THE POTENTIAL OF PODS FROM TREE LEGUMES AS SUPPLEMENT TO LOW QUALITY ROUGHAGES FOR RUMINANTS

by

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A fact of life

" All the good things which an animal likes have the wrong kind of swallow or too many spikes."

A. A. Milne (1928)

ABSTRACT

The goal of the study was to examine the use of pods from tree legumes as supplements to poor quality roughage-based diets. Trials were carried out to address issues related to the nutrient content of the pods and their limitations as supplements due to the presence of anti-nutritional factors.

In the first trial (Chapter 3), the chemical, mineral and amino acid compositions of pods from six tree species were examined. The rumen degradation of the dry matter, nitrogen and cell wall constituents of the pods were evaluated, using the nylon bag technique. In trial 2 (Chapter 4), different rumen ecologies were created in fistulated sheep by feeding pasture hay in combination with different pod meals and alfalfa (50:50), in order to examine the effects of anti-nutritional factors (present in the pods) on the degradation of dry matter and fibre constituents by ruminal microorganisms. Trial 3 (Chapter 5) further examined the effects of anti-nutritional factors on the production of volatile fatty acids (VFAs) and on the activities of microbial enzymes in the rumen. In trial 4 (Chapter 6), ensilage was examined as a means of detoxification of cyanogenic glycosides in the pods of *Acacia sieberiana* and molasses as well urea were evaluated as preservatives that could improve the aerobic stability of the silage produced from the pods of *A. sieberiana*. In trial 5 (Chapter 7), the silage and four other feeds (3 pod meals and alfalfa) were used in a choice feeding trial to study the effect of anti-nutritional factors on the palatability and intake of the feeds by goats and sheep, using hay as the standard feed for comparison. Trial 6 (Chapter 8), evaluated the use of the silage with or without wheat bran as supplements to a roughage basal diet fed to lambs. Intake, digestibility of dry matter, organic matter, cell wall

constituents, nitrogen retention and weight gain were considered as indices for examining the potential of the supplements. Chapter 9 presents a general discussion on the attributes and limitations of pods as supplements. It also leads to a conclusion on the importance of browse in tropics and raises the need for further research on this class of feeds.

The results of the work show that pods from tree legumes are rich in nitrogen and minerals and may provide sufficient ammonia (71-85 mg/l of rumen fluid) in the rumen that could enhance the growth and/or activity of rumen microorganisms. The maintenance of rumen pH by the pod meals at a range of 6.2-6.4 gives an additional advantage over other supplements that contain high concentrations of soluble carbohydrates because cellulolytic activity by rumen microbes is said to be optimal around this pH range. The importance of pods as supplements was however, reduced by the presence of anti-nutritional factors (especially condensed tannins) which had a very high concentration (28%QE) in the pods of *Acacia sieberiana*. The results of the feeding trial showed a positive correlation with the intake of the basal diet and weight gain when pods meals were included in the diet at moderate levels (30% of total dry matter intake). This is an indication that the pod diets were able to maintain a conducive rumen environment for microbial activity and at the same time, provided by-pass protein through the formation of protein-tannin complexes which leave the rumen at near neutral pH but in the abomasum (pH 3-4), the protein was liberated and digested by gastric enzymes to provide amino acids that were utilized by the host animal. However, when the pod meal was increased to 50% of total intake (Chapters 4 and 5), the concentration of condensed tannins in the diet depressed the degradation of cell wall constituents, production of VFAs and reduction in the activity of fibrolytic enzymes in the rumen.

Ensilage was found to be effective in reducing the concentration of cyanogenic glycosides by 80% when the pods of *A. sieberiana* were ensiled for a period of 35 days. The resulting silage was relished by both goats and sheep in a choice feeding trial, an indication that besides the benefit of reducing this important anti-nutritional factor, ensilage enhanced the intake and palatability of these pods. Molasses and urea improved the fermentation process, the nutritive quality and the aerobic stability of the silage.

The important conclusions drawn from the results of this work are that, at low concentration, tannins are beneficial to ruminants by protecting plant proteins from excessive degradation in the rumen thus preventing bloat and increasing the quantity of dietary protein reaching the lower gastro-intestinal (GI) tract. However, at higher concentrations, the effect of condensed tannins is rather detrimental, both to the ruminal microorganisms and the host animal, mainly through their binding effect with proteins and structural carbohydrates and precipitation of both microbial and gastric enzymes, with the net effect of reducing the digestibility of the roughage-based diet.

Further research is necessary to better quantify the concentration of anti-nutritional factors in this class of feeds in order to optimize their utilization by rumen microorganisms and host animal, taking into account the fact that the concentration varies according to the plant species, stage of maturity of the plant and the environment in which the plant is found. It should also be born in mind that the biological effects of different tannins depend on the characteristics of the specific tannin (condensed or hydrolysable), the animal species and possibly the nutritional status of the animal, particularly whether the animal is tannin-naive or tannin-adapted.

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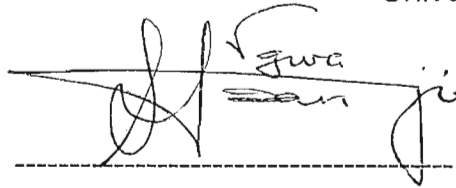
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This thesis is dedicated to my wife

Zita

and my children

Gerald, Adama, Dieudonné and Gloria

DECLARATION

I hereby certify that this research is the result of my own investigation, except as acknowledged herein, and that it has not been submitted for a higher degree in any other University.

Signed

Preface

The experimental work described in this thesis was conducted in the Discipline of Animal and Poultry Science, University of Natal, Pietermaritzburg, South Africa, under the supervision of Dr Nsahlai V. Ignatius, senior lecturer in the department. The thesis consist of six experimental chapters, each of which was prepared as an individual manuscript with the aim of publication in scientific journals. Some repetition in the thesis was therefore unavoidable. While some chapters have been published already, others have been accepted for publication and the rest are at various levels of the review process. Author citation and referencing were however, done in a unique way to give some degree of uniformity to the thesis.

Journal and conference papers from the thesis

Journal papers

1. **Ngwa AT, Nsahlai IV and Bonsi MLK 2002.** The rumen digestion of dry matter, nitrogen and cell wall constituents of the pods of *Leucaena leucocephala* and some Acacia species. *Journal of the Science of Food and Agriculture.*Vol 82: 98-106

2. **Ngwa AT, Nsahlai IV and Bonsi MLK, 2001.** The effect of feeding pods of multipurpose trees (MPTs) on the degradability of dry matter and cell wall constituents of maize stover and alfalfa incubated in the rumen of sheep. *Journal of the Science of Food and Agriculture.* Vol. 81: 1235-1243.

3. **Ngwa AT, Nsahlai IV and Bonsi MLK 2000.** The potential of legume pods as supplements to low quality roughages. *South African Journal of Animal Science* Vol. 30: 107-108.

4. **Ngwa AT, Nsahlai IV and Bonsi MLK, 2001.** Feed intake and dietary preferences of sheep and goats offered hay and pods from tree legumes. *Agroforestry Systems* Accepted for publication on July 10, 2001.

5. **Ngwa AT, Nsahlai IV and Iji P A, 2001.** The effect of feeding legume pods or alfalfa in combination with poor quality hay on microbial enzyme activity and production of volatile fatty acids in the rumen of South African Merino sheep. Small Ruminant Research. Submitted

6. **Ngwa AT, Nsahlai IV and Iji P A, 2001.** Effect of supplementing veld hay with a dry meal or silage from pods of *Acacia sieberiana* with or without wheat bran on voluntary intake, digestibility, excretion of purine derivatives, nitrogen utilization and weight gain in South African Merino sheep. *Livestock Production Science.* Accepted for publication on December 3, 2001.

7. **Ngwa AT, Nsahlai IV and Iji P, 2001.** Ensilage as a means of reducing the concentration of cyanogenic glycosides in the pods of *Acacia sieberiana. Animal Feed Science and Technology.* Submitted.

Conference papers

1. **Ngwa AT, Nsahlai IV and Iji PA, 2001.** Strategies for improving the palatability and nutritive value of the pods of *Acacia sieberiana* for livestock feeding. Agricultural Research Week, South Africa. South African day @ Cedara. Showcase of South African Agricultural Research, 20 May 2001.

2. **Ngwa AT and Nsahlai IV, 2001.** The supplementary value of forage from tree legumes in ruminant feeding. South African Society of Animal Science. KwaZulu-Natal Branch Symposium. Animal Production Research in KwaZulu-Natal Proceedings, 24 July 2001.

3. **Ngwa AT and Nsahlai IV, 2001.** Strategies for improving the palatability and nutritive value of the pods of *Acacia sieberiana.* Paper presented at the monthly seminar in the discipline of Animal Science, University of Natal, Pietermaritzburg, on 22 May, 2001.

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CHAPTER 1

INTRODUCTION

1.1 Background

Seasonal shortages and low nutritive value of available feed resources are considered the most widespread technical constraints to livestock production in the tropics (Minson, 1982a; 'tMannetje, 1982; Topps, 1992; Osuji and Odenyo, 1997; Kaitho, 1997). In most ruminant production systems in the developing world, native or natural pastures make up the bulk of the feed. The low quality and seasonal nature of forage supply, together with low intake by animals and the poor digestibility of forage, are factors contributing to the low productivity of ruminants (Remenyi and McWilliam, 1986).

Improving the nutritive value of low quality roughages through chemical treatment (Adebowale et al., 1989; Orskov, 1989), supplementation with urea (Umunna et al., 1982) or protein nitrogen and energy sources (Alawa et al., 1987; Silva and Orskov, 1988; Orskov, 1989; Steen, 1989) have been practised with success in developed countries. However, these methods have not been successful in African traditional communities because they are expensive and not practical (Meru and Uden, 1990) and conventional feed supplements are expensive and economically out of the reach of most farmers.

Over the past two decades, considerable emphasis has been placed on the role played by legumes in salvaging the problem of feed deficiency in ruminant livestock in particular (Le Houerou, 1980a; D'Mello, 1992; Osuji and Odenyo, 1997). This class of

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plants confers several nutritive advantages in ruminants. Their high protein content relative to grasses is widely recognized (Bonsi *et al.*, 1994; Nsahlai *et al.*, 1995a; Ben Salam *et al.*, 1997; Sawe *et al.*, 1998). Tree legumes in particular are capable of providing forage rich in proteins, vitamins and minerals (Le Houerou, 1980b; Gohl, 1981; Brewbaker, 1986; Ahn et al., 1989) during critical periods of the year when both quantity and quality of pasture grasses are limited. They also serve as an effective means of utilising marginal land on which crop production is ineffective owing to climatic, topographic and edaphic constraints. The Acacias in particular dominate the dry zone of Africa where they serve the rural population with multiple products such as fuel wood, fodder, medicines, fibres and gums and are thus placed among multipurpose trees.

Unfortunately the use of forage (browse) from these trees and shrubs as feed supplements has not been fully exploited in livestock feeding, especially in the small holder system, because their mode of utilization has not been well understood. Browse here refers not only to the tender shoots and twigs of woody plants but also includes fruits and pods. Browse forms an important component of the diet, especially for a variety of herbivores in Asia, Africa and the Pacific (Skerman, 1977; NAS, 1979; LeHouerou, 1980a). In ruminants it provides rumen degradable nitrogen and/or by-pass nitrogen, digestible energy and minerals when used as supplements or sole feed (Ash, 1990; Kaitho, 1992).

About 264 browse species have been documented (Mckay and Fradsen, 1969; Skerman, 1977; Lamprey *et al.*, 1980; Le Houerou, 1980a, 1980b 1980c; NAS, 1980)

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as useful animal fodders. Although not all browses are legumes, more than 200 species of leguminous trees are used as fodder (Kaitho, 1997) and the most commonly used species come from the genera Acacia, Albizia, Calliandra, Desmanthus, Desmodium, Gliricidia, Leucaena, Prosopis and Sesbania (Brewbaker, 1986). Past research work has concentrated mostly on the foliages of these species as feed for ruminants (Jones, 1979; Robertson,1988; Ahn, 1990; Ash, 1990; Goodchild, 1990; Reed et al., 1990; Siaw et al., 1993 ; Bonsi et al., 1995; Nsahlai et al., 1995a). This narrow focus has tended to relegate the important role played by fruits and pods of these tree species in animal feeding. Some reports (Tanner et al.,1990; Nsahlai et al., 1995b) show that Acacia pods tend to have lower values of rumen degradable nitrogen as compared to leaves but registered higher levels of neutral detergent fibre (NDF), NDF-nitrogen, soluble and insoluble phenolics. Jones et al.(1978) stated that the toxic principle, mimosine, is more concentrated in the seeds than in the leaves of L. leucocephala while Steyn (1943), Mitchel-Watt (1957) and Coates-Palgrave (1977) reported high levels of pruccic acid (cyanogenic glycocides) in some Acacia pods which cause toxicity problems in animals. Manifestation of toxicity ranges from severe reduction in feed intake and nutrient utilization to profound neurological problems and even death (D'Mello, 1991). The toxic factors may occur in all parts of the plant but the seed is normally the most concentrated source (D'Mello, 1991). Consequently, most legume grains are toxic to animals if fed without adequate processing. The Acacias in particular are widely known to contain both hydrolysable and condensed tannins (Kuma and Vaithiyanathan, 1990) and their major effect is to reduce fibre digestibility in ruminants.

Despite the presence of these toxic compounds, the Acacia pods still have a high

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nutritive value (Gwynne, 1969; Topps and Oliver, 1978; Nsahlai *et al.*, 1995b; D'Mello, 1991) and could serve as a potential source of protein for diets based on poor quality roughages and cereal crop residues. This is a compelling reason to research on methods of detoxification in order to improve the feeding value of this important feed resource.

Acacia sieberiana was chosen as the centre piece of this work based on the fact that very little is known of this species and its products even though it is widely distributed in Africa, extending from the Sudanian region to Ethiopia, Kenya, Zimbabwe, Zambia, Angola, Botzwana and the Transvaal (Skerman,1988).The size of the pods (10-20 cm long, 2-2.5 cm wide and 1-1.5 cm thick) and the enormous quantity of pods produced per tree gives an additional advantage over other pods.

1.2   Overall Goal

Evaluate the potentials and limitations of pods from tree legumes as supplement to low quality roughages for ruminant livestock.

1.3   Specific objectives

1. Determine the chemical and digestion properties of pods of *Acacia erioloba, A. karoo, A. nilotica, A.sieberiana, A. tortilis* and *Leucaena leucocephala* as protein supplements to roughage-based diets.

2. Study the effects of feeding pods from different tree legumes on the fermentation of dry matter and fibre constituents (NDF, ADF and hemicellulose) in the rumen of sheep given a poor quality roughage-based diet.

3. Study the effect of feeding pods on microbial enzyme activity (fibrolysis and

proteolysis) and production of volatile fatty acids (VFAs) in the rumen of sheep given a poor quality roughage-based diet.

4. Examine ensilage as a means of reducing the concentration of cyanogenic glycosides (an anti-nutritional factor) in the pods of *A. sieberiana* in order to improve the feeding value.

5. Evaluate the effect of condensed tannins and cyanogenic glycosides on the intake and palatability of various pods offered to sheep in a free choice situation.

6. Study the effect of supplementation using legume pods on the intake of a roughage-based diet, nitrogen utilization and productivity (liveweight gain).

The work was divided into six experimental protocols, each of which investigated one of the specific objectives above.

CHAPTER 2

LITERATURE   REVIEW

2.1   Tropical Pastures

In most tropical areas of the world, the natural food of herbivorous domestic animals is

forage from pastures. For a large part of the year, this food forms all or most of the diet.

Most of the pastures are natural grasslands which are not cultivated and consist largely

of a wide range of grasses, legumes and herbs. There are, however, a limited number

of cultivated (improved) pastures which may consist of a single or mixtures of relatively

small numbers of species.

2.1.1 Chemical composition of pasture plants

The chemical composition of the dry matter (DM) of pastures is very variable; for

example, the crude protein content may range from as little as 30 g/kg in mature

herbage to over 300 g/kg in young heavily fertilized grasses (McDonald *et al.*, 1988).

Fibre forms the bulk of most tropical forages and is considered as the sum of cellulose,

hemicellulose (xylans, mannans, glucomannans, arabino-galactans) and pectin and is

broadly related inversely to the crude protein content. Crude fibre may vary from 200

to as much as 400 g/kg in very mature samples. In straws the digestible cell contents

constitute usually less than 250 g/kg of the total dry matter (FAO, 1982) and therefore,

make a minor contribution to the nutritive value. Generally, cellulose content falls within

the range 200-300 g/kg DM and hemicellulose within the range 100-300 g/kg DM

(McDonald *et al.*, 1988). These polysaccharide components increase with the maturity

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of the plant. The lignin concentration increases in the same manner and adversely affects the digestibility of nutrients, except soluble carbohydrates (Akin and Benner, 1988).

Proteins are the main nitrogenous compounds in herbage. Although the total protein decreases with maturity, the relative proportions of amino acids do not alter greatly. Similarly, the amino acid composition of proteins varies little among grass species. This is not surprising as up to half of the cellular protein in grasses is in the form of a single enzyme, ribulose-1,5-biphosphate carboxylase (McDonald *et al.*, 1998). This enzyme plays an important role in the photosynthetic fixation of carbon dioxide. Grass proteins are particularly rich in the amino acid, arginine, and also contain appreciable amounts of glutamic acid and lysine. They have higher biological values for growth compared with seed proteins.

The non-protein nitrogenous fraction of herbage varies with the physiological state of the plant. Generally, the more favourable the growth conditions the higher the non-protein N content and the total nitrogen value, and as the plants mature the contents of both decrease (Jones and Wilson, 1987; Ngwa, 1988). The main components of the non-protein N fraction are amino-acids, and amides such as glutamine and asparagine, which are involved in protein synthesis (McDonald *et al.*, 1998). Nitrates might also be present and considerable attention has been given to the presence of these in pasture herbage because of their toxic effects on farm animals (Holmes, 1989). The nitrate *per se* is relatively non-toxic to animals. The toxic effect in ruminants is caused by the reduction of nitrate to nitrite in the rumen. Nitrites oxidise the ferrous iron of

haemoglobin to the ferric state, producing methaemoglobin, which is incapable of transporting oxygen to the body tissues (McDonald et al., 1998). Toxic symptoms include trembling, staggering, rapid respiration and death.

The lipid content of grasses is comparatively low and rarely exceeds 60 g/kg DM (Leng, 1990; Minson, 1990). The components of this fraction include triglycerols, glycolipids, waxes, phospholipids and sterols. Linolenic acid is the main fatty acid, composing between 60 and 70% of the total fatty acids present, with linoleic and palmitic acids the next most abundant (McDonald et al., 1998).

The mineral content of tropical pastures is very variable, depending on the species, stage of growth, soil type, cultivation conditions and fertilizer application (Whiteman, 1980). The concentration of Ca in dry grass varies from 1.5-3.0 g/kgDM while that of P is less than 1 g/kgDM (Le Houerou, 1978). The ash fraction of most straws in particular, is made up mainly of silica and most essential minerals (Ca, P, Mg, Na) and trace elements (Cu, Mn, Cu, Mn, Fe, Zn) are either absent or present in very low concentrations (McDonald et al., 1998).

Green forage, however, is an exceptionally rich source of carotene, the precursor of Vitamin A, and quantities as high as 550 mg/kg may be present in the dry matter of young green crops (Butler and Bailey, 1973; McDonald et al., 1998). Herbage of this type supplies about 100 times the requirement of a grazing cow when eaten at voluntary intake levels.

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2.1.2 Physico-chemical factors that characterise and determine the nutritive value of tropical forages

In the tropics, the quality of forage at the beginning of the wet (rainy) season is high but because of high temperatures, rapid physiological maturation takes place leading to early lignification with the protein and phosphorus contents falling to very low levels and the fibre content rising (Ngwa, 1988; Becker and Lohrmann, 1992; Nyamangara and Ndlovu, 1995; McDonald *et al.*, 1998). Lignification is the major cause of the resistance by forage fibre to mechanical and microbial degradation in the rumen and this explains the long retention time of tropical forages in the rumen. Long retention time facilitates rumen fill and consequently, decreases feed intake (Thorton and Minson, 1973; Aitchison *et al.*, 1986). Other physical characteristics of tropical forages include the presence of the cuticle, waxes, suberins and hairs on plant cell walls.

The plant cell wall has been shown to be the primary restrictive determinant of forage intake (Van Soest, 1994). Tropical and sub-tropical forages are more stemmy and have more cell wall than temperate species (Meissner, 1997). This results in low digestibility, slow fermentation rate and particle size reduction which slows down the passage rate of residue from the rumen, and thereby reduces intake (Minson, 1982a; Meissner, 1988). Cell wall constituents that have been shown to be correlated with intake in South Africa include NDF (Meissner *et al.*, 1991a), ADF (Cilliers and Van der Merwe, 1993) and acid detergent lignin (ADL) (Pietersen *et al.*, 1993). Meissner *et al.* (1991b) reported that intake was limited above NDF concentrations of 550-600g DM but not below. Non-cell constituents that limit the intake and digestibility of tropical and sub-tropical forages include phenolics (ferulic, deferulic, p-coumaric acids and vanillin)

acetyl terpenoids, flavonoids, alkaloids and aromatic compounds (Meissner and Paulsmeier, 1995), low DM content (Meissner et al., 1992), high ash content (Meissner and Paulsmeier, 1995) and poor N to energy ratio limiting microbial protein production (Meissner et al., 1993).

Most temperate grass species belong to the C3 category of plants in which the 3-carbon compound, phosphoglycerate, acts as an important intermediate in the photosynthetic fixation of carbon dioxide (Hatch and Slack, 1970). The tropical species on the other hand follow the C4 pathway of photosynthesis in which carbon dioxide is first fixed in a reaction involving the 4-carbon compound, oxalate. The low protein and sulphur contents often found in tropical grasses are an inherent characteristic of C4 plant metabolism (Egan, 1986) which is associated with survival under conditions of low soil fertility. In temperate grasses, fructans are the main storage carbohydrates while in tropical species, these are replaced by starch.

Another factor of nutritional importance is that the mesophyll cells in the leaves of tropical grasses are more densely packed than those in temperate grasses (McDonald et al., 1988) and intercellular air spaces represent only 3-12 percent of leaf volume compared with 10-35 percent in temperate species. This might partly explain why tropical grasses have a higher tensile strength than the temperate ones, a feature which results in both a slow mechanical and microbial degradation in the rumen and low voluntary dry matter intake by ruminants consuming these plants. Tropical leguminous species however, differ from the grasses in that they are able to fix atmospheric nitrogen (N) which in time might become available to the host plant, companion grasses, soil

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organic matter and to grazing animals (Whiteman, 1980). The fixation of atmospheric N is most probably responsible for the difference in chemical composition between tropical legumes and grasses as elucidated in the next section.

2.2   Tropical forage legumes

2.2.1 Chemical composition and nutritive value

A large number of legumes have been documented as useful livestock fodder. The main features of forage legumes are their high contents of soluble dry matter, crude protein and mineral content (Whiteman, 1980). The crude protein percentages reported for tropical legumes vary from 5.6 for a cut of *Stylosanthes humilis* (Newman, 1968) to 35.8 for the leafier parts of *Leucaena leucocephala* (Hutton and Bonner, 1960), with a mean crude protein percentage of 17.2 (Skerman *et al.*, 1988) for all legumes. These levels of crude protein are much higher than those found in tropical grasses, for example, the mean protein content for the samples reviewed by Butterworth (1967) was 7.7 percent for good quality hay.

As tropical legumes mature, there is usually a decrease in the crude protein percentage (Milford, 1967; Milford and Minson, 1968; Fisher, 1969). The apparent digestibility has also been measured by several researchers (Miller *et al.*, 1964; Peixoto *et al.*, 1966; Milford, 1967; Minson and Milford, 1967; Newman, 1968) and the values vary considerably, but this variation is mainly associated with the level of crude protein in the legume and other factors like genetic make-up, environment and crop management (Nsahlai *et al.*, 1998d). Crude fibre percentages in legumes range from 12.4 for *L.*

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leucocephala (Farinas, 1970) to 43.4 for *Macroptilium lathyroides* (Milford, 1967) with a mean of 30.6 percent. This value for tropical legumes is slightly lower than the mean value of 33.4 percent for tropical grasses reported by Butterworth (1967). The crude fibre content tends to increase with increasing maturity and decreasing dry matter digestibility. Where facilities are not available for the *in vivo or in vitro* determination of digestibility, the level of crude fibre (CF) can provide a rough estimate of the dry matter digestibility, using the regression equation proposed by Skerman *et al.* (1988):

DM Digestibility $= 84.5 - 0.94CF$ $r = -0.79$ $RSD = \pm 4.1$

More recently, Nsahlai *et al.* (1998b) proposed the following regression equation for apparent digestible nitrogen (ADN): $ADN = -4.5(0.63) + 0.87(0.051)N$ ($n = 39$; Adj-R^2 = 0.89; $p < 0.0001$) where N is the nitrogen content of the feed. The equation suggests a metabolic faecal nitrogen (MFN) loss of 4.5 g/kgDM which lies within the range 4.3 - 5.4 reported in a previous investigation (Wiegand *et al.*, 1996).

$MFN = 0.15(0.359) + 0.012(0.00082)FDMO$ ($n = 39$; Adj-R^2 = 0.75; $p < 0.0001$) where FDMO is faecal DM output (Nsahlai *et al.*, 1998b).

An interesting empirical prediction equation of forage intake was developed by Meissner and Paulsmeier (1995). They showed that intake of non-lactating ruminant species feeding on grasses, legumes, hays, silages, shrubs and even supplemented forages could be predicted with the relationship from the ratio between *in vitro* digestibility of organic matter (IVDOM) and NDF:

OM intake (g/kg.W $^{0.9}$/day) $= 70 - 97^{-0.975(IVDOM/NDF)}$

$r = 0.82$; Error of estimate 5 g/kg.$W^{0.9}$/day

Two aspects feature prominently in the equation: the body mass of the animal is raised

to the power 0.9 instead of the usual 0.75. The authors justified this with the reason that the intake of most forages was controlled by a combination of physical limiting factors and chemical constituents sensed in the blood or at the tissue level. Secondly, they also added that the law of diminishing returns should feature, as intake will be severely limited at low ratios of IVDOM to NDF (ie low digestibility and high cell wall). An advantage of the equation is that it can be used to distinguish between cell wall-induced and other limiting factors. It also complies with the notion that feed intake of forages is controlled by physical constraints, primarily rumen fill and the rate of removal of digesta from the rumen (Roux and Meissner, 1984). Removal of digesta is a function of fermentation rate and the rate of outflow from the rumen. A mechanistic model approach to predict intake therefore, would have to include these rates in a combined equation.

2.2.2 Forage from tree legumes: attributes and limitations to utilization by ruminants

Browse or forage from tree legumes is defined as the leaves, shoots and sprouts including tender twigs and stems of woody plants which are cropped to a varying extent by domestic and game animals (Gutteridge and Shelton, 1994). This definition could be extended to include fruits, pods and seeds which are often more valuable than the foliage, especially if the tree is deciduous. Compared to herbaceous legumes, browse legumes are easy to establish and sustain and therefore ecologically more appropriate. At least 75% of trees and shrubs of Africa serve as browse plants to a certain extent and many of these are leguminous (Le Houerou, 1980a). Such trees and shrubs have been regarded as sources of reserve feed for use in drought or prolonged dry season conditions.

The importance of browse in the diet of herbivores is reflected in reports from Africa, Latin America, Asia and Australia. For example, in northern Africa, browse constitutes 60-70% of rangeland production and 40% of the total available animal feeds in the region (Le Houerou, 1980c; Kaitho, 1997). In India, browse is the principal feed for goats and meets over 60-70% of forage requirements (Devendra, 1995) while in the Shrub Steppes of southern Australia, it constitutes 100% of the sheep's diet (Wilson and Harrington, 1980).

The beneficial effects of feeding browses to ruminants include increased metabolizable energy intake, increased nitrogen intake, better animal performance and feed efficiency, increased availability of minerals and vitamins, improved rumen function and a laxative influence on the alimentary system (Kaitho, 1997). In this context, forage from tree legumes is often used as a buffer to overcome feed gaps that arise from seasonal fluctuations in the productivity of other fodder resources. For example during the dry season or in times of drought, trees provide green feed that is rich in proteins, minerals and vitamins while the herbaceous cover provides only poor quality straw. Some differences in the nutritive value of browse and dry grass during the dry season in the Sahel are presented in Table 2.1 to elucidate the importance of browse supplementation during such periods. The capacity of browse in the provision of these nutrients is however limited by the presence of a diverse array of natural compounds (anti-nutritional factors) which are not directly involved in the process of plant growth, but act as deterrents to attack by bacteria, fungi, insects, animal and even man (Brewbaker, 1986).

Table 2.1 Comparison of feed value of dry grass and browse during the dry season in the Sahel (Le Houerou, 1978) and requirements for growing lambs

	Net Energy (MJ kg^{-1} DM)	Digestible prot (g kg^{-1} DM)	P (g kg^{-1} DM	Ca (g kg^{-1} DM)	Carotene (mg kg^{-1} DM)
Dry grass hay	2.5-3.4	≤ 1	≤ 1	1.5-3.0	≤ 1
Browse	1.7-2.9	50-300	1.5-2.5	2.5-20.0	50-800
Maintenance needs	2.9	50	1.3	2.5	1.5

These compounds are capable of producing toxic effects in animals (cyanide, fluoro-acetate); depress intake and/or utilization of feed components (high tannin content) or may enhance feed nutritive value (anti-protozoa properties) (Bird, 1991; Leng et al., 1992; Odenyo et al., 1997). Manifestation of toxicity in animals may range from a marked reduction in animal performance and nutrient utilization to profound neurological effects and increased mortality (D'Mello, 1992). The following sections of this review highlight the major advantages obtained from browse utilization in the animal industry, identify the primary anti-nutritional factors in tropical legumes and evaluate the prospects of their detoxification.

2.2.3 Chemical composition of browses

The chemical composition of browses has been reported by various authors in different parts of the world: Africa (Rose-Innes and Mabey, 1964; Le Houerou, 1980b; Nsahlai et al., 1995a), Asia and Pacific (Devendra, 1995), Australia (Wilson, 1977) and other developing countries (NRC, 1981, Kearl, 1982). A summary of the chemical composition of a selected range of browse species is presented in Table 2.2. From the table it is

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Table 2.2 The chemical composition (g / kg DM) of leaves and pods of some browse species

Species	CP	Ash	NDF	ADF	Lignin	Tannin	References
Acacia albida (pod)	143	27	374	279	45		Tanner et al., 1990
Acacia aneura	110	35	511	396	206	70	Goodchild, 1990
	114	48	498	194			Leche et al., 1982
Acacia mangium	120		619	610	422		Blair et al., 1988
Acacia nilotica (pod)	130	49	316	225	53		Tanner et al., 1990
Acacia saligna	128		573	429	207		Nsahlai et al., 1995a
Acacia sieberiana (pod)	127	52	370	282	58		Tanner et al., 1990
Acacia seyal	206		228	172	69		Reed et al., 1990
Acacia tortilis (pod)	136	47	324	242	48		Tanner et al., 1990
Albizia chinensis	263	46	603	348	145	33	Robertson, 1988
Cajanus cajan	158	55	314	292	100		Bamualin et al., 1980
Calliandra callothyrus	212	42	259	209	96	111	Robertson, 1988
	173	40	302	229	84	96	Ahn, 1990
Chamaecytisus palmensis	178		342	235	64		Nsahlai et al., 1995a
Desmanthus virgatus	146	85	256	195	91		Bamualin et al., 1980
Enterolobium cyclocarpum	168		361	296	141		Blair et al., 1988
Erythrina bentipoene	155		495	393	65		Nsahlai et al., 1995a
Erythrina variegata	175		532	425	68		Nsahlai et al., 1995a
Gliricidia sepium	150	55	272	212	55		Bamualin et al., 1980
	275	60	255	216	41		Robertson, 1988
Leucaena diversifolia	308	75	246	114	41		Siaw et al., 1993
Leucaena leucocephala	294	83	216	104	32		Siaw et al., 1993
	258	69	309	234	87	55	Good child, 1990
	267	57	312	226	99	37	Robertson, 1988
Leucaena pallida	206		420	245	105		Nsahlai et al., 1995a
	326	73	211	113	38		Siaw et al., 1993
Leucaena pulverulenta	227	87	192	107	37		Siaw et al., 1993
	269		405	234	93		Nsahlai et al., 1995a
Sesbania sesban	356	94	203	108	24		Siaw et al., 1993
	241		206	141	25		Bonsi, 1996
Vernonia amygdalina	148		312	277	61	47	Bonsi, 1996

seen that crude protein (CP) varies from a low of 110 g kg^{-1} for *Acacia aneura* to a high of 356 g kg^{-1} for *Sesbania seban* with an average of 239 gkg^{-1} for all browse legumes (Devendra, 1995). As it is the case with most grasses, the CP of most tropical browse legumes decreases with maturity (Milford, 1967; Milford and Minson, 1968). The neutral detergent fibre (NDF) varies from 192 g kg^{-1} for *Leucaena pulverulenta* to 656 gkg^{-1} for *Gliricidia sepium* with an average of 296 g kg^{-1}, a figure which is less than the value of 334 gkg^{-1} reported for tropical grasses (Butterworth, 1967). The acid detergent fibre (ADF) varies from 104 g kg^{-1} for the leaves of *L. leucocephala* to 610 g kg^{-1} for *A. mangium*. The ash content and concentration of lignin vary from 35 to 94 g kg^{-1} and 25 to 422 g kg^{-1} respectively. The CP content of 11-36% is quite high compared to 3-10% for mature grasses. The high ADF and lignin contents are an indication that browse legumes have low levels of hemicellulose, an assertion supported by Topps (1992). The high variability in chemical constituents could be attributed to the stage of maturity of the plant, plant part, harvesting regimen, leaf-stem ratio in the forage, season, location and type of the browse plant.

Table 2.3 highlights some differences in chemical composition between the leaves and pods of some Acacia species, examined by different researchers in separate locations. A general tendency is for leaves to have higher concentrations of crude protein, ash and NDF than pods which are richer in crude fibre. The table also shows high variability in chemical composition within the same species found at different locations and this may partly be due to the age of the plant and/or harvesting regimen. This variability can affect the nutritive value of the plant, ruminal microbes and the host animal (D'Mello, 1992). The nutritional differences can also be attributed to the concentration of anti-

Table 2.3 Comparison between the chemical composition of leaves and pods of some Acacia species

Species	Plant part	Chemical composition g / kgDM							Reference
		Ash	CP	CF	NDF	ADF	Sol. Phen	Insol. Phen	
A. albida	Leave	57	147	210					Le Houerou, 1980
	Pod	32	115	234	551				Verboom, 1965
		22	143		374	279	365	27	Tanner et al., 1990
		39	131		427		270	24	Nsahlai et al., 1995b
		44	113		420		⟩		Fall-Toure et al., 1998
A. brevispica	Leave	65	188	180	511				Sawe et al., 1998
	Pod	46	175	240	528				Dougal & Bogdan, 1958
		39	214	260	511	411			Sawe et al., 1998
A. nilotica	Leave	87	224	202					Le Houerou, 1980
	Pod	49.2	130		316	225	436	89	Tanner et al., 1990
		35	135	128	227	207			Sawe et al., 1998
A. nubica	Leave	89	325	158	412				Dougal & Bogdan, 1958
	Pod	75	151.7	374	380				Dougal & Bogdan, 1958
A. polycantha	Leave	56	155	169	605				van Rensburg, 1968
	Pod	79	104	378	431				van Rensburg, 1968
A. senegal	Leave	87	224	202					Le Houerou, 1980
	Pod	53	170	296	433				Wilson & Bredon, 1963
A. sieberiana	Leave	67.6	211	247	426				Verboom, 1965
			215		412	340	83	62	Nsahlai et al., 1995a
	Pod	51	127		370	282	406	37	Tanner et al., 1990
		55	163	165					Kibon & Maina, 1993
		52	128		427		327	50	Nsahlai et al., 1995b
A. tortilis	Leave	10	108	245					Dugmore & du Toit, 1988
	Pod	47	136		324	242	373	31	Tanner et al., 1990
		42	134		425		240	21	Nsahlai et al., 1995b

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nutritional factors (secondary compounds) and form (fresh or dried) at the moment of feeding (Burns, 1985; Mahyuddin *et al.* 1988; Palmer and Schlink, 1992; Bonsi *et al.*, 1995; Bonsi, 1996).These factors determine their chemical composition, palatability, intake, the extent and rate of degradation, digestibility and nutrient utilisation by ruminants fed predominantly low quality roughages. The screening of browses as sole feed or supplement based on their chemical composition by qualitative methods may therefore, lead to some erroneous conclusions if not supported by feeding trials.

2.2.4 Mineral composition

The information on mineral composition of browses is presented in Table 2.4 but is rather sketchy particularly for local species. There is little information on trace elements (Cu, Mn, Zn, Co, I) and only fragmentary data on macro-elements. However, browses are generally high in Ca though P levels are low (Le Houerou, 1980a, Lamprey *et al.*, 1980; Brewbaker, 1986; Bonsi, 1996).Ca levels vary from 1.0 to 37 gkg^{-1}DM and thus are higher than in tropical grasses. Contents of P vary from 0.7 gkg^{-1}DM for *A. aneura* (Everist, 1969) to 39 gkg^{-1}DM for whole pods of *A. senegal* (Wilson and Bredon, 1963).These concentrations decrease with increasing maturity of the plant (Fisher, 1969) and are also influenced by fertilizer application (Shaw *et al.*, 1966). It is therefore important to define the agronomic background of the samples tested to enable valid comparisons within legumes. Mg and K are also found in excess requirements in browses and are seldom a limiting factor in ruminants (Kaitho, 1997). Cases of Na deficiency have been reported but ruminants counteract this deficiency by conserving tissue Na (saliva) through recycling in the rumen (Norton, 1994).

Table 2.4   The concentration of minerals (g/kgDM) in the leaves of some browse species

Species	Minerals							Reference
	N	S	P	N:P	K	Ca	Ca:P	
Acacia aneura	24.9	1.2	1.3	20.8				Ahn et al., 1989
	20	1.4	0.8	25				Entwistle & Baird, 1976
Acacia anguistisima	36	1.4	1.3	24.7				Ahn et al., 1989
Acacia albida	23.5		1.8	13.1	12.5	9.2	5.11	Le Houerou, 1980a
	31.5		2.3	13.7		10	4.3	Gohl, 1981
Albizia chinensis	42.2	1.8	2	21.1				Ahn et al., 1989
	42.1	1.8	1.9	22.2				Robertson, 1988
	24.2		1.4	17.3		11.7	8.4	Gohl, 1981
Albizia lebbeck	38.5	2	1.6	24.1				Ahn et al., 1989
	46.7		2	23.4		18	9	Brewbaker, 1986
Calliandra calothyrsus	36.8	1.9	1.5	24.5				Ahn et al., 1989
	33.9	2	1.4	24.2				Robertson, 1988
Cajanus cajan	25.3		2.2	11.5				Bamualin et al., 1980
	34.2		2.4	14.3		8.9	3.7	Gohl, 1981
Chamaecytisus palmensis	34		1.4	24.3	5.3	4.5	3.2	Borens & Poppi, 1990
	29.5	1.5	1.2	24.6	5.9	10.5	8.8	Bonsi, 1996
Desmanthus virgatus	23.4		1.9	12.3				Bamualin et al., 1980
Enterolobium cyclocarpum	40	2.7	1.7	23.5				Ahn et al., 1989
	26.9		1.4	19		6.7	4.8	Gohl, 1981
Gliricidia sepium	44.2		1.9	23.3	27.5	11.9	6.3	Chadhoker, 1982
	41.8		2.8	14.9	33.1	10.5	3.8	Carew, 1983
Leucaena leucocephala	42	2.2	1.6	26.3				Ahn et al., 1989
	32.5		1.6	20.3	13	23	14.4	Brewbaker, 1986
	40.6	2.3	1.8	22.6	11.4	20	11.1	Bonsi, 1996
Sesbania grandiflora	55.7		3.3	16.9		23.3	7.1	Brewbaker, 1986
	41.5		4.7	8.8		13.2	2.8	Gohl, 1981
Sesbania sesban	42.2	2.7	2.4	15.6				Ahn et al., 1989
	38.5	2.1	2.7	14.6	14.6	17.4	6.4	Bonsi, 1996
Vernonia amygdalina	23.7	2.2	0.7	33.9	21.1	11.7	16.7	Bonsi, 1996

N, nitrogen; S, sulphur; P, phosphorus; Ca, calcium; K, potassium

2.2.5 Voluntary intake of browse

The nutritive value of any feed depends on the voluntary intake of that feed and on the extent to which its dry matter (DM) can supply dietary energy, protein, minerals and vitamins when eaten by the animal. Many factors influence the intake of browse fodder but the most important are the physical characteristics (hairiness, presence of waxes, bulkiness, height of the foliage layer) and the presence of chemical compounds which may influence taste and palatability e.g. volatile oils, soluble carbohydrates and anti-nutritional factors. Considerable variation exists in dry matter intakes between provenances, families within provenances and individual plants within the same family in some browse legumes such as G. sepium, and this is reflected in the range of reported intake, 30.2 to 63.3 g $kg^{-0.73}$ (Kass et al., 1992). Based on maintenance requirements of cattle, sheep and goats (Devendra, 1995), browse, with an energy value which varies from 2.95 to 5.31 MJ ME kg^{-1} DM, may not as sole feed, ensure the maintenance requirements of cattle (6.02 MJ ME kg^{-1} DM). However, it can ensure the maintenance of sheep (5.17MJ ME kg^{-1} DM) without providing for production; while in goats, maintenance and production may be provided on a pure browse diet (4.72MJ ME kg^{-1} DM). The data explains why only goats, camels and some wild herbivores can survive on depleted rangelands, where browse constitutes most of the feed. It also explains why goats and camels are less affected by catastrophic droughts in the Sahel of Africa, compared to sheep and cattle (Le Houerou, 1978).

Browse however, is seldom used exclusively. In most situations, its practical use is as a supplement to enhance the intake and utilization of other fibrous crop residues like cereal straws and hays, and thus meets the requirements for maintenance and variable levels of production (Kaitho, 1992; Oosting, 1993).

2.2.6 Browse as feed supplement

The aim of supplementation is to use judicious amounts of one feed or feed additives to optimize the utilization of the least expensive material and increase productivity. Among other factors, feed supplements should be palatable, provide rumen degradable protein, maximize the outflow of microbial protein from the rumen, provide by-pass protein to augment the supply of amino acids from microbial protein, increase energy intake and increase the efficiency of absorption of nutrients in the intestines. It should also enhance the intake of the basal diet or at least maintain its intake (McMeniman *et al.*, 1988). The value of browses as supplements is mainly dependent on their capacity to provide nutrients that are deficient in the basal diet. This includes their ability to provide essential nutrients to the rumen microbial population and/or critical nutrients to meet the host animal's requirements thus increasing the efficiency of feed utilization (Elliot and McMeniman, 1987). There is extensive literature on the effects of leguminous browse supplementation on the productivity of cattle, sheep and goats. Some of the results are summarized in Table 2.5. It is seen that in all cases where the basal diet was supplemented with browse, there was an improvement in voluntary dry matter intake, dry matter digestibility and liveweight gain irrespective of the nature or type of the basal feed.Feeding of non-supplemented roughages such as maize stover, rice straw and teff straw resulted in liveweight losses in sheep and cattle while others like pasture hay

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Table 2.5 The effects of supplementation with forage from tree legumes, on the intake of low quality forages and productivity of cattle, sheep and goats

Browse	Plant part	Animal	Basal diet	Voluntary intake (gkg^{-1}LWT)		DMD (%)	LWG (g d^{-1})	Reference
				Browse	Basal			
L.leucocephala	Leaf	Goat	M.stover	0	10.3	46		Banda and Ayoade, 1986
	Leaf	Goat	M.stover	5.5 D	15.8	51		Banda and Ayoade, 1986
	Leaf	Sheep	M. stover	0	24.6	41.7	-44	Goodchild, 1990
	Leaf	Sheep	M. stover	5.9 D	32.8	46.7	85	Goodchild, 1990
	Leaf	Cattle	Grass	0	20.2	42	-20	Wahyuni et al., 1982
	Leaf	Cattle	Grass	5.2 D	26.1	44	290	Wahyuni et al., 1982
	Leaf	Cattle	Rice straw	0	13.6	18.3		Moran et al., 1983
	Leaf	Cattle	Rice straw	6.8 D	15.9	40.3	37.6	Moran et al., 1983
	Leaf	sheep	Teff straw	0	28.2	49	-22.8	Bonsi et al., 1996
	Leaf	sheep	Teff straw	10.7 D	30.2	52.1	30.9	Bonsi et al., 1996
Gliricidia sepium	Leaf	Goat	0	32.6 F	0	56.3	60-75	Murugan et al., 1985
	Leaf	Sheep	0	33.9 F	0	50.1	39	Murugan et al., 1985
	Leaf	Cattle	Rice straw	0	27	47	-113	Doyle et al., 1986
	Leaf	Cattle	Rice straw	11.0 D	22	55	10	Doyle et al. 1986
Calliandra	Leaf	Sheep	B straw	6.8 F	14.5	36.3		Ahn, 1990
calothyrsus	Leaf	Sheep	B straw	6.8 D	22.9	59		Ahn, 1990
Sesbania sesban	Leaf	sheep	Teff straw	10.7 D	52.4	52.4	26.5	Bonsi et al., 1996
	Leaf	Goats	Pasture	0	39.6	60.9	49.6	Sawe et al., 1998
Acacia nilotica	Pod	Goats	Pasture	8.1 D	34.8	65.7	65.5	Sawe et al., 1998
A. tortilis	Pod	Goats	Pasture	8.1 D	30.2	56.7	68.5	Sawe et al., 1998
A. brevispica	Pod	Goats	Pasture	8.1 D	37.8	58.9	56	Sawe et al., 1998
A. browni	Leaf	Goats	Pasture	8.1 D	32	40.3	61.6	Sawe et al., 1998
B. discolor	Leaf	Goats	Pasture	8.1 D	35	61.9	59.1	Sawe et al., 1998
A. tortilis	Pod	Sheep	M. stover	9.8	19	58	32	Tanner et al., 1990
A. albida	Pod	Sheep	M. stover	9.2	18	55	21	Tanner et al., 1990
A. nilotica	Pod	Sheep	M. stover	9.7	17	59	16	Tanner et al., 1990
A. sieberiana	Pod	Sheep	M. stover	10.1	15	53	4	Tanner et al., 1990

F, fresh; D, dry: M, Maize; B, Barley

elicited modest levels of gains. It is therefore possible that in order to meet requirements for a given level of productivity, the amount of supplement is dependent on the quality of the basal roughage.

Leucaena and Gliricidia, in particular have been used as supplements to a wide range of forages and agricultural by-products (Hegarty et al., 1964; Jones , 1979; Jones and Megarity, 1986; Bamualin et al., 1984a,b; Bamualin, 1986; Van Eys et al., 1986; Reynolds and Adediran, 1987; D'Mello and Acamovic, 1989; Vadiveloo, 1989; Tangendjaja et al., 1990; Adejumo and Ademusun, 1991; Girdhar et al., 1991; Bonsi et al., 1994; Richards et al., 1994; Abdulrazak et al., 1997). These species grow very easily. Jones (1979) reported that the leaves of Leucaena were comparable with lucerne leaf material in terms of protein and minerals as well as yield. However, the leaves and pods of other browse species have also been implicated in ruminant feeding, either as supplements to low quality roughages or as a means of enhancing the rumen degradability of the basal diet. Examples are: Sesbania sesban (Khalili and Varvikko, 1992; Bonsi et al. 1994; Shahjalal et al. 1994; Umunna et al., 1995; Wiegand et al., 1995; Odenyo et al., 1997), foliage from Acacia spp (Woodward and Reed, 1989; Reed et al., 1990; Ben Salem et al, 1994, 1997; Newbold et al., 1994; Chriyaa et al., 1997; Newbold et al., 1997; Osuji and Odenyo, 1997), pods of Acacia spp (Dugmore et al., 1988; Ibeauchi and Adamu, 1990; Tanner et al., 1990; Kibon and Maina, 1993; Fall-Toure et, 1998; Sawe et al., 1998), Chamaecytisus palmensis (Borens and Poppi, 1990; Bonsi et al., 1995; Nsahlai et al., 1998b), Calliandra calothyrsus (Ahn, 1990; Kaitho et al., 1993), just to mention a few. These studies all show that browse plays an important role in the provision of critical nutrients in the nutrition of herbivores.

2.3   Limitations to the nutritive value of browses

2.3.1 Anti-nutritional factors (ANFs)

Many plants produce chemicals which are not directly involved in the process of plant growth, but act as deterrents to bacteria, fungi, insect and animal attack thus ensuring survival and dissemination of the plant species (Norton, 1994). Although some of these compounds are known to evoke an immediate violent reaction, much more subtle effects are commonly noticed due to prolonged ingestion. Such effects might include reduction in feed intake, diminution of digestive process or utilization of nutrients resulting to a decrease in growth, a goitrogenic response or damage to vital organs. Therefore, these are termed "anti-nutritional factors" (ANFs) or deleterious substances. The effect of an ANF is not an intrinsic characteristic of the compound but depends on the digestive process of the ingesting animal species. Non- ruminants (pigs, poultry and horses) are usually more susceptible to toxicity than ruminants. In this regards, Kumar (1992) defined ANFs as those substances generated in natural feedstuffs by the normal metabolism of the species and by different mechanisms which exert effects contrary to optimum nutrition.

The nature and action of ANFs in plants have been the subject of several reviews (Duke, 1977; Rosenthal and Jansen, 1979; Hegarty, 1982; Reed *et al.*, 1985; Seawright *et al.*, 1985; Barry and Blaney, 1987; Muller-Harvey *et al.*, 1987; D'Mello and Acamovic, 1989; D'Mello, 1992) . Table 2.6 provides a summary of compounds which have been found in tree legumes species which may affect animal productivity. The list is not exhaustive and some compounds listed may not be toxic. Most anti-nutritional factors

belong to a group of related compounds with similar mode of action. There are about 8000 phenols, 270 non-protein amino acids, 32 cyanogens, 10000 alkaloids and several saponins which have been reported to occur in plant species (Kumar, 1992). Information on toxic substances in browses is well documented (Reed et al., 1985; Reed, 1986, Muller-Harvey et al., 1987; D'Mello and Acamovic,1989; Ash, 1990; D'Mello, 1995). The effect of these toxins on animals may depend on the pre-feeding treatment applied (Ahn et al., 1989), age of the plant foliage, site of the harvest or location (D'Mello and Acamovic,1989) and the plant part fed (leaves or pods). The effects of these compounds becomes more pronounced when browse constitutes a high proportion of the diet.

The Acacia species generally have low nutritive value due to the limitation imposed by anti-nutritional factors and as sole feed, they hardly meet the maintenance requirements (Tanner et al., 1990; Kibon and Maina, 1993; Nsahlai et al., 1995b; Kaitho, 1997) of livestock. McMeniman (1976) showed that sheep fed Mulga (Acacia aneura) responded to the addition of urea to their diet even though the diet contained more than the minimum level of crude protein. Sheep fed polyethylene glycol (PEG) in addition to mulga (Pritchard et al., 1988) showed marked increases in intake, weight gain and wool growth. The low response by sheep to supplementation with mulga is therefore related to its content of condensed tannins and their capacity to bind proteins. These bound proteins are poorly digested in the rumen. The addition of PEG preferentially binds the tannins thereby making the plant proteins available for ruminal digestion. Only toxic compounds considered to be of economic importance will be discussed in this review.

Table 2.6 Anti- nutritional factors found in some browse legume species

Species	Plant part	Compound	Reference
Acacia aneura	leaf	condensed tannins, oxalates	Gartner & Hurwood, 1976
Acacia cambagei	leaf	Cyanogenic glycosides (CG) CG hydrolase	Cunningham et al.,1981
Acacia cyanophylla	leaf	condensed tannins	Reed et al., 1990
Acacia cana	leaf, stem	selenium	Cunningham et al., 1981
Acacia doratoxylon	leaf, stem	Cyanogenic glycoside	Cunningham et al., 1981
Acacia georgina	leaf, stem	CG hydrolase, (no CG), fluoroacetate	Cunningham et al., 1981
Acacia nilotica	pods	condensed tannins	Tanner et al., 1990
Acacia salicina	leaf, back	tannins	Everist, 1969
	pods	saponins	Hall et al., 1972
Acacia sieberiana	leaf, pods	tannins, CG	Tanner et al., 1990
Albizia chinensis	bark	echinocystic acid, glycosides	Rawat et al., 1989
	leaf	condensed tannins	Ahn et al., 1989
Albizia lebbeck	flowers	various sterols	Asif et al., 1986
	leaf	pipecolic acid derivatives	Romeo, 1984
	root	echinocystic acid	Sotelo et al., 1986
Calliandra calothyrsus	leaf	condensed tannins	Kaitho, 1992
Calliandra portoricensis	leaf	tannins, saponins, flavonoids, glycosides	Aguwa & Lawal, 1987
Gliricidia sepium	leaf	pitinol	Calle et al., 1987
	leaf	condensed tannins	Ahn et al., 1989
	leaf	coumarins, melitotic acid	Griffiths, 1962
	leaf	CG, nitrates	Manidool, 1985
	seed	canavanine, heat stable toxin	Sotelo et al., 1986
Leucaena leucocephala	leaf	mimosine	Hegarthy et al., 1964
	leaf	condensed tannins	Ahn et al., 1989
	leaf	flavono-glycosides	Lowry et al., 1984
	leaf	saponins	Tangendijaja et al., 1990
Sesbania grandiflora	leaf, seed	tannins, glycosides	Andal & Sulochana, 1986
	flowers	methyl oleanolate	Kalyanaguranathan et al., 1985
Sesbania sesban	leaf	saponin	Dorsaz et al., 1988
	leaf	saponin, heat stable toxin	Shqueir et al., 1989
Chamaecystisus. palmensis	leaf	tannins, alkaloids	Borens & Poppi, 1990

2.3.2 Effects of anti- nutritional factors

Tannins

Tannins are complex polyphenolic compounds of plant origin which are soluble in polar solutions and have an ability to precipitate a range of biomolecules, including proteins, carbohydrates and minerals (Ndlovu, 2000). They constitute one of the most widespread and diverse group of secondary metabolites (Kumar, 1992). Their ability to precipitate biomolecules is responsible for their varied biological impacts. Tannins can be broadly classified into two distinct types which may be found in variable concentrations in the same plant species or in different species: hydrolysable (HT) and condensed (CT) tannins. HTs are polyesters of gallic acid (gallotannins) or hexahydrodiphenic acid (ellagitannins) with various individual sugars. They are susceptible to hydrolysis by acids, bases or esterases to yield carbohydrates and the constituent phenolic acids (Haslam, 1989). CTs are oligomers or polymers of cathechin-like units (flavanoid phenols) which are linked by carbon-carbon bonds that are not susceptible to cleavage upon hydrolysis (Ndlovu, 2000). CTs are sometimes called proanthocyanidins (PAs) because they are degraded to form monomeric anthocyanidin (e.g. cyanidins, delphinidins) pigments upon heating in strong acid (Porter *et al.*, 1986; Haslam, 1989). Anthocyanidin pigments are responsible for the wide array of colours in flowers, leaves, fruits, fruit juices and wines and are responsible for the astringency taste (Horvath, 1981; Haslam, 1989; Lawrence, 1991). Tannins are widespread in plants used for feed and food in tropical and sub-tropical areas and are abundant in feed resources that are readily available to ruminant livestock in extensive production systems.

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Condensed tannins have a greater effect on the digestibility of dietary nutrients than hydrolysable tannins, while the latter may cause varied toxic manifestations due to hydrolysis in the rumen. The binding of condensed tannins with dietary proteins inhibits the fermentation of structural carbohydrates (D'Mello, 1992) in the rumen and reduces protein availability to the rumen microbes. A high tannin content decreases feed consumption due to impaired palatability. Tannins have a harsh astringent taste and produce a feeling of constriction, dryness and roughness in the mouth (Haslam, 1979). They also reduce the digestibility of dietary protein and carbohydrate as well as the absorption of nitrogen, resulting to a higher faecal nitrogen output, lower urine nitrogen excretion and reduction in nitrogen retention (Barry and Duncan, 1984; Barry and Manley, 1984; Barry et al., 1986; Reed et al., 1990; Reed, 1995, Mbatha, 2001). Free condensed tannins are highly reactive and were reported to inactivate and precipitate microbial enzymes responsible for the degradation of cell wall constituents (McLeod, 1974; Lohan et al., 1981). Condensed tannins are known to induce changes in the morphology of cellulolytic microorganisms (Scalbert, 1991; Jones et al., 1994). They also bind with long chain carbohydrates (cellulose and hemicellulose), forming ruminal undegradable complexes (Makkar, 1993; Waghorn et al., 1994a,b; Fall-Toure et al, 1998) and are said to inhibit the action of endoglucanase which enables fungi to digest and colonize ligno-cellulolytic tissues not degraded by bacteria (Akin et al., 1983; McAllister et al., 1994b).

Mbatha (2001) reported nodule like lesions, a decrease in the height of microvilli, increase in pit and goblet cells, excess mucous secretion and an erosion of the epithelial tissue of the gastro-intestinal tract of Boar goats when a high level (10-20%)

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of commercial tannins was included in the diet of the goats. The consequence was a decrease in nutrient absorption.

Apart from these negative effects on digestibility, CTs are known to suppress bloat and to prevent excessive degradation of proteins in the rumen (Kumar and D'Mello, 1995). The protein-tannin complexes formed may dissociate in the post ruminal gut providing additional protein for digestion, absorption and utilization by the animal (Barry, 1989; McNabb et al., 1996; Karchi, 1998). They have also been shown to increase the performance of lambs infected with internal parasites (Niezen et al., 1995; Butter et al, 2000). Even though the mechanism is not yet elucidated, this has a potential application in smallholder systems where financial resources for the purchase of anthelmintics are limited. It may also be beneficial to large scale commercial livestock production systems in which resistance to anthelmintics is prevalent. Unfortunately, we have not been able to replicate this effect using commercial tannins which may have the same effect and are easier to acquire.

Mimosine

Mimosine, a non-protein amino-acid (B-[N-(3-hydroxy-4-oxopyridyl)]-a-amino propanoic acid) structurally similar to tyrosine, occurs in a few species of Mimosa and all species of genus Leucaena. The presence of this compound has restricted the exploitation of L. leucocephala as a forage legume in some regions of the tropics. Concentrations of mimosine in the leaf range from 10 to 25 g kg^{-1} DM with even higher quantities of up to 145 g kg^{-1} DM in the seeds (D'Mello, 1991). Mimosine may be regarded as a structural analogue of the nutritionally important amino acid, tyrosine and its neurotransmitter

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products, dopamine and noradrenaline, found in the brain (D'Mello, 1991). However, the structural relationship between mimosine and the other non-protein amino-acids present in the seeds of certain tropical legumes is not clearly defined. Like other amino acids, mimosine undergoes degradation in the rumen and the extent of degradation by the rumen microflora determines the nature and severity of toxic manifestations in the host animal (D'Mello, 1991, 1992).

During degradation, 3-Hydroxy-4(1H)-pyridone (3,4-DHP) is produced. In addition, 3,4-DHP also arises from post-harvest enzymatic action in the leaf. Animals grazing Leucaena forage are thus able to promote the synthesis of 3,4-DHP by maceration since the requisite enzyme for this reaction exist in the legume (Lowry et al., 1983). Studies on DHP-degrading enzymes have shown that DHP is toxic on its own right and that 3,4-DHP is a potent goitrogen (Hegarty et al., 1979; Jones, 1979; Jones et al., 1976). The main symptoms of toxicity of mimosine and its toxic metabolite, 3,4-DHP in ruminants are poor growth, loss of hair and wool, swollen and raw coronets above the hooves, lameness, mouth and oesophageal lesions, depressed serum thyroxine levels and goitre, excessive salivation, lethargy, weight loss, depressed appetite, poor growth, cataracts and death (Jones, 1979; Jones et al., 1976; Jones et al., 1978; Jones and Hegarty, 1984). Mimosine is also known to have antimitotic activity that blocks the cell cycle at late gametogenesis (Boehme and Lenardo, 1993) and to arrest cell division in cultured human cells (Hoffman et al., 1991). Incidences of still-born calves and low birth weight were reported by Hamilton et al. (1971) and Pratchett et al. (1991) for cows that were fed exclusively on Leucaena or that grazed irrigated Leucaena-pangula pastures while Holmes et al. (1981) reported a very low conception rate for heifers grazing L.

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leucocephala. However, the most significant factor affecting the susceptibility of cattle and goats to *L. leucocephala* toxicity resides in the geographical differences in the distribution of DHP-degrading ruminal bacteria. In some regions of the tropics (Papua New Guinea, Australia, USA, Kenya) ruminants lack these bacteria and consequently, succumb easily to effects of toxicity while in other parts (Central America, Hawaii and Indonesia) where the plant is indigenous or naturalized, animals possess the requisite bacteria for DHP degradation and this accounts for the absence of Leucaena toxicity in these countries (Holmes *et al.*, 1981; Jones, 1985; Hammond *et al.*, 1989; Semenye, 1990). The absence of any detectable adverse effects and non-incidence of symptoms of mimosine toxicity in a flock of South African indigenous goats grazing Leucaena/grass pastures and inoculated with DHP-degrading rumen bacteria (Akingbade *et al.*, 2001) is proof of the success story of the DHP-degrading bacteria.

Cyanogens

Cyanogenic glycosides

A good number of plants contain glycosides which are capable of releasing prussic acid or hydrogen cyanide (HCN) upon hydrolysis and the process is known as cyanogenesis. Cyanogenic glycosides contain an aglycone linked by an ether (-o-) bond to a carbohydrate fraction. Glycosides are metabolized by enzymatic action to release the aglycone from the carbohydrate. The aglycone may then be metabolized further. Cyanogenic glycosides are those that contain cyanide as part of the aglycone. When they are metabolized, free cyanide, a deadly poison is released (McDonald *et al.*, 1988). Cyanogenic glycosides are said to be common among the Acacia species and

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numerous reports of positive cyanide test have appeared in literature, especially from Australia (Bentham, 1964) and South Africa (Steyn and Remington, 1935; Steyn, 1943). Among the species identified in South Africa were *A. sieberiana, A. erioloba* and *A. lasiopetala*. The cyanogen identified was known as acacipetalin ($C_{11}H_{17}NO$) (Steyn and Remington, 1935) and a related compound, dihydro-acacipetalin was reported by Seigler *et al.* (1975) in the leaves and young stems of *A. sieberiana*. The latter compound occurs in the ratio of 1:3 with acacipetalin. Two enzymes present in the tissue of cyanogenic plants are said to be responsible for the release of HCN when the plant tissue is disrupted. These are a substrate-specific β-glucosidase which, when brought in contact with its substrate, produces glucose and an α-hydroxynitrile that, in turn, dissociates rapidly in the presence of hydroxynitrile lyase to form HCN (Conn *et al.* 1989). Cyanide is very toxic to cellular metabolism (D'Mello, 1991). It combines with haemoglobin in the blood and inhibits respiratory enzymes, ultimately causing death. Apart from reports of acute intoxication and death (Steyn and Remington, 1935) there is strong evidence that goitre and cretinism (due to iodine deficiency) are exacerbated and neurological disorders are caused by long term ingestion of feed containing cyanogenic glycosides (Delange and Ahluwalia 1983).

The response of ruminants to cyanogenic glycosides ingestion varies. In the rumen HCN is converted to thiocyanate using available sulphur, and thiocyanate is absorbed and excreted. Thiocyanate is a goitrogen, inhibiting the action of the thyroid gland and often the effect of cyanogenic glycosides ingestion is seen as the development of the goitre (Jones *et al.*, 1978). Iodine supplementation overcomes this effect. In the rumen, the catabolism of cyanogenic glycosides to liberate HCN does not require the presence

of the plant enzymes because rumen microorganisms are capable of producing the same enzymes (Majak and Cheng, 1984) and the lethal dose for cattle and sheep was reported to be 2 mg HCN/kg body weight (Coop and Blakley, 1950).

Saponins

Saponins are glycosides containing a polycyclic aglycone of either C27 steroid or C30 triterpenoid attached to the carbohydrate (Kumar and D'Mello, 1995). They occur in alfalfa and many other different plants. Saponins have a characteristic bitter taste, foam in water and can cause haemolysis of red blood cells (Hanson et al., 1973). They have also been implicated in causing bloat (acute tympanites) in ruminants (Lindahl et al., 1957a; Birk, 1969) and reduced ruminal mobility (Lindahl et al, 1957b) in sheep. Studies by Lu et al. (1987) and Lu and Jorgensen (1987) suggested that saponins inhibit microbial synthesis in the rumen and altered the site and extent of nutrient digestion in ruminants. The saponin, stigmasta-galactopyranoside was isolated from *Sesbanian sesban* seeds and shown to have spermicidal and haemolytic activity (Kholi, 1988). Factors such as genetic differences among animals, species differences and physiological status of animals can influence the response of animals to saponins (Kumar and D'Mello, 1995).

2.3.3 Methods of alleviating antinutritional factors in browses

The uncertainty of quantification and the imperfectly understood biological effects of anti-nutritional factors impede the development of methods to alleviate their effects. The simplest approach of dilution (supplementation) may reduce the effect of toxicity but the required degree of dilution is difficult to recommend because of the difficulties involved

in determining the exact concentrations in forages. Toxicity depends on the concentration of the deleterious compounds in the fodder and the rate at which it is eaten. An amount of the plant eaten quickly, for example, in one hour could be fatal whereas the same amount eaten slowly over a longer period could be harmless (Storrs, 1982; Raghaven, 1990). It has also been noticed that as leaves mature, their concentration of anti-nutritional factors and nutrients decrease (Singh, 1982).

Another method of reducing nutritional problems associated with anti-nutritional factors is drying. Swain (1979) found that drying at 50°C irreversibly fixed tannins to other cell polymers while Ahn et al.(1997) reported the complete disappearance of tannins in G. sepium and Tipuana tipu when the leaves of these species were oven dried. Brewbaker (1986) reported an increase of 35% in rumen nitrogen digestibility of the leaves of A. aneura, A. angustissima, A. chinensis and C. calothyrsus after oven drying and associated this improvement to a reduction in active tannins. Drying however, increased cell wall constituents (Mahyuddin et al., 1988; Terril et al., 1992; Bonsi et al., 1995; Norton and Ahn, 1997) due to the disappearance of soluble carbohydrates, proteins and organic acids, but despite this negative effect, these results show that drying could be an effective and an economically viable way of reducing tannins in browse.

Another approach is the inclusion of polyethylene glycol (PEG) or polyvinyl pyrrolidone (PVP) in the diet of animals fed tannin-rich forage (Kumar and Singh, 1984). This could be done by spraying the absorbents on the forage before feeding. Barry (1985) and Pritchard et al. (1988) reported increases in liveweight and wool growth in sheep when forages from Lotus pedunculatus and A. aneura were supplemented with 100 and 24

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g d^{-1} of PEG respectively. Free condensed tannins bind PEG in preference to protein and this renders dietary protein free for digestion. In addition, activities of endogenous proteins and enzymes are not affected (Kumar and Vaithiyanathan, 1990). However, a report by McNeill *et al.* (1999) indicated that dietary protein complexed with tannin was made available in the abomasum and digested in the intestines but the tannin released from the protein-tannin complexes reacted with non-dietary protein (including digestive enzymes) as it passes along the intestines thus partially counteracting the benefits of by-pass dietary protein. Using PEG in routine feeding may not also be economical.

Mimosine toxicity can be avoided or drastically reduced if animals are allowed to go through an adaptation period during which Leucaena is offered to animals in small quantities with other feeds although Hegarty *et al.*(1964) stated that this adaptation period has to be long. Other studies (Moog, 1983; Van Eys *et al.*, 1986; Jones *et al.*, 1989; Goodchild and McMeniman, 1994) have shown that apart from the benefit of averting toxicity, there was an increase in dry matter intake as well as growth rate when *Leucaena* was given as supplement to sheep feeding on a low quality roughage diet or when cattle were allowed to graze *L. leucocephla*-grass pastures.

Molasses supplementation has also been shown to increase the degradation of mimosine to DHP which is then excreted in conjugated form in faeces (Elliott *et al.*, 1985). Jones *et al.* (1978) also demonstrated that supplementation with minerals (Fe, Cu, Zn) can decrease hair loss and skin lessions in cattle fed on *L. leucocephala*. Pre-treatment of fresh *Leucaena* forage with hot water at 60°C for 15 min before drying or freezing and thawing produces material that is mimosine-free (Lowry *et al.*,1983). In

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areas where *L. leucocephala* is not indigenous, toxicity may be prevented by dosing animals fed on this forage with DHP-degrading bacteria. An example of a success story was the transfer of this bacteria from animals in Hawaii to Australia cattle (Jones and Megarity, 1986; Quirk *et al.*, 1988). Transfer of this bacteria from animal to animal is very rapid (Quirk *et al.*, 1988) which implies that inoculation of just a few animals in the herd or flock may be all that is necessary to prevent toxicity. This method, therefore, offers a viable strategy for the extended use of *L. leucocephala*.

Post harvest wilting of forage containing cyanogenic glycosides may reduce cyanide toxicity and the treatment of animals suffering from cyanide toxicity with sodium nitrate and sodium thiosulphate has proved to be successful (Kumar and D'Mello, 1995). Inhibition of microbial β-glycosidase in the rumen might provide a means of protecting ruminants from toxic glycosides but this method needs to be examined in detail because this enzyme plays a fundamental role in the bio-transformation of cyanogenic glycosides.

The adverse effects of saponins could be overcome by repeated washing of the feed in water. This renders the feed palatable because the bitterness associated with saponins is reduced (Joshi *et al.*, 1989). It should, however, be noted that nutrients (soluble carbohydrates and protein) also leach out with washing. The problem of leaching becomes even more critical with fast degrading forages like *Sesbania sesban* with a wash value of up to 419 g kg^{-1} DM (Bonsi *et al.*, 1995).

2.4 The effect of anti-nutritional factors on palatability, intake, digestion, microbial activity, nitrogen balance and animal performance

2.4.1 Palatability

Palatability is a complex phenomenon which depends on the animal, plant and environmental factors (Marten, 1978). The palatability of a forage is determined by its ability to provide stimuli to the oropharyngeal senses of the animal eg taste, odour and texture (Kaitho *et al.*, 1997). It has been widely used in the initial evaluation of feeds as an index of their intake by ruminants. Results from palatability trials have been used to rank feeds but this depends on seasonality (Schultze-Kraft *et al.*, 1989), which could be explained by the availability of edible biomass and/or influence of environmental factors.

Forage legumes contain an array of compounds which confer upon them anti-nutrional attributes which may directly influence palatability while others affect, principally, rumen microorganisms and thus digestibility in the rumen (El Hassan, 1994). These include tannins, saponins and flavonoids. The inverse relationship between tannin levels in forages and palatability, voluntary intake or N retention in some mammalian herbivores is well established (Robbins *et al.*, 1987; Silanikove *et al.*, 1994; Silanikove *et al.*, 1996). The correlation between palatability and alkaloid concentration in different forage species was also reported to be high and negative ($r = -0.83$ to -0.95, Simons and Marten, 1971).

The form in which browse is fed influences palatability. Nitis (1986) observed an improvement in the acceptance of *G. sepium* after prolonged wilting and drying, while

Palmer and Schlink (1992) reported a significant (p< 0.05) difference between voluntary intake of fresh and wilted *C. calothyrsus*. Kaitho *et al.* (1996) , however, demonstrated wide variations in potential value and acceptability of 40 multi-purpose tree species in sheep. The same authors (1997), contrary to earlier reports by Heady (1964) and Martz *et al.* (1967), reported poor relationships between palatability and chemical constituents and stated that MPTs with good nutritive value such as *L. leucocephala* , *C. palmensis* and *A. perciflora* had consistently high intake and palatability values irrespective of the form offered to both sheep and goats. On the other hand, *Samanea saman* and *A. polycantha* had strong odours which affected palatability. Drying of these MPTs reduced the odours, leading to an improvement in palatability. Others (*A. nilotica* and *A. perciflora*) were more preferred wilted by both sheep and goats.

Correlation and regression analyses indicated a poor predictability of palatability of sheep using goat palatability indices. Kaitho *et al.* (1997) reported that the indices for goats were more than two-fold those of sheep and suggested that goats should be preferred to sheep when assessing palatability. The differences may be related to the fact that goats are "super browsers" and therefore, have no peers using browse and forbs (Ensminger *et al.*, 1990). Sheep were considered more resistant to alkaloids than cattle (Cheeke,1988) and in numerous instances, goats are more resistant than sheep to poisonous pants (Cheeke and Shull, 1985; Kellerman *et al.*, 1988). To a considerable extent, these differences among ruminants may account for their feeding behaviour.

Palatability however, depends on the period of adaptation. Kaitho *et al.* (1996) observed an erratic behaviour on the first day, a preference rating after four days but high

correlations indicated that a period of 5 to 8 days was ideal. This concurs with earlier reports by Ben Salem et al. (1994).

2.4.2 Intake

The effect of anti-nutritional factors on intake of forage legumes appears to be more contentious. Some researchers (Greenhalgh and Reid, 1971; Butler and Bailey, 1973; Weston and Davis, 1986; Ologunde and Ayorinde, 1990) reported that forage legumes contained anti-nutritional factors that may confer astringency that reduced palatability and may subsequently influence intake. Astringency is the sensation caused by the precipitation of salivary proteins and mucopolysaccharides in the mouth (Luck et al., 1994). Astringency may increase salivation and decrease palatability (Reed, 1995). Gherardi et al. (1991) observed that rumen digesta load and apparent fractional rate of digestion were more associated to intake than palatability while Aitchison (1986) reported that bulkiness was an important determinant of intake.

In a study using goats, Holechek et al. (1990) reported reduced total organic matter (OM) and digestible OM intake when high tannin shrubs were fed compared to low tannin diets. Woodward and Reed (1989) also reported reduced feed intake when browses high in tannins were compared to those low in tannin content. Reduced palatability, low rate of evacuation of digesta out of the rumen and toxic effects were considered as some of the negative effects of ANFs on feed intake in ruminants (Kumar and Singh, 1984, Provenza, 1995; Reed, 1995). However, Dube (1992) observed no relation between condensed tannin levels in four browse species and intake of the browses by goats. In this study, nitrogen was the most important factor affecting browse

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intake. The author attributed the lack of a significant relationship between tannin content and intake to the fact that the animals were adapted to the browses.

Intake like palatability, also depends on animal species. Goats have been reported to have higher DM intake and digest the fibre component of the diet better than sheep (El Hag, 1976; Howe *et al.*, 1988). Dominique *et al.* (1991) made similar observations when they fed a low quality roughage to goats and sheep. A possible explanation to the above contentions (goats vs sheep) is that goats possess a proline rich protein (PRP) (Robbins *et al.*, 1987) and because of the high affinity for tannins, PRP might displace protein in tannin-protein complexes making protein available. The consequence would be increased fibre digestibility (Wilson,1977), high rate of passage of feed out of the rumen and higher intake. Rumen ammonia-nitrogen and volatile fatty acids (VFAs) were also higher in goats (Dominique *et al.*, 1991).

Using the same feeds and animals, degradability constants measured by nylon bag disappearance (Orskov *et al.*, 1988) and gas production (Blummel and Orskov, 1993; Siaw *et al.*, 1993; Nsahlai *et al.*, 1994; Kaitho, 1997) have been shown to relate very strongly to intake. However, the phenolic components were more related to DM degradation and gas production than palatability and DM intake (Kaitho, 1997).

2.4.3 Digestion

Depending on the concentration and type, phenolic compounds in tree foliage are accredited with both beneficial and adverse effects in ruminant digestion (Barry, 1989; Cheeke and Palo, 1995). Beneficial attributes include the suppression of bloat (Jones

and Mangan, 1977), prevention of excessive degradation of high-leaf protein in the rumen, consequently increasing the supply of high quality nutrients entering the duodenum (Mangan, 1988; Wang et al., 1994; McNabb et al., 1996; Perez-Maldonado and Norton, 1996) and reduction in the effects of intestinal nematodes (Niezen et al., 1993). These beneficial effects are the result of the fact that tannins, under certain temperature and pH conditions, form complexes with protein, which may subsequently be digested in the lower gut.

At high concentrations of condensed tannins, intake and apparent digestion of protein and carbohydrates are depressed through the inhibition of bacterial enzymes and/or forming indigestible complexes with cell wall carbohydrates (McLeod, 1974; Barry and Manley, 1984). *In vitro, in sacco* and *in vivo* studies have been undertaken to quantify the effects of tannins on digestion and several workers reported tannin-induced reductions in digestibility (Mahyuddin et al., 1988; Ahn et al., 1989; van Hoven and Furstenburg, 1992; Dzowela et al., 1995).

Depressed digestibility of DM, NDF and ADF (Rafique et al. 1993), and of OM and NDF (Holechek et al., 1990; Tanner et al., 1990) was observed when tanniferous forages were fed as sole or as supplements to poor quality roughages. Weigand et al. (1995) measured NDF, ADF, acid detergent lignin (ADL) and neutral detergent insoluble lignin (NDIN) digestibility in sheep fed browses with high and low levels of condensed tannins and observed negative digestibility coefficients for ADL and NDIN. The authors attributed the gain in ADL and NDIN along the digestive tract to the formation of detergent insoluble complexes. As a result, condensed contains (proanthocyanidins)

were positively correlated with faecal ADL and NDIN. The effects of tannins are however, depended highly on tannin concentration in the diet. Waghorn *et al.* (1987) observed no effect of medium tannin concentrations (33 g kg^{-1} DM) of *L. corniculatus* on the digestibility of DM and NDF in sheep. Similar observations were made by Chiquette *et al.* (1989). In another study, Waghorn *et al.* (1994a) observed no effects of *L. pendunculatus* tannins on the digestibility of hemicellulose, cellulose and ash.

Rumen microbial fermentation has also been shown to be affected by tannins. Using the gas production technique, Nsahlai *et al.* (1994) observed significant negative correlations between NDF-bound proanthocyanidins (PA) and the extent of gas production. Similar responses were observed by Langland *et al.* (1995). In the latter study, the authors reported that total phenolic content had more pronounced effects than tannins alone. Tannins have also been shown to depress the production of volatile fatty acids (Chiquette *et al.*, 1989; Terril *et al.*, 1992; Waghorn *et al.*, 1994a) while increasing the molar proportion of butyrate (Terril *et al.*, 1992) and isobutyrate (Waghorn *et al.*, 1994a).

2.4.4 Nitrogen metabolism

Since browse is best utilized as protein supplement, tannins thus have a profound influence on the nutritive value of the browse via their effect on nitrogen metabolism. Tannins affect nitrogen metabolism at each stage of digestion (Woodward and Reed, 1989). The concentration of rumen ammonia nitrogen (NH_3-N) is a measure of proteolytic activity in the rumen. This pool of ammonia-N is reduced with an increase in tannin content of the diet (Waghorn *et al.*, 1987; Kraiem *et al.*, 1990; Waghorn *et al.*,

1994b). Data presented by Woodward and Reed (1989) also indicated a reduction in rumen ammonia-N levels with increase in the tannin content of diets. The reduced protein degradation in the rumen could be due to enzyme inhibition and/or substrate deprivation (Reed, 1995). Several studies have reported reduced activity for proteases, urease and glutamate ammonia ligase in the presence of tannins (Lohan et al., 1981; Makkar et al., 1988; Makkar et al., 1990; Tanner et al., 1994).

The reduction in the activity of these enzymes may lead to excess ammonia-N not utilized by the microbes in the rumen. This excess is absorbed across the rumen wall and converted to urea in the liver before excretion in urine or recycling via saliva. Woodward and Reed (1989) observed higher plasma urea when tanniferous forages were fed to sheep. On the other hand, Waghorn et al. (1994b) observed an increased flow of dietary non-ammonia N (NAN) to the abomasum in animals fed high tannin diets compared to those fed the same diet supplemented with PEG. Microbial NAN was not affected by tannins and total tract N digestibility was reduced. In the same study there was a net gain (18 %) of essential amino acids (EAA) and a loss of non-essential amino acids (NEAA) in the rumen. It appears, therefore, that ammonia production is poorly correlated to apparent microbial digestion of N for animals fed tanniferous forages. This could be due to the different rates and efficiencies of incorporation of ammonia-N into microbial protein as carbohydrate is fermented (McSweeney et al. (1999).

In another study, Nsahlai et al. (1995a) reported that CT depressed the solubility, rate and effective degradability of DM and N of samples incubated in nylon bags in the rumen of sheep. However, in a further study, Nsahlai and Umunna (1996) added that

the effect of tannins on *in sacco* degradability may be underestimated because of a dilution effect of the rumen, considering the size of the sample (3 g per bag) relative to the total rumen volume. It is thus probable that the effect of anti-fermentation factors may be reflected in the gas production (Menke *et al.*, 1979) than the nylon bag technique. This may explain why gas production (GP) was superior to degradability in predicting the *in vivo* DMD of legumes. In an earlier study, Siaw *et al.* (1993) stated that the differences between DM degradability and GP lie on the fact that the gas produced was from the fermentation of both the soluble and insoluble portions of the incubated feed.

Another limitation of the nylon bag technique arises from the fact that although *in sacco* losses of protein generally correlate with degradability, this may not be the case with tanniferous feeds because protein loss from the bag may either be in the form of tannin-protein complexes or interact with tannins in the rumen to form ruminal undegradable complexes (McNabb *et al.*, 1996; Perez-Maldonado and Norton, 1996). It is therefore expected that the release of tannins in the digestion medium will depress ammonia concentration.

Condensed tannins were also reported to reduce the absorption of amino acids in the intestine (Waghorn *et al.*, 1994b) even though they added that the reduction was compensated by a higher influx of amino acids from the rumen. A reduction in the absorption of amino acids may have major implications because the intestines utilizes a considerable amount of amino acids during absorption, the remainder being available for protein synthesis by other tissues (Tagari and Bergman, 1978). Mechanisms by

which CT may affect amino acid absorption include: direct effects on endogenous enzymes by binding with the enzyme and reducing its activity; binding with digesta proteins and reducing the activity of endogenous proteolytic enzymes to cleave off peptides and amino acids and association with the intestinal mucosa, thus reducing transport and absorption of peptides and amino acids. Horigome *et al.* (1988) demonstrated an inhibition of 50 to 60% of trypsin, α-amylase and lipase activity when CT were added to purified enzymes and crude extracts from rat intestines. The binding effect of tannins in the intestine is however, driven by pH. Jones and Mangan (1977) reported that although tannin-protein complexes dissociate at a pH of 3.0 to 3.5 in the abomasum, they may reform once the pH increases to 5.5 in the intestine.

The value of any feed as a source of N is usually indicated by the nitrogen retained in the animal. In general, when compared with low tannin diets, high tannin diets result in lower N retention (Woodward and Reed, 1989; Holechek *et al.*,1990; Tanner *et al.*, 1990; Dube, 1992). In most of these studies, supplementation with high tannin browses resulted in higher N retention than when poor quality roughage were fed alone. Therefore, tanniferous browses have a role to play as supplements to these roughages.

2.4.5 Rumen microbes

The tannin-induced reduction in rumen fermentation rate reported in studies cited in the preceding paragraphs is a reflection of effects of tannins on rumen bacteria, fungi and protozoa. Tannins cause reduced fermentation via one or all of the following: bacterial enzyme inhibition (McLeod 1974; Lohan *et al.* 1981; Mueller Harvey *et al.* 1988; Reed *et al.* 1990; Weigand 1991; Makkar, 1993, Reed, 1995), formation of tannin-substrate

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complexes (Makkar 1993; Waghorn *et al.* 1994a,b; Fall-Toure *et al.* 1998), and inducing morphological changes on microbes (Scalbert, 1991).

Microbes respond differently to tannins. Jones *et al.* (1994) observed inhibition of cell-associated proteases of *Streptococcus bovis* and *Butyrivibrio fibrisolvens* but not of *Prevotella ruminocola* and *Ruminicoccus albus*. Changes in morphology were observed in *B. Fibrisolvens* and *R. amylophilus*. In a study with anaerobic fungi, McAllister *et al.* (1994b) reported higher inhibition of endoglucanase in *Orpinomyces joyonii, Pyromyces communis* and *Neocallimastix patriciarum* compared to *Neocallimastix frontalis*. These fungi were able to grow in PA media concentrations which have been shown to be lethal to bacteria. The authors suggested that PA formed complexes with fungal proteins and constituted filamentous material which covered the rhizoids of all the species. The physiological changes associated with the changes in morphology observed by the various authors are probably more important than the morphological changes *per se*.

2.4.5 Animal performance

A wide variation in liveweight gain of animals given roughages and supplemented with forage legumes was reported (Nsahlai *et al.*, 1998d). This variation may be a consequence of the quality of the basal roughage (Umunna *et al.*, 1995), quality of the forage legume (Woodward and Reed, 1989; Wiegand *et al.*, 1995), the productive stage, breed or species of animal considered. From literature reviewed, it may be difficult to draw conclusions on the effects of tannins on animal performance. Tanner *et al.* (1990) reported lower growth for animals given high tannin forages than those on low tannin forages. Similarly, Pritchard *et al.* (1988) and Niezen *et al.* (1993) observed

higher growth rates and higher wool yields when they reduced tannin effects by adding PEG to *A. aneura* and *L. pendunculatus* respectively. In contrast, Terril *et al.* (1992) reported better wool growth when they fed tanniferous *Hedysarum coronarium* (sulla) alone to sheep compared with feeding it with PEG but liveweight gain and carcass yield were however, not affected by tannins. The authors explained the increase in wool growth as due to an increase in the flow of the sulphur amino acids (methionine and cystine) from the rumen to the abomasum. McNabb *et al.* (1993) reported similar findings.

Wang *et al.* (1996) reported that lambs grazing *Lotus corniculatus* registered lower voluntary feed intake (VFI) than those grazing lucerne (*Medicago sativa*) but had higher liveweight gain (LWG), carcass weight gain, carcass dressing-out percentage and wool growth. In their work, addition of PEG in the diet of lambs reduced wool growth, slightly reduced LWG but increased rumen ammonia concentrations and the molar proportions of iso-butyric, iso-valeric and n-valeric acids as well as protozoa numbers in rumen fluid. Terril *et al.* (1992) earlier reported reduced carcass fatness in sheep grazing sulla. Contrary to the above observations, Priolo *et al.* (2000) noticed lower growth rates and poorer feed efficiencies when 2.5 % condensed tannins (DM basis) was included in the diet of lambs, as compared to the treatment that was offered the same amount of tannins but in addition received 40 g of PEG kg^{-1} of feed. Lambs fed the tannin diet had a lower carcass yield and had less fat. The digestibility of DM, N and fibre was reduced in the animals fed the tannin diet. The authors concluded that the inclusion of PEG eliminated the effects of condensed tannins such that lamb performance and meat quality were similar to lambs given a maize diet.

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In another study, sheep fed a high tannin basal diet (bird-resistant sorghum stover) and supplemented with a rapidly fermentable oilseed (*Guizotia abyssinica*) cake registered lower weight gains than those that were supplemented with a slowly fermentable oilseed (cottonseed) cake (Nsahlai *et al.*, 1998c). The authors attributed the lower weight gains to a greater loss of N in faeces and urine, and excessive excretion of urine by animals, probably in a bid to eliminate toxic substances. In a later study, the same authors (1999) reported linear increases in weight gain when the proportion of Acacia pods in a supplement mixture of oilseed cake and pods was given at increasing levels. The increases were attributed to increased OM and N intake and/or efficient use of nutrients. This may imply that the addition of pods in the diet decreased the degradation of protein in the rumen due to the formation of tannin-protein complexes which leave the rumen undegraded and dissociate in the abomasum to release high quality protein which is then digested by intestinal enzymes to give amino acids used for tissue growth.

A lower nematode load was also reported in sheep fed tanniferous forage (Purchas and Keogh, 1984; Niezen *et al.* 1995). This attribute of tannins, if replicable, will reduce the use of commercial drugs in the control of gastro-intestinal parasites. Long term feeding and use of other animal species may be required before conclusive results are obtained. In this regards, the use of commercial tannins might be more practical in the long run.

2.5 Inferences from literature review

Constraints that limit the productivity of ruminants in the tropics were highlighted at the beginning of the review and prospects of the use of forage from tree legumes were

viewed as a possible solution of reducing the problem of feed shortages and quality. In this regard, browse utilization and the constraints to its utilization in the nutrition of herbivores constituted the main body of the review. The chemical composition and nutritive value of shoots, leaves and pods of browse species have been considered. It is clear that browses have a distinct advantage over tropical grasses in terms of their superior nutritional value particularly during the dry season.

Although many different and some potentially dangerous compounds have been isolated from some of the most useful fodder trees, not much is known about the specific effects of these compounds on ruminant metabolism. Tannins have been considered as the most important deleterious compounds in browses. It is, therefore, necessary to carry out more research to determine appropriate methods of alleviating these deleterious effects in order to upgrade the quality of these feeds. The long term effects of these substances on rumen microbes and the host animal need to be investigated jointly by nutritionists, rumen microbiologists, toxicologists, phytochemists and physiologists.

For optimum utilization of browses, it is essential that details of agronomic characteristics, palatability and nutritive value of some prominent species are determined. Studies addressing the quantity of degradable and by-pass protein in the rumen as well as protein digestion in the small intestines should also receive more attention. Although a lot has been said about browse utilization, either as sole feed or supplement, there is still a dearth of information on the utilization of pods from tree legumes as supplements to roughage based diets for ruminants. Pods can easily be

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harvested or drop from the trees when ripe and tend to be available for consumption by ruminants at periods when the foliage (leaves) is scarce (particularly for deciduous species) or found at heights above animal reach. The ease to store pods and their less bulky nature give them an extra advantage over foliage.

This study will evaluate the attributes and identify limitations of some pods from tree legumes as protein supplement to poor quality roughages. Particular attention will be paid to their chemical and degradation properties, their content in some anti-nutritional factors and methods of detoxification. Evaluation of the effects of anti-nutritional factors on palatability, rumen microbial activity and post-ruminal digestion will also constitute an integral part of the study. The results are expected to partially close the gap of information and enhance productivity of ruminants, particularly at the level of small-scale livestock farmers.

CHAPTER 3

The rumen digestion of dry matter, nitrogen and cell wall constituents of the pods of *Leucaena leucocephala* and some *Acacia* species

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3.1 Abstract

The disappearance of dry matter, nitrogen and cell wall constituents of the pods of *Leucaena* and some *Acacia* species incubated in the rumen of Jersey cattle were studied. The chemical compositions of the pods (seeds + husks), seeds and husks were examined separately. The amino acid and lipid analyses were limited to the pods and seeds while mineral and tannin analyses were done on the pods alone. The seeds had a higher crude protein (CP) content than pods and values ranged from 200-300 g CP kg^{-1}DM. The *Leucaena* pods and seeds had higher ($p<0.001$) CP contents than the *Acacia* species. The NDF concentrations of the husks were higher than those of the pods and seeds with the pods of *Acacia erioloba* having the highest and those of *Acacia nilotica*, the lowest values, respectively. These values were highly influenced by the seed-husk ratio. *Acacia sieberiana* and *Leucaena leucocephala* had the highest and lowest concentrations of condensed tannins, respectively. The pods of *Acacia tortilis* and *Acacia karoo* were particularly rich in macro- and micro-minerals while the others contained sufficient quantities that could contribute substantially to the dietary requirements of small ruminants. The amino acid concentrations differed significantly($p< 0.001$) among the pods, with the *Leucaena* pods having the highest concentrations. The *Leucaena* pods and seeds also had higher ($p<0.001$) lipid contents than the *Acacia* species. Dry matter and nitrogen degradabilities were higher ($p< 0.001$) for pods of *A. nilotica* and *A. sieberiana* while *A. karoo* had the lowest value. The cell wall constituents of the pods of *A. tortilis* and *A. erioloba* were the most and the least susceptible to microbial degradation. The rates of degradation of the fibre fractions as well as the lag time were similar ($p> 0.05$) among the pods. The results show that these pods can serve as a valuable source of nitrogen and minerals to ruminants fed roughage-based diets.

Keywords: Legume pods, chemical composition, degradation, supplements, ruminants

3.2 INTRODUCTION

Browse has been defined as the leaves, shoots and sprouts including tender twigs and stems of woody plants which are cropped to a varying extent by domestic and game animals (Gutteridge and Shelton, 1994).This definition could be extended to include fruits, pods and seeds which are often more valuable than the foliage, especially if the tree is deciduous. The importance of browse in the diet of herbivores is reflected in reports from Africa, Latin America, Asia and Australia. In northern Africa, browse forms 60-70% of rangeland production and 40% of the total available animal feeds in the region (Le Houerou, 1980; Kaitho, 1997). In India, browse is the principal feed for goats and meets over 60-70% of forage requirements (Devendra, 1995) while in the Shrub Steppes of southern Australia, it constitutes 100% of the sheep's diet (Wilson, 1977).

In most dry zones of Africa, pods and fruits from tree legumes constitute an important component of the diet of ruminants. For example, in the semi-arid zone of northern Kenya the flowers and fruits of *Acacia* species contributed up to 40 and 20 percent respectively in the diets of goats during the dry season (Schwartz and Said, 1981). Livestock farmers in such areas have developed strategies which make maximum use of these products, particularly during the dry season when pastures are deficient both in quantity and quality. Domestic ruminants are often allowed to harvest the pods directly from the trees or pick those that have fallen on the ground. In some areas, the pods are collected by herdsmen and taken home for later use. The *Acacia* species are predominant in most dry zones of Africa and serve as an important source of feed for ruminants as well as having other uses (Le Houerou, 1980). *Leucaena* is a widely grown legume which performs well on fertile soils but its yield on acid and water-logged soils

is rather poor (Shelton and Brewbaker, 1994). The excellent nutritive value of its foliage is well reported (Jones, 1979; Bonsi *et al.*, 1994; Gutteridge and Shelton, 1994). A lot of research has equally been done on the nutritive value of the foliage of some *Acacia* species (Kaitho,1997; Goodchild,1990; Nsahlai *et al.*, 1995a) but little is known of the nutritive value of the pods. However, some authors reported the feeding value of pods to be good, especially when given in combination with other supplements (Gwynne, 1969; Ibeawuchi and Adamu, 1990; Tanner *et al.*, 1990; Kibon and Maina, 1993; Nsahlai *et al.*, 1995b; Nsahlai *et al.*, 1995c).

The objective of this work was to examine the chemical and degradation properties of pods from six tree legumes; *Acacia erioloba, A. karoo, A. nilotica, A. sieberiana, A. tortilis* and *Leucaena leucocephala*. These six were selected based on the quantity of pods that each species could produce, the density of the species in the veld and their distribution in southern Africa.

3.3 MATERIALS AND METHODS

Collection of pods

Pods containing seeds were harvested from six tree legumes just before winter fall when most of the pods were dry. Most of the *Acacia* pods were harvested at different sites on the outskirts of Pietermaritzburg but some came from the Zululand thornveld, east of the Drakensburg mountains, along the coastal zone where the soils are heavily textured and rainfall varies between 500 and 700mm (Walker, 1980).The *Leucaena* pods were harvested mainly from the research farm of the University of Natal. Harvesting was facilitated by using a metal sisal fitted on to the end of a long (6m) stick.

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The *Acacia* pods from different sites were bulked after each harvest, according to species and sun dried for two days before milling them. To determine the seed-husk ratio of the pods (husk + seeds), one kg of each pod species was separated manually and the ratio was calculated on dry matter basis. Samples for laboratory analysis and degradability studies were ground through a 1-mm and 2-mm screen respectively in a laboratory hammer mill (Retch). The chemical analysis of the pods, seeds and husks was done twice following the period of harvest for the purpose of a proper comparison.

Animals

Two adult Jersey cows (about 400kg liveweight), fitted with ruminal cannulae (120 mm id) were kept in a roofed shed with a concrete floor and fed *ad libitum* on pasture hay (*Themeda triandra*), supplemented with 2 kg of cotton seed cake per animal each day. The supplement was given at 08.00h in the morning and the animals had free access to water. A two-week period of adaptation was allowed prior to incubation.

In sacco **degradation**

In order to determine the *in sacco* disappearance of dry matter (DM) and nitrogen (N), about 4 g of each ground pod sample was weighed into nylon bags (bag size 8 x 14 cm). The bags were incubated in the rumen of the cows in duplicates following the method described by Mehrez and Orskov (1977). Bags were added sequentially and were incubated for 144, 120, 96, 72, 48, 24, 12, 6 and 3 h. After removal from the rumen, the bags, including the zero-hour ones which had not been incubated, were washed in a semi- automatic washing machine six times in cycles of 5 minutes. The washed bags were then dried in a force draught oven at 60° C for 48 hours, cooled in

a desiccator and weighed. The residues were subsequently analysed for nitrogen.

The same process except the last step was repeated to determine the degradability of the cell wall constituents. After weighing the residues for the determination of DM loss, the bags were boiled in a neutral detergent solution (NDS) for one hour and then washed, dried and weighed as described above. This was followed by boiling in acid detergent solution (ADS) for one hour. The bags were washed, dried and weighed for the estimation of ADF. The neutral and acid detergent solutions were prepared following the method described by Van Soest et al (1991) To validate our method, samples were analysed in parallel using the method described by Van Soest et al (1991) . The difference between the two methods of extraction was expressed as a percentage of the result from the Van Soest's method and was equal 6.7 ± 2.31.

Chemical analysis

The DM, organic matter (OM) and N contents of the feed and nylon bag residues were determined using the methods of the AOAC (1990). The neutral detergent fibre (NDF) and acid detergent fibre (ADF) of the pod samples were measured by the method of Van Soest et al. (1991) while hemicellulose was calculated as the difference between NDF and ADF. Pod samples were also sent to the Jacob Blaustein Center for Desert Biodiversity, Ben Gurion University of Negev Desert, in Israel for the analysis of condensed tannins and the method of determination was that described by Hagerman (1995). Amino acid analysis was done using an amino acid analyser (Technicon Auto-analyzer II). After acid digestion, phosphorus was determined colorimetrically while calcium was determined using a Varian spectra AA-200 Atomic Absorption

spectrophotometer. Na and K concentrations were determined using a flame photometer (Gallenkamp) and Mg, Fe, Cu, Mn, Se and Zn using an atomic absorption spectrophotometer (AOAC, 1990).

Calculations and statistical analysis

The disappearance of DM, N and detergent fibre-fractions was estimated by fitting the non-linear model proposed by McDonald (1981) and modified by Dhanoa (1988) to the degradation data for each component; variables were determined using the Secant Method (DUD), (SAS,1987).

$$Y = W + B[1-e^{-C(T-LT)}]$$

where Y is the disappearance of DM, N or fibre fraction at time T, W = washing loss or solubility, B = degradable part of the insoluble fraction, C = rate of degradation of B and LT, the lag time. The potential degradability (PD) was calculated as W + B. A passage rate (k) of 0.03 /h was assumed in order to calculate the effective degradabilities (ED) of DM and N (Bonsi *et al.*, 1994; Nsahlai *et al.*, 1998a) The equation used was that proposed by McDonald (1981): ED =0.8w + b x c /(k+c)

In calculating the effective degradability of the cell wall constituents (NDF, ADF, hemicellulose) it was assumed that their degradability was the same as that of dry matter but the passage rate of fine particles of the fibre fractions was equal to that of the liquid phase in the rumen. The equation used was as modified by Nsahlai *et al* (unpublished):

ED= fw + b x c /(k+c) where fw = (w x c)/(c + k_1) (disappearance of fine particles) and k_1 was passage rate of liquid phase and was estimated to be 0.05 for sheep.

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The effects of pod species on all variables were obtained by subjecting the data to a one-way analysis of variance using the general linear model (SAS, 1987). Contrasts between pods were done by applying the probability of difference (PDIFF) option of the LSmeans Statement available in the GLM. Only samples that were supplied in duplicates for chemical analysis were analysed statistically.

3.4 RESULTS

Chemical composition

The chemical composition of the pods, seeds and husks of the six browse species is shown in Table 3.1. The results show that all six pod species as well as their components differ significantly in their chemical composition. The seeds had higher crude protein contents than the pods with values ranging from 200-300 g CP kg^{-1} DM. The CP contents of the *Leucaena* pods and seeds were higher than those of the *Acacias*. The concentrations of the detergent fibre fractions (NDF and ADF) of the husks were generally higher than those of the pods and seeds with the *Leucaena* husks having exceptionally high values. In terms of fibre-bound nitrogen, the pods of *L. leucocephala* and *A. karoo* had similar ADF-N concentrations, which were about twice the corresponding values in the other four species of *Acacia*. The NDF-N concentrations varied narrowly among species. The hay fed to the fistulated cows was equally analyzed and its CP and NDF contents were 40 and 700 g kg^{-1} DM respectively.

The lipid contents of the pods and seeds of *L. leucocephala* were higher than those of the *Acacia* species and for all species, the seeds had higher lipid contents than the pods. The concentration of condensed tannins in the pods, expressed in Quebracho

Table 1 Chemical composition of the pods, seeds and husks of *Leucaena leucocephala,*
Acacia erioloba, Acacia karoo, Acacia nilotica, Acacia sieberiana and *Acacia tortilis*

Consti tuent	Plant part	Plant species							
		L.leuc o- cephala	Acacia					SED	*p* value
			erioloba	karoo	nilotic a	sieberiana	tortilis		
CP [1]	Pod	246.9^a	124.1 ^{b}	193.2^c	149.1^d	174.9^e	191.2^c	1.56	.0.001
	Seed	303.5^a	233.4 ^{b}	281.2^c	196.7^d	223.3^e	319.8^f	3.99	0.001
	Husk	84.1^a	103.8 ^{b}	110.8 ^{b}	132.0^c	148.3^d	129.5^c	4.48	0.001
NDF [1]	Pod	408.9^a	477.0 ^{b}	459.7 ^{b}	224.7^c	·346.5^b	398.9^a	9.30	0.001
	Seed	333.4^a	260.1 ^{b}	294.9^c	323.6^a	318.8^a	264.7 ^{b}	7.35	0.002
	Husk	740.1^a	494.8 ^{b}	561.9^c	213.7^d	366.9^e	470.4^f	9.05	0.001
ADF [1]	Pod	284.7^a	335.2 ^{b}	384.2^c	180.8^d	285.0^a	294.9^a	4.63	0.001
	Seed	164.8^a	175.8^a	213.8 ^{b}	248.8^c	210.8 ^{b}	158.7^a	5.28	0.001
	Husk	660.5^a	355.8 ^{b}	477.6^c	157.1^d	290.1^e	361.4^b	6.59	0.001 ·
HEM [1]	Pod	124.2^{af}	141.8^a	75.5 ^{b}	43.9^c	61.5 ^{b}	104.0 ^{f}	9.51	0.001
	Seed	168.6^a	84.3 ^{b}	80.1 ^{b}	75.5 ^{b}	108^c	105.74^c	5.75	0.001
	Husk	79.6^a	139.0 ^{b}	84.3 ^{c}	56.6^d	76.8 ^{a}	109.3^{ab}	14.3	0.01
ASH [1]	Pod	45.4^a	46.5^a	63.7 ^{b}	40.7^c	44.2^d	76.4^c	0.20	0.001
	Seed	40.8^a	42.6 ^{b}	50.7 ^{c}	50.6^c	45.0^d	54.3^c	0.040	0.001
	Husk	47.4^a	47.3^a	73.1 ^{b}	54.3^c	58.2^d	87.7^e	0.65	0.001
NDF-N [2]	Pod	7.0^a	5.2 ^{b}	8.0 ^{c}	6.3^d	5.5 ^{b}	5.6 ^{b}	0.27	0.001
ADF-N [3]	Pod	10.2^a	4.9 ^{b}	9.5 ^{c}	5.3 ^{b}	4.7 ^{b}	4.1^d	0.19	0.001
LIPID [1]	Pod	67.7^a	14.1 ^{b}	19.9 ^{c}	13.7 ^{b}	10.6^d	28.0^e	0.70	0.001
	Seed	72.6^a	41.3 ^{b}	50.2 ^{c}	34.3^d	20.5^e	41.8 ^{b}	0.70	0.001
CT (%QE)	Pod	1.8	13.0	9.2	17.4	28.3	3.6	-	-
S-H ratio (% wt of pod)		60-40	22-78	53-47	31-69	27-73	45-55	-	-

Means in the same row with different superscripts differ significantly [1] g/kgDM, [2] g/kgNDF, · [3] g/kgADF, QE Quebracho equivalents.
equivalents (QE) (Hagerman and Butler, 1982; Martin and Martin, 1982; Wisdom *et al.*

equivalents (QE) (Hagerman and Butler, 1982; Martin and Martin, 1982; Wisdom et al. 1987), indicated that the Leucaena pods and those of A. tortilis had the lowest concentration of condensed tannins while A. sieberiana had the highest. In between the two, were those of A. karoo, A. erioloba and A. nilotica in increasing order.

Mineral composition of the pods

The mineral composition of the pods is shown in Table 3.2. Two species; A. tortilis and A. karoo had high concentrations of Ca while the highest concentration of P was noticed in the pods of L. leucocephala. The values for Mg, Na and K were similar among all pods except A. erioloba which had the lowest concentration of all macro-minerals except Ca. Among the micro-minerals, only Fe was present in high concentration in all pods. The concentrations of Zn and Mn were similar among all pods except A. erioloba which was equally deficient in micro-minerals.

Table 3.2 Mineral composition of the pods of Leucaena leucocephala, Acacia erioloba, Acacia karoo, Acacia nilotica, Acacia sieberiana and Acacia tortilis.

Pod	Minerals									
	gkg^{-1}DM					mgkg^{-1} DM				
	Ca	P	Mg	Na	K	Fe	Zn	Cu	Mn	Se
L. leucocephala	4.5	3	2.7	0.3	0.3	118	29.5	8.4	23.2	0.08
A. erioloba	6.1	1.5	1.2	0.1	0.3	167	18.1	4.3	7.5	0.04
A. karoo	10.7	2.7	2.5	1.3	0.3	195	33.2	10.7	18.2	0.17
A. nilotica	6.4	1.9	1.6	0.2	0.3	112	29.9	10.7	17.1	0.07
A. sieberiana	7.2	2.2	2.4	0.5	0.3	68.3	29.9	4.3	17.1	0.14
A. tortilis	10.9	2.8	3.5	0.3	0.4	196	30.9	4.3	18.1	0.17

The pods of *A. sieberiana* and *A. tortilis* had high concentrations of Se but their Cu concentrations were rather low.

Amino acid composition of the pods and seeds

Tables 3.3 and 3.4 show the amino acid compositions of the pods and seeds respectively. The six pod species differed in their amino acid compositions. The concentration of amino acids in the *Leucaena* pods was considerably higher than those of the *Acacia* species with the exception of aspartic acid, proline and tyrosine.

Table 3.3 Amino acid concentration of the pods of *Leucaena leucocephala*, *Acacia erioloba* *Acacia karoo*, *Acacia nilotica*, *Acacia sieberiana* and *Acacia tortilis*.

Amino acid	Concentration (gkg^{-1}DM)							
	L. leuco-	*Acacia*						
	cephala	*erioloba*	*karoo*	*nilotica*	*sieberiana*	*tortilis*	SED	*p* value
Aspartic acid	25.08 [a]	10.9 [b]	22.83 [c]	16.25 [d]	33.96 [e]	23.42 [f]	0.025	0.001
Threonine	5.39 [a]	2.97 [b]	4.23 [c]	2.85 [d]	2.46 [e]	4.08 [f]	0.01	0.001
Serine	5.2 [a]	3.12 [b]	4.43 [c]	2.83 [d]	2.22 [e]	3.91 [f]	0.01	0.001
Glutamine	33.29 [a]	13.71 [b]	21.13 [c]	12.57 [d]	11.7 [d]	21.28 [c]	0.043	0.001
Proline	9.37 [a]	20.71 [b]	11.86 [c]	22.16 [d]	22.95 [d]	17.02 [e]	0.048	0.001
Glycine	10.6 [a]	4.57 [b]	8.31 [c]	5.93 [d]	5.80 [d]	8.82 [e]	0.011	0.001
Alanine	8.43 [a]	3.95 [b]	6.62 [c]	5.24 [d]	3.6 [e]	6.52 [c]	0.01	0.001
Valine	10.13 [a]	4.97 [b]	8.37 [c]	5.95 [d]	4.59 [b]	8.79 [c]	0.03	0.001
Methionine	1.74 [a]	0.32 [b]	0.47 [b]	1.02 [c]	0.20 [b]	0.55 [b]	0.01	0.001
Isoleusine	8.36 [a]	3.60 [b]	5.93 [c]	3.71 [b]	2.9 [d]	5.69 [e]	0.01	0.001
Leucine	13.8 [a]	6.15 [b]	10.81 [c]	6.41 [d]	5.18 [e]	10.59 [e]	0.01	0.001
Tyrosine	3.1 [a]	1.81 [b]	2.04 [b]	3.29 [a]	0.91 [c]	2.54 [d]	0.018	0.001
Phenylalanine	8.63 [a]	3.43 [b]	6.28 [c]	3.76 [d]	2.83 [e]	5.8 [f]	0.01	0.001
Histidine	5.47 [a]	2.71 [b]	5.02 [c]	3.58 [d]	3.24 [e]	4.6 [f]	0	0.001
Lysine	14.34 [a]	5.51 [b]	9.16 [c]	6.03 [d]	4.76 [e]	8.92 [f]	0.01	0.001
Arginine	14.55 [a]	4.56 [b]	11.74 [c]	8.13 [d]	6.64 [e]	9.84 [f]	0.019	0.001

Means in the same row with different superscripts differ significantly

The pods of *A. sieberiana* had the lowest concentrations of eleven of the sixteen amino acids analyzed but however recorded the highest concentrations of two (aspartic acid and proline) non-essential amino acids while the pods of *A. nilotica* had the highest concentration of tyrosine. The concentrations of aspartic acid, glycine, histidine and arginine were lowest in the pods of *A. erioloba*. In descending order of concentration of amino acids in the pods, *L. leucocephala* was followed by *A. tortilis, A. karoo, A. nilotica, A. erioloba* and *A. sieberiana*. The concentration of the sulphur amino acid, methionine when compared with the others was quite low in all the pods with *A. sieberiana* having the lowest concentration. On the other hand, the dicarboxylic acids (aspartic and glutamic acids) were found in high concentration in all the pods with *A. sieberiana* and *L. leucocephala* having the highest values of aspartic and glutamic acids respectively. The concentrations of the basic (arginine, lysine and histidine) acids were equally high in most pods; *L. leucocephala* having the highest and *A. erioloba* and *A. sieberiana* having the lowest values. Of the nine essential amino acids determined, those with high concentrations in most pods were arginine, lysine, leucine and valine while methionine and histidine were only present in low concentrations.

The trend of amino acid concentration in the seeds (Table 3.4) showed a similar pattern as in the pods with the exception of the seeds of *A. nilotica* which were rather deficient in most of the amino acids. In order of decreasing content of the amino acids found in seeds, *L. leucocephala* was followed by *A. tortilis, A. karoo, A.erioloba, A. sieberiana* and *A. nilotica*. The seeds like the pods, were rich in the dicarboxylic acids and low in the concentration of methionine. The concentrations of the monocarboxylic (threonine, serine, glycine, alanine, valine, isoleucine and leucine) and the aromatic (proline,

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phenylalanine and tyrosine) amino acids were between the two extremes. The range of the basic acids was lower than that of the dicarboxylic acids but higher than that of the monocarboxylic acids. All the seeds except those of *A. nilotica* were rich in most of the essential amino acids.

Table 3.4 Amino acid concentration of the seeds of *Leucaena leucocephala, Acacia erioloba Acacia karoo, Acacia nilotica, Acacia sieberiana* and *Acacia tortilis*

Amino acid	Concentration (gkg⁻¹DM)							
	L. leuco-	Acacia						
	cephala	erioloba	karoo	nilotica	sieberiana	tortilis	SED	p value
Aspartic acid	31.63 [a]	20.84 [b]	29.33 [c]	16.25 [d]	22.71 [e]	28.27 [c]	0.064	0.001
Threonine	7.00 [a]	4.99 [b]	6.05 [c]	3.72 [d]	3.97 [e]	6.19 [c]	0.009	0.001
Serine	6.73 [a]	5.45 [b]	6.75 [a]	4.03 [c]	4.40 [d]	6.81 [a]	0.011	0.001
Glutamine	43.93 [a]	32.69 [b]	35.39 [b]	22.11 [c]	24.29 [c]	38.37 [d]	0.122	0.001
Proline	10.95 [a]	17.45 [b]	14.94 [b]	9.85 [a]	10.21 [a]	15.83 [b]	0.109	0.002
Glycine	13.38 [a]	11.53 [b]	13.56 [a]	11.6 [a]	14.83 [d]	16.22 [e]	0.032	0.001
Alanine	10.97 [a]	9.18 [b]	10.26 [a]	6.45 [c]	6.92 [c]	10.93 [a]	0.023	0.001
Valine	12.22 [a]	10.72 [b]	12.56 [a]	7.83 [c]	7.97 [c]	12.73 [a]	0.021	0.001
Methionine	2.70 [a]	1.04 [b]	2.21 [c]	0.34 [d]	1.16 [b]	1.07 [b]	0.014	0.001
Isoleusine	10.45 [a]	8.41 [b]	9.51 [c]	5.71 [d]	5.99 [d]	9.76 [c]	0.015	0.001
Leucine	17.6 [a]	16.14 [b]	18.46 [c]	11.17 [d]	11.91 [e]	19.4 [f]	0.022	0.001
Tyrosine	3.04	2.88	2.58	2.29	2.43	2.78	0.015	0.018
Phenylalanine	11.06 [a]	8.44 [b]	10.25 [c]	5.87 [d]	5.93 [d]	10.34 [c]	0.015	0.001
Histidine	7.24 [a]	5.31 [b]	7.73 [c]	5.09 [b]	4.93 [b]	7.62 [c]	0.013	0.001
Lysine	15.68 [a]	11.61 [b]	14.1 [c]	9.00 [d]	9.36 [d]	14.82 [c]	0.014	0.001
Arginine	19.56 [a]	14.63 [b]	18.89 [a]	13.04 [c]	19.12 [a]	19.47 [a]	0.012	0.001

Means in the same row with different superscripts differ significantly

Degradation of dry matter, nitrogen and fibre fractions

The degradabilities of dry matter (DM) and other constituents are shown in Table 3.5. The slowly degradable fraction (B), potential degradability (PD), effective degradability (ED) and rate of degradation (C) of DM differed ($p<0.001$) but the lag time (LT) that preceded degradation was similar ($p>0.05$) among all pods. The pods of *A. nilotica* had the highest PD and ED while those of *A. tortilis* had the highest B value, closely followed by *A. karoo* and *A sieberiana*. The lowest values were seen with the pods of *A. erioloba*. The *Leucaena* pods degraded faster ($p<0.001$) than the *Acacia* ones which had similar rates.

The pattern of degradation of nitrogen was similar to that of dry matter. The pods of *A. tortilis* had the highest B value for nitrogen, closely followed by *A. karoo* while those of *A. sieberiana* had the lowest value. The B values for the *L. leucocephala*, *A. erioloba* and *A. nilotica* were similar ($p>0.05$) and were intermediate between the highest and lowest values. PD values were also similar ($p>0.05$) among the pods of *L. leucocephala*, *A.erioloba* and *A. karoo* but lower than the values of the pods of *A. nilotica, A. sieberiana* and *A. tortilis* which had similar values. The N of the *Leucaena* pods degraded faster ($p<0.01$) than that of the *Acacia* pods which all had similar rates of degradation. The effective degradabilities of the pods of *L. leucocephala* and *A. sieberiana* were similar ($p>0.05$) but higher ($p<0.01$) than those of the other *Acacia* species which had similar values. In descending order of effective degradability, *Leucaena* and *A. sieberiana* were followed by *A. tortilis, A. nilotica, A. erioloba* and *A. karoo*. LT was similar among all pods.

Table 3.5 Disappearance of DM, N and cell wall constituents of the pods of *Leucana leucocephala*, *Acacia erioloba*, *A. karoo*, *A. nilotica*, *A. sieberiana* and *A. tortilis* incubated in the rumen of Jersey cattle.

Comp onent	Para meter	Browse species						SED	p-value
		L. leucoce phala	Acacia						
			erioloba	karoo	nilotica	sieberiana	tortilis		
DM	W (gkg⁻¹DM)	266.6ᵃ	310.8	229.5ᶜ	473.4	374.1	304.5ᵇ	5.75	0.001
	B (gkg⁻¹DM)	372.1ᵃ	318.5ᵇ	425.9ᶜ	381.7ᵃᶜ	415.4ᵃᶜ	506.7ᵈ	17.42	0.001
	PD (gkg⁻¹DM)	638.7ᵃ	629.3ᵃ	655.4ᵃ	855.1ᵇ	789.5ᶜ	811.2ᶜ	13.99	0.001
	ED (gkg⁻¹DM)	477.3ᵃ	415.0ᵇ	402.4ᵇ	549.6ᶜ	521.7ᵈ	511.7ᵈ	13.81	0.001
	C (h⁻¹)	0.063ᵃ	0.028ᵇ	0.026ᵇ	0.022ᵇ	0.027ᵇ	0.028ᵇ	0.004	0.001
	LT (h)	0.306	1.783	-0.878	-2.688	1.161	0.948	1.285	0.227
N	W (gkg⁻¹DM)	567.4ᵃ	610.0ᵇ	459.5ᶜ	636.8ᵈ	685.8	470.3ᶜ	14.42	0.001
	B (gkg⁻¹DM)	324.9ᵃ	278.6ᵇ	408.3ᶜ	299.6ᵃᵇ	255.9ᵇ	468.5ᵈ	7.88	0.001
	PD (gkg⁻¹DM)	892.3ᵃ	888.6ᵃ	867.8ᵃ	936.4ᵇ	941.7ᵇ	938.8ᵇ	7.36	0.003
	ED (gkg⁻¹DM)	713.9ᵃ	647.2ᵇ	624.2ᶜ	658.4ᵇ	694.2ᵃ	671.1ᵇ	9.73	0.003
	C (h⁻¹)	0.100ᵃ	0.034ᵇ	0.043ᵇ	0.026ᵇ	0.033ᵇ	0.043ᵇ	0.005	0.001
	LT (h)	3.17	4.75	-3.03	-4.44	-0.31	-0.54	2.252	0.175
NDF	W (gkg⁻¹DM)	92.2ᵃ	150.7ᵇ	31.8ᶜ	153.1ᵇ	66.7ᵃᶜ	35.1ᶜ	21.33	0.001
	B (gkg⁻¹DM)	391.3ᵃ	332.7ᵇ	541.4ᶜ	496.2ᵈ	563.4ᶜ	684.2ᶜ	10.54	0.001
	PD (gkg⁻¹DM)	483.5ᵃ	483.4ᵃ	573.2ᵇ	649.3ᶜ	645.5ᶜ	719.3ᶜ	5.11	0.001
	ED (gkg⁻¹DM)	286.1ᵃ	228.5ᵇ	306.0ᵃ	294.8ᵃ	325.3ᵃ	378.1ᶜ	17.09	0.019
	C (h⁻¹)	0.043	0.028	0.03	0.025	0.026	0.029	0.004	0.149
	LT (h)	-0.44	6.13	1.28	4.66	13.61	3.91	1.919	0.082
ADF	W (gkg⁻¹DM)	34.4ᵃ	121.6ᵇ	17.3ᵃ	72.1ᵇ	26.4ᵃ	-14.5ᵃ	36.36	0.025
	B (gkg⁻¹DM)	355.1ᵃ	337.6ᵃ	524.9ᵇ	526.1ᵇ	586.6ᶜ	718.8ᵈ	16.87	0.001
	PD (gkg⁻¹DM)	389.5ᵃ	459.2ᵇ	542.2ᶜ	598.2ᵈ	613.0ᵈ	704.3ᵈ	13.25	0.001
	ED (gkg⁻¹DM)	205.9ᵃ	204.2ᵃ	281.3ᵇ	275.4ᵇ	315.1ᶜ	349.5ᵈ	13.43	0.003
	C (h⁻¹)	0.031	0.024	0.028	0.024	0.028	0.024	0.004	0.574
	LT (h)	5.35	6.3	-0.32	5.8	15.2	2.19	2.36	0.455
HEM	W (gkg⁻¹DM)	213.3ᵃ	238.2ᵃ	83.3ᵇ	336.1ᶜ	213.2ᵃ	167.0ᵃ	39.98	0.003
	B (gkg⁻¹DM)	507.0ᵃ	333.0ᵇ	605.5ᶜ	431.7ᵃ	436.3ᵃ	609.6ᶜ	23.98	0.007
	PD (gkg⁻¹DM)	720.0ᵃ	571.2ᵇ	688.8ᵃ	767.8ᶜ	649.5ᵃᵇ	776.6ᶜ	8.63	0.001
	ED (gkg⁻¹DM)	454	318.2	392.1	348.3	428.7	480.4	37.94	0.148
	C (h⁻¹)	0.063	0.044	0.036	0.029	0.074	0.048	0.0151	0.694
	LT (h)	-10.7	5.06	4.81	1.48	11.06	6.65	5.75	0.348

With respect to the degradation of NDF, the pods of *A. erioloba, A. karoo, A. nilotica,* and *L. leucocephala* had similar (p>0.05) B values but those of *A. sieberiana* and *A. tortilis* were lower (p<0.05). The latter had the highest potential (PD)and effective (ED) degradabilities. These values were rather low for the pods of *A. erioloba* and those of *L. leucocephala.* The rates of degradation of NDF were similar (p>0.05) among all pods while LT was higher (p<0.05) for the pods of *A. sieberiana.*

With respect to the degradation of ADF, *A. tortilis* had the highest B, PD and ED values while Leucaena had the lowest. In between the two extremes, were the pods of *A. sieberiana, A. nilotica, A. karoo* and *A. erioloba,* in descending order. The rates of degradation of ADF were similar (p>0.05) among the pods while lag time was higher for the pods of *A. sieberiana.* The ED, C and LT were equally similar (p>0.05) among the pods in the degradation of hemicellulose. The pods of *A. tortilis,* like in the case of NDF and ADF, had the highest B and PD values, followed in descending order by those of *Leucaena, A. karoo, A. nilotica, A. sieberiana* and *A. erioloba.*

The ED, C and LT were equally similar (p>0.05) among the pods in the degradation of hemicellulose. The pods of *A. tortilis,* like in the case of NDF and ADF, had the highest B and PD values, followed in descending order by those of *Leucaena, A. karoo, A. nilotica, A. sieberiana* and *A. erioloba.*

3.5 DISCUSSION

Chemical composition

The chemical compositions of the pods, seeds and husks were analysed separately in order to better appreciate what the animal takes at any given time considering the fact that some of these pods (*L. leucocephala, A. karoo* and *A. tortilis*) split easily when they are dry and the seeds generally fall off before the husks. It should however be mentioned that separating seeds from husks before feeding is not practical. The results presented in Tables 1 to 4 show that the pods of six browse species differ significantly in their chemical, mineral and amino acid compositions. The crude protein values for the pods of *A. tortilis* and *A. sieberiana* were similar to those reported by Sawe *et al* (1998) but higher than those reported by others (Tanner *et al.*, 1990; Nsahlai *et al.*, 1995b). These disparities may be attributed to the site and within species differences (Le Houerou, 1980). It is also apparent from Table 1 that the CP content of the pods was largely due to a high contribution from the seeds while the high levels of the detergent fibres were mainly attributed to the husks. As a result, the pods with high seed:husk ratio (*L. leucocephala, A. karoo* and *A. tortilis*) had higher CP contents while those with low ratios (*A. erioloba*) had low CP values and high concentrations of NDF. It should however be noted that the *Leucaena* pods had high concentrations of fibre fractions despite their high seed:husk ratio because the husks were particularly rich in NDF and ADF.

The results presented in Tables 3 and 4 indicate that the concentration of amino acids was reasonably high in the pods although methionine appeared to be deficient. The range of amino acid concentrations in the pods is lower (about one-third) than the range

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in oil seed (soya, sunflower, cotton and groundnut) cakes but comparable to what is seen in alfalfa (*Medicago sativa*), beans (*Vicia faba*) and peas (*Pisum sativum*) (McDonald *et al.*, 1998). Although the analysis for cysteine and tryptophane could not be done due to high costs involved, the results of the sixteen amino acids analyzed showed a consistent superiority of the pods and seeds of three species (*L. Leucocephala, A. tortilis and A. karoo*) over the others in amino acid concentrations. Taminga (1979) reported that arginine, aspartic acid, glutamic acid, proline and alanine degraded to a larger extent in the rumen, while methionine, serine, glycine, tyrosine and cystine degraded to a lesser extent. Incidentally those that degrade readily in the rumen had higher concentrations in all the pods. This may suggest that animals consuming these pods could have more rumen degradable nitrogen resulting from the break down of amino acids. This expectation may however be impaired by the high concentration of condensed tannins in these pods, which tend to bind with protein forming rumen undegradable complexes (Reed *et al.*, 1990; Reed, 1995). It is also possible to reduce the degradation of amino acids in the forestomachs in order to obtain greater absorption from the intestines by grinding the pods before feeding or treating them with chemicals such as aldehydes or volatile fatty acids (Taminga, 1979; Chalupa, 1976; Scheifinger *et al.*, 1976).

All the pods were rich in Ca, P and Mg as well as the micro-minerals but deficient in Na and K. However it is seen from Table 2 that supplementing a diet of poor quality hay fed to a sheep (35 kg liveweight, gaining about 150 g a day) with 0.5 kg of pod meal could meet most of the mineral requirements of the animal but for P and Zn for all pods and Se in the case of *L. leucocephala, A. erioloba* and *A. nilotica* (McDonald *et al.*, 1998).

The Ca values of the pods are higher when compared to those of oil seed (cotton, groundnut and soya) meals while the values for P, Mg, Na and the micro-minerals are rather lower. However, in the absence of the oilseed cakes, as it is generally the case in most rural areas, the pod meals could be considered as a good replacement.

Degradation properties

With respect to degradability, the Leucaena and Acacia pods had variable degradation characteristics for DM, N and the fibre fractions. The high rate of degradation of DM of the Leucaena pods relative to those of the Acacia pods may be linked to the fact that the former had a higher seed:husk ratio. Since the seeds degrade faster than the husks, it is possible that the rate of degradation of DM of the Leucaena pods could be directly linked to that of the seeds. The high ED of DM for the pods of *A. nilotica* and *A. sieberiana* could be linked to their low NDF concentrations since the ED of the pods was found to be negatively correlated ($r = -0.91$, $p<0.01$) to NDF. This observation confirms a report that DM digestibility was positively correlated to CP content and negatively correlated to crude fibre, NDF and ADF (Minson, 1982b). This may also explain why *A. erioloba* and *A. karoo* had low effective dry matter degradabilities. The high PD values for the pods of *A. nilotica* and *A. sieberiana* resulted from very high washing losses of these pods. The low PD values for the pods of *L. leucocephala, A. erioloba, A karoo* and *A. tortilis* are closely linked to their low values for washing losses. The slow rate of degradation of DM in the pods of *A. nilotica* may be attributed to the presence of (+)- cattechin gallates which are known to be toxic to rumen bacteria and the host animal (Self *et al.*, 1986; Muller-Harvey *et al.*, 1987). It was noticed that goats consuming high quantities of the pods of *A. nilotica* showed hypoglobulinaemia,

abortion, tachycardia, ruminal atony, hyperglycaemia, liver and kidney disruptions (Terblanche *et al.*, 1967), although the toxic compound was not linked to catechins at the time.

The high B, PD and ED values of the NDF, ADF and hemicellulose in the pods of *A. tortilis* may be attributed to low levels of soluble phenolics and proanthocyanidins as seen in this study and confirmed by other authors (Tanner *et al.*, 1990; Nsahlai *et al.*, 1995b). These compounds are present in most tropical legume forages (Mueller-Harvey *et al.*, 1987; Reed *et al.*, 1985; D'Mello, 1992; Kumar, 1992) and were reported to be toxic to rumen microbes, consequently limiting the degradability of cell walls (Akin, 1982; Borneman *et al.*, 1986; Jung, 1985, 1988). It has also been reported that extracts from *Leucaena leucocephala* delayed the growth of cellulolytic bacteria especially, *Fibrobacter succinogenes, Ruminicoccus albus* and *R. flavesciens* (ILCA, 1994; Nsahlai *et al.*, 1994). This delay probably explains the depression in the digestion of ADF in the *Leucaena* pods. The high level of proanthocyanidins in the pods of *A. sieberiana* may be responsible for the delay in the onset of fermentation in these pods. It is also possible that the high ash content in the pods of *A karoo* was responsible for the depression in digestion of fibre in these pods since silica is negatively correlated to digestibility (Hoover, 1986). Furthermore, lignin (though not measured in this study) also plays an important role in depressing fibre degradability in the pods. Variation in lignin was reported to have had a greater effect on NDF degradability of legumes than the variation in structural polysaccharides (Buxton *et al.*, 1987). Lignin equally influnces the degradability of hemicellulose (Wedig *et al.*, 1986; Albrecht *et al.*,1987; Fort and Elliott, 1987; Hatfield, 1989). Non-lignified tissues may

also be poorly degraded due to binding with low molecular weight phenolic compounds (Vadiveloo and Fadel, 1992).

The disappearance of N from the nylon bags ranged from a low of 62 to 71 per cent for the pods of *A. karoo* and *L. leucocephala* respectively. The low extent of N degradability in the pods of *A. karoo* may be explained by the fact that a high proportion (0.31) of N is ADF- bound. It may however be misleading to consider the disappearance of N from the nylon bags as the quantity that was effectively degraded. Some studies have shown that although *in sacco* losses of protein generally correlate with degradability, this may not necessarily be the case with tanniferous feeds because part of protein loss from nylon bags may be in the form of tannin-protein complexes which are not degradable in the rumen (McNabb *et al.*, 1996; Perez-Maldonado and Norton, 1996; Nsahlai *et al.*, 1999). These interactions reduce the availability of proteins to microbes in the rumen. However, calculations of effective rumen degradable protein (ERDP) and digestible undegraded protein (DUP) indicated that feeding animals with 0.5 kg of pod meal could provide 38-45 g of metabolizable protein (MP) per day. Even though these amounts fall short of the stipulated value of 83g for a growing sheep of 35 kg liveweight (McDonald *et al.*, 1998), they can sustain maintenance and promote growth. In this regards, it is seen that legume pods can constitute a possible source of protein for ruminants.

3.6 CONCLUSION

Despite the possible nutritional limitations imposed by the presence of condensed tannins, results from this work show that the CP content of the pods (12.4-24.7%), the high mineral and amino acid contents, the effective degradabilities of pod DM of 0.48-

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0.64 and the possible provision of MP levels of up to 44 g per day give positive indications that pods have a high potential of reducing the problem of feed shortages in areas where pastures are deficient. Their low degree of bulkiness as compared to foliages gives them an additional advantage over the latter in that they can promote higher intake of roughage when fed as supplements.

The effect of feeding pods of multipurpose trees (MPTs) on the degradability of dry matter and cell wall constituents of maize stover and alfalfa incubated in the rumen of sheep

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4.1 Abstract

The study evaluated the effects of feeding alfalfa and pods of tree legumes on rumen pH and ammonia concentration as well as *in sacco* degradability of dry matter and fibre constituents of maize stover, alfalfa and their neutral detergent extracts. The feeds were incubated in the rumens of five South African Merino sheep fed individually in an incomplete (5x4) Latin square design, using five diets. The diets comprised of equal proportions of pasture hay and either alfalfa (Alfalfa diet), pods of *Acacia sieberiana* (Sieberiana diet), *Acacia nilotica* (Nilotica diet) or *Leucaena leucocephala* (Leucaena diet). The fifth diet composed of pasture hay alone (Hay diet) served as a negative control. Supplementation of hay with either legume pods or alfalfa hay significantly (p<0.05) increased rumen ammonia concentration from 56 to a maximum of 86mgL^{-1}. The pH of the rumen fluid ranged between 6.2 and 6.5 for all diets but variations in pH were not significant. Diet did not affect (p>0.05) degradability constants; lag time (LT), slowly degradable fraction (B), potential degradability (PD), effective degradability (ED) or rate of degradation (C) of dry matter but significantly (p<0.01) affected the ED of the fibre constituents of the incubated feeds. The effective degradabilities of the incubated feed differed (p<0.001) with alfalfa and maize stover having average values of 555 and 318 gkg^{-1} DM respectively. The rate of degradation of alfalfa was also higher (p<0.01) than that of maize stover. Neutral detergent extraction improved ED of the cell wall constituents of maize stover but rather produced opposite effects for alfalfa. The interactions between incubation feed and extract were significant (p<0.01) for ED and C of NDF and hemicellulose. The observations show that pods from tree legumes are comparable to alfalfa in the provision of rumen ammonia but limit microbial activity in the degradability of fibre constituents. They also show that maize stover and alfalfa differ in their degradabilities and the differences may be attributed to their cell wall chemistry.

Key words: Legume pods, rumen ammonia, dry matter, fibre constituents, degradability.

4.2 INTRODUCTION

Leaves and pods of leguminous browses provide a good source of protein supplement to ruminants in tropical Africa. The *Acacia* species in particular are very common in the agrosilvipastoral systems of sub-Saharan Africa (SSA) where they supply livestock with fodder and shade and benefit crop production by fixing nitrogen and improving soil fertility. Previous research has shown that *Acacia* pods are of high nutritive value and could serve as a potential source of protein for diets based on crop residues (Gwynne,1969; Topps, 1992). Unfortunately some of the leaves and pods have a high concentration of tannins and fibre-bound nitrogen which are not digested (Tanner *et al.*, 1990; Reed, 1995), consequently, their concentration increases along the digestive tract of the animal (Reed *et al.*, 1990). This phenomenon favours high faecal nitrogen and low urinary nitrogen excretion (Ebong, 1989; Woodward and Reed, 1989). The slow degradability of dry matter (DM) and nitrogen (N) of *Acacia* pods as compared to leaves of *Sesbania* (Nsahlai *et al.*, 1995a), is also an indication that if ruminants are offered *Acacia* pods as sole supplement, it is possible that the degradability of the pods may be a limiting factor in the supply of rumen ammonia for microbial activity. This could partly explain the low digestion of fibre following supplementation with fruits of *Acacia* species (Tanner *et al.*, 1990).

This study examined the effects of feeding pods of *Acacia sieberiana*, *Acacia nilotica* and *Leucaena leucocephala* on rumen pH, rumen ammonia concentration and *in sacco* degradability of DM and fibre constituents of maize stover, alfalfa hay and their neutral detergent extracts. The objective of extraction was to evaluate the activity of fibrolytic microorganisms on fibre constituents in the absence of cell solubles and examine any

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form of synergy in the activity of fibrolytic and amylolytic microorganisms in the degradation of fibre constituents in the rumen.

4.3 MATERIALS AND METHODS

Animals, diet and experimental design

Five rumen fistulated Merino sheep (all males) weighing 48.8 (SD = ± 4.76) kg were used in the experiment. Each animal was assigned to a dietary treatment. The diets comprised of equal proportions (dry matter weight) of pasture hay and either alfalfa (Alfalfa diet), pods of *Acacia sieberiana* (Sieberiana diet), *Acacia nilotica* (Nilotica diet) or *Leucaena leucocephala* (Leucaena diet). The fifth diet was composed of pasture hay alone (Hay diet) and served as a negative control. The feeds were ground through a 3-mm screen of a hammer mill and mixed, using an industrial mixer. Each feed was given at the rate of 450 g day^{-1} per animal partitioned into equal portions of 150 g each and offered at 08.00, 12.00 and 16.00h each day. An incomplete Latin square design (5 x 4) was adopted and the diets were offered in four periods of 15 days each, comprising 7 days of adaptation, followed by 7 days of incubation and one day of rumen fluid collection.

Dry matter and fibre degradability

Two roughages, maize stover and alfalfa hay, were chosen as incubation material to study the degradability of DM and fibre constituents. Each roughage was ground through a 2- mm screen in a hammer mill and then divided into two portions. One portion was used to examine DM degradability and in this regards, 3 g of each sample was weighed into nylon bags (41 μm pore size; bag size 6 x 12 cm; Polygon

Switzerland). The second portion was weighed and pre-treated with neutral detergent solution (NDS) to extract soluble cell fractions before incubation (Nsahlai *et al.*, 1995c). The NDS was prepared using the method described by Van Soest *et al* (1991). During the extraction process, 500 g of ground material was weighed into a 10l pot to which 4l of NDS was added. Using an electric heater, the mixture was quickly brought to bubbling and then regulated to boil gently by reducing the heat. At this point, 1 ml of heat stable amylase (Sigma A-3306) was added and the mixture was allowed to boil for one hour, then poured into a silk cloth bag and rinsed with tap water while squeezing until the colour of the solution was clear. The residue was put back into the pot and the process was repeated once. The final residue was dried at 40°C in a force draught oven for 24 hours and 2 g of the residue was weighed into nylon bags.

At each incubation period, four bags, two containing ground samples of the roughages and two containing the neutral detergent extracts of the same samples were put into the rumen of each sheep through the cannula using the method described by Mehrez and Orskov (1977). The method of sequential addition was adopted while ensuring that each time the number of bags reached a maximum of 12, incubation was suspended until those incubated were removed. Bags were withdrawn from the rumen after incubating for 168, 144, 120, 96, 72, 48, 24, 12, 6 and 3 hours. To determine losses at zero hour, a set of bags were not incubated but were washed with the incubated ones in a semi automatic machine (Hoovermatic) for 30 min (five cycles of 6 min each). The bags were dried in a force draught oven at 60° C for 48 hours, cooled in a desiccator and weighed. To remove any microbial contamination and digested soluble material, the intact bags were put into a pot to which 4l of NDS and 1 ml of amylase were added and boiled for

one hour. This was followed by washing, drying and weighing as described above. The variation in this method of extraction compared to the standard Van Soest's (1991) method was 5 percent.

Extraction of acid detergent fibre (ADF)

ADF was obtained by boiling the intact bags with the residue obtained above in 4l of acid detergent solution (ADS) prepared as described by Van Soest *et al* (1991). After boiling for one hour, the bags were washed, dried and weighed as described above. Hemicellulose was estimated as the difference between NDF and ADF.

Estimation of rumen ammonia and pH

After each incubation period, animals were maintained on the same diets and rumen fluid was collected from the rumen of each animal using a mechanical suction pump for the analysis of rumen ammonia and determination of rumen pH. Collection was conducted at 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, and 24 hours after feeding in the morning at 08.00h. The second feeding was conducted at 12.00h and the third at 16.00h. Immediately after collection, the rumen fluid was strained through a cheese cloth and this was closely followed by the reading of the pH. Samples of about 100ml used for determination of rumen ammonia were put in 250 ml containers to which 3 drops of concentrated sulphuric acid were added and stored in a freezer maintained at -20°C until they were needed for analysis.

Chemical analyses

The dry matter (DM), organic matter (OM) and nitrogen (N) contents of the feeds were determined using the method outlined by AOAC (1990). Neutral detergent fibre (NDF) and acid detergent fibre (ADF) of the feeds were determined by the method of Van Soest *et al.* (1991) while hemicellulose was estimated as the difference between the NDF and ADF. After acid digestion, phosphorus was determined colorimetrically while calcium was determined using a Varian spectra AA-200 Atomic Absorption spectrophotometer, AOAC (1990). Pod samples were also sent to the Jacob Blaustein Center for Desert Biodiversity, Ben Gurion University of Negev Desert, in Israel for the analysis of condensed tannins and the method of determination was that described by Hagerman (1995).

Calculations and statistical analyses

The degradation of DM and detergent fibre fractions was estimated by fitting the non-linear model proposed by McDonald (1981) and modified by Dhanoa (1988) to the degradation data of each component:

$$Y = A + B[1-e^{-C(T-LT)}]$$

where Y is the disappearance of DM or fibre fraction at time T, A = washing loss or solubility, B = degradable part of the insoluble fraction, C = rate of degradation of B and LT, the lag time. The four data sets obtained from four animals over the four periods were used to fit one curve, consequently, the period and animal effects were eliminated. The potential degradability (PD) was calculated as A + B . A passage rate (k) of 0.03 h^{-1} was assumed in order to calculate the effective degradabilities (ED) of DM (Bonsi *et al.*, 1994; Nsahlai *et al.*, 1998a):

ED = A + B x C /(k+C).

In the calculation of the ED of the fibre fractions, it was assumed that the degradability of the fibre fractions was the same as that of DM but the passage rate of fine particles of the fibre fractions was equal to that of the liquid phase in the rumen. The equation was therefore modified as such:

ED= fA + B x C /(k+C), where fA = (A x C)/(C + k_1) and k_1 is passage rate of liquid phase and is equal to 0.05.

The effects of diet, incubation feed and extraction as well as their interaction were considered in the model that was used in the analysis of variance to which the data was subjected. Contrasts between diets, incubation feeds and extracts were obtained by applying the probability of difference (PDIFF) option of the LSmeans Statement available in the GLM (SAS, 1987).

4.4 RESULTS

Chemical composition

The chemical composition of the ration ingredients is shown in Table 4.1 Among the feeds considered as protein sources, the pods of *Leucaena leucocephala* had the highest crude protein content, followed by alfalfa, *A. sieberiana* and *A. nilotica* in descending order. NDF concentrations were similar for alfalfa and the leucaena pods but lower for the acacia pods. The concentration of ADF in alfalfa, when compared to that of the pods, was higher. The ADF values for *Leucaena leucocephala*, *A. sieberiana* and *A. nilotica* were 0.83, 0.85 and 0.53 times the value for alfalfa. The concentration of hemicellulose in alfalfa was comparable to that of the acacia pods but just about half the value of the leucaena pods. The ash content in alfalfa was about two times the

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Table 4.1 The chemical and mineral compositions of the pods of *Leucaena leucocephala, Acacia nilotica, Acacia sieberiana,* alfalfa, maize stover and veld hay.

Feed	DM	OM	CP	NDF	ADF	HEM	Ca	P	CT
	gkg^{-1}				gkg^{-1}DM				%QE
L. leucocephala (Pod)	948	953	247	409	285	124	4.5	3	1.8
A. nilotica (Pod)	937	953	149	225	181	43.9	6.4	1.9	17.4
A. sieberiana (Pod)	937	946	175	367	290	76.8	7.2	2.2	28.3
Alfalfa (Hay)	927	899	220	402	341	61.3	1	0.3	--
Maize stover	943	929	55.4	523	360	162	0.3	0	-
Pasture Hay	952	925	41.7	701	472	229	0.3	0	-

QE, Quabracho Equivalent; DM, dry matter; OM, organic matter; CP, crude protein; NDF, neutral detergent fibre; ADF, acid detergent fibre; HEM, hemicellulose; Ca, calcium; P, phosphorus; CT, condensed tannins

times the amount in the pods. Ca and P contents were higher in the pods than in alfalfa. As expected, maize stover and pasture hay had high fibre (NDF, ADF and hemicellulose) but were deficient in crude protein and minerals. The results of the analysis of condensed tannins were expressed in percentage Quebracho equivalents (QE) (Hagerman, 1995; Wisdom *et al.*, 1987) and indicated that the *Leucaena* pods had the lowest concentration of condensed tannins while *A. sieberiana* had the highest. The value for *A. nilotica* was in between the two.

pH and ammonia concentration in the rumen

Dietary and temporal variations in rumen ammonia concentration and pH of sheep are presented in Figure 4.1, while the daily means are presented in Table 4.2. The pH curves for all diets were undulating with small deviations from the mean of 6.4 which were not significant (p>0.05) over the period of measurement. However, all the curves showed a slight decline from the 1st to the 12th hour after feeding.

Table 4.2 Daily means of rumen ammonia concentration and pH in the rumen of South African Merino sheep fed Hay, Alfalfa, Leucaena, Sieberiana and Nilotica diets

Parameter	Diet					SE
	Hay	Alfalfa	Leucaena	Nilotica	Sieberiana	
NH_3 conc. (mgl^{-1})	56.1[a]	86.0[b]	82.6[b]	84.8[b]	71.5[b]	10.97
pH	6.5	6.4	6.4	6.2	6.3	0.45

Means that differ in superscript are significantly different (p < 0.05)

Rumen ammonia concentrations ranged from a minimum of 46-60 to a maximum of 65-107 mgl^{-1} for the Hay and Alfalfa diets respectively. The range in the pod diets was in between the two. The ammonia concentration for the Alfalfa diet rose to a maximum level one hour after feeding and then decreased gradually to a minimum level twelve hours later. The pattern for pod diets was different. There was a small increase in ammonia concentration one hour after feeding but this increase was followed by a gradual drop to a minimum level, 4-5 hours after feeding. From then on the concentration gradually rose to a maximum level at the 10th hour before decreasing again. The profile of ammonia concentration of the Hay diet was maintained at a lower level than for other diets throughout the period of measurement. Significant effects of diet on ammonia concentration were only noticed at the 6th and 9th hours after feeding. At these hours, the concentration of the Hay diet was lower (p<0.05) than those of Alfalfa, Sieberiana and Nilotica diets.

Figure 4.1 Effect of diet on variations in pH and ammonia concentration in the rumen of South African Merino sheep fed Hay, Alfalfa, Leucaena, Nilotica and Sieberiana diets.

Dry matter degradability of incubated feeds

The DM degradability of the incubation feeds (maize stover and alfalfa) is shown in Table 4.3. The lag time (LT), slowly degradable fraction (B), potential degradability (PD), effective degradability (ED) and rate of degradation (C) of B of DM of incubation feeds were not affected by diet. However, differences in ED and C (p<0.001) were evident between incubated feeds with alfalfa having higher values. The other parameters (B, PD and LT) were similar (p>0.05).

Table 4.3 The effect of diet and incubation feed on dry matter degradability of maize stover and alfalfa hay incubated in the rumen of South African Merino sheep

Diet	Incubation	Degradation Parameter				
	Feed	A (gkg⁻¹)	B (gkg⁻¹)	ED (gkg⁻¹)	C (h⁻¹)	LT (h)
Hay	MS	117	642	336	0.013	-2.88
Alfalfa	MS	117	613	329	0.013	-5.39
Leucaena	MS	117	780	299	0.007	-6.9
Nilotica	MS	117	442	294	0.016	-3.61
Sieberiana	MS	117	555	330	0.016	-1.1
Hay	ALF	288	426	578	0.053	-1.51
Alfalfa	ALF	288	432	565	0.045	-2.88
Leucaena	ALF	288	432	548	0.037	-3.85
Nilotica	ALF	288	403	545	0.044	-2.61
Sieberiana	ALF	288	428	540	0.036	-4.62
SED			60.7	8.2	0.0114	1.297
Diet			NS	NS	NS	NS
Incubation feed			NS	***	**	NS

A, wash value; B, slowly degradable fraction; ED, effective degradability; C, rate of degradation of B; LT, lag time; h, hour; ALF, alfalfa; MS, maize stove

$* = p < 0.05$; $** = p < 0.01$; $*** = p < 0.001$

Degradability of the fibre fractions of the incubated feeds and their extracts

Diet did not affect LT, B, PD, and C of the NDF of the incubated feeds (Table 4.4) but

significantly affected the ED, with the Alfalfa and Hay diets having higher (p<0.01)

values than the *Leucaena, Sieberiana* and *Nilotica* diets. The B and PD values of the

NDF of maize stover were higher (p<0.01) than those of alfalfa but NDF obtained from

alfalfa degraded faster (p<0.001) than that of maize stover. The LT and ED of NDF of

the two feeds were similar (p>0.05). The NDF of the extracted material had a longer

(p<0.001) LT preceding degradation but lower B (p<0.01) and ED (p<0.05) values when

compared to that of the feeds. The PD and C were similar (p<0.05) for both feed

extracts. The interaction of feed type and extract was significant for ED (p<0.001) and

C (p<0.05), with maize stover having lower ED and C values than its extract and alfalfa

the reverse.

Diet had no effect on the LT that preceded the degradation of the ADF fraction but

significantly affected B, PD, C and ED (Table 4.5). The B and PD values of ADF of the

Nilotica diet were lower (p<0.05) than the corresponding values of the other diets.

Degradation rates were similar (p>0.05) for the Leucaena and Sieberiana diets but

lower (p<0.05) than the corresponding values of the Alfalfa, Hay and Nilotica diets. The

ED values of ADF were similar for the Alfalfa and Hay diets but lower (p<0.05) for the

pod diets. The ADF of maize stover had higher (p<0.001) B and PD values than that of

alfalfa but the latter degraded faster (p<0.01) and had a longer LT. The ED of ADF was

similar (p>0.05) between the incubation feeds.

Table 4.4 The effect of diet, incubation feed and extraction on the degradability of NDF in maize stover, alfalfa and their detergent extracts incubated in the rumen of South African Merino sheep

Diet	Inc. feed	Degradation Parameters				
		A (gkg⁻¹)	B (gkg⁻¹)	ED (gkg⁻¹)	C (h⁻¹)	LT (h)
Hay	MS	71	630	255	0.015	3.5
Alfalfa	MS	71	682	230	0.012	-1.54
Leucaena	MS	71	623	183	0.005	-0.55
Nilotica	MS	71	427	184	0.016	2.47
Sieberiana	MS	71	563	203	0.016	4.95
Hay	MSE	102	552	270	0.019	6.48
Alfalfa	MSE	102	490	272	0.024	4.67
Leucaena	MSE	102	568	215	0.013	7.87
Nilotica	MSE	102	498	194	0.013	5.9
Sieberiana	MSE	102	460	231	0.02	7.62
Hay	ALF	40	485	267	0.027	-2.99
Alfalfa	ALF	40	449	298	0.041	-3.38
Leucaena	ALF	40	473	266	0.028	2.2
Nilotica	ALF	40	439	252	0.029	0.87
Sieberiana	ALF	40	481	244	0.023	-1.04
Hay	ALFE	179	306	227	0.028	10.7
Alfalfa	ALFE	179	324	203	0.021	-1.77
Leucaena	ALFE	179	282	194	0.024	0.7
Nilotica	ALFE	179	260	190	0.025	9.96
Sieberiana	ALFE	179	304	196	0.022	8.23
SED			92.5	14.3	0.0039	2.411
Diet			NS	**	NS	NS
Incubation feed			***	NS	***	NS
Extract			**	*	NS	***
Incubatin feed x Extract			NS	***	*	NS

Inc., Incubation; A, wash value; B, slowly degradable fraction; ED, effective degradability; C, rate of degradation of B; LT, lag time; h, hour; ALF, alfalfa; MS, maize stover; MSE, maize stover extracted; ALFE, alfalfa extracted * = $p < 0.05$; ** = $p < 0.01$; *** = $p < 0.001$

Table 4.5 The effect of diet, incubation feed and extraction on the degradability of ADF in maize stover, alfalfa and their detergent extracts incubated in the rumen of South African Merino sheep

Diet	Inc. feed	Degradation Parameters				
		A (gkg⁻¹)	B (gkg⁻¹)	ED (gkg⁻¹)	C (h⁻¹)	L (h)
Hay	MS	9	648	288	0.02	-1.17
Alfalfa	MS	9	855	217	0.009	-12.55
Leucaena	MS	9	986	207	0.007	-6.95
Nilotica	MS	9	474	194	0.017	-11.22
Sieberiana	MS	9	862	178	0.007	-11.74
Hay	MSE	22	628	259	0.017	-2.78
Alfalfa	MSE	22	639	236	0.014	-3.8
Leucaena	MSE	22	705	187	0.009	-3.54
Nilotica	MSE	22	451	197	0.018	-3.45
Sieberiana	MSE	22	535	206	0.015	-2.68
Hay	ALF	25	479	235	0.023	-6.35
Alfalfa	ALF	25	444	266	0.034	-5.24
Leucaena	ALF	25	490	223	0.02	5.92
Nilotica	ALF	25	398	211	0.026	7.11
Sieberiana	ALF	25	443	229	0.025	5.6
Hay	ALFE	129	312	237	0.036	9.75
Alfalfa	ALFE	129	307	209	0.028	-5.24
Leucaena	ALFE	129	330	156	0.0155	-7.39
Nilotica	ALFE	129	248	198	0.035	12.49
Sieberiana	ALFE	129	330	197	0.023	4.49
SED			73	16.6	0.0037	4.843
Diet			*	**	*	NS
Incubation feed			***	NS	***	**
Extract			**	NS	*	NS
Incubation feed x Extract			NS	NS	NS	NS

Inc., incubation; A, wash value; B, slowly degradable fraction; ED, effective degradability; C, rate of degradation of B; LT, lag time; h, hour; ALF, alfalfa; MS, maize stover; MSE, maize stover extracted; ALFE, alfalfa extracted * = p < 0.05; ** = p < 0.01; *** = p < 0.001

Table 4.6 The effect of diet, incubation feed and extraction on the degradability of Hemicellulose in maize stover, alfalfa and their detergent extracts incubated in the rumen of South African Merino sheep

Diet	Inc. feed	Degradation Parameters				
		A (gkg⁻¹)	B (gkg⁻¹)	ED (gkg⁻¹)	C (h⁻¹)	L (h)
Hay	MS	66	654	273	0.016	1.53
Alfalfa	MS	66	622	254	0.155	3.75
Leucaena	MS	66	694	224	0.011	7.82
Nilotica	MS	66	438	198	0.018	6.61
Sieberiana	MS	66	582	233	0.015	2.83
Hay	MSE	178	450	277	0.024	19.02
Alfalfa	MSE	178	381	308	0.038	14.36
Leucaena	MSE	178	646	196	0.009	23.76
Nilotica	MSE	178	361	201	0.018	21.73
Sieberiana	MSE	178	422	208	0.016	20.69
Hay	ALF	270	289	311	0.044	10.87
Alfalfa	ALF	270	277	357	0.067	7.88
Leucaena	ALF	270	256	256	0.034	8.18
Nilotica	ALF	270	301	279	0.033	7.76
Sieberiana	ALF	270	307	245	0.025	10.73
Hay	ALFE	325	303	212	0.018	-0.49
Alfalfa	ALFE	325	361	179	0.012	-6.96
Leucaena	ALFE	325	214	238	0.03	0.49
Nilotica	ALFE	325	555	99	0.004	-33.33
Sieberiana	ALFE	325	255	211	0.021	6.96
SED			92.9	29.5	0.0086	6.721
Diet			NS	*	NS	NS
Incubation feed			***	NS	*	**
Extract			NS	**	NS	NS
Incubation feed x Extract			NS	**	**	***

Inc., incubation; A, wash value; B, slowly degradable fraction; ED, effective degradability; C, rate of degradation of B; LT, lag time; h, hour; ALF, alfalfa; MS, maize stover; MSE, maize stover extracted; ALFE, alfalfa extracted * = $p < 0.05$; ** = $p < 0.01$; *** = $p < 0.001$

The extract had higher B (p<0.01) and PD (p<0.05) values than the feed but the ED, C and LT were similar between the feed and extract. The incubation feed by extract interaction was not significant (p>0.05) for any of the degradation parameters.

With respect to the degradation of hemicellulose (Table 4.6), diet had no effect on LT, B, PD and C constants but significantly (p<0.05) affected the ED, with Alfalfa and Hay diets having higher ED values of hemicellulose than pod diets, as was the case with NDF and ADF. The hemicellulose of maize stover had a higher (p<0.001) B value and a longer (p<0.01) LT than that of alfalfa but the latter degraded faster (p<0.05). The PD and ED of hemicellulose of both feeds were similar (p<0.05). The ED of the hemicellulose of the extract was lower (p<0.01) than the corresponding value in the feed. All the other parameters were similar (p>0.05). The feed type by extract interaction was significant (p<0.01) for ED, C and LT. The LT, C and ED values were similar (p>0.05) for maize stover and its extract but this was not the case for alfalfa for which the ED and C values were higher for the feed than the extract.

4.5 DISCUSSION

The high ammonia concentration of the Alfalfa diet just one hour after feeding may be explained by the fact that alfalfa contains a high proportion of soluble proteins which undergo microbial digestion shortly after ingestion. An important proportion of the crude protein of the pods was found in a previous study to be equally soluble (Ngwa et al., 2002) but since the pods are tanniferous in nature, part of the solubilized component may have escaped microbial digestion due to the formation of tannin-protein complexes which are ruminally undegradable thus decreasing the ammonia concentration in the

rumen (McNabb *et al.*, 1996; Perez-Maldonado and Norton, 1996). The gradual increase in ammonia concentration from the 5th hour after feeding for the pod diets, could be attributed to microbial degradation of the slowly degradable fraction of the protein in the pods. The small but progressive decreases in pH six hours after feeding for all diets, may be linked to the presence of volatile fatty acids (VFAs) produced as end products of rumen fermentation of the ingested feeds (Ngwa *et al.*, in press).

Effect of diet on the degradability of DM and fibre fractions of incubated feeds

The fact that diets had no effect on DM degradability of the incubated feeds may be an indication that rumen ammonia concentration was not a limiting factor in any of the diets. This assertion is confirmed by Satter and Slyter (1974), who reported that increasing ammonia concentration beyond 50 mg l^{-1} had no effect on microbial protein production. The ammonia concentration of the Hay diet, although lower than the concentrations of the other diets, was above the 50 mg l^{-1} threshold and thus could not have limited the degradability of DM. Further evidence was given by Orskov *et al* (1972) who reported that maximum growth of rumen microbes occurred when abomasal fluid contained between 40 and 80 mg NH_3-N l^{-1} of fluid. The Alfalfa diet produced the highest concentration of rumen ammonia but its failure to improve the DM degradability of the incubated feeds may relate to the fact alfalfa contains saponins which inhibit microbial synthesis and are said to alter the site and extent of nutrient digestion in ruminants (Lu and Jorgenson, 1987). In addition, young alfalfa stems are highly digestible but as they mature, they become less digestible and this contributes primarily to the reduced digestibility of the plant (Jung and Fahey, 1984; Albrecht *et al.*, 1987).

The decrease in the degradability of fibre constituents in the rumen of animals fed pod diets could be due to the presence of anti-nutritional factors present in the pods, which affect fibrolytic microorganisms. The pods of *A. sieberiana* and *A. nilotica* have a high level of condensed tannins (Table4.1) which have been reported to limit the growth and activity of fibrolytic microorganisms (Reed, 1995; Mueller Harvey *et al.*, 1987; Wiegand, 1991). Free condensed tannins are highly reactive and were reported to inactivate and precipitate microbial enzymes responsible for the degradation of cell wall constituents (McLeod, 1974; Lohan *et al.*, 1981). Condensed tannins are known to induce changes in the morphology of cellulolytic microorganisms (Scalbert, 1991; Jones *et al.*, 1994). They also bind with long chain carbohydrates (cellulose and hemicellulose), forming ruminal undegradable complexes (Makkar,1993; Waghorn *et al.*, 1994b; Fall-Toure *et al.*, 1998) and are said to inhibit the action of endoglucanase which enables fungi to digest and colonize ligno-cellulolytic tissues not degraded by bacteria (Akin *et al.*, 1983; McAllister *et al.*, 1994a).

Although the pods of *L. leucocephala* had a low level of condensed tannins, it is possible that the reactivity of the tannins in the rumen could be quite high or the pods may contain other anti-nutritional factors different from condensed tannins which also limit the action of fibrolytic microbes. This assertion is supported by the results of other researchers at ILCA (1994) who worked with pure cultures and reported that extracts of *L. leucocephala* limited the growth of cellulolytic microorganisms especially, *Fibrobacter succinogenes, Ruminicoccus albus* and *R. flavesciens*. It was also reported that the digesta from *Leucaena* species delayed the onset of digestion and slowed down the rate of digestion of NDF obtained from Napier grass (Nsahlai *et al.*, 1995c). The

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decrease in the degradability of cell wall constituents was however, masked during the degradability of DM. This may be due to the preference for soluble carbohydrates by rumen microorganisms leading to reduced action by cellulolytic bacteria (Mould and Orskov, 1984).

Effect of incubation feed on the degradability of dry matter and fibre fractions

The differences observed in the rates and extent of degradation of the dry matter of maize stover and alfalfa may be related mainly to the proportion of soluble contents in the two feeds. Alfalfa has a soluble proportion of 0.6 [(DM-NDF)/DM] while the same proportion in maize stover is only 0.48. This most probably explains why the wash value (A) of alfalfa was about 2.5 times higher than the corresponding value in maize stover. In respect to the degradability of cell wall constituents, alfalfa contains a readily fermentable cell wall (Ndlovu and Buchanan-Smith, 1985). In addition, a sizable proportion of alfalfa cell walls consists of rapidly degradable pectins found in non-lignified tissues (Hatfield and Weimer, 1995; Jung *et al.*, 2000). Maize stover unlike alfalfa, consists of cell walls that have undergone secondary thickening (Adebowale and Nakashima, 1992; Jung and Allen, 1995; Wilson and Martens, 1995) consequently they are very slowly degraded. This may explain why the rates of degradation of NDF and ADF in alfalfa were almost two times higher than in maize stover. The low rate of degradation of the hemicellulose of maize stover could be attributed to the fact that maize stover, like most cereal crop residues, contains a high concentration of lignin which is bonded to hemicellulose, and is negatively correlated to the digestibility of the latter (Hoover, 1986). The high B and PD values of NDF and ADF of maize stover are indications that this feed might respond more to increasing time of fermentation (Jung

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et al., 2000), and the decrease in the effects of tannins due to increasing time of fermentation (Makkar *et al.*, 1995) could enhance this process in animals supplemented with pod meals.

Effect of extraction on the degradability of fibre constituents

Extraction improved the rate and extent of degradation of NDF and hemicellulose of maize stover but rather produced opposite effects in alfalfa. It is possible that boiling in NDS may have weakened the crystalline structure of cellulose and hemicellulose in the feeds, rendering it more permeable to fibrolytic microorganisms (Wilson and Martens, 1995). Also, boiling in NDS might have reduced the effect of lignin on the degradability of fibre constituents through the hydrolysis of ether bonds that link lignin to hemicellulose as well as reducing the tensile strength of the cuticular wall. This could facilitate the penetration of rumen microbes. These effects might have been more pronounced on the cell wall constituents of maize stover than those of alfalfa because of the chemical nature of the cell wall constituents of the two feeds as discussed above.

4.6 CONCLUSION

The results from this study show that pods from tree legumes are comparable to alfalfa in the provision of rumen ammonia. However, some of the nitrogen may not be available to rumen microorganisms due to binding with condensed tannins, common in these class of feeds. Although the presence of this compound tends to limit the activity of fibrolytic microorganisms, its action cannot overrule the value of these pods as a supplier of nitrogen particularly in areas where pastures are deficient in nitrogen. The high rate and extent of degradation of the dry matter of alfalfa compared to maize stover

may be expected considering the chemical composition of the two feeds. The same

reason may also explain the differences in the rate and effective degradability of the

extracts from these feeds.

CHAPTER 5

Effect of feeding legume pods or alfalfa in combination with poor quality hay on microbial enzyme activity and production of volatile fatty acids in the rumen of South African Merino sheep

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5.1 Abstract

Two studies were carried out between February and May, 2001, to investigate the effects of feeding alfalfa and pods from tree legumes on microbial activity in the rumen of fistulated South African Merino rams. The diets comprised of equal proportions (dry matter weight) of pasture hay and either alfalfa (Alfalfa diet), pods of *Acacia sieberiana* (Sieberiana diet), *Acacia nilotica* (Nilotica diet) or *Leucaena leucocephala* (Leucaena diet). The fifth diet was composed of pasture hay alone (Hay diet) and served as a negative control. In the first study, the diets were offered to 5 fistulated rams individually for periods of 15 days using an incomplete (5x4) Latin square design. Rumen fluid was collected on the last day of each period for measurement of pH and volatile fatty acids (VFAs). In the second, a randomised block design was adopted where 3 fistulated rams were blocked and the same diets were assigned to the animals. Diets were offered for periods of 10 days and this was followed on the eleventh day by the collection of rumen fluid and removal of extracted hay incubated in nylon bags in the rumen of the sheep for 24 hours, for the evaluation of enzyme activity and degradation characteristics of dry matter and cell wall constituents of the hay in the rumen. The pH and total concentration of VFAs in rumen fluid ranged from 6.2 to 6.5 and 30-53 mmoles/l while degradation of DM and NDF varied from 126 to 269 g/kgDM and 233-309 g/kgNDF respectively among diets. Variations in pH were not significant ($p > 0.05$) but diet influenced ($p < 0.01$) the concentration of VFAs, degradation of DM and cell wall constituents as well as enzyme activities of proteolytic and fibrolytic microorganisms. The results show that the differences between the alfalfa and pod diets may have been due mainly to the presence of tannins in the pods. These compounds limit the growth and/or activity of ruminal microorganisms. However, a limitation in the supply of energy imposed by the pod diets may have reduced the efficiency of N capture by microorganisms leading to a depression in microbial activity.

Key words: Legume pods, enzyme activity, rumen fermentation, volatile fatty acids

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5.2 INTRODUCTION

Feedstuffs consumed by ruminants are all exposed initially to a fermentative activity in the rumen prior to gastric and intestinal digestion. Dietary polysaccharides and protein are generally degraded by the ruminal microorganisms into characteristic end products, which in turn provide nutrients for metabolism by the host animal. The extent and type of transformation of feedstuffs in the rumen thus determine the productive performance of the host animal. Pods from tree legumes are known to provide high levels of rumen degradable nitrogen (Jones, 1979; Kibon and Maina 1993; Nsahlai et al., 1995b; Sawe et al., 1998). This class of feeds has, however, been reported to contain high concentrations of anti-nutritional factors which in addition to other toxic effects in the animal, limit the growth and/or activity of rumen microbes (Barry and Duncan, 1984; D'Mello, 1991, 1992; Scalbert, 1991; Kumar, 1992; Jones et al., 1994; Reed, 1995). The anti-nutritional factors range from tannins to alkaloids, goitrogens, saponins, mimosine and cyanogens, to mention a few.

In many instances, the ruminant forestomach performs a protective function whereby ruminal microflora effectively degrades a wide variety of toxic compounds (Jones, 1985). In some cases the opposite can occur with a production of toxic metabolites from the innocuous compounds, which are harmful to the rumen microflora and host animal (D'Mello, 1991; Reed, 1995). In the rumen, volatile fatty acids (VFAs) are produced as end products of fermentation of organic matter (OM) by microorganisms. The rate and extent to which these acids are produced could be indicative of microbial activity in the rumen. The predominant VFAs in the rumen fluid are acetic, propionic and butyric acids with isobutyric, isovaleric, valeric and other acids generally present in small amounts.

The concentration and relative proportions of VFAs are related to the nature of the feed (Bergman, 1990). Since both types and number of microorganisms vary with diet (Warner, 1965), it is possible that differences between feeds, especially with regard to the degradability of protein in the rumen may, at least in part, be due to diet-induced differences in the efficiency of digestion in the rumen, resulting from changes in the microbial population. This study was therefore undertaken to examine the effect of diet on fibrolytic and proteolytic activities of rumen microbes and the concentration of VFAs in the rumen of sheep fed poor quality hay supplemented with tanniferous feeds (pods of *Acacia sieberiana, Acacia nilotica* and *Leucaena leucocephala*), in comparison with alfalfa (*Medicago sativa*), as a positive control.

5.3 MATERIALS AND METHODS

Animals, diet and experimental design

The experiment was conducted in two phases. In the first phase, five rumen fistulated Merino rams weighing 48.8 ± 4.76 kg (sd) were used. An incomplete (5×4) Latin square design was adopted and each animal was assigned to a dietary treatment. The diets comprised of equal proportions (dry matter weight) of pasture hay and either alfalfa (Alfalfa diet), pods of *Acacia sieberiana* (Sieberiana diet), *Acacia nilotica* (Nilotica diet) or *Leucaena leucocephala* (Leucaena diet). The fifth diet was composed of pasture hay (*Themeda triandra*) alone (Hay diet) and served as a negative control. The pods were ground through the 3-mm screen of a hammer mill, the hay through a 30-mm screen and alfalfa was purchased from local producers in chopped (about 3 cm length) form. The feeds were mixed, using an industrial mixer. Each feed was given at the rate of 450

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g/animal/day partitioned into three equal portions of 150 g each and offered at 08.00, 12.00 and 16.00h each day. The diets were offered in four periods of 15 days each. At the end of each period (Day 15), rumen fluid was collected for VFA and pH analysis. The fluid was collected from the rumen of each animal using a mechanical suction pump. Collection was conducted at 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, and 24 hours after feeding in the morning at 08.00h. Immediately after collection, the rumen fluid was strained through a cheese cloth and this was closely followed by reading of pH. Samples of about 100 ml destined for determination of VFAs were put in 250 ml polythene containers to which 5 ml of 10% NaOH was added. The samples were stored in a freezer (-20 °C) until they were analysed. For analysis, the frozen samples were thawed, transferred into centrifuge tubes and centrifuged at 3000 x g for 20min. A sub-sample of the supernatant (1.5 ml) was pipetted into another tube containing 1 ml of internal standard solution (10ml of 1.6% isocaproic acid, 10 ml of 12% metaphosphoric acid and 30 ml of distilled water).The mixture was homogenized using a vortex mixer and centrifuged at 3000 x g for 10 min. From the supernatant, 0.9 ml was transferred into Eppendorf tubes containing 0.1 ml of formic acid. The mixture was again homogenised and centrifuged in a bench-top centrifuge (Eppendorf centrifuge 5410) at 12000 x g for 15 min. The supernatant was then used for the determination of VFAs using gas chromatography (GC).

In the second phase, three rumen fistulated Merino rams weighing 53.8 ±12.27 kg (sd) were used in the experiment and five diets were prepared in the same way as in phase 1. A randomized block design was adopted where the 3 fistulated rams were blocked and the feeds were assigned to the animals, making sure that each animal was

assigned to only one diet during each period. The diets were offered for periods of 10 days each and sample collection was on the 11th day. Each feed was given at the rate of 0.6, 0.7, 0.8, 0.9 and 1 kg on days 1 and 2, 3 and 4, 5 and 6, 7 and 8 and 9 and 10 respectively. Incubation of test material was done on day 10 and removed on day 11 after 24 hours.

In sacco degradation

The incubation material used to study the degradability of fibre fractions by fibrolytic microorganisms was extracted hay. The hay was ground to pass through a 2-mm screen. Extraction was done by boiling 200 g of ground hay for 1 hour in 4 litres of neutral detergent solution (Van Soest *et al.,* 1991), in a 10 litre metal vat. After boiling, the material was rinsed under tap water and oven-dried at 40 °C for 24 hours. About 3 g of the extracted hay was weighed into nylon bags (ANKOM Co, Fairport, New York, USA; internal dimensions: 5cm x 9cm; pore size 50μm) and incubated in duplicate, in the rumen of fistulated sheep on day 10 of each feeding period, for 24 hours. After removal from the rumen, the bags, including the zero-hour ones (6 in number) which were not incubated, were washed in a semi-automatic washing machine six times in cycles of 5 minutes. The washed bags were dried in a force draught oven at 60 °C for 48 hours, cooled in a desiccator and weighed. The residues were subsequently analysed for NDF and ADF according to the method of Van Soest *et al.* (1991) and hemicellulose was calculated as the difference between NDF and ADF. The fractions, ADF and (NDF-ADF) were considered as cellulose and hemicellulose, respectively.

In order to determine the *in sacco* disappearance of dry matter (DM) of the dietary

ingredients, about 4 g of each ground sample was weighed into nylon bags (ANKOM Co, Fairport, New York, USA; internal dimensions: 5cm x 9cm; pore size $50\mu m$). The bags were incubated in the rumen of two Jersey cows in duplicates following the method described by Mehrez and Orskov (1977). Bags were added sequentially and were incubated for 144, 120, 96, 72, 48, 24, 12, 6 and 3 h. After removal from the rumen, the bags, including the zero-hour ones which had not been incubated, were washed in a semi- automatic washing machine six times in cycles of 5 minutes. The washed bags were then dried in a force draught oven at 60 °C for 48 hours, cooled in a desiccator and weighed. Prior to incubation, the two cows were adapted on a diet of pasture hay (*Themeda triandra*), fed *ad libitum* and supplemented with 2 kg of cotton seed cake per animal per day for two weeks.

Evaluating the fibrolytic activity of solid-associated microoganisms (SAM)

Only the SAM population, which forms most of the total rumen microbial population in terms of mass (Craig *et al.*, 1987; Legay-Carmier and Bauchart, 1989; McAllister *et al.*, 1994a) and enzyme activity (Martin *et al.*, 1993; Martin and Michalet-Doreau, 1995) was considered to study fibrolysis. In order to study the relationship between microbial fibrolytic activity and *in sacco* degradability, the extracted hay was weighed into two other bags, additional to those used for *in sacco* degradability studies and incubated alongside the latter, for the same period of time, with the assumption that fibrolytic activity was maximum between 2 and 24 hours after incubation (Williams *et al.*, 1989; Nosiere *et al.*, 1996). On removal, the bags destined for the measurement of fibrolytic activity were washed manually in an anaerobic (0.025M 2- (N-morpholino) ethane sulphonic acid (MES) and 0.001M DL-dithiothreitol (DTT, pH 6.5) buffer (Coleman,

1978) to remove non-adherent microorganisms from the particles. After washing, the samples were removed from the bags, frozen in liquid nitrogen and stored at -20 °C, until needed for analysis.

Before analysis, samples were ground in liquid nitrogen and about 2 g (fresh weight) was put in test tubes and reconstituted with 10 ml of pre-cooled (4°C) anaerobic buffer. The mixture was centrifuged at 15000 x g for 15minutes at 4 °C and the supernatant containing soluble proteins was pipetted into Eppendorf tubes and stored at -20 °C. A portion of each ground sample was weighed and placed in a pre-warmed oven (60°C) for 48 hours to determine the dry matter content of the samples. The activities of hemicellulolytic and cellulolytic microbes were assessed by measuring the activities of β-D-galactosidase and β-D-glucosidase, repectively (Noziere et al., 1996). β-glucosidase and β-galactosidase activities were determined by measuring the amount of p-nitrophenol released from either 5 mM p-nitrophenyl glucoside or galactoside (Sigma N8016 and N-1252), respectively, in MES/DTT buffer. The reaction was initiated by incubating 1 ml of the substrate with 0.1 ml of supernatant at 37 °C for 45 min and was terminated with 1.1 ml glycine-NaOH solution (0.4 M glycine, pH 8.5). The amount of p-nitrophenol produced was quantified spectrophotometrically at 420 nm. The standard was p-nitrophenol (Sigma 104-1).

Protein was assayed following the modified technique of Bradford (1976). The reaction was started by adding 2 ml of the Bradford reagent to 40 µl to dilute samples. The resultant colour development was assessed at 595 nm after 5 minutes but within 1 hour. Data generated were analysed with the aid of a computer software, Lowry (Elsevier

BIOSOFT, Cambridge, UK).

Measurement of proteolytic activity

Rumen fluid was collected at the moment that the bags were removed from the rumen. The proteolytic activity of ruminal bacteria was measured following the method described by Kopecny and Wallace (1982). The substrate used was 0.4 % azo-casein (sulphamide-azocasein, Sigma A-2765), in distilled water. One ml of enzyme preparation was incubated with 1 ml of substrate and 2 ml of 100 mM KH_2PO_4, 2mM DTT buffer (pH 7.5) at 37°C for four hours. The reaction was terminated with 1 ml of 25% (w/v) TCA. The mixture was centrifuged at 31,000 x g for 10 minutes. One ml of the supernatant was transferred into fresh test tubes and mixed with an equal volume of 0.5 M NaOH. Absorbance was read at 440 nm using azo-casein as standard.

Chemical analyses

The dry matter (DM), organic matter (OM) and nitrogen (N) contents of the feeds were determined using the method outlined by AOAC (1990). Neutral detergent fibre (NDF) and acid detergent fibre (ADF) of the feeds were determined by the method of Van Soest *et al.* (1991) while hemicellulose was estimated as the difference between the NDF and ADF. Pod samples were also sent to the Jacob Blaustein Center for Desert Biodiversity, Ben Gurion University of Negev Desert, in Israel for the analysis of condensed tannins and the method of determination was that described by Hagerman (1995).

Calculations and statistical analyses

The degradation of DM of the extracted hay was estimated by fitting the non- linear model proposed by McDonald (1981) and modified by Dhanoa (1988) to the degradation data:

$$Y = A + B[1 - e^{-C(T-LT)}]$$

where Y is the disappearance of DM at time T, A = washing loss or solubility, B = degradable part of the insoluble fraction, C = rate of degradation of B and LT, the lag time. The potential degradability (PD) was calculated as A + B . A passage rate (k) of 0.03/h was assumed in order to calculate the effective degradabilities (ED) of DM (Bonsi et al., 1994; Nsahlai et al., 1998a).

$$ED = A + B \times C /(k+C).$$

Data were analyzed using the analysis of variance technique (SAS 1987) for a complete randomized block design and contrasts between diets and incubation feeds were obtained by applying the probability of difference (PDIFF) option of the LSmeans Statement available in the GLM.

5.4 RESULTS

Chemical Composition

The chemical and degradation properties of the dietary ingredients are presented in Table 5.1. Among the feeds considered as protein sources, the pods of Leucaena had the highest crude protein content, followed by alfalfa, A. sieberiana and A. nilotica in descending order. NDF concentrations were similar for alfalfa and the leucaena pods but lower for the acacia pods. The concentration of ADF in alfalfa, compared to that of the pods, was higher. The ADF values for Leucaena, A. sieberiana and A. nilotica were

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Table 5.1 The chemical and degradation properties of dietary ingredients

Parameter	Dietary ingredients				
	L. leucocephala	*A. nilotica*	*A. sieberiana*	Alfalfa	Hay
DM (gkg^{-1}DM)	948	937	937	927	952
OM (gkg^{-1}DM)	952.6	952.7	945.8	898.8	924.6
CP (gkg^{-1}DM)	246.9	149.1	174.9	220.2	41.7
NDF (gkg^{-1}DM)	408.9	224.7	366.9	402.4	700.8
ADF (gkg^{-1}DM)	284.7	180.8	290.1	341.1	472.3
HEM (gkg^{-1}DM)	124.2	43.9	76.8	61.3	228.5
CT (%QE)	1.8	17.4	28.3	-	-
A (gkg^{-1}DM)	266.6	473.4	374.1	288.2	61.2
B (gkg^{-1}DM)	372.1	381.7	415.4	454.9	576
PD (gkg^{-1}DM)	638.7	855.1	789.5	743.1	637.2
ED (gkg^{-1}DM)	477.3	549.6	521.7	591	291
C (h^{-1})	0.063	0.022	0.027	0.06	0.022
LT (h)	0.31	-2.69	1.16	-1.74	-1.08

QE, Quabracho Equivalent; DM, dry matter; OM, organic matter; CP, crude protein; NDF, neutral detergent fibre; ADF, acid detergent fibre; HEM, hemicellulose; A, wash value; B, slowly degradable fraction; ED, effective degradability; C, rate of degradation of B; LT, lag time; h, hour

respectivly 0.83, 0.85 and 0.53 times the value for alfalfa. The concentration of hemicellulose in alfalfa was comparable to that of the acacia pods but just about half the value of leucaena pods. The ash content of alfalfa was about two times the amount in the legume pods. The pasture hay was very rich in NDF, ADF and hemicellulose but deficient in crude protein. The results of the analysis of condensed tannins were expressed in percentage Quebracho equivalents (QE) and indicated that the leucaena pods had the lowest concentration of condensed tannins while *A. sieberiana* had the highest. The value for *A. nilotica* was intermediate.

Among the protein supplements, the pods of *A. nilotica* had the highest solubility (wash value), followed by *A. sieberiana*, alfalfa and *L. leucocephala* in decreasing order. The potential degradability (PD) and effective degradability (ED) had similar trends like the solubility. The rate of degradation (C) of the slowly degradable portion (B) of alfalfa was similar to that of Leucaena but the values for *A. nilotica* and *A. sieberiana* were 0.3 and 0.5 times lower, respectively. Lag time was similar for all four supplements.

Rumen pH and concentration of VFAs

Dietary and temporal variations in rumen pH of sheep are presented in Figure 4.1 (Chapter 4). The pH curves for all diets fluctuated with small deviations from the mean of 6.4 and there were no significant ($p > 0.05$) differences among diets over the period of measurement. However, all the curves showed a slight decline from the 1st to the 12th hour after feeding.

The mean totals and molar proportions of individual VFAs are presented in Table 5.2 while temporal variations are shown in Figures 5.1 and 5.2, respectively. The total concentration of VFAs in sheep fed on the Alfalfa diet was higher ($p < 0.01$) than that of those on the pod and Hay diets. Among the pod diets, the Nilotica diet promoted a higher ($p < 0.05$) concentration of total VFAs in sheep than the Sieberiana and Leucaena diets. In order of decreasing concentration, the Alfalfa diet was followed by the Nilotica, Sieberiana, Hay and Leucaena diets, respectively. These concentrations were maintained (Figure 5.2) throughout the observation period with only slight fluctuations. All VFA concentrations rose during the first hour after feeding. In the second hour, while

the upward trend continued for the Alfalfa and Leucaena diets, a drop was noticed for the others and this was followed by an undulating trend. Peaks and depressions were not consistent for the individual acids. Differences in molar proportions were not significant (p>0.05) among dietary treatments. Acetic acid had the highest molar proportion (ranging from 78-83 %) followed by propionic, butyric, isovaleric, valeric and isobutyric acids in decreasing order. Sheep on the Leucaena diet tended to have higher molar proportions of the iso-acids than those on other diets. In the daily variations of acetic acid, the Sieberiana diet showed a profile which was lower than all the others but this was compensated for by a high profile for propionic and valeric acids. As a result, sheep on this diet had the lowest acetate:propionate ratio (5.4:1) while the Nilotica diet had the highest (8.2:1) ratio. The Hay diet promoted a low profile for all the acids except acetic acid.

Table5. 2 Daily mean totals and molar proportions of VFA concentrations in the rumen of South African Merino sheep fed Alfalfa, Hay, Leucaena, Nilotica or Sieberiana diets

Diet	Totals (mmole/ l of R_f)	Molar proportions of VFAs (%)					
		Acetic	Propionic	Isobutyric	Butyric	Isovaleric	Valeric
Hay	38.16	82.85	11.13	0.25	4.34	0.93	0.49
Afalfa	53.2	82.16	11.9	0.28	4.36	0.77	0.53
Leucaena	30.25	80.87	11.74	0.31	5.07	1.43	0.56
Nilotica	46.15	82.69	10.06	0.18	5.62	0.76	0.6
Sieberiana	40.67	78.23	14.37	0.26	5.29	1.18	0.65
SED	4.076	2.357	1.416	0.058	0.612	0.299	0.093
SIG	**	NS	NS	NS	NS	NS	NS

R_f = rumen fluid; ** = significant at $p < 0.01$, NS = non-significant; SED = standard error of difference; SIG = significance
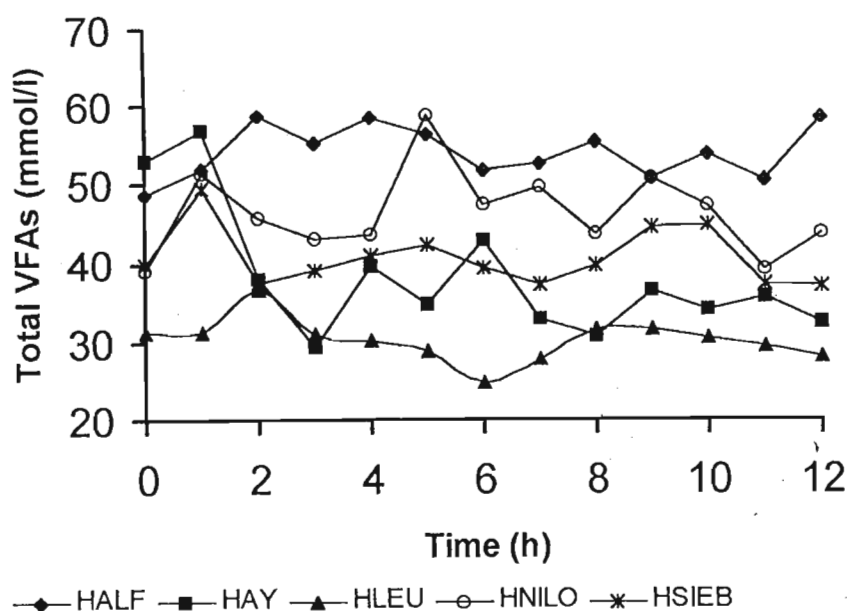
Figure 5.1 Dietary and temporal variation in the concentration of VFAs in the rumen of South African Merino sheep. (HALF is Alfalfa; HAY, Hay; HLEU, Leucaena; HNILO, Nilotica and HSIEB, Sieberiana diets respectively).

Effect of diet on the degradability of DM, NDF, ADF and HEM

The degradabilities of DM and cell wall constituents of the hay extract incubated in the rumen of sheep for a period of 24 hours are presented in Table 5.3. The Alfalfa and Hay diets had similar ($p>0.05$) degradability values for DM, NDF, ADF and HEM of the extract and these were higher ($p<0.001$) than the corresponding values for the pod (Leucaena, Nilotica and Sieberiana) diets. Among the pod diets, the Leucaena and Sieberiana diets had similar ($p>0.05$) degradability values for the extract while those of the Nilotica diet were lower ($p<0.001$).

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Acetic acid

Butyric acid

Isobutyric acid

Isovaleric acid

Propionic acid

Valeric acid
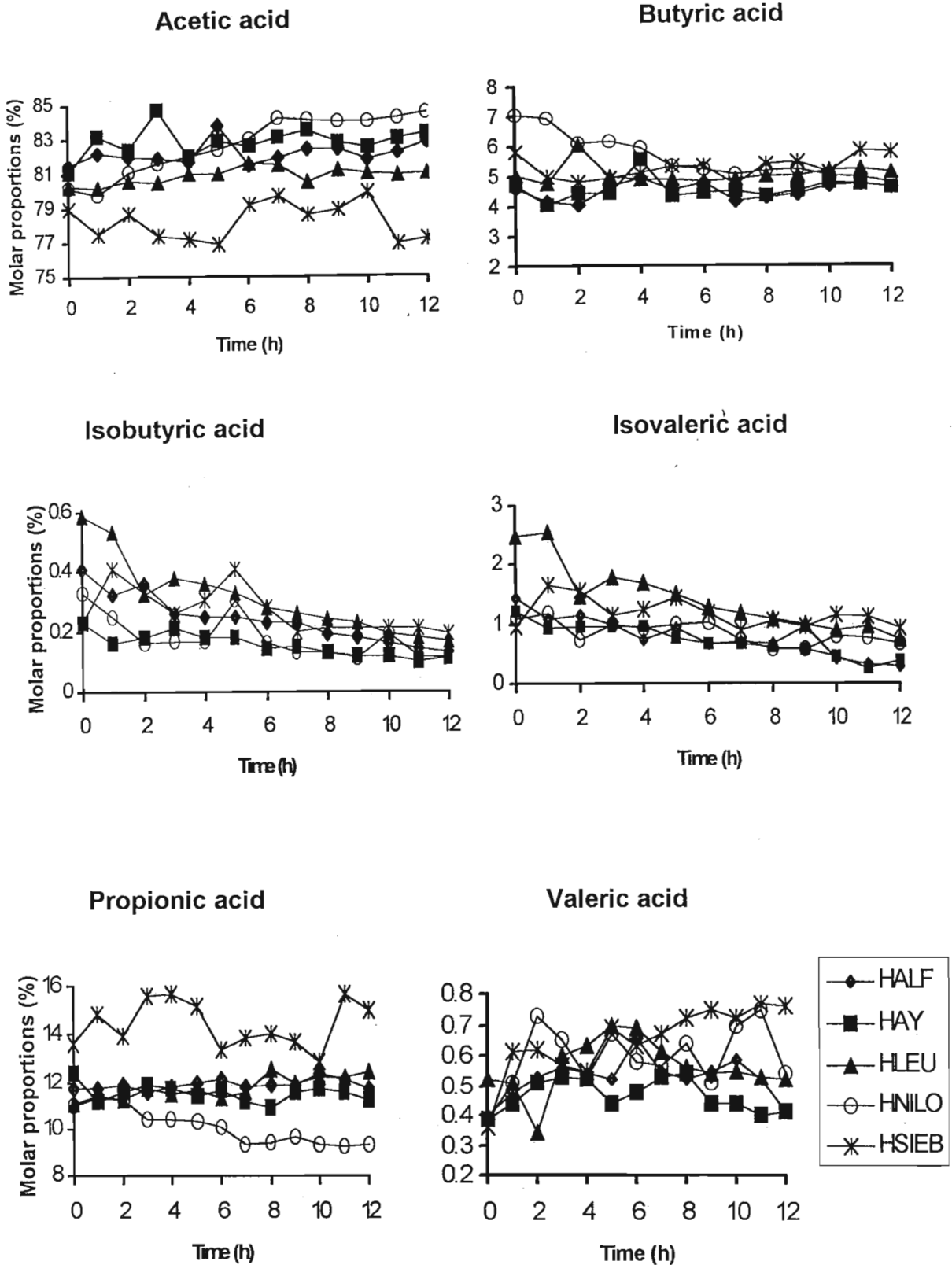
Figure 5.2 Dietary and temporal variation in the concentration of VFAs in the ruemn of South African Merino sheep. (HALF is Alfalfa diet; HAY, hay diet; HLEU, Leucaena diet; HNILO, Nilotica diet; HSIEB, Sieberiana diet)

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Table 5.3 Effect of diet on the degradability of DM, NDF, ADF and HEM of extracted hay incubated for a period of 24 hours in the rumen of South African Merino sheep fed Alfalfa, Hay, Leucaena, Nilotica or Sieberiana diet

Diet	Amount of constituent degraded			
	DM (g/kgDM)	NDF (g/kgNDF)	ADF (g/kgADF)	HEM (g/kgHEM)
Hay	269.7 [a]	303.5 [a]	275.6 [a]	348.2 [a]
Alfalfa	269.0 [a]	309.2 [a]	293.7 [a]	334.0 [a]
Leucaena	197.4 [b]	226.5 [b]	217.4 [b]	241.3 [b]
Nilotica	126.2 [c]	144.3 [c]	129.1 [c]	168.5 [c]
Sieberiana	199.6 [b]	233.3 [b]	216.5 [b]	260.2 [b]
SED	22.34	26.6	27.09	32.29
p- value	0.001	0.001	0.001	0.001

DM, dry matter; NDF, neutral detergent fibre; ADF, acid; Hem, hemicellulose; SED, standard error of difference. Means in the same column with different superscripts are significantly different ($p < 0.001$)

Protein concentration, fibrolytic and protease activities

Table 5.4 shows the effect of diet on protein concentration and enzyme activity in the rumen fluid of experimental animals and incubated samples. The concentrations of protein in the rumen fluid of animals that were fed Alfalfa, Hay and Leucaena diets were similar ($p > 0.05$) but the corresponding values for Nilotica and Sieberiana diets were lower ($p < 0.01$). The same trend was observed for protein concentrations in the samples that were incubated in nylon bags in the rumen.

β-glucosidase activities in extracts of solid-associated microorganisms (SAM) were higher ($p < 0.01$) for sheep on the Alfalfa and Leucaena diets, intermediate for the Hay diets and low for the Nilotica and Sieberiana diets. Galactosidase activities in SAM extracts were similar ($p > 0.05$) for the Hay, Leucaena, Nilotica and Sieberiana diets but higher ($p < 0.01$) for the Alfalfa diet. Protease activities were similar ($p > 0.05$) in sheep

Table 5.4 The effect of diet on protein concentration and activities of Glucosidase, Galactosidase and protease in nylon bag residues and rumen fluid of fistulated South African Merino sheep fed Alfalfa, Hay, Leucaena, Nilotica or Sieberiana diets

Diet	Protein concentration		Enzyme Activity		
	Rumen Fluid (mg/l)	Incubated material (mg/gDM)	Glucosidase (µmole P/mg prot/min)	Galactosidase (µmole P/mg prot/min)	Protease (U)
Hay	668.3[a]	4.11[a]	110.3[b]	68.7[b]	4.12[a]
Alfalfa	691.7[a]	4.16[a]	158.2[a]	93.1[a]	4.18[a]
Leucaena	621.7[a]	4.70[a]	124.2[a]	59.6[b]	4.19[a]
Nilotica	396.7[b]	2.36[b]	75.2[c]	61.7[b]	3.54[b]
Sieberiana	368.3[b]	2.08[b]	98.7[c]	78.0[ab]	3.80[c]
SED	96.09	0.576	18.36	9.43	0.088
p-value	0.015	0.001	0.003	0.009	0.001

U = 1 mg of azo-casein digested/min; P = p-nitrophenol released and quantified spectrophotometrically.

on the Hay, Alfalfa and Leucaena diets but lower for the Nilotica and Sieberiana diets. In general, sheep on the Nilotica diet had the lowest values for protein concentration and enzyme activity while the opposite was observed for the Alfalfa diet.

5.5 DISCUSSION

Volatile fatty acids constitute the major source of energy for ruminants (Phillipson and McAnally, 1942; Barcroft et al., 1944), providing up to 0.7-0.8 of all their energy requirements (Warner, 1964; Bergman et al., 1965; Annison and Armstrong, 1970). Although ruminal fermentation of dietary proteins contributes to the production of VFAs (iso-acids), in roughage-fed ruminants, acetate, propionate and butyrate originate mainly from the fermentation of dietary carbohydrates (van Houtert, 1993). The quantity of VFAs produced in the rumen depends on the hay to concentrate ratio, the physical

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and chemical characteristics of the diet, and the level of intake (Orskov et al., 1968). The low concentration range of 30 to 60 mmol VFA /l of rumen fluid obtained in this experiment (Table 5.2) as compared to the normal range of 70 to 150 mmol/l reported by McDonald et al. (1998) may be attributed to these reasons, given the fact that only 450 g of feed was offered to each animal per day and that some of the feeds were tanniferous in nature.

Several reasons could be accountable for the differences in the concentration of total VFAs observed among the dietary treatments. Firstly, the amount of VFA produced apparently depended on the extent (effective degradability) of the feed ingested by the animals which subsequently determines the amount of substrate available for fermentation (Firkins et al., 1986; Robinson et al., 1986; 1991). The effective degradabilities of the diet ingredients in decreasing order were as follows: Alfalfa > Nilotica > Sieberiana > Leucaena > Hay. The concentration of VFAs in the rumen of sheep originating from the OM fermentation of the five diets followed the same order but for the Leucaena diet which switched positions with the Hay diet. The reasons for the switch may be attributed to the fact that the degradation of the leucaena pods rather yielded more peptides and/or amino acids which were incorporated directly into microbial protein synthesis (Nolan and Leng, 1972), a scenario which is seen with the fermentation of most protein supplements (Elsden and Phillipson, 1948; Annison, 1954; Wang et al., 1996). It should however, be emphasized that the Leucaena diet most probably promoted the synthesis of non-fibrolytic (floating) microorganisms and/or limited the activity of fibrolytic microorganisms since the diet depressed fibrolysis. This may also explain why the Leucaena diet promoted similar protein concentrations and

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protease activity (Table 5.4) as the Alfalfa diet which had a higher effective degradability. The relatively higher proportions of iso-acids found in the rumen fluid of sheep that fed on the Leucaena diet further supports the fact that the fermentation of the organic matter of this diet might have followed a slightly different pattern when compared to the others.

The presence of condensed tannins (CT) in the legume pods may partly be responsible for the depression in the concentration of VFAs in the rumen of animals that fed on the pod diets. The formation of undegradable complexes (Makkar, 1993; Waghorn et al., 1994; Fall-Toure et al., 1998) between CT and protein and/or carbohydrates may have reduced the amount of substrate available for fermentation. The escape of these undegradable complexes from the rumen to the abomasum (Reed et al., 1990, Reed, 1995; Weigand et al., 1995) could also explain the low concentrations of protein in the rumen fluid and SAM extracts of animals that fed on the Nilotica and Sieberiana diets in particular since these diets were rich in CT. A low concentration of protein in the rumen fluid disfavours microbial growth and activity, and this partly explains the low degradation of DM and cell wall constituents of the hay extract incubated in the rumen of animals fed Nilotica and Sieberiana diets.

Apart from the formation of undegradable complexes, CT also precipitate microbial enzymes which are responsible for proteolysis and degradation of cell wall constituents (McLeod, 1974; Lohan et al., 1981; Jones et al., 1994).The results presented in Table 5.4 illustrate this assertion. A decrease in microbial enzyme activity leads to reduced fermentation of structural carbohydrates and therefore explains in part, the depression

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in the concentration of VFAs in the rumen of sheep fed pod diets. Consequently, inclusion of polythene glycol (which preferentially binds to, and attenuates the effects of tannins) in tannin-rich diets fed to sheep greatly improved the digestibilities of DM, N and cell wall constituents (Priolo *et al.*, 2000) and total VFA concentration in the rumen (Wang *et al.*, 1996; McSweeney *et al.*, 1999).

The efficiency with which N is captured by rumen microorganisms however, depends not only on the rate and extent of breakdown of feed but also on the synchronous provision of readily available, utilizable source of energy to fuel the synthesis of microbial protein. Failure to achieve this balance may result in rapid and extensive breakdown of the synthetic powers of the rumen microorganisms (McDonald *et al.*, 1998). It is therefore possible that although the Leucaena diet promoted a similar protein concentration in the rumen like the Alfalfa diet, a deficit in energy could have led to a wastage, since excess ammonia could have been absorbed and excreted as urea. The conversion of ammonia to urea requires 4 moles of ATP per mole of urea formed (Nsahlai *et al.*, 1998c). Furthermore, diets containing phenolic compounds require substrates like gluthathione, glucuronic acid, sulphate, glucose or cysteine, which conjugate with the phenolic compounds to form water-soluble catabolic products for excretion (Cheeke and Palo, 1995). The combined effect of these two processes could accentuate the energy deficit and cause an imbalance in the amino acids that are supplied for microbial synthesis. This effect when added to the direct impact of tannins on rumen microbes and their enzymes could seriously impair microbial activity and may explain the large differences noticed between the pod diets and the Alfalfa or hay diets in the degradation of DM and cell wall constituents of the material incubated in the

rumen of sheep for 24 hours.

Grinding of forages has also been reported to markly depress the intensity of fibrolysis (Journet and Holden, 1973; Shaver et al., 1986; Fadlalla et al., 1987; Le Liboux and Peyraud, 1998, 1999) and reduce both OM and cell wall digestibility (Rode et al., 1985; Woodfort and Murphy, 1988). This was unlikely to be the case in this work since the amount of feed offered was very restricted and thus could not significantly influence rumen pH nor retention time of the feeds in the rumen. The absence of significant differences in molar proportions of VFAs suggest that although dietary treatment may not have altered the structure of the microbial population, it affected the proportion of fermented nutrients.

5.6 CONCLUSION

The results of the study have highlighted the importance of feedtype in fueling the activities (proteolysis, fibrolysis, fermentation) of microorganisms in the rumen. The chemical composition of the feed was shown to be the driving force to microbial growth and activity and the presence of condensed tannins was once more seen to impair degradability through the formation of undegradable complexes and reduction in the activities of proteolases and fibrolases. The concentration of VFAs in the rumen was seen to depend on the amount and type of substrate available for fermentation and therefore on the effective degradability of the ingested feed. A reduction in microbial activity also emphasized the need for a synchrony between energy and protein to improve the efficiency of nitrogen capture by rumen microorganisms, an imbalance of which may lead to ammonia wastage.

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Evaluating the process of ensilage as a means of reducing the concentration of cyanogenic glycosides in the pods of *Acacia sieberiana*

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6.1 Abstract

Cyanogenic glycosides are anti-nutritional factors capable of liberating hydrogen cyanide, a respiratory poison that has been reported to be responsible for the death of livestock browsing some Acacia species. This study examined (1) ensiling as a means of reducing contents of cyanogenic glycosides and improving the nutritive value of the pods of *Acacia sieberiana* and (2) the influence of levels of molasses and urea on the aerobic stability of the silage. In experiment 1, laboratory silages were prepared by mixing ground pods with water in two ratios; 60:40 and 40: 60 (weight of pod material: weight of water). The mixtures were put in 250 ml polythene bottles, ensiled for 4, 7, 14, 21, 28 or 35 days and analyzed for cyanide. In experiment 2, silages were prepared as described above using only one of the ratios (40:60), but in addition, 3 levels of molasses (0, 3 or 6 %) and urea (0, 0.25 or 0.5 %) were included in a 3 x 3 factorial design, to study the effect of additives on the stability of the silage. The period of ensilage was 14 days. In experiment 3, the concentrations of molasses and urea were modified to 0, 4.5 or 9 % and 0, 0.75 or 1.5 % respectively and the period of ensilage was extended to 45 days to further examine the effect of higher concentrations and extended period of ensilage on silage quality.

The parent material was found to contain 130.6 mg CN/ kg DM and ensiling the material for 35 days reduced the concentration to 18.1 mgCN/kg DM. Increased moisture content at ensiling significantly ($p < 0.001$) decreased the concentration of cyanide in the silages during the first 4 weeks. Silages opened after 14 days were unstable irrespective of the additives included at the moment of ensiling. However those with combined urea and molasses additives showed a slower rate of deterioration and lower fungal counts. Extending the period of ensiling to 45 days improved the aerobic stability of all the silages. The addition of urea significantly ($p < 0.001$) increased the pH, crude protein content, gross energy and fermentation acids while molasses treatment increased ($p < 0.001$) the lactic acid and ash content of the silages. The results show that ensiling ground pods for 45 days was enough to reduce the cyanide content of the pods to non-toxic levels and produced a silage which was aerobically stable while the inclusion of additives further improved the quality of the silages.

Key words: Livestock, *Acacia sieberiana*, cyanogenic glycosides, toxicity, ensilage, aerobic stability

6.1 INTRODUCTION

The Acacias are the most predominant browse species in Sub-Saharan Africa (Le Houerou, 1980). They are found in all agro-pastoral systems where they provide forage and shade to livestock at critical periods of the year when pastures are deficient both in quantity and quality. The high protein content of the forage relative to grasses is widely recognized (Nsahlai et al., 1995a; Bonsi et al., 1994; Kaitho 1997; Sawe et al., 1998). Browse from these species is also capable of providing forage rich in vitamins and minerals (Le Houerou, 1980; Gohl, 1981; Brewbaker,1986; Ahn et al., 1989). Previous research (Gwynne, 1969; Topps, 1992) has shown that Acacia pods have a high nutritive value and could serve as a source of protein for diets based on crop residues and poor quality roughages.

Acacia sieberiana is one of the most dominant species of Acacia in South Africa. It is also found in all the southern as well as east African countries (Carr, 1976; Skerman et al., 1988). The pods of this species are brown when mature, 10-15 cm long, 2-2.5 cm broad and 1-1.5 cm thick. They are indihescent, contain 12-15 seeds arranged in a row and embedded in a spongy tissue. Mature pods drop during the dry season and are readily eaten by livestock and game (Dougal and Bogdan, 1958; Lamprey, 1967). In a good fruiting season, a yield of 100-200 kg of pods per tree is common (personal observation). The seeds and husks have a CP content of 330 and 148 g/kgDM respectively and the pods were found to be moderately rich in minerals and amino acids (Ngwa et al., 2002). These indices make the pods of A. sieberiana, a suitable source of nitrogen and minerals to ruminants fed roughage based diets. This potential is however, limited by the presence of a high level of tannins (Tanner et al., 1990; Nsahlai

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et al., 1995b; Ngwa *et al.*, 2002) and cyanogenic glycosides (Steyn and Remington, 1935; Steyn, 1943; Seigler *et al.*, 1975).

Cyanogenic glycosides (CG) are carbohydrate molecules which are linked by ether bonds to a non-carbohydrate fraction called an aglycone. Glycosides are metabolized by enzymatic action to release the aglycone from the carbohydrate. The aglycone contains cyanide as part of the molecule and when it is metabolized, free cyanide, a deadly poison is released (McDonald *et al.*, 1988). Cyanide is very toxic to cellular metabolism (D'Mello, 1991). It combines with haemoglobin in the blood and inhibits respiratory enzymes, ultimately causing death. Brabdbury *et al.* (1991) reported a dose rate of 0.5-3.5 mg HCN/ kg body weight to be lethal while Seddon and King (1930) stated that fresh plants, which contain 0.02 per cent prussic acid (CG), were capable of causing fatality in sheep if they consumed an average of 500 g of the plant. Apart from reports of acute intoxication and death, there is strong evidence that goitre and cretinism (due to iodine deficiency) are exacerbated and neurological disorders are caused by long term ingestion of feed containing CG (Delange and Ahluwalia, 1983).

In ruminants, mastication and subsequent rumen digestion results in the release of HCN from cyanogenic glycosides. Ruminal microorganisms have the capacity to degrade cyanogenic glycosides and intraruminal release of HCN does not require the presence of plant enzymes (Majak and Cheng, 1984, 1987). The released HCN, once absorbed into the blood stream from the rumen, inhibits cytochrome oxidase of the mitochondrial respiratory system with which it forms a stable complex thus causing asphyxiation at the cellular level and death (Kingsbury, 1964; Boundoux *et al.*, 1980).

Under grazing conditions, there is little danger that animals will be poisoned, primarily because an animal will seldom consume large quantities of these pods. However, cases of cyanide poisoning have occurred under field conditions in animals that had been previously starved (Steyn, 1943). The danger of poisoning is greater when the pods are offered as supplements, particularly if they are ground before feeding.

Many African traditional communities that consume cassava (*Manihot esculenta Crantz*) which has been reported to contain high levels of CG (Wood, 1965; Izomkun-Etiobhio and Ugochukwu 1984; Bradbury *et al.*, 1991) use fermentation as a means of detoxification of the compound in this food crop. This method was found to be less successful for the pods of *A. sieberiana* (Nsahlai *et al.*, unpublished). The pods of *Acacia sieberiana* like most legumes, have a higher buffering capacity than grasses due to low sugar contents and some difficulties in ensiling such a feed have been attributed to this deficiency. They require a higher level of soluble carbohydrates (WSC) to support sufficient fermentation which can adequately lower the pH to about 4, at which point, further bacteria growth is inhibited and a stable silage is produced (Cheeke, 1999). Ammonia application increases the non-protein nitrogen content which promotes the growth of fermentative microbes (Cheeke, 1999), thus leading to a better fermentation. It also improves the nutrient content and fibre degradability of the silage through its alkaline properties (Colenbrander *et al.*,1971;Robertson, 1983; Tedlow, 1884/84). This may justify the choice of molasses and urea as additives in this study.

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The study was therefore undertaken to examine ensilage as a means of reducing the content of cyanogenic glycosides in the pods of *A. sieberiana* and to evaluate the effects of additives on the aerobic stability of the end product (silage). The chemical composition of the silages was also analysed in order to evaluate the effects of fermentation and additives on other nutrients.

6.3 MATERIALS AND METHODS

Ensiling Procedures

In order to study the effect of ensilage on the concentration of cyanogenic glycosides, laboratory silages were prepared by mixing dry ground pods of *A. sieberiana* with water in two ratios: 60:40 and 40:60 (weight of pod material:weight of water). The resulting mixtures were put in polythene bottles (250 ml), care being taken to ensure that filling was as tight as possible to maximize exclusion of air. Bottles were filled in duplicates and stored in a 200 l drum, half-filled with saw dust. After placing the bottles in the drum, a thick layer of saw dust was added, followed by a layer of moist soil. The first samples were removed after 4 days and the rest on weekly basis for five weeks.

To study the effects of molasses and urea on the aerobic stability of the silages, the ensiling process was divided into two phases. In the first phase, laboratory silages were prepared by mixing ground pods of *A. sieberiana* with water at the ratio of 40:60 (w:w) and the resulting mixture was separated into 9 treatments to which 3 levels of molasses (0, 3, 6 %) and urea (0, 0.25, 0.5 %0) were added in a 3 x 3 factorial design. The mixtures were put in 1-litre polythene bottles in duplicates, covered and ensiled as described above. The silos were opened after a period of 14 days. After opening,

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samples were taken immediately from each bottle for fungal analysis and for the determination of pH, dry matter (DM), crude protein (CP), gross energy (GE), lactic acid (LA) and volatile fatty acids (VFAs). The rest of the silage samples in the bottles were left in the laboratory exposed to air for a period 4 days in order to evaluate the rate of deterioration. After this period, three samples were taken from each bottle, the first from the surface layer, the second from the middle and the third from the bottom layer and sent for fungal analysis. Due to the unstable nature of the silage that was produced, a third phase of the work was designed in which the concentrations of molasses were increased to 4.5 and 9 and urea to 0.75 and 1.5 % respectively. The period of ensilage was also extended from 14 days to 45 days, to determine whether a longer period of ensilage could improve the stability of the silage after exposure to aerobic conditions. The silage produced was evaluated as described above.

Chemical analysis

Determination of cyanide

The non-enzymatic method described by Bradbury *et al.* (1991) was adopted for the determination of cyanide. Samples (about 10g) of ground pods (zero-day) or ensiled pod meal were weighed in 250 ml beakers and blended with 75 ml of 0.1M H_3PO_4 using a magnetic rod for 30 minutes. The resulting mixture was filtered using suction through a Whatman No 1 filter paper and washed twice with 0.1M H_3PO_4. The filtrate was made up to 100 ml with the same acid in a volumetric flask and stored at 4°C overnight. To 2 ml of the solution was added 2 ml of 4M H_2SO_4 in a glass stoppered tube. The resulting solution was placed in a boiling water bath for 50 min and then cooled on ice for 5 min. To the cold solution, 5 ml of 3.6M NaOH was added, mixed and filtered. The filtrate was

analyzed for cyanide (CN⁻) in a Skalar San Plus Auto-Analyzer using a segmented flow technique (Skalar, 1993). The system used comprised of three essential components where the sample was mixed with phosphoric acid and exposed to UV digestion during which all complex cyanides and thiocyanates were converted to hydrocyanic acid which was then distilled. The distillate was converted to cyanogen chloride by reacting with chloramine-T. Cyanogen chloride subsequently reacted with iso-nicotinic acid and barbituric acid to form a strongly coloured polymetine dye which was read at a wavelength of 600 nm. The standard was potassium cyanide (KCN) (A R grade) in 0.2M NaOH.

The gross energy (GE), dry matter (DM), organic matter (OM) and nitrogen (N) contents of the parent material and silage samples were determined using the methods of the AOAC (1990). The neutral detergent fibre (NDF) and acid detergent fibre (ADF) were determined using the method of Van Soest *et al.* (1991), lactic acid using the method described by Pryce (1969) and modified by Palic *et al.* (1998), VFAs by gas chromatography and pH using the method described by MAFF (1986). The mineral, amino acid, condensed tannin content as well as degradation properties of the parent material had been examined in a previous study (Ngwa *et al.*, 2002).

Statistical analysis

The data were analysed using the general linear model (GLM) of the Statistical Analysis System (SAS, 1987).Treatment effects on the variables were obtained by subjecting the data to an analysis of variance and contrasts between treatments were done by applying the probability of difference (PDIFF) option of the least square means statement available in the GLM.

6.4 RESULTS

Chemical properties of the parent material

The chemical composition of the pods (parent material) is presented in Table 6.1. The pods have a relatively high OM and CP content. The concentration of cell wall constituents is low and the mineral concentration is moderate when compared to other Acacia pods. However, the pods contain a high level of condense tannins.

Effect of ensilage on the concentration of cyanide and CP in silage samples

The concentration of cyanide significantly ($p<0.001$) dropped from 130.6 mgCN/kg DM in the dry pods (non-ensiled) to 30.9 and 52.2 mgCN/kg DM for the 60:40 and 40:60 pod:water mixture ratios respectively, after 4 days of ensilage. The measurement of cyanide after 7 days showed a further drop to 23.8 and 39.7 mgCN/kg DM in the same order. After this sharp drop in the first week, the decrease became more gradual in the subsequent weeks (Figure 6.1).

Increased moisture at ensiling significantly ($p<0.001$) decreased the concentration of cyanide in the silages during the first phase of the ensiling process but measurements after 28 days of ensiling showed similar concentrations of cyanide for both ensiling mixtures and no further reductions. The CP content showed a very gradual but more or less linear decrease (Figure 6.2) although the effect of period of ensilage on CP was significant ($p = 0.0387$). The effect of mixture ratio on CP content tended to be significant ($p = 0.0517$).

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Table 6.1 Chemical, mineral and amino acid compositions of the pods of *A. sieberiana*

Constituent	Concn	Mineral	Conc.	Amino acid	Conc. (g/kgDM)
OM (g/kgDM)	945.8	Ca	7.2 [a]	Threonine	2.5
Ash (g/kgDM)	44.2	P	2.2 [a]	Valine	4.6
GE MJ/ kgDM)	16.8	Mg	2.4 [a]	Methionine	0.2
CP (g/kgDM)	174.9	Na	0.5 [a]	Isoleucine	2.9
NDF (g/kgDM)	366.9	Fe	118.1 [b]	Leucine	5.2
ADF (g/kgDM)	290.1	Zn	29.5 [b]	Phenylalanine	2.8
HEM (g/kgDM)	76.8	Cu	8.4 [b]	Histidine	3.2
LIPID (g/kgDM)	10.6	Mn	23.2 [b]	Lysine	4.8
CT(%QE)	28.3	Se	0.1 [b]	Arginine	6

[a] g/kgDM; [b] mg/kgDM; OM, organic matter; CP, crude protein; NDF, neutral detergent fibre; ADF, accid detergent fibre and HEM, hemicellulose ; CT, condensed tannins ; QE, quebracho equivalents
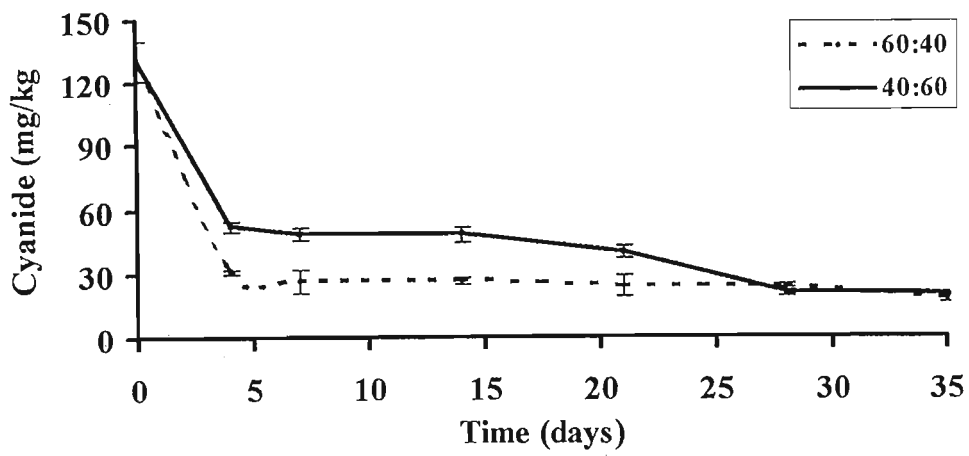
Figure 6.1 Variation in cyanide content following period of ensilage. Vertical lines indicate variation between replicate samples, expressed as standard deviations.
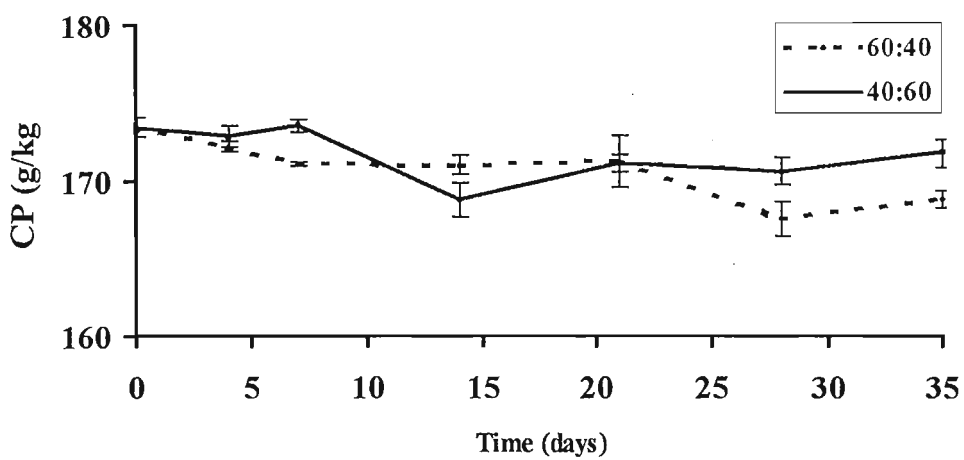
Figure 6.2 Variation in crude protein content following period of ensilage. Vertical lines indicate variation between replicate samples, expressed as standard deviations.

Effect of additives on the aerobic deterioration of silages

All silages had a desirable aroma and there were no apparent differences in colour or odour when the silos were opened. The silages appeared to have been well preserved. Two types of fungi (Penicillium and yeasts) were used as indices of aerobic deterioration because of their high numbers. Other types were identified but their numbers were relatively low (Tables 6.2 and 6.3). Samples examined immediately after the 14-day silages were opened had very low counts (<1000 propagules/g) of Penicillium (Table 6.2) and yeast was not detected in most of the samples. After 4 days of exposure, the silages quickly deteriorated and the surface layer of silages that had either molasses or urea additives as well as the control (no additives) were visibly (naked eye) very mouldy while treatments containing both urea and molasses showed signs of moulds but to a lesser extent. After this period of exposure, there was a very rapid multiplication of Penicillium and yeast, particularly for the surface layers of all the silages. However, Penicillium counts were lower for the middle and bottom layers of the silages treated either with molasses or molasses and urea combined as compared to the corresponding layers of the silages with urea only or without additives.

The silages opened after ensiling for 45 days, had a higher yeast count than Penicillium on the first day and the urea treated silages had higher total fungal counts than those treated with molasses alone (Table 6.3). However, all silages were aerobically stable. After 4 days of exposure, the silages showed no visible signs of moulds. Fungal counts

Table 6.2 Fungal screening results on silage (propagules/g $\times 10^3$) opened after 14 days

Period of exp.	Fungal species	Layer	0	0	0	3	3	3	6	6	6
Molasses (%)											
Urea (%)			0	0.25	0.5	0	0.25	0.5	0	0.25	0.5
0	Penicil	Mixed	0.1	0.8	0.9	0.6	0.5	0.4	0.3	0.5	0.5
	Yeast	Mixed	ND	ND	ND	ND	20	ND	ND	ND	ND
	A. fum	Mixed	ND	ND	ND	ND	ND	0.2	ND	ND	0.8
	A. flav	Mixed	0	ND	ND	0.1	ND	ND	ND	ND	ND
	Rhizop	Mixed	ND	0	ND	ND	ND	ND	0.01	ND	ND
4	Penicil	SL	>5000	>50000	>50000	1000	1	0.2	>50000	0.05	0.2
	Penicil	ML	>5000	5000	30000	30	0.4	0.2	40	0.1	0.2
	Penicil	BL	1000	1000	60	10	ND	0.2	30	0.05	ND
4	Yeast	SL	>5000	>50000	>50000	>5000	>50000	>50000	20000	>50000	>50000
	Yeast	ML	>5000	>50000	20000	10	10	10	40	20	3000
	Yeast	BL	>5000	10000	4000	10	12000	10	30	80	2000
4	Rhzop	SL	1000	ND	30	ND	ND	ND	1000	ND	ND
	Rhzop	ML	10	ND	0.4	ND	ND	ND	0.3	ND	ND

SL, surface layer; ML, middle layer; BL, bottom layer; ND, not detected; Penicil, Penicilium; A. fum, Aspergillus fumigatus; A. flav, Aspergillus flavus; Rhizop, Rhizopus

recorded after 8 days of exposure when moulds began to appear on the surface of the silages indicated high yeast counts for all silages but high Penicillium counts were observed only for silages treated with urea alone, in the case of the surface layer. The middle and bottom layers had very low (<1000 propagules/g) counts for most treatments. Yeast counts were very high (>10^5 propagules/g) for the surface layer but were considerably lower or not detected for the middle and bottom layers. In general, the urea treated silages had higher fungal counts than those treated with molasses and in all the silages, yeasts were present in higher numbers than the Penicillium. Analysis for mycotoxins could not be done because of the high costs involved.

Table 6.3 Fungal screening results on silage (propagules/g $\times 10^3$) opened after 45 days

			Treatments								
Molasses (%)			0	0	0	4.5	4.5	4.5	9	9	9
Urea (%)			0	0.75	1.5	0	0.75	1.5	0	0.75	1.5
Period of exp.	Fungal species	Layer									
0	Penicil	Mixed	ND	80	0.3	0.5	ND	20	0.8	30	20
	Yeast	Mixed	3000	40	30	10	30	35	ND	10	60
	Mucor	Mixed	10	ND	0.1	ND	0.5	ND	ND	3	10
8	Penicil	SL	ND	>50000	20000	6000	60	ND	40	ND	ND
	Penicil	ML	0.2	1	30	1000	0.1	ND	0.4	0.05	ND
	Penicil	BL	0.2	1	1	0.8	0.05·	ND	ND	ND	0.1
8	Yeast	SL	20000	1000	20000	>50000	10000	10000	2000	20000	20000
	Yeast	ML	ND	ND	12000	15000	0.5	1500	ND	7000	ND
	Yeast	BL	ND	ND	6000	10	ND	2000	ND	1000	ND
8	Mucor	SL	6000	ND	ND	ND	5000	ND	ND	30000	10000
	Mucor	ML	10	ND	ND	ND	0.2	ND	ND	ND	10000
	Mucor	BL	0.1	ND	ND	ND	0.1	ND	ND	0.1	0.1

SL is surfacce layer; ML, middle layer; BL, bottom layer; ND, not detected; Penicil, Penicilium

The chemical composition of the 14 day silages, as influenced by the various levels of additives is presented in Table 6.4. Molasses treatment decreased (p = 0.005) the GE content of the silages while the opposite effect (p = 0.033) was noticed with urea treatment. The addition of urea either singly or in combination with molasses increased (p = 0.001) the CP content of the silages. The NDF concentrations of the silages were not affected by the addition of molasses or urea but the two in combination decreased (p = 0.029) its contents in the silages.

Table 6.4 The effect of molasses and urea additives on the chemical composition of silages ensiled for a period of 14 days

Comp	Treatment									SED
Mol. (%)	0	0	0	3	3	3	6	6	6	
Urea (%)	0	0.25	0.5	0	0.25	0.5	0	0.25	0.5	
GE[1]	17.1^a	17.1^a	17.2^b	16.9^c	16.9^c	17.0^a	16.8^c	17.0^a	17.0^a	0.05
CP[2]	134.7^a	143.3^b	147.2^c	133.2^a	137.9^d	138.6^d	134.2^a	146.4^c	144.6^c	1.5
NDF[2]	425.8^a	442.2^b	447.0^b	413.6^a	403.4^c	401.0^c	404.6^c	396.6^c	412.0^a	11.15
ADF[2]	360.3^a	390.1	387.2	348.9^a	348.9^a	348.1^a	341.6^a	343.3^a	344.5^a	12.62
Ash[2]	45.4^a	44.6^a	45.7^a	50.8^b	49.3^b	50.7^b	55.2^c	51.2^c	54.9^c	0.58
LA[2]	0.7^a	0.9^b	1.0^{bc}	0.8^a	0.9^b	1.0^b	1.1^{bc}	1.6^d	1.2^c	0.36
AcA[2]	1.3^a	2.2^b	2.4^c	1.3^a	1.7^a	1.8^a	1.9^a	2.7^c	2.2^b	0.36
IValA[2]	0.6	0.6	0.63	0.57	0.55	0.52	0.59	0.52	0.52	0.04
pH	4.5^a	4.8^b	5.3^c	4.5^a	4.7^b	4.7^b	4.5^a	4.7^b	4.8^b	0.08

[1] MJ/kg DM; [2] g/kgDM; Comp, component; LA, lactic acid; AcA, acetic acid; iValA, iso-valeric acid; Mol, molasses; NDF, neutral detergent fibre; ADF, acid detergent fibre; CP, crude protein

The addition of molasses increased ($p = 0.001$) the ash content of the silages. The concentration of lactic acid (LA) increased ($p = 0.003$) with the presence of additives included either singly or combined. The same pattern was seen for the concentration of acetic acid. The concentration of iso-valeric acid was not influenced by treatment with additives. Other VFAs (propionic, butyric, iso-butyric and valeric acids) were not detected. Urea treatment significantly increased ($p = 0.001$) the pH of the silages.

The 45-day silages had similar ($p>0.05$) GE contents except one treatment (0 molasses, 1.5% urea) which had a higher ($p = 0.03$) value (Table 6.5). The addition of urea increased ($p<0.01$) the CP and acetic acid contents of the silages but had no effect on the NDF and ADF contents. Molasses increased ($p<0.001$) the ash content.

Table 6.5 The effect of molasses and urea additives on the chemical composition of silages ensiled for a period of 45 days

Comp	Treatment									SED
Mol. (%)	0	0	0	4.5	4.5	4.5	9.0	9.0	9	
Urea (%)	0	0.75	1.5	0	0.75	1.5	0	0.75	1.5	
GE[1]	17.0^a	17.1^a	17.4^b	17.3^{ab}	17.0^a	16.9^a	17.3^{ab}	16.9^a	17.0^a	0.53
CP[2]	137.5^a	152.9^b	174.8^c	142.3^a	155.3^b	166.2^d	140.1^a	157.5^b	174.3^c	3.21
NDF[2]	411.4	437.8	441	404.4	429	433.4	406.8	406	404.4	15.5
ADF[2]	346.1	379.1	362.2	364.2	339.8	357.5	334.6	326.5	32	17.01
Ash[2]	47.8^a	47.9^a	48.8^a	57.4^c	54.0^c	54.1^c	63.0^d	61.0^d	60.4^d	1.53
LA[2]	0.8^a	2.5^b	3.1^c	1.0^d	2	2	1.4^f	3.7^g	2.4^b	0.05
AcA[2]	1.7^a	2.8^b	3.1^b	1.7^a	2.2^{ab}	2.4^{ab}	2.4^{ab}	3.7^b	2.9^b	0.5
IValA[2]	0.8	0.8	0.7	0.6	0.7	0.7	0.8	0.7	0.7	0.05
pH	4.5^a	6.4^b	7.1^c	4.6^a	5.5^d	7.3^c	4.5^a	5.6^d	5.2^d	0.1

[1] MJ/kg DM; [2]g/kgDM; Comp, component; LA, lactic acid; AcA, acetic acid; iValA, iso-valeric acid; Mol, molasses; NDF, neutral detergent fibre; ADF, acid detergent fibre; CP, crude protein

Both additives increased ($p<0.001$) the concentration of lactic acid, with the silage that was treated with 9% molasses and 0.75% urea, producing the highest concentration. The concentration of iso-valeric acid was not affected by treatment with additives. Other VFAs were not detected. Treatment with molasses did not affect the pH but urea significantly ($p<0.001$) increased the pH of the silages.

6.5 DISCUSSION

The results of this study show that the concentration of cyanide in the dry pods of A. sieberiana, though lower than the concentrations reported by Steyn and Remington (1935), may have fatal effects if a sheep weighing about 40 kg liveweight is fed 0.5 kg of pod meal (1.625 mgCN/kg liveweight). This falls within the lethal dose of 0.5-3.5

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mgCN/kg liveweight reported by Coop and Blankley (1950) and Bradbury *et al.* (1991).

Ensiling the pod material for a period of 35 days reduced the concentration to 18

mgCN/kg DM of pod meal or 0.4 mgCN/kg liveweight, if the same animal was fed 1 kg

DM of silage. This significantly increases the quantity of the feed that the animal could

consume without fear of toxicity.

The difference in cyanide concentration from this work and that reported by Steyn and

Remington (1935) could be explained by the fact the latter used fresh leaves and pods

which are said to contain higher concentrations. Torres *et al.* (1988) reported that dry

plant material may appear to lack cyanogenic activity because of the denaturation of the

enzyme, β-glucosidase or decomposition of cyanogenic glycosides. CG content also

varies from one species to another and within the same species, may vary from one

location to another. The concentrations of cyanide in the silage samples indicated that

ensiling effectively reduced the cyanide content in the feed and that the period of 35

days was sufficient to bring down the concentration to non-toxic levels. However,

ensiling did not completely eliminate the cyanide in the pods. This may relate to the fact

that cyanide in most plants exists in two forms: CG which are metabolized by

microorganisms during the process of fermentation and non-glycosidic cyanide which

is not metabolized (Izomkun-Etiobhio and Ugochukwu,1984). Butler and Bailey (1973)

described the non-glycosidic cyanide as HCN gas which is entrapped by a reaction with

metal ions and/or carbonyl groups of hexoses to form cyanohydrins. The differences in

the rate at which cyanide concentration decreased between the two mixture ratios at the

onset of fermentation may be due to an increase in DM at ensiling which tends to

improve fermentation, an observation confirmed by Moseley and Ramanathan (1989).

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The slight reduction in CP content during the first phase of the trial when additives were not included, was probably due to the breakdown of amino acids during the process of fermentation (Langston et al., 1962) but this decrease in practice may be compensated for by offering more of the ensiled feed to animals or adding urea to the pod material before ensiling, the effects of which are discussed in detail in the next section.

The choice of Penicillium and yeast as parameters for indexing the degree of deterioration in this study was based on the fact that these two constituted the bulk of the fungal population in the silages. However, a few other genera (Aspergillus, Mucor and Rhizopus) were also detected in smaller numbers. The presence of moulds produces undesirable effects in silage because they break down, not only sugars and lactic acid via normal respiratory pathways, but also hydrolyze and metabolize cellulose and other cell wall components (McDonald 1981). Many other authors (Ohyama et al., 1977; Woolford, 1978; Hara and Ohyama, 1979; McDonald, 1981; McDonald et al., 1998) reported that moulds and yeasts seem to be mainly responsible for the deterioration of silages exposed to air. Furthermore some moulds produce mycotoxins which are harmful to animals and humans (Palmisano and Barlez, 1996; McDonald et al., 1998).

The low Penicillium counts observed just after the silages were opened could be explained by the fact that most types of moulds depend on oxygen for their growth and propagation, however, minute quantities (at least 10% of normal oxygen of the air)are sufficient to maintain the growth and metabolism of the moulds (Pahlow, 1991). Yeasts also multiply rapidly in the presence of oxygen and play a great role in the deterioration

of silages (Woolford, 1990). Silages with yeast counts greater than 10^5 propagules/g are likely to be unstable (Woolford, 1990). The rapid deterioration of the 14d silages was an indication that the process of fermentation was not completed after 14 days, the consequence being the production of an unstable silage. Increasing the concentration of additives and/or extending the period of ensiling promoted the growth of more fermentative microorganisms and the production of a higher quantity of fermentation acids, necessary for stabilizing the silage. The growth of fermentative bacteria especially lactic acid bateria (LAB) depends on the availability of nitrogen (Chaudhry et al., 1993; Al-Rokayan et al., 1998) but the fact that the molasses-urea treatments produced more acids than the treatments with urea alone points to the fact that these microbes equally need a source of energy (ATP) for adequate multiplication and this was provided by the fermentation of molasses to lactic acid. The drop in the concentration of acids in treatments that were treated with 6% molasses, 0.5% urea and 9% molasses, 1.5% urea indicates that there is a threshold beyond which, increases in the concentration of urea begins to play a negative role in the fermentation process, a fact supported by Rasool and Gilani (1996).

The increase in the level of urea produced linear increases in pH despite the increases in fermentation acids. This may be due to a strong buffering capacity resulting from the ammonia released from urea. Secondly, acetic acid which is a weaker acid was higher in concentration than lactic acid and thus the acidity was not strong enough to neutralize the buffering capacity of ammonia. Similar observations were made in other trials (Langston et al., 1962) and it may be concluded that pH alone is not always a good criterion for determining silage quality.

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The low concentrations of lactic acid, compared to acetic acid could be attributed to the activity of heterofermentative lactic acid bacteria that tend to be dominant in silages with low WSC (McDonald, 1981) and produce in addition to lactic acid, acetic acid, ethanol, mannitol and carbon dioxide. The low values of lactic acid obtained as compared to those reported by other workers (Chaudhry et al., 1993; Kato et al., 1994; Sibanda et al., 1997; Al-Rokayan et al., 1998) could be attributed to the type and nature (dry pods) of the ensiled material. However, the low concentrations of acids and the high pH values are not an indication that clostridial fermentation took place because no butyric acid typical of clostridial silages (McDonald et al., 1998) was detected.

The increases in GE values observed in some of the 45d silages as compared to the 14d silages may suggest the formation of high energy compounds such as alcohol (McDonald et al., 1973; McDonald et al., 1998). The increases in CP content of the urea-treated silages were as expected. Chaudhry et al. (1993), Kato et al. (1994) and Al-Rokayan et al. (1998) reported the same linear increases in CP with increased levels of broiler litter. The increases in NDF and ADF of the silages as compared to the contents of the same fibres in the parent material are explained by the hydrolysis of the soluble matter during the fermentation process while lower values of NDF of the 45d silages when compared to those opened after 14 days could have been due to the hydrolysis of hemicellulose to monosaccharides for lactic acid fermentation (Thomas and Thomas, 1985; Muck, 1988). The increase in ash content of the molasses-treated silages is in conformity with other reports that molasses treatment increases the ash content of silages (Castle and Watson, 1985; Sibanda et al., 1997). The production of iso-valeric acid could be attributed to the catabolism of amino acids (McDonald, 1981)

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and this acid is known to have beneficial effects in preserving silages exposed to air because it inhibits the growth of organisms responsible for the aerobic deterioration of silages (Ohyama and McDonald, 1975).

6.6 CONCLUSION

The results from this study clearly show that the process of ensiling could be a cheap and easy method of detoxifying of CG and that four to five weeks is long enough to reduce the concentration of CG to non-toxic levels. This should enable an adult sheep to consume up to 1 kg of the ensiled material without fear of toxicity. This is extremely important in most dry zones of Africa where pastures are completely depleted during the dry season, and ruminants depend on forage (leaves and pods) from browse species for survival. Molasses and urea improved the fermentation process as a result of increases in fermentation acids and fermentative microorganisms. The quality and aerobic stability of the silages were also improved by the addition of additives. However, in the absence of the latter, the best method of preventing a rapid deterioration of the silage may rely on using the silage without lengthy exposure to aerobic conditions. The most obvious and effective method of preventing aerobic deterioration would be to ensure that the silage is consumed by animals as soon as it is removed from the silo. In this regards, ensiling in small bales may be the recommended method for small scale farmers. This technique was evaluated in an *in vivo* study that is reported in the next chapters.

Feed intake and dietary preferences of sheep and goats offered hay and pods from tree legumes

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7.1 Abstract

Two experiments were designed to investigate the intake and relative palatability of six feeds. In the first experiment, the feeds offered were pasture hay (*Themeda triandra*), alfalfa (*Medicago sativa*), pods of *Acacia sieberiana*, *Acacia nilotica* and *Leucaena leucocephala*. In the second, silage from the pods of *A. sieberiana* was included as the 6th feed in order to examine the effect of ensiling on intake and palatability since this process was devised as a means of detoxifying cyanogenic glocosides known to be present in the leaves and pods of this species. Intake (T_i) and relative palatability (P_i) were determined using 3 goats and 3 sheep in each experiment. Since the feeds had different dry matter contents, intake was expressed as a ratio of the quantity offered (T_i/A_i). Using the hay offered (A_1) and intake (T_1), as standard, relative palatability indices were calculated as $P_i = (T_i/A_i)/(T_1/A_1)$ where i represented the other feeds.

In experiment 1, goats consumed more hay than alfalfa or pods. An increasing trend in intake was observed for leucaena while sieberiana and nilotica were poorly consumed. Sheep consumed more leucaena than all the other feeds. In experiment 2, the trends were similar but the intake of all feeds except nilotica increased over time. The effect of animal species and weight on intake were not significant ($p > 0.05$). Hay and leucaena had the highest P_i for goats and sheep, respectively, in experiment 1 but in experiment 2, the silage from pods of *A. sieberiana* had the highest P_i for goats. The nilotica meal had the lowest P_i in both experiments. P_i was affected ($p < 0.0001$) only by feed type in experiment 1 but in experiment 2, feed type, animal species and weight significantly ($p < 0.01$) affected P_i. The results show that the pods of *L. leucocephala* and *A. nilotica* were the most and least preferred respectively, and ensiling significantly improved the intake and palatability of the pods of *A. sieberiana*. However, some feeds that are least preferred in times of abundance and variety could be relished during periods of scarcity and severe feed shortages, based on animal survival instinct.

Key words: Legume pods, intake, palatability, small ruminants

7.2 INTRODUCTION

When grazing, ruminants are faced with a complex array of plant materials differing in nutrient type and quality as well as level of toxic substances from which they make a propitious combination in order to maximize their biological performance. Goats and sheep in particular are known for feeding on a wide spectrum of feeds and are said to select those that meet their nutritional needs and avoid those that can cause toxicosis (Provenza and Balph, 1990; Provenza, 1995). Feed selection depends on palatability and the latter depends on both plant and animal factors. Plant factors that influence palatability include species, intraspecific variation, chemical composition, morphology or physical traits, succulence or maturation and form of the forage (Marten, 1978). Phenolics, alkaloids, tannins and aromatic compounds are some of the chemical compounds known to alter palatability and intake, irrespective of the nutritional value of the feed. Animal factors include the senses, species or breeds, individual variation, previous experience and physiological condition (Marten, 1978). Flavour (taste and odour) is considered to be the most important food cue (Garcia, 1989) and according to Personius et al. (1987), herbivores are able to detect some toxic compounds by smell before eating or immediately after the first bite.

A number of trials have been carried out to evaluate the palatability of browses during grazing (Johnston,1988; Rutagwenda et al., 1990; Becker and Lohrmann, 1992). However, other authors pointed out that conventional methods used to assess classic forage preference (oesophageal fistula technique, stomach content and faecal analyses) are not convenient for evaluating palatability because they are laborious, costly and complicated. Methods based on direct feeding observation and intake of

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plant species either at pasture or in stall feeding seem to be more suitable for palatability studies (Olson, 1991; Ben Salem *et al*, 1994; Kaitho *et al.*, 1996; Ngwa *et al.*, 2000). This study was thus designed to evaluate the intake and palatability of six feeds: pasture hay (*Themeda triandra*), alfalfa (*Medicago sativa*), pods of *Acacia sieberiana* (both in dry and silage form), *Acacia nilotica* and *Leucaena leucocephala* offered to goats and sheep in a stall feeding system. In addition differences in preference attitudes of the two animal species were also assessed.

7.3 MATERIALS AND METHODS

Animals and housing

Three Merino ewes and three does of the Nguni breed in South Africa (averaging 22 months of age) and weighing 32.3 (s.d. 1.26) and 23.0 (s.d. 1.8) kg respectively were used in the first experiment. In the second experiment, three ewes and three does of the same breeds, weighing 41.2 (s.d. 2.18) and 36.2 (s.d. 3.01) kg respectively were used. In both experiments, animals were housed in a roofed shed in individual pens (4 x 6 m) for the duration of the experiments. The floor was cemented and the pens were equipped with automatic water troughs. Five feed troughs were placed in each pen during the first experiment and the number was increased to six during the second experiment. The troughs were attached to the wire mesh separating the pens to avoid spillage of feed. Prior to the beginning of the experiment, the animals were grazing on an improved pasture made up principally of *Cynodon dactylon*.

Feeds

Collection

Pasture hay was harvested from improved pastures of the Range and Forage unit of the

University of Natal, dried and baled. Alfalfa was bought from local feed manufacturers while pods containing seeds were harvested from six tree legumes just before winter fall when most of the pods were dry. Most of the *Acacia* pods were harvested at different sites on the outskirts of Pietermaritzburg while the *Leucaena* pods were harvested from the University farm. Harvesting was facilitated by using a metal sisal fitted on to the end of a long (6m) stick. The hay was ground through a 10-mm screen in an industrial grinder while the pods were ground through a 3-mm screen using a smaller grinder.

Feeding

Five feeds, pasture hay (Hay), alfalfa, pods of *L. leucocephala* (leucaena meal), *A. nilotica* (nilotica meal), and *A. sieberiana* (sieberiana meal) were used in the first experiment. In the second, silage made from the pods of *A. sieberiana* was added. The silage was prepared by mixing ground pods of *A. sieberiana* with water at a ratio of 40:60 (w:w) and the resulting mixture was put into polythene bags in amounts of about 15 kg per bag. The bags were well tied to ensure maximum exclusion of air, placed in 200-l drums in which the bottom and top layers were covered with saw dust and the material was ensiled for a period of 45 days. Only one bag was removed at a time for feeding animals. During the first experimental period, animals were offered 500g of each feed in separate troughs every morning at 08:00h. In the second experiment, animals were offered 300g of each feed for the first six days and 500g during the last six days of data collection. The order of placement of feeds in the troughs was randomized daily to avoid 'habit reflex'.

Chemical analyses

The DM, organic matter (OM) and N contents of the feed were determined using the methods of the AOAC (1990). The neutral detergent fibre (NDF) and acid detergent fibre (ADF) of the pod samples were measured by the method of Van Soest *et al.* (1991) while hemicellulose was calculated as the difference between NDF and ADF. After acid digestion, phosphorus was determined colorimetrically while calcium was determined using a Varian spectra AA-200 Atomic Absorption spectrophotometer, AOAC (1990). Pod samples were also sent to the Jacob Blaustein Center for Desert Biodiversity, Ben Gurion University of Negev Desert, in Israel for the analysis of condensed tannins and the method of determination was that described by Hagerman(1995).

Measurements, calculations and statistical analysis

The experiments ran for a period of 12 days each. The feeds offered and refusals were weighed and recorded for each animal on a daily basis. A modification of the methods described by Ben Salem *et al.* (1994) and Kaitho *et al.* (1996) was adopted for our calculations and variables were determined taking the intake of pasture hay (*Themeda triandra*) henceforth called hay, as the basis of comparing the intake and palatability of the other feeds. All calculations were done on dry matter basis. The following parameters were adopted:

$T1$ = Average daily intake of hay;

Ti = Average daily intake of the ith feeds where i = 2, 3, 4, 5 or 6 representing alfalfa, leucaena meal, nilotica meal, sieberiana meal and silage respectively.

$A1$ = Quantity of hay offered; and

Ai = Quantity of the other feeds offered as listed above.

Relative palatability indices (Pi) which described palatability of individual feeds in relation to hay were calculated as:

P1 = (T1/A1)/(T1/A1) for hay

Pi = (Ti/Ai)/(T1/A1) where i = 2, 3, 4, 5 or 6.

In this way Pi was calculated for each feed on daily basis for the period of data collection. To reduce large variations in Pi among the feeds, the Pi values were log-transformed and from the values obtained, palatability curves were drawn for each feed. Pi for the whole experimental period was computed using the cumulative intake and feed offered. An analysis of variance (SAS, 1987) was used to compare effects of animal species and feed type on intake and palatability.

7.4 RESULTS

The chemical composition of the feeds is presented in Table 7.1. There was a wide variation in the chemical composition of the six feeds. Crude protein (CP) varied from a low of 42 g/kg for hay to a high of 247 g/kg for Leucaena. In between the two, were alfalfa, sieberiana, nilotica and silage in decreasing order. The hay had the highest concentrations of the detergent fibres (NDF and ADF) and hemicellulose. The lowest concentrations of these components were noticed with nilotica. The ash content of alfalfa was at least 2 times the contents in the pods and 1.5 times that of the hay. Condensed tannins were examined for the pods only and the results indicated that sieberiana had the highest concentration while Leucaena had the lowest. The value for nilotica was in between the two.

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Table 7.1 The chemical and mineral compositions of the pods of *Leucaena leucocephala, Acacia nilotica, Acacia sieberiana,* alfalfa and veld hay.

Feed	DM	OM	CP	NDF	ADF	HEM	Ca	P	CT
	gkg⁻¹				gkg⁻¹DM				%QE
L. leucocephala (Pod)	948	952.6	246.9	408.9	285	124.2	4.5	3	1.8
A. nilotica (Pod)	937	952.7	149.1	224.7	181	43.9	6.4	1.9	17.4
A. sieberiana (Pod)	937	945.8	174.9	366.9	290	76.8	7.2	2.2	28.3
Alfalfa (Hay)	927	898.8	220.2	402.4	341	61.3	1.04	0.3	-
Pasture Hay	952	924.6	41.7	700.8	472	228.5	0.3	0	-
Silage	331	944.9	147.6	449.8	347	1.3.1	4.6	2.9	-

QE, Quabracho Equivalent; DM, dry matter; OM, organic matter; CP, crude protein; NDF, neutral detergent fibre; ADF, acid detergent fibre; HEM, hemicellulose; Ca, calcium; P, phosphorus; CT, condensed tannins

Intake of feeds

The average DM intake of the feeds and their daily variations are presented in Table 7.2 and Figure 7.1 respectively. In experiment 1, goats consumed more hay than either alfalfa or pods. An increasing trend in daily intake by goats was observed for leucaena. The intake of hay initially increased during the first 10 days of the experimental period but dropped drastically during the last 2 days, while the reverse trend was seen for alfalfa. The intake of sieberiana by goats was fluctuating but the general trend portrayed a decreasing trend over time. The nilotica meal was the least consumed. Sheep consumed more leucaena than all the other feeds and its intake had an increasing trend, though with slight fluctuation over the experimental period. The intake of hay had an increasing trend during the first 10 days but dropped during the last 2 days. The intake of alfalfa initially increased, then dropped from day 4 to day 6 and then increased rapidly during the second half of the experimental period.

Table 7.2 Average daily feed intake by goats and sheep in experiments 1 and 2

Feed type	Intake (g DM)			
	Experiment 1		Experiment 2	
	Goats	Sheep	Goats	Sheep
Alfalfa	196.8	263.6	252.7	295.1
Hay	312.8	294.9	294	188
Leucaena	253.2	308	162.3	375.1
Nilotica	48.7	37.9	15	36.7
Sieberiana	89.6	106.3	257.8	232.6
Silage	-	-	119.4	93.1
Total	901.1	1010.7	1101.2	1220.6
SED	31.03		49.58	
Feed type	***		***	
Anim species	NS		NS	
Spec x Feed	NS		NS	
Animal weight	NS		NS	

*** significance at $p < 0.0001$; NS, non-significant

The sieberiana and nilotica meals were poorly consumed by sheep. In terms of total daily DM intake, goats had a slightly lower value than sheep but the effects of animal species or live weight on intake were not significant ($p>0.05$). However, DM intake was significantly ($p<0.0001$) influenced by feed type.

In experiment 2, the intake of all feeds except nilotica increased over time. In terms of DM intake, hay was the most consumed feed, followed in decreasing order by sieberiana, alfalfa, silage and nilotica, as far as goats were concerned. Sheep on the other hand, consumed more Leucaena than all the other feeds. The intake of hay and

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Figure 7.1 Daily variation in intake (g DM/day) for sheep and goats in experiments 1 and 2

Expt 1

Goats

Sheep

Expt 2

—◆—Alf —■—Hay —▲—Leu —●—Nilo —✱—Sieb —⊛—Sil
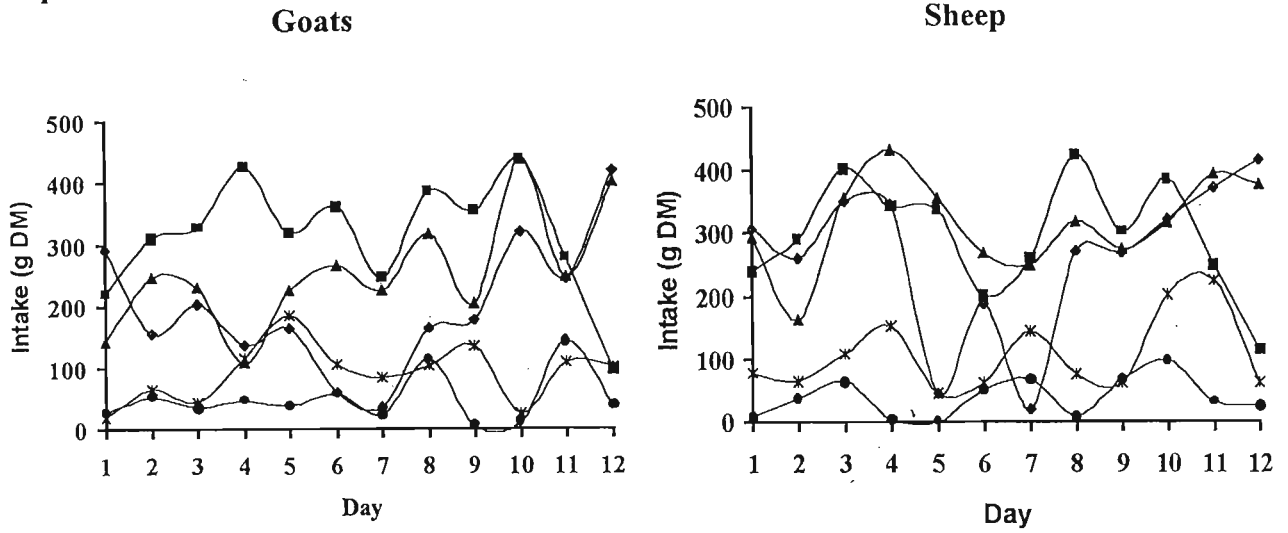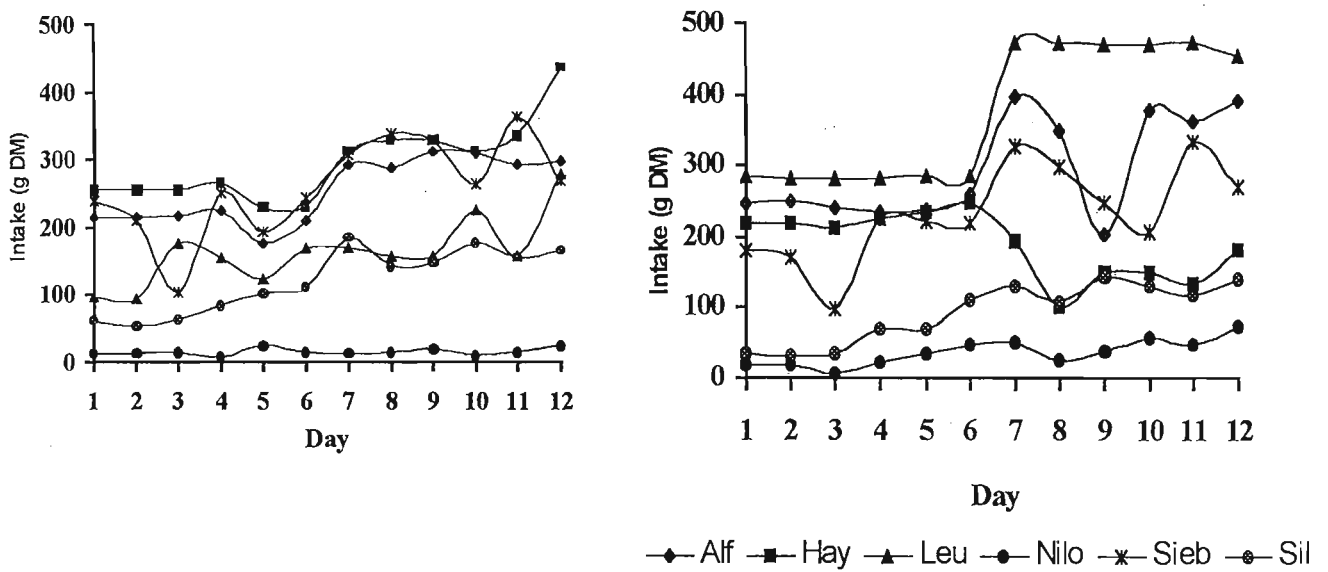
Alf is alfalfa; Leu, leucaena; Nilo, nilotica; Sieb, sieberiana and Sil, silage meals

alfalfa by sheep was relatively constant during the first six days of data collection, but decreased sharply when the quantities of feed offered were increased from 300g to 500g per day, probably because of the very high consumption of leucaena. However, the intake of alfalfa increased again during the last 3 days of the experiment while that of hay remained low. The intake of both the sieberiana meal and its silage were quite high and had an increasing trend throughout the period of observation. As in experiment 1, the intake of nilotica was very low for both species. The daily DM intake was slightly higher for sheep than for goats but the difference was not significant ($p>0.05$). The effect of animal weight on intake was not significant ($p>0.05$) but the type of feed consumed significantly ($p<0.0001$) influenced intake.

Palatability

Palatability indices of the feeds offered are presented in Table 7.3 and the daily variations in figure 7.2. In experiment 1, hay had the highest palatability index (Pi) in the case of goats. This was followed in decreasing order by leucaena, alfalfa, sieberiana and nilotica. The situation was different for sheep, for which leucaena had the highest Pi value, followed in decreasing order by hay, alfalfa, sieberiana and nilotica. Pi was neither affected by animal species nor by weight ($p>0.05$) but was highly influenced by the type of feed offered. In experiment 2, the scenario was different with the inclusion of silage made from the pods of *A. sieberiana*. The silage was well cherished by goats and this resulted to the silage having the highest Pi for this species. This was closely followed by alfalfa, then sieberiana, hay, leucaena and nilotica in decreasing order. In the case of sheep, leucaena had the highest Pi, followed in decreasing order by alfalfa, silage, sieberiana, hay and nilotica .

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Table 7.3 Palatability indices of feeds offered to goats and sheep in experiments 1 and 2

Feed type	Palatability index (Pi)			
	Experiment 1		Experiment 2	
	Goats	Sheep	Goats	Sheep
Alfalfa	0.65	0.92	1.06	1.9
Hay	1	1	1	1
Leucaena	0.84	1.07	0.86	2.28
Nilotica	0.16	0.12	0.09	0.18
Sieberiana	0.3	0.37	1.01	1.44
Silage	-	-	1.13	1.44
SED	0.114		0.316	
Feed type	***		**	
Anim species	NS		***	
Spec x Feed	NS		NS	
Animal weight	NS		**	

*** significance at $p < 0.0001$; ** $p < 0.01$; NS, non-significant

Unlike in experiment 1, the effect of animal species and weight significantly ($p<0.01$) influenced palatability. Pi was also highly affected ($p<0.0001$) by the type of feed offered.

Figure 7.2 Daily variation in palatability index (Pi) for sheep and goats in experiments 1 and 2

Expt 1 **Goats** **Sheep**
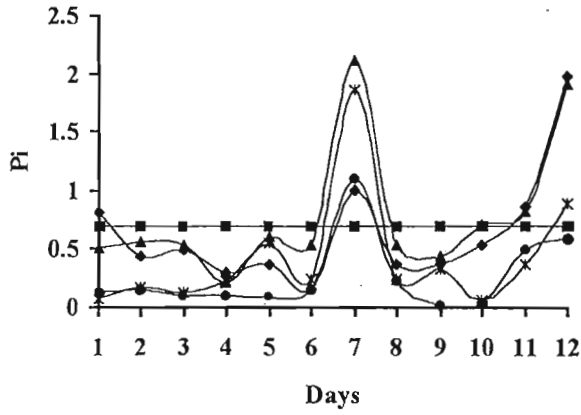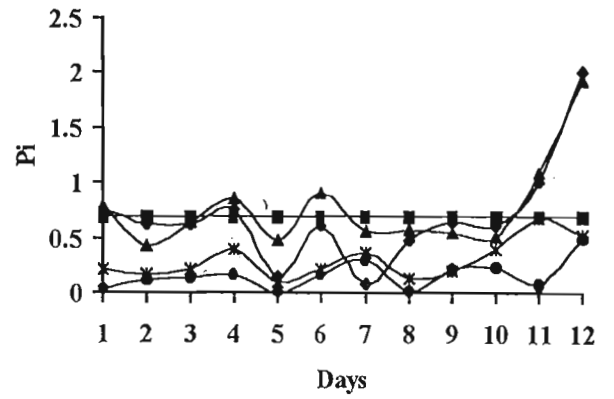
Expt 2
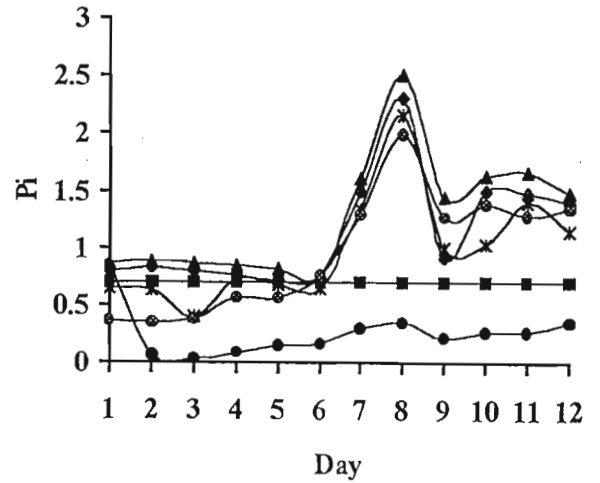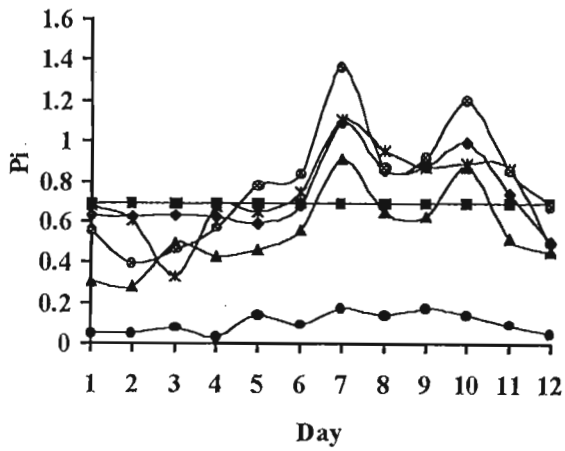
7.5 DISCUSSION

Intake

The two experiments show that apart from the hay, the intake of most of the feeds had a decreasing tendency during the first 3 days beyond which the pattern changed and the intake of some e.g. alfalfa and leucaena (experiment1) and alfalfa, leucaena, sieberiana and silage (experiment 2) began to increase rapidly. The intake of nilotica did not improve throughout the experimental period. This pattern of intake was explained by Provenza *et al.* (1995) when they reported that ruminants prefer familiar to novel foods and that they sample novel foods with much caution. The higher values of total intake noticed in experiment 2 might have resulted from the fact that the animals in this experiment were heavier. However, body weight did not influence intake between the two animal species in both experiments. It is also possible that the period during which the experiments took place (end of winter for experiment 1 and summer for experiment 2) could have influence the intake of the various feeds.

The wide differences in intake values observed among feeds is proof of the fact animals have different preferences for feeds that are offered simultaneously.

Although the effect of species on intake was not significant, it is clear from Figure 7.1 that while goats consumed more hay, sheep tended to cherish leucaena in both experiments. The similarities in intake pattern between the two species might have risen because of the fluctuations in the daily intake of each feed. The high consumption of hay in both experiments may be associated to the familiarity of this feed to both species of animals and not the chemical composition. Although the DM intake of roughages has been reported to be inversely proportional to the filling capacity (Van Soest, 1965;

Campling, 1970; Forbes, 1995) and NDF concentration (Waldo, 1986), this does not seem to be the case when ruminants are offered more than two feeds that vary in their chemical composition as was the case in this study. The hay had the highest NDF concentration but was consumed in higher amounts than alfalfa and the pod meals which had lower NDF concentrations and higher digestibilities (Ngwa *et al.*, 2001). These observations confirm other reports that ruminants acquire strong preferences for foods consumed early in life, and prefer those foods as adults, whereas they are reluctant to eat food that they have not experienced (Distel and Provenza, 1991; Ramos and Tennessen, 1992; Walker *et al.*, 1992). Burritt and Provenza (1996) added that the situation gets even worse when novel foods are offered in novel environments.

The low intakes of the *Acacia* pods may also be attributed to the high level of secondary metabolites such as tannins (as seen in Table 1), volatile oils and alkaloids said to be present in this class of feeds (Malechek and Provenza, 1983; Mueller Harvey *et al.*, 1987; Reed, 1995). Terblanche *et al.* (1967) noticed that goats consuming high quantities of the pods of *A. nilotica* suffered from globinaemia, abortion, dysnoa, tarchycardia, ruminal atony, hyperglycaemia, liver and kidney disruptions. The low intake of this particular feed by both species in the two experiments is an indication that these metabolites could be producing a taste or smell which is not cherished by the animals. High tannin concentrations have also been reported to reduce the intake of legumes by decreasing palatability (Barry and Duncan, 1984). This may explain why leucaena with a moderate tannin concentration was more cherished among the pod meals.

The pods of A. sieberiana contain in addition to high levels of tannins, cyanogenic glycosides (Steyn and Remington, 1935; Seigler et al., 1975) which release hydrogen cyanide (HCN) when degraded by rumen microorganisms (Majak and Cheng, 1984, 1987). The released HCN is absorbed directly into the blood stream (Kingsbury, 1964) where it inhibits cytochrome oxidase of the mitochondrial respiratory system, with which it forms a stable complex thus causing asphyxiation at cellular level and death (Kingsbury, 1964; Boundoux et al., 1980). In an earlier study Ngwa et al. (in press) found that ensiling the ground pods of A. sieberiana for a period of 35 days reduced the cyanide content of the pods from 130 to 18 mg/kgDM. The present study examined whether ensilage affects the palatability and/or intake of the pods of A. sieberiana. The results show that while the palatability of the silage was higher than that of the dry pods in the case of goats, sheep had similar palatabilities for the two feeds (dry pods and silage). It should, however, be mentioned here that the low DM intake values of the silage as compared to the parent material or the other feeds was due mainly to the high moisture content of the silage and not the amount of feed consumed per se.

It was also observed that there were increments in intake for most feeds during the course of the two experiments (figure 7.1). This tendency may be explained by an adaptation effect of the animals. It may also explain why the intake of highly palatable species like leucaena (Bonsi et al., 1994; Kaitho et al., 1996) was erratic at the beginning of each experiment, before taking an upward trend. The fluctuations noticed in Figure 7.1 may be due to the fact that the intake of nutritious foods containing toxins is often cyclical, with sharp declines, followed by gradual increases (Pfister et al., 1990). Thus when food ingestion is followed by an aversive feedback, the aversion to the food

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diminishes with time because the recuperative process gradually counter-conditions the aversion (Garcia, 1989). Early and Provenza (1998) reported that eating even a nutritively balanced diet to satiety may equally result to a mild aversion and transient decrease in preference. Finally, intake is closely related to the amount of feed offered (Osafo et al., 1992). This was illustrated in experiment 2 (Figure 7.1) where the intake of some of the feeds increased dramatically when the amounts of feed offered were increased from 300 to 500 g/animal/day on day 7.

Relative palatability

Relative palatability is defined as a plant characteristic eliciting a proportional choice among two or more feeds conditioned by plant, animal and environmental factors which stimulate a selective intake response by the animal (Marten, 1978). This characteristic may also be described in terms of acceptability, preference, selective grazing and relish, conditioned by a sensory impulse. However, in the present study, acceptability may not be a proper synonym because it represents both palatability and intake. While palatability might at times influence voluntary intake (units of feed consumed per unit time per animal), relative palatability is considered here as a measure of selection of one feed relative to a standard feed at a given period of time. The ratio including the standard feed (hay) seems to be a suitable index for the palatability ranking of the pod meals. The choice of such an index was justified by the necessity of a roughage source in the diets.

The low palatability indices noticed for most of the feeds at the beginning of each experiment (Figure 7. 2) point to the fact that if palatability is measured, especially for

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novel foods, on the first day that the feeds are offered, the results may be misleading. Ben Salem *et al.* (1994) and Kaitho *et al.* (1996) reported that periods of less than 5 days led to palatability results which were highly variable while 5 to 12 days gave reliable results with r^2 values of up to 0.99. In experiment 1, the relative palatabilities of alfalfa and the pod meals were generally lower when compared to the standard feed, both for goats and sheep but in experiment 2, the situation was slightly different. While the sheep demonstrated a particular relish for leucaena as shown by the high Pi, the goats rather portrayed a taste which cut across all the feeds except *A. nilotica*. However, the silage had the highest Pi for goats but scored third in decreasing order for sheep. This is an indication that besides the benefit of detoxifying cyanogenic glycosides, ensiling might have enhanced the palatability of this feed.

The sharp increases in Pi on day 7 in experiment 1 (Figure 7. 2) did not reflect an increase in palatability of the alfalfa and pod meals but rather due to a very small amount of hay consumed by one of the goats. This led to a sharp drop in the denominator of the formula stated in the section on materials and methods and an increase in Pi values. In experiment 2, the increase in the feed offered from 300 to 500 g on day 7 led to an increase in intake of the other feeds (except nilotica) and thus an increase in Pi values at the expense of the standard meal (hay). As already explained above, the cyclical pattern of Pi in the subsequent days could be explained by the post-ingestive feed back mechanism described by Pfister *et al.* (1990) and Provenza (1995) for feeds that contain toxins.

Contrary to what was observed in experiment 1, the pods of A. *sieberiana* had a higher palatability index in the second experiment and appeared to have been quite cherished by both goats and sheep. This points to the fact that diet selection by ruminants does not seem to follow an optimal foraging strategy, an assertion supported by other authors (Kenny and Black, 1984; Cropper, 1987; Newman *et al.*, 1992). Numerous associations reported between plant characteristics and plant palatability to ruminant animals have proven to be situation-specific, making them worthless as general selection criteria. Among these are concentrations of sugars or soluble carbohydrates, protein or nitrogen, fibre or cell wall constituents, ether extracts, individual minerals or total ash, carotene, vitamins, organic acids, tannins and silica (Marten, 1969). The same author (1978) reported that the relative palatability of any feed depends on the nature of the associated feeds on offer.

It is therefore possible that the effects of toxins in the pods of A. *sieberiana* could have been minimized by the ingestion of the other feeds. However, this did not seem to be the case with nilotica that maintained a very low Pi for both goats and sheep throughout the study. This suggests that taste, smell or feel may be playing an important role in determining the palatability of each feed. The sniffing reaction of the animals at each attempt to consume the nilotica meal could be related directly to taste or smell. Arnold (1966a, 1966b) reported that taste, smell, feel and sight all contributed to forage selectivity and went further to demonstrate that the surgical impairment of the sense of taste increased the scope of selection. The resentful taste and/or smell exhibited by nilotica may be due to specific metabolites or phenolic compounds that require further investigation. Its however worth mentioning that some foods which are least preferred

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in times of abundance and variety could be relished during periods of scarcity and severe feed shortages, based on animal survival instinct.

Effect of supplementing veld hay with a dry meal or silage from pods of *Acacia sieberiana* with or without wheat bran on voluntary intake, digestibility, excretion of purine derivatives, nitrogen utilization and weight gain in South African Merino sheep

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8.1 Abstract

The study evaluated the synergism between wheat bran and tanniferous feeds (dry meal or silage from pods of *Acacia sieberiana*) on intake and digestibility of various nutrients as well as weight gain in sheep fed a basal diet of veld hay (*Cynodon dactylon*). Dietary treatments comprised veld hay given *ad libitum* and supplemented with: 270 g of wheat bran (WB) alone (T_1), 203 g of WB plus 83 g of silage(T_2), 135 g of WB plus 165 g of silage (T_3), 330 g silage without WB (T_4) or 306 g of dry pods without WB (T_5). The silage was prepared by mixing ground pods with water in a ratio of 40:60 (weight of pod:weight of water) and ensiling was done in 200-litre drums for 35-45 days. Thirty young South African Merino sheep (15 males and 15 females), averaging 24 kg liveweight and 8 months of age, were blocked by sex and weight and within block, randomly distributed to the dietary treatments. A growth study was followed by a metabolism phase. All animals were slaughtered at the end of the trial to evaluate the weight of the dressed carcasses and some body organs. Faecal samples were examined at the beginning and at the end of the trial in order to evaluate the effect of tannins on endoparasites. Dry matter intakes for treatments 1, 2, 3, 4 and 5 were 1017, 1087, 1169, 1238 and 1214 g, respectively. Average liveweight gains (ADG) were 97, 114, 114, 132 and 123 g in the same order. The addition of silage increased ($p<0.01$) hay and DM intake but had no effect ($p>0.05$) on liveweight gain and feed conversion efficiency. Increasing the levels of silage in the diet linearly ($p<0.01$) increased ADG. Sex did not influence intake or ADG. The digestibilities of DM, OM and detergent fibres as well as excretion of purine derivatives were similar ($p>0.05$) among treatments but the WB diet promoted higher N digestibility. The pod and silage diets increased ($p<0.01$) faecal N and lowered ($p<0.05$) urinary N excretion by sheep. N retention was similar among treatments. Carcass, liver and kidney weights were similar ($p>0.05$) among treatments and the organs had no lesions. The results show that combining WB and silage did not show significant synergism but condensed tannins present in the silage and pod supplements produced beneficial effects probably by forming tannin-protein

complexes which by-passed the rumen and were digested at the lower segments of the gastro-intestinal tract. The results of the study have shown that the pods of *Acacia sieberiana* can constitute an important source of nitrogen to livestock and the liveweight gains are indications that if legume pods are well managed, they can reduce weight loss in animals grazing low quality rangelands and enhance production.

Key words: *Acacia sieberiana*, silage, intake, digestibility, tannins, beneficial effects

8.2 INTRODUCTION

The demand for animal protein in the diet of the rapidly growing human population of Sub-Saharan Africa emphasizes the need to improve on the productivity of ruminant livestock in this region. This can only be achieved through a proper management of renewable resources that are not used by non-ruminants and humans. There is, therefore, a need to research on cheap alternative feed resources which can sustain and increase ruminant production on rangelands not suitable for crop production. Pastures from such rangelands are deficient in nitrogen, energy and some minerals ('tMannetje, 1982; Kaitho, 1997) and thus cannot adequately support ruminant production. Browse plants such as the *Acacia* species which are widely distributed in the region, produce fruits (pods) which contain substantial amounts of these nutrients and can serve as supplements to poor quality pastures.

Acacia sieberiana is one of the species that produce enormous amounts of pods and in a good fruiting season, a yield of 100-200 kg of pods per tree is common. The pods (entire fruit), seeds and husks have crude protein contents of 179, 330 and 148 g/kgDM,

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respectively, and the pods were found to be moderately rich in minerals and amino acids (Ngwa et al., 2002). The nutritive value of these pods is, however, limited by the presence of anti-nutritional factors such as tannins (Tanner et al., 1990; Nsahlai et al., 1995b, Ngwa et al., 2001), cyanogenic glycosides (Steyn and Remington, 1935; Steyn, 1943; Seigler et al., 1975) and fibre-bound nitrogen (NDF-N) which in some cases constitutes a high proportion of total nitrogen (N) that is not digested (Tanner et al., 1990; Ngwa et al., 2002). This phenomenon favours high faecal nitrogen and low urinary N excretion (Ebong, 1989; Woodward and Reed, 1989; Weigand, 1991). Therefore, in circumstances where animals are offered fruits from tanniferous trees as sole supplement, it is possible that the fruits may degrade only to a limited extent and low ammonia concentration may still limit microbial activity.

Current research suggests that extensive interactions occur between free condensed tannins and protein in the rumen which increase the digestion of protein post-ruminally (McNabb et al., 1996; Nsahlai et al., 1998c). Supplementation with a protein source and tanniferous feeds in combination may enhance the formation of complexes between tannins and dietary protein possibly sparing microbial enzymes and increasing rumen degradability of the protein. It is, however, not certain whether this interaction could be harnessed to benefit the productivity of the ruminant livestock. This study was therefore, conducted to examine the effects of feeding dry pods of Acacia sieberiana as sole supplement and silage from this material fed in combination with wheat bran, on intake, digestibility, nitrogen utilization and growth of sheep fed poor quality hay. The combination of wheat bran and ensiled pod meal was offered with the hypothesis that feeding mixtures of these supplements could reduce rumen degradability of the dietary

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protein without depressing the rumen digestibility of the basal roughage diet. The purpose of introducing the non-ensiled material was to determine if reduced content of cyanogenic glycosides in the pods of *Acacia sieberiana* can be associated with improved animal performance.

8.3 MATERIALS AND METHODS

Feeds

Pods containing seeds from *A. sieberiana* trees were harvested just before winter fall when most of the pods were dry, at the outskirts of Pietermaritzburg, South Africa (22-34°S, 14-32°E). Harvesting was facilitated by using a metal sisal fitted on to the end of a long (6m) stick. After harvest, pods were sun-dried for two days before milling. The silage was prepared by mixing ground pods of *A. sieberiana* with water at a ratio of 40:60 (w:w) and the resulting mixture was put into polythene bags in amounts of about 15 kg per bag. The bags were well tied to ensure maximum exclusion of air, placed in 200-litre drums in which the bottom and top layers were covered with saw dust and ensiled for 35-45 days. Silages were prepared at 10-day intervals to ensure that at feeding, they had not exceeded 35-45 days in the silos. Only one bag was removed at a time for feeding animals.

Animals, diets and experimental design

Thirty young lambs of the South African Merino breed (15 males and 15 females), housed in a roofed shed, were blocked according to sex and initial liveweight and then within block, randomly assigned to five dietary treatments. Each group of six animals was offered a basal diet of pasture hay (*Cynodon dactylon*) served *ad libitum* and a

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supplement in which silage from ground pods of *A. sieberiana* replaced wheat bran at the rate of 0, 25, 50 and 100 percent, respectively. The fifth supplement was comprised of ground non-ensiled pods. The supplements were formulated such that they had the same effective rumen degradable protein (ERDP) as seen in Table 8.1. The animals were weighed before commencing a 14-day period of adaptation to the experimental diets. The supplements and hay were weighed daily and offered to the animals in individual feeding pens at 08.00h and 10.00h, respectively. Refusals of the previous day were collected each morning, pooled over a period of one week and then weighed. The pens were also equipped with automatic water troughs. The animals were weighed every fortnight throughout the trial period of 56 days.

Following the growth trial, 3 lambs (males) from each treatment were placed in metabolic crates. Feeding was done as in the preceding growth trial. The animals were allowed to adapt in their crates for a period of 3 days, following which total faeces and urine were collected for 7 days. The daily output of faeces by each sheep was recorded and 10% was sub-sampled, pooled on animal basis and frozen pending chemical analysis. The urine was collected in plastic buckets containing a solution of 100 ml of 10% H_2SO_4 to prevent ammonia-N loss. The daily output from each animal was weighed and 10% sub-sampled, pooled on animal basis and stored at 4°C until required for the analysis of purine derivatives (PD) and nitrogen. The PD of microbial origin (x; mmol) was estimated according to Chen *et al.* (1990):

PD = $0.84x + Ce^{-25}$, where PD (mmol) is the total urinary PD, C = 0.150 $BW^{0.75}$, the metabolic correction factor that estimates the endogenous losses and BW is the liveweight of the lamb.

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Table 8.1 Composition of diets (dry matter basis)

Feed	Diets				
	1	2	3	4	5
Wheat bran (g/day)	270	202.5	135	0	0
Silage (g/day)	0	82.5	165	330	0
Dry pods (g/day)	0	0	0	0	305.5
Hay	ad lib	ad lib	ad lib	ad lib	ad lib
ERDP (g/day)	37	37	37	37	37

ERDP, effective rumen degradable protein of the supplemental portion

Rectal faecal samples were taken from all animals at the beginning of the study to evaluate the degree of infestation with endoparasites as indexed by egg count. All animals were treated with Ivomec after rectal faecal sampling. At the end of the growth trial (before animals were taken to metabolic crates), another set of rectal faecal samples was taken for egg count. At the end of the metabolic trial, the animals were all slaughtered. The dressed carcass, liver and kidneys of each animal were weighed. The organs were also examined for lesions by visual appreciation, taking into account the degree of paleness, tissue erosion and ratio of organ to carcass weight.

In sacco degradation

The degradability of the feeds was determined prior to the growth study. Two adult Jersey cows (about 400 kg liveweight), fitted with ruminal cannulae (120 mm id) were kept in a roofed shed with a concrete floor and fed *ad libitum* on pasture hay (*Themeda triandra*), supplemented with 2 kg of cotton seed cake per animal each day. The supplement was given at 08.00h in the morning and the animals had free access to water. A two-week period of adaptation was allowed prior to incubation. About 4 g of

each ration ingredient was weighed into nylon bags (ANKOM Co, Fairport, New York, USA; internal dimensions: 5cm x 9cm; pore size 50μm). The silage was weighed fresh. The bags were incubated in the rumen of the cows in duplicates following the method described by Mehrez and Orskov (1977). Bags were added sequentially and were incubated for 120, 96, 72, 48, 24, 12, 6 and 3 h. After removal from the rumen, the bags, including the zero-hour ones which had not been incubated, were washed in a semi-automatic washing machine (Hoovermatic) six times in cycles of 5 minutes. The washed bags were then dried in a force draught oven at 60 °C for 48 hours, cooled in a desiccator and weighed. The residues were subsequently analysed for nitrogen.

Chemical analysis

Faecal samples were dried to constant weight and like the feed samples were ground to pass through a 1-mm screen for chemical analysis. Standard AOAC (1990) procedures were used to determine the dry matter (DM), organic matter (OM), N, and the detergent fibres (NDF and ADF) by the method of Van Soest *et al.* (1991). The tannin content of the pods was determined by the method of Hagerman (1995). Urine samples were also analyzed for N.

Statistical analysis

The degradation of DM and N was estimated by fitting the non-linear model proposed by McDonald (1981) and modified by Dhanoa (1988) to the degradation data of each component:

$$Y = A + B[1-e^{-C(T-LT)}]$$

where Y is the disappearance of DM or N at time T, A = washing loss or solubility, B = degradable part of the insoluble fraction, C = rate of degradation of B and LT, the lag

time.The potential degradability (PD) was calculated as A + B. A passage rate (k) of 0.03/h was assumed (Bonsi *et al.*, 1994; Nsahlai *et al.*, 1998a) in order to calculate the effective degradabilities (ED) of DM:

ED = A + B x C /(k+C).

Weight gain for each group was determined by regression of liveweight on time and efficiency was calculated as weight gain/dry matter intake. Data from the growth and metabolism trial were analyzed using the analysis of variance technique (SAS, 1987) for a randomized complete block design and treatment effects were separated using the general linear model (GLM). The initial body weight was used as a co-variate for all variables. Contrasts among treatments were done by applying the probability of difference (PDIFF) option of the least square means (LSMEANS) statement available in the GLM. The treatment sums of squares were further partitioned into linear and quadratic orthogonal contrasts by applying the contrast statement of the GLM.

8.4 RESULTS

Chemical and degradation properties of dietary ingredients

The dietary ingredients are presented in Table 8.1 while their chemical and degradation properties are presented in Table 8.2. Among the supplementary feeds, the pods of *A. sieberiana* had the highest crude protein content, followed by wheat bran (WB) and silage in decreasing order but the differences were not significant. Silage had higher NDF and ADF concentrations than pods and WB. The ash content of WB was higher than the values for silage and pods which were similar. The results of the analysis of condensed tannins were expressed in percentage Quebracho equivalents (QE) (Hagerman, 1995) and the value for the dry pods was 28.3.

Table 8.2 Chemical and degradation properties of dietary ingredients

Parameter	Ration ingredients			
	Wheat bran	Dry pods	Silage	Hay
Chemical properties				
OM (g/kg DM)	923.7	945.8	944.9	952.4
GE (MJ/kgDM)	18.92	18.4	17.17	18.04
CP (g/kg DM)	167.1	174.9	161.3	56.4
NDF (g/kg DM)	387.7	366.9	449.8	702.6
ADF (g/kg DM)	121.2	290.1	346.7	449.4
HEM (g/kg DM)	266.5	76.8	103.1	253.2
CT (%QE)	-	28.3	-	-
Degradation properties				
A (g/kg DM)	406.8	374.1	198	73
DMD (g/kg DM)	715.7	521.7	460.7	291
ND (g/kg DM)	839	694	683	n.d
CDM (/h)	0.056	0.027	0.038	0.022
CN (/h)	0.084	0.033	0.03	n.d

OM, organic matter; GE, gross energy; CP, crude protein; NDF, neutral detergent fibre; ADF, acid detergent fibre; HEM, hemicellulose; CT, condensed tannins; QE quebracho equivalents; A, soluble fraction; DMD, dry matter degradability; ND, nitrogen degradability; CDM, rate of degradation of dry matter; CN, rate of degradation of nitrogen; nd, not determined.

WB dry matter (DM) was 1.5 and 1.4 times more degradable in the rumen than the DM of the pods and silage, respectively, while its nitrogen (N) was 1.2 times more degradable than that of the other two. The rate of degradation of total DM of WB was 1.4 and 2 times higher than the rates for silage and pods, respectively.

Growth Study

All the animals remained in good health throughout the experimental period and the

supplements offered were entirely consumed. The average hay intake, DM intake, average daily weight gain (ADG), efficiency of feed conversion (E) and faecal egg count are presented in Table 8.3. Animals that received WB alone as supplement (Treatment 1) consumed lower amounts of hay and total DM than those of the other four treatments. Hay and DM intake increased linearly ($p < 0.01$) as the proportion of silage in the diet increased. ADG was similar ($p > 0.05$) among all dietary treatments but increased linearly ($p < 0.05$) as the level of silage increased in the diets. The efficiency of feed conversion was similar ($p > 0.05$) among treatments. The final weights (FWT) of the animals were influenced ($p < 0.001$) by the initial weights (IWT) at the start of the experiment. Ewe lambs were lighter ($p < 0.01$) in weight than the male lambs at the start (23.4 vs 24.6 kg) but the difference leveled out (30.6 vs 30.8 kg) at the end of the experiment. DM intake, ADG and E were however, not influenced ($p > 0.05$) by sex. Neither intake nor weight gain was affected by endoparasitic burden but the initial egg count significantly ($p < 0.001$) influenced the final egg count. Ensilage (Treatment 4 vs Treatment 5) did not influence ($p > 0.05$) DM intake, ADG, E nor worm burden.

Metabolism study

The intake and digestibility of various feed components during the metabolism trial are presented in Table 8.4. Like in the growth trial, animals that received WB alone as supplement consumed lower amounts of hay and total DM than those of the other four treatments. The addition of silage increased DM intake linearly ($p < 0.01$). This trend was also reflected in the intake of OM, N, NDF and ADF. There were no significant

Table 8.3 The effect of diet on intake and weight gain of South African Merino sheep fed pasture hay supplemented with wheat bran and/or silage or dry pods of *Acacia sieberiana*

Parameter	Diet					SED	Diet	Silage		IWT	Sex	Eggs	4 vs 5
	1	2	3	4	5			Linear	Quad				
WB:SIL R	4:0	3:1	1:1	0:4	NEP	n = 6							
Hay (g/day)	746.9[a]	801.9[ab]	869.4[ab]	907.6[b]	908.7[b]	50.82	0.006	0.004	0.492	0.088	0.173	0.637	0.9836
DM (g/day)	1016.9[a]	1086.9[a]	1169.4[b]	1237.6[b]	1214.2[b]	50.82	0.002	0.002	0.633	0.088	0.173	0.637	0.6549
SWT (kg)	24.4	24.5	24.6	23.3	23.4								
FWT (kg)	29.6	31.9	31.2	30.8	30.8	0.88	0.447	0.151	0.170	0.001	0.740	0.605	0.9510
ADG (g/day)	96.9	113.9	114.1	131.5	123.3	13.80	0.199	0.025	0.838	0.363	0.360	0.765	0.5628
E (g gain/g feed)	0.097	0.105	0.099	0.106	0.101	0.0076	0.844	0.441	0.790	0.641	0.816	0.908	0.6457
EGGf (10^3)	8.3	7.9	7.4	8.6	8.1	0.54	0.318	0.776	0.030	0.497	0.204	0.001	0.3466

WB:SIL R, wheat bran: silage ratio: IWT, weight at adaptation; STW, weight after adaptation; FWT, final weight; ADG, average daily gain; DM, dry matter; E, efficiency of feed conversion; Quad, quadratic;Eggs, egg count at the beginning of adaptation; EGGf, egg count at the end of the experiment; NEP, non-ensiled pods

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Table 8.4 Feed intake and digestibility of DM, OM, N, NDF, ADF and HEM South African Merino sheep fed pasture hay supplemented with wheat bran and/or silage or dry pods of *Acacia sieberiana*

Constituent	Diet					SED	Significance (p - value)			
	1	2	3	4	5					
WB-SIL R	4: 0	0.125694	0.04236	0.0028	NEP	n = 3	Diet	Linear	Quadrat	4 vs 5
Intake (g/d)										
DM	956	1109	1129	1206	1149	68.5	0.042	0.002	0.339	0.419
DM (g/kgW$^{0.75}$)	80.1	80.9	84.1	97.4	90.3	6.56	0.115	0.039	0.247	0.306
OM	902	1050	1070	1146	1092	65.1	0.036	0.001	0.347	0.421
N	13.4	15	15.3	16.4	16.2	0.62	0.005	0.001	0.482	0.675
NDF	587	694	709	764	704	48.2	0.045	0.002	0.346	0.241
ADF	341	423	446	508	467	30.7	0.003	0.001	0.552	0.215
HEM	245	271	263	256	337	21.3	0.368	0.607	0.122	0.309
Digestibility										
DM	610	571	529	615	573	32.6	0.132	0.832	0.039	0.224
OM	625	582	541	625	583	31.9	0.117	0.721	0.035	0.221
N	612	543	462	503	513	33.7	0.013	0.007	0.058	0.975
NDF	572	511	468	573	506	35.4	0.055	0.755	0.017	0.087
ADF	588	516	459	544	487	37.9	0.051	0.199	0.031	0.166
HEM	550	503	483	631	542	33.8	0.013	0.089	0.006	0.024

WB:SIL R, wheat bran:silge ratio; Quatrat, quadratic; W, bodyweight; NEP, non-ensiled pods; DM,dry matter; OM, organic matter; N, nitrogen; ADF, acid detergent fibre; HEM, hemicellulose

Table 8. 5 Purine derivative (Allantoin and total uric acid) (PD) excretion, microbial purine derivative (MPD), microbial nitrogen supply (MN), faecal nitrogen

differences ($p>0.05$) among dietary treatments in the digestibilities of DM, OM, NDF and ADF but the WB supplemented diet promoted higher ($p<0.05$) digestibility of N while diet 4 promoted that of hemicellulose (HEM). The digestibility of all components (DM, OM, N, NDF, ADF and HEM) decreased quadratically ($p<0.05$) as the proportion of silage increased in the diet with diet 3 (equal proportions of WB and silage) having the lowest values. Apart from HEM whose digestibility was improved ($p<05$) by the process of ensilage, there were no differences ($p>0.05$) in the intake and digestibility of the other components (DM, OM, N, NDF AND ADF) when diet 4 was compared with diet 5.

Excretion of purine derivatives and microbial protein supply

The allantoin excreted in urine was similar ($p>0.05$) among treatments but total uric acid (uric acid + xanthine + hypoxanthine) differed ($p<05$) among dietary treatments with animals on diet 4 having the lowest value (Table 8.5). However, the sum of purine derivatives (allantoin + total uric acid) excreted per day did not vary among dietary treatments. Microbial purine derivatives (MPD), microbial nitrogen (MN), efficiency of microbial nitrogen synthesis per kg of organic matter digested in the rumen (MN/kgDOMR) as well as the efficiency of microbial supply per kg of digestible organic matter (MN/kgDOM) were similar ($p>0.05$) among dietary treatments. WB offered as sole supplement had the highest urinary nitrogen (UN) which decreased linearly with increasing level of silage in the diet. The opposite effect was observed in the excretion of faecal nitrogen (FN). Nitrogen retention was similar ($p>0.05$) among treatments. There were no differences ($p>0.05$) in the excretion of nitrogenous products, nitrogen supply and nitrogen retention between animals in treatments 4 and 5.

Table 8. 5 Purine derivative (Allantoin and total uric acid) (PD) excretion, microbial purine derivative (MPD), microbial nitrogen supply (MN), faecal nitrogen (FN) urinary nitrogen (UN) and nitrogen retention in South African Merino sheep fed pasture hay supplemented with wheat bran and/or silage or pods of *Acacia sieberiana*

Parameter	Diet						Significance			
	1	2	3	4	5	SED		*p*-value		
WB:SIL R	0.16667	0.125694	0.04236	0.00278	NEP	n = 3	Diet	Linear	Quadrat	4 vs 5
Allantoin (mmol/d)	9.19	8.51	9.44	8.96	8.9	0.92	0.222	0.082	0.234	0.952
Uric acid (mmol/d)	1.67	2.13	1.65	1.18	1.58	0.762	0.048	0.054	0.041	0.149
PD (mmol/d)	10.6	10.64	11.09	10.14	10.48	0.905	0.216	0.207	0.085	0.715
MPD (mmol/d)	10.38	12.57	13.1	11.96	12.37	1.105	0.215	0.204	0.085	0.715
MN (g/d)	7.55	9.14	9.53	8.69	8.99	0.803	0.215	0.204	0.085	0.716
MN/DOMR (g/kg)	18.02	20.11	22.47	16.25	18.95	2.997	0.367	0.785	0.11	0.389
MN/DOM (g/kg)	13.51	15.09	16.85	12.19	14.22	2.248	0.367	0.785	0.11	0.389
FN (g/d)	5.2	6.84	8.25	8.15	7.89	0.626	0.003	0.001	0.057	0.695
UN (g/d)	6.11	5.08	4.6	3.15	3.71	0.776	0.025	0.008	0.737	0.487
N retention	2.09	3.05	2.5	5.12	4.55	1.111	0.085	0.057	0.358	0.615

WB:SIL R, wheat silage ratio; PD, purine derivatives; MPD, microbial purine derivatives; MN, microbial nitrogen; DOMR, organic matter digested in the rumen; DOM, digestible organic matter; FN, faecal nitrogen; UN, urinary nitrogen; NEP, non-ensiled pods.
DOMR, organic matter digested in the rumen = 0.75 of digestible organic matter (Osuji et al., 1993, Nsahlai and Umunna, 1996)

Effect of diet and sex on carcass and organ weights

The effects of diet and sex on carcass and organ weights are shown on Table 8.6. Diet had no effect on the carcass, liver or kidney weights but the female carcasses were heavier ($p < 0.001$) than those of the males. The appearance of all organs was normal and there were no apparent lesions.

Table 8.6 The effect of diet and sex on carcass, liver and kidney weights of South Africa Merino sheep fed dry pods and silage from the pods of *Acacia sieberiana* with or without wheat bran

Parameter	Diet					SED	Effects (p-value)	
	1	2	3	4	5		diet	sex
Carcass (%LWT)	36.5	35.8	36.3	35.7	34.8	1.65	0.787	0
Liver (g/kgLWT)	14.8	13.7	14.3	14.5	13.7	1.15	0.277	0.97
Kidney (g/kgLWT)	2.5	2.3	2.3	2.3	2.2	0.17	0.277	0.878

8.5 DISCUSSION

A supplement should primarily increase critical nutrients lacking in the basal diet, and create an environment conducive to optimizing the release and utilization of other nutrients in the basal diet (McMeniman *et al.*, 1988). In this trial, it was however noticed that the WB elicited lower intake and digestibility of the basal diet (hay) than the silage and dry pods of *A. sieberiana*. This effect was also apparent during the metabolism trial. This is probably a consequence of depressed ruminal pH since WB undergoes rapid fermentation and contains a high level of soluble carbohydrates. The depression in intake is in line with other reports (Henning *et al.*, 1980; van der Linden *et al.*, 1984; Miller and Muntifering, 1985; Meissner *et al.*, 1991a, Osuji *et al.*, 1993; Nsahlai *et al.*,

1998b). Hoover (1986) reported that as little as 10-15% of readily fermentable carbohydrate (RFC) is enough to impair fibre digestion and that if the concentration of RFC increased to 30%, serious depressions in the intake of the basal diet would occur. Several theories have been advanced to explain the depressive effect of RFC on fibre digestion (Hoover, 1986; Osuji et al., 1993; Nsahlai and Umunna, 1996) but the following have received greater attention: a preference by rumen microbes for RFC rather than fibre components, a decrease in ruminal pH caused by rapid RFC and competition for essential nutrients resulting in preferential proliferation of RFC digesting microbes (Mould and Orskov, 1984; Hoover, 1986). In most studies (Stewart, 1977; Mould and Orskov, 1984; Mould et al., 1984), reduced pH has been shown to have a major impact on fibre digestion. Cheng et al. (1984) reported that low ruminal pH appeared to prevent a strong attachment of bacteria to plant cell walls, resulting to lower fibre digestion. This was also supported by Shriver et al. (1986) who reported that at pH 5.8, the quantity of microbes associated with fibre particles was reduced by 43% and NDF digestibility at this pH was 8.1% compared to an average of 32.5% at a higher pH. A fall in pH below 6.0 resulted in a precipitous loss of fibrolytic activity and complete cessation of fibre digestion between 4.5 and 5.0 (Simpson et al., 1977; Stewart, 1977; Hoover et al., 1984) in view of reduced growth of several species of ruminal bacteria (Russel et al., 1979) and to a flush out of cellulolytic microbes from continuous cultures (Russel and Dombrowski, 1980).

The high intake and digestibility of DM and OM by animals consuming diets 4 and 5 are in line with other reports (McMeniman et al., 1988; Bonsi et al., 1994; Nsahlai et al., 1998b) that supplementation of roughage-based diets with various types of legumes

increases the rate and extent of DM degradation of the basal diet. These increases in degradation are associated with increased rate of passage (Umunna et al., 1996) and thus, of DM disappearance from the rumen. Since dietary supplements were made to provide the same amount of rumen degradable nitrogen, the positive linear effects of silage on basal roughage intake may be attributed to improved fibrolytic activity.

This interpretation may seem to conflict with reports that supplementation of roughage-based diets with tanniferrous feeds depresses microbial activity in the rumen (Lohan et al., 1981; Reed et al,. 1990; Tanner et al., 1990; Makker et al., 1993; Meissner et al., 1993; Jones et al., 1994; Nsahlai et al., 1994). The nutritional effects of tannins are associated with their ability to bind with proteins (dietary and enzymes), structural carbohydrate polymers found in plant cell walls and minerals with an overall effect of lowering the bioavailability of nutrients at specific sites in the gastro-intestinal tract (Ndlovu, 2000). Other workers (Barry et al., 1986; Waghorn, 1990; Lee et al., 1992; Waghorn and Shelton, 1992; Wang et al., 1994) have, however, reported that at low concentrations tannins are beneficial to ruminants because they protect plant proteins from degradation in the rumen and increase the quantity of dietary protein reaching the lower gastro-intestinal tract (GIT). At low concentrations, condensed tannins bind with plant protein at near neutral pH in the mouth and rumen to form tannin-protein complexes which are stable and insoluble at pH 3.5-7.0, but dissociate and release protein at pH<3.5 in the abomasum with little effect on DM, OM and fibre apparent digestibility (Jones and Mangan, 1977; Wang et al., 1996). The latter also reported that feed conversion efficiency was higher for sheep that were fed a tanniferous legume (Lotus penduncalatus) than for those that were fed lucerne. A similar observation was

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noticed in this study although differences among treatments were not significant.

The positive linear response of ADG to incremental levels of dietary silage is similar to observations reported by Kibon and Maina (1993) when ground pods of A. sieberiana replaced 0-45% of maize offal in the diet of sheep, and the authors interpreted this as an increase in digestible crude protein. This is an indication that the binding effect of tannins produced a beneficial effect by supplying protein to the lower GIT which is digested by gastric enzymes to form amino acids which are absorbed and used for tissue growth. Studies with Lotus penduncalatus (McNabb et al., 1993; Wang et al., 1994) showed that condensed tannins reduce the degradation of sulphur amino acids in the rumen, increased the irreversible loss of cystine from plasma and increased the flow of cystine to body synthetic reactions.

Correlations between the concentration of tannin fractions in the plant and reductions in digestibility are varied (Khazaal and Orskov, 1994; Khazaal et al., 1994; Balogun et al., 1998), thus tannin concentration is probably unreliable in predicting effects on nutritive value. This probably explains the quadratic effects noticed in the digestibilities of DM, OM, N and fibre fractions. The addition of tannin-binding compounds such as polyethylene glycol (PEG) and polyvinyl pyrrolidon (PVP) may provide a better measure of the effects of tannins on the digestibility of nutrients. Unfortunately this was not incorporated in this study. A report by McNeill et al. (1999) indicated that dietary protein complexed with tannin was made available in the abomasum and digested in the intestines but tannin released from the protein-tannin complexes may react with non-dietary protein (including digestive enzymes) as it passes along the intestines thus

counteracting the benefits of by-pass dietary protein. This may explain the slight decreases in the digestibility of dietary components when the level of silage increased although the trend changed when WB was completely replaced by silage in the diets. However, linear increase in ADG as levels of silage increased is probably an indication that the effect of by-pass protein was stronger than the binding of non-dietary protein to tannins liberated in the abomasum. The slower rate of degradation of protein from the silage and pod supplements in the rumen compared to WB may also be a desirable attribute since excessive fermentation of protein would be reduced while adequate amounts of ammonia resulting from the degraded portion would be available for growth of cellulolytic bacteria in the rumen.

Some researchers (Waghorn *et al.*, 1994b; Niezen *et al.*, 1995) have reported that tannins have nematocidal properties and sheep consuming tanniferous plants (*Hedysarum coronarium*) or sulla were found to have fewer egg counts than those that fed on lucerne (*Medicago sativa*). On the contrary, egg counts obtained in this study show that tannins did not have any effect on nematode infestation. This may probably be the results of the fact that effects of tannins depend on the type, concentration and reactivity.

In an earlier study, ensiling ground pods of *A. sieberiana* reduced the concentration of cyanogenic glycosides (CG) in the pods. Comparison between diet 4 (silage alone) and diet 5 (non-ensiled pods) was intended to evaluate the effect of detoxification of dry pods on ADG of the animals. In addition, examination of internal organs (liver and kidneys) that detoxify toxic substances in the body did not reveal any signs of

inflamation or lesions in the animals supplemented with pods. The reasons may be two-fold; the quantity of pods (0.3 kg/animal/day) was not high enough to provoke toxicity problems and/or the feeding period was not long enough to produce significant effects. Furthermore, the toxic effects of CG are greater when animals are starved prior to the consumption of feeds containing CG (Majak et al., 1990).

Total urinary purine derivatives (allantoin, uric acid, xanthine and hypoxanthine) were used to estimate microbial biomass supply (Rys et al., 1975; Zinn and Owens, 1986; Chen et al., 1990; Chen et al., 1992) and hence protein supply. The values obtained in this study are similar to those reported by Nsahlai and Umunna (1996) and Bonsi and Osuji (1997) when sheep feeding on teff (Eragrostis tef) straw were supplemented with a mixed ration of crushed maize grain and legume forages. Increasing the levels of silage in the diet had no significant effects on the urinary excretion of purine derivatives or microbial PDs, consequently, microbial protein synthesis was not influenced by diet. However, the yields of 16 to 22g of microbial nitrogen (MN) per kg of organic matter apparently digested in the rumen (DOMR) fall within the desired range of 14 to 49g per kg DOMR reported by ARC (1984). Though the efficiency of microbial N supply was expected to increase with increased DM intake (Chen et al., 1992) it was not the case in this study.

The increases in faecal nitrogen (FN) and corresponding decreases in urinary nitrogen as the level of silage increased in the diets accords with the observation that tannins favour high FN and low UN excretion (Ebong, 1989; Woodward and Reed, 1989; Nsahlai et al., 1995a; Reed, 1995). The high excretion of UN by animals in treatment

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1 could be attributed to the rapid degradation of WB in the rumen (Nsahlai *et al.*, 1998b). However, the positive values seen for N retention in all dietary treatments indicate that supplementary feeds met the animals' needs for maintenance.

8.6 CONCLUSION

The results of this study have shown that pods from tree legumes can constitute an important source of nitrogen to livestock consuming low quality forage diets. The liveweight gains are indications that if legume pods are well managed, they can reduce weight loss in animals grazing low quality rangelands and enhance production. This is extremely important for low-resource farmers who cannot afford the purchase of conventional protein supplements like the oil seed cakes. The capacity of the legume supplements to produce an ammonia-rich rumen environment and by-pass protein that provides amino acids to the lower GIT gives an additional advantage to this type of feed. Supplementation with either silage or dry pods increased DM and OM intake and promoted higher weight gains than the WB supplement. The major difference between the two types of supplements lies in their degradabilities in the rumen. The high degradation of WB caused a drop in rumen pH and consequently depressed the digestibility of fibre constituents and ruminal undegradable protein. In this regards, the pod meals had an edge over WB as supplements to the roughage diet. The presence of cyanogenic glycosides in the pods did not provoke toxic effects probably because of their low concentrations and the failure of tannins in the pod to show any nematocidal effects may be attributed to the same reason.

GENERAL DISCUSSION

9.1 Summary

The problems encountered in ruminant livestock feeding in the tropics were highlighted at the beginning of this work. These include: the seasonal availability of pastures, low intake and digestibility due to the fibrous nature of forage from pastures and crop residues, high stock density relative to available forage resources, impracticability of chemical methods to delignify low quality roughages, high cost and non-availability of conventional protein supplements in developing countries. More than 340 million tonnes of fibrous crop residues are produced in Africa per year (Kossila, 1984), the great majority of which are from cereals. The utilization of these low quality roughages (defined as forages which are less than 55% digestible, having less than 8% crude protein and low in soluble sugars) (Leng, 1990) as sole feed by ruminants is limited because they are high in ligno-cellulose compounds and low in nitrogen and thus do not meet the maintenance requirements of ruminants, let alone those for production, due to their low protein content (Wilman *et al.*, 1999). In this work, forage from tree legumes (with particular emphasis on pods) was proposed as a source of protein supplement to poor quality roughage diets especially for low income-farmers. The series of experiments reported in this thesis were aimed at examining the potential of pods from tree legumes as protein supplement to roughage-based diets for ruminants.

Many authors have reported on browse as an important source of protein and minerals for ruminant animals (Jones, 1979; Le Houerou, 1980; Brewbaker, 1986; Ahn, 1990,

Abdulrazak *et al.*, 1997) but the information on pods is rather scanty. The first experiment was therefore designed to answer questions on the chemical, mineral and amino acid compositions of pods from six (*A. erioloba, A. karoo, A. nilotica, A. sieberiana, A. tortilis* and *L. leucocehpha*) tree legumes. To better appreciate the contribution of these pods to effective rumen degradable protein (ERDP) and subsequently to microbial protein (MCP), the degradabilities of the pods were determined and on the basis of the information obtained, metabolisable protein (MP) was calculated (AFRC, 1992). All six species differed in their chemical compositions and this highly influenced their degradabilities in the rumen. Pods of *A. nilotica* and *A. sieberiana* had the highest DM and N degradabilities but were very rich in condensed tannins. The Leucaena pods were richer in amino acids than the Acacia pods and had the lowest tannin contents.

Tannins have been reported to affect the growth and/or activity of rumen microbes as well as post-ruminal digestion (Barry and Manley, 1984; Reed *et al.*, 1990; Waghorn *et al.*, 1994a,b). In this regards, experiments 2 and 3 were aimed at examining the effects of tannins on rumen dynamics (pH, enzyme activity, fibre degradation, concentrations of ammonia and VFAs) in the rumen of South African Merino sheep fed three pod diets. The fourth experiment further examined the effects of tannins on post-ruminal digestion by feeding pod meals as supplements to a roughage-based diet. Focus was concentrated on roughage intake, fibre digestion, nitrogen retention, microbial synthesis and growth response of South African Merino lambs. At high levels (50 % of DMI), the pod diets depressed microbial enzyme activity and consequently limited fibre fermentation and production of VFAs. At low concentrations (< 30 % of DMI), the diets

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promoted higher intake of the roughage basal diet and weight gain in lambs.

Apart from high concentrations of tannins, the pods of A. *sieberiana* have been reported to contain high levels of cyanogenic glycoside (CG) (Seigler *et al.*, 1975), a cyanide precursor which has been reported to cause mortalities in livestock (Steyn and Remington, 1935; Coop and Blaney, 1950; Vogel *et al.*, 1987). The fifth experiment was thus designed to evaluate ensilage as a means of reducing the concentration of CG in the pods. The method was found to be effective in reducing the concentration of this compound by 80%. Unfortunately the ensuing silage was aerobically unstable, most probably because of poor fermentation since the parent material (dry pods) contained a very low level of soluble carbohydrates that are transformed to lactic acid which stabilizes the silage at a near pH of 4. To ensure stability, another study evaluated the potential of molasses and urea as preservatives. The latter proved to be effective in stabilizing the silage and in addition, improved the nutritive value of the silage.

Previous studies have shown that ANFs limit the palatabilty and/or intake of feeds (Barry and Duncan, 1984; Provenza and Balph, 1990; Provenza, 1995). Experiment 6 was designed to confirm this assertion using the silage produced in the previous experiment as an example. The latter was offered along side five other feeds (hay, alfalfa, dry pods of A. *sieberiana, A. nilotica* and L. *leucocephala*) to sheep and goats in a choice feeding trial, to examine palatability and intake. From the results obtained, one could conclude that ensilage effectively improved the palatability and intake of the pods. This was confirmed by a high relish for the silage by both sheep and goats.

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9.2 Mineral and chemical compositions of pods

9.2.1 Mineral concentration in pods

Low digestion of fibre and organic matter has been attributed to the deficiency of N, S and P (Durand and Komisarczuk, 1988). Insufficient Ca, P, K, S, Na, Cl, and Mg have been associated (to varying extents) to a limitation in microbial growth (Durand and Kawashima, 1980; Mackie and Therion, 1984; Petri et al., 1988; Leng, 1990) especially, P and S which are implicated in the metabolism of nucleic acids and amino acids respectively in microbes. A deficiency in Mg can reduce the digestibility and intake of forage (Wilson and Minson, 1980) because it is essential for all rumen microorganisms, particularly cellulolytic microbes (Pettipher and Latham, 1979). The calculated means of mineral concentrations in six pods (Chapter 3) show that apart from Ca whose content was very high in the pods, the contents of the other minerals (P, Mg, Na, Zn, Mn, Se) were about half of the corresponding values in cotton seed cake and soya meals (Table 9.1). However, considering a rumen digestibility of 0.5 (Table 3.5), the pods will be capable of supplying the mineral requirements of sheep and goats (especially in the major minerals) if the animal is fed 1.0 kg of pod meal per day. This is important for small livestock farmers who are unable to purchase mineral concentrates (licks) for their livestock, due to high cost. Deficit in Na which was particularly low in the pods can easily be covered by offering kitchen salt (NaCl) to animals, a practice which is common among livestock farmers.

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Table 9.1 Mineral content of pods compared to those of groundnut cake, cotton seed cake, soya bean cake and alfalfa, and daily requirements for sheep

Mineral	Pods *	Gnut cake	CSC	SB cake	Alfalfa	Mineral requirement	
						20 kgLWT	35 kgLWT
Ca (g)	7.6	2.9	1.9	3.5	21.9	0.7	0.9
P (g)	2.4	6.8	12.4	6.8	3.3	0.5	0.9
Mg (g)	2.3	1.7	5	3	2.7	0.38	0.62
Na (g)	0.5	0.8	0.6	0.4	2.1	0.57	0.99
Zn (mg)	28.6	22	79	61	-	40	40
Cu (mg)	7.1	17	16	25	11	3	3
Mn (mg)	16.9	29	25	32	41	40	40
Se (mg)	0.11	-	-	0.55	0.05	0.1	0.1

* values represent the mean of six pod types; Gnut, groundnut; CSC, cotton seed cake; SB, soya bean

9.2.2 Interrelationship between rumen digestion and chemical composition of pods

In chapter 3, an attempt was made to establish any form of interrelationship between chemical composition and the degradation of dry matter, nitrogen and cell wall constituents. The pods from the different tree species differed widely (402 to 550 g/kgDM) in the degradability of their respective dry matter in the rumen but the gap narrowed for the degradabilities of N and cell wall constituents. The data on degradability indicated possible tannin-induced as well as NDF-N and ADF-N bound depressions among the pods. Differences in solubility and potential degradability among the pods were primarily attributed to their NDF and N concentrations. Correlation analysis showed that NDF and ADF negatively ($r = -0.91$) affected DM degradation. This was in accordance with previous reports (Minson, 1982b; Nsahlai et al., 1994; Nsahlai et al., 1995b) on the negative effects of NDF and ADF on forage digestion. The strong

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relationship (r = 0.98 and r = 0.86) between rates and extents of degradation of DM and N respectively revealed the possibility of predicting N degradability using DM degradability characteristics.

9.3 Effects of supplementation with tannin-rich pods on fermentation dynamics in the rumen

9.3.1 Rumen pH

The rumen pH range of 6.2 to 6.4 observed after feeding pod diets (Chapter 4) supports a conducive rumen environment for fibrolysis. This is a major attribute of forage from tree legumes, an assertion supported by Bonsi (1996). Most supplements, especially grains and water soluble carbohydrates reduce the rumen pH below the cellulolytic threshold of 6.2 (Chamberlain *et al.*, 1985; Hutanen, 1988, Khalili, 1992). *In vitro* experiments have indicated a positive correlation between pH and cellulolysis (Stewart, 1977) and fibre digestion was reported to be depressed by a reduced pH (Mould and Orskov, 1984; Mould *et al.*, 1984). The depressions in the degradability of fibre fractions in this work (chapters 4 and 5) may not be linked to rumen pH since there were no significant differences in pH between the alfalfa and pod diets. As such, differences in the concentration of VFAs among diets were rather attributed to other factors (ED of feeds, energy deficiency and presence of tannins) and not pH.

9.3.2 Protease activity and rumen ammonia concentration

Microbial protease activity in the rumen results to the breakdown of protein to peptides and amino acids but some amino acids are further degraded to ammonia (Chapter 4), organic acids (Chapter 5) and CO_2. The ammonia produced, together with some small

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peptides and amino acids found in rumen liquor is utilized by rumen microbes to synthesize microbial protein (Nolan and Leng (1972). Since tannins have been accused of suppressing proteolysis either by the formation of tannin-protein complexes (Makker, 1993, Reed,1995; Ngwa et al., 2001)), precipitation of proteolases or reduction in enzyme activities due to changes in the morphology of microorganisms (Scalbert, 1991; McAllister et al., 1994b), the expectation after feeding tanniferous pod diets would have been a drop in ammonia concentration. The results presented in Table 4.2 show that this was not the case. Two reasons may be advanced for the results obtained; the inability of rumen microbes to effectively use rumen ammonia for the synthesis of microbial protein due to a deficiency in energy and/or change in the morphology of microbes as explained above. Secondly, a lack of direct relationship in protease activity and ammonia concentration may tally with reports by others (Khazaal and Orskov, 1994; Khazaal et al., 1994; Balogun et al., 1998) on the failure to establish correlations between the concentration of tannin fractions in forage and reductions in digestibility. This renders tannin concentrations in diets probably unreliable for predicting the nutritive value of feeds and production of rumen ammonia.

An increase in fractional outflow rate of liquid from the rumen (Czerkawski, 1986), microbial utilization, absorption across the wall and recycling of nitrogen through the rumen wall or from saliva are also some of the factors that can affect ammonia measurements and interpretations (Hennessy et al., 1983).

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9.3.3 Rumen degradation of pods and metabolizable protein

In order to obtain a satisfactory digestion of any feed in the rumen, ruminal microbes must grow and multiply and this involves a large scale synthesis of microbial protein (Leng, 1990). The degradation and ability to enhance microbial growth by legume pod supplements are of major importance in the N economy of the host animal since this determines the nature of the amino acid mix made available for protein synthesis at tissue level (McDonald et al., 1998). The quantity of microbial crude protein (MCP) produced from any dietary protein depends on the quantity of OM fermented and the ratio of effective rumen degradable protein (ERDP) to fermentable metabolizable energy (FME) estimated to be 10 for a growing ruminant (Beever, 1996).The ERDP:FME ratios obtained for pod meals (Table 9.2) are obviously higher indicating a limitation in fermentable energy but since pods or cakes are usually fed as supplements, energy emanating from the fermentation of the basal diet (roughage) could compensate for the deficit.

The MP values for the pod meals were about half the corresponding value in cotton seedcake (CSC) but comparable to those of the soya bean meal (SBM) (Table 9.2). It is estimated that a lamb of 20 kg LW growing at 0.2 kg /day requires 55 g MP/day to take care of its N endogenous losses, tissue and fleece growth (McDonald et al., 1998). This amount of MP could be provided for by supplementing a lamb on a roughage diet, with about 0.6 kg of pod meal per day, an indication that this class of feed is a credible source of protein supplement.

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Table 9.2 Estimation of the metabolizable protein of the pods of *L. leucocephala, A. erioloba, A. karoo, A.nilotica, A. sieberiana* and *A. tortilis* compared to cotton seed cake (CSC) and soya bean meal (SBM)

PODS OR CAKE	FME MJ/kgDM	ERDP g/kg	ERDP/FME g/MJDM	DUP g/kg	DMP g/kg	MP g/kg
L.leucocephala	5.12	176.3	34.45	47.67	32.62	80.29
A. erioloba	5.8	80.3	13.85	45.68	36.96	82.64
A. karoo	4.71	120.6	25.62	45.32	30.01	75.33
A. nilotica	7.87	98.1	12.47	39.54	50.15	86.68
A. sieberiana	6.85	121.4	17.72	39.59	43.67	83.27
A. tortilis	6.32	128.3	20.29	47.2	40.31	87.51
CSC (decorticated)	9.2	265	28.8	125	58.7	183.7
SBM (decortucated)	10	335	33.5	54	63.8	117.8
SBM (undecorticated)	6.7	232	34.6	45	42.8	87.8

FME = Fermentable metabolizable energy

ERDP = Effective rumen degradable protein

DUP = Digestible undegradable protein

DMP = Digestible microbial protein

MP = Metabolizable protein

9.3.4 Relationship between N source, N excretion and weight gain in sheep

To investigate the response of sheep (in terms of liveweight gain) to different N supplements, data were pooled from chapters 3, 6 and 8 and a number of regressions were done to highlight the effects of tannins on the digestibility of protein. Digestible CP (% DM) was regressed against dietary CP (% DM), linear and quadratic terms of tannin content (T, T^2) and the following result was obtained:

DCP = -0.81 (S.E. = 3.603) + 0.71 (S.E. = 0.412) TCP — 0.40 (S.E. = 0.115) T + 0.03 (S.E. = 0.012) T^2

(n= 15, RMSE = 0.36, Adjusted R^2 = 0.64).

This relationship shows that DCP followed a quadratic decrease with increasing tannin content. An equation without intercept was fitted to data because the intercept was not different from zero (P=0.82) with the following result:

$$DCP = 0.62 \ (S.E. = 0.022) \ TCP - 0.41 \ (S.E. = 0.101) \ T + 0.03 \ (S.E. = 0.010) \ T^2$$

$(n= 15, RMSE = 0.34, Adjusted \ R^2 = 0.99)$.

The decrease in DCP could be the result of tannins increasing faecal N excretion, as is given in the following regression of faecal N (FN; % TCP) against the linear and quadratic level of tannin content (T, T^2):

$$FN = 38.2 \ (S.E. = 2.22) + 4.9 \ (S.E. = 1.21) \ T - 0.40 \ (S.E. = 0.121) \ T^2$$

$(n= 15, RMSE = 4.02, Adjusted \ R^2 = 0.58)$.

The increase in FN as the tannin content increased in the diet may be due to one or a combination of the following factors: free condensed tannins form complexes with dietary protein at neutral pH in the rumen which later dissociate to release the protein at low pH in the abomasum (Barry, 1989; D'Mello, 1992). The released protein may then be digested in the intestines but the tannin released from the protein-tannin complexes may again complex with dietary and non-dietary protein (including digestive enzymes) as it passes along the intestines (McNeill et al. 1999). CT may also reduce the absorption of amino acids through their effects on endogenous enzymes. Their binding with endogenous enzymes reduces the activity of proteolytic enzymes to cleave off peptides and amino acids which are in association with the intestinal mucosa, thus reducing transport and absorption of peptides and amino acids (Horigome et al., 1988). The bound complexes are subsequently eliminated with faeces and this may therefore explain the diversion of N from urine to faeces.

Given, that tannins increase faecal N excretion, it is expected that increased dietary tannin would decrease urinary N excretion (UNT; % TCP) as in the equation:

UNT = 0.43 (S.E. = 0.026) - 0.03 (S.E. = 0.004) T

(n= 15, RMSE = 0.06, Adjusted R^2 = 0.71).

This observation was also reported by Bravo et al. (1993) and Nsahlai et al. (1998c). The negative effect of tannin persisted even after urinary N was expressed as a percentage of digestible N (UND; % digestible N) as in the following regression equation:

UND = 0.76 (S.E. = 0.063) - 0.04 (S.E. = 0.010) T

(n= 15, RMSE = 0.14, Adjusted R^2 = 0.45).

The implication of the shift of N excretion from urine to faeces as seen in Chapter 8, is important especially to low resource farmers, natural resource management and sustainability. The advantages of tannin complexing dietary nutrients especially protein, are obvious for the small holder, who depends to a large extent on tannin-rich forages for protein supply to his livestock and on the faeces to improve soil fertility for his crops. Part of the N excreted in urine comes from purine derivatives, a greater proportion of which is thought to be of microbial origin (Chen et al., 1990). Although it is expected that tannins would have reduced microbial growth through their effects on the fermentation of the supplemental dietary protein (Akin et al., 1988), the evidence presented on Table 8.5 on ruminal microbial synthesis does not seem to support this expectation. One may therefore conclude that the concentration of tannins in the diets (Chapter 8) favoured the formation of tannin-protein complexes that were subsequently translated to the

linear increases in ADG after digestion in the small intestines, but was not high enough to affect substrate fermentation.

9.4 Effect of feeding pod supplements on fibre digestion

The effects of the pod meals on fibre digestion were illustrated in chapters 4, 5 and 8. Depressions in the degradation of cell wall constituents, fibrolytic enzyme activity and concentration of VFAs in the rumen were attributed mainly to the presence of CT. The adverse effects on the digestibility of NDF after supplementing the diet of sheep with tanniferous forages have also been reported by many authors: *A. cyanophylla* (Reed et al. 1990); *C. palmensis* (Varvikko and Khalili, 1993); MPTs (Nsahlai et al., 1995c); *S. sesban* and *S. goetzi* (Weigand et al., 1995, 1996). Two main reasons have been advanced for these observations: (1) the formation of complexes between tannins and structural carbohydrates that leave the rumen undegraded (Akin et al., 1988; Cherney et al., 1992; Waghorn et al., 1994a; Makkar et al., 1995; Fall Toure et al., 1998) and (2) the inactivation of microbial fibrolytic enzymes by CT rendering them unavailable for fibrolysis (McLeod, 1974; Lohan et al., 1981). These effects are clearly illustrated by the results presented in Tables 4.4, 5.5 and 5.4. The resultant of these two effects is a reduction in the fermentation of fibre and consequently a drop in the production of VFAs in the rumen (McSweeney et al., 1999).

The effects of tannins on post-ruminal digestion appear to be more contentious. The results on the digestibility of cell wall constituents (Table 8.4) evolved quadratically as the concentration of tannins (increase in pod meal) increased in the diet. This places some doubt on the prediction of fibre digestibility from the concentration of tannins in

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the diet. To better appreciate the effect of tannins in a diet, it may be important to run parallel studies with the inclusion of tannin binding compounds such as PEG and PVP. Significant improvement in *in vitro* fermentation of DM and OM (Makkar *et al.*, 1995; Balogun *et al.*, 1998) and on *in vitro* gas production (Khazaal *et al.*, 1996) were reported after the inclusion of PEG and PVP in analytical samples. McSweeney *et al.* (1999) however, reported that the increase in both DM and NDF digestibilities that occurred with the inclusion of PEG may be underestimated because the addition of tannin-binding agents could reduce the true digestibility value by binding with NDF and thus being included as "artefact NDF and DM" (Makkar *et al.*, 1995).

9.5 Cyanogenesis and effect of detoxification of CG on palatability and intake

9.5.1 Effect of rumen pH on cyanogenesis

Previous studies indicated that the optimum pH of cyanogenesis in ovine ruminal fluid was 6.5 (Majak *et al.*, 1990). The same authors reported that at a pH<6.0, the production of HCN was negligible and this was attributed to the enzyme β-glucosidase whose activity was optimal at a pH of 6.5 and drops by 80% when the pH drops to 5.0. This report agrees with other findings (Simpson *et al.*, 1977; Stewart, 1977; Hoover *et al.*, 1984) on the activity of β-glucosidase on fibrolysis. The effects of pH< 6.0 were also shown by Russel *et al.* (1979) to be related to reductions in the growth of several species of ruminal bacteria. The rate of cyanogenesis in ruminal fluid also depends on the diet, post prandial time and the chemical nature of the cyanogenic glycoside. For example, the aromatic hydroxynitrile, prunasin, showed higher rates of HCN production than the aliphatic hydroxynitrile, linamarin (Majak *et al.*, 1990). Cyanogenesis occurred

most rapidly in prefeeding ruminal fluid; hence animals are probably more susceptible to poisoning by plants containing CG after a 24 hour fast or starvation. Inocula from grain diets yielded the lowest rates of HCN production, whereas the highest rates were obtained with fresh alfalfa herbage and cubed alfalfa hay diets (Majak et al., 1990).

The effects of cyanide toxicity may therefore be averted by including feeds that have high contents of soluble carbohydrates in diets with high levels of CG. This will however, depress fibrolysis and thus, not convenient for roughage based diets. Ensiling feeds rich in CG may therefore serve as a cheap and effective means of reducing their contents of CG.

9.5.2 Effect of CG on selenium utilization in sheep

Low levels of selenium and/or vitamin E are the principal causes of nutritional myopathy in herbivorous farm animals (Gutzwiller, 1993). Rudert and Lewis (1978) also reported that chronic cyanide intake by pregnant ewes caused nutritional myopathy in their offspring. The authors came to the conclusion that cyanide or its metabolite, thiocyanate affects the metabolism of Se and possibly Vitamin E. Although the effect of cyanide on Se metabolism in ruminants is not well documented, there is ample evidence that the cyanide metabolite, thiocyanate inhibits the iodine uptake of the thyroid gland (Kaneto, 1989; Ellis et al., 1984). It has also been reported that Se deficiency mitigates hypothyroximia in iodine-deficient humans (Vanderpas et al., 1993). The intake of CG was reported to profoundly affect the erythrocyte glutathione peroxidase (Gpx) activities of ewes and their lambs (Gutzwiller, 1993), an indication that Se status was affected. Belstein and Whanger (1984) and Elzubeir and Davis (1988) reported that chronic

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cyanide intake affected the Se status of chicks and rats. The exact mechanism by which this is done has not been elucidated, however, rats that were exposed to cyanide, excreted more Se in urine while faecal Se was not affected (Belstein and Whanger, 1984).

The interaction between cyanide of dietary origin and Se may be more complicated in ruminants than in monogastrics because part of the cyanide is detoxified to thiocyanate in the rumen (Onwuka *et al.*, 1992). It is possible that selenocyanate is also formed in the rumen (Gutzwiller, 1993) which in turn might affect the absorption of Se. The Se requirement of sheep, and possibly of other ruminants may increase when the intake of CG is high. The use of feed with high concentration of CG may therefore increase the incidence of Se deficiency diseases when the Se intake is marginal. This situation is unlikely to be serious in sheep fed pods of *A. sieberiana* because these pods have a relatively high content of Se (Chapter 3).

9.5.3 Effects of detoxification of CG on palatability and intake

The efficiency of nutrient utilization in ruminant production enterprises generally increases with the quality and quantity of feed intake (Weston, 1996). Accordingly, high efficiencies prevail with forage feeding at or near *ad libitum* and the achievement of higher production goals may often be largely dependent on an enhancement of forage intake. The quantities of forage diets selected at pastures or consumed indoors may fail to provide sufficient nutrients to meet the ruminant's needs for maximum production. Factors contributing to this limitation include sparse distribution of plants and

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prehension difficulties but the key determinants of forage intake are palatability, essential nutrient inadequacy and the presence of deleterious secondary compounds. A knowledge on how intake is regulated and information on the extent to which these factors can act to impair palatability and intake is important for improving productivity. It is in this context that the choice feeding trial was set up with the main objective of looking at ANFs on palatabilty and intake.

Phenolics, alkaloids, tannins, cyanogenic glycosides and aromatic compounds are some of the chemical compounds known to alter palatability (Marten, 1973). From experience, animals have learned to associate the sensory properties of feeds with their metabolic consequences. Provenza (1995) reported that aversions were acquired on the basis of interactions between sensory receptors (taste and odour of particular foods) and post-ingestive feedback (e.g. amount or frequency of malaise) which depend on the nutritional and toxicological characteristics of the diet. The palatability trends observed in chapter 7 may validate this assertion.

The time acquired for the onset of an aversion, the degree to which the aversion is manifested and the duration over which a particular food is avoided will depend on the flavour of the food, the amount and the frequency of the malaise. The erratic feeding behaviour of the experimental animals and the subsequent established preference rating in the intake of feeds after five days (Chapter 7) may have been influenced by intolerance to some of these ANFs. The reduction of the concentration of CG in the pods of A. sieberiana by ensiling may have elicited a higher palatability and intake since this constituted the major difference in chemical composition between the silage and dry

pods. This is an incentive for more research in order to arrive at practical methods of detoxification of ANFs in order to improve on the palatability of forage from tree legumes.

The between-species differences observed in the second part of the trial (Chapter 7) highlight differences among animal species when it comes to palatability and point to the fact that the palatability indices of goats cannot be used to predict those of sheep and vice versa. Robbins *et al.* (1987) attributed the differences in palatability between sheep and goats to the ability of the latter to secret proline-rich protein in saliva and the high affinity of proline-rich proteins for tannins, a process which reduces astringency.

GENERAL CONCLUSION

The results of this work have confirmed reports by many other authors that forage from tree legumes have protein and mineral concentrations that can maintain and sustain small ruminants through periods of feed scarcity. The pods that were examined had Ca concentrations which are higher than what is found in oilseed cakes even though the phosphorus contents were lower. The concentrations of the other minerals however, show amounts which will be sufficient to meet the mineral requirements of small ruminants in full production if the pod meals are fed at the rate of 1 kg/animal/day.

The CP content of the Leucaena pods was higher than that of alfalfa but those of the Acacia pods were lower. The NDF concentrations ranged from 200 g/kgDM for *A. nilotica* to 400 g/kgDM for the Leucaena pods. Dry matter degradability of the pods was

about 500 g/kgDM while the nitrogen degradability was higher, ranging from 600 g/kgDM for the Acacia pods to 700 g/kgDM for the Leucaena pods. Ammonia concentrations in the rumen of sheep fed pod diets ranged from 70 to 80 mg/l of rumen fluid and the values were similar to what was provided by the alfalfa diet. It is possible that these values would have been higher if the feed was not restricted to less than 500 g/animal/day.

A comparison of the pod diets with an alfalfa diet and hay offered unsupplemented to sheep indicated that the pod diets depressed proteolytic and fibrolytic enzyme activities, degradation of DM and cell wall constituents and the production of VFAs in the rumen. These depressive effects were attributed mainly to the presence of condensed tannins present in the pods. The presence of these phenolic compounds also negatively influenced the palatability and intake of the pod meals in a choice feeding trial. These observations are in conformity with reports from other authors on the effect of ANFs on microbial fermentation, palatability and intake of feed by host animal.

Another ANF which was given elaborate attention is cyanogenic glycoside which is present in some Acacia species and has been reported to be responsible for the mortality of some grazing livestock species. Ensiling effectively reduced the concentration of this ANF in the pods of *A. sieberiana* and improved their palatability and intake. The addition of molasses and urea to the parent material at ensiling further improved the fermentation process, the aerobic stability and the nutritive value of the silage.

The use of the ensuing silage as a supplement in a growth trial improved the intake of a roughage basal diet, total DM intake and liveweight gain in sheep when compared to a wheat bran supplemented diet. These positive attributes show that despite the presence of ANFs (mainly tannins), legume pods in general and the silage in particular, can be considered as a good provider of nitrogen and minerals to ruminants if well managed. Improved management of this class of feeds will go a long way to benefit the small-scale farmer who depends to a large extent on tannin-rich forages for protein supply to his stock and on the animal faeces to improve soil fertility for his crops.

IMPLICATIONS

The work described in this thesis was aimed at evaluating the attributes and limitations of pods from tree legumes as supplements to ruminants fed roughage-based diets. The rumen degradability of the pods and their ability to provide both rumen degradable and undegradable protein was evaluated. The influence of tannins and cyanogens on rumen fermentation and palatability were also examined. Further development of standard methods of quantification and assessment of the biological effects of tannins and other anti-nutritional factors needs more attention as the current methods seem inadequate. More attention needs to be given to the evaluation of pods as livestock feed since this class of feeds has been neglected in the past. The elimination of anti-nutritional factors in the pods in particular and browse in general still remains a core issue and a long term solution to this problem will require the joined efforts of nutritionists, phytotoxicologists, plant breeders and biochemists.

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Appendices

Appendix 1
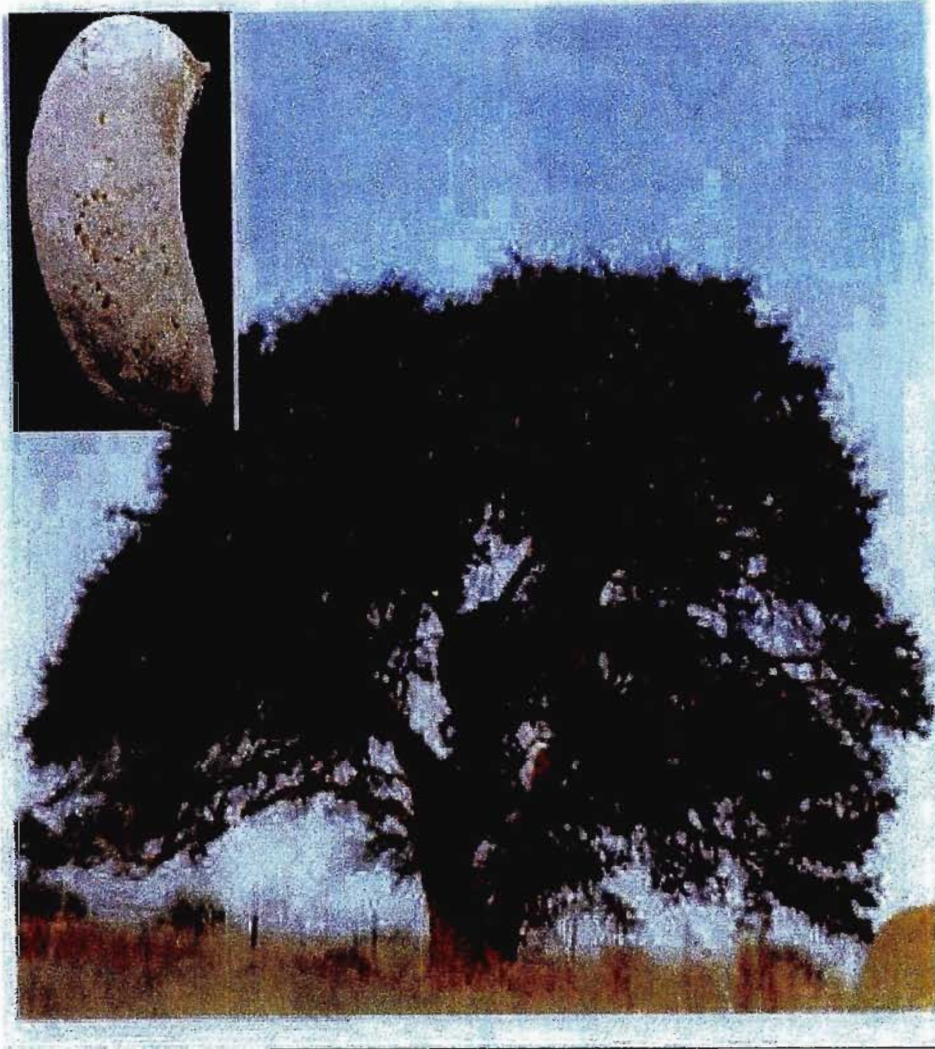
Acacia erioloba (Synonym , *A. giraffe* ;   Common name, Camel thorn).

It is very widespread and dominant in desert regions. Erioloba means half moon and this refers to the shape of the pod. The tree usually has a single stem but this frequently divides into several stems at low level. The height can be up to 12m, averagely ranging from 6-7m with a trunk diameter of 1.5m and a spread of as much as 22m. It has a flattish spreading crown and terminal branches that trail appreciably, especially where regrowth has been vigorous. The tree is semi-deciduous. Leaves vary from 1-7 per node and up to 6cm long with 1-3 pairs of pinnae. The pods, young shoots and flowers are relished by stock and game. The flowers are browsed off the tree and picked off the ground by stock and game. The fresh green foliage, green pods and ripe pods can all contain dangerous quantities of cyanogenic glycosides. The forage contains the most and the ripe pods the least and this varies with the sample tree and the period of the year. *A. erioloba* pods can be fed safely to stock provided small quantities are fed at time.

Acacia karoo (Synonym, *A. natalitia* ; Common name, Sweet thorn or Soedoring).

This species is the most widely distributed and occurs in varying climatic conditions in all provinces in South Africa, Eastern Botswana, Swaziland and Lesotho. The species varies considerably in overall appearance but the typical form is made up of a tree which is either single or several stemmed, branching well clear off the ground to give a rounded outline and having a height of 5-10m. The species can easily be identified by the pods which have a characteristic sisal-shape. The foliage, flowers , green pods and mistletoes of *A. karoo* are important sources of browse for livestock and game in southern Africa. At high leaf densities goats fed almost exclusively on *A. karoo* in a woodland pasture in the Eastern Cape of South Africa. In the Transvaal, South Africa, *A. karoo* is particularly susceptible to losses of soft green shoots due to browsing animals in spring. Fresh leaves, flowers and immature pods were found to be free from cyanogenic glycosides. This makes *Acacia karoo* very suitable for stock feed.
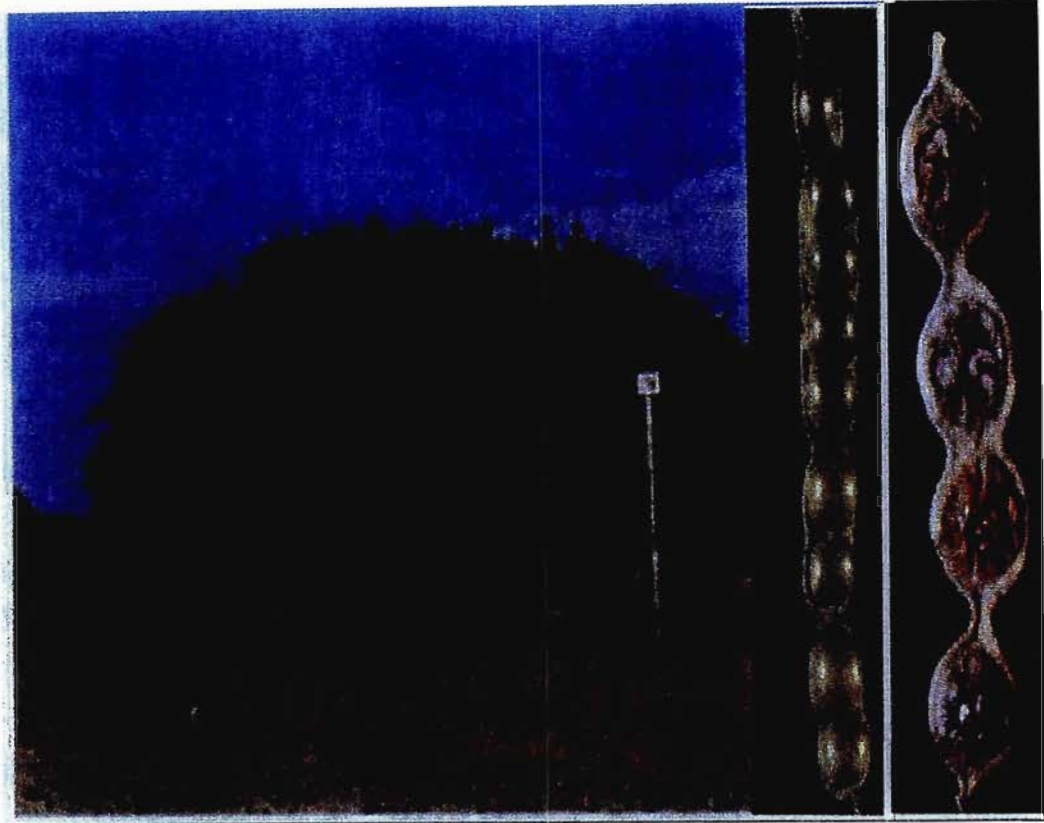
Acacia nilotica (Synonym, *A. arabica*, Common name, Scented thorn)

It is usually singled stem (5-6m high) but generates branches from low down to form a compact rounded to flattened crown with a spread appreciably exceeding the height. In a silhouette, it resembles *Acacia tortilis* but the crown is generally more rounded. It is readily identifiable when the black, pendent, beaded and sweet- smelling pods are present. The tree is semi-deciduous and when in full leaf provides good shade. The stem and branchlets are usually dark coloured. The leaves are bright green and occur at nodes mostly in threes or fours, but sometimes there may be as many as eight. Pinnae are usually in 3 to 12 pairs; leaflets in 10-30 pairs, linear oblong, about 0.4cm long. Flowers are yellow. Pods are grey, thick, softly tomentose , straight or slightly curved., 10-15cm on a pedicel, 0.5 -1.2cm wide with constrictions between the seeds giving a necklace appearance, fleshy when young, becoming black and hard at maturity. The fleshy pods are readily eaten by sheep, goats and cattle but some tribes believe that this causes bloat. Because of the spiny nature of the young trees, the species is considered noxious.

Acacia sieberiana
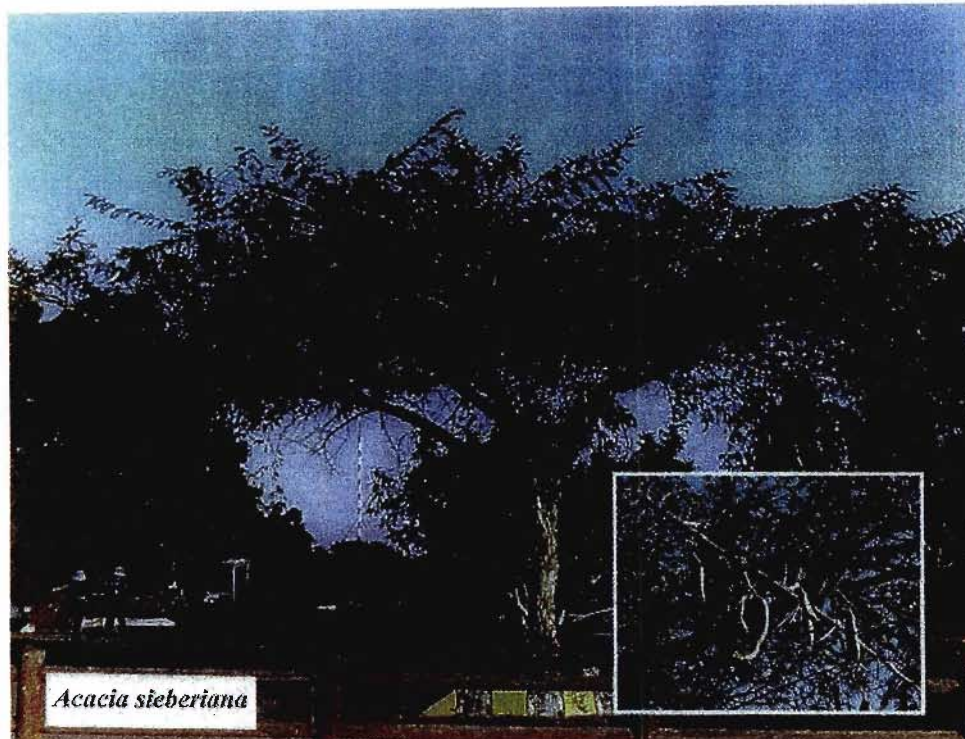
Acacia sieberiana (Synonym: *A. woodii* Common name: White thorn).

This species is the most widely distributed and occurs in varying climatic conditions in all provinces in South Africa, Eastern Botswana, Swaziland and Lesotho. The species varies considerably in overall appearance but the typical form is made up of a tree which is either single or several stemmed, branching well clear off the ground to give a rounded outline and having a height of 5-10m. The foliage, flowers , green pods and mistletoes of *A. karoo* are important sources of browse for livestock and game in southern Africa. At high leaf densities goats fed almost exclusively on *A. karoo* in a woodland pasture in the Eastern Cape of South Africa. In the Transvaal, South Africa, *A. karoo* is particularly susceptible to losses of soft green shoots due to browsing animals in spring. Fresh leaves, flowers and immature pods were found to be free from cyanogenic glycosides. This makes *Acacia karoo* very suitable for stock feed.
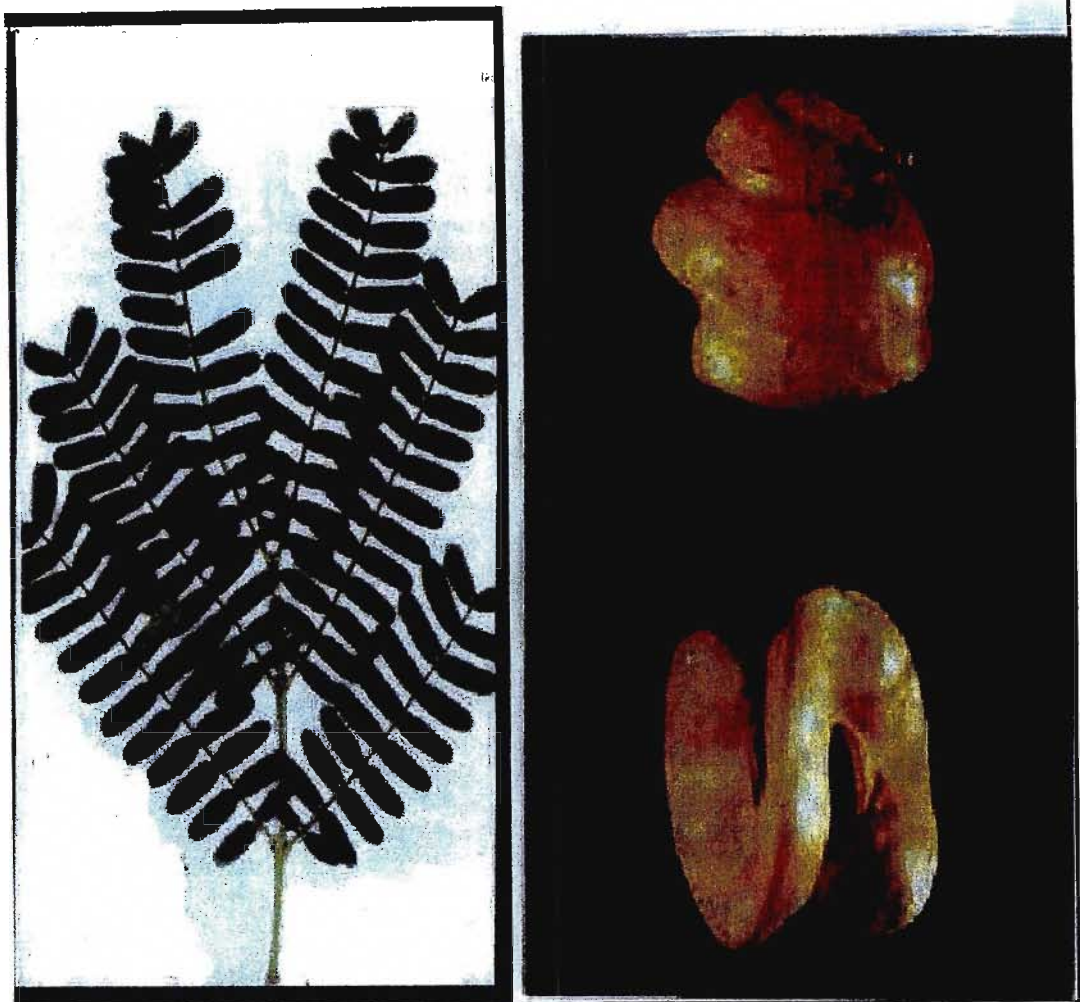
Acacia tortilis (Synonym , *A.spirocarpa* ; Common name, Umbrella thorn)

It is a distinctive and thus easily identified species. It has a dense, compact, flattened crown or umbrella shaped, fine foliage, contorted pods and both hooked and straight thorns. The tree is usually singled stemmed and 3-4m high, though branching from quite low down to give a spread which may appreciably exceed the height. Terminal branchlets characteristically droop initially but are upturned at their extremities. There are usually 2-6 leaves per node. Pinnae are in 3-10 pairs , leaflets in 7-15 pairs. Flower heads are white to cream. Pod yellow- brown, pubescent, spirally twisted, slightly constricted between the seeds, circular in cross section , 7.15-15cm long, 0.6-0.8cm thick. Leaves of young trees are browsed by goats and sheep, but the main value of this species is in its pods, which can be very numerous and are picked up from the ground and eaten by African livestock. When the pods are mature, they are often the main source of feed for cattle, sheep and goats.

Leucaena leucocephala

It is probably the most widely used tree legume in the world. It is highly nutritive and a great variety of other uses (food, wood, fertilizer) have contributed to its worldwide success. The thornless nature of the tree makes the foliage very accessible to browsing animals. The leaves are bipinnate. Originally, Leucaena is from Central America and Mexico. Leucaena does well on fertile soils but performs poorly in acid and waterlogged soils. It is highly palatable to livestock and its dry matter digestibility were reported to range from 500 to over 800 g/kg. However, Leucaena can be toxic to livestock. The non-protein amino acid, mimosine found in the foliage and pods can be broken down by rumen microbes to 3 hydroxy-4-(1H)-pyridone (DHP) and the latter is goitrogenous and causes alopecia, loss of appetite, excessive salivation and weight loss.
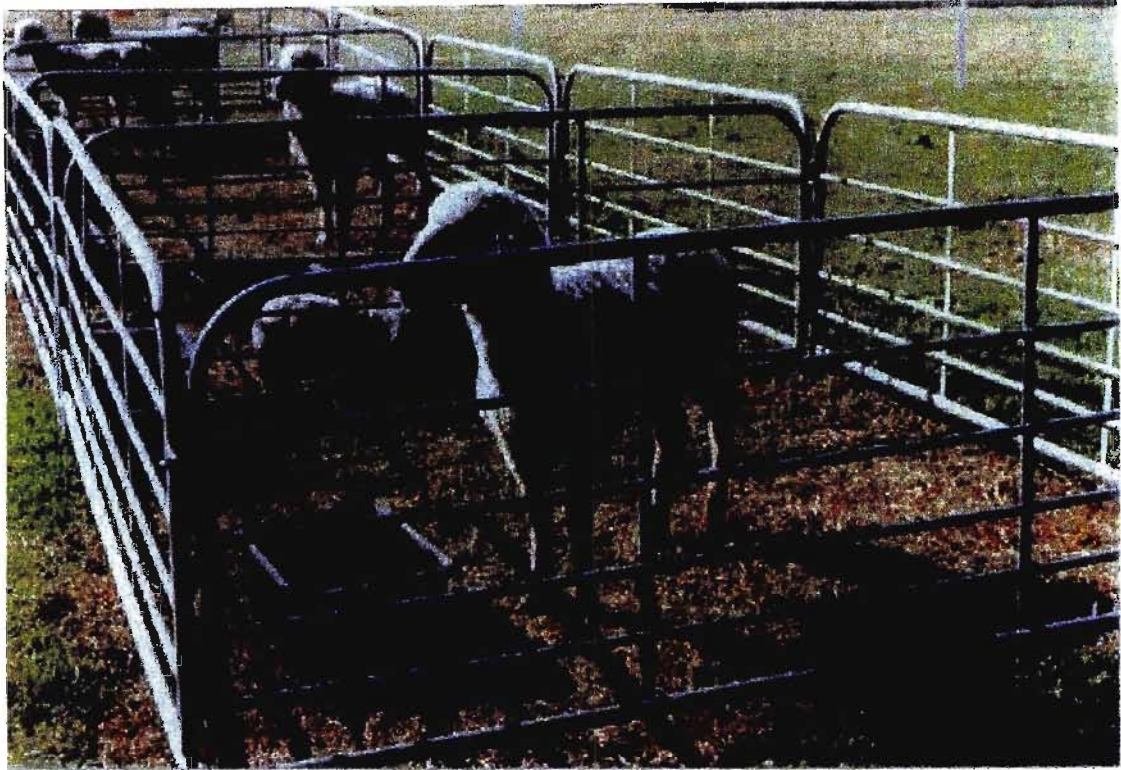
Fistulated South African Merino rams

The rumen of each ram can carry up to 12 nylon bags and the animal does not seem to be really bothered with the activity of putting and removing of bags as long as there is something nice in the trough to feed on and some water to cool its thirst later.