Research note: Nutritive value of a range of tropical forage legumes

M.J. VALARINI AND R.A. POSSENTI
Instituto de Zootecnia, Nova Odessa/SP, Brazil

Abstract

Shoot material from accessions of *Centrosema pubescens*, *Galactia striata*, *Macrotyloma axillare*, *Neonotonia wightii* and *Stylosanthes guianensis* was evaluated for chemical composition and ruminal digestion of dry matter (DM), crude protein (CP) and neutral detergent fibre (NDF) using *in situ* degradability. N and CP concentrations for the species ranged from 2.7 to 3.5% and 167 to 243 g/kg DM, respectively. Overall, the legumes produced forage with high crude protein, low NDF, and low phenolic concentrations. By the root nodulation, shoot N concentration and N status of the soil, the legumes appeared to demonstrate significant N_2-fixation capability. Except for Ca, mineral concentrations were in agreement with values reported in the literature. The effective degradabilities (k=5%/h) ranged from 49.8 to 64.0%, with highest values for *M. axillare* and *N. wightii*. In general, the herbage legumes studied showed great potential as ruminant feed.

Introduction

Low levels of animal production in tropical areas are usually associated with the low N-concentration and digestibility of tropical grasses, which make up the natural pastures. The low N-concentration is often a function of the low N-status of the soils. There is a need for forage species, specifically legumes, which can be introduced into these pastures to improve both quality and dry matter yields of the pastures.

One of the mechanisms by which this enhancement occurs is through an improvement in soil fertility as a result of biological N-fixation by the legumes (Olanite *et al*. 2004). About 65% of nitrogen input into global agriculture comes from N-fixation (Vance and Graham 1995) and introduction of legumes has been promoted as a feasible option for improving the sustainability of livestock production in tropical and subtropical zones (Lascano 1991; Olanite *et al*. 2004). Good-quality forage legumes (2–4% N, Harricharan *et al*. 1988) can be either grazed or harvested and hand-fed as a high-protein supplement to grasses. Herbaceous and shrub legumes are promising sources of protein for supplementing diets of ruminants consuming low-quality forages (Hess *et al*. 2003). Chemical analyses, particularly in combination with *in vitro* digestibility and *in situ* degradability, can provide a suitable evaluation of nutritive value of forage legumes (El Hassan *et al*. 2000).

In this study, the tropical forage legumes, *Centrosema pubescens*, *Galactia striata*, *Macrotyloma axillare*, *Neonotonia wightii* and *Stylosanthes guianensis*, inoculated with recommended strains of rhizobium, were evaluated for forage nutritive value and *in sacco* degradability in the rumen.

Materials and methods

Leguminous species and growth conditions

A pasture on the Experimental Station of Instituto de Zootecnia in Nova Odessa, São Paulo State, Brazil (22°42′S, 47°18′W; mean annual rainfall 1185 mm), which had been under grass without fertiliser for more than 15 years, was cultivated and sown to the following legumes (*C. pubescens* cv. Deodora Br1 Ac No 241, *G. striata* cv. Yarana Ac No 1922, *M. axillare* cv. Guatá Ac No 356, *N. wightii* cv. Tinaroo Ac No 779 and *S. guianensis* var. *guianensis* Ac No 1336) in December 1999. The seed was obtained from the Instituto de Zootecnia and was scarified in concentrated

Correspondence to: M.J. Valarini, Instituto de Zootecnia, Caixa Postal 60, Nova Odessa/SP 13460-000, Brazil, E-mail: mjvalarini@iz.sp.gov.br

sulphuric acid for 20 minutes and inoculated with a mixture of the recommended *Bradyrhizobium* spp. strains, SEMIA 6146, 6149 and 6155 from CPFBN/MIRCEN culture collection, before planting. Sowing rates were: 4.0 kg/ha for *Macrotyloma axillare* (25% germination); 2.0 kg/ha for *Neonotonia wightii* (65% germination); 3.0 kg/ha for *Centrosema pubescens* (55% germination); 4.0 kg/ha for *Galactia striata* (60% germination); and 2.0 kg/ha for *Stylosanthes guianensis* (20% germination). The soil was a clayey oxisol with chemical composition: organic matter — 30 g/dm^3; P — 24 mg/dm^3; mmol$_c$/dm^3 K — 5.6; Ca — 19; Mg — 14; H+Al — 22; pH ($CaCl_2$) — 5.5; CEC — 61.5 mmol$_c$/dm^3. At planting, the soil was fertilised with 66 kg/ha P + 50 kg/ha K. An individual plot area of 18 m^2 was used in a completely randomised design with 4 replicates for each accession.

When plants were 3 months old, herbaceous stems were harvested 15 cm above the ground for DM determination and chemical analyses. To estimate DM yield, 2 random 1 m^2 quadrats per plot were cut at a height of 15 cm and 200 g sub-samples were taken and oven-dried at 65°C to constant weight (48h). At the same time, root samples were collected with the help of a mattock, nodules detached, washed, oven-dried at 65°C and dry weights determined.

Chemical analyses

The dry matter (DM), ether-soluble compounds and ash concentrations of each leguminous sample were determined according to AOAC (1980). Neutral-detergent fibre (NDF), acid-detergent lignin (ADL), NDF residues and *in sacco* undegraded residues were analysed according to Goering and Van Soest (1970). N concentration in the dry matter was determined by the micro-Kjeldahl procedure (AOAC 1980). P and K concentrations were measured by Photoelectric Colorimeter and Burning Photometer, respectively, and Ca and Mg concentrations by Atomic Absorption Spectrometer. Phenolic components in the samples were extracted in 70% acetone solution. Total extractable phenolic (TEPH) and total extractable tannin (TETA) concentrations, expressed in tannic acid equivalent, of the supernatant were determined by the Folin-Ciocalteau method, and the extractable condensed tannins (TCTA), expressed in leucocyanidine equivalent, by using butanol-HCl assay according to Makkar *et al*. (1993). The *in vitro* digestion of DM was determined as described by Tilley and Terry (1963). All chemical analyses were performed in triplicate.

In sacco *dry matter and protein degradability analysis*

In sacco degradability analysis was carried out according to Mehrez and Ørskov (1977). About 5 g samples were transferred into nylon bags and incubated, in triplicate, in 3 fistulated Holstein steers fitted with rumen cannulae. The steers were fed, at maintenance level, twice daily on a mixed diet of coast-cross hay and legume hay (1:1) plus mineral supplement. The legume hay was composed of a mixture of the 5 leguminous species studied in this work. The nylon bags were withdrawn after 6, 24, 48, 72 and 96 h of incubation, thoroughly washed with tap water until rinse water was clear and dried at 60°C for 48 h. Three nylon bags for each leguminous species were soaked in water at 39°C for 10 min, washed and dried to determine the washing loss. The dried and washed residues for each incubation time were bulked and further ground through a 1-mm sieve for N and NDF determination. Data for *in sacco* dry matter, protein and NDF degradability were fitted by the exponential equation $P = a + b(1–e^{ct})$, were a, b and c are constants, and P is the degradability at time t (Ørskov and McDonald 1979).

Statistical analysis

In situ degradability values for the legumes were compared using a General Linear Model Procedure (SAS 1994); data are the result of triplicate analysis. Nodule dry weights are analysed using log-transformed data.

Results

Proximate analyses of the plant samples (stem+leaf) are presented in Table 1. There was significant variation between species for most components, the most pronounced being for extractable condensed tannins and total extractable tannins. Highest levels of both total and condensed tannins were

found in *G. striata* and *S. guianensis*. Data on *in situ* degradability of plant samples (Table 2) show that effective degradability of dry matter varied (50–64%), and there was good agreement between values obtained for *in vitro* digestibility and effective degradability of dry matter. *N. wightii*

Table 1. Means and standard errors (in brackets) for nutritive composition of shoot (leaf+stem) samples from five tropical forage legume species.

Component	*C. pubescens*	*G. striata*	*M. axillare*	*N. wightii*	*S. guyanensis*
			(%)		
Dry matter	92.3 (1.2)	91.6 (1.0)	91.8 (0.9)	91.8 (0.8)	92.0 (1.3)
Crude protein	22.1 (0.5)	24.3 (0.1)	16.7 (0.2)	23.7 (0.2)	17.9 (0.3)
Ash	5.1 (0.1)	7.2 (0.1)	4.8 (0.2)	7.7 (0.2)	6.8 (0.1)
Ca	0.61 (0.001)	0.80 (0.0)	0.53 (0.001)	0.50 (0.001)	0.74 (0.001)
P	0.18 (0.001)	0.22 (0.001)	0.20 (0.001)	0.17 (0.0)	0.15 (0.0)
Ca:P	3.4 (0.001)	3.6 (0.001)	2.7 (0.001)	2.9 (0.001)	4.9 (0.001)
Mg	0.32 (0.0)	0.43 (0.001)	0.33 (0.001)	0.39 (0.002)	0.63 (0.001)
K	1.5 (0.01)	2.0 (0.01)	1.9 (0.03)	1.4 (0.01)	2.3 (0.02)
Fat	2.4 (0.03)	2.2 (0.02)	2.2 (0.01)	2.1 (0.01)	4.7 (0.04)
Neutral-detergent fibre	53.2 (0.9)	51.0 (0.6)	46.3 (0.5)	46.6 (0.7)	48.6 (0.5)
Acid-detergent fibre	40.1 (0.5)	36.2 (0.7)	35.6 (0.6)	33.3 (0.5)	37.1 (0.5)
Cellulose	30.5 (0.5)	25.2 (0.6)	25.4 (0.6)	24.9 (0.5)	27.7 (0.5)
Lignin	9.4 (0.8)	10.7 (0.5)	7.7 (0.8)	7.7 (0.9)	8.4 (1.0)
In vitro dry matter digestibility	53.0 (1.5)	52.0 (1.9)	65.0 (1.8)	64.0 (1.5)	57.0 (1.2)
Total extractable phenolics	2.7 (0.001)	6.2 (0.001)	2.3 (0.001)	1.8 (0.001)	6.2 (0.001)
Total extractable tannins	2.0 (0.001)	4.9 (0.001)	1.9 (0.001)	1.2 (0.001)	4.9 (0.002)
Extractable condensed tannins	0.03 (0.001)	5.1 (0.001)	0.05 (0.0)	0.03 (0.0)	3.5 (0.006)

Table 2. Parameters of the exponential equation of Ørskov and McDonald (1979) for the *in sacco* dry matter, protein and neutral detergent fibre degradability of shoot (leaf+stem) samples from 5 tropical forage legumes

	a[1]	b[2]	$(a+b)$[4]	(c)[3]	Ef.d[5]
		(%)		(%/h)	
Species			Dry matter		
Centrosema pubescens	16.3c[6]	48.8c	65.1d	10.9c	49.8e
Galactia striata	12.0d	59.2b	71.2c	10.6c	52.2d
Macrotyloma axillare	28.3a	47.6c	75.9a	15.0a	64.0a
Neonotonia wightii	27.2b	48.0c	75.1a	12.7b	61.6b
Stylosanthes guianensis	11.4e	61.9a	73.2b	10.7c	53.5c
CV (%)	1.1	1.3	0.7	4.7	0.7
			Crude protein		
Centrosema pubescens	35.7a	54.7e	90.4c	19.8a	79.4a
Galactia striata	20.6c	69.5c	89.7c	11.0c	68.1e
Macrotyloma axillare	11.3e	82.7a	94.0ab	14.1b	72.3d
Neonotonia wightii	33.9b	59.4d	93.4b	12.5bc	76.3b
Stylosanthes guianensis	20.1d	74.3b	94.4a	13.9b	74.8c
CV (%)	0.6	0.7	0.4	5.3	0.7
			Neutral detergent fibre		
Centrosema pubescens	0.3bc	46.0d	46.3c	6.5c	25.7c
Galactia striata	0.2bc	55.7b	55.9b	7.9b	34.3b
Macrotyloma axillare	0.1c	57.8a	57.9a	9.2a	37.5a
Neonotonia wightii	0.7ab	58.2a	58.8a	8.3ab	36.9a
Stylosanthes guianensis	0.9a	53.5c	54.4b	9.0a	35.3b
CV (%)	16.8	1.2	1.2	4.8	1.4

[1] Soluble fraction.
[2] Potentially degradable fraction.
[3] Rate of degradation of DM, crude protein and ADF, (percentage per hour) of disappearance of fraction *b.*
[4] Potential degradability — Pd = $(a+b)$.
[5] Effective degradability of DM, CP and ADF, calculated for k = 5%/h solid outflow rates.
[6] Values in columns and parameters followed by different letters differ ($P<0.05$) by the Tukey test.

and *M. axillare* were more digestible than the remaining legumes. Overall, samples showed high N concentrations (2.7–3.9%) (Table 3). Nodule dry weight showed broad variation with *C. pubescens* and *M. axillare* producing the greatest weight of nodules (Table 3). Dry matter yields were highest ($P<0.05$) for *G. striata* and *C. pubescens*, while no differences in yield occurred for the other legumes (Table 3).

Table 3. Means of nodulation data and dry matter production of five 3-month-old tropical legumes species inoculated with recommended rhizobial strains.

Species	Nodule dry weight (g/plant)	N content (%)	DM (kg/ha)
C. pubescens	1.32a[1]	3.5 a	4250 a
G. striata	1.09c	3.9 ab	4758 a
M. axillare	1.32a	2.7 b	3040 b
N. wightii	1.15b	3.8 ab	2570 b
S. guianensis	1.02d	2.8 b	2795 b
CV(%)	3	15	19.5

[1] Means in the same column followed by the same letter are not significantly different ($P>0.05$) by Duncan's multiple range test.

Discussion

The data obtained in this study are from small plots, for a single harvest at a specific site, so cannot be assumed to be representative of values that might be obtained more widely and at different stages of maturity. However, they do represent a set of data for nutritive value of shoot samples (15 cm above ground) of 5 species of tropical leguminous forages.

As would be expected for growing shoots of leguminous plants, N concentrations in all legumes were high and well above the dietary level of 1%, below which DM intake becomes depressed (Humphreys 1991). They were even well above the 11% considered adequate to meet the protein requirements of growing beef cattle. All of these legumes, as a component of a legume-grass pasture or as a dietary supplement to a low-N diet, would boost dry matter intake of the diet of ruminants. In addition, the inclusion of these legumes in a grass-legume pasture could be expected to raise the N concentrations in the grass through biological nitrogen fixation by the legume. The wide variation in nodule production by the various species (0.01–0.12 g DW/plant) suggests that there was considerable variation in the ability of the rhizobia to infect the roots of the different species. It would appear that *C. pubescens* and *M. axillare* were adequately infected but that the rhizobial strains were less effective in infecting the remaining species. However, the contribution of BNF appears to have been significant, since the N status of the soil was considered low, while N concentrations in the legumes were high.

The condensed tannin (CT) values for *C. pubescens*, *M. axillare* and *N. wightii* were lower than those for *G. striata* and *S. guianensis*, and were comparable with those reported for tropical fodder legumes (Nozella 2001; Vitti *et al*. 2005). The presence of CT in legumes can have beneficial effects by protecting some protein from ruminal degradation but overprotection, which prevents digestion in the small intestine, can be detrimental. We are unable to draw any conclusions on the likely beneficial/detrimental effects of tannins present in these species. Generalisations based only on amounts of tannin can be misleading; protein reactivity of CT in diets is known to vary with the CT chemistry (Mueller-Harvey and McAllan 1992), which is species-dependent.

The use of forage legumes in agropastoral systems holds great promise for the tropics and is considered one of the sustainable land use options.

References

AOAC (1980) *Official methods of analysis*. 10th Edn. (Association of Official Analytical Chemists: Washington, DC).

El hassan, S. M., Kassi, A. L., Newbold, C.J. and Wallace, R.J. (2000) Chemical composition and degradation characteristics of foliage of some African multipurpose trees. *Animal Feed Science and Technology*, **86**, 27–37.

Goering, K. and Van Soest, P.J. (1970) Forage fibre analysis. Apparatus, reagents, procedures and some applications. *Agricultural Handbook,* **379**, 1–20.

Harricharan, H., Morris, J. and Denvers, C. (1988) Mineral content of some tropical forage legumes. *Tropical Agriculture* (Trinidad), **65**, 132–136.

Hess, H.D., Monsalve, L.M., Lascano, C.E., Carulla, J.E., Díaz, T.E. and Kreuzer, M. (2003) Supplementation of a tropical grass diet with forage legumes and *Sapindus saponaria* fruits: effects on *in vitro* ruminal nitrogen turnover and methanogenesis. *Australian Journal of Agricultural Research*, **54**, 703–713.

Humphreys, L.R. (1991) *Tropical Pasture Utilization*. (Cambridge University Press: Cambridge, UK).

Lascano, C.E. (1991) Managing the grazing resource for animal production in savannas of tropical America. *Tropical Grasslands*, **25**, 66–72.

Makkar, H.P.S., Blümmel, M., Norbert, K.B. and Becker, K. (1993) Gravimetric determination of tannins and their correlations with chemical and protein precipitation methods. *Journal of Science and Food Agriculture*, **61**, 141–150.

Mehrez, A.Z. and Orskov, E.R. (1977) A study of the artificial fibre bag technique for determining the digestibility of feeds in the rumen. *Journal of Agricultural Science*, **88**, 645–650.

MUELLER-HARVEY, I. AND MCALLAN, A.B. (1992) Tannins: Their biochemistry and nutritional properties. In: *Advances in Plant Cell Biochemistry and Biotechnology*. Vol. 1, pp. 151–217. (JAI Press Ltd: Greenwich).

NOZELLA, E.F. (2001) *Determinação de taninos em plantas com potencial forrageiro para ruminantes*. M.Sc. Dissertation. Universidade de São Paulo, São Paulo, Brazil.

OLANITE, J.A., TARAWALI, S.A. AND AKEN'OVA, M.E. (2004) Biomass yield, quality and acceptability of selected grass-legume mixtures in the moist savanna of west Africa. *Tropical Grasslands*, **38**, 117–128.

ØRSKOV, E.R. AND MCDONALD, I. (1979) The estimation of the protein degradability in the rumen from incubation measurements weighted according to rate of passage. *Journal of Agricultural Science,* Cambridge, **92**, 499–503.

SAS (1994) *SAS User's Guide: Statistics*. (SAS Institute, Inc.: Cary).

TILLEY, J.M.A. AND TERRY, R.A. (1963) A two-stage technique for the *in vitro* digestion of forage crops. *Journal of British Grassland Society,* **18**, 104–111.

VANCE, C.P. AND GRAHAM, P.H. (1995) Nitrogen fixation in agriculture: application and perspectives. In: TIKHONOVICH, I.A. *et al*. (eds) *Nitrogen Fixation: Fundamentals and Applications*. pp. 77–86. (Kluwer Publications: Dordrecht).

VITTI, D.M.S.S., ABDALLA, A.L., BUENO, I.C.S., SILVA FILHO, J.C., COSTA, C., BUENO, M.S., NOZELLA, E.F., LONGO, C., VIEIRA, E.Q., CABRAL FILHO, S.L.S., GODOY, P.B., AND MUELLER-HARVEY, I. (2005) Do all tannins have similar nutritional effects? A comparison of three Brazilian fodder legumes. *Animal Feed Science and Technology*, **119**, 345–361.

(Received for publication December 16, 2004; accepted May 4, 2006)