these low CP levels.

Rumen Liquor

Significant treatment and trial effects existed for all rumen liquor components for cows and steers (Table 2) when measured 20 hr post-feeding. Nitrogen concentrations of rumen components of both cows and steers increased significantly and linearly as the CP treatment increased. The N concentrations of all rumen components measured generally increased as the trials progressed, possibly due to acclimation of the microbial population from a Coastal bermudagrass *(Cynodon dactylon)* forage diet to the cottonseed hull based diet.

Total N and protein N levels were approximately twice as high in the 7.3% CP diet as compared to the 4.3% CP diet at 20 hours post-feeding. Microbial protein N increased approximately 50% and NPN increased over 9 times from the lowest to the highest CP diet. NPN in the rumen liquor of cows and steers was reduced to extremely low levels on the lowest CP diet. The ratio of NPN to total N was significantly less on the 4.3% CP diet than from the 5.2% to 7.1% CP diets for both cows and steers (Table 3).

Discussion

Blood

Dietary CP could not be accurately predicted by BUN of cows or steers fed diets ranging from 4.3 to 7.1% CP in this study. It would appear that a larger sample size would be required to detect BUN differences in the low range. Linear relationships between dietary CP and BUN levels have been reported by Prewitt et al. (1971) and Preston et al. (1965) with dairy cattle, and by Muir et al. (1972) with sheep. Metabolic profiles based on blood parameters are subject to great herd differences. In a survey of 2,400 dairy cows from 13 herds mean BUN value was 14.9 with a 95% confidence limit of 9.5 – 20.5 (Payne et al. 1970).

Linear relationships exist between BUN and diet CP content at maintenance level and higher since almost all of the ammonia is converted to urea (Lewis 1962). However, this relationship was not detected with submaintenance CP diets when samples were collected 20 hours post-feeding. All of the values from this study were much lower than values reported for whitetailed deer *(Odocoileus virginianus)* (Seal et al. 1983) and dairy cattle (Payne et al. 1970). Hewett (et al. 1975) concluded BUN appeared to be a fairly exact reflection of protein intake when energy and roughage remained constant. While BUN does not appear to be precise as a general indicator of dietary protein, the low levels found in this study reflect the low energy and protein contents of the diets. If BUN is used as an indicator, the animals should be fasted over night and handling stress minimized.

Rumen Liquor

Elliot et al. (1965) found rumen microbial activity increased with either protein-rich or protein and carbohydrate-rich concentrates. However, carbohydrate-rich supplements alone did not increase rumen microbial activity. This would explain the low in vivo OMD of the 4.3% CP diet fed in this study even though molasses was added in an attempt to provide an adequate energy source (Table 2). The additional N in the higher CP diets apparently stimulated microbial activity, resulting in a two-fold increase in the in vivo OMD. However, the ratio of microbial protein N to total protein N was not significantly affected by dietary CP treatment (Table 3).

Rumen N components were sensitive indicators of the CP content of these diets. Concentrations of microbial protein N and total protein N increased as diet CP increased (Table 3). Since the rumen is the major site of accumulation and initial degradation of plant protein, it is logical that the level of diet nitrogen would be reflected by total nitrogen levels in the rumen liquor. Davis and Stallcup (1964) found this same relationship on a cottonseed hull based ration and soybean meal.

Total N is routinely analyzed in most nutrition labs and is more feasible for rapid analysis than the other N components analyzed in this study. Although cows and steers had approximately the same total N concentrations with the 4.3% CP diet, the steers had over 20% higher total N concentrations for the 7.3% CP diet. This suggests that the cows were more efficient in either absorption of N or translocation of the N to the lower GIT. Studies by Weller et al. (1962) and Hogan (1973) indicated that 50 to 80% of the N in digesta passing from the rumen was of microbial origin.

Since the major source of N for the microbial population is ammonia, NPN was at critically low levels for microbial production on the diet containing 4.3% CP. Moir and Harris (1962) found a decrease in bacterial and ammonia N in sheep as the N in feed decreased. They observed that there was frequently no measurable ammonia N present in the rumen liquor at dietary CP levels of

Table 3. Concentrations and ratios of selected rumen liquor components of cows and steers on four dietary crude protein (DCP) treatments.

Rumen liquor components	Cows, DCP (%)				Steers, DCP (%)			
	4.3	5.2	6.2	7.1	4.3	5.2	6.2	7.1
Total nitrogen (mg/100ml)	21.4[a]	28.0[b]	40.0[c]	48.8[d]	22.9[w]	39.2[wx]	46.3[x]	59.6[x]
Protein nitrogen (mg/100ml) (PN)	19.7[a]	21.0[a]	28.9[b]	32.5[b]	20.2[w]	30.1[wx]	33.4[wx]	38.6[x]
Microbial protein nitrogen (mg/10ml) (MPN)	11.7[a]	14.1[ab]	15.5[bc]	18.0[c]	13.1[w]	15.9[w]	17.8[wx]	22.2[x]
Non-protein nitrogen (mg/100ml) (NPN)	1.7[a]	7.0[b]	11.1[b]	16.3[c]	2.8[w]	9.2[w]	12.9[xy]	21.0[y]
MPN:PN	.59	.67	.54	.55	.59	.67	.54	.55
NPN (%)	7.9[a]	25.0[b]	27.8[b]	31.4[b]	12.2[w]	23.5[x]	27.9[x]	35.2[x]

[a,b,c,d,w,x,y]Means in the same row with no common superscript differ ($P<.05$).

2.0% Wiedmeier et al. (1983) found a linear decrease in mean ammonia nitrogen in response to a decrease in diet nitrogen for animals fed wheat straw. They also noted an increase in bacteria numbers up to 8 hr post-feeding when diet nitrogen was increased.

Conclusions

Periodic checks of BUN and total protein level in the rumen liquor after an over-night fast could provide reliable information to monitor trends of protein intake. These techniques do not provide a high level of precision and accuracy, but samples collected across seasons should identify critical nitrogen deficient periods and indicate when levels of supplementation are adequate. To extend the results from pen-studies to intact free-grazing livestock or wildlife, further work is needed. These techniques show promise for monitoring the concentration of protein in the diets of grazing animals.

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