Concentration and degradation of nitrogen and fibre fractions in selected tropical grasses and legumes

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Abstract

Newer systems of protein evaluation partition feedstuff N into the proportion degraded in the rumen and that which escapes ruminal degradation. Protein and fibre degradation characteristics were measured in the grasses Pensacola bahiagrass (*Paspalum notatum*) and Bigalta limpograss (*Hemarthria altissima*), and the legumes aeschynomene (*Aeschynomene americana*) and hairy indigo (*Indigofera hirsuta*). Nitrogen concentration of legumes (30-39 g/kg) was greater than that of grasses (7-13 g/kg). Legumes contained greater absolute amounts of N soluble in a buffer solution, and potentially ruminally degradable N than grasses. Ruminal degradation rate of the potentially degradable N fraction was greater in legumes (24-44%/hr) than in grasses (6-18%/hr), leading to a greater estimated escape N as a percentage of total N in grasses (12.8-25.0%) than in legumes (6.6-11.8%). Major differences in cell wall structure between grasses and legumes occurred in hemicellulose (HC) concentration, with legumes containing much less HC than grasses. Nitrogen in these tropical grasses and legumes appears to undergo rapid and extensive ruminal degradation. Although ruminal degradation of N in these grasses was extensive, low absolute quantities of ruminally soluble and degradable N may limit microbial protein synthesis in ruminants fed tropical grass diets. Legume addition to tropical grass diets may enhance digestion by providing N for rumen function, but a combination of ruminally degradable and escape proteins may be required for optimal animal performance.

Resumen

*Sistemas nuevos para la evaluación de la proteína y la partición del N de los alimentos en las porciones degradadas en el rumen y aquellas que escapan de la degradación ruminal. Las caractersticas de la degradación de la proteína y la fibra fueron medidas en las gramíneas Pensacola bahiagrass (*Paspalum notatum*) y Bigalta limpograss (*Hemarthria altissima*) y en las leguminosas aeschynomene (*Aeschynomene americana*) y hairy indigo (*Indigofera hirsuta*). La concentración del N en las leguminosas (30-39 g/kg) fue superior al de las gramíneas (7-13 g/kg). Las leguminosas tuvieron una mayor cantidad absoluta de N soluble en solución buffer y potencialmente una mayor degradación ruminal del N que las gramíneas. La tasa de degradación ruminal de la fracción de N potencialmente degradable fue mayor en las leguminosas (24-44%/h) que en las gramíneas (6-18%/h), lo que condujo a una estimación elevada del porcentaje del N total sobrepasante en las gramíneas (12.8-25.0%) que en las leguminosas (6.6-11.8%). Las mayores diferencias entre las estructuras de las paredes celulares de las gramíneas y de las leguminosas fue en la concentración de la hemicelulosa (HC), la cual fue menor en las leguminosas que en las gramíneas. Parece ser que el N en estas gramíneas y leguminosas sufre una rápida y extensa degradación ruminal. A pesar que la degradación ruminal de estas gramíneas es extensa, las cantidades absolutas de N soluble y degradable en el rumen podrían limitar la síntesis de proteína microbiana de los rumiantes alimentados con gramíneas tropicales. La adición de leguminosas en las dietas con gramíneas tropicales podrían aumentar la digestión debido a la provisión de N para el funcionamiento del rumen, pero para un óptimo desempeño animal se podría requerir una combinación de proteína degradable y sobrepasante.*

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Introduction

Recently developed systems of protein evaluation (ARC 1980; NRC 1985) partition feedstuff N into the proportion degraded in the rumen and that which escapes ruminal degradation. Microbial protein production is influenced by the proportion of feedstuff N that is soluble and degradable in the rumen, in addition to the digestible energy available to fuel incorporation of ammonia and degradable protein into microbial protein. Protein available for absorption postruminally is influenced by the proportion of feedstuff N that is resistant to ruminal degradation plus microbial protein.

Protein composition and quality of oil meals and other protein-rich feeds have been evaluated extensively (Goetsch and Owens 1985), but information concerning the quality of forage protein is limited (Flores *et al.* 1979; Aii and Stobbs 1980). Research suggests that some forage proteins are degraded to a large degree in the rumen (Ulyatt *et al.* 1975; Anderson *et al.* 1988), and ruminants fed fresh forage have responded positively to supplementation with slowly degraded proteins (Penning and Treacher 1982). van Eys *et al.* (1986) associated the increased weight gain observed in goats supplemented with *Gliricidia, Leucaena* or *Sesbania* with the proportion and characteristics of ruminally degradable protein in the legumes and its effect on microbial protein production. However, Aii and Stobbs (1980) suggested that a considerable quantity of N in *Leucaena* may be resistant to ruminal degradation supporting the observation of Flores *et al.* (1979) that a supplement of *Leucaena* increased milk production to an extent similar to that of formaldehyde-treated casein. Variation may exist in the protein degradation characteristics of tropical forages.

Crude protein concentration and energy value of many tropical grasses are below animal requirements during much of the year (ARC 1980; NRC 1984), and legumes have been incorporated into tropical grass pastures to improve the nutritional status of ruminants. With all-forage or forage-based diets, protein quality of each dietary component is important in evaluating responses to supplementation. Objectives of this research were to evaluate the composition and degradation characteristics of nitrogen and fibre components in the tropical grasses Pensacola bahiagrass (*Paspalum notatum*) and Bigalta limpograss (*Hemarthria altissima*), and the tropical legumes aeschynomene (*Aeschynomene americana*) and hairy indigo (*Indigofera hirsuta*).

Materials and Methods

Growing and harvesting procedures, and sample preparation for the four forages are presented in a companion paper (Brown *et al.* 1991). Subsamples of the four forages were analyzed for laboratory dry matter (DM), organic matter (OM), and total N according to AOAC (1975) procedures. Quantities of N bound to the neutral detergent fibre (NDF) and acid detergent fibre (ADF) fractions were measured as the amount of N remaining after NDF and ADF refluxing (Goering and Van Soest 1970), respectively. All analyses were conducted in triplicate.

Solubilities of DM and total N were determined according to the following procedures. Triplicate samples (approximately 0.5 g DM) of each forage were incubated in 25 ml of McDougall's buffer (McDougall 1948) at 39 °C. Following incubation, forage residue was recovered on previously dried and weighed filter paper, then dried at 100 °C for 24 h and weighed. Soluble DM was calculated as the DM loss occurring during incubation as a percentage of the DM originally incubated. Filter paper and forage residue were then digested for total N analysis (AOAC 1975). Soluble N was calculated as the N loss occurring during incubation as a percentage of the N originally incubated. Preliminary experiments indicated that DM or N solubilities were not influenced by incubation time. For this experiment, an incubation time of 30 min was used. Insoluble, potentially degradable N was calculated as total N minus soluble N minus ADF-N, with all values expressed on a DM basis.

Degradability of the insoluble, potentially degradable N fraction was determined by the *in situ* procedure of Anderson *et al.* (1988). Polyester (35 to 80 um pore size) bags measuring 11 X 15 cm were filled with either 1.5 g of intact forage or an amount of ADF residue such that cellulose in the ADF was equivalent to cellulose plus hemicellulose of the intact forage sample. Triplicate bags containing intact forage or the corresponding ADF residue were made for each forage X sampling time combination. Incubations took place in the rumen of two steers (*Bos taurus* X *Bos indicus*) fed stargrass (*Cynodon nlemfuensis*) hay, with sampling times of 4, 8, 12, 24, 48,

and 72 h. At the appropriate time, bags were removed from the rumen and throughly washed with water. Contents of each bag were recovered and analyzed for total N (AOAC 1975). Correction for bacterial contamination of samples, and N degradation rates were calculated following the procedures of Anderson *et al.* (1988).

Rate of *in vitro* ruminal ammonia release from the four forages was measured following procedures of Britton *et al.* (1978) with the following modifications. Triplicate samples of 20 mg N from each forage were incubated with 50 ml of a ruminal fluid:McDougall's buffer (1:4) solution (McDougall 1948) for 4, 8, 12, 24 and 48 h. At the appropriate time, fermentation was halted by the addition of 2 ml of 50 g/kg $HgCl_2$. Concentration of NH_3-N was measured colorimetrically utilizing the phenol/hypochlorite reaction described by Broderick and Kang (1980).

Concentrations of NDF, ADF, and acid detergent lignin (ADL) in each forage were determined in triplicate following the procedures of Goering and Van Soest (1970). Hemicellulose (HC) was determined in triplicate by the sequential NDF, ADF extraction procedure of Van Soest and Robertson (1980). Separate samples were used for NDF, ADF, HC, and ADL analyses so that values could be expressed on an ash-free basis. Cellulose concentration was calculated as the difference between ADF and ADL.

For all *in vitro* digestion measurements, ruminal fluid was collected from a ruminally fistulated steer (*Bos taurus* X *Bos indicus*) fed stargrass hay plus approximately 0.5 kg soybean (*Glycine max*) meal daily. All *in vitro* digestion analyses were conducted in triplicate runs, with duplicate tubes within a run. *In vitro* organic matter digestion (IVOMD) was determined by a modification (Moore and Mott 1974) of the Tilley and Terry (1963) technique. For *in vitro* NDF and ADF digestion, an approximate 0.5 g sample was incubated for 48 h with 50 ml of a ruminal fluid:McDougall's buffer (1:1) solution (McDougall 1948). Fermentation was halted by the addition of 2 ml of 50 g/kg $HgCl_2$. Inoculum and residue were refluxed in NDF or ADF solution (Goering and Van Soest 1970) for measurement of NDF or ADF digestion, respectively. *In vitro* rate of NDF digestion was determined by incubating samples for 0, 4, 8, 12, 24, 36, 48, 72 and 96 h with 50 ml of a ruminal fluid: McDougall's buffer (1:1) solution (McDougall 1948). At the appropriate time, fermentation was halted by the addition of 2 ml of 50 g/kg $HgCl_2$. The 0 h samples were inoculated and $HgCl_2$ added immediately. Residue and inoculum were refluxed in NDF solution (Goering and Van Soest 1970) within 1 d after removal from the incubator. Rate and extent of NDF digestion, and lag time were calculated by nonlinear least squares (Mertens and Loften 1980).

For each nutritive value variable, a mean value was calculated using individual laboratory replications. A standard error of the overall mean for each nutritive value variable was also calculated.

Results

Greater amounts of DM were soluble in legumes (240-250 g/kg) than in grasses (150-160 g/kg; Table 1). Legumes contained greater total N concentration than grasses. Within the legumes, aeschynomene contained more total N than hairy indigo, and within the grasses, bahiagrass contained more total N than limpograss. Absolute quantities of soluble N, and N bound to the NDF and ADF fractions were greater in legumes than in grasses. When expressed as a percentage of total N however, these N characteristics were greater in grasses than in legumes. Legumes contained greater amounts of insoluble potentially degradable N than grasses when expressed as a function of DM or as a percentage of total N.

A lag time for the initiation of N degradation was observed for limpograss (11.5 h) and hairy indigo (7.5 h), but not for bahiagrass or aeschynomene (Table 2). Nitrogen degradation rate was faster in legumes than in grasses. Within the legumes, N degradation rate was faster for aeschynomene than for hairy indigo, and within the grasses was faster for bahiagrass than for limpograss. Using a rate of passage of 5%/h as in Anderson *et al.* (1988), a greater percentage of the potentially degradable N fraction was estimated to escape ruminal degradation in grasses (22.0-47.3%) than in legumes (10.3-17.3%). Estimated escape N as a percentage of total N ranged from 6.6-25.0%, suggesting that ruminal degradation of protein in these forages was rapid and extensive. Estimated quantity of insoluble potentially degradable N degraded in the rumen was much greater for legumes (17.1-22.5 g/kg) than for grasses (1.9-5.8 g/kg). Use of a faster rate of passage, as has been observed in diets containing legumes (Thornton and Minson 1973), would increase the estimated

Table 1. Dry matter solubility and N profiles of selected tropical grasses and legumes

Item[2]	Forage species[1]				s.e.
	Bahia	Limpo	Aeschynomene	Hairy Indigo	
DM solubility, g/kg	151.4	161.1	251.9	245.0	5.46
Total N, g/kg of DM	13.3	6.8	39.2	30.4	3.84
Soluble N					
g/kg of DM	4.7	2.7	12.7	8.5	1.16
% of total N	35.2	39.0	32.4	27.9	1.28
NDF-N					
g/kg of DM	7.2	3.7	11.8	9.2	1.27
% of total N	54.1	54.4	30.1	30.3	3.89
ADF-N					
g/kg of DM	1.1	0.5	1.4	1.2	0.28
% of total N	8.3	7.4	3.6	4.0	0.55
Insoluble, potentially degradable N[3]					
g/kg of DM	7.5	3.6	25.1	20.7	
% of total N	56.4	52.9	64.0	68.1	

[1]Bahia = *Paspalum notatum,* Limpo = *Hemarthria altissima,* Aeschynomene = *Aeschynomene americana,* Hairy Indigo = *Indigofera hirsuta*
[2]DM = dry matter, NDF = neutral detergent fibre, ADF = acid detergent fibre
[3]Calculated as: total N minus soluble N minus ADF-N

Table 2. N degradation characteristics of selected tropical grasses and legumes

Item	Forage species[1]				s.e.
	Bahia	Limpo	Aeschynomene	Hairy Indigo	
N degradation kinetics					
Lag time, h	0.0	11.5	0.0	7.5	1.90
Degradation rate, %/h	17.7	5.6	43.5	23.9	5.21
Prediction of escape N					
Insoluble potentially degradable N, g/kg DM[2]	7.5	3.6	25.1	20.7	
Escape N[3]	22.0	47.3	10.3	17.3	
Amount of escape N[4]	1.7	1.7	2.6	3.6	
Estimated escape N[5]	12.8	25.0	6.6	11.8	
Ruminally degraded N[6]	5.8	1.9	22.5	17.1	

[1]Bahia = *Paspalum notatum,* Limpo = *Hemarthria altissima,* Aeschynomene = *Aeschynomene americana,* Hairy Indigo = *Indigofera hirsuta*
[2]Values from Table 1
[3]From Anderson *et al.* (1988). Estimate of the amount of a dietary component that may exit the rumen before being digested. K_p (rate of passage) = 5%/h. Escape % = $K_p/(K_p + K_d)$. K_d = degradation rate of the rapidly degraded pool. Values represent the percentage of the potentially degradable N fraction escaping ruminal degradation
[4]Estimated quantity (g/kg DM) of insoluble potentially degradable N escaping ruminal degradation
[5]Expressed as a percentage of total N
[6]Estimated quantity (g/kg DM) of insoluble potentially degradable N degraded in the rumen.

percentage of potentially degradable N escaping ruminal degradation for legumes, and reduce the estimated quantity of N degraded in the rumen for legumes.

Greatest amounts of *in vitro* ruminal ammonia release were observed from aeschynomene, particularly early in the fermentation (4-12 h; Table 3). Limpograss had the least amount of ammonia release, with little or no ammonia production at 4 and 8 h of fermentation. Ammonia production from hairy indigo was less than that from bahiagrass, even though size of the soluble and potentially degradable N pools and degradation rate of the potentially degradable N fraction was greater for hairy indigo than for bahiagrass.

Legumes contained lower concentrations of NDF than did grasses (Table 4). Within the legumes, hairy indigo contained less NDF than aeschynomene, and within the grasses, bahiagrass contained slightly less NDF than limpograss.

Table 3. *In vitro* ruminal ammonia release (mg NH_3-N/l) from selected tropical grasses and legumes

Item[1]	Time, h					s.e.
	4	8	12	24	48	
Bahia	27.7	46.2	56.1	73.1	107.5	9.04
Limpo	0.0	3.1	32.7	46.7	74.7	9.37
Aeschynomene	52.6	85.1	93.2	108.5	122.2	7.91
Hairy Indigo	22.1	38.4	51.5	60.2	86.1	7.25

[1]Bahia = *Paspalum notatum,* Limpo = *Hemarthria altissima,* Aeschynomene = *Aeschynomene americana,* Hairy Indigo = *Indigofera hirsuta*

Table 4. Forage component concentration of selected tropical grasses and legumes

Item[1]	Forage species[2]				s.e.
	Bahia	Limpo	Aeschynomene	Hairy Indigo	
NDF	730	762	427	347	55.1
ADF	393	352	308	242	16.9
Cellulose	352	302	250	201	17.1
HC	338	415	130	83	41.8
ADL	41	49	58	41	2.7

[1]NDF = neutral detergent fibre, ADF = acid detergent fibre, HC = hemicellulose, ADL = acid detergent lignin. All values are expressed as g/kg ash free, dry matter basis
[2]Bahia = *Paspalum notatum,* Limpo = *Hemarthria altissima,* Aeschynomene = *Aeschynomene americana,* Hairy Indigo = *Indigofera hirsuta*

Table 5. *In vitro* digestion of selected tropical grasses and legumes

Item[1]	Forage species[2]				s.e.
	Bahia	Limpo	Aeschynomene	Hairy Indigo	
IVOMD	48.7	61.3	65.6	46.0	2.40
NDF digestion	44.0	64.3	45.2	20.3	5.93
ADF digestion	40.7	50.4	40.6	36.4	4.51
NDF digestion kinetics					
Lag time, h	14.1	13.8	5.9	17.3	1.46
Rate of NDF digestion, %/h	4.6	4.1	4.9	3.4	0.19
Extent of NDF digestion, %[3]	65.0	71.6	55.4	33.8	2.38

[1]IVOMD = *in vitro* organic matter digestion (%), NDF = neutral detergent fibre, ADF = acid detergent fibre. NDF and ADF digestion values are expressed as % ash free, dry matter basis following a 48 h fermentation
[2]Bahia = *Paspalum notatum,* Limpo = *Hemarthria altissima,* Aeschynomene = *Aeschynomene americana,* Hairy Indigo = *Indigofera hirsuta*
[3]Values are expressed as % ash free, dry matter basis following a 96 h fermentation

Legumes contained less ADF and cellulose than did grasses, but the magnitude of these differences were not as great as those observed for NDF concentration. Major differences in cell wall structure between grasses and legumes occurred in HC concentration, with legumes containing much less HC than grasses. Aeschynomene was greatest in ADL concentration, with only small differences among the other forages.

Greater variation occurred in *in vitro* digestion characteristics among grasses and among legumes (Table 5) than in chemical composition data (Table 4). Aeschynomene and limpograss were similar in IVOMD, and were greater in IVOMD than bahiagrass and hairy indigo, which were similar in IVOMD. *In vitro* digestion of NDF and ADF were greatest for limpograss, were similar between bahiagrass and aeschynomene, and were lowest for hairy indigo. Lag time for the initiation of NDF digestion was least for aeschynomene, was similar for bahiagrass and limpograss, and was greatest for hairy indigo. Aeschynomene had the fastest rate of *in vitro* NDF digestion, followed by bahiagrass, limpograss and then hairy indigo. Legumes had a lower extent of *in vitro* NDF digestion than did grasses.

Discussion

Forages used in this experiment were selected to represent a range in chemical composition and nutritive value within tropical grasses and within tropical legumes currently used in livestock production systems. Bahiagrass was greater in total N concentration, but lower in *in vitro* OM and fibre digestion characteristics than limpograss. In an evaluation of livestock producer hays, Brown *et al.* (1990) found that limpograss averaged greater in IVOMD but lower in total N than other tropical grasses. Moore *et al.* (1984) found that at a given regrowth interval, bahiagrass contained more total N than limpograss.

Consistent with the results of Flores *et al.* (1979) and van Eys *et al.* (1986) absolute quantities of soluble N were greater in legumes than in grasses. Greater size, and faster degradation rate, of the potentially degradable N fraction in legumes than in grasses were also reported by Nocek and Grant (1987) and van Eys *et al.* (1986). This resulted in a greater amount of ruminally soluble and degradable N from legumes than from grasses, and suggests that some tropical legumes may complement low N concentration tropical grasses by providing soluble and degradable N for rumen function.

In vitro ruminal ammonia release was consistent with N solubility and N degradation characteristics of these forages. Greater amounts of ammonia produced from aeschynomene early in the fermentation (4-12 h) was consistent with the greater amount of soluble N, and the faster degradation rate of aeschynomene N. The lag time observed for initiation of N degradation in limpograss (Table 2) was supported by little or no ammonia production at 4 h and 8 h of fermentation (Table 3). Low total N concentration, size and degradation rate of the potentially degradable N fraction in limpograss likely limited ammonia production from limpograss.

Ammonia production from hairy indigo was less than that from bahiagrass, even though size of the soluble and potentially degradable N pools and degradation rate of the potentially degradable N fraction was greater for hairy indigo than for bahiagrass. Tannin concentration or other anti-quality factors were not measured in these forages, however elevated tannin levels have been found in some tropical legumes (Ahn *et al.* 1989), including some *Indigofera* species (Minson and Hegarty 1984), leading to formation of a tannin: protein complex rendering forage protein unavailable to ruminal microbial degradation. These factors may have led to lower than expected *in vitro* ruminal ammonia production and *in situ* N degradation characteristics in hairy indigo. indigo.

High ruminal solubility and degradability of forage N could cause ruminants with high nutrient requirements which are fed forage-based diets to be deficient in metabolizable protein. In sheep fed white clover or ryegrass, Ulyatt *et al.* (1975) found that duodenal N flow was less than N intake, suggesting that forage proteins were degraded to a large degree in the rumen. Stobbs *et al.* (1977) found increased milk production when cows grazing N-fertilized tropical grass pastures (3.5% N) were supplemented with formaldehyde treated casein, indicating that although the grass pasture was high in total N concentration, it may have been inadequate in providing amino acids for absorption at the small intestine.

The above research was conducted with forages that were high in ruminally soluble and degradable N, which may have provided adequate N levels for optimal rumen function. Our data suggest that although ruminal degradation of N in these tropical grasses was extensive, low quantities of ruminally soluble and degradable N may limit the pool of ruminal N available for microbial synthesis. Brown *et al.* (1991) found positive associative effects on *in vitro* NDF digestion in bahiagrass-aeschynomene or limpograss-aeschynomene mixtures. Magnitude of the associative effects were similar to the improvement in *in vitro* NDF digestion of pure bahiagrass or pure limpograss by using ruminal fluid from a steer fed low quality hay plus supplemental protein compared to ruminal fluid from a steer fed low quality hay only. This suggests that *in vitro* NDF digestion of these low N grasses was limited by the quantity of soluble and/or degradable N in the grasses, and that aeschynomene stimulated *in vitro* NDF digestion of the grasses by providing soluble and/or degradable N to the *in vitro* fermentation.

Similar animal performance by cattle grazing limpograss compared to other tropical grass pastures, even though IVOMD of limpograss pastures is usually greater, also suggests an involvement of both composition and concentration of N components (Pitman *et al.* 1984; Sollenberger *et al.* 1988). Holderbaum (1989) found that cattle grazing limpograss pastures and supplemented with urea had a greater daily gain than unsupplemented controls, indicating that

limpograss was not providing adequate quantities of soluble N for the given level of energy intake. Also in that trial, cattle grazing limpograss-aeschynomene pastures had a similar daily gain to cattle grazing limpograss pastures and supplemented with urea, suggesting that aeschynomene provided soluble and/or degradable N for rumen function.

van Eys *et al.* (1986) found that formaldehyde-treated soybean meal (F-SBM) supplementation increased the daily gain of goats fed napiergrass. In that trial, increased daily gain by supplementation with F-SBM plus *Leucaena* or *Sesbania* above that obtained by F-SBM supplementation was associated with ruminal degradation characteristics of the legume protein. However, Aii and Stobbs (1980) suggested that the protein in *Leucaena* may be relatively resistant to ruminal degradation supporting the observation of Flores *et al.* (1979) that supplementation with *Leucaena* increased milk production to a similar degree as that for formaldehyde-treated casein. Our results suggest that N in some tropical grasses and legumes may be degraded rapidly and to a large degree in the rumen. In addition, because of low total N in many tropical grasses perhaps limiting the pool of ruminal N available for microbial protein synthesis, protein supplements containing a combination of ruminally degradable and escape proteins may be required for optimal animal performance.

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