

## Chemical and physical parameters of forage legume species introduced in the Capricorn region of Limpopo Province, South Africa

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### Abstract

The purpose of the study was to evaluate the nutritive value of the forage legume species, *Centrosema pascuorum*, *Lablab purpureus*, *Macroptilium bracteatum*, *Macroptilium gracile* and *Vigna oblongifolia* as feed for ruminants in the Capricorn region of Limpopo Province, South Africa. It was hypothesized that forage legumes grown in the Province will be high in fibre and polyphenolics because of the high temperatures and low rainfall in the region. The experimental field was divided into four replications with the five different legume species within each replication, using a randomized complete block design. The plots were hand-harvested 16 weeks after planting, the material sampled, air-dried in the laboratory and analysed for physical parameters, chemical composition and *in vitro* enzymatic degradability. *Lablab purpureus* had the highest dry matter (DM) yield of 8 tons/ha followed by *C. pascuorum* with 2 tons/ha, whilst the other three species had a similar DM yield of approximately 1 ton/ha each. Physical characteristics varied from 2.88 to 4.45 ml/g for packed volume and 6.79 to 9.42 g/g for water retention. The crude protein composition varied from 236.8 to 259.6 g/kg DM, neutral detergent fibre from 418.3 to 505.8 g/kg DM, acid detergent fibre from 335.0 to 374.6 g/kg DM, the DM ruminal degradability from 611.1 to 712.9 g/kg DM and the organic matter (OM) degradability from 586.4 to 688.7 g/kg DM. The concentrations of total phenolics and extracted condensed tannins varied from 19.35 to 48.37 g/kg DM and from 176.6 to 334.5 g/kg DM, respectively. It was concluded that the biotic environment in the Limpopo Province did not impact negatively on the potential nutritive value of these forage legumes since the concentrations of fibre and phenolics were below levels expected to have detrimental effects on animals, and the ruminal degradability of their DM and OM was high.

**Keywords:** Forage, legume, tannins, nutritive value, physical parameters

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### Introduction

The level of animal production achieved in any environment is related to quantity, quality and continuity of supply of feed throughout the year. These are in turn related to rainfall, temperature and soil type and fertility. The Limpopo Province is characterised by low rainfall, which results in low herbage yields that are often inadequate for the nutritional needs of the livestock in most of the rural grazing areas. A major problem facing livestock producers in Limpopo Province is proper nutrition for their animals during the dry season when pastures, cereal residues and maize stover provide a limited supply of protein. Consequently, new high producing grasses are replacing slow growing traditional varieties, and exotic forage legumes, shrubs and fodder trees are being promoted as more productive sources of feed than indigenous varieties and species. Although forage legumes generally have a high concentration of nitrogen (N), the critical nutrient in the dry season (Kelly *et al.*, 1976; Dube, 1993), a major limitation is the presence of secondary plant compounds, such as hydrolysable and condensed tannins, which can depress feed intake and utilisation by animals (Norton, 1994). The depression is related to the ability of the tannins to form a complex with and precipitate proteins and other macromolecules such as polysaccharides (Reed, 1995). The amount and type of tannin present in forage legumes are influenced by the climate and soils of the locality in which they are grown (Van Soest, 1995; Waghorn *et al.*, 1997). Hot climates, low rainfall and/or low soil fertility tend to increase the amounts of these allelochemicals in forages. These ecological conditions are prevalent in the Limpopo Province and it was thus hypothesised that forage legumes grown in the province would be high in secondary plant metabolites such as fibre and tannins and low in digestibility. There is a lack of local information on the nutritional quality of legume species that have recently been introduced in the Capricorn region of Limpopo Province, South Africa. This information is necessary for the efficient incorporation of these forage legume species into the current ruminant feeding systems.

The main objective of this study was to evaluate the nutritive value of legume species based on physical parameters, chemical composition and *in vitro* enzymatic degradability.

### Materials and Methods

The experiment was conducted at Syferkuil, the experimental farm of the University of the North. Five different legume species, *Centrosema pascuorum*, *Lablab purpureus*, *Macroptilium bracteatum*, *Macroptilium gracile* and *Vigna oblongifolia*, were grown in small plots, four replications per species. They were allocated to the plots according to a randomised complete block design.

The plots were hand-weeded regularly and all available herbage in each plot was harvested 16 weeks after planting, when all species had reasonable amounts of herbage. After harvesting, the herbage was weighed wet and then left to dry at room temperature in a well-ventilated laboratory until a constant dry weight was reached, and then stored in brown paper bags pending grinding. Dry herbage was ground to pass through a 1 mm screen for analysis of organic matter (OM), N and detergent fibres. Samples for polyphenolic analysis were further ground to pass through a 0.2 mm screen.

Packed volume and water retention were determined using the methods described by Seoane *et al.* (1981). Forage samples were analysed for N concentration, using the Kjeldahl procedure (AOAC, 1984). The dry matter (DM) content was used to calculate DM yield per hectare as follows: (Area of plot in m<sup>2</sup> / 10 000) X (wet weight of herbage) X DM % of herbage. Neutral detergent fibre (NDF) and acid detergent fibre (ADF) were determined by the method of Van Soest (1983) as modified by Van Soest *et al.* (1991). *In vitro* enzymatic degradability was determined as described by Aufrere & Michalet-Doreau (1988). Total phenolics were determined, using Folin-Ciocalteu methods and expressed as tannic acid equivalent (g/kg DM) (Waterman & Mole, 1994). Condensed tannin (CT) was determined, using the butanol-HCl method and expressed as leucocyanidin equivalent (g/kg DM) (Porter *et al.*, 1986). Hydrolysable tannin was determined using potassium iodate (Willis & Allen, 1998). Protein binding capacity was determined, using the filter paper assay (Dawra *et al.*, 1988). The determination of the amount of polyethylene-glycol (PEG) precipitated by tannins was carried out according to the method of Silanikove *et al.* (1996) as modified by Boithumelo (1999) for material that does not contain radioactive label.

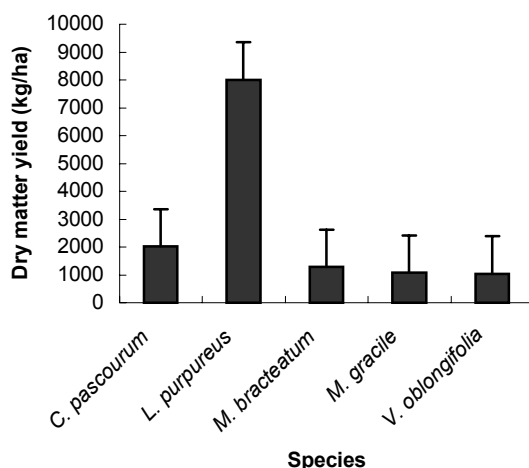
Analyses of variance were used to test for effect of species on chemical composition, physical characteristics and *in vitro* enzymatic degradability, using the general linear model procedure of the statistical analysis systems (SAS, 1998), for randomised complete block designs. If significant P values occurred, separation of means was done using the probability of difference (pdiff) facility of SAS. Correlation was used to measure the degree of association between variables.

### Results

There were significant species effects on DM yield (Figure 1). Species *L. purpureus* had the highest DM yield of approximately 8 tons/ha followed by species *C. pascuorum* with approximately 2 tons and the other species had a similar yield of about 1 ton/ha. There were significant ( $P < 0.05$ ) species effects on packed volume and water retention (Table 1). The *M. bracteatum* species had the highest packed volume and water retention, namely 51.3% and 38.6%, respectively.

Crude protein (CP) levels did not differ ( $P > 0.05$ ) between the five different species (Table 2). Neutral detergent fibre differed by approximately 20.9% between the species with the highest (*M. bracteatum*) and the lowest levels (*V. oblongifolia*). Species *M. gracile* contained the highest ADF level, which was 11.81% more than that in *V. oblongifolia*. There were significant species effects on total phenolics and extracted CT concentrations and non-significant effects ( $P > 0.05$ ) on protein binding capacity and PEG (Table 2). *Lablab purpureus* had the higher total phenolic concentrations as compared to *M. bracteatum* (Table 2). On the whole, all species had total phenolic concentrations that were less than 6% of the DM (Table 2). Extracted CT differed by approximately 89% between the species with the highest concentration (*L. purpureus*) and the lowest (*M. gracile*).

From the determination of hydrolysable tannin using the potassium iodate assay, all the species had a short reaction time (2-3 min.) compared to the two standards, gallic and tannic acids (Figure 2). All the species had absorbance values that were lower than those of tannic acid and gallic acid. The curves were flat like that of gallic acid in contrast to that of tannic acid that was sharp (Figure 2).



**Figure 1** Mean (s.e.) dry matter yield (kg/ha) of five legume forage species grown in the Capricorn region of Limpopo Province

**Table 1** Mean packed volume and water retention of five legume species grown in the Capricorn region of Limpopo Province

Species	Packed volume (ml/g)	Water retention (g/g)
<i>C. pascuorum</i>	3.43 <sup>c</sup>	7.44 <sup>c</sup>
<i>L. purpureus</i>	3.79 <sup>b</sup>	8.23 <sup>b</sup>
<i>M. bracteatum</i>	4.35 <sup>a</sup>	9.42 <sup>a</sup>
<i>M. gracile</i>	3.68 <sup>b</sup>	7.35 <sup>cd</sup>
<i>V. oblongifolia</i>	2.88 <sup>d</sup>	6.79 <sup>d</sup>
s.e.	0.172	0.191

<sup>a, b, c, d</sup> Column means with common superscripts do not differ ( $P > 0.05$ )

**Table 2** Mean concentration of dry matter (DM), organic (OM), crude protein (CP), neutral detergent fibre (NDF), acid detergent fibre (ADF), total phenolics, extracted condensed tannins (CT), protein binding capacity (PBC) and amount of polyethylene glycol (PEG) precipitated by tannins in the five legume species

Species	DM (g/kg)	OM (g/kg DM)	CP (g/kg DM)	NDF (g/kg DM)	ADF (g/kg DM)	Total phenolics (g/kg DM)	Extracted CT (g/kg DM)	PBC (g/kg DM)	PEG (g/kg DM)
<i>C. pascuorum</i>	873.5 <sup>ab</sup>	792.5 <sup>a</sup>	236.8	493.7 <sup>a</sup>	363.7 <sup>a</sup>	42.7 <sup>b</sup>	20.85 <sup>b</sup>	0.54	63.1
<i>L. purpureus</i>	873.8 <sup>ab</sup>	776.6 <sup>ab</sup>	244.6	487.1 <sup>ab</sup>	370.9 <sup>a</sup>	48.4 <sup>a</sup>	33.45 <sup>a</sup>	0.42	50.5
<i>M. bracteatum</i>	862.3 <sup>b</sup>	759.4 <sup>b</sup>	259.6	505.8 <sup>a</sup>	371.3 <sup>a</sup>	19.3 <sup>c</sup>	20.26 <sup>b</sup>	0.53	49.3
<i>M. gracile</i>	881.6 <sup>a</sup>	782.4 <sup>a</sup>	249.8	445.1 <sup>bc</sup>	374.6 <sup>a</sup>	43.6 <sup>ab</sup>	17.66 <sup>b</sup>	0.69	60.6
<i>V. oblongifolia</i>	885.6 <sup>a</sup>	794.7 <sup>a</sup>	241.5	418.3 <sup>c</sup>	335.0 <sup>b</sup>	24.1 <sup>c</sup>	21.60 <sup>b</sup>	0.53	46.9
s.e.	6.20	7.90	8.37	13.56	5.54	2.36	2.034	0.064	5.10

<sup>a, b, c</sup> Column means with common superscripts do not differ ( $P > 0.05$ )

There were significant species effects on *in vitro* enzymatic DM and OM degradabilities (Table 3). Species *V. oblongifolia*, *L. purpureus* and *M. gracile* had DM degradability and OM degradability that were

similar ( $P > 0.05$ ) and approximately 17 and 18% higher ( $P < 0.05$ ) than those of *C. pascuorum* and *M. bracteatum*. However, all species had degradabilities that were greater than 60%.

The DM yield was not correlated ( $P > 0.05$ ) to any of the parameters whilst DM degradability was negatively correlated to NDF and packed volume, but weakly positively correlated to CP (Table 4). Crude protein, on the other hand, was strongly negatively ( $P < 0.05$ ) correlated to NDF and packed volume.

**Table 3** Mean *in vitro* enzymatic dry (DMD) and organic matter degradability (OMD) of five legume species grown in the Capricorn region of Limpopo Province

Species	DMD (g/kg DM)	OMD (g/kg DM)
<i>C. pascuorum</i>	614.4 <sup>b</sup>	607.5 <sup>b</sup>
<i>L. purpureus</i>	686.5 <sup>a</sup>	672.6 <sup>a</sup>
<i>M. bracteatum</i>	611.1 <sup>b</sup>	586.4 <sup>b</sup>
<i>M. gracile</i>	679.3 <sup>a</sup>	660.6 <sup>a</sup>
<i>V. oblongifolia</i>	712.9 <sup>a</sup>	688.7 <sup>a</sup>
s.e.	10.95	13.71

<sup>a, b</sup> Column means with common superscripts do not differ ( $P > 0.05$ )

**Table 4** Pairwise correlation coefficients for chemical and physical parameters of forage legumes

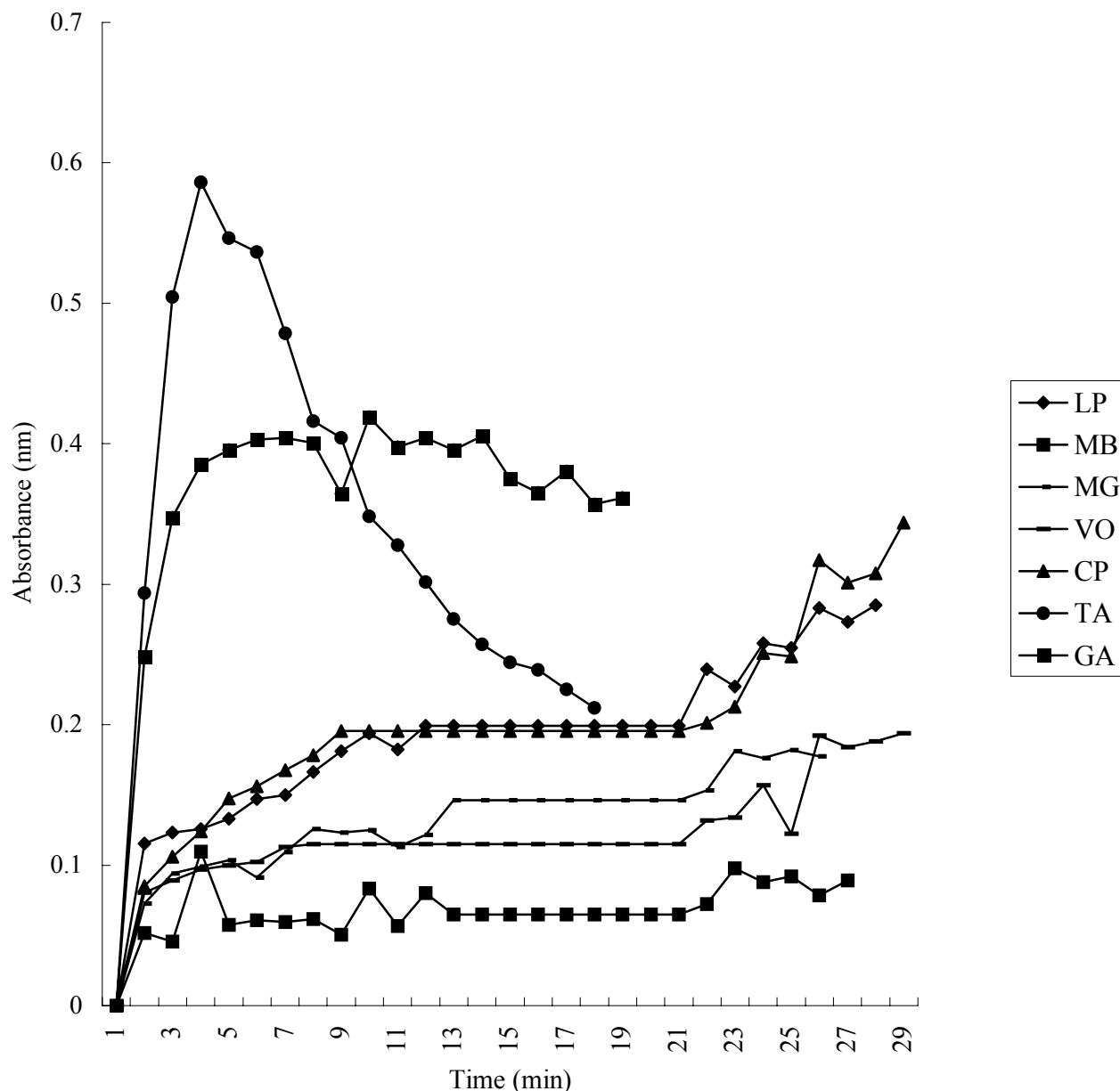
Parameters	Dry matter (DM) yield	Crude protein (CP)	Packed volume	Neutral detergent fibre (NDF)	DMD
DM yield	-				
CP	NS	-			
Packed volume	NS	- 0.93*	-		
NDF	NS	- 0.72*	NS	-	
DMD	NS	0.41*	- 0.64*	- 0.89*	-

DMD - *in vitro* enzymatic DM degradability; NS - Non-significant; \* $P < 0.05$

## Discussion

All forages were harvested after 16 weeks of growth when all species showed signs of flower buds and herbage yield was assumed to be optimal for all species at this stage. This was based on results elsewhere with other legume species (Gupta & Pradhan, 1993). Earlier harvests were not carried out because of low herbage yield for species *M. bracteatum*, *M. gracile* and *V. oblongifolia*. The Capricorn region is frost-prone and thus it was not possible to leave the forages growing for longer than 16 weeks. The harvesting at a fixed stage could have had an impact on both nutritional quality and content of polyphenolics. In general, young herbage is more nutritious than older herbage (Van Soest, 1995) and polyphenolic concentration increases with age (Makkar *et al.*, 1988; Chesselet *et al.*, 1992), though important exceptions exist (Waghorn *et al.*, 1997).

The choice of method of drying forage material before polyphenolic assays has been the subject of debate in the literature (Waterman & Mole, 1994; Reed, 1995; Mueller-Harvey, 2001) because of the potential alteration of tannin structure and content as a result of the method used. Freeze-drying is the method of choice but it is often not practical or feasible. Alternative methods include sun-drying, shade-drying, oven-drying at low (<55 °C) or high temperatures. Sun-drying results in rapid decomposition of phenolic compounds (Okuda *et al.*, 1989) and is discouraged whilst oven-drying at temperatures above 55 °C may cause polymerisation (Waterman & Mole, 1994). Oven-drying using temperatures of 50-52 °C for 48 h has been recommended (Makkar *et al.*, 1995), but compared to shade- and sun-drying it results in increased polymerisation, formation of tannin-protein and tannin-carbohydrate complexes and increased cell wall detergent fibre fractions (Terril *et al.*, 1994; Jackson *et al.*, 1996). Air-drying under shade has been criticised as it requires prolonged time intervals for drying samples during which time the enzymes present in plant leaves may cause polymerisation and oxidation of phenolic compounds (Reed, 1995). Despite this drawback, shade air-drying is the most practical method as it corresponds to the manner in which farmers are likely to manage the tree leaves when using them as feed supplements and is generally recommended (Mueller-Harvey, 2001).



**Figure 2** Absorbance hydrolysable tannin (nm) of tannic acid (TA), gallic acid (GA) and five legume species (*Centrosema pascuorum* (CP), *Lablab purpureus* (LP), *Macroptilium bracteatum* (MB), *Macroptilium gracile* (MG) and *Vigna oblongifolia* (VO))

When evaluating forages, plant yield at harvest is an important element to consider. This work has demonstrated that *L. purpureus* produced good yields of approximately 8 tons/ha DM, which fall within the range of 5 to 14 tons/ha suggested by Mullen (1999) as are adequate yield for a good forage legume. Based on subjective observations of their germination rates and the vigour of their growth, the species, *L. purpureus*, *V. oblongifolia* and *M. gracile* were found to have the tenacity to establish themselves during the summer season in the Limpopo Province.

The feeding of forage to livestock is the most accurate method of forage evaluation. While the accuracy of feeding and digestion trials cannot be denied, they are time consuming and expensive. Chemical and physical analyses have been suggested as alternative ways to determine quality of forage (Van Soest, 1983). Forages with a high packed volume and/or a high water retention capacity are associated with low intake potential because of “rumen-fill effect” (Van Soest, 1995). It has been postulated that legume species with packed volume of less than 2.98 ml/g and water retention of less than 6.45 g/g will have high intakes

(Seoane *et al.*, 1981). From our study only *V. oblongifolia* met the packed volume requirement and none of the species met the water retention criterion. However, Seoane *et al.* (1981) based their estimates on temperate legume species. Higher values may be expected of tropical legumes (Van Soest, 1995). No data relating these physical parameters to intake for tropical legumes were found in the literature and consequently there is a need to establish the relationship between water retention and packed volume on the one hand, and voluntary intake of tropical forage legumes on the other. The measurement of the two parameters is simple and fast and could provide a very robust method of assessing feed value of forages in environments with limited resources for carrying out chemical evaluation of forages.

The CP concentration should be high in the diet in order to meet the requirement of animals. The CP concentration of the five legume species that were evaluated in the current study (213 to 263 g/kg DM) is in excess of that proposed as the minimum requirements for lactation (120 g/kg CP DM) and growth (113 g/kg DM) in ruminants (ARC, 1984). High ADF and NDF concentrations reduce digestibility of plants and hence can affect the availability of protein even in forages with a high concentration of protein (Linn & Kuehn, 1993). For the five legume species evaluated, the level of NDF ranged from 418.3 to 505.8 g/kg DM, which falls within the range where NDF is expected to be digestible (Belyea & Ricketts, 1993) and would therefore not negatively impact on the bio-availability of CP.

Polyphenolics in feeds for ruminants may interfere with intake and digestibility of the feeds and may reduce availability of protein. All species contained low concentrations of total phenolics and consequently the phenolic levels would not be expected to have toxic effects on ruminants. However, it is probably more important from a nutritional perspective to consider tannins in terms of CTs and hydrolysable tannins (HTs), rather than in terms of total phenolics (Mueller-Harvey, 2001). Condensed tannins, also called proanthocyanidins, are polymers of flavan-3-ol monomeric units that are most frequently linked either at C-4/C-8 or at C-4/C-6. They are expressed in a wide range of herbage, especially legumes and browse, and affect animal performance in many environments (Waghorn *et al.*, 1997). At high levels, CTs have negative effects on protein metabolism and decrease palatability of feeds (Barry & Manley, 1986), but at very low levels most are beneficial (Foo *et al.*, 1996). The ideal CT concentration for ruminant nutrition has been suggested to be in the range 20 to 40 g/kg DM, based on the butanol-HCl method (Barry & Duncan, 1984). All species assessed in this experiment had high concentrations of CT (200 to 335 g/kg) compared to the range considered nutritionally safe (Barry & Manley, 1986; Reed, 1995). The high values of CT found in the current experiment have to be interpreted in the context of the fact that they are expressed as leucocyanidin equivalents. Leucocyanidins are monomer units of flavan-3,4-diols and may not have the same capacity compared to complex proteins and other macromolecules as anthocyanidins. The problems associated with the use of external standards in tannin analysis have recently been described in detail by among others Giner-Chavez *et al.* (1997), Mueller-Harvey (2001) and Schofield *et al.* (2001), among others, and will not be repeated here. Tannins from different plants are heterogeneous, chemically distinct and have different degrees of polymerization – all factors that affect the development of colour in the butanol-HCl method. The appropriateness of using a common standard for different varieties is thus questionable, but for convenience of inter-laboratory comparisons this is the accepted standard procedure. However, the results do indicate that *L. purpureus* yields more extractable CTs than the other species.

Adequate animal performance can be achieved even with relatively high levels of CT if the feeds are managed appropriately (Perez Maldonado & Norton, 1996; Woodward & Reed, 1997; Hove *et al.*, 2001). Addition of polyethylene glycol is one management tool to alleviate the effects of harmful tannins on protein utilization (Pritchard *et al.*, 1988; Silanikove *et al.*, 1996; 2001). The binding between PEG of various molecular weight and tannins is more efficient than that between polyvinyl pyrrolidone (PVP) and polyvinyl polypyrrolidone (PVPP) and tannins (Makkar *et al.*, 1995). This suggests that PEG should be preferred to other methods for complexing tannins.

Hydrolysable tannins are esters of a phenolic acid, which can be gallic acid or complex phenolic acids such as hexahydroxydiphenic acid and a polyol such as glucose. Esters of gallic acid are called gallotannins whilst esters of the more complex phenolic acids are called ellagitannins. The HTs have been found to have several physico-chemical properties such as inhibitory effects on various enzymes (Mueller-Harvey & McAllan, 1992). However, because they are hydrolysed in the rumen, their effect on nutrition is believed to be minimal although they are very potent toxicants as the products of their metabolism are absorbable (Mueller-Harvey, 2001). All the forage legumes used in this experiment had low values of HTs, mainly gallotannins, which have a lower sensitivity to potassium iodate and hence a lower absorbance than

ellagitannins (Willis & Allen, 1998) which react faster with potassium iodate, forming a red compound and deteriorate much more rapidly (as represented by the tannic acid curve in Figure 2).

Most of the negative nutritional effects of tannins are due to their ability to precipitate proteins (Reed, 1995) although they also precipitate other macromolecules like polysaccharides (Waghorn *et al.*, 1997) and chelated metal ions (Scalbert, 1991; 1992). Robbins *et al.* (1987) reported that a reduction in digestible proteins of feeds containing tannins was proportional to the protein-precipitating capacity of the plant tannins. This relationship suggested that the use of assays that measure the protein binding and precipitation capacity of plant proteins is more useful in evaluating anti-nutritional effects of tannins than the use of colorimetric methods such as butanol-HCl (Waterman & Mole, 1994). In our study the legumes contained 0.42 to 0.69 g/kg DM of protein binding capacity, which is much lower than the range of 1.2 to 15.6 g/kg DM obtained by Mupangwa *et al.* (2000) for similar forage legumes grown in Zimbabwe. Additionally, in our study the method failed to distinguish between the different varieties ( $P > 0.05$ ).

Dry matter degradability is a measure of the proportion of the legume DM that can be fermented in the rumen if the feed does not pass to the lower digestive tract before maximal degradation occurs (Mupangwa *et al.*, 1997). The degradability values obtained in the current study were higher than the degradability values necessary to provide maintenance requirements for adult ruminants (NRC, 1989; 1996), thus indicating that the polyphenolic and fibre levels of the forages did not negatively affect the nutritive value of the forages.

Forage legumes with high CP concentration and high DM degradability tend to have high intakes (Mero & Uden, 1998). The measured CP concentration and DM degradability were negatively correlated to both packed volume and NDF but positively correlated to one another. This means that both packed volume and NDF are inversely proportional to voluntary DM intake.

Results indicate that forage legumes grown in the Capricorn region of Limpopo Province have good potential as protein supplements to ruminants despite the high temperature and dry conditions that are prevalent in the Province. The practical implications are that smallholder ruminant production systems can benefit from a feed resource plan that includes growing and harvesting of these forage legumes in summer and utilizing them during the dry winter months. At this time of the year crop residues and very mature veld hay, which are low in N and digestibility, are the main feeds available to ruminants and tree leaf supplements can have a profound impact on animal productivity.

## Conclusion

Species *L. purpureus*, *M. gracile* and *V. oblongifolia* had the highest concentration of DM and OM degradability, CP and low levels of fibre, packed volume and water retention compared to *C. pascourum* and *M. bracteatum*. *Lablab purpureus*, *M. gracile* and *V. oblongifolia* adapted well to the agronomic conditions of the Capricorn region of Limpopo Province and did not produce secondary metabolites in quantities that might be detrimental to their use as livestock feed. Furthermore, *L. purpureus* produced large quantities of herbage, making it attractive for utilization by farmers.

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