CHAPTER 2

2 MATERIALS AND METHODS

2.1 INTRODUCTION

Ten forage legume species, which were planted in January 2003, were as follows: *Indigofera arrecta, I. amorphoides, I. viciodes, I. coerula, I. costata, I. trita*, *I. brevicalyx, I. vohemarensis, I. spicata and I. cryptantha*. The plots were 3 m × 1.5 m consisting of three plants rows with a 50 cm space between the rows.

2.1.1 Study location

The study was conducted at the University of Pretoria, Hatfield Experimental Farm in Pretoria, which is at an altitude of 1372 m. The area receives an average rainfall of 674 mm per annum. The average temperatures and rainfall data for the period January, February, March and April of 2003 and 2004 are presented in Table 2.1.

Table 2.1 Average temperature and rainfall for Hatfield Experimental Farm (Supplied by the Weather Bureau of South Africa)

	2003			
	January	February	March	April
Min. Temperature (°C)	17.0	18.2	14.3	11.9
Max. Temperature (°C)	28.9	28.6	28.2	25.8
Rainfall (mm)	32.5	110.8	68.7	0
	2004			
	January	February	March	April
Min. Temperature (°C)	16.9	16.0	15.4	11.6
Max. Temperature (°C)	26.3	25.6	22.2	24.7
Rainfall (mm)	64.8	160.7	168.3	32.8

2.1.2 Sample collection site

In the autumn of 2003 all the species were harvested at the same physiological stage. Five species were harvested in the autumn of 2004 i.e. *I. arrecta, I. cryptantha, I. costata, I. amorphoides and I. viciodes*. The third harvest was the spring re-growth after the winter of 2004, of the same species harvested in autumn 2004. The plants were harvested 10-15 cm from the ground. Only the leaves and stem with a diameter of <3 mm (edible material) were fed dry to sheep. Three plants from each of the five species were randomly harvested separately for chemical analysis.

The total dry matter accumulated for both *Indigofera* species and *Leucaena leucocephala* was 510-600kg which was used for an intake study using sheep. The sun dried plant material was fed to fifteen (15) Merino sheep, three times a day i.e. in the morning, midday, and in the afternoon. They were adapted for 10 days before the actual experiment of 7 days started and fed 2 kg DM/animal/day. The voluntary intake of *Indigofera* species and *Leucaena leucocephala* was then compared with that of *Medicago sativa*.

2.1.3 Sample preparation

All five plant species were dried and milled to pass through a 1 mm sieve size for chemical analysis. The following parameters were determined: dry matter (DM), ash, nitrogen (N), crude protein (CP), neutral detergent fibre (NDF), *in vitro* digestibility (IVDOM) and minerals (Ca, P, Mg, Cu, Zn and Mn).

2.2 Chemical analysis

2.2.1 Dry matter determination

A crucible was cleaned and dried in the oven for an hour. After an hour the crucible was removed and allowed to cool for at least half an hour in a dessicator. The crucible was then weighed to determine the dry mass. One gram (g) of the sample was then weighed in to the crucible. The sample and the crucible were dried for 18-24 hours at 100ºC. The crucible and sample were then placed in a dessicator for half an hour to cool, before weighing (AOAC, 1990).

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The dry matter % was calculated as follows:

$$DM\ \% = \frac{\text{Dry mass (g)}}{\text{Sample mass (g)}} \times 100$$

2.2.2 Ash determination

The crucible with dry sample was placed in a cold incinerating oven and then switched on at 600ºC for four (4) hours. The oven was allowed to cool down for two hours and then placed in a dessicator to cool for another half an hour. The crucible and ash was then weighed. Ash % was calculated as follows:

$$Ash\ \% = \frac{\text{Ash mass (g)}}{\text{Sample mass (g)}} \times 100$$

2.2.3 Neutral detergent fibre determination

The NDF concentration was determined according to Robertson and Van Soest (1981) using the "tector fibertec system". A one gram sample was weighed in a filter crucible and placed in a hot extraction unit, and then a neutral detergent solution (NDS) was added into the crucible and boiled for an hour. Solution was removed by washing with hot distilled water. The residues were dried at 100ºC and then cooled in a dessicator for half an hour and weighed. They were placed in a furnace at 600ºC for three hours to be ashed. The oven was allowed to cool and the crucible with residue was placed in a dessicator to cool.

NDF was calculated as follows:

$$NDF\ \% = \frac{W1 - W2}{W3} \times 100$$

Where: W1= dry mass of sample after NDS extraction

W2= Mass of ash

W3= Sample mass

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2.2.4. Leaf: stem ratio

Subsamples of all five *Indigofera* species which were harvested were used for this aspect. The leaves of each plant species were separated from the stem. The mass of each component was then determined and the dry matter content determined to establish the leaf:stem ratio. The leaf:stem ratio was calculated as follows:

$$\text{Leaf \%} = \frac{\text{Dry leaf weight}}{\text{Dry leaf weight + dry stem weight}} \times 100$$

$$\text{Stem \%} = \frac{\text{Dry stem weight}}{\text{Dry stem weight + dry leaf weight}} \times 100$$

2.2.5 Nitrogen and Crude protein determination

The nitrogen content of pasture sample was determined by macro Kjedahl method (AOAC, 1990), using a block digester and a Tecator kjeltec Model 1002.

$$\text{CP \%} = \text{N\%} \times 6.25.$$

2.2.6 Organic Matter (OM)

The organic matter concentration for calculating the *in vitro* digestibility was calculated as follows:

$$\text{OM} = \frac{\text{DM (g) - Ash (g)}}{\text{Sample mass}} \times 100$$

2.2.7 *In vitro* digestibility of organic matter (IVDOM)

The *in vitro* technique requires rumen fluid which was obtained from rumen fistulated sheep fed with 100% lucerne. The method is based on Tilley and Terry (1963) as modified by Engels and Van der Merwe (1967) with 0.2 g samples being fermented anaerobically with rumen fluid, urea solution, artificial saliva mixture and carbon dioxide

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for 48 hours at 39º C. The tubes were centrifuged at 2500 RPM for 15 minutes and then the clear liquid was decanted.

The dry matter residue was hydrolyzed with 20 ml of HCl and acid pepsin for a further 48 hours. After 48 hours, tubes were centrifuged at 2500 RPM for 15 minutes, then decanted, warm water added and centrifuged and clear liquid decanted as in stage one. The undigested residues were placed in the oven at 100º C for 18 hours. They were then cooled in a dessicator and weighed. The undigested residue was then placed in a furnace at 550º C for three (3) hours, cooled and weighed. A *Panicum maximum* with an IVDOM of 70- 75 % was used as a standard.

IVDOM was calculated as follows:

$$\text{IVDOM (\%)} = \frac{100[\text{OM sample- (OM residue-OM blank)}]}{\text{OM mass of sample}}$$

2.3 Minerals

The following mineral contents in all the samples were analyzed: calcium (Ca), phosphorus (P), magnesium (Mg), copper (Cu), zinc (Zn), and manganese (Mn).
A 0.5g sample was weighed in duplicate and digested in a block digester at 240º C using the wet digestion technique. After the samples had been weighed, 25 ml of Nitric acid was added and tubes placed on a block digester for approximately 10- 15 minutes, and then taken off to cool for 5 minutes.

Then 10 ml of perchloric acid ($HCLO_4$) was added and placed back on the block for another 20 minutes, until it was clear. Allowed to cool and then diluted with 50 ml of distilled water, before being capped into the bottles. Calcium concentration was determined on a 5100PC Atomic Absorption Spectrophotometer, whereas magnesium, copper, zinc and manganese were determined on a GBC 905AA Atomic Absorption

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Spectrophotometer. Phosphorus concentration was determined on a Technicon Auto Analyzer with the concentration determined from calibration curve.

The laboratory standards and controls with known concentration were also used in all the minerals analyzed to get accurate figures. Macro minerals were calculated as follows:

$$\% = \frac{\text{Reading ppm} \times \text{Initial Volume} \times \text{Dilution}}{\text{Sample mass} \times 10\,000}$$

Trace minerals were calculated as follows:

$$\% = \frac{\text{Reading ppm} \times \text{Initial Volume}}{\text{Sample mass}}$$

2.4 Voluntary intake trial

2.4.1 Feeding of experimental animals

A total of fifteen Döhne-merino sheep (wethers) were used. Before the trial, the animals were weighed and starved overnight. The animals were fed a mixture of five *Indigofera* species, which was compared with *Leucaena leucocephala* and lucerne as a control. They were fed *ad libitum*, three times a day at six-hour intervals i.e. 06H00, 12H00 and 18H00 in feed bins and had free access to fresh water. The animals were adapted for ten days in the metabolic house and during this period the voluntary intake was determined. Faichney (1992) suggested that the animals should to be maintained in a steady state by feeding continuously or at short regular intervals during the trial period for the most accurate results. During this period the orts were collected before the next feeding. After the adaptation period, the animals were fitted with faecal bags and kept individually in metabolic cages in the metabolic house for the actual experiment.

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2.4.1.1 Feed sample

Feed samples were taken of the fresh feed offered daily and placed in a plastic bag and frozen. The orts were also taken before the next feeding and frozen for individual sheep. At the end of the experiment the sub-sample (10%) for individual animals was analyzed for DM, ash, and NDF.

2.4.1.2 Faeces sample

The faeces excreted daily by individual sheep was collected in faecal bags, weighed and a 10% grab sample then frozen in a plastic bag at -10ºC. After the trial the faeces for individual sheep were mixed to obtain a representative sample. The initial dry matter content of each sheep was determined by drying 50g faeces sample at 100°C. The other 200g of faeces sample were dried at 60°C and ground through a 1mm sieve for laboratory analysis.

2.5 Statistical analysis

An analysis of variance with the GLM procedure (Statistical Analysis System, 2001) was used to determine the significant differences between different treatments and years for the balanced data. Means and standard deviation (SD) were also calculated. Significance of difference (5%) between means was determined by Bonferroni test (Samuels, 1989). The species and years interactions were also taken into account in the statistical analysis.