# EVALUATION OF STOVER MANAGEMENT PRACTICES OR AMELIORATIVE STRATEGIES ON THE UTILIZATION OF TANNIFEROUS FEEDS BY RUMINANTS

A Thesis

Presented to

The Faculty of Graduate Studies

of

The University of Guelph

by

WAME SHIMANE BOITUMELO

In partial fulfilment of requirements

for the degree of

Doctor of Philosophy

August, 1999

# ABSTRACT

## EVALUATION OF STOVER MANAGEMENT PRACTICES OR AMELIORATIVE STRATEGIES ON THE UTILIZATION OF TANNIFEROUS FEEDS BY RUMINANTS



Wame Shimane Boitumelo PhD
University of Guelph, 1999

Advisors:
Professor J. H. Burton
Professor V. Umunna



Seven experiments which included one field/agronomy, four in vitro and two in vivo trials were carried out to (i) determine the optimum time of harvesting and storage methods that preserved the nutritive quality of maize and sorghum stovers, (ii) examine strategies for enhancing the utilization of high tannin feeds (bird-resistant (BR) sorghum stover supplemented with forage legumes (FLs) with varying tannin concentrations) by using ameliorants (polyethylene glycol MW 4000 (PEG-4000), urea or sulphur). It was determined that nutritive value was significantly ($P<0.001$) influenced by crop type, period of harvest and storage method. The highest nutrient content was recorded at 2 weeks (-2 weeks) before physiological dead ripe stage of grain and when the stover was harvested immediately after grain harvest and stored under shade. Addition of ameliorants significantly improved DMD and gas production in vitro. Urea and sulphur were as effective as PEG in alleviating the anti-nutritive effects of high tannin in feeds. The optimum levels of PEG required to alleviate the anti-nutritive effects of CTs varied with forages. Nevertheless, a ratio of 2:1 (PEG: CT) was found to be optimum for the alleviation of the adverse effects of CTs. Forage legumes with high concentrations of CTs, when used as supplements to BR sorghum stover, depressed animal performance (feed intake, growth and growth efficiency). The response assumed both a linear and quadratic pattern, suggesting a threshold point beyond which response decreased.

Mixing FL (*Lablab purpureus, Chamaecytisus palmensis, Sesbania goetzei 15007, Sesbania sesban 15019, Desmodium intortum* and *Acacia angustissima*) with different nutritional attributes at 1:2 or 2:1 ratio significantly improved gas and ammonia production in vitro. Thus there is potential for improving the utilization of some forages that would normally pose problems (e.g. *A. angustissima*) if used by themselves. It was possible to assess the reactivity (biological activity) of tannins by reacting them with PEG and relating it to radial diffusion assay (protein precipitation assay). Results showed a high correlation ($r = 0.87$, $P<0.01$) between PEG-CT reactions and radial diffusion assay, thus indicating that PEG-CTs reaction may be an effective method of indexing tannin reactivity.

## ACKNOWLEDGEMENTS

I am grateful to my University advisor Dr John Burton for guiding me through the Ph.D. studies, even though he was geographically far from my research site, he continued to guide me either by telephone or electronic mail. The rest of the professors who served on my advisory committee are also recognized for their help, Dr Don Grieve, Dr Jim Atkinson and Dr Jim Shute. I would like to express my sincere gratitude to the International Livestock Research Institute (ILRI) for according me a fellowship to carry out this research at Debre Zeit Research Station. In particular the Director of SPAN, Dr Michael Smalley, Assistant to the Director of SPAN, Ms Letty Padolina and Ms Elizabeth Getachew (the previous and current incumbents) and the rest of the training department staff. Professor N. N. Umunna at ILRI-Debre-Zeit Research Station guided me during the development, implementation and write-up of this work as my ILRI supervisor. I am indebted to him for his guidance and support during these trying times. His patience and fatherly advice has seen me through. The rest of the scientists at the Debre Zeit Research Station, Dr Pascal Osuji, Dr Azage Tegegne and Dr Agnes Odenyo "our mother" are also recognized for their untiring advice and support. Dr Ignatius Nsahlai was my co-supervisor at ILRI DZ before he left for the University of Natal, South Africa from where he continued communicating electronically. I am indebted to his untiring advice. The research technicians Asfaw Yilmegnuhal, Dawit Negassa, Abebe Tessema were all very supportive. My fellow graduate fellows, Drs Robert Kaitho, Denis Mpairwe, Ato Workeneh Abebe, Sarah Ossiya and Ato Yoseph Mekasha provided the homely atmosphere and academically-enriching environment. All the technical staff who provided all the required support are recognized. From the fields in particular Aberra Adie,

Mohammed Yasin, very special thanks to Fikru Getahun and Salamawit Lemma who sacrificed their time to help me also in the laboratory. From the barns Tesfaye Gashaw, Kebede Melese, Amare Feleke and their colleagues. I would like in particular to mention for recognition Dawit Negassa, Almeshet Zewde, Berhanu Wadajo, Tigist Amenu, Bethlem Getachew, Negussie Sefu and Tigist Reggasa (Debre Zeit laboratory); Mebrahtu Ogbai, Genet Asefa and Mulugeta Habte-Michael (Addis Ababa Nutrition Laboratory); Ato Aklilu Bogale and Amare Atale DZ Biometry section for data entry and analyses respectively; and Messrs Mamadou Diedhiou and Zerihun Tadesse Biometrics Unit, ILRI, Addis Ababa for guidance during the experimental setup and during data analyses. From the secretarial services, Atkilt Getahun, Etenesh Yitna, Azeb Asmamaw and Liya Zewdie deserve special mention for their contribution. Dereje Kebede has been a brother and a friend and I will always cheerish his friendship. The rest of all DZ Research Station staff and ILRI-Ethiopia staff thank you very much and God bless you.

I made friends in Ethiopia, especially among the diplomatic community, in particular the Batswana and Basotho diplomats in Addis Ababa adopted me as their brother, their hospitality is highly recognised. To mention a few, His excellency the Ambassador of the Kingdom of Lesotho, Mr Leteka and his family, my friends: Thabang Lekhela, Andrew Moche, Lizzy Khampepe, Joalane Mokone, Princess Attie Nwako, Mr and Mrs Motlhatlhedi, Dr Marosi Molomo (assisted me in the laboratory), Mr and Mrs Motjoka Ramonono Andrew Nkhwa, Bisto Phole, Mr and Mrs Diabi Mmualefe (they read some of the chapters), Basadi Modisane, Mighty Mohurutshe and Elizabeth Macheka (she used her office management skills to edit the chapters).

In Botswana, my sincere thanks goes to the Government of Botswana for allowing me a long study leave. In particular Dr Lucas Gakale Director of Agricultural Research is recognized for his understanding and support. Mr Ricky Madibela kept me busy with the up dates of what was going on at home and at the Department. All other colleagues who have been supportive are recognized. All the friends that I made through my over 10 years in Canada are recognized for their hospitality. It can be long list if I can try list all of them. But in particular, the Ball family, Dave and Matshego Charne, Price and Diana Teeter And Dr John Afele.

Lastly but not least, my family sacrificed a lot during my absence, all of them are recognized for their great support. Special recognition goes to my dearest wife Onneile who bore the burden of raising our children alone and to the children for perseverance.

When I started the Ph.D. program at the University of Guelph, I told myself that I should do my research in an African setting. Little did I know that I will finally 'land in Ethiopia and at Debre Zeit in particular'. The experience has been challenging and enriching. My dream came true and I undertook my research in a true International animal research institute focussing on the developing world. The experience will go a long way to assisting the peoples of Botswana and the rest of the developing world. Finally, the financial support of International Development Research Centre (IDRC-Canada), International Livestock Research Institute (ILRI) and (GoB) Botswana Government is appreciated.

## TABLE OF CONTENTS

Chapter Page

## LIST OF TABLES

## LIST OF FIGURES

Table Page

## APPENDIX TABLES

Table Page

# CHAPTER ONE

## 1.0 GENERAL INTRODUCTION

The steady increase in the human population of the sub-tropical and tropical regions has led to a concomitant increase in demand for cereal grains, which are the staple foods. In order to meet the increasing food demand, marginal lands previously under grazing are being put into cultivation of food crops, leading to a steady decrease in grazing land. In addition, development projects such as roads, houses, airfields and industries are steadily taking up useful agricultural land. Consequently, livestock production in these regions increasingly depends on limited grazing and crop residues obtained after harvest. Seasonal fluctuations of food resources in the tropics follow the pattern of vegetation growth that is controlled by availability of moisture (rainfall). Because of this, the available feed resources are generally of low nutritional quality, and have a variable supply pattern. As green forage/fodder is only available for up to a maximum of five months in the year, ruminant animals have to depend on poor quality standing "straw" and harvested (crop) residues for subsistence. Quantitatively, cereal residues are the major feed resource for smallholder farmers in these regions, especially during the dry season. Depending on the particular ecoregion, different cereal crops are grown. In the drier parts of the sub-tropics and tropics, where birds are also major pests, sorghum is the most important cereal grain. Therefore, sorghum stover is the most important cereal crop residue as a feed resource for ruminants.

In a bid to increase grain yield, sorghum varieties have been selected based on bird-resistance to deter birds which to a considerable extent can devastate a sorghum crop. Bird resistance is related to the presence of condensed tannins in the grain and in the straw. Condensed tannins in the crop deter the birds from destroying the grain, but also decrease

the nutritive value of the stover by forming complexes with proteins, carbohydrates and minerals. In addition, the nutritive quality of sorghum stover is affected by other factors including genetics, environment and management. Because of these factors, the rate and extent of digestion of sorghum stover are marginal and thus the level of intake is so low that it can hardly support maintenance, let alone a reasonable level of production. The quality of sorghum stover, notably bird-resistant varieties, will therefore need enhancement when fed to livestock, if meaningful production levels are expected.

The nutrient deficiencies of low quality roughages have been addressed through several strategies, which include better stover management practices (harvest time, genetic selection), pre-treatment and supplementation with high protein agro-industrial by-products, forage legumes and other nitrogen supplements. Pre-treatments, which include physical and chemical treatments, are often expensive and technically not suitable for smallholder farmers. Grains and other conventional supplements are often scarce and too expensive to many of the smallholder farmers. Forage legumes (leguminous trees (multi-purpose trees (MPTs)) and shrubs plants) that establish easily without requiring extensive agronomic inputs are potential alternatives to provide sustainable sources of limiting nutrients in low quality roughage based feeding systems. Many of these have high fodder production in addition to high protein content, which could improve livestock productivity. Forage legumes however, synthesize secondary plant compounds (antinutritional factors) as defense mechanisms against herbivory and insect pests, which can affect animal performance. Tannins appear to be the major constraint on the use of forage legumes as animal fodders because of their effect on intake, digestibility and the animal's metabolism. Condensed tannins in ruminant feeds have both detrimental and beneficial effects. For example, voluntary intake and digestion of

organic matter of feeds containing large amounts of condensed tannins may be depressed. This is so because tannins combine with proteins to produce complexes resistant to microbial attack in the rumen. Despite the adverse effects on intake and digestibility, tannins may also protect protein from rapid degradation by rumen microorganisms and improve the quality of amino acids absorbed in the intestines. At a moderate level of tannins favorable effects on animal performance can be realized. Nevertheless, large differences in response have been reported when these fodders are fed as protein supplements. These differences have been attributed to differences in levels and may be reactivity of the phenolic (tannins) compounds.

In order to realize the benefits of supplementation, quality of the basal feed is important. Quality of cereal stover is however affected by several factors; some may be beyond the farmer's capacity to control. But there are some factors within farmers control, that are mainly management related. For example, the harvesting time and storage methods of stover after grain harvest are important and may greatly influence the quality of stover. Information available with regard to variation as a result of management in quality of sorghum and maize stover is very scanty.

Since sorghum stover from bird-resistant varieties contains ample quantities of condensed tannins and together with forage legumes constitute the feed resources that are available in the semi-arid zones; it is possible that the use of both in diets may accentuate antinutritional toxicity problems. Unfortunately, given the low resource base of most smallholders, they depend heavily on these leguminous crops as protein supplements. Any attempt to improve performance of livestock by the use of these forages must first overcome the problems posed by high content of tannins in the forages. Tannins are seen as plant protectants possessing a "toxic" effect. The risk of poisoning in unconfined animals having

a choice of diet seems very small. It is not small, however, when high-tannin food is the only choice, as has been indicated by fatalities in such animals. In areas where high-tannin forages form an important source of feed, tannin-protein interactions create a nutritional problem of applied nature. Therefore, practical strategies to overcome the antinutritive nature of condensed tannins are required in order to fully utilize these feed resources.

Significant progress in removing tannins from ruminant feed has been made in recent years. However, some aspects of their economical field application of such procedures remains to be elucidated. First, the cost of chemicals used can be prohibitive; second, they may be detrimental to the farmers; third, loss of dry matter during the removal process may not be in the economic interest of the livestock industry. The study reported here investigated ways of improving the utilization of bird resistant sorghum stover. The work included the development of strategies for enhancing the utilization of sorghum stover through supplementation with forage legumes. The work further explored practical ways of mitigating the antinutritive nature of condensed tannins in the feeds used. The major goal of this study was therefore, to develop strategies for better utilization of residues from sorghum, which is the main cereal residue of the semi-arid and arid regions of the sub-tropics and tropics.

# CHAPTER TWO

## 2.0 LITERATURE REVIEW

In order to meet the increasing food demand, marginal lands previously under grazing are being put into cultivation of food crops, leading to a steady decrease in grazing land. The decrease in grazing lands has forced livestock production to depend for sustenance on limited grazing and crop residues obtained after grain harvest. Seasonal shortages and low nutritional value of available feed resources are considered the most widespread technical constraints to livestock production in smallholder system, in sub-tropical and tropical regions. The availability of food resources in the tropics follows the pattern of vegetation growth that is modified by availability of rainfall. Green forage/fodder is available for up to a maximum of five months in the year, and for the rest of the time ruminant animals have to depend on the poor quality standing native "hay" and crop residues for subsistence. Availability of cereal crop residue depends on the cereal crop grown. In the drier parts of the sub-tropics and tropics, sorghum is the main cereal grown for human consumption; therefore, sorghum stover is the most important cereal residue feed resource for ruminants. But often the rate and extent of digestion of sorghum stover are marginal because of its inherent nature and thus the level of intake is so low that it can hardly support maintenance, let alone a reasonable level of production.

In order to enhance the utilization of low quality roughages, supplementation with deficient nutrients is necessary. Supplementation strategies include among others, supplementation with high protein agro-industrial by-products, forage legumes (FLs) and other nitrogen supplements. Availability and cost of high protein agro-industrial by-products limit their wide use by the smallholders. As a result, attention has been given to the use of

legume fodder (multi-purpose trees, MPT's, browses, and herbaceous forages) as sustainable sources of limiting nutrients in low quality roughage based feeding systems. Forage legumes, however, synthesize secondary plant compounds (antinutritional factors, (ANFs)) as defense mechanism against herbivory and insect pests, and consequently negatively affect animal performance. Because of the low resource base of smallholders in the tropics, they do not have many options except to rely on the leguminous crops (MPTs, FLs) and browse as protein supplements. In order to improve the performance of livestock fed to these feed resources, it is necessary to preserve high quality in the basal feed and overcome the problems posed by high content of tannins (and possibly other ANFs) in supplementary forages. This chapter reviews research and development reports that are relevant to the utilization of sorghum residues supplemented with FLs and browses.

**2.1 Importance of sorghum in the semi-arid and arid zones.**

Sorghum *(Sorghum bicolor)* is one of the four major food grains of the world. As a source of human nutrition, only wheat, rice and maize surpass it. It is the staple food for millions of the poorest people in the semi-arid, drought-prone areas of the sub-tropics and tropics, supplying over 10 million tonnes of grain annually (Hulse *et al.*, 1980). Sorghum and millet are the most important food crops of semi-arid tropics. 'Semi-arid' cannot be precisely defined, but in general, it describes those regions in which evapo-transpiration exceeds rainfall for more than half the year. The territories classified as semi-arid tropics include large areas of Africa, embracing most countries surrounding the Sahara, much of East and Southern Africa (Malton, 1990), a large area of central India (Badve *et al.*, 1994) and some regions of Southern Asia and Latin America. These areas cover the International Livestock Research Institute (ILRI) mandate zone. The estimated area under sorghum cultivation in the

semi-arid ecozones in 1981 was 47.8 million ha with 16 million ha in India, 15.3 million ha in Africa and the rest in other sub-tropical areas (FAO, 1981). In 1990, FAO estimated that 18 million ha were planted with grain sorghum in Africa (FAO, 1991).

The practical utility of sorghum arises from its adaptive merits above those of other related cereals (e.g. maize) (Aboud, 1991). Sorghum grows in areas of relatively low and erratic rainfall distribution. Annual rainfall of 500 mm is adequate to ensure substantial grain yield at this moisture level, where other cereal crops may fail (Malton, 1990). Sorghum tolerates drought (Jordan and Sullivan, 1981), water logging (Wilson and Eastin, 1982) and insect pests (Davies, 1982) better than maize. It also requires less pesticide and fertilizer inputs as compared to other cereal crops (Turner, 1981). Sorghum out-yielded maize in three out of five years under limited moisture conditions (Doggett and Jowett, 1966; Doggett, 1988). Sorghum grain yields, however, can be comparatively higher in areas with rainfall of 725-860 mm (Kalyankar *et al.*, 1989). The ability of sorghum to out-yield other cereals in water-stressed environments therefore makes it an important crop in the semi-arid areas of the sub-tropics and tropics (Doggett and Jowett, 1966; Malton, 1990).

**2.2 Importance of crop residues with special reference to sorghum**

In the sub-tropical and tropical regions, 40 to 80% of the livestock are associated with mixed crop-livestock farming systems, e. g. Africa 60 % (Brumby, 1987; World Bank, 1987). Emphasizing crop-livestock relationship, McDowell and Hildebrand (1980) identified the prevailing systems on small, mixed farms in Africa, Asia and Latin America. In these systems, crop residues and by-products from human food processing provided 30 to 90 % of livestock feed (McDowell, 1988). The need to improve utilization of crop residues in these regions has therefore, received considerable attention in recent years, but there have been few

studies on the availability of fibrous crop residues in relation to their potential for feeding livestock (Kossila, 1988). Cereal crop residues constitute a major feed resource for working animals in most African countries (Reed and Goe, 1989). For example, maize stover is the main feed resource in Kenya, Lesotho, Malawi, Zambia and Zimbabwe (Chabala, 1984; Molapo *et al.*, 1984; Shumba, 1984; Tessema, 1984; Watson *et al* 1984), while in Botswana and Tanzania, sorghum and millet stover are the main crop residues available for working and other livestock (Mayer, 1983; Urio, 1985). In Northern Nigeria, sorghum stover is the principal source of dry season stubble grazing (Olayiwole and Olurunju, 1986). Powell (1984) also reported that sorghum and millet stover are the principal feed sources for grazing animals during the first two months of the 6 month dry period in the sub-humid zone of Nigeria.

The amounts of available cereal crop residues could be estimated from grain yield. For sorghum, maize and millet, the straw/stover yield is about twice the weight of the grain yield (Umunna and Agishi, 1988), although multiplier values of 4 (Alhassan, 1985), and 5 (Kossila, 1988) have been used to estimate stover yield from sorghum grain yield. Kossila (1988) used a multiplier of 5, and estimated that in 1981, 55.2 million tonnes of residues were produced from sorghum in Africa. Assuming a digestibility of 45% and 20% wastage, the annual maintenance requirements of 39 million tropical livestock units (250 kg liveweight) could be met by sorghum and millet crop residues supplemented with low levels of protein or non-protein nitrogen (Kossila, 1988). Unfortunately there has been no recent estimates of these residues, but it is expected that the area under these cereals has increased over the years and as such the quantity has also increased.

There are several reports on the use of sorghum stover as ruminant animal feed, but most of these reports suggest varied levels of response (Reed *et al.*, 1988; Kiflewahid and Mosimanyana, 1989; Chesworth *et al.*, 1989; Aboud, 1991; Adu *et al.*, 1992; Osafo, 1993). The problem could be due to the wide diversity of sorghum varieties (Doggett, 1988) and different forms and methods in which the stover was offered. Reed *et al.* (1988) showed that there is large variation in both chemical and botanical composition among the common varieties of sorghum cultivated in Ethiopia. They found that *in vitro* dry matter digestibility of stover varied by 15% among the different varieties. Mosimanyana and Kiflewahid (1987) showed that in Botswana, even within the same variety, *in vitro* dry matter digestibility varied by as much as 10% depending on the time and conditions during harvesting.

**2.3 Factors affecting stover utilization (in particular sorghum) by ruminants**

Cereal straws and stovers are potential energy feeds for ruminants because of their high cellulose and hemicellulose contents. However, this energy is only partly available to animals because of poor digestibility due to inhibitory elements in stovers. Sorghum crop residues like other low quality roughages are high in fibrous carbohydrates (cell wall), low in soluble carbohydrates (cell contents), protein, and phosphorus, marginal in calcium and high in lignin (Table 2.1). This causes ruminal fermentation rate to be low and barely adequate to feed rumen bacteria near their maintenance requirements, thus severely limiting microbial yield for the animal to use. As a result, digestion is slow, rate of passage is low and voluntary intake is limited. Low quality roughages thus require adequate energy, protein and mineral supplementation in order to be degraded efficiently by microflora in the rumen (Preston and Leng, 1987).

**Table 1. Chemical composition and degradability of sorghum stover fed with or without supplements**

| Sorghum stover Variety | Animal species | Supplement | Crude protein | Neutral detergent fibre | Ash | Dry matter degradability | Source |
|---|---|---|---|---|---|---|---|
| | | | g/kg DM | | | | |
| Not indicated | Cattle | Mineral mix | 48 | 828 | 61 | 524 | Olayiwole and Olorunju, 1986 |
| Not indicated | Cattle | None | 56 | | 84 | 486 | Mosimanyana and Kiflewahid, 1987 |
| Not indicated | Cattle | None | 64 | | 84 | 598 | Mahabile *et al.*, 1990 |
| White | Sheep | 100g cotton | 54 | 762 | 65 | 548 | Ncube and Smith, 1992 |
| Red | Sheep | Seed cake | 56 | 765 | 67 | 502 | " " |
| Not indicated | Sheep | None | 46 | 697 | 94 | 594 | Aboud, 1991 |
| Not indicated | Goats | None | 56 | 699 | 85 | 492 | Macala *et al.*, 1992 |
| | | Lablab | 101 | 655 | | 595 | " " |
| | | Mophane | 79 | 522 | | 451 | " " |
| Not indicated | | Alone | 53 | 760 | 78 | 457 | Adu *et al.*, 1992 |
| BR | Sheep | Alone | 45 | 706 | 88 | 459 | Nsahlai *et al.*, 1998 |
| NBR | & goats | | 59 | 683 | 79 | 516 | |

Fibre quality is defined in terms of its ability to promote efficient rumen fermentation, and includes the potential digestibility and rate of fermentation of cellulosic carbohydrates, particle size and strength and cation exchange capacity (Van Soest, 1994). Graminaceous straws tend to be poor in these factors, although considerable variation exists (Van Soest, 1988). The quality of a feed is considerably modified by physical characteristics, which may be relatively independent of chemical composition. Factors such as caloric density, particle size, solubility in rumen liquor, buffering capacity, and the surface properties of fiber particles (i.e. hydration capacity and cation exchange) influence the physiological effects of ingesta on the gastrointestinal tract (Van Soest, 1994) and these factors are likely to be changed by feed processing.

Comparatively, cereal stovers are characterized by higher NDF and hemicellulose contents than legumes. As a result, intake of stover is lower than that of legumes at a given digestibility (Van Soest, 1988). Intake of sorghum stover has been reported to be 43% less than that of grass hay (McDowell, 1988). As indicated earlier, due to bird pest problems, sorghum varieties have been selected based on their bird-resistance trait, which lowers the stover quality. Variability in botanical and chemical attributes of sorghum stover is one of the factors determining the form and problems of its use as an animal feed. The nutritive value of sorghum stover will vary with the variety because of the differences in botanical composition (Mueller-Harvey and Dhanoa, 1991).

Work done at ILRI, formerly International Livestock Centre for Africa (ILCA), with sorghum stover have shown large varietal differences in digestibility. Reed and Hoefs (1987) have reported an extensive variation in the chemical and morphological characteristics among common sorghum varieties cultivated in eastern Africa. Low digestibility

of sorghum stover appeared to be associated with bird resistance in the grain (Reed *et al.*, 1987). The reluctance of farmers to adopt certain sorghum hybrids (especially the bird-resistant varieties) is believed to be associated with the botanical and chemical attributes of stover from these varieties (Reed *et al.*, 1988). The nutritional constraints of high tannin content in bird-resistant (BR) sorghum has been reviewed by Reed *et al.* (1988) and the implication of tannins on ruminant nutrition have been reviewed by Nsahlai *et al.* (1998). Butler (1982) through *in vitro* studies, suggested that there could be similar *in vivo* limitations to digestibility and intake when BR stover is offered to animals.

The reasons that have been given to explain why sorghum stover is poorly utilized include problems related to management practices (harvesting, collection, transport and storage), genetic, environmental, chemical and physical qualities (low nutrient content and high fibre and lignin) (Mlay, 1986; Owen and Jayasuriya, 1989; Ebong, 1989). These constraints will be briefly reviewed in the following section.

### 2.3.1 Management factors

Stover quality depends on how it is managed after grain harvest. It has been shown that DM content of stover decreases over time if the stover is left standing in the field for an extended period after grain harvest (Mosienyane, 1983). Sorghum stover is usually cut and stacked or shocked to reduce leaf loss from leaching or wind damage (Osafo, 1993). Assembling or storing crop residues is necessary where cropland is highly fragmented, the family is dependent on manure as fuel, for example in India and Ethiopian highlands or where marauding animals have access to crop residues during the off-crop season. When these elements are not pressing, farmers may prefer to graze the residues to reduce labor for storage or transport of manure to the fields. In this situation, the quality of the stover is likely

to be affected by the length of time the stover is left standing in the field. Parts of the stover which usually have higher nutritional quality like leaves and leaf sheaths may be lost through leaching by rain or damage by wind. The lower internodes and leaves are usually more lignified and less digestible than the upper internodes and leaves (Pearce *et al.*, 1988). Capper *et al.* (1988) observed that combine harvested barley straw tended to be more digestible than hand harvested. This was due to higher cutting heights by combine harvesting, thus cutting more of the upper internodes and leaves. Whether quality attributes like condensed tannins content of the stover left for an extended period in the field change or not is unknown. The conditions and methods under which stover is stored post-grain harvest may have large effects on stover quality and the possible consequences need to be elucidated.

### 2.3.2 Genetic and environmental factors

Differences exist between varieties in nutrient content and in other chemical constituents that can influence the value of sorghum stover as ruminant feed. This causes great variation in both the rate and extent of digestion of sorghum varieties, which could influence the level of nutrient intake and animal production. Such variations have been attributed in part to the influence of genotype on the leaf: stem ratio (McDonald *et al.*, 1981; Reed *et al.*, 1988) (Table 2.2). Part of the variation between varieties is controlled by genetic influences on chemical composition of different plant parts (Reed *et al.*, 1988) (Table 2.2.). Leaves and stems of the same plant tend to differ in nitrogen and cell wall contents, and nitrogen tends to be positively correlated with digestibility of straws (Erickson *et al.*, 1982; White *et al.*, 1981).

Table 2.2: The effect of site and bird resistance on content of neutral detergent fibre (NDF), digestibility of NDF (DNDF), content of lignin, soluble red pigments (A550 sol.uble) and insoluble proanthocyanidins (A550 insol.uble) in leaf blades, leaf sheaths and stems from the stover of bird resistant (BR, N=6) and non-BR (NBR, N=8) sorghum varieties

| | Debre Zeit | | | | Melkasa | | | | Significance | |
|---|---|---|---|---|---|---|---|---|---|---|
| | BR | | NBR | | BR | | NBR | | | |
| | Mean | SD | Mean | SD | Mean | SD | Mean | SD | Site | Resist |
| **Leaf blades** | | | | | | | | | | |
| NDF (% OM) | 62.3 | 3.4 | 64.6 | 3.2 | 60.6 | 3.2 | 60.0 | 3.2 | ** | NS |
| DNDF (%) | 57.1 | 4.2 | 61.5 | 3.8 | 61.9 | 5.5 | 61.9 | 4.5 | ** | NS |
| Lignin (% OM) | 3.5 | 0.4 | 4.2 | 0.6 | 4.0 | 0.6 | 4.0 | 0.6 | NS | NS |
| A550 soluble. | 0.08 | 0.02 | 0.05 | 0.01 | 0.07 | 0.07 | 0.07 | 0.02 | *** | *** |
| A550 insoluble. | 0.04 | 0.01 | 0.04 | 0.01 | 0.06 | 0.07 | 0.06 | 0.05 | NS | NS |
| **Leaf sheaths** | | | | | | | | | | |
| NDF (% OM) | 79.1 | 2.3 | 79.4 | 1.5 | 77.0 | 2.9 | 78.3 | 2.6 | NS | ** |
| DNDF (%) | 51.2 | 3.8 | 56.6 | 2.6 | 42.8 | 10.1 | 55.3 | 5.3 | *** | * |
| Lignin (% OM) | 6.3 | 0.9 | 5.7 | 0.5 | 6.1 | 0.8 | 5.8 | 0.7 | *** | NS |
| A550 soluble | 0.14 | 0.05 | 0.03 | 0.01 | 0.57 | 0.20 | 0.05 | 0.03 | *** | *** |
| A550 insoluble | 0.04 | 0.01 | 0.02 | 0.01 | 0.19 | 0.11 | 0.03 | 0.02 | *** | *** |
| **Stems** | | | | | | | | | | |
| NDF (% OM) | 72.2 | 7.4 | 74.5 | 5.5 | 78.4 | 6.2 | 79.8 | 3.3 | *** | ** |
| DNDF (%) | 52.9 | 5.6 | 54.1 | 3.9 | 57.4 | 5.0 | 57.0 | 4.7 | ** | NS |
| Lignin (% OM) | 6.8 | 1.4 | 7.0 | 1.3 | 6.7 | 1.1 | 6.6 | 0.8 | NS | NS |
| A550 soluble. | - | - | - | - | - | - | - | - | - | - |
| A550 insoluble. | 0.03 | 0.01 | 0.02 | 0.01 | 0.01 | 0.00 | 0.01 | 0.00 | *** | *** |

NS=not significant; **=P<0.01; ***=P<0.001. A550 = absorbance at 550 nm

Adapted from Reed *et al.*, 1988.

The genetic influence on straw is relatively clear in sorghum where the difference in fibre digestibility in leaves of BR and non-bird resistant (NBR) varieties has shown strong correlation with the levels of insoluble proanthocyanidins in these plant parts (Reed *et al.*, 1987) (Table 2.2). Akin (1988) cited experiments where genetic manipulations have produced mutants of maize and sorghum that differed in phenolic composition but had similar total phenolic contents. The quality of straw from these mutants was also improved by the genetic manipulation. Besides genetic factors that affect the nutritive value of stover, environmental conditions may partly account for the varied concentrations of deleterious substances in sorghum varieties. In selecting which sorghum varieties to grow, plant breeders have been concerned with selecting for varieties that are early maturing in order to reduce the risk of drought effect on grain yield. For example, in a trial with 15 sorghum varieties grown over a two-year period of low (342 mm) and high (570 mm) rainfall in Ethiopia, Kebede *et al.* (1990) obtained mean grain yields of 2.4 and 3.7 t/ha for the low and high rainfall years, respectively. The authors concluded that low stress-susceptible genotypes, which were early maturing, had a high potential under drought conditions. Significant interactions between variety x year and between variety x location have also been observed in other cereals like barley (Orskov, 1988; Erickson *et al.*, 1982). But the consistent ranking of varieties based on digestibility of their straw indicate that the variations are due to environmentally modified genetic potential of the varieties. Depending on the influence on the leaf:stem ratios and on the distribution of soluble nutrients between stems and leaves, soil fertility can have a significant influence on the nutritive value of straws.

Adverse weather and moisture stress tend to increase lignification of the parenchyma cells and this will lower stover quality (Akin, 1988). Environment is also likely to affect the nature and amount of cell wall phenolics leading to substantial variation in digestibility.

Erickson *et al.* (1982) observed that the quality of barley straws produced in locations where the grain yields were low were higher than the quality of straw of the same cultivar produced in locations where grain yields were high. They also noted that nitrogen content was higher in straws of malt barley than in straws from grain barley cultivars. They attributed this effect to the differences in translocation of nitrogen between the grain and vegetative parts. It has been reported that crops which were subjected to drought before anthesis had better quality straw because of interference in nutrient translocation for grain filling (Pearce *et al*, 1988). Since sorghum falls within the graminaceous family, it is expected that it will also be affected by the above environmental factors in the same way.

### 2.3.3 Sorghum grain and bird resistance

In areas where sorghum is produced, birds are the major pest and limit grain production from this cereal (Bullard and Elias, 1980). At least 600,000 tonnes of cereal grains (about 1.0 % of total cereal grains production in Sub-Saharan Africa) are lost annually through bird predation (Doggett, 1988). In a bid to increase grain yield, sorghum varieties have been selected based on bird-resistance to deter birds which to a considerable extent could devastate the sorghum crop. The bird-resistant trait is related to the presence of proanthocyanidins (condensed tannins) in the grain (Gupta and Haslam, 1980) and residues (Reed *et al.*, 1988) of improved varieties. The phenolic compounds in the new varieties resulted in bitter tasting sorghum grain as well as stover whose nutritive value has been questioned (Reed *et al.*, 1987). The phenolic content of the vegetative components of BR and

forage varieties is negatively associated with digestibility (Saini *et al.*, 1977). The differences between BR and NBR varieties is in the content of phenolics and their influence on the utilization of sorghum stover by ruminants will be discussed later.

Although knowledge of cell chemistry is still incomplete, it is known that most phenolic compounds are an integral part of the hemicellulose fraction of the plant (Mueller-Harvey *et al.*, 1988). Some tropical crop residues contain about 3% simple phenolic acids by weight (Mueller-Harvey *et al.*, 1988). Because sorghum can synthesize many different phenolic compounds in large quantities compared to other cereals (Butler, 1988), they have been investigated quite extensively (Reed *et al.*, 1988; Cherney *et al.*, 1992). Results showed that concentrations differed between varieties and that contents are higher in both leaves and stems of BR than in material from NBR varieties (Reed *et al.*, 1987; Ebong, 1989; Osafo, 1993; Nsahlai *et al.*, 1998). Thus, phenolic compounds, in particular condensed tannins, may be responsible for the differences in feeding values observed between BR and NBR sorghum varieties (Nsahlai *et al.*, 1998).

**2.4 Performance of livestock fed sorghum stover**

Intake is the most important variable affecting productivity in ruminants and is affected by palatability, digestibility, fill, passage rate and texture. Intake of sorghum stover based diets is usually below the level required to maintain the animal's body weight (Table 2.3). Its tough texture, poor digestibility and nutrient deficiencies all contribute to its low level of consumption. Stovers are often chopped in order to reduce bulkiness, increase consumption and reduce wastage. This practice forces the animal to eat more of the low quality parts and reduces the nutritive value of what is actually consumed.

**Table 2.3 Performance of sheep fed sorghum stover supplemented with urea or Lablab (*Lablab purpureus*)**

| | Stover alone | Stover + 90 g<br>10 % lablab | Stover + 180 g<br>20 % lablab | Stover + 270<br>30 % lablab | Stover + Urea |
|---|---|---|---|---|---|
| Stover intake (g/day) | 401.3 | 367.1 | 372.3 | 223.5 | 427.0 |
| Total DM intake (g/day) | 401.0 | 452.2 | 542.2 | 478.8 | 427.0 |
| Total DM intake (g/kgW.75/day) | 41.3 | 45.4 | 57.2 | 47.7 | 43.6 |
| Liveweight gain (g/day) | -20.9 | 15.9 | 36.1 | 47.7 | 9.3 |
| Feed efficiency | | 28.4 | 15.0 | 10.0 | 45.9 |

Adapted from Adu *et al.* 1992

Intake of sorghum stover which has high levels of tannin may be reduced by astringency (Bate-Smith, 1973). Table 2.4 shows the effect of variety on intake of sorghum crop residue in mature highland Zebu oxen. High levels of tannin may depress the feed intake in two ways. Firstly, they may slow down the digestion of dry matter (DM) in the rumen, react with the outer gut mucosa, and thus diminish the permeability of the gut wall. This will stimulate signals of physical distension, an important feedback signal in ruminants for controlling feed intake. Secondly, the depression in feed intake may just be due to unpalatability. Many studies however, draw the conclusion that depression in intake is due to the phenolic content of sorghum stover, but this may not necessarily be so (Murdiati and Mahyudin, 1985).

Most of the energy obtained by ruminants fed sorghum crop residue comes from the rumen fermentation of cell wall carbohydrates. Factors that limit the fermentation of these carbohydrates would have the greatest influence on differences in nutritive value between varieties after nitrogen deficiencies have been corrected. For example, leaf blades and leaf sheaths from BR varieties have higher levels of insoluble proanthocyanidins and soluble pigments than those of NBR varieties (Reed *et al.*, 1988). Leaf sheaths from BR varieties are higher in lignin than those from NBR varieties. In leaves, lignin, insoluble proanthocyanidins and soluble pigments contents are negatively correlated with the rate and extent of NDF digestion and digestibility at 48 hours, and positively correlated with indigestible NDF (Reed *et al.*, 1988).

Digestibility of straws is often limited by lignification. Lignin-polysaccharide interactions in the cell wall are the most important factors affecting straw quality (Chesson, 1988). Tables 2.2 and 2.4 show the effects of variety of sorghum on lignin, NDF and tannin

**Table 2.4: The effect of sorghum variety on intake and digestibility of crop residue by highland zebu oxen**

| Sorghum Variety | Percent leaves | DMI (kg/d) | DMD (g/kg) | OMD (g/kg) | NDF Dig. (g/kg) |
|---|---|---|---|---|---|
| MW5020 (BR) | 43.7 | 4.11 | 540.0 | 590.0 | 660.0 |
| Buraihi (NBR) | 23.2 | 4.43 | 580.0 | 600.0 | 680.0 |
| 2KX17 (BR) | 37.9 | 4.90 | 590.0 | 590.0 | 640.0 |
| Melkamash (NBR) | 39.3 | 4.96 | 570.0 | 600.0 | 660.0 |
| 5DX-160 (BR) | 35.3 | 5.18 | 580.0 | 610.0 | 630.0 |

Adapted from Reed and Hoefs, 1987; Reed *et al.*, 1988
BR = Bird resistant, NBR = non-bird resistant.

contents and also on intake and digestibility of stover. Tannin bound to protein and fibre in the feed may diminish digestibility of these components. Insufficient levels of degradable nitrogen in sorghum stover diets containing tannin may result in reduced digestibility of DM and fibre (Mueller-Harvey *et al.,* 1988). It is known that tannin can form complexes with dietary proteins (Vaithiyanathan and Kumar, 1993) as well as endogenous proteins including enzymes. The protein bound to tannin is most unlikely to undergo normal metabolism. Furthermore, tannin-enzyme interaction would inhibit the enzyme activity. Tannin has been shown to inhibit a broad spectrum of enzymes in *in vitro* assays (Horrigome *et al.,* 1988; Kumar, 1992). Aboud *et al.* (1991) fed two varieties of sorghum stover (BR and NBR varieties) to growing sheep and found that dry matter digestibility (DMD) was lower for BR sorghum stover compared to NBR stover. The authors suggested that the lower digestibility of DM of BR stover could not be attributed to the high phenolic contents in the BR variety alone but that another factor such as leaf:stem ratio of varieties could be important in explaining the differences found.

Blytt *et al.* (1988) observed that digestive enzymes might retain full activity in the presence of tannin *in vivo*, possibly due to the presence of detergents and unfavorable pH conditions that prevent tannin from binding to proteins. Decreased digestibility of organic matter and fibre has been attributed to increasing levels of tannin in the diet. Several studies (Barry and Manley, 1984; Reed *et al.*, 1990) reported a significant reduction in the extent of organic matter digestion in the rumen of sheep fed fresh forage legumes (FLs), which was partially attributed to the irreversible binding of tannin to fiber fractions. Despite the lower fiber digestion, whole tract digestibility was compensated by an increase in net protein outflow from the rumen (Barry and Manley, 1984) to be digested in the lower gut.

## 2.5 Measures to improve utilization of sorghum stover.

Fibrous crop residues have been used as ruminant feed from time immemorial. As noted above, residues are low in readily available energy, nitrogen, minerals and vitamins and do not provide adequate amounts of nutrients even to maintain the animal's body condition because of low intake and digestibility (Preston and Leng, 1984). Since the stover is short in both nitrogen and available energy, its improvement as animal feed can be achieved through pre-treatments or supplementation with deficient nutrients at the time of feeding or the combination of physical and chemical treatments and nutrient supplementation.

### 2.5.1 Physical treatment

Physical treatment affects the ultrastructural make up of fibrous material such as stover. Physical form and chemical composition of feed affect intake among others. Chopping and grinding reduce particle size though the size after chopping varies considerably (Sharma *et al.*, 1993) and this increases digestibility. However, dry matter intake (DMI) and rate of passage of liquid digesta may not be affected by chopping. For example, Singhal *et al.* (1991), working with oat straw found that chopping had no effect on DMI and rate of passage of liquid digesta. Feeding of chopped or long stover has also been reported not to affect DMI and digestibility in sheep by Devendra (1983). The effect of grinding on intake is inversely related to the quality of roughage, intake being more in case of crop residues compared to dried grasses (Sharma *et al.*, 1993). These effects may result because of increased rate of passage as well as better fermentation in the rumen (Sundstol and Owen, 1984). Grinding and pelleting increase voluntary intake, but frequently depress digestibility (McDonald *et al.*, 1984). Except for the decrease in digestibility of crude fibre,

grinding and pelleting appear to significantly improve the utilization of other constituents (Labuda *et al.*, 1979).

### 2.5.2 Chemical treatment

Chemical treatments have been imposed with the objective of improving the accessibility of structural carbohydrates to microbial enzymes in the rumen. Most attention has been given to treatment with alkali but digestibility may also be increased by treatment with oxidizing agents such as chlorine gas, ozone, hydrogen peroxide and sodium peroxide (Jackson, 1977). Ben Ghedalia and Miron (1981) have reported that treatment of straw with gaseous sulphur dioxide at 70% increased the *in vitro* organic matter digestibility (OMD) of the straw.

Improvement in digestibility due to chemical treatments is highly variable. In some cases digestibility decreased, apparently as a result of moulding or other fermentation and an increase in net lignin and phenolic absorbance from the formation of Maillard products from heating (Van Soest, 1988). However, much of the variation in the efficiency of treatment may be due to buffering capacity, which differed widely among the straws (Van Soest, 1988).

Chemical treatments may increase lignin solubility, reduce cell wall volume and increase cell content digestibility. The most commonly used chemicals are sodium hydroxide (NaOH), calcium hydroxide ($Ca(OH)_2$) and urea-ammonia (Tessema and Emojong, 1984 ; Ebong, 1989). Sodium hydroxide treatment of straw results in greater digestibility and animal performance than ammonia treatment (Males, 1987). Jayasuriya (1984) has pointed out that digestibility increases between 10 and 20%-units and intake increases of 30 - 50% can be expected when roughages are treated with NaOH. However, ammonia treated straws

have the advantage of added nitrogen (N) which reduces the need for supplemented N (Males, 1987). Males (1987) reported improvement in feed intake and increased DMD *in vivo* caused by NaOH-treatment of straw, associated with a decrease in retention time. Sodium hydroxide also increased solubilization of cellulose (Males, 1987).

Ebong (1989) reported improvement of *in vitro* digestibility of sorghum stover when it was treated with 80 g NaOH/kg DM. Reddy *et al.* (1989) treated sorghum stover with various concentrations of ammonia and reported that ammoniation improved voluntary intake in cattle by up to 230 g/kg DM. Digestibility of DM and OM were improved by 85 and 89 g/kg respectively by ammoniation of sorghum stover (Reddy *et al.*, 1989). Treating sorghum stover with urine may improve its palatability or may actually improve its nutritive value as a whole.

While improvement of feeding value of crop residues including sorghum stover by chemicals is technically feasible, generally, chemical treatments lack quality control and are expensive relative to the increase in nutritive value obtained (Tessema and Emojong, 1984; Wilkinson, 1984). This severely limits their application, and it may be more realistic to supplement a nutrient-deficient stover than to treat it. Preston and Leng (1987) noted that the methods for chemical treatments are rarely economical, difficult to apply and potentially hazardous to animals and people. Smallholders in the sub-tropics and tropics are unlikely to adopt this technology and therefore other suitable technologies have to be developed for improving crop residues.

### 2.5.3 Plant breeding and genetic selection

Cereal lines in breeding trials are usually evaluated on mean grain yield, although attention is sometimes given to yield stability and grain quality. Mean grain yield is most relevant where there is only one product of interest, such as grain, but may be misleading where there are joint products, especially if uses of the products differ. This is a dilemma, which is now faced by the sorghum breeders. The goal of improving stover quality through this strategy may not be feasible in the short term.

### 2.5.4 Plant, management and animal factors

#### 2.5.4.1 Plant factors

In studying other cereals like barley, it has been shown that straw botanical fractions vary in amount and nutritive value (Ramazin *et al.*, 1986; Tuah *et al.*, 1986; Petersen, 1988). In most straws except for rice, the degradation of leaf and sheath fractions is greater than that of stem (Wanapat and Devendra, 1985). Studies on degradation of sorghum stover fractions are very limited, but, the few studies reviewed reveal that the degradation pattern follows that of other cereals (Aboud *et al.*, 1991; Osafo, 1993; Nsahlai *et al.*, 1998). Mueller-Harvey and Dhanoa (1991) in a detailed study of sorghum stover using finger-prints in combination with pattern-recognition analysis, observed that the botanical fractions of leaf blade, leaf sheath and stem separated into distinct groups of HPLC chromatograms, indicated differences in the phenolic content of these fractions. Leaf sheath phenolics had highest variation. They further reported that at some sites environmental conditions were more important in influencing the phenolic content of the botanical fractions. Osafo (1993) found higher *in vitro* digestibility of NDF in leaves compared to the sheath and this was attributed to higher N and lower NDF content of leaves. Reed *et al.* (1987) working with sorghum stover

fractions also reported higher digestibility of leaves than of other fractions. It is possible that completion of the biosynthesis of leaf phenolics, which occurred earlier in the leaves than in the sheath (Khazaal *et al.*, 1993) might explain the higher digestibility of leaves, observed by Osafo (1993). Different varieties of sorghum stover have varying amounts of botanical fractions and thus the nutritive value of varieties are likely to be different on account of their botanical composition. These differences may ultimately contribute to nutritional differences between the sorghum varieties.

A lot of biochemical changes take place as a plant matures. For instance, cell wall contents decrease and lignification of parenchyma cells increase and hence the nutritive value of a plant decreases with age (Pearce *et al.*, 1988). Mosienyane (1983) reported that *in vitro* DMD for any residue harvested during stages earlier than full maturity was higher than 58%, and thus well above the critical level for maintenance of ruminant livestock. Aganga *et al.* (1996) observed that dry matter degradability of different varieties of sorghum and millet declined with advancing maturity. Wilson and Eastin (1982) have described the three different physiological growth stages of sorghum. The 50% flowering stage was described as the days (from planting) during which 50% of the plants flowered. The second, black layer stage is when a red mark or black layer appeared at the base of the grain. At this stage the plant had reached physiological maturity but may not be dry enough to be harvested. The third, harvest stage, is when the panicle is normally harvested. The NDF content in sorghum leaves and sheath is likely to increase with maturity and therefore, the decline in *in vitro* digestibility observed as the plants aged by Osafo (1993) and Aganga *et al.* (1996) is not surprising. Osafo (1993) found that digestibility of all fractions was highest at the 50% flower stage, which shows that quality will decline with maturity. Osafo (1993) also

observed that the phenolic content was higher for leaf and sheath at the black layer stage. The author attributed this observation to the fact that biosynthesis of phenolic compounds may have been higher at that stage. The influence of changes in the content of phenolic compounds in sorghum due to different physiological stages of growth on the nutritive value of the stover are not known. It is possible that these changes may have a profound effect on nutritive value of the stover.

**2.5.4.2 Management factors**

In order to realize the benefits of supplementation, quality of the basal feed is important. Quality of cereal stovers is, however, affected by several factors, some of which may be beyond the farmer's capacity to control. But there are factors within a farmers' control, and these are mainly management related. Information available with regard to variation in quality of sorghum stover as a result of stover management after grain harvest is very scanty.

As indicated earlier, stover is usually stored by various methods post grain harvest. The storage methods used include leaving stover as 'standing hay' in the field or harvesting and storing the stover in the field as a stack (Osafo, 1993). At times, farmers harvest the stover and have it transported to their homestead where the stover is either stored under cover or in the open as a stack. All these methods of storage would presumably influence the quality of stover. For example, stover left as 'standing hay' is likely to develop tillers if left long enough in the field and if there is enough moisture. The new growth of tillers could increase the nutritive value of the stover. But in the case where there is no moisture, stover left standing for extended periods after grain harvest will lose nutritive quality (Mosienyane, 1983) mainly due to leaf loss. Stover left standing and grazed *in situ* will have lower quality

because of loss of leaves as animals knock down and trample over the plants during grazing. Improvement in the quality of sorghum stover as animal feed can therefore be achieved in part through management practices that will minimize the leaf loss and other factors that influence quality.

**2.5.4.3 Animal factors**

Cereal crop residues are known to have low nutritive value (Owen and Jayasuriya, 1989). Most of the reports on nutritive evaluation of crop residues concerned the whole plant residue (Alhassan *et al.*, 1987). But it has become more evident that botanical fractions of crop residues differ in amount (Petersen, 1988) and nutritive value (Ramazin *et al.*, 1986). The leaf and sheath of most cereal crop residues are more digestible than the stem because leaf and sheath contain more nitrogen and are less lignified. The nutritive value of crop residues therefore will depend on the quantities of the separate fractions and their chemical composition (Petersen, 1988).

Livestock have peculiar foraging behavior, in that they will select the best plant parts if given the chance to do so. It is possible that intake could be enhanced by exploiting selective feeding behavior through generous allowances. University of Reading workers (Wahed and Owen, 1986) questioned the conventional way of *ad libitum* feeding where livestock are allowed to refuse 20% of feed offered. Owen *et al.*, (1989) drew on the evidence of improved intake reported by Gibb and Treacher, (1976) and Zemmelink, (1980) with grazing animals. These authors reported increased intake in ruminants when herbage allowance was increased up to four times that consumed. Increasing the amount of straw offered increased the quantities of botanical fractions available to the animal. The animals are thus presented with an opportunity to select the more digestible components and thus

increase intake (Wahed and Owen, 1986; Alimon, 1989). However, although this strategy may sound simple and practical, it has problems related to logistics. Offering judicious amounts of stover to livestock in smallholder systems may not always be possible. The land areas are too small to produce stover in amounts that would enable farmers to offer livestock the large quantities of stover required at a time.

### 2.5.5 Rumen environment alteration

The provision of N and non-protein nitrogen (NPN) such as urea and poultry waste are some of the strategies that have been used to manipulate the rumen environment to enhance roughage digestion and intake. The supplements work by providing additional nitrogen and minerals for rumen microbes to function more efficiently (Preston and Leng, 1987). The net effect of this strategy is to increase intake and improve digestibility of roughages (Leng, 1990). The use of this strategy is however limited by availability and cost of the materials (supplements).

### 2.5.6 Supplementation of sorghum stover

The qualities of sorghum stovers, notably the bird resistant varieties, are low (Reed *et al.*, 1987) and may need enhancement when fed to animals if any meaningful production level is expected. Physical and chemical treatments of stovers are technologically possible, but are often expensive and technically not suitable for smallholder farmers. Supplementation with deficient nutrients is an alternative approach to improve the utilization of cereal crop residues.

#### 2.5.6.1 Supplementation with agro-industrial by-products

Alternative strategies to improve the utilization of stovers that can be technically feasible are available. They include supplementation with high protein oilseed cakes such

as soybean, cottonseed and noug or other concentrate supplements e.g. wheat bran and sorghum bran (Krebs and Leng, 1984; Preston, 1986; Kiflewahid and Mosimanyana, 1987; Ebro, 1994). The addition of small quantities of highly digestible concentrates to low digestible roughages markedly increases the total energy intake by the animal (Hunter, 1988). Supplementing forage-based diets with concentrates can improve rumen fermentation, fibre digestibility and basal roughage intake (Nsahlai *et al.*, 1996). The primary role of N and energy supplementation is the provision of rumen degradable nutrients in order to increase intake and digestibility (Preston and Leng, 1987). Conservation of crop residues as animal feed, however, may be attractive where protein concentrates are available at modest prices. Indeed, in the Ethiopian highlands the price differential between roughages and oilseed cakes was marginal during the early nineties and as a result, the use of agro-industrial by-products was recommended (Alemu *et al.*, 1991). The general use of this strategy is constrained more by cost than availability.

**2.5.6.2 Forage legumes**

The cost of conventional N supplements such as oilseed cakes and other agro-industrial by-products prohibits the wide use of this technology, especially by smallholder farmers in tropical countries. Thus attention has been given to the use of legume fodder (MPTs, browses and herbaceous forages) as sustainable sources of limiting nutrients in roughage-based feeding systems (Mosi and Butterworth, 1985; Borens and Poppi, 1990; Varvikko and Khalili, 1993; Devendra, 1993). Strategies in this category that are economically feasible include intercropping of compatible cereal and legumes (Umunna *et al.*, 1995a), supplementation with browses (Reed *et al.*, 1990; Tanner *et al.*, 1990; Bonsi *et al.*, 1994) and with herbaceous legumes (Butterworth and Mosi, 1986; Mosi and

Butterworth, 1985; Said and Tolera, 1993; Abule *et al.*, 1995). Energy, N and mineral contents of legume fodder crops and browses make them attractive alternatives to expensive concentrates as supplements for poor quality stover.

A large number of legume shrubs and tree species have been documented as useful livestock fodders. Forage trees are readily available and have been integrated into farming systems (Atta-Krah and Okali, 1986). Along with diversity in size, there is a range of agronomic characteristics which has enabled establishment and growth to occur in different agroecological zones (Ivory, 1990). These include, for example, several *Sesbania sesban* accessions, *Sesbania goetzei, Leucaena leucocephala, Chamaecytisus palmensis* and several *acacia* species. These fodder trees are widespread in the sub-tropical and tropical ecoregions and have high potential as ruminant feed (Borens and Poppi, 1990; Lambert *et al,* 1989). Some forage legumes (FLs) such as *Desmodium intortum, Macrotyloma axillare, Vigna unguiculata, Lablab purpureus* and *Stylosanthes guianensis* were shown in agronomic trials to be adaptable and productive; these forages were further evaluated in feeding and digestibility trials and were found to be useful supplements to crop residue based diets (Boitumelo and Mahabile, 1992; Varvikko *et al.*, 1992; Getachew *et al.*, 1994; Abule *et al.*, 1995). For example, effect of MPTs on growth was examined using 40 Friesian x Zebu (Boran) crossbred cattle. Comparison was made among wheat middlings, *Vigna unguiculata Leucaena leucocephala* and *Chamaecytisus palmensis*. With exception of *Chamaecytisus palmensis,* the other FLs promoted higher growth (Varvikko *et al.*, 1992).

Fodder trees have nutritional diversity in terms of chemical composition and their effects on rumen microbes or the host animal (D'Mello, 1992) and these characteristics could influence intake and nutrient utilization by ruminants. These nutritional differences can be

attributed to maturity, concentration of antinutritional factors (ANFs) and form of presentation (fresh or dried) which determine their chemical composition, palatability, the extent and rates of degradation and passage out of the rumen. For example, most fodder trees have high rumen degradable protein (Ash, 1990), while others are bitter due to the presence of ANFs (Butler and Bailey, 1973), and therefore may need to be processed before feeding.

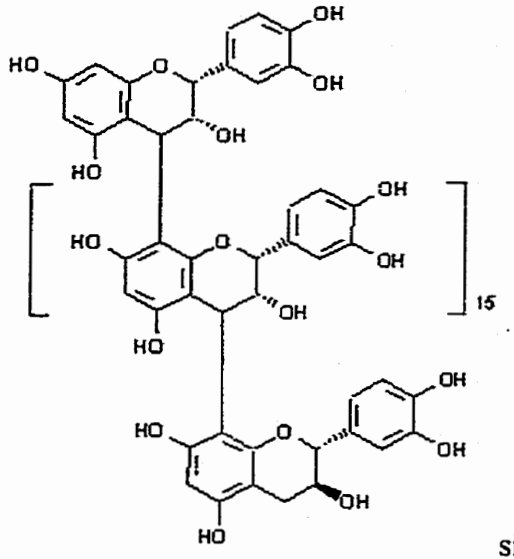
Forage legumes are well integrated into smallholder farming systems because of their versatility in terms of ease of establishment, adaptation, fertilizer and moisture requirements (Atta-Krah and Okali, 1986). However, some of these FLs synthesize secondary plant compounds (ANFs) as defense mechanisms against herbivory (D'Mello, 1992). Prominent among these are polyphenols, especially tannins, which could be considered as the most important deleterious principle in MPT's. They frequently occur at levels of 10-20% of the DM (D'Mello, 1992), while Reed (1986) in a study of 17 species of MPTs in East Africa reported contents of 13-50%. Other secondary compounds (e.g. cyanide, citrate, fluoro-acetate, alkaloids and terpenes) may produce toxic effects in ruminant animals as well as depress intake and/or utilization of feed components.

**2.5.6.3 Tannins and their effects**

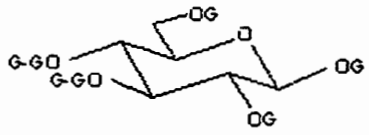
Tannins are polymers whose monomeric units are phenols and occur almost in all vascular plants. They constitute one of the widespread and diverse group of secondary metabolites (Kumar, 1992). The two major structural classes are hydrolysable and condensed tannins (Figure 2.1). Hydrolysable tannins are characterized by having carbohydrate molecules partially or totally esterified with monomer, dimers or higher oligomers of phenolic groups like gallic acid (gallotannins) or ellagic acid (ellagitannins). Hydrolysable tannins are susceptible to hydrolysis by acids, bases or esterases to yield carbohydrate and

Figure 2.1: Structural chemistry of tannins

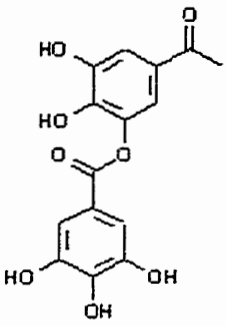
Structural chemistry of simple condensed and hydrolyzable tannin are shown below.



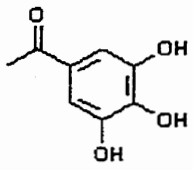
simple procyanidin from Sorghum.



G-G =



G =



Tannic acid is comprised of a mixture of polygalloyl esters of glucose like that shown here.

Courtesy : Ann E. Hagerman (1998). Structural chemistry of tannins. Internet information

the constituent phenolic acids (Haslam, 1989). Condensed tannins in nature are more widely distributed than hydrolysable tannins. Condensed tannins are oligomers and polymers of flavonoid units linked by carbon-carbon bonds that are not susceptible to cleavage upon hydrolysis. Condensed tannins are also called proanthocyanidins because they are degraded to form monomeric anthocyanidins (e.g. cyanidin, delphinidin) 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 astringency taste (Haslam, 1989).

The most commonly recognised property of tannins is that they bind proteins, a characteristic that has been recognised for centuries. Nevertheless, the tannin-binding mechanisms and the nutritional effects of this binding are not fully elucidated (McLeod, 1974; Reed *et al.*, 1990). Historically it was believed that tannins bind and precipitate all proteins nonspecifically, but it is now well recognised that tannin-proteins are specific and depend on the structure of both the protein and tannin (Hagerman et al., 1992). Condensed tannins have a more profound digestibility-reducing effect than hydrolysable tannins, whereas the latter may cause varied toxic manifestations due to hydrolysis in the rumen. The binding of condensed tannins with proteins inhibits fermentation of structural carbohydrates (D'Mello, 1992) in the rumen and reduces protein availability to rumen microbes.

Complex phenolic compounds (tannins and flavanols) are widespread, abundant and appear to be the major constraint of legumes as forages because of their effect on intake, digestibility and animal metabolism (Kumar and Singh, 1984). Free condensed tannins may bind protein and make it less soluble in neutral detergent and increase the content of NDF (Reed *et al.*, 1987). It has been noted that the large faecal N excretions in ruminants on MPTs

are invariably associated with consumption of high amounts of tannin in the diet.

Tannins in FLs have both negative and positive effects on nutritive value (Reed *et al.*, 1990; Mueller-Harvey and McAllan, 1992). Tannins in high concentrations reduce intake, digestibility of protein and carbohydrates, and animal performance (Barry and Manley, 1984; Barry, 1985; D'Mello, 1992). Despite the adverse effects on intake and digestibility, tannin may also protect protein from extensive degradation by rumen microorganisms and improve the quality of amino acids absorbed in the post-rumen gut (Waghorn *et al.*, 1987; D'Mello, 1992). The inverse relationship between tannin level in the forage and palatability, voluntary intake, digestibility, and N retention in mammalian herbivores is well established (Robbins *et al.*, 1987).

Depressed performance is an indication of an allelochemical effect (Lowry, 1990). Species like *Calliandra calothyrsus* and *Acacia angustissima* that have high phenolic content are known to depress performance (Lowry *et al.*, 1983). Many plant chemicals have effects, mainly inhibitory, on animal reproduction (Cheeke and Palo, 1995). It has been observed that reproductive performance, in particular reproductive structures/sperm cells, sperm volume and motility in sheep fed *L. leucocephala* (Senani *et al.*, 1996) or *L. pallida* and *S. sesban* accession 1198 (Azage, personal communication) were adversely affected. It is not clear however, whether this sterility is temporary and can be reversed. The implication of these observations in relation to female animals is still uncertain. Farmers in some parts of Ethiopia are reluctant to feed some of these FLs to livestock because they are allegedly implicated in reproductive problems. This may partly explain the low adoption rate of FLs within the farming systems.

Tannins may complex protein at normal rumen pH and protect the protein from microbial enzyme attack. These complexes are unstable at the acid pH of the abomasum where the protein becomes available for digestion (Jones and Mangan, 1977). For example, condensed tannins in *L. pedunculatus* have been associated with an increased flux of essential amino acids through the abomasum and increased net absorption of these amino acids (Waghorn *et al.*, 1987). It is therefore generally believed that at low to moderate concentrations, tannins increase the flow of non-ammonia N and essential amino acids from the rumen (Egan and Ulyatt, 1980; Barry and Manley, 1984; Waghorn *et al.*, 1987).

Tannins also promote metabolism of endogenous N in the digestive tract (Barry and Manley, 1984). Tannins lower the rate of protein degradation and deamination in the rumen and therefore lower ruminal ammonia ($NH_3$) (Woodward and Reed, 1990). The same author also observed that plasma urea nitrogen, ruminal $NH_3$, and urinary N loss were lower when sheep and goats were fed legumes that contained tannins (Woodward and Reed, 1989).

Tannin-protein complex that escaped rumen degradation can disassociate in the small intestine, where the majority of nutrient absorption took place. In non-ruminants, it has been shown that high tannins depressed performance (Potter *et al.*, 1967). For example, in pigs, Fan *et al.* (1997) reported that the adverse effects of extractable polyphenols in feeds on the digestive utilization of dietary carbohydrates and proteins were partly due to their direct interference with the normal functions of the small intestinal brush border membrane bound digestive enzymes. Tannins were found to adversely affect the intestinal mucosa causing disruption of brush borders and thus affecting absorption of nutrients and consequently poor performance (Mitjavila *et al.*, 1977). The inference from the above reports on effects of tannins in non-ruminants may suggest the potential problems of tannins that disassociate in

the small intestine of ruminants. There is limited data on this subject regarding ruminants. However, a marked reduction in milk yield and milk protein has been reported where cows were fed tannin-rich diet (West et al., 1993). This reduction was associated with the tannin level in the diet, thus pointing to a potential impairment of nutrient utilization.

It is likely that FLs rich in tannins will be superior as sources of bypass protein since tannins link with proteins during mastication, and appear to reduce microbial degradation of plant proteins (Reid *et al.*, 1984). The high levels of tannin in *L. pedunculatus*, whilst protecting protein from degradation, reduce digestibility of fibre by inhibiting the activity of bacterial enzymes (Chesson *et al.*, 1982) and fungi (Akin and Rigsby, 1988). Barry (1985) considered that the ideal concentration of condensed tannins was 0-40 g/kg diet dry matter; higher levels (76-90 g/kg) were detrimental. He also reported that sheep could adapt to high tannin levels provided that tannin-rich plants are only used as supplements (e.g. at less than 25% of the diet dry matter). At such levels, there is no serious problem and their presence in the diet may well be beneficial (Barry and Manley, 1984).

In conclusion, the seemingly negative attribute of tannins can be harnessed and used strategically to improve the value of certain proteins. Since tannins bind with proteins, they can be useful in "protecting" proteins of high solubility and degradability from rapid degradation in the rumen by allowing their digestion post ruminally for more efficient utilization. Since some FLs are highly rumen degradable while others are not, this attribute can be harnessed to improve the utilization of both forages.

An important secondary benefit of FLs production from fallow land is the improved subsequent crop yield obtained because of legume-fixed nitrogen. Mosi and Butterworth (1985) have argued that the use of legumes is also without the dangers of environmental

pollution caused by the excretion of large quantities of sodium; thus the danger of environmental pollution will be reduced by not using industrial chemical fertilizers. Availability of good quality forages for supplementation may lessen the attractiveness of other pre-treatment strategies such as physical and chemical treatment and this will reduce the risks associated with the use of some these strategies.

Large differences in response when FLs are fed as protein supplements have been reported and this has been attributed to the differences in the levels (concentrations) of phenolic compounds. Some research reports (Jackson *et al.* 1996; Khazaal *et al.* 1996; Kaitho *et al.*, 1998) suggest that the concentration of condensed tannins *per se* may not be the only factor impacting on the nutritive value of tropical forages. Although, Jackson *et al.* (1996) used the concentration of condensed tannins in forages in proposing a guide to the use of tanniferous forages, the authors cautioned that besides concentration "the biological activity (reactivity) of condensed tannins depend on other criteria such as chemical structure and degree of polymerization" (Horigome *et al.*, 1988). Indeed Khazaal *et al.* (1996) observed that biological response to polyphenolic compounds depends on their biological nature and this varies with plant species. This implies that considerable variation in animal response could occur when similar quantities of forage supplements from different species containing similar concentrations of condensed tannins are offered to livestock. It is recognized that other plant characteristics (such as NDF and ADF concentrations) could mediate the same response (Jackson *et al.*, 1996). The ability of tannins to form strong complexes with proteins is the most important aspect of their nutritional and toxicological effects (Hagerman and Butler, 1981). The strength of these complexes depends on characteristics of both tannins and protein such as molecular weight, tertiary structure,

isoelectric point, and compatibility of binding sites (Reed, 1995). Tannins have a large number of free phenolic hydroxyl groups that form hydrogen bonds with proteins and carbohydrates (Haslam, 1989). Tannins may also complex with protein through hydrophobic interaction (Oh *et al.*, 1980). In addition, tannins form covalent bonds with proteins through oxidative polymerization reactions as a result of heating, exposure to ultraviolet radiation, and the action of polyphenol oxidize (Reed, 1995). The above attributes in addition to concentration could affect the reactivity of tannins with proteins.

**2.6 Methods to alleviate ANF's in the forages**

Since BR sorghum variety contains ample quantities of phenolic compounds and with FLs (also rich in phenolics) constitute a substantial feed resources base in the semi-arid zones, it is possible that the use of both in diets may accentuate antinutritional toxicity problems since their total phenolics concentration may be above the threshold level for animals. Thus, any attempt to improve performance of livestock must first overcome the problems posed by high contents of tannins in the forages. Tannins hinder the utilization of forages and concentrates and thus affect the productivity of livestock. To obtain optimum animal performance, the animal rather than the plant must be considered and the ability of the animal to tolerate and use these forages must be maximized, with or without ameliorative measures (Lowry, 1990). Therefore, a major concern regarding the utilization of FLs (and other tanniferous feeds) is how to overcome allelochemical effects in order to increase the effective use of these forages. It has been observed that animals reduce chances of poisoning by consumption of mixtures of forages (Dicko and Sikena, 1992). But it is also recognized that sometimes animals show low preference for plants that are nontoxic but of high feed quality. Regardless, Le Houerou (1980) reported that consumption of mixed shrubs was

higher than that of a single species.

It therefore seems that if there is a problem with allelopathy, the question will be how to reduce it. One obvious response is to deal with the plant, perhaps to select for lower levels of allelochemicals or replace it in the feeding system with a more innocuous species (Lowry, 1990). However, plant toxins are an essential aspect of the plant's fitness for its particular environment. Without them the plant may be much less productive. If maximum primary productivity from FLs in the tropics is to be achieved, we must be prepared to accept that they will contain an array of antinutrients, which must be dealt with. Different strategies such as supplementation with adsorbent chemicals, soaking in water, heat treatment, dilution, genetic manipulation and use of rumen microorganisms have been used to alleviate the antinutritional attributes. These methods will be briefly discussed in the following sections.

### 2.6.1 Drying

Drying of forages may modify the nutritional effects of tannins (D'Mello, 1992). Terrill *et al.* (1989) observed that field drying of *Lespedeza cuneata* (high in tannin) decreased its assayable tannin concentration and this resulted in improved intake and increased N and fibre digestibility, while a low-tannin *L. cuneata* did not show similar effects. Palmer and Schlink (1992) reported that wilting (25 C for 24h) of *Calliandra* depressed feed intake in sheep when given as the sole diet over an 8 h period. Ahn *et al.* (1989) observed that oven-drying (50 C) of *Acacia aneura, A. angustissima, A. chinensis* and *C. calothyrsus* caused a reduction in 'active' tannin concentration and this improved N digestibility in the rumen by 35%. Table 2.5 shows the results from an experiment that examined the effects of drying supplemental *Gliricidia* and *Calliandra* leaf on the intake and utilization of barley straw by sheep. Drying alone increased straw intake and the digestibility

**Table 2.5. The effects of drying supplemental *Gliricidia* and *Calliandra* leaf on the intake and utilization of barley straw by sheep**

| Component | *Gliricidia* | | *Calliandra* | |
|---|---|---|---|---|
| | Fresh | Dried | Fresh | Dried |
| (a) Intake (g/d) | | | | |
| Tannins | 4.0 | 0 | 23.9 | 16.4 |
| Barley Straw | 392[a] | 680[b] | 436[a] | 691[b] |
| Browse | 200 | 200 | 204 | 200 |
| Total | 593[a] | 880[b] | 640[a] | 891[b] |
| (b) Digestibility | | | | |
| Dry matter | 42.3[a] | 60.5[b] | 36.3[a] | 59.0[b] |
| Nitrogen | 24.6[a] | 47.4[b] | 7.3[c] | 39.9[d] |
| (c) N utilization | | | | |
| N intake | 8.3[a] | 8.5[a] | 9.2[b] | 9.7[b] |
| N in faeces | 6.3[a] | 4.5[b] | 8.6 | 5.8[a] |
| N in urine | 2.9 | 2.6 | 2.4 | 1.4 |
| N balance | -0.9[a] | 1.4[b] | -1.7[a] | 2.5[b] |

Adapted from Ahn, 1990.

Different superscripts across the same line are significantly different

of neutral detergent fibre. Bonsi *et al.* (1994) observed high nitrogen degradation parameters for fresh compared to dry foliages and attributed this to the reduction in N solubility due to drying. Palmer and Schlink (1992) reported that fresh *C. calothyrsus* material was more rapidly digested than wilted, dried or freeze-dried material and the authors recommended the use of fresh forages for their maximum utilization.

Ahn (1990) found that drying and PEG infusion increased the digestibility of N, particularly in the rumen, which resulted in higher urinary N losses as urea. It was concluded from the study that the presence of both tannins and a heat labile compound in fresh *Calliandra* depressed feed utilization, and that drying removed the factor (not tannin) which depressed the digestibility (and intake) of barley straw by sheep.

Drying of MPTs reduced odours, leading to an improvement in palatability and intake (Bonsi *et al.*, 1996; Kaitho *et al.*, 1996). It is generally agreed that *Gliricidia* is a high quality fodder, but with low palatability when first introduced to animals (Norton, 1994). The odour of the leaves has been implicated in the initial reluctance of the animals to eat it (Brewbaker, 1986). However, once adapted, there appears to be no long term detrimental effects on sheep and goats consuming MPTs (Kaitho *et al.*, 1996). Ahn (1990) reported that drying removed all extractable tannins from *Gliricidia*, increased straw intake, dry matter and N digestibility and N balance in sheep. However, it was not possible to decide whether these effects were due to the tannins or some other factor removed or inactivated during drying.

Even though drying reduces tannins and improves nutritive value, sheep still consume some dried forage MPTs with reluctance e.g. *Gliricidia*, suggesting that the factor(s) associated with palatability were not completely removed by drying. Therefore, the efficacy of wilting and drying treatments in reversing the deleterious effects of tannins warrants further investigation (Kumar and D'Mello, 1995).

The effects of drying on some forage tannins are still not clear. For example, the level of tannins in fresh leaf of *Gliricidia* was much lower than that in *Calliandra* and also, drying removed all extractable tannins (Ahn *et al.*, 1997). Drying reduced true protein degradability, which may be explained by greater binding of tannins to proteins and/or a decreased solubility of plant protein arising from heat denaturation (Beever *et al.*, 1976). The strategy of drying may therefore not be useful in all forages.

### 2.6.2 Soaking in water

Simple soaking, washing and boiling with water removed up to 80% of tannins from sal seed meal (Singh and Arora, 1978). There are inherent problems related with this strategy. Firstly, there is considerable dry matter loss during the washing process which can result in loss of essential nutrients (Kumar and Singh, 1984). Secondly, in some areas, especially the sorghum growing regions (semi-arid ecozones), there is a general water shortage. In such areas, the strategy of using large quantities of water and extra labour will not be feasible.

### 2.6.3 Genetic manipulation

In plant kingdom, tannin content is simply inherited through one or two genes and it is not difficult to eliminate the tannin factor (Marshall *et al.*, 1981). Since tannins occur in many valuable sources of animal feed, including forage and tree foliage, it has been

suggested that the digestibility of feeds could be improved by breeding or developing low-tannin crop lines. A question arises as to whether in selecting for low tannin content a breeder may not lose important agronomic advantages of the high-tannin line. For example, high tannin sorghum with an open panicle structure appears to prevent bird depredation (Harris, 1969). Tannins are also responsible for plant resistance to pathogens and insects (Feeney, 1976; Schultz and Baldwin, 1982). Another desirable characteristic of tannins is weather resistance, particularly in retardation of preharvest seed germination and moulding (Harris and Burns, 1970). Given the protective function of tannins in the plant, however, such a procedure could lead to a loss of resistance to pests and diseases, thereby replacing a nutritional problem with one of agronomic or ecologic concerns.

**2.6.4 Rumen microbiology**

Degradation of condensed tannins by anaerobic microbes (Field and Lettinga, 1992) and of hydrolyzable tannin (Nelson *et al.*, 1995) by rumen microbes (Murdiati *et al.*, 1987) has been demonstrated. Recently Odenyo *et al.* (1997) working with *A. angustissima* showed that some rumen bacteria developed capability to detoxify the antinutritional attributes in the plant. Furthermore, occurrence of tannin-protein complex degrading *Streptococcus bovis* in the faeces of browsing animals has also been confirmed (Tsai and Jones, 1975). These studies indicate the potential of microbes in alleviating the deleterious effects of tannins or other phenolic compounds. Therefore, research is now being directed towards the identification and characterization of various rumen microbes capable of metabolizing tannin and testing their survivability in the rumen of unadapted animals. If these microbes could cohabit with normal microflora, tannin would be usefully fermented in the rumen. Though this is a long-term venture, it offers one possible strategy of

overcoming the antinutritive nature of these forages. As in the case of other suggested strategies, it is also not very practical at this moment to a smallholder farmer.

### 2.6.5 Reduction of toxicity by dilution

A simple approach to reducing tannin toxicity is to feed the toxic plant in mixture with other plants, thus diluting the effective level of each compound (Lowry, 1990). Although this technique is simple, it may not be easy to implement. The success of the technique will depend on the diversity of available plants and content of particular compounds that need to be diluted. The chemistry of secondary compounds is diverse and in general their effects may or may not interact in the animal (Kumar and Singh, 1984). Most compounds have an acceptable level below which no adverse effects are apparent; if so, the plant can then be fed safely as a set proportion of the diet. For example, a series of experiments showed that *Leucaena leucocephala* was an extremely useful feed with no toxic problems, providing intake was kept below 40% of the total diet (Jones, 1979). Therefore, it should be possible to select a set of several species, each possibly containing a different antinutrient factor, none of which can be fed as the sole diet but which may make an acceptable mixture (Lowry, 1990).

Forage tannins are complex phenolic polymers that vary in chemical structure and biological activity. More information is required on optimum dietary levels of forage supplements in mixtures appropriate to various ruminant species, methods to reduce the incidence and development of suitable mixtures of these in economic feeding systems for individual ruminants. Moreover, the required degree of dilution may be difficult to recommend because of the uncertainty of quantification of the antinutrients. It is also possible that differences between animal species exist in the response to tannins content in

the concerned portions and these need to be determined.

Several studies indicate that tannin-rich leaves, in combination with concentrate rations, can be fed to animals without any adverse effect (Kumar and Singh, 1984; Prasad *et al.*, 1997). This happens because animals consume protein in excess of their requirement from the concentrate and therefore the anti-nutritional effects of tannins are masked. This approach is not feasible since as much as twice the protein required by the animal is needed to alleviate the problems of the tannins present (Kumar and Singh, 1984). It may be possible that mixing FLs with different rumen protein degradabilities can elicit the same effects as providing extra N in the rumen for the animal. Feeding such a mixed diet to ruminants would present practical but solvable problems. The major constraint is that, in most cases, farmers do not have a choice of several forages and are usually dependent on one or two which they are able to grow on their farms or can be harvested from the bushes.

**2.6.6 Removal by supplementation with adsorbents**

Other strategies that may reduce the detrimental effects of phenolic compounds on the utilization of feed resources by ruminants have been employed. Polyethylene glycol (PEG) and polyvinylpyrrolidone (PVP) have been used to bind phenolics instead of protein and displace protein from preformed phenolic-protein complexes (Jones and Mangan, 1977; Garrido *et al.*, 1991). Recent studies have shown that PEG-4000 is more effective than PVP in displacing protein from the preformed complexes in a wider range of pH (3-7) (Makkar *et al.*, 1995). Tannins bind PEG-4000 in preference to protein; this renders the dietary protein free for digestion and, in addition, activities of endogenous proteins and enzymes are not affected (Kumar and D'Mello, 1995).

Polyethylene glycol (PEG), with a molecular weight of 4000 (PEG-4000) is a

nonionic detergent which forms complexes with hydrolyzable and condensed tannins over a wide pH range (2-8.5)(Jones, 1965). Spraying PEG on tannin-rich green chop, mixing with harvested leaves, infusion into the rumen, and drenching the animals has been reported to increase feed intake, digestibility, and wool growth in sheep (Kumar and Vaithiyanathan, 1990; Pritchard *et al.*, 1992; Terrill *et al.*, 1992). This improvement in animal performance was attributed to the protein released from protein-tannin complex by exchange reaction with PEG (Jones and Mangan, 1977). PEG-tannin complexes are insoluble in boiling water, most organic solvents, and neutral and acidic detergent solutions (Makkar *et al.*, 1995). Such complexes do not respond to most colorimetric methods for determination of tannins (Jones and Mangan, 1977; Makkar *et al.*, 1995). Hydrogen bonding between oxygen (ether) of the PEG chain and the phenolic hydroxyl group on the tannin moiety might explain the precipitation phenomena (Silanikove *et al.*, 1996). It suggests that there is a considerable analogy between PEG-tannin and protein-tannin complexation (Jones, 1965). Thus, the information obtained from the amount of PEG binding to plant sample might be analogous to that obtained from protein precipitation capacity.

Feeding experiments with sheep showed that condensed tannins of *Lotus corniculatus* (sainfoin) inhibited the release of soluble protein in the rumen and, when PEG-4000 was added, a large quantity of protein was released (Reid *et al.*, 1974). Jones and Mangan (1977) demonstrated *in vivo* with sheep and *in vitro* that PEG breaks the complex of protein with condensed tannins. Barry and Duncan (1984) applied this principle using PEG to *Lotus penduculutus* (which is high in condensed tannins) in laboratory studies and found that PEG was effective in reversing all the effects of condensed tannins.

However, researchers differ on the level of PEG required to release of protein from tannins and protein complexes or to prevent the complex from forming. For example, levels used in various sheep and goat feeding trials ranged from 0.22-6.67 g PEG per g of condensed tannins (Table 2.6) (Pritchard *et al.*, 1992; Silanikove *et al* 1994; Silanikove *et al.*, 1996; Silanikove *et al.*, 1997). In situations where PEG is employed to complex tannins, any uncomplexed or free tannins are likely to have a deleterious effects on the nutrition of the animal, the severity of which is related to both the quantity and reactivity of the uncomplexed free tannins. Therefore, if the beneficial effects of PEG are to be fully realized, it is necessary to establish the appropriate levels to be used in feeding trials. However, using PEG in routine animal feeding may not be economical and practical for resource poor farmers (Silanikove *et al.*, 1996).

### 2.6.7 Supplementation with other chemicals

#### 2.6.7.1 Sulphur

Since routine use of PEG is impractical and uneconomical in smallholder production systems, other practical strategies are required. Some suggested strategies for overcoming the antinutritive nature of phenolic compounds include the provision of appropriate supplements. This approach has a biological basis in the pathways by which the compounds are metabolized and excreted (Lowry, 1990). Most absorbed toxic compounds after metabolism in the liver are usually detoxified by means of excretion as a conjugate with glycine, glucuronic acid or sulphate (Lowry, 1990). In *A. aneura* (mulga), which contains high levels of phenolics and sulphur (McMeniman and Little, 1974), beneficial effects of sulphur supplementation have been demonstrated (Gartner and Niven, 1978). Gartner and Hurwood (1976) suggested that a considerable portion of the sulphur (as amino acids) in

**Table 2.6 Various amounts of Polyethylene glycol (PEG) used in animal feeding experiments by different researchers**

| | | Intake | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Condensed tannins | | PEG | | Feed DM | | PEG | Reference |
| Animal | Feed | g/kg DM | g/day | g/day | g/kg DM | g/day | g/kg $^{W.75}$ | g/g CT | |
| Sheep | *A. aneura* | 96 | 35.3 | 0 | 0.0 | 368 | 23.1 | 0.00 | 1 |
| Sheep | " | 96 | 55.2 | 12 | 20.9 | 575 | 36.2 | 0.22 | " |
| Sheep | " | 96 | 62.9 | 24 | 36.6 | 655 | 41.2 | 0.38 | 2 |
| Goats | *Ceratonia* | 5 | 4.0 | 0 | 0.0 | 799 | 55.5 | 0.00 | " |
| Goats | *siliqua* | 5 | 4.4 | 5 | 5.7 | 870 | 60.5 | 1.15 | " |
| Goats | *(Carob)* | 5 | 6.0 | 10 | 8.3 | 1200 | 83.4 | 1.67 | " |
| Goats | " | 5 | 6.3 | 20 | 16.0 | 1250 | 86.9 | 3.20 | " |
| Goats | " | 5 | 6.4 | 30 | 23.4 | 1280 | 89.0 | 4.69 | " |
| Goats | " | 5 | 6.0 | 40 | 33.3 | 1200 | 83.4 | 6.67 | " |
| Goats | *Quercus* | 9.5 | 6.3 | 0 | 0.0 | 664 | 46.1 | 0.00 | " |
| Goats | *calliprinos* | 9.5 | 7.1 | 5 | 6.7 | 750 | 52.1 | 0.70 | " |
| Goats | *(Oak)* | 9.5 | 8.1 | 10 | 11.8 | 850 | 59.1 | 1.24 | " |
| Goats | " | 9.5 | 8.3 | 20 | 23.0 | 870 | 60.5 | 2.42 | " |
| Goats | " | 9.5 | 8.6 | 30 | 33.3 | 900 | 62.5 | 3.51 | " |
| Goats | *Pistacia* | 20.5 | 9.5 | 0 | 0.0 | 465 | 32.3 | 0.00 | " |
| Goats | *lentiscus* | 20.5 | 14.6 | 10 | 14.1 | 710 | 49.3 | 0.69 | " |
| Goats | " | 20.5 | 16.8 | 20 | 24.4 | 820 | 57.0 | 1.19 | " |
| Goats | " | 20.5 | 17.0 | 30 | 36.1 | 830 | 57.7 | 1.76 | " |
| Goats | " | 20.5 | 16.4 | 40 | 50.0 | 800 | 55.6 | 2.44 | " |
| Sheep | *Ceratonia* | 27.5 | 15.5 | 0 | 0.0 | 562 | 31.3 | 0.00 | 3 |
| Sheep | *siliqua* | 27.5 | 20.9 | 12.5 | 16.4 | 760 | 42.3 | 0.60 | " |
| Sheep | *(Carob)* | 27.5 | 26.7 | 25 | 25.7 | 972 | 54.1 | 0.94 | " |
| Sheep | " | 27.5 | 26.8 | 25 | 25.7 | 974 | 54.3 | 0.93 | " |
| Sheep | " | 27.5 | 29.5 | 32 | 29.8 | 1074 | 59.8 | 1.08 | " |
| Sheep | " | 27.5 | 30.7 | 50 | 44.8 | 1116 | 62.2 | 1.629 | " |
| Goats | *Quercus* | 47 | 31.2 | 0 | 0.0 | 664 | 46.1 | 0.00 | 4 |
| Goats | *Calliprinos* | 47 | 39.3 | 10 | 12.0 | 836 | 58.1 | 0.25 | " |

1. Pritchard *et al*, 1992; 2. Silanikove *et al.*, 1994; 3. Silanikove *et al.*, 1996; 4. Silanikove *et al.*, 1997.

mulga was unavailable for digestion because of the complexing of tannins with proteins.

Pritchard *et al.* (1992) found that sheep improved in rate of gain when PEG was included in the diet based on tanniferous feeds. This improvement was attributed to the increasing levels of sulphur availability in the rumen with subsequent improvement in sulphur balance and digestibility. Ruminants require a dietary sulphur:nitrogen ratio of at least 1:10 to maximise digestibility (ARC, 1980) and responses are greater in sheep than in cattle (ARC, 1980). Sulphur is of similar importance to ammonia in the rumen, and bacteria derive at least half of their sulphur from the rumen sulphide pool (McMeniman *et al.*, 1976).

Sulphur is of special significance in the digestion of low-quality roughages, as loss in faeces is inversely related to roughage digestibility (Langlands *et al.*, 1973), and rumen anaerobic fungal colonization responds to the sulphur content of roughages (Akin *et al.*, 1983). The use of tanniferous diets may interfere with sulphur availability and provision of extra sulphur may alleviate some of the antinutritional problems. Thus one strategy worth investigating is the effect of supplemental sulphur in alleviating the deleterious effects of tannin-rich diets on ruminants.

**2.6.7.2 Urea**

If the interaction of dietary protein and tannins leading to the formation of indigestible protein-tannin complexes were the direct effect of dietary tannins, then supplementation of the diet with extra protein should eliminate it (Kumar and Singh, 1984). In this case, large quantities of protein would be required to annul the effect. Hagerman and Butler (1978) showed that for total incorporation of tannins in tannin-protein complex, at least twice as much protein as tannin (by weight) would be required. It is therefore, necessary to try other cheaper nitrogen sources as chemicals to annul the effects of tannins in high tanniferous feeds. Russell

and Lolley (1989) observed that tannin in high tannin sorghums can be deactivated rapidly and completely by reconstitution with aqueous urea. Treatment of high moisture sorghum with urea increased grain pH due to hydrolysis of urea to ammonia and was shown to be effective in the preservation of stored grain (Russell and Lolley, 1989).

Goodchild and McMeniman (1994) demonstrated the importance of minerals in maximizing the intake and digestibility of sorghum stover and in maximizing response to urea where the ration was deficient in N. It is thought that urea supplementation of tannin-rich feed can improve the feed quality by providing extra N for microbial synthesis and by its chemical activity. Urea supplementation of tannin-rich food has been a subject for research. Deoiled sal seed meal has a negative crude protein digestibility and the digestibility of a concentrate diet containing 20% deoiled sal seed meal was not affected in the presence of 10% urea (Kumar and Singh, 1984). There are mixed observations regarding the effectiveness of urea in high and low tannin foods (Kumar and Singh, 1984). As a result Kumar and Singh (1984) suggested that quantitative relationship between tannins and urea for improving the feed quality had to be worked out. From the practical point of view, supplementation procedures will be more applicable in smallholder farmer situations. Therefore, practical strategies to overcome the antinutritive effects of phenolic compounds suitable for smallholder use need to be developed. Concentration should be on chemicals on-farm readily available, especially chemicals that can be safely used by resource poor farmers.

## 2.7 SUMMARY OF LITERATURE REVIEW

Feeding of ruminant livestock in the tropics is based on crop residues and low quality native hay or pasture that are often deficient in both protein and energy. Cereal residues contribute the majority of feed resources for smallholder farmers, especially during the dry season. In the drier regions, sorghum is the most important cereal crop where birds are also major pests. Sorghum improvement programs have as such selected varieties that can resist the bird pest problem. Bird resistance, however, is related to presence of condensed tannins in the grain and residues. As such, condensed tannins in sorghum stover decrease nutritive value by complexing protein, carbohydrates and minerals. Therefore condensed tannins together with other management factors render the quality of the sorghum stover low and influence its utilization. There is, as a result, constant need for supplementary feeding to meet nutrient requirements even for maintenance. Quality of the cereal stover, which forms the major basal diet in the smallholder farms of the tropics and sub-tropics, is affected by several factors. Some of these factors may be beyond the farmers' capacity to control. But there are some factors, which the farmers are able to control and these are mainly management related. For example, the harvesting times and storage methods of stover after grain harvest are important and can greatly influence the quality of the stover.

From the review of literature, it is apparent that availability and cost of supplementation with high protein agro-industrial by-products are limiting factors among smallholders; thus attention has been given to the use of legume fodder (MPTs, browses, herbaceous forages) as sustainable sources of limiting nutrients. Many of these have high fodder production in addition to high protein and mineral content, which could improve livestock production. However these leguminous trees and herbaceous plants also synthesize

secondary plant compounds (antinutritional factors), which can influence their utilization by the animals. Among these compounds, condensed tannins appear to be the major constraint on the use of forage legumes as animal fodders. The literature suggests that condensed tannins in ruminant feeds have both detrimental and beneficial effects on voluntary intake and digestion of the nutrients of feeds. Tannins combine with proteins to produce complexes resistant to microbial attack in the rumen and the behavior of these complexes will depend on how tannins and nutrients interact. There is growing evidence to suggests that the biological activity of condensed tannins depends on other criteria as well as their concentration. The implication is that when forage supplements with similar concentrations of condensed tannins are offered at similar intake levels, considerable variation in animal response results.

Since sorghum stover from bird-resistant varieties contains ample quantities of condensed tannins and together with forage legumes constitute the feed resources that are available in the semi-arid zones, it is possible that the use of both in diets may accentuate antinutritional toxicity problems. Because of the low resource base of smallholders in the tropics, they depend on these leguminous crops as protein supplements. Practical strategies to overcome the antinutritive effects of the polyphenolic compounds are required in order to fully utilize these feed resources.

Several experiments were therefore carried out to (i) determine the optimum time of harvesting and better storage method for cereal crop residues, (ii) examine strategies for enhancing the utilization of high tannin feeds (bird-resistant (BR)sorghum stover supplemented with forage legumes (FLs) with varying tannin concentrations) by using ameliorants (polyethylene glycol (PEG), urea or sulphur).

# CHAPTER THREE

## 3.0 EFFECTS OF STAGE AT HARVEST AND POST - GRAIN - HARVEST MANAGEMENT PRACTICES ON THE NUTRITIVE VALUE OF CEREAL (SORGHUM AND MAIZE) STOVER.


### ABSTRACT

This study examined changes in the nutritive value of three cereal crops as influenced by period of harvest (relative to the physiological dead ripe stage of the grain) and storage method of the stover. Storage methods assessed the effects of leaving stover rooted in the field for extended periods post grain harvest, in comparison with harvesting the whole crop at various physiological growth stages and storing it under shade or in the open with or without grain heads or cobs. Three cereal crops (maize, (*Zea mays*), and two sorghum (*Sorghum bicolor*) varieties (Non-bird-resistant (NBR), Bird-resistant (BR)) were evaluated for fodder DM yield, chemical composition, N, NDF and CTs contents and nylon bag DM degradability. Stover DM yield, N, NDF and CTs contents were significantly ($P<0.001$) influenced period of harvest and storage method. There was significant ($P<0.001$) period of harvest x storage method interaction for stover DM yield, N and CT contents. DM degradability was also significantly influenced by period of harvest and storage methods ($P<0.01$). The highest nutrient concentration was recorded in stovers harvested at -2 week period of harvest (two weeks before physiological dead ripe stage of grain). Stover left rooted in the field after grain harvest had higher ($P<0.001$) stover DM yield but lower ($P<0.01$) N content compared to those harvested (cut) immediately after grain harvest. Storage of stover under shade resulted in higher ($P<0.05$) N and CT contents and reduced ($P<0.05$) stover DM yield. After harvesting, storing the whole crop with grain decreased ($P<0.05$) DM yield and N content but did not affect CT and NDF concentrations. Stover DM

degradability was affected by storage method ($P<0.001$). Storage under shade resulted in higher ($P<0.001$) degradability than storage in the field. Condensed tannin concentrations of the sorghum varieties also followed the same pattern as the DM and NDF, although the BR sorghum variety had significantly ($P<0.001$) higher CT content than NBR sorghum variety. DM degradability was affected by the physiological stage of growth. The two sorghum varieties had lower DM degradability than maize. It is concluded that timely harvesting of sorghum and maize and proper storage (whole crop cut with heads and cobs removed) in the open or under shade, can provide stover of considerable nutritive value. Therefore, a better utilization, for example, of otherwise tannin-rich BR sorghum stover may be possible through post-grain-harvest management of stover.

## 3.1 INTRODUCTION

There is a wide variation in the nutritional attributes of roughages available to ruminant livestock in the sub-tropics and tropics especially in the dry season. This variation in the feeding value of roughages or crop residues is influenced by the type of crop, soil fertility, morphological composition, variations within cultivars and harvesting and storage methods (Thiago and Kellaway, 1982; Pearce, 1983; Alhassan, *et al.,* 1987). For example, the management of stover after grain harvest may influence its quality. Harvesting, handling and storing systems should minimize loss of the more nutritious leaf and leaf sheath. In this regard, delayed harvesting would be expected to cause greater loss of leaf and leaf sheath, with a consequent reduction in nutritive value (Owen and Aboud, 1988).

Generally, information on the influence of post harvest management practices on the nutritive value of cereal crop residues like sorghum stover is scanty. It is possible that quality attributes can be affected by the length of time that stover is left in the field after grain harvest. In addition, sorghum can synthesize many different phenolic compounds (in particular condensed tannins) in large quantities (Butler, 1982) as compared to other cereals. Concentration of condensed tannins differs between sorghum varieties and is higher in both leaves and stems of the bird resistant (BR) than in the corresponding fractions of the non-bird resistant (NBR) varieties (Reed *et al.*, 1987; Ebong, 1989; Nsahlai *et al.*, 1998). Condensed tannins are known to affect the nutritive value of sorghum stover by depressing both intake and digestibility (Reed *et al.*, 1987; Nsahlai *et al.*, 1998). It is not clear whether quality attributes like condensed tannins content of the stover left standing for extended periods in the field would change or not. The different methods of managing stover post grain harvest could affect the quality of stover but such changes have not been documented.

The objectives of this trial were;

i) To monitor changes in the nutritive factors during different physiological growth stages of sorghum and maize crops;

ii) To assess the effects of stage at harvest and post grain - harvest - management methods on changes in the nutritive value of sorghum and maize stovers.

## 3.2 MATERIALS AND METHODS

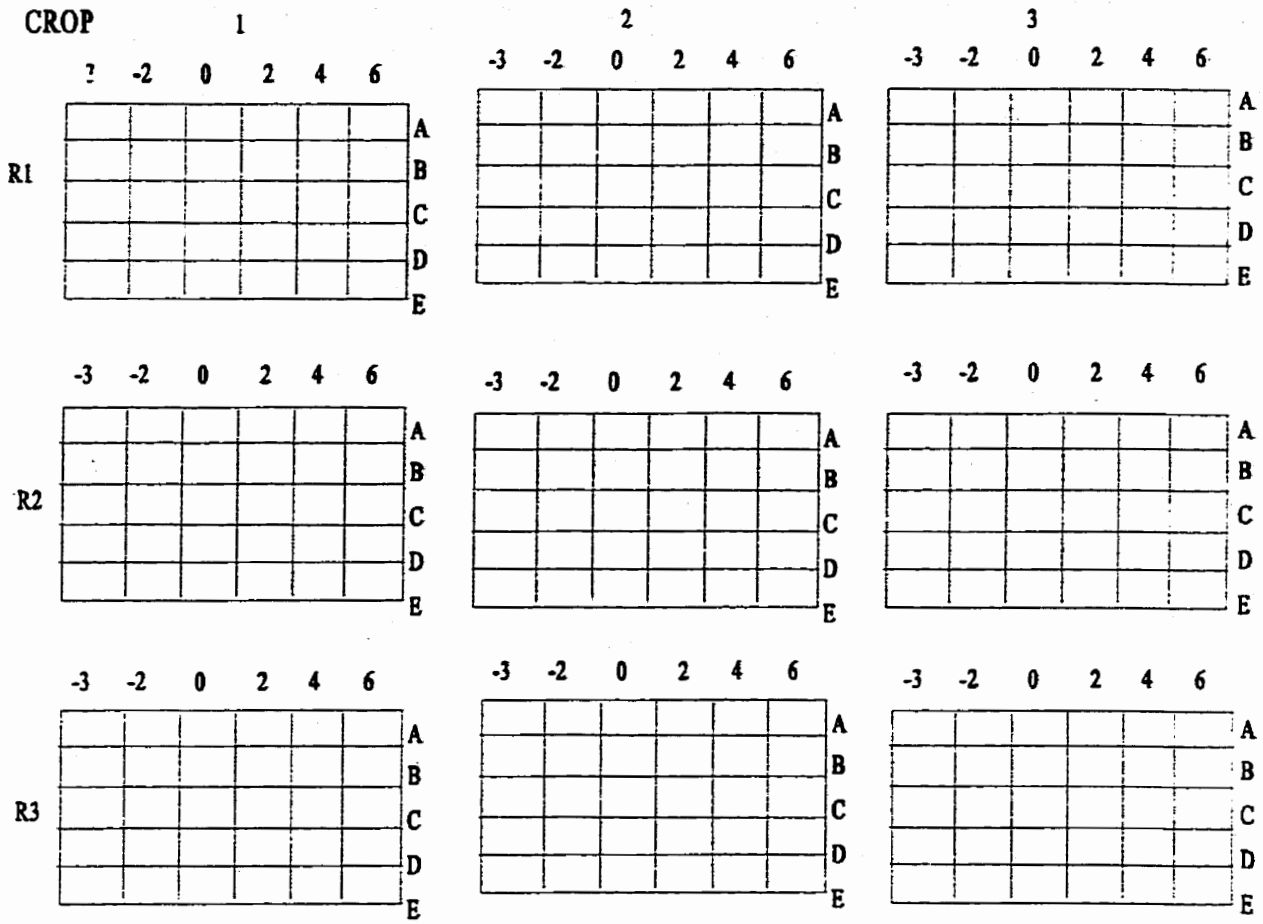
### 3.2.1 Experimental location and site

The experiment was conducted in the 1996/97 growing season (June-December 1996), at the Debre Zeit Research station of the International Livestock Research Institute (ILRI). Debre Zeit research station is in the Ethiopian highlands, at an altitude of about 1850 m above sea level. The average annual rainfall in Debre Zeit is 866 mm, of which 84% falls in the main rainy season extending from June to September. Average annual temperature is 18.7° C with maximum of 26° C and minimum of 11.3° C. The soil of the experimental site is light black soil (alfisol).

### 3.2.2 Land preparation, planting and crop management.

The field was ploughed and harrowed with a tractor and then demarcated into plots measuring 7m x 3m (21 square metre) in order to obtain 7 rows per plot (Figure 3.1). Diammonium phosphate (DAP) was applied by drilling prior to planting, at the rate of 100 kg/ha. Urea was applied as top dress two months later at the rate of 50 kg/ha. Three cereal crops, *Sorghum bicolor* (Bird-resistant (BR) and Non bird-resistant (NBR)), and *Zea mays* (maize) were planted in three replicates, on June 11, 1996. Seeding rate for both sorghum varieties was 10 kg/ha while maize was seeded at a rate of 30 kg/ha. After thinning, the distance between plants within rows for all the three crops was 25 cm while that between rows was 75 cm resulting in a plant density of 54,000 plants/ha for all the three crops.

## Figure 3.1 Experimental layout




R1, R2 and R3 are replicates of each of the crops

Crops 1=Non-bird resistant sorghum, 2=bird resistant sorghum and 3=maize

(-3,-2,0,2,4,6) are different times of harvesting grain and stover

-3 or -2 are weeks before harvest time and 2,4,6 are weeks after

A, B, C, D, E for different storage methods

### 3.2.3 Effects of stage at harvest and storage methods

#### 3.2.3.1 Stage at harvest

Stover harvest was carried out at six different periods (in weeks) before and after physiological dead ripe stage: -3, -2, 0, 2, 4, 6. The physiological dead ripe stage corresponds to the time when the seeds are physiologically mature and have started to dry out. The physiological maturity stage was when a red mark or black layer appeared at the base of the grain and this corresponded to -3 period of harvest. Harvesting started on October 14th, 1996 and ended on December 16th, 1996. Samples were collected for laboratory analysis at the various periods and dry matter determined on them immediately after harvest.

#### 3.2.3.2 Storage methods

During stover harvesting session, 5 storage methods were applied.

(A) Maize cobs and sorghum grain heads were removed and the stovers left rooted in the field until about 85% dry.

(B) The whole plant was cut and dried with cobs or grain heads in the field. Cobs or grain heads were later removed after drying to about 85 % DM.

(C) The whole plant was cut and dried without cobs or grain heads in the field.

(D) The whole plant was cut and dried with cobs or grain heads under shade. Cobs or grain heads were later removed after drying to about 85 % DM.

(E) The whole plant was cut and dried without cobs or grain heads under shade.

### 3.2.4 Effects of physiological growth stage on nutritive quality

Fresh samples of maize or sorghum plants were collected at three stages of growth (boot, milk and dough stages) to study nutritive quality changes at different physiological growth stages. Harvesting that corresponded to these physiological growth stages started on August 27, 1996 and ended on October 14, 1996 for BR sorghum variety, while for NBR

variety and maize, harvesting started on September 3, 1996 and ended on October 29 and October 21, 1996 respectively.

### 3.2.5 Measurements

Before harvesting, each plot was divided into eight subplots and seven rows. The eight subplots represented the periods of harvest, while the rows represented the storage methods. The outer subplots and rows were used as guard rows. Fodder yield of each crop was estimated by harvesting and weighing the entire plot (excluding guard rows) of the three replicates. At each harvest period, all the three crops were harvested on the same day at ground level. After harvesting and weighing each subplot, sub-samples of plants were taken, cut to small pieces (about 2-4 cm), put in plastic bags and taken to the laboratory to determine the DM content by weighing 100 g of samples and drying in an oven at 100°C for 48 h. Samples for chemical analyses (nitrogen (N), neutral detergent fibre (NDF), condensed tannins (CTs)) and for *in vivo* nylon bag DM degradability were collected after the plants were subjected to the above storage procedures.

For the nylon bag study, dry weight of sample before and after incubation for 24 and 48 h were noted and disappearance of dry matter calculated by difference. The feed samples were incubated in the rumen of three fistulated steers fed grass hay (900g organic matter (OM), 9 g nitrogen (N) and 730 g neutral detergent fibre (N) per kg DM) *ad libitum* and supplemented with 2 kg per head per day cottonseed cake (910 g OM, 86 g N and 380 g NDF per kg DM).

### 3.2.6 Laboratory analysis

Samples of crop residues were dried in the oven at 40°C to constant weight. Samples for chemical analyses were then milled to pass through a 1-mm and 2-mm sieve. The DM and N content of samples and nylon bag residues were determined using the standard

methods (Association of Official Analytical Chemists, 1980). NDF was estimated by the method of Goering and Van Soest (1970). Total CTs were estimated by the method of Giner-Chavez *et al.* (1997). Soluble CTs were determined by extracting 200 mg of plant material with 8ml of 70% aqueous acetone 3 times for twenty minutes. Thereafter, 1 ml of the extract was added to 5 ml n-butanol/HCL (95/5, v/v) and the solution was then placed in a water bath at 100°C for 1 hour, following which absorbance of the solution was read at 550 nm . A subsample of 10 mg of residue from the above was placed in culture tubes with 1 ml of 70 % aqueous acetone (v/v) and n-butanol/HCL (95/5, v/v, 5ml) was added. The insoluble CTs were determined using the same procedure described for soluble tannins. The fibre bound CTs were measured in triplicate as described by Reed *et al.* (1982). A subsample of 10 mg of NDF was placed in culture tubes with 1 ml of 70 % aqueous acetone (v/v). The sample was heated for 1 hour at 100°C in n-butanol/HCL (95/5, v/v) then absorbance read at 550nm. The total amount of CTs present in a sample was calculated by adding the amounts of soluble, insoluble and fibre bound.

### 3.2.7 Statistical analysis

Data were statistically analysed using a split plot design in SAS package (SAS, 1987). Analysis of variance to test for main effects of crop, period of harvest and storage methods and crop x period of harvest, crop x storage methods, period of harvest x storage methods and crop x period of harvest x storage methods interactions were performed. When significance of main effects were detected, non-orthogonal contrasts were carried out for: BC vs DE to test for the shade effect, BD vs CE to test for the effect of drying with grain and A vs C to test for the effect of crop standing in field after grain harvest.

## 3.3 RESULTS

### 3.3.1 Effects of period of harvest and storage methods

#### 3.3.1.1 Stover DM yield

Stover DM yield (Figure 3.2 and Table 3.1) was significantly ($P<0.001$) influenced period of harvest (period) and storage methods (storage).There was significant ($P<0.001$) period x storage interaction. The highest stover DM yield (8.2 tonnes DM/ha) was recorded at the -2 week (period 2) period of harvest. Thereafter, the yields significantly ($P<0.001$) declined. Maize had the highest stover DM yield (7.8 tons DM/ha) followed in order by BR sorghum variety (7.6 tons DM/ha) and NBR sorghum variety (7.4 tons DM/ha) (Appendix Table 1.). The three crops tended to follow the same pattern across the different periods of harvest except for the 0 week period when BR sorghum yielded the highest DM.

Delayed harvesting of stover after grain removal affected stover DM yield. Leaving the stover rooted in the field for extended periods compared to cutting the whole crop immediately after grain harvest (field drying effect) produced significantly ($P<0.001$) higher stover DM yield (Table 3.1) for the sorghum varieties ($P<0.001$) and for maize ($P<0.01$) at all periods of harvest. Storage of stover under shade (shade effect) significantly reduced yield for sorghum varieties ($P<0.001$) and maize ($P<0.05$). Storing stover with cobs or grain heads (grain effect) until dry increased ($P<0.05$) stover yield for maize and reduced ($P<0.05$) yield for NBR sorghum variety, while yield for BR sorghum varieties was not affected ($P>0.05$)

#### 3.3.1.2 Nitrogen content

Stover N content was significantly ($P<0.001$) affected period of harvest and storage methods. There was also significant ($P<0.001$) period x storage interaction (Figure 3.3, appendix Table 3.2). Nitrogen content was highest at the -3 week (period 1) period of harvest but steadily decreased with time until the last period of harvest (Table 3.2). The N

Figure 3.2 Effect of period of harvest and storage methods on yield (tonnes DM/ha) of cereal stover

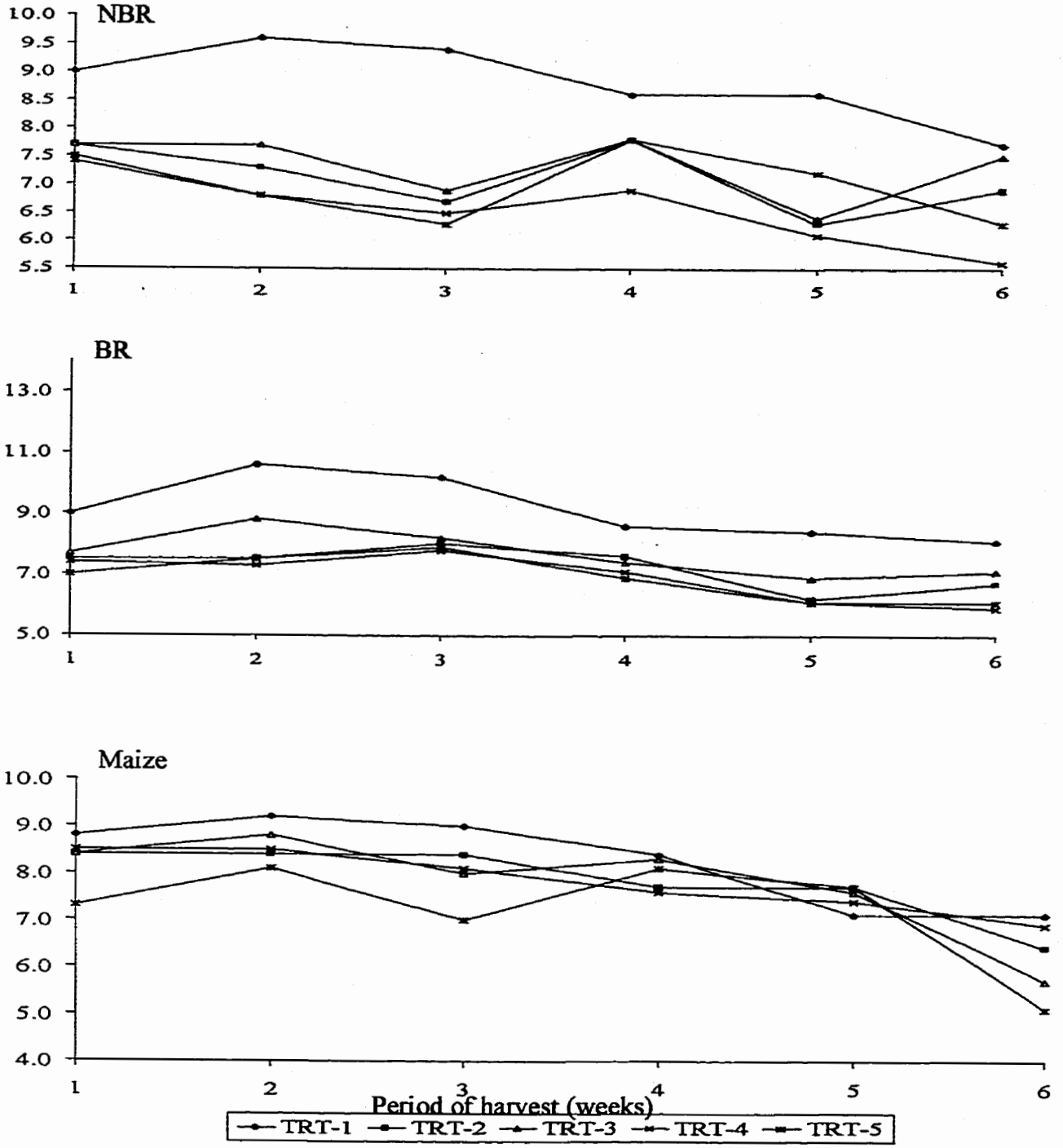


Table 3.1. Analysis on changes in yield over period of harvest

| CROP | Storage methods | Period of harvest LSMEAN | Standard error LSMEAN | Pr > \|T\| H0:LSMEAN=0 |
|---|---|---|---|---|
| MAIZE | 1 | -0.2360 | 0.0305 | 0.0001 |
| MAIZE | 2 | -0.1905 | 0.0305 | 0.0001 |
| MAIZE | 3 | -0.2583 | 0.0305 | 0.0001 |
| MAIZE | 4 | -0.1781 | 0.0305 | 0.0001 |
| MAIZE | 5 | -0.1840 | 0.0305 | 0.0001 |
| SORGHUM(BR) | 1 | -0.2070 | 0.0305 | 0.0001 |
| SORGHUM(BR) | 2 | -0.1328 | 0.0305 | 0.0002 |
| SORGHUM(BR) | 3 | -0.1515 | 0.0305 | 0.0001 |
| SORGHUM(BR) | 4 | -0.1813 | 0.0305 | 0.0001 |
| SORGHUM(BR) | 5 | -0.1538 | 0.0305 | 0.0001 |
| SORGHUM(NBR) | 1 | -0.1612 | 0.0305 | 0.0001 |
| SORGHUM(NBR) | 2 | -0.0890 | 0.0305 | 0.0075 |
| SORGHUM(NBR) | 3 | -0.0608 | 0.0305 | 0.0576 |
| SORGHUM(NBR) | 4 | -0.1576 | 0.0305 | 0.0001 |
| SORGHUM(NBR) | 5 | -0.0445 | 0.0305 | 0.1571 |

BR = Bird resistant, NBR = Non-bird resistant

| Contrast | DF | Contrast SS | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| maize: shade effect | 1 | 0.2486 | 0.2486 | 5.7700 | 0.0244 |
| Sorghum (BR): shade effect | 1 | 0.8788 | 0.8788 | 20.3900 | 0.0001 |
| Sorghum (NBR): shade effect | 1 | 0.6760 | 0.6760 | 15.6900 | 0.0006 |
| Maize: grain effect | 1 | 0.3339 | 0.3339 | 7.7500 | 0.0103 |
| Sorghum (BR): grain effect | 1 | 0.1248 | 0.1248 | 2.9000 | 0.1017 |
| Sorghum (NBR):grain effect | 1 | 0.3094 | 0.3094 | 7.1800 | 0.0131 |
| maize: field drying effect | 1 | 0.3386 | 0.3386 | 7.8600 | 0.0099 |
| Sorghum (BR):Field drying effect | 1 | 3.1205 | 3.1205 | 72.4200 | 0.0001 |
| Sorghum (NBR):Field drying effect | 1 | 3.0475 | 3.0475 | 70.7200 | 0.0001 |

**Figure 3.3: Effect of period of harvest and storage methods on nitrogen content of stover (g/gkDM)**

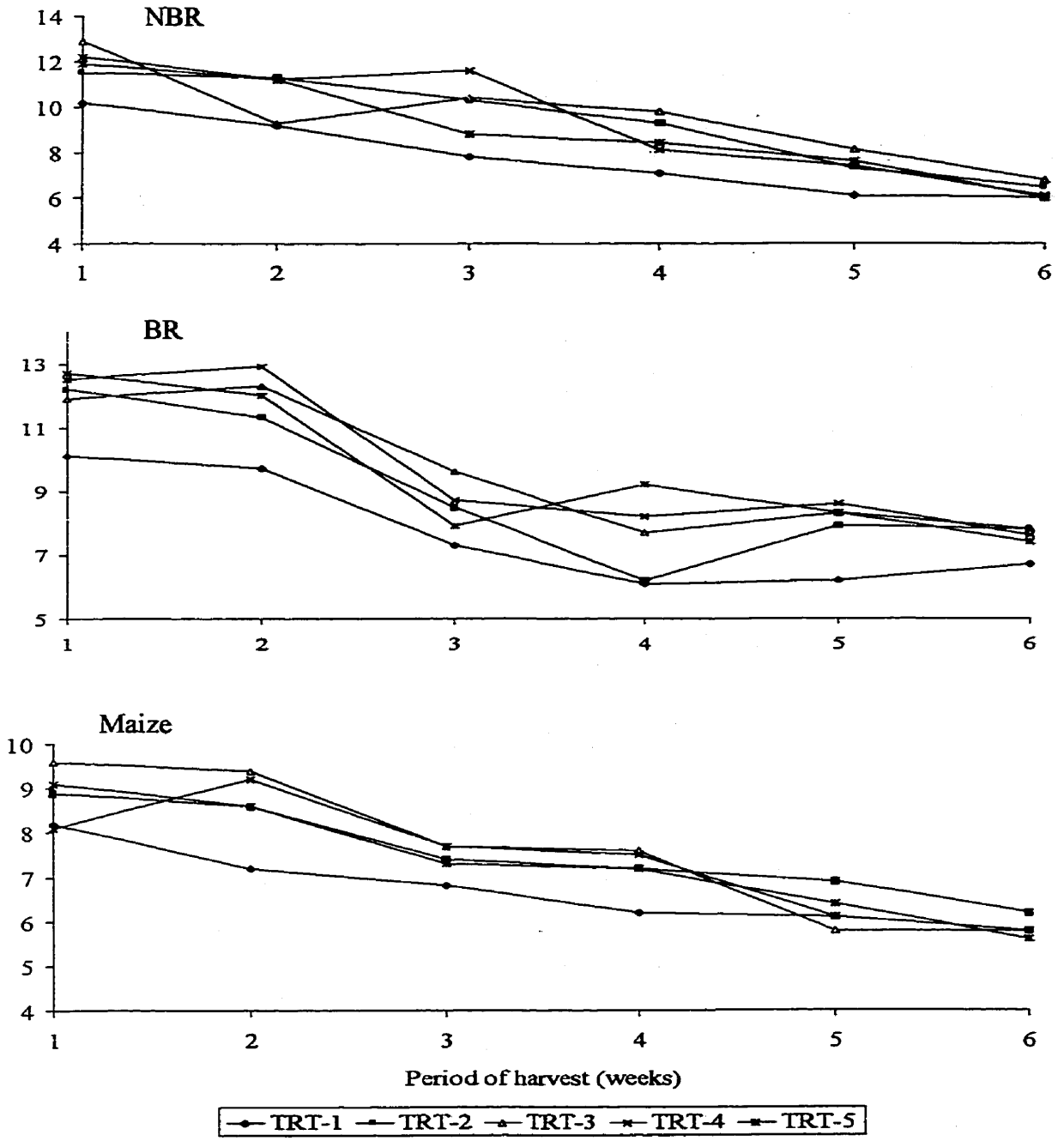


Table 3.2. Analysis on changes in nitrogen content over period of harvest

| CROP | Storage methods | Period of harvest LSMEAN | Standard error LSMEAN | Pr > \|T\| H0:LSMEAN=0 |
|---|---|---|---|---|
| MAIZE | 1 | -0.2466 | 0.0478 | 0.0001 |
| MAIZE | 2 | -0.2895 | 0.0478 | 0.0001 |
| MAIZE | 3 | -0.4645 | 0.0478 | 0.0001 |
| MAIZE | 4 | -0.3369 | 0.0478 | 0.0001 |
| MAIZE | 5 | -0.3749 | 0.0478 | 0.0001 |
| SORGHUM(BR) | 1 | -0.4357 | 0.0478 | 0.0001 |
| SORGHUM(BR) | 2 | -0.5113 | 0.0478 | 0.0001 |
| SORGHUM(BR) | 3 | -0.5222 | 0.0478 | 0.0001 |
| SORGHUM(BR) | 4 | -0.5806 | 0.0478 | 0.0001 |
| SORGHUM(BR) | 5 | -0.5780 | 0.0478 | 0.0001 |
| SORGHUM(NBR) | 1 | -0.4732 | 0.0478 | 0.0001 |
| SORGHUM(NBR) | 2 | -0.5886 | 0.0478 | 0.0001 |
| SORGHUM(NBR) | 3 | -0.5242 | 0.0478 | 0.0001 |
| SORGHUM(NBR) | 4 | -0.6991 | 0.0478 | 0.0001 |
| SORGHUM(NBR) | 5 | -0.6226 | 0.0478 | 0.0001 |

BR = Bird resistant, NBR = Non-bird resistant

| Contrast | DF | Contrast SS | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| maize: shade effect | 1 | 0.1041 | 0.1041 | 3.9700 | 0.0577 |
| Sorghum (BR): shade effect | 1 | 0.7702 | 0.7702 | 29.4000 | 0.0001 |
| Sorghum (NBR): shade effect | 1 | 0.1815 | 0.1815 | 6.9300 | 0.0146 |
| Maize: grain effect | 1 | 0.0110 | 0.0110 | 0.4200 | 0.5228 |
| Sorghum (BR): grain effect | 1 | 0.3569 | 0.3569 | 13.6200 | 0.0011 |
| Sorghum (NBR):grain effect | 1 | 0.0410 | 0.0410 | 1.5600 | 0.2231 |
| maize: field drying effect | 1 | 1.2896 | 1.2896 | 49.2200 | 0.0001 |
| Sorghum (BR):Field drying effect | 1 | 5.4679 | 5.4679 | 208.7000 | 0.0001 |
| Sorghum (NBR):Field drying effect | 1 | 4.8851 | 4.8851 | 186.4600 | 0.0001 |

content tended to be highest for BR sorghum variety at all periods, followed by the NBR sorghum variety and maize.

Leaving the stover rooted in the field for extended periods (field drying effect) significantly ($P<0.001$) reduced the N content of the stover (Table 3.2). Storing stover under shade (shade effect) compared to storing it in the open did not ($P>0.05$) affect N content of maize stover, but significantly ($P<0.001$) increased N content in the BR sorghum stover and reduced ($P<0.05$) N content in the NBR variety. Storage of stover with grain significantly ($P<0.001$) reduced N content of BR sorghum stover, but did not have any significant ($P>0.05$) effect on the N content of maize and NBR sorghum.

**3.3.1.3 NDF content**

NDF content was significantly ($P<0.001$) influenced by period of harvest (Figure 3.4 and Table 3.3). There was a significant ($P<0.01$) crop x period interaction. Delayed harvest resulted in significantly ($P<0.001$) higher NDF content of stover (Table.3.3). The maximum value for the three crops was obtained at the 6 week period of harvest. Maize had significantly ($P<0.05$) higher NDF (565 g/kg DM) than the two sorghum varieties (495 g/kgDM for BR and 490 g/kgDM for NBR) (Appendix Table 3.3).

Storage methods did not have significantly ($P>0.05$) influenced the NDF content of stover. However, field drying and shade effects had significant ($P<0.05$) influence on the BR sorghum stover, shade reduced NDF content while grained increased NDF content.

**3.3.1.4 Condensed tannins content**

The CT concentration of sorghum stover was significantly ($P<0.001$) influenced by period of harvest and storage methods (Figure 3.5 and Table 3.4). There was significant ($P<0.001$) period x storage methods interaction. Bird resistant sorghum stover had significantly ($P<0.001$) higher CT concentration across all the periods of harvest compared

Figure 3.4: Effect of period of harvest and storage methods on neutral detergent fibre of cereal stover (g/kgDM)

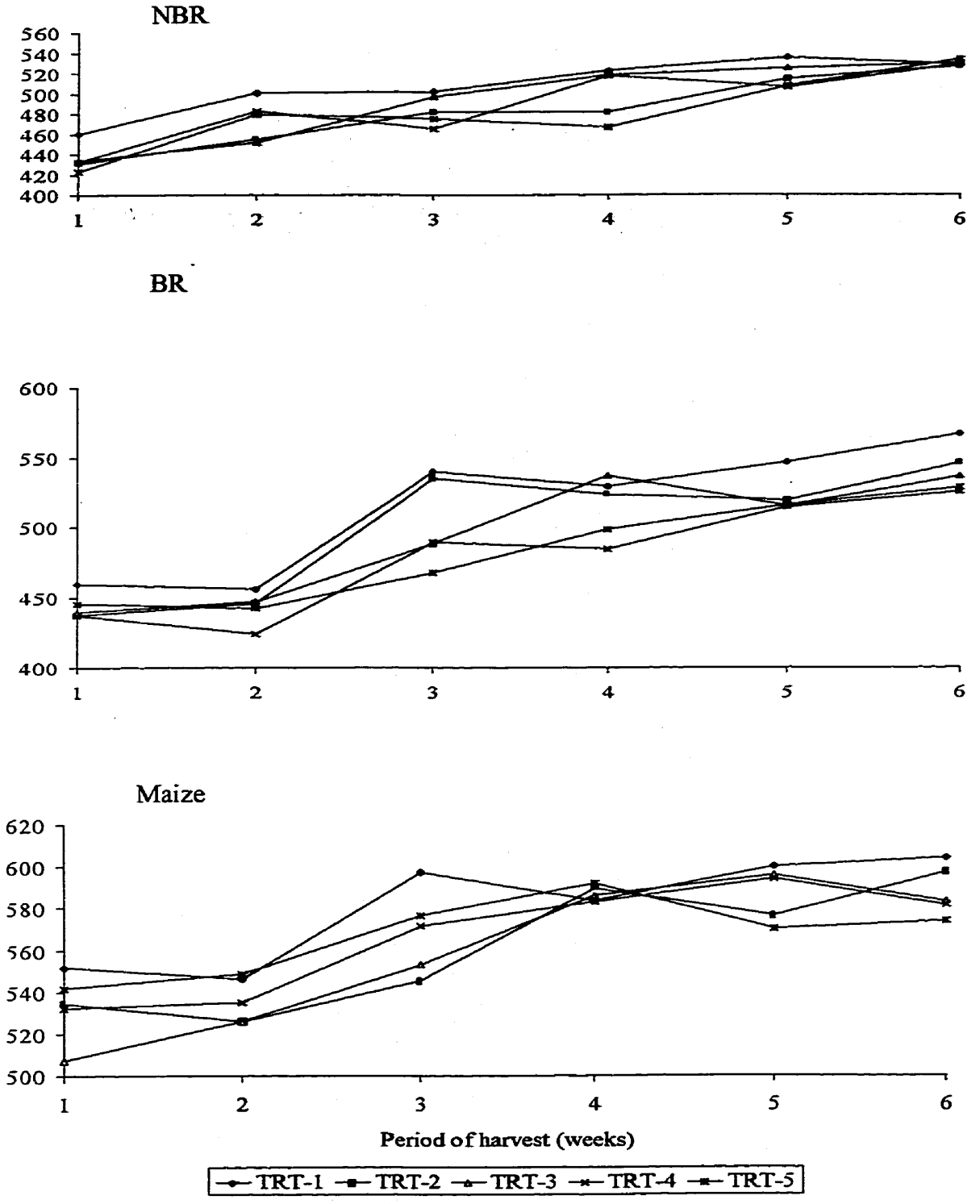


Table 3.3. Analysis on changes in neutral detergent fibre content over period of harvest

| CROP | Storage methods | Period of harvest LSMEAN | Standard error LSMEAN | Pr > \|T\| H0:LSMEAN=0 |
|---|---|---|---|---|
| MAIZE | 1 | 5.4358 | 2.2101 | 0.0215 |
| MAIZE | 2 | 7.9755 | 2.2101 | 0.0014 |
| MAIZE | 3 | 9.2532 | 2.2101 | 0.0003 |
| MAIZE | 4 | 8.6154 | 2.2101 | 0.0007 |
| MAIZE | 5 | 3.5270 | 2.2101 | 0.1236 |
| SORGHUM(BR) | 1 | 12.1667 | 2.2101 | 0.0001 |
| SORGHUM(BR) | 2 | 11.0866 | 2.2101 | 0.0001 |
| SORGHUM(BR) | 3 | 11.2301 | 2.2101 | 0.0001 |
| SORGHUM(BR) | 4 | 10.9322 | 2.2101 | 0.0001 |
| SORGHUM(BR) | 5 | 10.2892 | 2.2101 | 0.0001 |
| SORGHUM(NBR) | 1 | 6.8353 | 2.2101 | 0.0050 |
| SORGHUM(NBR) | 2 | 10.0337 | 2.2101 | 0.0001 |
| SORGHUM(NBR) | 3 | 10.8166 | 2.2101 | 0.0001 |
| SORGHUM(NBR) | 4 | 8.8803 | 2.2101 | 0.0005 |
| SORGHUM(NBR) | 5 | 9.5817 | 2.2101 | 0.0002 |

BR = Bird resistant, NBR = Non-bird resistant

| Contrast | DF | Contrast SS | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| maize: shade effect | 1 | 48.7666 | 48.7666 | 0.3400 | 0.5674 |
| Sorghum (BR): shade effect | 1 | 910.8797 | 910.8797 | 6.2800 | 0.0194 |
| Sorghum (NBR): shade effect | 1 | 19.2956 | 19.2956 | 0.1300 | 0.7185 |
| Maize: grain effect | 1 | 5.7369 | 5.7369 | 0.0400 | 0.8440 |
| Sorghum (BR): grain effect | 1 | 20.8803 | 20.8803 | 0.1400 | 0.7077 |
| Sorghum (NBR):grain effect | 1 | 3.3993 | 3.3993 | 0.0200 | 0.8796 |
| maize: field drying effect | 1 | 585.9636 | 585.9636 | 4.0400 | 0.0558 |
| Sorghum (BR):Field drying effect | 1 | 756.5246 | 756.5246 | 5.2200 | 0.0315 |
| Sorghum (NBR):Field drying effect | 1 | 328.4260 | 328.4260 | 2.2600 | 0.1454 |

Figure 3.5: Effect of period of harvest and storage methods on total condensed tannins content (g/kgDM)

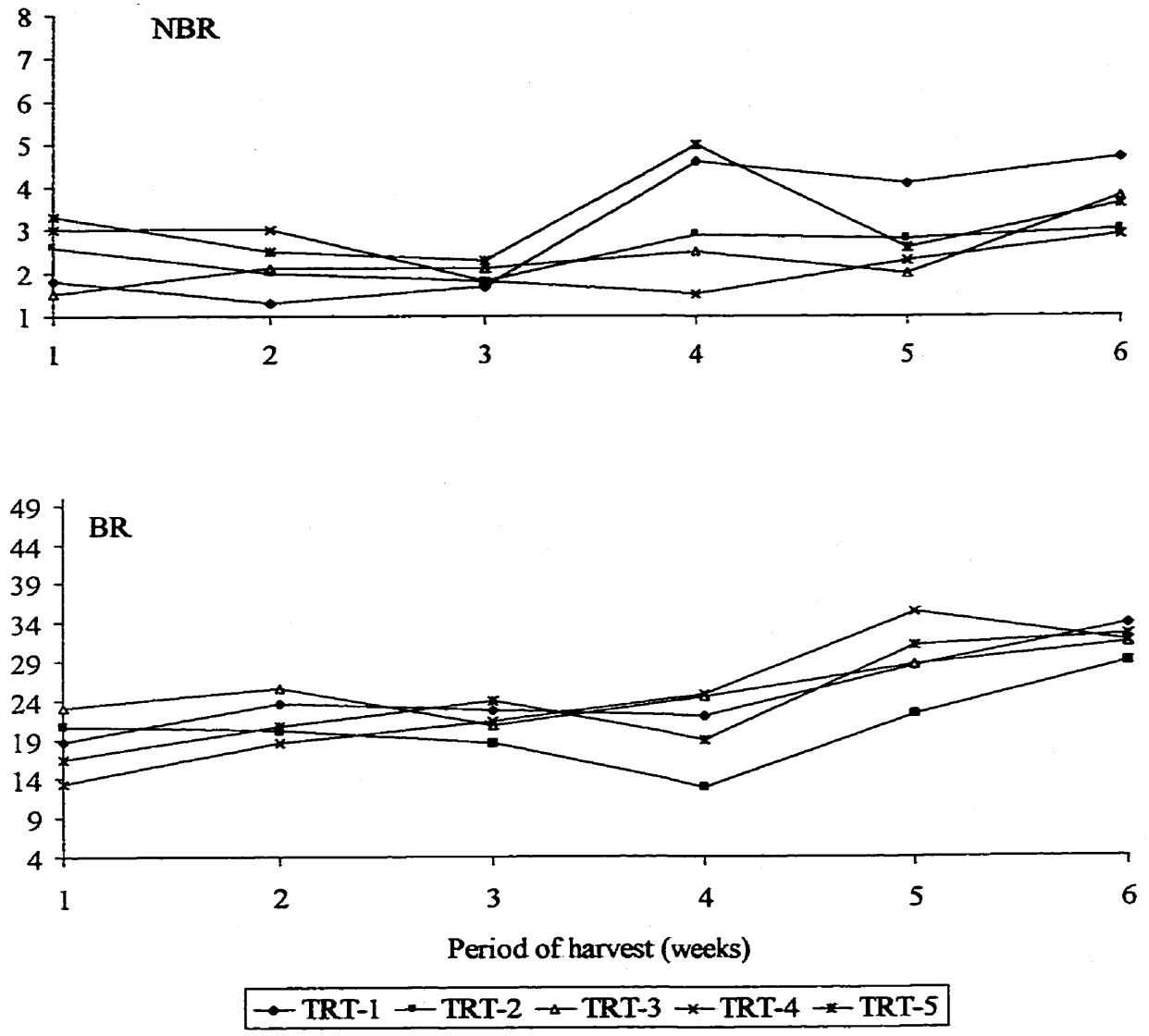


Table 3.4. Analysis on changes in total condensed tannin content over period of harvest

| CROP | TREAT | PERIOD LSMEAN | Std Err LSMEAN | Pr > \|T\| H0:LSMEAN=0 |
|---|---|---|---|---|
| SORGHUM(BR) | 1 | 1.3621 | 0.1188 | 0.0001 |
| SORGHUM(BR) | 2 | 0.7015 | 0.1188 | 0.0001 |
| SORGHUM(BR) | 3 | 0.8612 | 0.1188 | 0.0001 |
| SORGHUM(BR) | 4 | 2.2120 | 0.1188 | 0.0001 |
| SORGHUM(BR) | 5 | 1.6116 | 0.1188 | 0.0001 |
| SORGHUM(NBR) | 1 | 0.4027 | 0.1188 | 0.0037 |
| SORGHUM(NBR) | 2 | 0.0930 | 0.1188 | 0.4449 |
| SORGHUM(NBR) | 3 | 0.2697 | 0.1188 | 0.0373 |
| SORGHUM(NBR) | 4 | -0.0370 | 0.1188 | 0.7596 |
| SORGHUM(NBR) | 5 | 0.0429 | 0.1188 | 0.7230 |

BR = Bird resistant, NBR = Non bird resistant

| Contrast | DF | Contrast SS | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| Sorghum (NBR): shade | 1 | 3.8341 | 3.8341 | 90.5800 | 0.0001 |
| Sorghum (BR): shade | 1 | 0.0955 | 0.0955 | 2.2600 | 0.1525 |
| Sorghum (NBR): grain | 1 | 0.1457 | 0.1457 | 3.4400 | 0.0820 |
| Sorghum (BR): grain | 1 | 0.0494 | 0.0494 | 1.1700 | 0.2962 |
| Sorghum (NBR): field drying effect | 1 | 0.3764 | 0.3764 | 8.8900 | 0.0088 |
| Sorghum (BR): Field drying effect | 1 | 0.0265 | 0.0265 | 0.6300 | 0.4401 |

to the NBR variety. Condensed tannin concentrations tended to increase with plant maturity especially for BR at 4 and 6 weeks post harvest.

Leaving the stover rooted for extended period (field drying effect) of time in the field significantly ($P<0.01$) increased CT content of the NBR sorghum stover. Storing stover under shade (shade effect) significantly ($P<0.01$) increased CT content of NBR sorghum stover (Appendix Table 3.4).

**3.3.1.5 DM degradability**

Dry matter degradability of stover was significantly ($P<0.001$) influenced period and storage methods (Figure 3.6 and Table 3.5).There was significant ($P<0.001$) period x storage interaction. DM degradability was highest at the -3 and -2 week period compared to the rest of the harvest periods. Dry matter degradability for the two sorghum varieties (385 for BR and 401g/kg DM for NBR) were lower ($P<0.05$) than for maize (453 g/kg DM) (Appendix Table 3.5). Similarly the BR stover had lower ($P<0.01$) DM degradability than the NBR variety.

Stover that was left in the field for extended periods had lower DM degradability ($P<0.01$) compared to when stover was cut and removed (field drying effect). Storing stover in the open (shade effect) affected ($P<0.05$) the stover DM degradability (Table 3.5). Storing stover with grain heads (grain effect) did not influence ($P>0.05$) DM degradability of the BR sorghum stover. However, storing BR sorghum stover with grain promoted higher ($P<0.05$) DM degradability, while grain reduced ($P<0.05$) DM degradability in maize stover.

**3.3.2 Effects of physiological age at harvest**

Dry matter content of crops increased significantly ($P<0.001$) as the plant matured (Table 3.6). Whereas the DM contents of sorghum varieties were significantly ($P<0.001$) higher than of maize, NDF content followed a reverse pattern. However the NBR sorghum

Figure 3.6 Effect of period of harvest and storagemethods on DM degradability of cereal stover (g/kgDM)

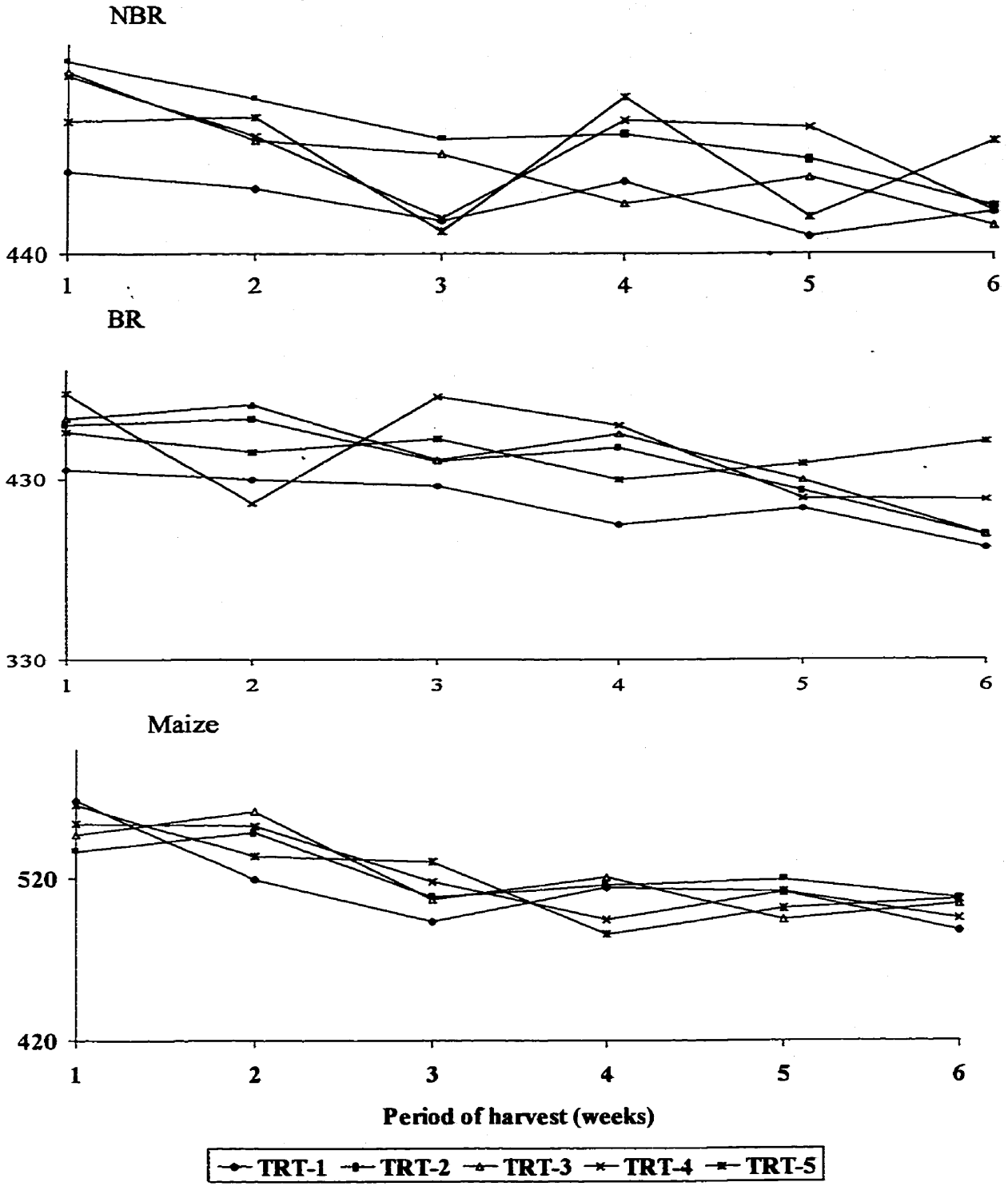


Table 3.5. Analysis on changes in DM degradability of stover over period of harvest

| CROP | Storage methods | Period of harvest LSMEAN | Standard error LSMEAN | Pr > \|T\| H0:LSMEAN=0 |
|---|---|---|---|---|
| MAIZE | 1 | -0.2360 | 0.0305 | 0.0001 |
| MAIZE | 2 | -0.1905 | 0.0305 | 0.0001 |
| MAIZE | 3 | -0.2583 | 0.0305 | 0.0001 |
| MAIZE | 4 | -0.1781 | 0.0305 | 0.0001 |
| MAIZE | 5 | -0.1840 | 0.0305 | 0.0001 |
| SORGHUM(BR) | 1 | -0.2070 | 0.0305 | 0.0001 |
| SORGHUM(BR) | 2 | -0.1328 | 0.0305 | 0.0002 |
| SORGHUM(BR) | 3 | -0.1515 | 0.0305 | 0.0001 |
| SORGHUM(BR) | 4 | -0.1813 | 0.0305 | 0.0001 |
| SORGHUM(BR) | 5 | -0.1538 | 0.0305 | 0.0001 |
| SORGHUM(NBR) | 1 | -0.1612 | 0.0305 | 0.0001 |
| SORGHUM(NBR) | 2 | -0.0890 | 0.0305 | 0.0075 |
| SORGHUM(NBR) | 3 | -0.0608 | 0.0305 | 0.0576 |
| SORGHUM(NBR) | 4 | -0.1576 | 0.0305 | 0.0001 |
| SORGHUM(NBR) | 5 | -0.0445 | 0.0305 | 0.1571 |

BR = Bird resistant, NBR = Non-bird resistant

| Contrast | DF | Contrast SS | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| maize: shade effect | 1 | 0.2486 | 0.2486 | 5.7700 | 0.0244 |
| Sorghum (BR): shade effect | 1 | 0.8788 | 0.8788 | 20.3900 | 0.0001 |
| Sorghum (NBR): shade effect | 1 | 0.6760 | 0.6760 | 15.6900 | 0.0006 |
| Maize: grain effect | 1 | 0.3339 | 0.3339 | 7.7500 | 0.0103 |
| Sorghum (BR): grain effect | 1 | 0.1248 | 0.1248 | 2.9000 | 0.1017 |
| Sorghum (NBR):grain effect | 1 | 0.3094 | 0.3094 | 7.1800 | 0.0131 |
| maize: field drying effect | 1 | 0.3386 | 0.3386 | 7.8600 | 0.0099 |
| Sorghum (BR):Field drying effect | 1 | 3.1205 | 3.1205 | 72.4200 | 0.0001 |
| Sorghum (NBR):Field drying effect | 1 | 3.0475 | 3.0475 | 70.7200 | 0.0001 |

variety had higher ($P<0.05$) NDF than the BR variety. The N concentration was higher at the boot stage ($P < 0.001$) when compared to the milk and dough stages of growth for the three crops. The BR sorghum variety had higher ($P<0.05$) N content than the NBR variety, which in turn had higher values than maize. Tannins were significantly ($P<0.001$) higher in the BR variety than in the NBR sorghum.

Physiological age at harvest influenced DM degradability at 48 h of incubation. Dry matter degradability of maize, was higher than that of sorghum ($P<0.01$), however, the NBR variety tended to have higher DM degradability than the BR variety.

Table 3.6: Effects of physiological stage of harvest on chemical composition and DM degradability of stover (g/kgDM).

| Crops | Stage | DM | N | NDF | CTs | DM degradability |
|---|---|---|---|---|---|---|
| Maize | Boot | 125 $^{c}$ | 16 $^{a}$ | 533 $^{c}$ | ND | 576 |
| | Milk | 180 $^{b}$ | 12 $^{b}$ | 556 $^{b}$ | ND | 567 |
| | Dough | 352 $^{a}$ | 10 $^{c}$ | 651 $^{a}$ | ND | 555 |
| | Mean | 219 | 13 | 588 | ND | 566 |
| BR sorghum | Boot | 162 $^{c}$ | 18 $^{a}$ | 465 $^{c}$ | 25 $^{c}$ | 554 |
| | Milk | 280 $^{b}$ | 15 $^{b}$ | 535 $^{b}$ | 40 $^{b}$ | 496 |
| | Dough | 381 $^{a}$ | 11 $^{c}$ | 589 $^{a}$ | 42 $^{a}$ | 457 |
| | Mean | 275 | 15 | 530 | 35 | 503 |
| NBR sorghum | Boot | 160 $^{c}$ | 19 $^{a}$ | 496 $^{c}$ | 0 | 540 |
| | Milk | 309 $^{b}$ | 12 $^{b}$ | 523 $^{b}$ | 0 | 528 |
| | Dough | 424 $^{a}$ | 8 $^{c}$ | 628 $^{a}$ | 0 | 509 |
| | Mean | 298 | 13 | 549 | 0 | 526 |
| | **Overall mean** | **264** | **14** | **555** | **16.3** | **532** |
| | | | | SED | | |
| | Crop | 1.64 $^{***}$ | 1.1 $^{***}$ | 11.4 $^{***}$ | 0.6 $^{***}$ | 5.6 $^{***}$ |
| | Stage | 1.64 $^{***}$ | 1.1 $^{***}$ | 11.4 $^{***}$ | 0.7 $^{***}$ | 5.6 $^{***}$ |
| | Crop x stage | 2.3 $^{***}$ | 1.3 $^{***}$ | 19.9 $^{***}$ | 1.0 $^{***}$ | 9.8 $^{***}$ |

[3] Level of significance: NS P>0.05, * P<0.05, ** P<0.01, *** P<0.001

## 3.4 DISCUSSION

The delayed regimen in harvesting time imposed on the three cereal crops after reaching physiological maturity decreased both the DM yield and N content and increased the CT and NDF contents. Some of these are in line with the well known physiological phenomena that as a plant matures the nutritive quality declines. Therefore in order to obtain high quality stover, it is imperative that crops are harvested at the right stage of growth. Nutrients usually reach their maximum concentration in cereals at the critical point of heading or incipient flowering, and this may vary with environment and plant species (Van Soest, 1994). Stage of maturity of a crop can significantly influence the stover quantity and quality. Harika and Sharma (1994) found that a delay in harvesting maize at physiological maturity to dead ripe stage reduced the crude protein content and DM degradability with associated small increases in the NDF and ADF contents of leaf and stem fractions of the stover. Maize harvested at physiological maturity had higher crude protein content, leaf: stem ratio and DM degradability and lower NDF and ADF contents than that harvested at dead ripe stage of the grain. These observations are in agreement with the findings in this experiment. The increase in DM content with maturity observed in this experiment, is also similar to those reported by other researchers (Osafo, 1993; Aganga *et al.,* 1996).

The low nutritive value of the stovers with increasing maturity was a result of the lowered N content and increased NDF, CT concentrations and most probably lignin particularly for the BR sorghum variety. The N content decreased with maturity both because of decreases in N in leaves and stems, and because stems, with their lower N concentration, make up a larger portion of the herbage in more mature forage. The increase in phenolic compounds content may be due to moisture (and other) stress as well as continued

translocation of soluble cell contents from leaves and stems to the grain. Stress conditions cause more phenolic precursors to be synthesized and specific plant enzymes cause their polymerization (Mueller-Harvey *et al.*, 1988). The highest CT content observed at 6 weeks after physiological mature stage may be explained by the fact that biosynthesis of phenolic compounds are highest at this stage (Khazaal *et al.*, 1993). This observation offers an opportunity for managing tannin content of sorghum crop through early harvest.

Aganga *et al.* (1996) observed that DM degradability of both sorghum and millet declined with advancing maturity. The increase in cell wall concentration in stems and usually in leaves that occurs during maturation has a large influence on forage digestibility (Buxton, 1996). Badve *et al* (1994) showed that the variation in digestibility of sorghum stover was influenced by neutral detergent soluble content and differences in the digestibility of NDF components, indicating that differences in cell wall composition and structure are important. Stems of most forages have a higher concentration of cell walls than leaves. Thus stems usually are lower in digestibility than leaves, and stem digestibility declines more rapidly with increased plant maturity than does that of leaves.

Delayed harvest after grain removal reduced the nutritive value of the stover (A vs C). The reduction in nutritive quality was mainly manifested in increased NDF and decreased N contents of the stovers. This increase in NDF content may be the result of moisture and other stresses. Since the crop was left standing rooted (alive), it is possible that the stresses will be more severe than if the crop was cut immediately. The lower nutritive value of stover left rooted in the field could also occur as a result of changes in the botanical composition due to shedding dried leaves.

Storing of stover under shade (D&E vs B&C) preserved nutrients much better than storing in the open field. However shading did not prevent all nutrient loss. It is possible that the plant remained alive for several days under the shade and respiration continued for several days and as such affected the cell soluble in the stover. Storing BR sorghum stover with grain (B&D) did not affect CT concentration and but had higher DM yield of the stover. This may mean that the most commonly practiced method of storing stover in the open may be appropriate. The observed changes in the concentrations of CTs with storage methods and stage of growth could allow for feeding programs that take advantage of the plant when concentration of tannins are low.

All three crops showed a progressive decline in nutritive value with delay in harvesting time. These trends have been reported (Ward, *et al.*, 1979; Alhassan *et al.*, 1987). The decline however differed among the crops. This difference may indicate differences in biochemical pathways among the crops and their ability to respond differently to environmental stresses. Bird-resistant sorghum variety is known to synthesize CTs in large quantities (Butler, 1982), which may indicate its genetic ability to respond to environmental stresses. The sorghum stover left rooted in the field had higher DM yield than that harvested immediately at the different stages of grain harvest. This difference may have been caused by the fact that at the time the stover was harvested, it was still green (alive) and some new green shoots and leaves would have grown. In contrast, maize stover was already dry and there was evidence of loss of leaves. This observation is in support of the known physiological differences between maize and sorghum. Sorghum has more secondary roots than maize and as such tolerates drought better. It is therefore not surprising that sorghum stover had more intact leaves than maize stover at harvest time. In accordance with this

observation, sorghum stover had better nutritive quality than maize, which was especially true for the stover left rooted for extended periods in the field after grain harvest. This, therefore, suggests that different strategies for harvesting and storing maize and sorghum crops should be applied.

## 3.5 CONCLUSION

These results confirmed loss of nutrient with advancing maturity; similarly stover rooted in the field for extended period of time after grain harvest had nutrient losses. It is concluded that crop residues of sorghum and maize when harvested timely and stored appropriately (whole crop or grain heads removed and stored in the open), can be of considerable nutritive value. Because of differences in the physiology of sorghum and maize, different harvest and storage strategies should be applied. Better utilization of otherwise tannin-rich BR sorghum stover could be possible if the harvest is carried out when the tannin content is lowest. In this experiment, this period was at 2 weeks before physiological dead ripe stage of the grain. The grain at this time was not completely dry and therefore would need further drying in order to keep well in storage.

# CHAPTER FOUR

## 4.0 *IN VITRO* COMPARISON OF UREA AND SULPHUR WITH POLYETHYLENE GLYCOL-4000 (PEG) AS EFFECTIVE AMELIORANTS TO PHENOLICS-RELATED ANTINUTRITIVE EFFECTS


### ABSTRACT

Effects were studied on *in vitro* dry matter disappearance (IVDMD) and gas production (GP) when PEG, urea and sulphur were added as ameliorants to BR sorghum stover supplemented with forage legumes (FLs). BR sorghum stover basal roughage was supplemented with *Lablab purpureus* (lablab), *Desmodium intortum* (desmodium), *Sesbania goetzei 15007* (goetzei) or *Leucaena leucocephala* (leucaena). These were incubated with or without PEG, urea or sulphur as ameliorants according to modified Tilley and Terry (1963) procedure for dry matter digestibility (DMD) and Menke et al. (1979) technique for gas production. Steers fed natural grass hay or BR sorghum stover basal diets served as sources of rumen fluid inoculum. Source of inoculum did not significantly ($P>0.05$) influence IVDMD and extent of gas produced ($P>0.05$), but significantly influenced gas production at 24 h (GP24) ($P<0.01$) and 48 h (GP48) ($P<0.05$) as well as rate of gas production ($P<0.01$) by promoting higher response. *In vitro* DMD ($P<0.001$), GP24 and GP48 ($P<0.01$) and rate of gas production ($P<0.05$) were influenced by supplemental forage type. *Lablab* and *leucaena* promoted higher IVDMD, GP24, GP48 and rate of gas production than desmodium and goetzei. Ameliorants significantly ($P<0.001$) improved IVDMD but did not affect ($P>0.05$) gas production. The results showed that the ameliorants increased or depressed gas production in some samples, and the depression was not related to the concentration of CTs. Urea and sulphur had similar effects as PEG on IVDMD. It is possible

that rumen fluid from steers fed sorghum stover contained free condensed tannins and as such when used with high tannin forages, produced an accumulation of tannins, thus suggesting the possible detrimental effects of using a high tannin basal diet supplemented with high tannin forages.

## 4.1 INTRODUCTION

Ruminants in smallholdings in sub-tropical and tropical countries subsist predominantly on crop residues and low quality native hay or pasture that are often deficient in both protein and energy. As a result, there is constant need for supplementary feeding to meet nutrient requirements even for maintenance. Availability and cost of supplementation with high protein agro-industrial by-products are limiting factors for smallholder farmers. Thus attention has been given to the use of legume fodder (MPTs, browses, herbaceous forages) as sustainable sources of limiting nutrients in low quality roughage based feeding systems (Borens and Poppi, 1986; Butterworth and Mosi, 1986). Many of the legume fodder are high in fodder production in addition to high protein and mineral contents, which would improve livestock production (Khalili and Varvikko, 1992; Bonsi *et al.*, 1994). Forage legumes (FLs) however, synthesize secondary plant compounds (antinutritional factors), such as tannins (polyphenolics), saponins and flavonoids, which can affect animal performance (Woodward and Reed, 1989; D'Mello, 1992). Condensed tannins (CTs) appear to be the major constraint to the use of FLs as fodder because of their effect on intake, digestibility (Reed, 1995) and animal metabolism (Cheeke and Palo, 1995). Similarly sorghum, an important crop in the semi-arid regions of the world, exhibits varying degrees of pest resistance. This trait is positively correlated with CTs in the grain (Reed *et al.* 1988) and in the straw (Reed *et al.*, 1987; Ebong, 1989; Aboud *et al.*, 1990). Since stover from bird-resistant (BR) varieties contains significant quantities of CTs (Reed *et al.*, 1988), and together with FLs constitute the principal feed resources that are available in the semi-arid zones, it is possible that the use of both in diets may accentuate antinutritional toxicity

problems. Unfortunately, given the low resource base of smallholders in the tropics, they depend more on these FLs as protein supplements. Thus, any attempt to improve the nutrition of livestock with these feed resources must first overcome the problems posed by the high tannin content of these forages. Several strategies available for overcoming the problems posed by high tannins in forages include the following; addition of chemicals, such as polyethylene glycol (PEG) to bind tannins in the diet; development of breeding and selection programs for plants with high potential to increase animal productivity, but low in CTs concentration; avoiding the use of high tannin plants or feeding such at controlled or reduced levels; and the use of microbes to detoxify the CTs (Odenyo *et al.*, 1997). These strategies are not necessarily practical for smallholders who may have no other choice of feeds but forages high in CTs. Practical strategies to overcome the antinutritive effects of polyphenolic compounds are therefore required to fully utilize these feed resources.

This study had three objectives: first, to assess the effects of supplementing BR sorghum stover with FLs varying in CT concentration, secondly, to explore practical ways of mitigating the antinutritive effects of CTs with ameliorants on *in vitro* dry matter disappearance and gas production and thirdly, to determine dietary combinations (treatments) to be used in a sheep feeding (growth) trial (chapter 7).

## 4.2 MATERIALS AND METHODS

### 4.2.1 Feeds

Bird resistant sorghum stover was used as the basal roughage source and was supplemented with *Dolichos lablab* (lablab), *Desmodium intortum* (desmodium), *Sesbania goetzei* Accession 15007 (goetzei) or *Leucaena leucocephala* (leucaena). The four FLs were

chosen because they are widely available in the semi-arid tropical regions. *Lablab* and *leucaena* were chosen to represent low to medium tannin FLs, while *desmodium* and *goetzei* were chosen to represent high tannin FLs. Supplements were added at 30% of basal roughage dry matter (DM). These were then incubated with or without polyethylene glycol (PEG), urea or sulphur as ameliorants (Table 4.1) according to procedures for *in vitro* dry matter digestibility (DMD) and gas production. Ameliorants were first dissolved in distilled water and then added at a concentration of 3% of the total DM weighed (15 mg for IVDMD and 7 mg for gas production).

### 4.2.2 *In vitro* DMD procedure

*In vitro* DM digestibility (IVDMD) determination followed a modified method of Tilley and Terry (1963) whereby pepsin-HCl digestion was replaced with neutral detergent extraction (Van Soest, 1982). Two runs of IVDMD were carried out. In the first run rumen fluid inoculum was obtained from steers fed grass hay (900 g organic matter (OM), 9 g nitrogen (N) and 730 g neutral detergent fibre (NDF) per kg DM) ad libitum and supplemented with 2 kg per head per day cottonseed cake (910 g OM, 86 g N and 380 g NDF per kg DM). In the second run, the inoculum came from steers that had received BR sorghum stover ad libitum and supplemented with 2 kg per head per day cottonseed cake. About 500 mg (1 mm size) of forage and forage combination (350 mg of basal + 150 mg of supplement) were weighed into 125 ml Erlenmeyer flasks. Rumen fluid was collected from 3 fistulated steers in the morning before feeding. The rumen fluid was collected into a thermos flask flushed with carbon dioxide. The rumen fluid was filtered through two layers of cheesecloth under carbon dioxide. Thereafter, 40 ml of prepared medium (Tilley and Terry, 1963) was added to each flask before inoculating

Table 4.1 Treatment combinations

| Treatment combinations plus ameliorants (PEG, urea or sulphur) |
|---|
| 1. Bird resistant (BR) sorghum stover alone |
| 2. BR sorghum stover + Polyethylene glycol (PEG) |
| 3. BR sorghum stover + Urea |
| 4. BR sorghum stover + Sulphur |
| 5. BR sorghum stover + Urea + Sulphur |
| 6. BR sorghum stover + Dolichos lablab (lablab) alone |
| 7. BR sorghum stover + lablab + PEG |
| 8. BR sorghum stover + lablab + Urea |
| 9. BR sorghum stover + lablab + Sulphur |
| 10. BR sorghum stover + lablab + Urea + Sulphur |
| 11. BR sorghum stover + Desmodium intortum (desmodium) alone |
| 12. BR sorghum stover + desmodium + PEG |
| 13. BR sorghum stover + desmodium + Urea |
| 14. BR sorghum stover + desmodium + Sulphur |
| 15. BR sorghum stover + desmodium + Urea + Sulphur |
| 16. BR sorghum stover + Leucaena leucocephala (leucaena) alone |
| 17. BR sorghum stover + leucaena + PEG |
| 18. BR sorghum stover + leucaena + Urea |
| 19. BR sorghum stover + leucaena + Sulphur |
| 20. BR sorghum stover + leucaena + Urea + Sulphur |
| 21. BR sorghum stover + Sesbania goetzei 15007 (Goetzei) alone |
| 22. BR sorghum stover + Goetzei + PEG |
| 23. BR sorghum stover + Goetzei + Urea |
| 24. BR sorghum stover + Goetzei + Sulphur |
| 25. BR sorghum stover + Goetzei + Urea + Sulphur |

with 10 ml of rumen fluid. The tubes were sealed and incubated for 48 h according to the Tilley and Terry (1963) procedure. At the end of incubation, the residues were washed with 50 ml neutral detergent solution (NDS) (Van Soest, 1994) and refluxed for 1 h. Dry matter loss was then determined following the method described by Goering and Van Soest (1970) for neutral detergent fibre analysis.

### 4.2.3 Gas production

Fermentation was carried out in graduated glass syringes (100 ml capacity) following the procedure described by Menke *et al.* (1979). Rumen liquor was collected as described above. About 200 mg (1mm size) DM of each sample (basal alone 200 mg or basal 140 + 60 mg of supplement) was incubated in six glass syringes per treatment with 30 ml of the incubation medium prepared as described by Menke *et al.* (1979). Twelve other syringes containing either incubation medium only or incubation medium with the ameliorants were also incubated to correct for gas production due to microbial activity of rumen fluid alone. Gas production was read after 3, 6, 12, 24, 48, 72, 96 and 120 h of incubation. After each reading, the piston of the syringe was reset to 30 ml whenever it had gone beyond the 60-ml mark.

### 4.3.4 Chemical analyses

Dry matter, ash and N were analyzed using procedures described by the Association of Official Analytical Chemists (AOAC, 1980). NDF was analyzed by the method of Goering and Van Soest (1970). Total CTs were assayed using the method described by Giner-Chavez *et al.* (1997). Soluble CTs were determined by extracting 200 mg of plant sample with 8 ml of aqueous acetone 3 times for 20 min each. Thereafter 1 ml of the extract

was added to 5 ml of n-butanol/HCL (95/5, v/v) following which the solution was placed in a water bath (100 ° C, 1 h), and the absorbance of the solution read A550 nm. A subsample of 10 mg of residue from the above was placed in culture tubes to which 1 ml of 70 % aqueous acetone (v/v) and n-butanol/HCL (95/5, v/v, 5ml) was added. Insoluble CTs were determined by using the same procedures. The fibre bound CTs were measured in triplicate as described by Reed *et al.* (1982). A subsample of 10 mg of neutral detergent fibre was placed in culture tubes with 1 ml of 70 % aqueous acetone (v/v). The sample was heated for 1 h at 100° C in n-butanol/HCL (95/5, v/v) and the absorbance read at 550 nm. The total amount of CTs present in a sample was calculated by adding the amounts of soluble, insoluble and fibre bound.

**4.3.5 Statistical analysis**

The results were based on DM content and presented as replicates. The difference in IVDMD or gas production as a result of addition of ameliorants was calculated and this was expressed as percent (%) change. The IVDMD and gas production data were then subjected to analysis of variance for split plot design. Analyses were done to study the main effects (inoculum, FL and ameliorant sources) and their interactions. The volume of gas produced (ml per 200 mg DM) from the forages studied were calculated and gas production constants derived by fitting the non-linear model:

V (ml/200 mg DM) = $b(1-e^{-ct})$ (Nsahalai *et al.*, 1995) based on the argument that no gas is produced from feed that is not fermented. V is the gas produced at time t, b cumulative extent of volume of gas produced, c the rate of gas produced ($h^{-1}$). The statistical significance of the differences between means was tested using least significance difference

(LSD) and means were separated using Duncan multiple range procedure (SAS, 1987).

## 4.3 RESULTS

### 4.3.1 Chemical composition

The chemical compositions of feeds used in this experiment are shown in Table 4.2. Neutral detergent fibre and N contents varied widely among the forages. Nitrogen content was highest for *Leucaena* and lowest for the basal BR sorghum stover and ranged from 7.7 to 42.8 g/kg DM. The NDF content was highest for BR stover and lowest for *lablab*. Among the FLs, *lablab* and *leucaena* had lower NDF content compared to *desmodium* and *goetzei*. *Leucaena* had the lowest ash content compared to the other FLs. *Goetzei* had the highest content of CT, followed by *desmodium*, *leucaena* and *lablab*.

### 4.3.2 Effects of source of inoculum, forage type and ameliorants on IVDMD

Table 4.3 shows the effect of source of inoculum, forage type, ameliorants and their interactions on IVDMD. There was no significant ($P>0.05$) effect of source of inoculum on IVDMD. There were significant ($P<0.001$) inoculum x forage, forage x ameliorant and ($P<0.05$) inoculum x forage x ameliorant interactions. There was however no significant ($P>0.05$) inoculum x ameliorant interaction. Forage type significantly ($P<0.001$) influenced IVDMD. *Lablab* and *leucaena* promoted significantly ($P<0.001$) higher IVDMD than *desmodium* and *goetzei*. *Desmodium* promoted the lowest ($P<0.001$) IVDMD compared to the other FLs including the control treatment (BR stover alone). Based on IVDMD the forages ranked in the following descending order, *leucaena*, *lablab* > goetzei > *desmodium*.

Ameliorants significantly ($P<0.001$) improved IVDMD. PEG, urea or sulphur produced similar results which were higher (<0.001) than the results obtained with the combination of urea and sulphur or no ameliorant. In turn a combination of urea and sulphur

Table 4.2 Chemical composition (g/kg) of forage samples used in the experiment (g/kgDM)

| Feed sample | Dry matter | Organic matter | Nitrogen | Ash | Neutral detergent fibre | [1]Condensed tannins |
|---|---|---|---|---|---|---|
| BR[2] sorghum stover | 925 | 878 | 7.7 | 122 | 717 | 20.6 |
| Desmodium intortum | 920 | 891 | 32.2 | 109 | 180 | 1.9 |
| Sesbania goetzei 15007 | 913 | 892 | 29.6 | 108 | 426 | 100.7 |
| Dolichos lablab | 920 | 922 | 42.8 | 78 | 201 | 33.3 |
| Leucaena leucocephala | 913 | 896 | 29.5 | 104 | 421 | 224.5 |

[1]Determined according to Giner-Chavez et al. (1997)

[2] BR = bird-resistant

Table 4.3 Effects of inoculum, forage legumes and ameliorants on in vitro dry matter digestibility % (IVDMD)

| | | Ameliorants | | | | | |
|---|---|---|---|---|---|---|---|
| **Inoculum source** | **Forage legumes** | **None** | **PEG** | **Urea** | **Sulphur** | **Urea + Sulphur** | **Mean** |
| Grass hay | None | 49.1 | 50.6 | 50.9 | 50.4 | 49.2 | 50.0 |
| | *Desmodium intortum* | 46.7 | 50.2 | 50.0 | 50.0 | 46.7 | 48.7 |
| | *Sesbania goetzei 15007* | 52.9 | 53.5 | 53.3 | 53.8 | 52.4 | 53.2 |
| | *Dolichos lablab* | 52.2 | 52.6 | 52.3 | 52.6 | 51.8 | 52.3 |
| | *Leucaena leucocephala* | 56.4 | 55.6 | 55.3 | 56.0 | 54.7 | 55.6 |
| | Mean | 51.5 | 52.5 | 52.4 | 52.6 | 51.0 | 52.0 |
| [1]BR sorghum stover | | | | | | | |
| | None | 49.7 | 51.7 | 50.2 | 50.3 | 48.6 | 50.1 |
| | *Desmodium intortum* | 45.8 | 47.1 | 48.1 | 48.0 | 47.4 | 47.3 |
| | *Sesbania goetzei 15007* | 51.8 | 48.7 | 51.8 | 52.3 | 51.2 | 51.2 |
| | *Dolichos lablab* | 54.2 | 54.7 | 53.7 | 54.4 | 53.6 | 54.1 |
| | *Leucaena leucocephala* | 57.3 | 57.0 | 56.8 | 57.0 | 56.9 | 57.0 |
| | Mean | 51.8 | 51.9 | 52.1 | 52.4 | 51.8 | 52.0 |
| **OVERALL MEAN** | | **51.6** | **52.2** | **52.2** | **52.5** | **51.4** | **52.0** |

| **Effects** | **SED** | **[2]Significance** |
|---|---|---|
| Inoculum | 0.17 | NS |
| Forage | 0.16 | *** |
| Ameliorant | 0.16 | *** |
| Inoculum x forage | 0.38 | *** |
| Inoculum x ameliorant | 0.38 | NS |
| Forage x ameliorant | 0.61 | *** |
| Inoculum x forage x ameliorant | 0.86 | * |

[1]BR, bird-resistant sorghum stover
[2]Level of significance, NS P>0.05 *P<0.05, **P<0.01, ***P<0.001

did not result in any improvement over no ameliorant (none). Inoculum from steers fed grass hay promoted higher (48.7% for *desmodium*, 53.2 % for *goetzei*) response than inoculum from steers fed BR sorghum stover was the reverse true for the other two FLs. Generally source of inoculum had no effect on response to ameliorants; however, in some instances it influenced ameliorant effectiveness. For example, PEG depressed IVDMD of goetzei with inoculum from steers fed BR sorghum stover compared to inoculum from grass fed steers (48.7 vs 53.5).

### 4.3.3 Effects of source of inoculum, forage type and ameliorant on gas production at 24 (GP24) and 48 (GP48) h

Table 4.4 shows the effect of source of inoculum, forage type, ameliorants and their interactions on GP24 and GP48. Inoculum from steers fed grass hay significantly ($P<0.001$) promoted higher GP24 ($P<0.001$) and GP48 ($P<0.05$) compared with inoculum from steers fed BR stover. Forage type significantly ($P<0.001$) influenced gas production. *Lablab* and *leucaena* promoted significantly higher GP24 and GP48, compared to *desmodium* and *goetzei*. Generally, based on gas production the forages were ranked in the following descending order, *leucaena, lablab* > *goetzei, desmodium* for GP24 and GP48. Ameliorants did not affect ($P>0.05$) GP24 or GP48.

### 4.3.4 Effects of source of inoculum, forage type and ameliorant on extent of gas produced (b) and rate of gas production (c)

Table 4.5 shows the effect of source of inoculum, forage type, ameliorants and their interactions on extent of gas produced (b) and rate of gas produced (c). Source of inoculum Significantly ($P<0.01$) affected rate of gas production (c) but did not have any significant

Table 4.4 Effects of inoculum, forage legumes and ameliorant on gas production (ml/200mg DM) at 24 (GP24) and 48h (GP48)

| Inoculum source | Forage legumes | Ameliorants | | | | | |
|---|---|---|---|---|---|---|---|
| | **GP24** | **None** | **PEG** | **Urea** | **Sulphur** | **Urea + Sulphur** | **Mean** |
| Grass hay | None | 25.3 | 24.3 | 20.6 | 20.5 | 21.1 | 22.4 |
| | *Desmodium intortum* | 27.3 | 22.4 | 20.9 | 19.2 | 18.4 | 21.6 |
| | *Sesbania goetzei 15007* | 18.6 | 18.3 | 16.8 | 14.4 | 16.5 | 16.9 |
| | *Dolichos lablab* | 27.5 | 29.6 | 29.3 | 28.5 | 30.1 | 29.0 |
| | *Leucaena leucocephala* | 26.52 | 24.7 | 24.4 | 29.6 | 23.7 | 25.8 |
| | Mean | 525.0 | 23.9 | 22.4 | 22.4 | 22.0 | 23.1 |
| BR sorghum stover | | | | | | | |
| | None | 23.8 | 21.9 | 18.4 | 16.1 | 14.4 | 18.9 |
| | *Desmodium intortun* | 20.0 | 20.7 | 16.6 | 15.0 | 16.1 | 17.7 |
| | *Sesbania goetzei 15007* | 22.5 | 20.8 | 18.2 | 13.7 | 17.0 | 18.4 |
| | *Dolichos lablab* | 23.5 | 22.7 | 23.9 | 20.2 | 21.0 | 22.3 |
| | *Leucaena leucocephala* | 20.3 | 27.1 | 19.5 | 16.3 | 17.9 | 20.2 |
| | Mean | 22.0 | 22.6 | 19.3 | 16.3 | 17.3 | 19.5 |
| **OVERALL MEAN** | | **23.5** | **23.2** | **20.8** | **19.4** | **19.6** | **21.3** |

| | **GP48** | | | **Ameliorants** | | | |
|---|---|---|---|---|---|---|---|
| **Inoculum source** | **Forage legumes** | **None** | **PEG** | **Urea** | **Sulphur** | **Urea + Sulphur** | **Mean** |
| Grass hay | None | 42.1 | 40.1 | 38.6 | 39.4 | 39.9 | 40.0 |
| | *Desmodium intortun* | 45.0 | 40.2 | 37.1 | 36.3 | 34.3 | 38.6 |
| | *Sesbania goetzei 15007* | 36.8 | 25.8 | 32.9 | 29.8 | 32.1 | 31.5 |
| | *Dolichos lablab* | 44.1 | 48.3 | 45.9 | 47.3 | 47.3 | 46.6 |
| | *Leucaena leucocephala* | 42.9 | 41.3 | 40.9 | 50.5 | 40.3 | 43.2 |
| | Mean | 44.2 | 39.1 | 39.1 | 40.7 | 38.8 | 40.0 |
| [1]BR sorghum stover | None | 45.2 | 42.8 | 36.6 | 35.7 | 31.5 | 38.4 |
| | *Desmodium intortum* | 37.0 | 38.0 | 32.7 | 31.0 | 30.8 | 33.9 |
| | *Sesbania goetzei 15007* | 39.2 | 36.5 | 33.1 | 27.9 | 32.1 | 33.8 |
| | *Dolichos lablab* | 39.9 | 39.5 | 39.9 | 37.6 | 37.9 | 39.0 |
| | *Leucaena leucocephala* | 37.7 | 40.4 | 34.6 | 32.5 | 34.0 | 35.8 |
| | Mean | 39.8 | 39.4 | 35.4 | 32.9 | 33.2 | 36.2 |
| **OVERALL MEAN** | | **41.0** | **39.3** | **37.2** | **36.8** | **36.0** | **38.1** |

| **Effects** | **GP24 SED** | **GP24 Significance** | **GP48 SED** | **GP48 Significance** |
|---|---|---|---|---|
| Inoculum | 1.26 | ** | 1.70 | * |
| Forage | 2.00 | ** | 2.69 | ** |
| Ameriorant | 2.00 | NS | 2.69 | NS |
| Inoculum x forage | 2.83 | NS | 3.80 | NS |
| Inoculum x ameliorant | 2.83 | NS | 3.80 | NS |
| Forage x ameliorant | 4.47 | NS | 6.01 | NS |
| Inoculum x forage x ameliorant | 6.32 | NS | 8.50 | NS |

[1]BR, bird-resistant sorghum stover
[2]Level of significance, NS P>0.05, **P<0.05, **P<0.01, ***P<0.001

Table 4.5 Effects of inoculum, forage legumes and ameliorant on extent (ml/200mg DM) and rate ($h^{-1}$) of gas production

| Inoculum source | Forage legumes | Ameliorants | | | | | |
|---|---|---|---|---|---|---|---|
| | **Extent GP (h)** | **None** | **PEG** | **Urea** | **Sulphur** | **Urea + Sulphur** | **Mean** |
| Grass hay | None | 79.9 | 78.8 | 75.0 | 75.4 | 77.1 | 77.2 |
| | *Desmodium intortum* | 75.6 | 78.8 | 73.8 | 76.3 | 75.0 | 75.9 |
| | *Sesbania goetzei 15007* | 75.0 | 77.5 | 82.5 | 75.0 | 80.0 | 78.0 |
| | *Dolichos lablab* | 79.6 | 79.6 | 77.9 | 78.8 | 80.0 | 79.2 |
| | *Leucaena leucocephala* | 73.3 | 73.8 | 79.2 | 78.8 | 79.2 | 76.8 |
| | Mean | 76.7 | 77.7 | 77.7 | 76.8 | 78.3 | 77.4 |
| BR sorghum stover | | | | | | | |
| | None | 84.6 | 77.7 | 77.5 | 79.0 | 80.2 | 79.8 |
| | *Desmodium intortun* | 80.8 | 81.5 | 77.1 | 74.4 | 78.3 | 78.4 |
| | *Sesbania goetzei 15007* | 74.6 | 79.6 | 80.6 | 72.5 | 77.9 | 77.0 |
| | *Dolichos lablab* | 81.5 | 79.2 | 80.2 | 82.7 | 78.1 | 80.3 |
| | *Leucaena leucocephala* | 75.8 | 82.1 | 77.5 | 76.5 | 80.0 | 78.4 |
| | Mean | 79.5 | 80.0 | 78.6 | 77.0 | 78.9 | 78.7 |
| **OVERALL MEAN** | | **78.1** | **78.8** | **78.1** | **76.9** | **78.6** | **78.1** |

| | **Rate ( c )** | | | | **Ameliorants** | | |
|---|---|---|---|---|---|---|---|
| **Inoculum source** | **Forage legumes** | **None** | **PEG** | **Urea** | **Sulphur** | **Urea + Sulphur** | **Mean** |
| Grass hay | None | 0.017 | 0.016 | 0.015 | 0.015 | 0.015 | 0.015 |
| | *Desmodium intortun* | 0.021 | 0.015 | 0.015 | 0.013 | 0.012 | 0.015 |
| | *Sesbania goetzei 15007* | 0.013 | 0.010 | 0.010 | 0.010 | 0.010 | 0.011 |
| | *Dolichos lablab* | 0.019 | 0.021 | 0.020 | 0.020 | 0.021 | 0.020 |
| | *Leucaena leucocephala* | 0.021 | 0.018 | 0.016 | 0.021 | 0.015 | 0.018 |
| | Mean | 0.018 | 0.016 | 0.015 | 0.016 | 0.015 | 0.016 |
| [1]BR sorghum stover | None | 0.015 | 0.016 | 0.013 | 0.011 | 0.009 | 0.013 |
| | *Desmodium intortum* | 0.013 | 0.013 | 0.011 | 0.010 | 0.010 | 0.011 |
| | *Sesbania goetzei 15007* | 0.015 | 0.013 | 0.011 | 0.009 | 0.011 | 0.012 |
| | *Dolichos lablab* | 0.014 | 0.014 | 0.015 | 0.013 | 0.014 | 0.014 |
| | *Leucaena leucocephala* | 0.014 | 0.016 | 0.012 | 0.011 | 0.011 | 0.013 |
| | Mean | 0.014 | 0.014 | 0.012 | 0.011 | 0.011 | 0.013 |
| **OVERALL MEAN** | | **0.016** | **0.015** | **0.014** | **0.013** | **0.013** | **0.014** |

| | **Extent GP (b)** | | **Rate ( c )** | |
|---|---|---|---|---|
| **Effects** | **SED** | **Significance** | **SED** | **Significance** |
| Inoculum | 0.07 | NS | 0.000 | ** |
| Forage | 1.18 | NS | 0.002 | * |
| Ameriorant | 1.18 | NS | 0.002 | NS |
| Inoculum x forage | 1.67 | NS | 0.002 | NS |
| Inoculum x ameliorant | 1.67 | NS | 0.002 | NS |
| Forage x ameliorant | 2.63 | NS | 0.004 | NS |
| Inoculum x forage x ameliorant | 3.73 | NS | 0.005 | NS |

BR, bird-resistant sorghum stover; c, rate of gas production ($h^{-1}$); extent GP, extent of gas produced.
Level of significance, NS P>0.05, *P<0.05, **P<0.01, ***P<0.001.

(P>0.05) effect on extent of gas production (b). Inoculum from steers fed hay promoted a higher (P<0.001) rate of gas production. Forage type significantly (P<0.05) influenced rate of gas production but did not affect (P>0.05) the extent of gas produced. *Lablab* promoted (P<0.05) higher rate of gas production than *leucaena*, both of which promoted higher rates (P<0.05) than *desmodium* and *goetzei*. There were no significant (P>0.05) interactions.

## 4.5 DISCUSSION

Although quantitative chemical differences among FLs are frequent, analysis showed that the presence or absence of a particular component could differentiate the species. The major chemical differences among the species in this study were the presence of relatively large amounts of CTs and NDF in desmodium and goetzei and large amounts of N in both *lablab* and *leucaena*. Generally FLs contain more N and are more digestible than cereal residues on account of their low fibre and higher N.

Inoculum from steers fed grass hay promoted higher GP24 and GP48 and rate of GP than inoculum from steers fed BR sorghum stover. However, source of inoculum did not influence extent of GP and IVDMD. This may mean that source of rumen fluid inoculum may not be important for these variables. There was, however, an inoculum x FLs interaction since rumen fluid inoculum from BR sorghum stover fed steers depressed IVDMD of treatments containing FLs with high CTs (desmodium and goetzei). It is possible that rumen fluid from steers fed BR sorghum stover had free CTs such that when used in conjunction with tannin rich FLs it resulted in more free CTs binding with protein, carbohydrates and/or microbial extracellular enzymes and thus interfering with the rumen fermentation. Unfortunately, both N and free CT concentration of the rumen was not

measured.

Inclusion of FLs did not influence the extent of gas produced, but greatly affected IVDMD, GP24, GP48 and rate of gas production. *Lablab* and *leucaena* had more positive effects on these variables than desmodium and goetzei. Generally, supplementation of low quality roughages with FLs has been shown to improve the digestibility of DM (Mosi and Butterworth, 1985; Ndlovu and Buchanan-Smith, 1985; McMeniman *et al.*, 1988; Kaitho *et al.*, 1998). The positive effect of *leucaena* for example, on digestibility has been ascribed to the provision of protein, energy and sulphur to rumen bacteria (Devendra, 1993). However, the extent of this improvement in digestibility may be attenuated by the presence of antinutritional factors (ANFs). Waterman et al. (1980) for example, showed that *in vitro* DM disappearance of tree leaves declined with increasing tannin content. Van Hoven (1984) observed a decreased IVDMD of Lucerne with tannin concentration. Tannins interfere with DM digestibility by having bacteriostatic and bactericidal effects on rumen microbes and by inactivation of rumen microbial enzymes (Horigome *et al.*, 1988). On the other hand, tannin concentration *per se* may not be the only factor impacting on forage digestibility. The biological activity (reactivity) of CTs seems important also. For instance, *desmodium* had less CTs concentration (101 g/kg DM) than *goetzei* (225 g/kg DM), yet *desmodium* was less effective in improving IVDMD than *goetzei*.

The fact that extent of GP was not influenced by FLs was not surprising. Other workers (Khazaal *et al.*, 1993; Siaw *et al.*, 1993; Bonsi *et al.*, 1995; Nsahlai and Umunna, 1995) had previously demonstrated a poor relationship between GP and degradability. This may be an indication that some FLs degrade but yield proportionately less gas (Nsahlai *et*

*al.*,1995) as evidenced by the lack of relationship between the GP24 and *in vitro* DMD observed in their study. In this study, volume and rate of gas produced responded to inclusion of FLs. The results showed that varying quantities of gas evolved per unit of forage DM. Using correlation studies, this variation has partly been ascribed to composition of plant fibre such as NDF and polyphenolics (Nsahlai *et al.*, 1994; Nsahlai *et al.*, 1995). In the present study, FLs with high NDF and CTs contents as well as low N content negatively affected the fermentation of OM to release gases.

PEG was used as a standard phenolic binding agent which was compared against urea and sulphur (S) to determine whether the latter two could effectively alleviate the antinutritive effects of CTs. Ameliorants (PEG, urea and S) improved IVDMD but not GP. The effects of the ameliorants were statistically similar, indicating that they had similar alleviate capacity. An increased *in vitro* (Kumar and Vaithiyanathan, 1990) and *in vivo* (Jones and Mangan, 1977; Pritchard *et al.* 1992) digestibility of tannin-rich feeds has been observed with the addition of PEG-4000. Similarly, protein digestibility of Robinia pseudoacacia leaves in rats which was increased from 49.1 to 70.7% was attributed to the binding of dietary tannin to PEG (Horigome *et al.*, 1988).

The improved IVDMD with ameliorants, indicated that the ameliorants, to some degree, counteracted the effects of the antinutrients. Whereas the improved IVDMD with PEG could be attributed to the binding of dietary tannins to the PEG, that obtained with the addition of urea or S could be by the provision of extra nutrients (Kumar and Singh, 1984), which may have been limiting in the high tannin forages. For example, higher protein levels or inclusion of amino acids to provide favourable tannin:protein ratios in the diet have been

reported to alleviate some of the anti-nutritional effects of tannins (Mueller-Harvey and McAllan, 1992).

The use of *in vitro* GP technique in conjunction with phenolic binding agents has been indicated ( Khazaal and Orskov, 1994; Khazaal *et al.*, 1994; Makkar *et al.*, 1995; Khazaal *et al.*, 1996) as appropriate for the assessment of phenolics-related antinutritive factors in browse species. Such studies have shown an increase in the amount of gas produced by the binding of these compounds (ANFs) in the fermentation system with phenolic binding agents. This observation is not in agreement with those of Khazaal *et al.* (1994), Makkar *et al.* (1995) and Tolera *et al.* (1997). Although it was not easy to compare the CTs concentration of the FLs used in the above experiments, it would seem that the tannin contents in those studies were lower than the levels used in this study. There is equally the question of biological activity of different sources of tannins that will ultimately influence the tannin and phenolic binding agents interactions.

On the other hand, the lack of any improvement in GP with the ameliorants could indicate that not all phenolic compounds that bind to PEG affect GP, or that ANFs other than phenolics with no affinity to PEG and which cannot respond to the addition of urea or S are involved. It can therefore, be inferred that biological response to polyphenolic compounds depends on their nature and thus varies between plant species (Khazaal *et al.*, 1996). Factors such as pH, quantity and type of binding agent or phenolics are factors that can affect the binding efficiency to phenolics (Khazaal *et al.*, 1996). Garrido *et al.* (1991) reported that 2.8 mg PEG (molecular weight 4000) per mg of tannin was needed to obtain maximum increase in digestible crude protein of faba beans whereas 160 mg of insoluble polyvinyl pylorridone

was required to obtain maximum reduction in the levels of tannin in faba beans. In this experiment, 15 mg (IVDMD) or 7 mg (GP) of PEG was used which translated to (PEG:condensed tannin (mg/mg)) ratios of 15:9 15:12 15:22 15:41 for IVDMD or 7:3 7:5 7:9 7:17 for GP for the *lablab*, *leucaena*, desmodium and goetzei treatments respectively. The results suggest that the quantity of PEG used may not have been enough to promote GP. This argument is however countered by the fact that the same level of ameliorants effectively counteracted the antinutritive effects in these forages when used in Tilley and Terry *in vitro* system.

*In vitro* digestion techniques provide comparative estimates of DMD among feeds. These values may be used to rank the quality of feeds but usually underestimate values obtained from *in vivo* digestibility. A study by Getachew *et al.* (1994) showed that desmodium was readily eaten by sheep resulting in weight gains. Thus the high CTs content is apparently not undesirable, even though *in vitro* digestibilities indicate so. The fact that IVDMD was improved with the addition of ameliorants indicated that it could prove beneficial *in vivo*. The use of *in vitro* techniques like GP to study antinutritive effects of FLs may be useful, but it seems that it is also necessary to examine other fermentation indices like ammonia and VFA production and microbial protein synthesis in order to properly quantify these effects. Other results indicate that ammonia and VFA production (Odenyo *et al.*, 1997) and microbial protein synthesis (Nsahlai *et al.*, 1996) could provide better indices.

## 4.6 CONCLUSION

Inoculum from steers fed BR sorghum stover was less effective than inoculum from steers fed grass hay in promoting IVDMD in treatments containing high tannin forages, suggesting a cumulative effect of tannins from both sources (the inoculum and the forages) depressed fermentation. This observation points to the possible detrimental effects of using a high tannin basal diet together with high tannin forages as supplements. Generally, inclusion of FLs as supplements improved digestibility and FLs with lower CTs levels were superior to those with high tannin content. Ameliorants were effective in alleviating antinutritive effects of tannin as indexed by IVDMD. This improvement suggests that it is possible to use urea or sulphur as effective ameliorants to high tannin feeds. Gas production did not respond to the addition of ameliorants possibly indicating that biological response to polyphenolic compounds depends on their nature and this varies with plants. Future evaluation of the antinutritive properties in forages would benefit by the inclusion of other fermentation indices such as ammonia and VFA productions and microbial protein synthesis.

# CHAPTER FIVE

## 5.0 DETERMINATION OF OPTIMAL LEVEL OF POLYETHYLENE GLYCOL 4000 (PEG) TO ALLEVIATE PHENOLICS-RELATED ANTINUTRITIVE EFFECTS INDEXED BY *IN VITRO* GAS AND AMMONIA PRODUCTIONS, AND DRY MATTER LOSS IN FORAGE LEGUMES VARYING IN TANNIN CONCENTRATIONS

## ABSTRACT

In vitro dry matter disappearance (IVDMD), gas and ammonia production were used to index the optimal level of PEG required to alleviate phenolics-related antinutritive effects in forage legumes varying in tannin concentrations. Leaves from *lablab, tagasaste, leucaena, S. sesban 15019, S. sesban 15036, S. sesban 2024, goetzei, desmodium, acacia* and *calliandra* were studied. Based on the hydrolyzable tannins (HTs) content, the forages ranked in the order: *calliandra* > *acacia, leucaena* > *tagasaste* > *desmodium* > *S. sesban 15036* > *goetzei, S. sesban 15019* > *S. sesban 2024* > *lablab*. The ranking based on total CTs concentration was as follows: *goetzei* > *S. sesban 2024* > *desmodium* > *S. sesban 15036* > *calliandra* > *leucaena, acacia* > *S. sesban 15019* > *tagasaste, lablab*. Gas and ammonia production were significantly (P<0.001) influenced by forage type and PEG level. There was significant (P<0.001) forage x PEG level interaction. *Tagasaste* and *lablab* produced more gas than the other forages. Level of PEG had significant (P<0.001) linear and quadratic effects on gas and ammonia production. Gas production (GP) increased rapidly from PEG level 0:1 (PEG:CTs) to level 2:1 and thence increased gradually to a maximum at level 4:1 beyond which GP dropped drastically at level 5:1. Forage type and forage x PEG level interaction significantly (P<0.001) influenced DM loss. Incremental levels of PEG significantly (P<0.05) improved DM loss in *calliandra* and *desmodium* but significantly (P>0.05) reduced DM loss from *goetzei* and *tagasaste*. The optimal PEG levels to CTs ratios

as determined by linear and quadratic contrast for maximum gas and ammonia production and DM loss were 3.3:1, 2.3:1 and 3.2:1 respectively. The mean values for IVDMD obtained when comparing the optimum level of PEG that was determined with urea and sulphur were not significantly ($P<0.05$) different. However, forage type and ameliorant type significantly ($P<0.001$) influenced IVDMD. Because the forages required varied amounts of PEG to alleviate the antinutritive effects, and response did not depend on the CTs concentrations, it was concluded that the binding of PEG does not depend on CTs concentration alone. Thus suggesting that different tannins have different biological activity.

## 5.1 INTRODUCTION

Polyethylene glycol (PEG), applied either by spraying on tannin-rich green chop, mixing with harvested forages, infusion into the rumen, or by drenching of animals has been reported to increase feed intake, digestibility, and wool growth in sheep (Barry and Manley, 1984; Pritchard *et al.*, 1988; Pritchard *et al.*, 1992). This improvement was attributed to the protein released for the host animal to use from protein-tannin complexes by exchange reaction with PEG. PEG with a molecular weight of 4000 is a nonionic detergent that forms complexes with hydrolyzable and condensed tannins over a wide pH range (2.0 to 8.5) (Jones, 1976). However, researchers hold divergent views on the level of PEG per unit of tannin that will be most efficient. For example, levels used in various sheep and goat feeding trials ranged from 0.22-6.67 g PEG per g of condensed tannins (Pritchard *et al.*, 1992; Silanikove *et al* 1994; Silanikove *et al.*, 1996; Silanikove *et al.*, 1997). In situations where PEG is employed to complex tannins, any uncomplexed or free tannins are likely to impact their deleterious effect on the nutrition of the animal, the severity of this effect probably being related to both the quantity and reactivity of the uncomplexed tannins (Barry and Manley, 1984). Therefore, if the beneficial effects of PEG are to be fully realized, it is necessary to establish appropriate levels to be used in feeding trials.

In order to quantify the amounts of PEG to be used, it is important also to know the tannin content of the feed. Condensed tannins (proanthocyanidins) in forages can be quantified by oxidatively cleaving the polymer to form anthocyanidins using the acid butanol method (Porter *et al.*, 1986). Condensed tannin level in a plant sample can be determined either in comparative amounts by the use of a commercial standard (external standard), or in actual amounts by using purified extracts of the tannins of interest, isolated from the plant

under study (internal standard). The main problem with the use of a single compound in tannin standardization, is that one compound is chosen as a standard in assays which are actually measuring a range of reactive groups (Giner-Chavez *et al.*, 1997). Waterman and Mole (1994) suggested that the best solution for the problem of standards in tannin assays is to prepare large batches of tannin extracts from the plant to be assayed, and to use this material to generate internal standards for each plant species. This internal standard can then be used for between and within species comparisons. Standard selection is crucial for the interpretation of results in all tannin assays, as incorrect conclusions can easily be drawn (Giner-Chavez *et al.*, 1997).

Several studies have shown an increase in the amount of gas produced by removal of phenolic compounds from fermentation systems with polyvinylpyrrolidone (PVP) resin. Gas production was therefore used in this study to index the efficiency of PEG in complexing tannins.

The objectives of this study were to (i) extract and quantify condensed tannins from selected forage legumes, (ii) assess the effects of graded levels of PEG on in vitro gas and ammonia production, and dry matter disappearance of these forage legumes (that vary in tannins concentration) with the view of identifying the optimum levels of PEG required to alleviate phenolics-related antinutritive effects in these forages. The study also compared the optimal level of PEG (as indexed by the highest gas production level) with two other ameliorants, urea and sulphur to determine their effectiveness in counteracting phenolic-related antinutritive effects.

## 5.2. MATERIALS AND METHODS

### 5.2.1 Plant leaf material

Leaves were hand-harvested from a number of plants in single rows of 5-10 trees or herbaceous legumes on the same day from the Debre Zeit Research Station of the International Livestock Research Institute in Ethiopia. Debre Zeit research station is in the Ethiopian highlands, at an altitude of about 1850 m above sea level. The average annual rainfall in Debre Zeit is 866 mm, of which 84% falls in the main rainy season extending from June to September. Average annual temperature is 18.7° C with maximum of 26° C and minimum of 11.3° C. The soil of the experimental site is light black soil (alfisol). Leaves were collected from ten different plant species: *Lablab purpureus* (*lablab*), *Chamaecytisus palmensis* (*tagasaste*), *Leucaena leucocephala* (*leucaena), Sesbania sesban 15019* (*S. sesban 15019), S. sesban 15036, S. sesban 2024, S. goetzei 15007* (*goetzei*), *Desmodium intortum* (*desmodium*), *Acacia angustissima* 15132 (*acacia*) and *Calliandra callothyrsus* (*calliandra*). Leaves were oven dried at $40^{E}$ C until constant-weight, cooled in a dessicator and ground to pass through a 1-mm screen.

### 5.2.3 Measurements

#### 5.2.3.1 Effect of PEG on in vitro gas production

The *in vitro* gas production method described by Menke *et al.* (1979) was used to evaluate the effectiveness of PEG in alleviating phenolics-related antinutritive effects. Fermentation was carried out in graduated glass syringes (100-ml capacity). Rumen liquor was collected before morning feeding from two fistulated steers fed bird resistant sorghum (containing 890g organic matter (OM), 7 g nitrogen (N) and 740 g neutral detergent fibre (NDF) per kg DM) offered ad libitum and supplemented with 2 kg per head per day

cottonseed cake (910 g OM, 86 g N and 380 g NDF per kg DM) and mineral block. Feeds were ground to pass through a 1-mm screen and approximately 500 mg DM of each weighed into a graduated glass syringe into which PEG was added to meet one of the following PEG:tannin ratios: 0:1, 1:1, 2:1, 3:1, 4:1 and 5:1 (PEG levels 0, 1, 2, 3, 4 and 5). For each feed, each of the ratios was replicated three times in glass syringes into which was added 40 ml of the incubation medium prepared as described by Menke *et al.* (1979). The sample size (500 mg) was chosen to ensure that enough digesta residues were available after fermentation for the determination of ammonia production and DM loss. During incubation, the syringes and their contents were maintained at 38.5-39 ° C in a thermostatic circulating water bath (260, Precision Scientific, Fisher Scientific, Springfield, USA). Alongside, six other syringes containing only the incubation medium were placed in the water bath to correct for gas production due to the activity of rumen fluid alone. Gas production (GP) readings were taken after 3, 6, 9, 12 and 24 h of incubation. After each reading, the piston of the syringe was re-set to 40 ml whenever it had gone beyond the 60-ml mark. After the 24 h gas reading, ammonia concentration was determined in the incubation medium and dry mater disappearance estimated from the residue. DM left as residues after the 24 h incubation was determined. The residues were washed with neutral detergent solution (NDS) and refluxed for an hour to determine NDF according to the method described by Goering and Van Soest (1970).

#### 5.2.3.2 *In vitro* DMD procedure

*In vitro* DM disappearance (IVDMD) determination followed a modified method of Tilley and Terry (1963) whereby pepsin-HCl digestion was replaced with neutral detergent extraction (Van Soest, 1982). About 500 mg of forage samples with or without PEG, urea

or sulphur were weighed into 125-ml Erlenmeyer flasks. The level of PEG used in this trial was the level which produced the highest amount of gas volume determined in the gas production trial. Urea and sulphur were added at 3% of DM of the feed sample (3% of 500 mg DM i.e. 15 mg). Thereafter, 40 ml of prepared medium (Tilley and Terry, 1963) was added to each flask which was then inoculated with 10 ml of rumen fluid harvested as described in section 5.2.3.1.and incubated for 48 h. The 48 h residues were washed with neutral detergent solution (NDS) and refluxed for an hour to determine NDF according to the method described by Goering and Van Soest (1970); the dry matter disappearance (DM loss) was then calculated.

**5.2.4 Chemical analysis**

**5.2.4.1 Extraction of tannins from plant leaves**

A procedure based on the method of Giner-Chavez *et al.(*1997) was used for the extraction. One gram of ground plant material was extracted 3 times in a 50-ml snap-top plastic tube with 20 ml of 70% aqueous acetone (v/v) for 20 minutes in duplicate. After the last extraction, the plant residues were transferred to Gooch crucibles and rinsed with 40 ml of 70% aqueous acetone (v/v). The combined extract was purified by precipitation with trivalent ytterbium.

**5.2.4.2 Purification of crude condensed tannins extracts by trivalent ytterbium method**

The crude plant extract obtained above was washed 3 to 4 times with an equal volume of petroleum ether to remove chlorophyll. Traces of petroleum ether and acetone in the aqueous phase were evaporated under vacuum without allowing precipitates to form. If the solution turned hazy, a small amount of 70 % aqueous acetone (v/v) was added to clarify the solution. The aqueous solution was washed 3 times with ethyl acetate, and evaporated

under vacuum at less than 30E C to remove traces of ethyl acetate. The remaining solution was separated into 50 ml aliquots and placed in 125-ml Erlenmeyer flasks. Ytterbium acetate ($Yb(C_2H_2O_2)_3.4H_2O$, 0.1M, 2ml) was added to the aqueous solution of condensed tannins and stored overnight at 5E C. The stored solution was then centrifuged at 3,000 x g for 10 minutes at 5E C. The pellet was then washed and recentrifuged twice with 70 % aqueous acetone and once with acetone, then dried and pulverized.

**5.2.4.3 Preparation of condensed tannin Yb-ppt standard for acid butanol assay**

A known amount (20-30mg) of purified tannin Yb-ppt from the plant samples was solubilized in 10 ml of 70% aqueous acetone (v/v) containing 0.5% concentrated HCl to make a standard solution. Six serial dilutions (six concentrations) of 1 ml total volume were performed with standard solutions in 1.3 x 10 cm glass screw cap culture tubes. Thereafter, 6 ml of acid butanol reagent (n-butanol:concentrated HCl, 95:5 by volume) and 0.2 ml of iron reagent [2% ferric ammonium sulfate dodecahydrate salt ($FeNH_4(SO_4)_2.12H_2O$), in 2 N HCl] were added. Samples were vortexed for 30 seconds and placed in a water bath at 95E C for 50 minutes. Tubes were cooled to room temperature and absorbance of the samples measured at 550 nm. The standard calibration between tannin concentration and absorbance was performed in triplicate. Dry matter concentration of Yb-ppt in serial dilutions was corrected for ash. Condensed tannin Yb-ppt (20-30 mg) was weighed in a porcelain dish and ashed at 400EC for four hours in a muffle furnance. Thereafter, it was removed and placed in a dessicator to cool and reweighed to account for the ash content in the Yb-ppt. The amount of condensed tannins in a plant sample was estimated using the internal standard.

The DM and N concentrations of samples were determined using the method of the Association of Official Analytical Chemists (1980). Neutral detergent fibre was estimated by the method of Goering and Van Soest (1970). Soluble, insoluble and fiber-bound condensed tannin (CT) contents of plant samples were determined according to methods described by Giner-Chavez *et al.*(1997) as described in chapter 4 of this thesis. Total concentration of CTs was determined by adding the three values. hydrolyzable tannins (HTs) were assayed as total water-soluble phenolics after water extraction following the method of Kaitho *et al.* (1993). After filtration the tannins were transformed to a blue-coloured product by Folin-Denis reagent and sodium carbonate. The intensity of the colour was measured at 760 nm. The content of total water soluble phenolics was calculated using a calibrated standard line prepared using tannic acid (Sigma T0125) as the standard compound

A modified method of Chaney and Marbach (1962) that uses phenol and hypochlorite reagents was used to determine ammonia concentration. At the end of incubation, a 50 ml of sample was added to a test tube. Approximately 3 ml of hypochlorite reagent (25 g NaOH; 16.6 mL household bleach; 1 litre double distilled $H_2O$) was added and mixed, followed by the addition of 3 ml phenol reagent. The standard solutions had various concentrations of ammonium chloride. The samples and the standard solutions were incubated for 30 minutes at room temperature and the absorbance at 630 NM was read in a spectrophotometer 21D (Milton Roy, Brussels, Belgium).

**5.2.5 Statistical analysis**

Analysis of variance was carried out on in vitro gas production, ammonia production and on dry matter disappearance using statistical analysis package (SAS, 1987). Main effects of forage, PEG level, ameliorants and forage x PEG level interactions were tested.

Contrasts were performed to test the linear and quadratic effects of graded levels of PEG on the above. Regression equations to estimate the optimum level of PEG were formed through regression analysis.

## 5.3 RESULTS

### 5.3.1 Chemical composition of the forage samples

Table 5.1 shows the mean values for DM, OM, ash, N, NDF, hydrolyzable tannins (HTs), soluble, insoluble and fibre-bound condensed tannins (CTs) of the FLs. Figure 5.1 shows the standard calibration between tannin concentration and absorbance of the 10 forages studied. *S. sesban 15019* had the highest N content, while *calliandra* had the lowest N content. Neutral detergent fibre content was high in FLs that had low N content and high tannin content, for example *goetzei, desmodium* and *S. sesban 2024*. Table 5.2 shows the correlation coefficients between the chemical properties. Total DM content was significantly ($r=0.75$, $P<0.05$) correlated to HTs. The total CTs content also had a positive relationship ($r=0.69$, $P<0.05$) with the NDF content, while N content was negatively correlated ($r=-0.69$, $P<0.05$) to HTs.

### 5.3.2 Effect of level of PEG on 24 h gas production from various forage legumes

Table 5.3 shows the mean values of gas production (GP) and main effects of forage, PEG level and forage x PEG level interaction on the vclume of gas produced after 24 h of incubation. Gas production was significantly ($P<0.001$) influenced by the type of forage and PEG level. There was a significant ($P<0.001$) forage x PEG level interaction. For some forage (*goetzei, S. sesban 15036, S. sesban 2024* and *tagasaste*) GP responded negatively to incremental level of PEG, reaching a trough from whence it started to rise again. In the case of *leucaena, desmodium, S. sesban 15019* and *lablab* GP responded almost linearly to incremental levels of PEG. Still for a third group of forages (*acacia* and *calliandra*) GP responded positively following a quadratic pattern to incremental levels of PEG. An attempt was made to determine the optimal level of PEG required to produce maximum volume of

Table 5.1 The dry matter (g/kg) and chemical composition (g/kg DM) of leaves of forage legumes

| Forage legumes | Dry Matter | Organic matter | Ash | N | NDF | Water soluble tannins (HTs) | Soluble CTs | Insoluble CTs | NDF-bound CTs | Total CTs. |
|---|---|---|---|---|---|---|---|---|---|---|
| Acacia angustissima | 890 | 878 | 122 | 46.2$^{c}$ | 303$^{b}$ | 74.55$^{b}$ | 12.42 | 16.40 | 1.76 | 30.59$^{f}$ |
| Calliandra calothyrsus | 890 | 876 | 124 | 30.9$^{g}$ | 242$^{bc}$ | 88.17$^{a}$ | 13.66 | 25.28 | 0.20 | 39.13$^{e}$ |
| Desmodium intortum | 880 | 870 | 130 | 46.0$^{c}$ | 426$^{a}$ | 34.45$^{d}$ | 40.47 | 44.06 | 16.21 | 100.73$^{c}$ |
| Lablab purpureus | 880 | 860 | 140 | 50.5$^{b}$ | 180$^{e}$ | 16.35$^{g}$ | 0.47 | 0.47 | 0.93 | 1.88$^{h}$ |
| Leucaena leucocephala | 900 | 880 | 120 | 42.0$^{d}$ | 201$^{e}$ | 73.58$^{b}$ | 14.72 | 14.52 | 4.06 | 33.30$^{f}$ |
| Sesbania goetzei 15007 | 880 | 869 | 131 | 40.2$^{e}$ | 421$^{a}$ | 29.41$^{ef}$ | 15.19 | 118.71 | 90.60 | 224.49$^{a}$ |
| Sesbania sesban 15019 | 890 | 880 | 120 | 53.1$^{a}$ | 183$^{e}$ | 28.54$^{ef}$ | 0.52 | 8.56 | 3.49 | 12.57$^{g}$ |
| Sesbania sesban 15036 | 890 | 880 | 120 | 42.4$^{d}$ | 201$^{e}$ | 31.52$^{de}$ | 13.04 | 38.05 | 26.30 | 77.39$^{d}$ |
| Sesbania sesban 2024 | 890 | 876 | 124 | 51.1$^{b}$ | 307$^{b}$ | 25.98$^{f}$ | 19.06 | 75.10 | 58.12 | 152.28$^{b}$ |
| Chamaecytisus palmensis | 880 | 868 | 134 | 37.3$^{f}$ | 302$^{b}$ | 59.51$^{c}$ | -1.20 | 3.37 | -0.07 | 2.10$^{h}$ |
| MEAN | 886 | 874 | 127 | 44 | 276 | 40.69 | 12.84 | 34.45 | 20.16 | 67.45 |
| SED | | | | 0.48 | 29.7 | 1.34 | 0.34 | 1.37 | 1.34 | 1.89 |
| Level of significance | | | | *** | *** | *** | *** | *** | *** | *** |

Means with the same superscript are not significantly different.

Significance level: NS = P>0.05, * = P<0.05 ** = P<0.01, *** = P<0.001

N= nitrogen, NDF= neutral detergent fibre, CTs = condensed tannins, Water soluble tannins (HTs)= hydrolysable tannins

**Figure 5.1 Calibration lines of condensed tannin internal standards from 10 forage legumes in acid butanol assay. Sample-1represents Acacia angustissima (y=2297x+0.0078,$R^2$=0.9983), sample-2, Calliandra calothyrsus (y=0.2193x+0.0199,R2=0.9932), sample-3, Desmodium intortum (y=0.1701x+0.0126,R2=0.9901), sample-4, Dolichos lablab (y=0.0765x+0.0098, R2=0.9453), sample-5, Leucaena leucocephala (y=0.0535x+0.0087,R2=0.9112), sample-6, Sesbania goetzei 15007 (y=0.2067x+0.0152,R2=0.9893), sample-7, S. sesban 15019 (y=0.0973x0.0145,R2=898), sample-8, S. sesban 15036 (y=0.0578x+0.0067,R2=0.9973), sample-9, S. sesban 2024 (y=0.0268x+0.0083,R2=0.9991) and sample-10, Chamaecytisus palmensis (y=0.1318x+0.0072,R2=0.9909).**

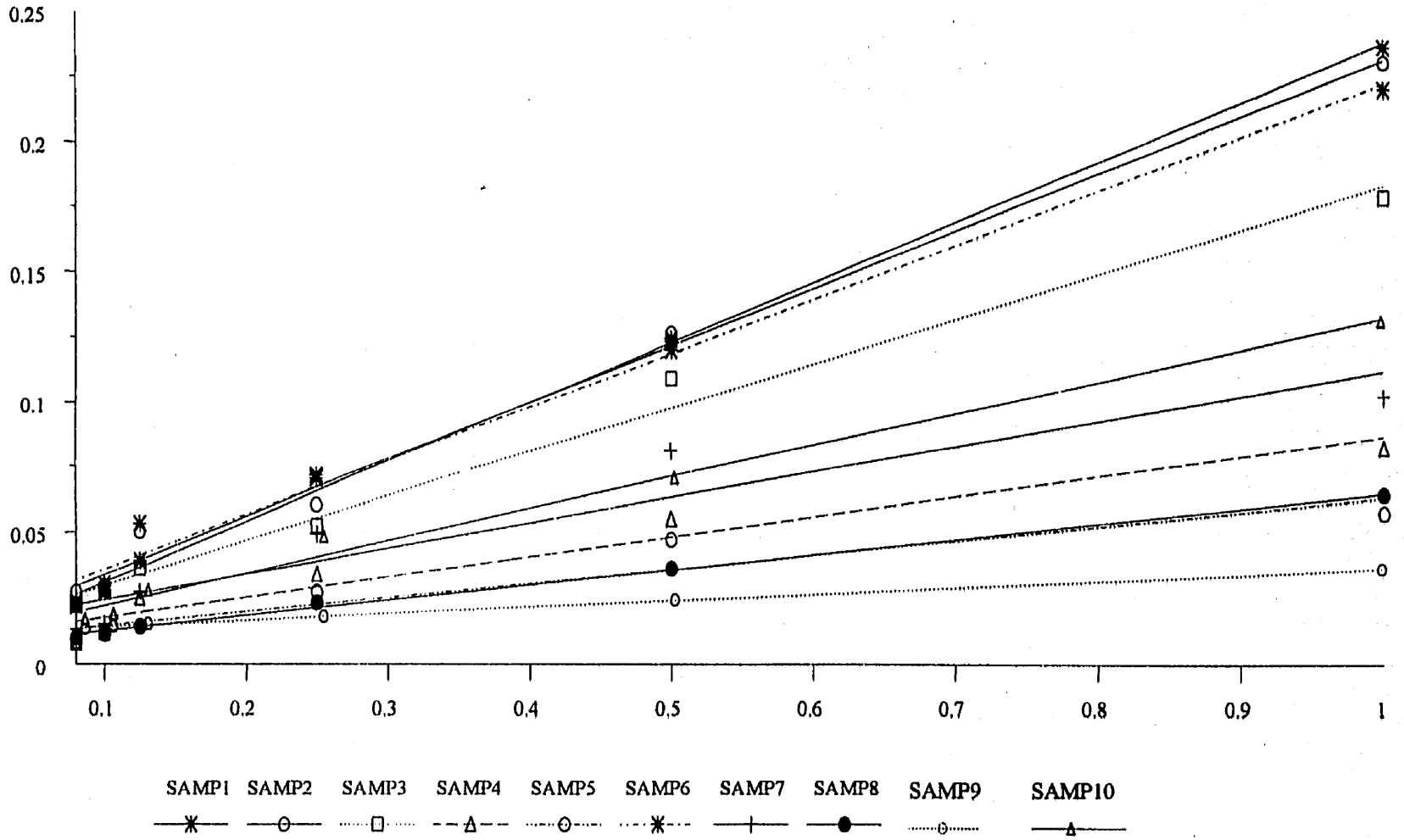


Table 5.2 The correlation coefficients between chemical properties.

| | DM | OM | N | NDF | CTs | HTs |
|---|---|---|---|---|---|---|
| Dry matter (DM) | | $-0.00^{NS}$ | $-0.24^{NS}$ | $-0.21^{NS}$ | $-0.22^{NS}$ | $0.75^{*}$ |
| Organic matter (OM) | | | $-0.33^{NS}$ | $-0.03^{NS}$ | $-0.04^{NS}$ | $0.17^{NS}$ |
| Nitrogen (N) | | | | $-0.16^{NS}$ | $-0.02^{NS}$ | $-0.69^{*}$ |
| Neutral detergent fibre (NDF) | | | | | $0.69^{*}$ | $-0.07^{NS}$ |
| Condensed tannins (CTs) | | | | | | $-0.36^{NS}$ |
| Water soluble tannins (HTs) | | | | | | |

Level of significance, NS = P>0.05, * = P<0.05.

Table 5.3 Effect of graded levels of Polyethylene glycol (PEG) on 24 h gas production (ml/500mg DM) from various forage legumes

| | PEG Level | | | | | | | Regression equation | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Forage legumes | 0 | 1 | 2 | 3 | 4 | 5 | Mean | a | b | c | P=[2] | X[3] |
| Acacia angustissima | 28.8 | 81.2 | 168 | 169.5 | 170.8 | 75.0 | 115.5 | 15.3 | 110.9 | -19.3 | 0.02 | 2.8 |
| Calliandra calothyrsus | 81.2 | 83.7 | 99.5 | 139.5 | 144.8 | 145.0 | 115.6 | 74.3 | 19.6 | -0.9 | 0.04 | 11.1 |
| Desmodium intortum | 160 | 176 | 177 | 177.2 | 179.2 | 177.2 | 174.4 | 162.7 | 10.1 | -1.5 | 0.05 | 3.4 |
| Lablab purpureus | 174 | 170 | 178 | 179.7 | 181.5 | 177.2 | 176.7 | 171.5 | 3.8 | -0.5 | 0.34 | 3.7 |
| Leucaena leucocephala | 171 | 173 | 174 | 176.0 | 176.2 | 179.3 | 174.8 | 170.8 | 1.5 | 0.01 | 0.01 | -75.0 |
| Sesbania goetzei 15007 | 170 | 168 | 165 | 167.3 | 167.3 | 164.8 | 167.0 | 169.5 | -1.7 | 0.2 | 0.35 | 4.9 |
| Sesbania sesban 15019 | 171 | 172 | 175 | 174.3 | 177.3 | 177.7 | 174.6 | 171.1 | 1.4 | -0.01 | 0.01 | 70.0 |
| Sesbania sesban 15036 | 170 | 172 | 171 | 168.3 | 168.7 | 170.7 | 170.1 | 171.3 | -1.0 | 0.1 | 0.65 | 3.2 |
| Sesbania sesban 2024 | 171 | 175 | 172 | 169.7 | 178.3 | 177.3 | 173.8 | 172.1 | -0.6 | 0.4 | 0.43 | 0.9 |
| Chamaecytisus palmensis | 194 | 188 | 185 | 174.2 | 176.8 | 181.7 | 183.3 | 195.1 | -9.5 | 1.3 | 0.06 | 3.7 |
| Mean | 149 | 156 | 166 | 169.6 | 172.1 | 162.6 | 163.1 | | | | | 3.3 |

| Main effects | SED | Level of significance |
|---|---|---|
| Forage type | 3.83 | *** |
| PEG level | 2.98 | *** |
| Forage x PEG level | 9.39 | *** |
| Contrasts: | | |
| Linear | | *** |
| Quadratic | | *** |

PEG level: 0= no PEG added, 1= PEG added at 1:1 ratio with condensed tannins etc.

Significance level: NS = P>0.05, * = P<0.05 ** = P<0.01, *** = P<0.001

[1]Terms a= constant, b= linear, c= quadratic,[2] p-values, [3] Predicted optimum level of PEG

gas. For the first group of forages, the PEG levels obtained (4.9, 3.2 , 0.9 and 3.7) were the worse and in this situation, it could be subjectively considered to be zero. For the group two forages, the values determined are quite scattered (-38.2 to 30.4) thus indicating the difficulty of determining their optimal level of PEG.. The third group had values of 2.8 and 11.1.

### 5.3.3 Effect of PEG level on DM loss from various FLs after 24 h incubation

Table 5.4 shows mean DM loss after 24 h of incubation. The type of forage and forage x PEG level interactions significantly ($P<0.001$) influenced DM loss. However, PEG level did not significantly ($P>0.05$) affect DM loss. For some forages *(lablab, leucaena, goetzei, S. sesban 15036, S. sesban 2024* and *tagasaste*) DM loss responded negatively to incremental level of PEG. The other forages *(acacia, calliandra, desmodium and S. sesban 15019*) DM loss responded almost linearly with the incremental level of PEG. The optimal level of PEG required to achieve greatest DM loss was derived for each of the forages (Table 5.4). *Desmodium, S. sesban 2024* and *goetzei* that had high CTs concentration required 0.2, 1.0 and 3.7g of PEG per g CT, respectively, while *S. sesban 15019* that was low in CTs content would require 8.3 g of PEG per g CT.

### 5.3.4 Effect of PEG level on ammonia production from various FLs after 24 h incubation

Mean values of ammonia production are shown in Table 5.5. The type of forage, level of PEG and the forage x level of PEG interaction significantly ($P<0.001$) affected ammonia production. Some forages (*goetzei, S. sesban 15019, S. sesban 15036, tagasaste* and *lablab*) responded negatively to the incremental level of PEG, reaching a trough whence it started to rise again. For the other group of forages (*acacia, calliandra, desmodium, leucaena* and *S. sesban 2024*) ammonia production responded positively in a quadratic

Table 5.4 Effect of graded levels of Polyethylene glycol (PEG) on DM loss after 24 h incubation

| | PEG level | | | | | | | Regression equation | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Forage legumes | 0 | 1 | 2 | 3 | 4 | 5 | Mean | a | b | c | P=[2] | X[3] |
| Acacia angustissima | 64.2 | 64.7 | 62.7 | 67.9 | 67.5 | 67.2 | 65.7 | 63.8 | 0.6 | 0.0 | 0.34 | -6.2 |
| Calliandra calothyrsus | 43.7 | 59.9 | 57.8 | 63.5 | 61.1 | 58.6 | 57.4 | 45.8 | 10.9 | -1.7 | 0.06 | 3.1 |
| Desmodium intortum | 68.1 | 67.7 | 68.8 | 67.2 | 66.6 | 66.5 | 67.5 | 68.1 | 0.1 | -0.1 | 0.17 | 0.2 |
| Lablab purpureus | 78.9 | 77.5 | 78.3 | 77.6 | 78.3 | 77.3 | 78.0 | 78.6 | -0.4 | 0.0 | 0.54 | 4.6 |
| Leucaena leucocephala | 78.3 | 77.4 | 77.5 | 77.4 | 77.0 | 77.1 | 77.5 | 78.2 | -0.5 | 0.1 | 0.06 | 2.5 |
| Sesbania goetzei 15007 | 59.2 | 53.1 | 51.1 | 50.0 | 48.9 | 49.8 | 52.0 | 58.6 | -5.2 | 0.7 | 0.004 | 3.7 |
| Sesbania sesban 15019 | 76.8 | 78.1 | 77.6 | 78.9 | 79.3 | 79.6 | 78.4 | 76.9 | 0.7 | -0.0 | 0.04 | 8.3 |
| Sesbania sesban 15036 | 77.2 | 75.7 | 74.9 | 72.8 | 74.6 | 73.6 | 74.8 | 77.2 | -1.8 | 0.2 | 0.08 | 3.7 |
| Sesbania sesban 2024 | 68.0 | 65.2 | 64.5 | 69.7 | 68.9 | 67.5 | 67.3 | 66.7 | -0.2 | 0.1 | 0.79 | 1.0 |
| Chamaecytisus palmensis | 67.4 | 66.6 | 63.0 | 64.3 | 58.8 | 64.0 | 64.0 | 68.1 | -3.1 | 0.4 | 0.26 | 3.9 |
| Mean | 68.2 | 68.6 | 67.6 | 68.9 | 68.1 | 68.1 | 68.3 | | | | | 3.2 |

| Main effects | SED | Level of significance |
|---|---|---|
| Forage type | 0.75 | *** |
| PEG level | 0.61 | NS |
| Forage x PEG level | 1.84 | *** |
| Contrasts: | | |
| Linear | | NS |
| Quadratic | | NS |

PEG level: 0= no PEG added, 1= PEG added at 1:1 ratio with condensed tannins etc.
Significance level: NS = P>0.05, * = P<0.05 ** = P<0.01, *** = P<0.001
[1]Terms a= constant, b= linear, c= quadratic,[2] p values, [3] Predicted optimum level of PEG

Table 5.5 Effect of graded levels of Polyethylene glycol (PEG) on ammonia production (ppm) after 24 hour incubation

| | PEG level | | | | | | Regression equation | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Forage legumes | 0 | 1 | 2 | 3 | 4 | 5 | Mean | a | b | c | P=[2] | X[3] |
| Acacia angustissima | 60.0 | 66.1 | 83.3 | 76.8 | 78.6 | 73.8 | 73.1 | 59.0 | 13.2 | -2.1 | 0.08 | 3.0 |
| Calliandra calothyrsus | 44.7 | 48.2 | 56.0 | 62.0 | 61.6 | 62.8 | 56.0 | 43.4 | 8.0 | -0.8 | 0.01 | 4.7 |
| Desmodium intortum | 56.2 | 90.0 | 83.7 | 89.9 | 88.4 | 85.9 | 82.3 | 62.0 | 18.7 | -2.9 | 0.13 | 3.2 |
| Lablab purpureus | 79.5 | 82.8 | 75.2 | 83.6 | 85.7 | 88.8 | 82.6 | 80.3 | -1.5 | 0.7 | 0.20 | 1.2 |
| Leucaena leucocephala | 57.6 | 56.2 | 61.6 | 57.5 | 56.4 | 56.2 | 57.6 | 57.1 | 1.5 | -0.4 | 0.58 | 2.1 |
| Sesbania goetzei 15007 | 72.8 | 91.5 | 77.8 | 82.2 | 83.8 | 91.8 | 83.3 | 78.3 | 1.5 | 0.1 | 0.59 | -5.3 |
| Sesbania sesban 15019 | 100.9 | 89.3 | 87.1 | 91.2 | 89.2 | 87.1 | 90.8 | 98.3 | -6.1 | 0.9 | 0.20 | 3.6 |
| Sesbania sesban 15036 | 81.5 | 57.9 | 56.7 | 43.7 | 56.5 | 74.1 | 61.7 | 81.2 | -25.0 | 4.7 | 0.02 | 2.6 |
| Sesbania sesban 2024 | 101.5 | 108.2 | 119.4 | 114.9 | 99.4 | 105.0 | 108.1 | 102.3 | 9.6 | -2.0 | 0.35 | 2.3 |
| Chamaecytisus palmensis | 74.5 | 47.9 | 56.9 | 49.3 | 45.2 | 49.3 | 53.9 | 70.0 | -13.1 | 1.8 | 0.17 | 3.7 |
| Mean | 72.9 | 73.8 | 75.8 | 75.1 | 74.5 | 77.5 | 75.0 | | | | | 3.2 |

| Main effects: | SED | Level of significance |
|---|---|---|
| Forage type | 1.10 | *** |
| PEG level | 0.83 | *** |
| Forage x PEG level | 2.62 | *** |
| Contrasts: | | |
| Linear | | *** |
| Quadratic | | ** |

PEG level: 0= no PEG added, 1= PEG added at 1:1 ratio with condensed tannins etc.

Significance level: NS = P>0.05, * = P<0.05 ** = P<0.01, *** = P<0.001

[1]Terms a= constant, b= linear, c= quadratic,[2] p values, [3] Predicted optimum level of PEG

pattern to the increase in level of PEG. For the first group of forages, the optimal PEG levels (-5.3, 3.6, 2.6, 1.2 and 3.7) were the worse and in this situation, it could again be subjectively considered to be zero. For the other group forages, the values determined ranged from 2.1 to 4.7 (3.0, 4.7, 3.2, 2.1, and 2.2).

### 5.3.5 Comparison of IVDMD between the PEG level that gave maximum GP with urea and sulphur

The mean values for the IVDMD obtained with the level of PEG that gave maximum gas volume, urea and sulphur are shown in Table 5.6. Forage type significantly ($P<0.001$) influenced IVDMD. There was also significant ($P<0.001$) influence on IVDMD due to the addition of ameliorants (PEG, urea and sulphur). There was however no significant ($P>0.05$) forage x ameliorant interaction on IVDMD. Based on the degree of IVDMD, the FLs were ranked in the following descending order *S. sesban 15019, lablab* > *leucaena* > *S. sesban 15036* > *tagasaste, S. sesban 2024* > *desmodium, calliandra* > *acacia* > *goetzei.* The ameliorants had the similar effect on IVDMD, but the greatest improvement was with *acacia* and *goetzei.*

## 5.4 DISCUSSION

The amount of PEG required to bind tannins varied greatly among the ten forage legumes studied. For example, *goetzei, S. sesban 2024* and *S. sesban 15036* that had high concentration of CTs, responded to less amounts of PEG to alleviate the antinutritive effects. On the other hand *acacia* and *calliandra* that had lower concentration of CTs than the above FLs responded to higher amounts of PEG (2:1 and 3:1 PEG:CTs) to alleviate the effects. The results showed that the binding capacity (reaction) of PEG does not depend on the concentration of CTs only, there may be other attributes responsible for this effect. The two

Table 5.6 Effect of ameliorant (PEG, urea or sulphur) on in vitro dry matter disappearance of forage legume leaves

| | Ameliorants | | | | |
|---|---|---|---|---|---|
| Forage legumes | None | PEG | Urea | Sulphur | Mean |
| Acacia angustissima | 52.80 | 62.00 | 57.80 | 60.40 | 58.3$^{e}$ |
| Calliandra calothyrsus | 54.50 | 56.90 | 63.20 | 63.80 | 59.6$^{de}$ |
| Desmodium intortum | 55.60 | 63.10 | 63.50 | 63.60 | 61.1$^{de}$ |
| Lablab purpureus | 67.20 | 74.80 | 74.90 | 74.10 | 72.3$^{ab}$ |
| Leucaena leucocephala | 65.00 | 72.10 | 70.50 | 69.60 | 69.2$^{bc}$ |
| Sesbania goetzei 15007 | 45.80 | 55.20 | 57.50 | 55.60 | 53.3$^{f}$ |
| Sesbania sesban 15019 | 64.40 | 73.50 | 79.20 | 72.10 | 73.0$^{a}$ |
| Sesbania sesban 15036 | 66.30 | 70.40 | 68.80 | 69.70 | 67.8$^{c}$ |
| Sesbania sesban 2024 | 53.40 | 63.70 | 67.20 | 66.40 | 62.3$^{d}$ |
| Chamaecytisus palmensis | 59.30 | 63.30 | 62.20 | 64.10 | 62.2$^{d}$ |
| Mean | 58.3$^{b}$ | 65.0$^{a}$ | 66.5$^{a}$ | 66.0$^{a}$ | 64.2 |

| Main effects | SED | Level of significance |
|---|---|---|
| Forage type | 1.7 | *** |
| Amelliorant | 1.0 | *** |
| Forage x ameliorant | 2.9 | NS |

Means in the same column or row with same superscript are not significantly different
Significance level: NS = P>0.05, * = P<0.05 ** = P<0.01, *** = P<0.001
PEG was added at the optimal level determined (3:1 mgPEG/mg condensed tannins)
Urea and sulphur added at 3 % of total dry matter (15 mg).

forages (*acacia* and *calliandra*) that responded to higher amounts of PEG as measured by GP had low (30.6 and 39.1g/kgDM, respectively)) CTs and high HTs (74.6 and 88.2 g/kg DM, respectively). Both gross chemical differences, such as those distinguishing CTs from HTs (*gallotannins*), and subtle differences such as molecular weight and stereochemical configuration (Clausen *et al.*, 1990) can influence the biological activity of tannins (Hagerman *et al.*, 1992). Hagerman *et al.* (1992) cautioned that generalizations about the effects and functions of tannins should not be based on studies with tannins from a single source, but should be drawn from studies that address the diversity of tannin chemistry. Tannins are chemically very heterogeneous (Porter, 1989), and tannin structure may influence the tannin-PEG interaction (Mcleod, 1974). The structural differences between gallotannins and CTs from different sources would influence the biological activities of the tannins (Hagerman *et al.*, 1998). The increase in gas evolution in vitro, upon addition of PEG to *acacia* and *calliandra* may indicate that more PEG was required to bind the tannins from these forages because of their chemical diversity. The fact that *S. sesban 15019* that had low CTs and high N had very high optimal PEG level suggests that PEG will bind to macromolecules other than CTs. The increase in DM loss and ammonia released also indicates that PEG precipitated tannins and spared protein to be degraded in the case of *acacia* and *calliandra*. While the lack of response with *S. sesban 15019* may suggest that PEG bound with protein and as such interfered with the protein degradation. This observation is supported by the fact that DM loss and ammonia production were also similarly depressed.

Addition of PEG to *goetzei, S. sesban 2024* and *S. sesban 15036* that had high CTs and low HTs was not effective in alleviating the antinutritive effects of these forages. The

structural differences between HTs and CTs may have influenced the tannin-PEG interaction in these forages by reducing or blocking the binding sites (Hagerman *et al.*, 1998). The reduction in apparent DM digestibility of the high tannin forages, *goetzei, S. sesban 2024* and to some extent *desmodium* could be due to the presence of PEG-tannin complex in the residues. This view is tenable in view of the observation by Makkar *et al.* (1995) that PEG-tannin and/or PVP-tannin complexes are insoluble in boiling water, most organic solvents, and neutral and acid detergent solutions. This implies that PEG-tannin complexes could have influenced the NDF values of the treatments with PEG. Garrido *et al.* (1991) using the in vitro systems to digest tannin-rich feeds showed that the treatment of digesta with HCl-pepsin resulted in digestibility values that were higher than when digesta were treated with NDS. The increase was attributed to the higher solubility of tannin-PEG complexes in HCl-pepsin than in NDS. The in vitro DMD procedure in this study used NDS instead of HCl-pepsin. This could explain why in vitro DMD was depressed for *goetzei* and *S. sesban 2024* that are rich in tannins and NDF.

Tannin-complexing agents like PEG have been used for various purposes such as quantification of tannins and alleviation of adverse effects of tannins in foods and feeds (Barry and Manley, 1984; Garrido *et al.*, 1991; Pritchard *et al.*, 1992; Makkar *et al.*, 1993). PEG-4000 in particular has been shown to alleviate the deleterious effect of tannins (Kumar, 1992). However, there has been little agreement regarding the optimum ratio of PEG to tannins that would alleviate the adverse effect of tannins on ruminal metabolism for animals fed on tannin-rich diets (Pritchard *et al.*, 1992; Silanikove *et al.*, 1994; Silanikove *et al.*, 1996). The results of this experiment also demonstrated this problem. Since PEG binds to tannins, a concomitant increase in GP on inclusion of PEG into the gas system, may

represent the potential adverse effect of tannins present in the feed (Makkar *et al.*, 1995). *Acacia* and *calliandra* responded to larger amounts of PEG suggesting that these two forages may have more of the phenolics-related antinutritive principles. On the other hand it is recognized that the biological activity (reactivity) of the tannins present may be a factor. The results showed that it is not adequate to consider CTs concentration only as having potential adverse effects to be alleviated with tannin-complexing agents; it seems the interaction of CTs and other attributes is important in determining the biological activity of tannins with PEG.

The PEG level, which gave the best gas production, was further compared to urea and sulphur as ameliorants. The ameliorants produced similar results, which indicated that the optimal level of PEG determined was adequate for the various forages and it is in agreement with the earlier in vitro results (chapter 4).

## 5.5 CONCLUSION

The effect of graded levels of PEG on in vitro dry matter digestibility of FLs varying in tannin concentrations was assessed and optimal levels of PEG required to alleviate phenolics-related antinutritive effects were determined. Optimal PEG level varied greatly among the ten FLs studied. Overall optimal ratios of PEG: CTs for GP, ammonia production and DM loss were 3.3:1, 2.3:1 and 3.2:1 respectively. As such the overall mean recommended ratio is 3:1 g PEG to g of CTs i.e. a ratio of 3:1. The results also showed that even though techniques like gas production may be useful in assessing phenolics-related antinutritive effects of tanniferous feeds, they need to be combined with the other fermentation by-products like DM loss and ammonia production to give a complete picture.

# CHAPTER SIX

## 6.0 EFFECTS OF SUPPLEMENTING A HIGH TANNIN BASAL DIET (BIRD-RESISTANT SORGHUM STOVER) WITH FORAGE LEGUMES VARYING IN CONDENSED TANNINS CONCENTRATIONS ON INTAKE, FEED UTILIZATION AND GROWTH EFFICIENCY BY SHEEP


### ABSTRACT

A study was conducted to i) measure extractable and bound condensed tannins (CTs) concentrations in forage legumes (FLs) and relate these to feed utilization and growth of sheep; ii) investigate the influence of CTs concentrations on utilization of a high CTs basal diet. In a study, which lasted 80 days, 70 rams were blocked on the basis of liveweight, and rams within blocks were assigned at random to the ten dietary treatments. The dietary treatments comprised two varieties of sorghum BR and NBR as control diets fed *ad libitum* without supplementation or BR sorghum stover supplemented with 150 g (as fed) of either *lablab, tagasaste, leucaena, desmodium, S. sesban ascensions 15019, 15036, 2024* or *goetzei 15007.* Intake of NBR sorghum stover was higher ($P<0.001$) than BR sorghum stover, but the group fed BR sorghum stover alone had higher ($P<0.05$) nutrient digestibility and N retention. Supplementation with FLs significantly ($P<0.001$) increased total DM and nutrient intake and digestibility ($P<0.01$). There was significant ($P<0.05$) linear and quadratic response due to supplementation, suggesting a threshold point beyond which response decreased. For example, FLs with high CTs content (*S. sesban 2024*, 154 g/kg DM) promoted similar growth (22 vs 16 g/day liveweight gain) as FLs with moderate CTs content (*desmodium*, 100 g/kg DM). Nevertheless, *goetzei* that had the highest CTs content (224 g/kg DM) depressed growth drastically (-20.5 g/day liveweight). Results of this experiment showed that those FLs with high concentrations of CTs, when used as supplements to BR sorghum stover, depressed animal performance (feed intake, growth and feed efficiency). The results also seem to reinforce the view that tannin (polyphenols) concentration *per se* may not be the only factor impacting on the nutritive value/utilization of forages.

## 6.1 INTRODUCTION

Generally tannins present in forages limit voluntary intake, diminish digestion and utilization of nutrients and cause toxicity, all of which negatively influence animal productivity. Condensed tannins (CTs) in ruminant feeds, however, have been shown to have both detrimental (Barry and Duncan, 1984; D'Mello, 1992) and beneficial effects (Barry and Manley, 1984; McNabb *et al.*, 1996; Perez-Maldonado and Norton, 1996). For example, voluntary intake and digestion of organic matter of feeds containing high amounts of CTs may be depressed because tannins combine with proteins and carbohydrates to produce complexes resistant to microbial attack in the rumen (Reed *et al.*, 1982). Large differences in response when forage legumes are fed as supplements have been reported and there have been attributed to the differences in the levels (concentrations) of phenolic compounds. Some research reports (Jackson *et al.*, 1996; Kaitho *et al.*, 1998) suggest that the concentration of CTs *per se* may not be the only factor impacting on the nutritive value of tropical forages and indicate that other factors besides the concentration, affect the nutritive value. One such factor is the biological activity (reactivity) of CTs, which depends on concentration, as well as on chemical structure and degree of polymerization (Horigome *et al.*, 1988). It is, however, recognized that other plant characteristics (such as NDF and ADF concentration) could mediate the response.

Besides leguminous forages, cereal crop residues, particularly sorghum stover, form a major part of the diet of livestock in the arid and semi-arid regions of the tropics. In an attempt to increase cereal crop yields, the bird resistant trait has been used as a selection criterion for sorghum. This trait is positively correlated with proanthocyanidins or CTs in the grain (Reed, 1987) and in the stover (Reed *et al.*, 1988; Aboud *et al.*, 1991; Nsahlai *et al.*,

1998). The beneficial effects of feeding a non-tanniferous feed in association with tanniferous forages has been reported (Feng Yu and Leng, 1991; Kaitho *et al.*, 1998; Nsahlai *et al.*, 1998). However, when a tanniferous basal diet is supplemented with tanniferous forages, the total CT load would be expected to be high and the tannin composition would be more varied. In an earlier *in vitro* experiment, there was an indication that the use of a high tannin FL supplement (*Sesbania goetzei 15007*) in association with high tannin basal substrate depressed IVDMD and gas production (chapter 4 of this thesis).Thus, it was postulated that a diet comprising BR stover and tannin-rich forages could accentuate anti-nutritional effects and toxicity problems since CTs could accumulate and exceed the threshold level that animals can tolerate. This study determined extractable and bound CTs concentrations in forage legumes (FLs) and the effect of feeding these FLs to sheep on the utilization of sorghum stover for growth.

## 6.2 MATERIALS AND METHODS

### 6.2.1 Animals and Feeds

In this study which lasted 80 days, 70 Ethiopian Menz rams (average initial weight, 16.2 kg SD=1.38 kg) were used. The rams were blocked on the basis of liveweight, and rams within blocks were assigned to the ten treatments described below. The feeds comprised of stover of two varieties of sorghum (bird-resistant (BR) and non-bird resistant (NBR)) and eight FLs (*Lablab purpureus* (lablab), *Chamaecytisus palmensis* (tagasaste), *Leucaena leucocephala* (leucaena), *Desmodium intortum* (desmodium) and four *Sesbania* accessions, *S. goetzei 15007* (goetzei), *S. sesban 15019, S. sesban 15036* and *S. sesban 2024*). The FLs were chosen on the basis of their content of CTs, from low to very high (Table 6.1).

**Table 6.1 Dry matter (g/kg) and chemical composition of experimental feeds (g/kg DM)**

| Experimental feeds | DM | OM | N | NDF | HTs | Soluble CTs | Insoluble CTs | NDF-bound CTs | Total CTs |
|---|---|---|---|---|---|---|---|---|---|
| *1.Lablab purpureus* | 880 | 862 | 32 | 180 | 16.4 | 0.5 | 0.5 | 0.9 | 1.9 |
| *2. Chamaecytisus palmensis* | 882 | 870 | 32 | 302 | 59.5 | -1.2 | 3.4 | -0.1 | 2.1 |
| *3. Leucaena leucocephala* | 902 | 887 | 43 | 201 | 73.6 | 14.7 | 14.5 | 4.1 | 33.3 |
| *4. Sesbania sesban (15019)* | 880 | 869 | 39 | 183 | 28.5 | 0.5 | 8.6 | 3.5 | 12.6 |
| *5. Sesbania sesban (15036)* | 888 | 861 | 45 | 201 | 31.5 | 13.0 | 38.1 | 26.3 | 77.4 |
| *6. Desmodium intortum* | 881 | 871 | 30 | 426 | 34.5 | 40.5 | 44.1 | 16.2 | 100.7 |
| *7. Sesbania sesban (2024)* | 888 | 873 | 39 | 307 | 26.0 | 19.1 | 75.1 | 58.1 | 152.3 |
| *8. Sesbania goetzei (15007)* | 881 | 870 | 29 | 421 | 29.4 | 15.2 | 118.7 | 90.6 | 224.5 |
| 9. [2]BR sorghum stover | 902 | 878 | 8 | 717 | ND | | | | 20.6 |
| 10. [3]NBR sorghum stover | 892 | 884 | 7 | 731 | ND | | | | 1.8 |

DM= dry matter, OM= organic matter, N= nitrogen, NDF= neutral detergent fibre, HTs = hydrolyzable tannins, CTs = condensed tannins

*Total CTs = soluble + insoluble + NDF bound condensed tannins.

[2]BR sorghum stover = Bird-resistant sorghum stover

[3]NBR = Non-bird-resistant sorghum stover

Daily feed (basal and supplements) on offer were weighed and recorded. Refusals were collected in the morning before 8:00 am, and samples were taken on animal basis daily. Both the initial and final weights were taken on two consecutive days and used as the mean starting and terminal weights, respectively. To keep abreast with the progress of the experiment, interim weights were taken biweekly. Feed and water were withheld for about 14 h before weighing. During the course of the experiment, two animals from the *goetzei* treatment group died. They were therefore not included in the analysis because the data was not complete.

### 6.2.2 Feeds and feeding

Sorghum stover comprised the basal diet and was offered *ad libitum* to allow for 20% refusal. Bird-resistant (BR) and NBR sorghum stovers were offered *ad libitum* to two groups without supplementation as the control treatments. The FL supplements were fed in the morning at 8:00 am and sheep were allowed 2 h to consume the supplements before offering the basal diet. Each sheep received 150 g of air dried leaves of supplement forage. Mineral block and water were provided *ad libitum*. The sheep were individually fed and watered.

### 6.2.3 Nutrient balance and digestibility study

Four sheep from each treatment were selected randomly, harnessed and fitted with faecal collection bags and moved into metabolism cages in the middle of the growth study. Since the sheep had been on the various diets for about 40 days, there was no need for an extended preliminary feeding period; just a 2-day preliminary period to get the rams adjusted to the stalls. Total faeces voided by each animal was collected, weighed daily and recorded for the 7 days of the trial. Urine produced daily by each sheep was collected in a bucket containing 100 ml of 0.1N sulphuric acid ($H_2SO_4$) and 10% of the daily amount was sampled,

bulked and kept frozen (-20 °C) for N analysis. Approximately 10 % of the daily faecal output for each animal was sampled, bulked separately and kept frozen until required for analysis. At the end of faeces and urine collections, rumen ammonia and pH were determined in rumen fluid collected at 0, 3, 6, 9, 12 and 24 h post parandium, with a stomach tube and suction pump. The pH was determined immediately with a pH meter (Kent EIL 7045/46, ABB, Kent-Taylor LTD) and sub-samples were collected in plastic bottles, preserved with a few drops of concentrated sulphuric acid before storing in the freezer for future analysis.

### 6.2.4 Nylon bag trial

Samples of the basal feeds and forage supplements were ground (2.5 mm screen) and DM degradability was studied (Mehrez and Orskov, 1977) using 2.5 g DM of sample per nylon bag (pore size 41 μm and measuring 6 x 12 cm; Polymon, Switzerland). Samples were incubated in the rumen of fistulated sheep for 0, 3, 6, 12, 24, 48, 96 and 120 h. The sheep were fed natural grass hay ((containing 900g organic matter (OM), 9 g nitrogen (N) and 730 g neutral detergent fibre (NDF) per kg DM) *ad libitum* and supplemented with 200 g per head per day cottonseed cake (910 g OM, 86 g N and 380 g NDF per kg DM). One bag of each feed was incubated per incubation period in three sheep. In addition zero hour bags (not incubated) were washed alongside other bags using a semi-automatic washing machine (Tefal alternatic, Finland) for 30 minutes. The bags were dried in a forced draught oven at 60° C for 48 h, cooled in a desiccator and weighed. The disappearance of DM and degradation parameters a, b, c and TL were estimated by fitting data to the Orskov and McDonald (1979) degradability model.

### 6.2.5..Chemical analyses

The DM, OM, N, rumen fluid ammonia-N and urinary-N were determined by the methods of the Association of Official Analytical Chemists (1980). Neutral detergent fibre was estimated by the method of Goering and Van Soest (1970). The total CTs were estimated by the methods of Giner-Chavez *et al.*(1997), the description of the methods in chapter 5. Hydrolysable tannins (HTs) were assayed by the method described by Kaitho *et al.* (1993).

### 6.2.6 Statistical analysis

Liveweight gains (LWG) for each animal over the experimental period were calculated by regression analysis. Liveweight changes, intake and *in vivo* digestibility data were subjected to analysis of variance using General Linear Model (GLM) procedure available in SAS (1987). Initial liveweight was used as a covariate in the analyses. Rumen ammonia and pH data were subjected to repeated measures analysis. Linear and quadratic effects of tannins on intake, liveweight, digestibility and degradability were tested by excluding the unsupplemented treatments from the analysis.

## 6.3 RESULTS

### 6.3.1 Chemical composition

The chemical composition data for the feeds used in the trial are presented in Table 6.1. Whereas the FL supplements had higher protein (184-278 g/kg DM) values than sorghum stover (BR and NBR) (41-48 g/kg DM), the NDF concentration was higher for the stovers (717-731 g/kg DM) than for the supplements (180-426 g/kg DM). Nitrogen content was negatively correlated to NDF content ($r=-0.94$, $P<0.001$) (Table 6.2). Otherwise there were no significant ($P>0.05$) relationships among the other chemical attributes.

### 6.3.2 Intake during growth trial

Treatment effects on intake and growth are given in Table 6.3. Intake of NBR sorghum stover was higher ($P<0.001$) than BR sorghum stover. Intake of sorghum stover alone was higher ($P<0.05$) than when supplemented with FLs. However, supplementation significantly ($P<0.001$) improved total DM intake which followed the order, *S. sesban 2024, tagasaste, leucaena* > *S. sesban 15019* > *lablab, S. sesban 15036, desmodium* and *goetzei.* There was however no significant ($P>0.05$) difference in intake of the basal stover among the supplemented treatments. There were significant ($P<0.05$) linear and quadratic effects of CTs on the intakes of OM, N, NDF (Table 6.3).

### 6.3.3 Growth rate

As expected, rams fed sorghum stover alone lost weight. The loss in weight was more severe ($P<0.05$) for the BR sorghum stover treatment (-30.8 g/d) than for NBR sorghum stover (-5.7) treatment. Forage legume supplements improved LWG. Consequently, with the exception of the *goetzei* treatment, LWG was significantly higher ($P<0.05$) for the supplemented compared to the unsupplemented treatments. However, with

**Table 6.2 The correlation coefficients between chemical properties.**

| | DM | OM | N | NDF | CT |
|---|---|---|---|---|---|
| Dry matter (DM) | - | -0.00$^{NS}$ | -0.32$^{NS}$ | 0.42$^{NS}$ | -0.08$^{NS}$ |
| Organic matter (OM) | | - | 0.41$^{NS}$ | -0.35$^{NS}$ | -0.02$^{NS}$ |
| Nitrogen (N) | | | - | -0.94$^{***}$ | 0.35$^{NS}$ |
| Neutral detergent fibre (NDF) | | | | - | -0.11$^{NS}$ |
| Condensed tannins (CTs) | | | | | - |

Level of significance, NS = P>0.05, * = P<0.05, ** = P<0.01, *** = P<0.001

**Table 6.3 Effect of supplementing BR sorghum stover with forage legumes on feed and nutrient intakes (g/day) and on lamb growth rate (g/day) (N=7).**

| | Total intake | | | | | | |
|---|---|---|---|---|---|---|---|
| Treatments | Basal | Supplement | DM | OM | N | NDF | Liveweight gain |
| BR sorghum stover + *lablab* | 367.4 | 133.7 | 501.0 | 439.4 | 7.4 | 283.0 | 24.5 |
| BR sorghum stover + *tagasaste* | 378.1 | 135.3 | 513.4 | 458.3 | 7.4 | 310.0 | 23.8 |
| BR sorghum stover + *leucaena* | 386.1 | 136.3 | 522.3 | 460.9 | 9.2 | 296.3 | 30.6 |
| BR sorghum stover + *S. sesban 15019* | 381.4 | 134.2 | 515.7 | 451.7 | 8.5 | 291.0 | 19.9 |
| BR sorghum stover + *S. sesban 15036* | 360.5 | 135.6 | 496.1 | 434.9 | 9.3 | 270.1 | 17.8 |
| BR sorghum stover + *desmodium* | 367.8 | 133.3 | 501.1 | 436.5 | 7.2 | 308.0 | 16.4 |
| BR sorghum stover + *S. sesban 2024* | 392.4 | 135.2 | 527.6 | 464.3 | 8.7 | 312.0 | 22.1 |
| BR sorghum stover + *goetzei* | 354.5 | 135.1 | 489.6 | 428.1 | 7.2 | 299.3 | -20.5 |
| BR sorghum stover | 416.2 | 0.0 | 416.2 | 360.1 | 3.7 | 284.8 | -30.8 |
| NBR sorghum stover | 489.1 | 0.0 | 489.1 | 439.4 | 3.3 | 352.0 | -5.7 |
| | | | | | | | |
| Mean | 390.8 | | 500.7 | 440.5 | 7.3 | 301.7 | 10.3 |
| SED | 5.20 | | 5.19 | 4.67 | 0.03 | 3.26 | 8.12 |
| | | | | | | | |
| Effects | | | | | | | |
| Forage | *** | | *** | *** | *** | *** | *** |
| INWT | *** | | *** | *** | *** | *** | NS |
| Linear | * | | * | *** | NS | ** | ** |
| Quadratic | NS | | NS | ** | *** | *** | * |

BR sorghum stover = Bird-resistant sorghum stover, NBR sorghum stover = Non-bird resistant sorghum stover.
Level of significance, NS = P>0.05, * = P<0.05, ** = P<0.01, *** = P<0.001. INWT= initial weight

the exception of rams supplemented with *goetzei*, the differences in LWG among supplemented treatments were not significant (P>0.05) (Table 6.3). The highest LWG was observed with rams fed *leucaena* (30.6 g/ day) followed by those fed *lablab* (24.5 g/day). On the contrary, rams fed *goetzei* (that is very high in CTs 224.5 g/kg DM) lost weight (-20.5 g/day). However, rams fed *S. sesban 2024* which had the next highest CT (152.3 g/kg DM) concentration had higher LWG (22.1g/day) than rams on some FL treatments which had lower CT concentration e. g *desmodium* (LWG =16.4g/day , CTs = 100.7g/kg DM).

### 6.3.4 Intake during digestibility trial

Voluntary intake and digestibility values are given in Table 6.4. Between the control groups, the NBR sorghum stover group had higher (P<0.05) nutrient intake than the BR sorghum stover group. However, nutrient digestibility was the reverse, being higher (P<0.05) for the BR sorghum stover group than for the NBR sorghum stover group. Supplementation with FLs significantly (P<0.001) influenced nutrient intake. Among the supplemented diets, N intake was highest (P<0.05) for *S. sesban 15036* and *leucaena* then followed in descending order by *S. sesban 2024* > *S. sesban 15019* > *lablab*, *tagasaste* > *goetzei*. Neutral detergent fibre intake was significantly (P<0.05) lower for rams supplemented with *S. sesban 15036*. There was a significant (P<0.001) quadratic effect of CT on N intake. Increasing levels of dietary CT (from 1.9 to 152.3 g/kg DM) in the diets greatly (P<0.001) influenced N intake. The intake increased from 7.6 to 9.4 g/day and beyond 152.3 g/kg DM CTs concentration, N intake dropped to 7.2 g/day. However, *S. sesban 2024* supplement that had the second highest CT (152.3 g/kg DM) concentration had higher N intake (8.9 g/day) than some FLs which had lower CTs concentration e. g. *desmodium* (100.7g/kg DM) (N intake = 7.3 g/day).

**Table 6.4 Effect of supplementing BR sorghum stover with forage legumes on nutrient intake (g/day), apparently digestibility (g/kg/DM) and N retention (g/day) by sheep (N=4)**

| | Total intake | | | | Digestibility | | | | |
|---|---|---|---|---|---|---|---|---|---|
| **Treatment** | DM | OM | N | NDF | DM | OM | N | NDF | N retention |
| BR sorghum stover + *lablab* | 531.0 | 465.4 | 7.6 | 303.3 | 509.8 | 531.9 | 650.5 | 432.7 | 2.5 |
| BR sorghum stover + *tagasaste* | 541.4 | 483.2 | 7.6 | 329.3 | 505.3 | 533.6 | 639.8 | 472.0 | 2.7 |
| BR sorghum stover + *leucaena* | 536.0 | 472.8 | 9.3 | 305.5 | 480.6 | 503.5 | 626.3 | 413.3 | 3.4 |
| BR sorghum stover + *S. sesban 15019* | 534.6 | 467.9 | 8.6 | 301.1 | 485.4 | 512.1 | 634.5 | 435.9 | 3.1 |
| BR sorghum stover + *S. sesban 15036* | 502.2 | 439.4 | 9.4 | 272.5 | 467.6 | 479.0 | 655.6 | 374.8 | 3.5 |
| BR sorghum stover + *desmodium* | 521.1 | 454.4 | 7.4 | 322.2 | 495.9 | 514.3 | 587.0 | 468.3 | 2.8 |
| BR sorghum stover + *S. sesban 2024* | 557.5 | 491.1 | 8.9 | 334.8 | 457.5 | 476.7 | 603.5 | 407.9 | 2.8 |
| BR sorghum stover + *goetzei* | 520.4 | 455.5 | 7.3 | 323.0 | 400.7 | 424.9 | 472.9 | 361.4 | 1.9 |
| BR sorghum stover | 430.1 | 371.2 | 3.9 | 292.0 | 472.9 | 486.6 | 371.5 | 487.9 | 0.1 |
| NBR sorghum stover | 505.1 | 454.2 | 3.4 | 363.4 | 432.4 | 474.7 | -30.7 | 504.2 | -1.0 |
| Mean | 519.0 | 456.5 | 7.4 | 313.4 | 458.3 | 482.4 | 505.1 | 422.0 | 2.1 |
| SED | 23.33 | 20.42 | 0.14 | 17.74 | 46.21 | 49.14 | 53.91 | 62.43 | 0.38 |
| Effects | | | | | | | | | |
| Forage | *** | *** | *** | * | *** | *** | *** | ** | *** |
| INWT | *** | *** | *** | *** | *** | *** | *** | *** | *** |
| Linear | * | ** | NS | NS | *** | *** | *** | ** | * |
| Quadratic | * | ** | *** | NS | *** | *** | *** | ** | NS |

BR sorghum stover = Bird-resistant sorghum stover, NBR sorghum stover = Non-bird resistant sorghum stover.
Level of significance, NS = P>0.05, * = P<0.05, ** = P<0.01, *** = P<0.001. INWT= initial weight

### 6.3.5 Digestibility and nitrogen utilization

Rams fed BR sorghum stover alone had higher ($P<0.05$) nutrient digestibility compared to those fed NBR sorghum stover alone. Supplementation improved ($P<0.01$) nutrient digestibility as compared to the unsupplemented groups. Among the supplemented groups, DM and N digestibility of the rams on *goetzei* (which had the highest level of CTs (224.5 g/kg DM)) treatment were significantly ($P<0.05$) lower than for the other supplemented rams. However, there was no significant ($P>0.05$) difference among the supplemented groups in OM digestibility. All treatments except for that supplemented with *goetzei* had similar ($P>0.05$) NDF digestibility. The concentration of CTs resulted in significant ($P<0.01$) linear and quadratic effects on nutrient digestibility. Nutrient digestibility tended to increase with increase in the concentration of CTs but dropped at high levels of CTs. Rams fed on the BR sorghum stover alone had higher ($P<0.05$) N retention than those fed on the NBR sorghum stover alone. Supplementation significantly ($P<0.001$) increased N retention compared to the control treatments. Rams supplemented with *goetzei* had lower ($P<0.05$) N retention compared to rams supplemented with the other FLs. Condensed tannins concentration in the diets had a linear ($P<0.05$) effect on N retention. However, N retention increased with increase in CTs concentration up to maximum and then dropped.

### 6.3.6 Rumen pH and ammonia nitrogen concentration

The mean pH values are given in Table 6.5. The rumen pH ranged from 6.8 to 7.4. There were no significant ($P>0.05$) treatment differences in pH at 0, 6, and 24 h of sampling but there were ($P<0.001$) differences at 3, 9 and 12 h sampling periods. Between 6 and 12 h, pH dropped for all the treatments, and returned to a high level at 24 h sampling period. There were no significant ($P<0.05$) treatment or treatment x time interaction and tannin effects on

**Table 6.5 Effect of supplementing BR sorghum stover with forage legumes on rumen pH (N=4)**

| Treatments | Sampling time (h) | | | | | |
|---|---|---|---|---|---|---|
| | 0 | 3 | 6 | 9 | 12 | 24 |
| BR sorghum stover + *lablab* | 7.2 | 7.1 | 7.1 | 6.9 | 6.8 | 7.3 |
| BR sorghum stover + *tagasaste* | 7.3 | 7.1 | 7.1 | 6.9 | 7.0 | 7.4 |
| BR sorghum stover + *leucaena* | 7.1 | 7.0 | 7.1 | 6.9 | 6.9 | 7.3 |
| BR sorghum stover + *S. sesban 15019* | 7.2 | 7.3 | 7.1 | 7.1 | 7.1 | 7.4 |
| BR sorghum stover + *S. sesban 15036* | 6.9 | 7.1 | 7.1 | 7.0 | 6.9 | 7.2 |
| BR sorghum stover + *desmodium* | 7.2 | 7.3 | 7.1 | 7.1 | 7.1 | 7.1 |
| BR sorghum stover + *S. sesban 2024* | 7.0 | 7.2 | 7.1 | 7.0 | 6.9 | 7.3 |
| BR sorghum stover + *goetzei* | 7.2 | 7.2 | 7.2 | 7.2 | 7.0 | 7.4 |
| BR sorghum stover | 7.2 | 7.3 | 7.1 | 7.0 | 7.0 | 7.1 |
| NBR sorghum stover | 7.2 | 7.2 | 7.2 | 7.1 | 6.9 | 7.1 |
| Mean | 7.1 | 7.2 | 7.1 | 7.0 | 7.0 | 7.3 |
| SED | 0.08 | 0.07 | 0.10 | 0.07 | 0.07 | 0.14 |
| Effects | | | | | | |
| Forage | NS | * | NS | ** | * | NS |
| Linear | NS | * | NS | *** | NS | NS |
| Quadratic | NS | NS | NS | ** | NS | NS |

BR sorghum stover = Bird-resistant sorghum stover, NBR sorghum stover = Non-bird resistant sorghum stover.
Level of significance, NS = P>0.05, * = P<0.05, ** = P<0.01, *** = P<0.001.

ruminal pH. pH levels were within the normal range.

Rumen ammonia concentrations (Table 6.6) at 6 h collection were lowest for rams fed NBR sorghum stover and differed significantly ($P<0.05$) from the other treatments except for *desmodium* and BR sorghum stover treatments. The highest ammonia level during the period 0-3 h was with rams supplemented with *lablab*, which was not significantly ($P>0.05$) different from the levels obtained with *leucaena, tagasaste* and *Sesbania 2024* and *15019*. Ammonia N decreased steadily between 3 and 12 h for all treatments and then increased across all treatments at 24 h. The increase was highly significant ($P<0.01$) for rams supplemented with *S. sesban 15036*. The lowest rumen ammonia (5.7 mg/100ml) concentration was obtained on the NBR sorghum stover treatment whereas the highest (28 mg/100ml) was obtained with the lablab supplemented treatment. The *leucaena, tagasaste*, and *S. sesban 2024* and *15019* treatments maintained high levels of rumen ammonia across all sampling times compared to the other treatments. As would be expected, the BR and NBR stover control treatments had the lowest ammonia concentrations. Dietary CTs levels had no effect on ammonia concentration except for the ($P<0.01$) difference between *S. sesban 15036* and *desmodium*.

### 6.3.7 Degradability trial

Dry matter degradability results are given in Tables 6.7 and 6.8. The BR sorghum stover had lower solubility and lower rate of degradation than the NBR stover (Table 6.8). The FL supplements had similar solubility except for leucaena that had significantly ($P<0.05$) higher solubility. Similarly, the supplements had similar degradability parameters except for *desmodium* which sustained the lowest potential degradability (PD) and the slowest rate (c) of degradation, while lablab and tagasaste had the fastest rates of degradation. Dietary

**Table 6.6 Effect of supplementing BR sorghum stover with forage legumes on rumen ammonia concentration (mg/100ml)** (N=4)

| | Sampling time (h) | | | | | | |
|---|---|---|---|---|---|---|---|
| Treatments | 0 | 3 | 6 | 9 | 12 | 24 | Mean |
| BR sorghum stover + *lablab* | 11.7 | 28.1 | 16.7 | 12.7 | 8.9 | 11.5 | 11.5 |
| BR sorghum stover + *tagasaste* | 12.8 | 26.2 | 18.0 | 14.7 | 11.3 | 14.1 | 14.1 |
| BR sorghum stover + *leucaena* | 15.3 | 27.5 | 18.7 | 12.8 | 10.3 | 13.8 | 13.8 |
| BR sorghum stover + *S. sesban 15019* | 14.0 | 23.3 | 12.5 | 13.3 | 8.8 | 11.5 | 11.5 |
| BR sorghum stover + *S. sesban 15036* | 9.2 | 18.2 | 11.7 | 10.5 | 8.5 | 12.2 | 12.2 |
| BR sorghum stover + *desmodium* | 11.1 | 15.3 | 8.0 | 6.6 | 6.8 | 10.5 | 10.5 |
| BR sorghum stover + *S. sesban 2024* | 13.6 | 26.6 | 16.1 | 12.5 | 10.4 | 12.4 | 12.4 |
| BR sorghum stover + *goetzei* | 11.3 | 20.4 | 11.4 | 9.1 | 7.3 | 8.7 | 8.7 |
| BR sorghum stover | 8.3 | 11.4 | 8.6 | 7.1 | 5.0 | 8.5 | 8.5 |
| NBR sorghum stover | 8.5 | 13.5 | 7.7 | 8.5 | 5.7 | 8.5 | 8.5 |
| Mean | 11.7 | 21.2 | 13.1 | 10.8 | 8.4 | 10.2 | 10.2 |
| SED | 1.6 | 2.7 | 1.7 | 1.5 | 1.2 | 1.5 | 1.5 |
| Effects | | | | | | | |
| Forage | ** | *** | *** | *** | *** | NS | NS |
| Linear | NS | *** | *** | *** | * | NS | NS |
| Quadratic | * | ** | *** | *** | * | NS | NS |

[2]BR sorghum stover = Bird-resistant sorghum stover, NBR sorghum stover = Non-bird resistant sorghum stover.
Level of significance, NS = P>0.05, * = P<0.05, ** = P<0.01, *** = P<0.001.

**Table 6.7 Mean dry matter disappearance (g/kg DM) of the experimental feeds from nylon bags at different incubation times.**

| | incubation time (h) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Treatments | 3 | 6 | 12 | 24 | 48 | 72 | 96 | 120 |
| *Lablab* | 393 | 525 | 689 | 777 | 781 | 804 | 779 | 790 |
| *Tagasaste* | 385 | 474 | 684 | 834 | 832 | 775 | 851 | 854 |
| *Leucaena* | 357 | 452 | 557 | 775 | 813 | 813 | 887 | 863 |
| *S. sesban 15019* | 383 | 459 | 654 | 859 | 851 | 879 | 909 | 897 |
| *S. sesban 15036* | 310 | 487 | 484 | 797 | 853 | 231 | 854 | 891 |
| *Desmodium* | 242 | 275 | 353 | 494 | 588 | 637 | 664 | 642 |
| *S. sesban 2024* | 314 | 406 | 513 | 793 | 805 | 729 | 838 | 835 |
| *goetzei* | 292 | 388 | 416 | 736 | 741 | 790 | 796 | 789 |
| BR sorghum stover | 170 | 202 | 283 | 359 | 473 | 527 | 577 | 622 |
| NBR sorghum stover | 222 | 254 | 325 | 407 | 518 | 618 | 584 | 641 |
| Mean | 306 | 390 | 495 | 681 | 722 | 692 | 772 | 781 |
| SED | 38.2 | 41.1 | 42.3 | 22.3 | 38.3 | 190.1 | 26.3 | 21.2 |
| Effects | | | | | | | | |
| Forage | *** | *** | *** | *** | *** | NS | *** | *** |
| Linear | *** | *** | *** | *** | ** | NS | ** | *** |
| Quadratic | *** | ** | *** | *** | *** | NS | *** | *** |

[2]BR sorghum stover = Bird-resistant sorghum stover, NBR sorghum stover = Non-bird resistant sorghum stover.
Level of significance, NS = P>0.05, * = P<0.05, ** = P<0.01, *** = P<0.001.

**Table 6.8 Degradability constants of the experimental feeds**

| Treatments | A | B | C | PD | TL |
|---|---|---|---|---|---|
| *Lablab* | 165.0 | 625.2 | 0.148 | 790.2 | 2.2 |
| *Tagasaste* | 179.7 | 686.9 | 0.124 | 837.8 | 2.7 |
| *Leucaena* | 285.6 | 625.6 | 0.071 | 855.9 | 2.7 |
| *S. sesban 15019* | 190.1 | 702.3 | 0.093 | 892.4 | 3.3 |
| *S. sesban 15036* | 171.6 | 711.1 | 0.063 | 882.8 | 2.4 |
| *Desmodium* | 163.2 | 493.4 | 0.043 | 667.0 | 0.9 |
| *S. sesban 2024* | 204.8 | 664.1 | 0.083 | 812.1 | 2.2 |
| *goetzei* | 194.5 | 634.7 | 0.065 | 796.7 | 0.9 |
| BR sorghum stover | 134.6 | 513.1 | 0.021 | 662.6 | -0.7 |
| NBR sorghum stover | 183.7 | 473.5 | 0.028 | 657.1 | -0.2 |
| | | | | | |
| Mean | 188 | 610 | 0.072 | 783 | 1.6 |
| SED | 26.6 | 50.3 | 0.0132 | 26.3 | 1.26 |
| | | | | | |
| Effects | | | | | |
| Forage | * | ** | *** | *** | NS |
| Linear | NS | NS | *** | ** | NS |
| Quadratic | NS | NS | *** | *** | NS |

[2]BR sorghum stover = Bird-resistant sorghum stover, NBR stover = Non-bird resistant sorghum stover.
Level of significance, NS = P>0.05, * = P<0.05, ** = P<0.01, *** = P<0.001.

tannins significantly ($P<0.001$) decreased PD and rate of degradation.

## 6.4 DISCUSSION

Forage legumes are important sources of protein and minerals that are usually limiting in low quality basal feeds. However, when FLs contain high levels of CTs, intake and apparent digestion of protein and carbohydrates are depressed (Barry and Duncan, 1984; Reed *et al.*, 1990). A beneficial effect of feeding a non- tanniferous feed in association with tanniferous forages have been reported (Feng Yu and Leng, 1991; Kaitho *et al.*, 1998; Nsahlai *et al.*, 1998). However, when a tanniferous basal diet is supplemented with tanniferous forages the total CTs load will be high and the tannin composition will be more varied. In an earlier *in vitro* experiment, it was shown that using high tannin FL supplements in association with a high tannin basal diet may accentuate anti-nutritional toxicity (chapter 4).Thus, it was postulated that a diet comprised of BR sorghum stover and tannin-rich forage will accentuate anti-nutritional or toxicity problems because CTs may accumulate and possibly exceed the threshold level that animals can tolerate.

The high NDF and low N content of sorghum stover are characteristics of low quality roughages and these could have contributed to the low fermentation of OM compared to the supplemented diets. Nutrient intake from BR sorghum stover was much lower than from NBR sorghum stover even though their nutrient compositions were similar. This result may be due to the higher level of CTs in BR than NBR sorghum stover obtained in this study (see Table 6.1). Higher levels of CTs have been previously reported in the leaf blades and leaf sheaths of BR than NBR sorghum stover (Reed *et al.*, 1988).The lower total intake may have resulted from the negative effects of CTs on fibre digestion, which in turn decreased the intake of feeds with high content of NDF. The faster degradation of NBR sorghum stover

compared to BR sorghum stover could be due to the lower tannin concentration in the former variety. As previously observed by Nsahlai *et al.* (1998), CTs may therefore largely account for the differences between the two varieties in degradability during the early hours of incubation.

Increases in total feed intake and in digestibility of the total diet arising from supplementation with FLs should be manifested in a significant increase in animal performance. In this experiment, unsupplemented diets depressed performance of the animals, while supplementation resulted in higher nutrient intake and digestibility. Rams fed the unsupplemented diets were critically deficient in rumen ammonia concentration (RAC), while supplementation promoted higher RAC, which in turn could have promoted higher digestibility and nutrient intake. Supplementation with FLs has been shown to improve RAC in sheep (Getachew *et al.*, 1994; Umunna *et al.*, 1995) and this increase was attributed principally to the N degradability from the FLs. In a study by Said and Tolera (1994), the legume with the lower N content (*Macrotyloma axillare*) gave higher RAC than *desmodium*, which had more crude protein but lower degradability. It needs to be remembered however, that tannins found in most FLs could influence this response since tannins form complexes with plant proteins and decrease the rate of protein degradation in the rumen, thereby decreasing RAC, which could be the case with the high CTs diets, especially *desmodium* and *goetzei* supplemented diets (Table 6.6).

Generally, the high intake and digestibility of nutrients across all the supplemented treatments were manifested in significant LWG. Total DM, OM and N intakes of *desmodium* and *goetzei* supplemented groups were significantly reduced. In addition, DM and N digestibility of the goetzei supplemented group were reduced. Although the N content

appeared to be a dominating factor, it should be recognised that legumes with high N content are likely to contain less fibre and the total OM may be more easily fermented. It may be that energy available to the microorganisms is a factor equal in significance to the supply of degradable N. The intake of nutrients appears to be a function of the forage chemical composition. Total nutrient intake and digestibility were significantly affected by CT level.

A decrease in nutrient digestibility of the *goetzei* diet compared to other supplemented diets such as *lablab*, *tagasaste*, and *desmodium* could have been caused by the high content of CTs in the former. The binding of CTs with proteins inhibits fermentation of structural carbohydrates (D'Mello, 1992) in the rumen and reduces protein availability to rumen microbes. It is therefore probable that CTs in *goetzei* bound with protein and affected its utilization. Condensed tannins also bind with other nutrients in the rumen, and these together with binding to protein are critical in enhancing the rumen ecosystem so to increase microbial growth, rate of fibre digestion, and escape of dietary protein.

Supplementation of BR sorghum stover diet with FLs with increasing levels of CTs improved performance up to a maximum at medium-high concentration (100-150 g/kg DM), beyond which performance was depressed, suggesting a threshold point beyond which animal response decreased. Higher LWG by rams fed *leucaena, lablab* and *tagasaste* may have been due to the low to moderate levels of CTs concentrations and NDF. Low concentrations of CTs could have provided protection against extensive rumen degradation of protein. The loss of live weight by rams supplemented with high CTs (224.4 g/kg DM) forage (*goetzei*) point to the anti-nutritional effect of high level of CTs. However, rams supplemented with *S. sesban 2024* that had the next highest CTs (152.3 g/kg DM) level to *goetzei*, had significantly higher LWG (22.1 g/day) than *desmodium* (16.4 g/day) that had

lower CTs (100.7 g/kg DM) content. This observation reinforces the concept that tannin (polyphenols) concentration per se may not be the only factor impacting on the nutritive value and utilization of tropical forages (Jackson *et al.*, 1996; Khazaal *et al.*, 1996; Kaitho *et al.*, 1998). The reaction of different tannins from different sources with other molecules is important.

The effect of accumulation of CTs when tannin-rich *goetzei* was used as a supplement may have been above the threshold level and as such affected animal performance. The basal feed intake, N intake and weight gain of rams supplemented with *goetzei* were significantly affected. In addition, all the rams in this treatment group lost weight and two animals died. Post mortem examination showed symptoms of severe malnutrition. This observation confirms the results from the *in vitro* experiment (chapter 4) where there was depression in IVDMD and gas production with goetzei as supplement incubated with rumen fluid from steers fed BR sorghum stover basal feed. The tannins present in various forages lower the voluntary feed intake, diminish the utilization of nutrients and cause toxicity and thus cumulatively have a negative influence upon animal productivity. This observation suggests that, the use of high tannin basal feed in conjunction with FLs high in CTs may lead to high accumulation of tannins above a threshold level that animals cannot tolerate and therefore accentuate anti-nutritional effects and toxicity problems.

## 6.5 CONCLUSION

The high content of CTs in some of the FLs created nutritional problems such as reduction in voluntary feed intake, digestibility of nutrients and liveweight gain. Therefore, it would not be advisable to feed a high tannin basal diet in association with very high tannin forage supplements without additional ameliorative measures. Rams supplemented with

forages varying in CT levels had increasing performance up to a certain level and then started to decline. This may indicate a threshold point beyond which animals may not be able to tolerate the tannin concentration without ameliorative measures. The fact that some FLs with higher CT (e.g. *S. sesban 2024*) concentration had higher animal performance than some with lower CTs concentration (*desmodium*) could mean that there were other antinutritive factors that affected animal performance besides CTs. The concentration of CTs *per se* may not be the only factor impacting on the nutritive value of tropical forages. It would seem that the content and perhaps the type of tannin mixtures in the diet jointly determine the chemical reactivity of the tannin that influences the direction of the effect. It would therefore be beneficial if some of these factors can be further explored in order to develop better strategies of utilizing BR sorghum and FLs available in the farming systems.

# CHAPTER SEVEN

## 7.0 EFFECTS OF SUPPLEMENTING BIRD RESISTANT SORGHUM STOVER WITH *LABLAB, DESMODIUM, SESBANIA* AND *LEUCAENA* WITH OR WITHOUT POLYETHYLENE GLYCOL-4000 (PEG), UREA OR SULPHUR AS AMELIORANTS ON VOLUNTARY INTAKE, DIGESTIBILITY AND GROWTH OF SHEEP

## ABSTRACT

Seventy two yearling male sheep were used in a randomized complete block design experiment that lasted 90 days to examine the effects of feeding BR sorghum stover supplemented with forage legumes varying in CT concentration on intake, digestibility and growth of sheep. Dietary treatments comprised *ad libitum* BR sorghum stover fed alone or in association with 190 g (as fed) of *lablab, desmodium, leucaena and goetzei.* Ameliorants, PEG, urea or sulphur were added to the *desmodium* and *goetzei* supplemented diets only. NBR sorghum stover was also given to one group as another control diet. There was no significant ($P>0.05$) intake difference between the control groups (NBR and BR sorghum stovers). Supplementation with FLs significantly ($P<0.01$) improved total dry matter (DM) and nutrient intake and liveweight gain (LWG). *Lablab* and *leucaena*, which are low in CTs, promoted higher ($P<0.05$) nutrient intake and LWG than *desmodium* and *goetzei*, which have high CTs. Ameliorants significantly ($P<0.001$) improved nutrient intake and nutrient utilization. Similarly, LWG improved (from -22 to 18 g/head/day) with *goetzei* ($P<0.01$) which had higher CTs concentration (224 g/kg DM) than *desmodium* (15 to 16 g/head/day) which had CTs concentration of 100 g/kg DM. PEG, urea and sulphur were all effective in alleviating phenolics-related antinutritive effects, although ameliorants did not have the same effect on utilization of high tannin feeds. This may be attributed to the different biological activity of the tannins from different sources. However, the use of PEG in routine feeding may not be economical but urea and sulphur that are available in the farming systems, would become practical alternatives with urea being the ameliorant of first choice since it was more efficient than sulphur.

## 7.1 INTRODUCTION

Quantitatively, sorghum is the most important cereal crop in the semi-arid and arid regions of the world. Therefore, sorghum stover is the most important potential feed resource for ruminants in these regions. However, sorghum can synthesize many different phenolic compounds (in particular condensed tannins (CTs)) in large quantities (Butler, 1982) as compared to other cereals. The concentration of CTs differ between sorghum varieties and are higher in both leaves and stems of the bird resistant (BR) than of the non-bird resistant (NBR) varieties (Reed *et al.*, 1987; Ebong, 1989; Nsahlai *et al.*, 1998). Condensed tannins are known to depress both intake and digestibility, thus affecting the use of sorghum stover (Reed *et al.*, 1987). Condensed tannins may therefore be responsible for the variation in feeding values between BR and NBR sorghum varieties (Reed *et al.*, 1987; Nsahlai *et al.*, 1998). Furthermore, since the quality of stover from the BR sorghum variety is low (Reed *et al.*, 1987), it needs enhancement to improve its feeding value to animals. Physical and chemical treatments are often expensive and technically not suitable for smallholder farmers. However, there are other technically and economically feasible strategies that can be employed to enhance stover quality. Supplementation with deficient nutrients is an easier approach to improve the use of the stovers. Given the low resource base of smallholder farmers, they depend more on leguminous crops as protein supplements. Energy and nitrogen contents in forage legumes (FLs) make them attractive alternatives to expensive concentrates as supplements for poor quality stovers. But often the intake and digestibility of FLs are reduced by their content of phenolic compounds, in particular CTs (Woodward and Reed, 1989; Wiegand *et al.*, 1995). These compounds also reduce the digestibility of sorghum

stover especially of the BR variety, which also is high in CTs. Despite the adverse effects of CTs on intake and digestibility, tannins may protect proteins from rapid degradation by rumen microorganisms thereby ensuring that amino acids are absorbed in the post ruminal gut (Barry and Manley, 1984).

The use of FLs high in CTs as nutrient supplements to BR sorghum stover could depress performance since the total tannin concentration could be above the animal's tolerance level. Some strategies, which could reduce the detrimental effects of tannins on nutritive value of feeds, have been employed and these include the following: addition of chemicals in the diet, for example polyethylene glycol (PEG) to bind tannins; development of breeding and selection programs for plants with high potential to increase animal productivity, but lower in CT concentration; avoiding the use of high tannin plants or feeding such at controlled or reduced levels, and the use of microbes to detoxify the CTs. Some of these strategies however may not be suitable for smallholder farm situations because of cost and difficulty of implementation. Some suggested practical strategies for overcoming the antinutritive nature of phenolic compounds include the provision of appropriate supplements. This approach has a biological basis in the pathways by which the compounds are metabolized and excreted (Lowry, 1990). Most absorbed toxic compounds after metabolism in the liver are detoxified by means of excretion as conjugation of hydroxyl group with glycine, glucuronic acid or sulphate (Lowry, 1990). The experiment reported here assessed the effects of supplementing BR stover with FLs with or without the addition of PEG, urea or sulphur as ameliorants to tannin depressive effects on animal performance. The experiment also tried to develop better strategies of feeding forages that are high in CTs concentration. The forages and treatments chosen are based on the earlier results from an *in*

*vitro* experiment reported in chapter four of this thesis.

Specifically the experiment assessed the effects of feeding BR sorghum stover supplemented with FLs with varying CTs concentration on intake, digestibility and growth of sheep, and investigated the efficiency of urea and sulphur compared to PEG in mitigating tannin-related antinutritive effects.

## 7.2 MATERIALS AND METHODS

### 7.2.1 General management of animals

Seventy-two male Ethiopian Menz type sheep of average initial weight of about 20.1 (SD=1.39) kg were used in this randomized block design experiment which lasted 90 days. Sheep were blocked by weight, and randomly assigned to 12 dietary treatments.

### 7.2.2 Feeding management

Treatments comprised bird-resistant (BR) sorghum stover fed alone or in association with four forage legumes *(Dolichos lablab (lablab), Desmodium intortum (desmodium), Leucaena leucocephala (leucaena), and Sesbania goetzei 15007 (goetzei)*). Ameliorants, PEG, urea or sulphur were added to diets supplemented with *desmodium* or *goetzei*. This was based on the results from an earlier *in vitro* experiment which showed no benefit in *IVDMD* and gas production with PEG, urea or sulphur as ameliorants in *lablab* and *leucaena* supplemented treatments (see chapter 4). In addition, non-bird-resistant (NBR) sorghum stover was fed to one treatment group as a low tannin control treatment. Treatments were as outlined in Table 7.1. Sorghum stover was fed *ad libitum* allowing for a 20% refusal rate. Supplements were fed at the rate of 180 g DM per sheep every morning at 0800h. Sheep were allowed 2 h before basal stover was provided. Ameliorants were fed in the form of dry pellets (pellets were made by mixing with 100ml of water and 10 g of wheat bran and then dried) in the morning prior to feeding legume supplements, at the following rates; 18 g/day

Table 7.1 Treatment combinations

| Treatments |
|---|
| 1. Non-bird resistant (NBR) sorghum stover alone |
| 2. Bird-resistant (BR) sorghum stover alone |
| 3. BR Sorghum stover + Lablab purpureus (lablab) |
| 4. BR Sorghum stover + Leucaena leucocephala (leucaena) |
| 5. BR Sorghum stover + Desmodium intortum (desmodium) |
| 6. BR Sorghum stover + Desmodium + Polyethylene glycol (PEG) |
| 7. BR Sorghum stover + Desmodium + Urea |
| 8. BR Sorghum stover + Desmodium + Sulphur |
| 9. BR Sorghum stover + Sesbania goetzei 15007 (goetzei) |
| 10. BR Sorghum stover + goetzei + PEG |
| 11. BR Sorghum stover + goetzei + Urea |
| 12. BR Sorghum stover + goetzei + Sulphur |

urea, 20 g/day of ammonium sulphate (sulphur) and 25 g/day PEG. The inclusion levels were determined in earlier in vitro experiments (chapter four of this thesis).

### 7.2.3 Nutrient balance and digestibility study

Towards the end of the growth trial, four sheep from each treatment were moved into metabolic crates for total faeces and urine collection. Since sheep had been on the various diets for about 60 days, there was no need for an extended preliminary period; a two-day preliminary period to get the sheep adjusted to the stalls was allowed. The amount of feed offered and refused were recorded daily and samples bulked separately for each feed and animal for the entire collection period. Faeces were collected into canvas bags and 10% of the daily output for each animal was bulked separately and kept frozen until required for analysis. Urine was also collected, sampled in the same way and stored frozen in plastic bottles until required for analysis.

### 7.2.4 Measurements

#### 7.2.4.1 Growth trial

After 14 days of adaptation, feed offered and refused were recorded daily until the end of the trial. Representative samples of the offered feed and refusals were taken daily from each animal, bulked and sub-samples taken on treatment basis for feed and on animal basis for refusals. The samples were stored in a freezer until analyzed. Both the initial and final weights of the sheep were taken on two consecutive days before and at the end of the experiment. The mean initial and final weights taken on two consecutive days were used as the starting and terminal weights, respectively. To keep abreast with the progress of the experiment, interim weights were also taken biweekly. All weights were taken after removal of feed and water overnight for about 14 hours.

### 7.2.4.2 Nitrogen balance and digestibility

Total daily faecal output of each sheep was weighed, thoroughly mixed and sub-sampled into a plastic bag and kept frozen at -20° C. Urine output from each sheep was collected in plastic buckets containing 100 ml of 10% sulphuric acid to keep the pH below 3. Total urine voided by each animal was measured daily and 2% of undiluted urine sub-sampled into plastic bottles for N analysis. Animals were weighed at the beginning and at the end of the trial. At the end of the collection period, rumen fluid was collected from each animal through a stomach tube the following morning before feeding, and thereafter at 3, 6, 9, 12 and 24 h post feeding. The pH was determined immediately with a pH meter (Kent EIL 7045/46, ABB, Kent-Taylor LTD) and a sub-sample was collected in a plastic bottle, preserved with few drops of concentrated sulphuric acid before storing in a freezer for future analysis.

### 7.2.5 Chemical analysis

Samples of sorghum stover, legume supplements (both offered and refusals) and faeces were analyzed for dry matter (DM), organic matter (OM), ash and nitrogen (N) according to methods described by AOAC (1980). Neutral detergent fibre (NDF) of feeds and faeces were analyzed according to Goering and Van Soest (1970). Urine and rumen fluid samples were analyzed for N, according to methods described by AOAC (1980). Condensed tannins were analyzed by the methods of Giner-Chavez *et al.* (1997) and outlined in chapter 4 of this thesis.

### 7.2.6 Statistical analysis

Liveweight changes (LWG) for each animal over the experimental period were calculated by regression analysis. The feed intake, digestibility and animal growth data were analyzed according to analysis of variance using dietary treatment and block as class variables, and initial live weight as a covariate. Analyses were done to study the main effects (forage legume and ameliorants sources) using SAS package (SAS, 1987). The FLs x ameliorants interactions were estimated through the general linear model (GLM) for the diets supplemented with *desmodium* and *goetzei* only using the SAS package (SAS, 1987)..

## 7.3 RESULTS

### 7.3.1 Chemical composition

The chemical composition of the experimental feeds is presented in Table 7.2. All the supplements had higher N values than sorghum stover (both BR and NBR), but NDF content was higher for sorghum stover than for the supplements. The feeds used in this experiment were similar to those used in the experiment reported in chapter six of this thesis.

### 7.3.2 Effects of FLs and ameliorants on intake during growth trial

The treatment effects on intake and growth are given in Table 7.3. Dry matter and nutrient intake between rams on the NBR and BR sorghum stover control treatments were not different ($P>0.05$). Intake of sorghum stover alone was higher ($P<0.05$) than when supplemented with FLs. As expected, the intake of nutrients was higher for the supplemented than for the unsupplemented treatments. There were significant ($P<0.001$) differences in total DM, OM and N intake due to FL supplementation. Sheep supplemented with *lablab* and *leucaena* had significantly ($P<0.001$) higher nutrient intake than those supplemented with *desmodium* and *goetzei*. Supplementation did not ($P>0.05$) affect NDF intake. Among treatments supplemented with FLs alone, rams on the *leucaena* treatment had the highest ($P<0.05$) N intake, followed by those on *lablab, desmodium* and *goetzei*.

Ameliorants significantly influenced total DM and NDF ($P<0.01$), OM and N ($P<0.001$) intakes. There were significant ($P<0.01$) forage x ameliorant interaction effects on DM, OM and N and for NDF ($P<0.05$) intakes. Addition of ameliorants to the *desmodium* treatment reduced total DM, OM and NDF intake but increased N intake.

Table 7.2 Dry matter (g/kg) and chemical composition of experimental feeds (g/kg DM).

| Feed | DM | OM | Ash | N | NDF | * CTs |
|---|---|---|---|---|---|---|
| Lablab purpureus | 880 | 862 | 138 | 32.2 | 180 | 1.9 |
| Leucaena leucocephala | 902 | 887 | 113 | 42.9 | 201 | 33.3 |
| Desmodium intortum | 881 | 871 | 129 | 29.6 | 426 | 100.7 |
| Sesbania goetzei (15007) | 881 | 870 | 130 | 29.4 | 421 | 224.5 |
| BR Sorghum stover | 902 | 878 | 122 | 7.7 | 731 | 20.6 |
| NBR Sorghum stover | 892 | 884 | 116 | 6.6 | 717 | 1.8 |

DM= dry matter, OM= organic matter, N= nitrogen, NDF= neutral detergent fibre, CTs = condensed tannins

*Total CTs = Soluble + insoluble + NDF bound condensed tannins.

BR = Bird resistant,

NBR = Non-bird resistant

Table 7.3 Effects of ameliorants on feed and nutrient intakes (g/day) and growth (g/day) rate of lambs (N ranges from 6 to 7)

| Treatments | Total intake | | | | | | Liveweight |
|---|---|---|---|---|---|---|---|
| | Basal | Supplement | DM | OM | N | NDF | gain |
| 1. NBR sorghum stover alone | 479 | 0 | 479 | 452 | 4.0 | 318 | -16.4[d] |
| 2. BR sorghum stover alone | 465 | 0 | 465 | 440 | 3.0 | 341 | -27.0[e] |
| 3. BR sorghum stover + lablab alone | 394 | 178 | 573 | 539 | 9.0 | 301 | 18.5[ab] |
| 4. BR sorghum stover + leucaena alone | 381 | 181 | 563 | 537 | 11.0 | 297 | 25.1[a] |
| 5. BR sorghum stover + desmodium alone | 391 | 162 | 553 | 517 | 8.1 | 334 | 15.6[ab] |
| 6. BR sorghum stover + desmodium + PEG | 376 | 161 | 537 | 501 | 7.9 | 326 | 16.1[ab] |
| 7. BR sorghum stover + desmodium + urea | 368 | 162 | 530 | 494 | 16.1 | 312 | 14.4[ab] |
| 8. BR sorghum stover + desmodium + sulphur | 319 | 162 | 480 | 450 | 11.6 | 277 | 7.1[bc] |
| 9. BR sorghum stover + goetzei alone | 305 | 179 | 484 | 462 | 7.9 | 267 | -22.7[d] |
| 10. BR sorghum stover + goetzei + PEG | 409 | 180 | 589 | 555 | 8.7 | 357 | 18.2[ab] |
| 11. BR sorghum stover + goetzei + urea | 308 | 177 | 485 | 463 | 16.1 | 288 | 2.3[c] |
| 12. BR sorghum stover + goetzei + sulphur | 324 | 179 | 503 | 469 | 12.3 | 287 | -0.4[c] |
| Mean | 377 | 144 | 520 | 490 | 9.6 | 309 | 4.3 |
| SED | 24.6 | 0.58 | 24.9 | 21.8 | 0.21 | 22.9 | 3.6 |
| Effects | | | | | | | |
| Forage | *** | | *** | *** | *** | NS | *** |
| Ameliorants | ** | | ** | *** | *** | ** | *** |
| Forage x ameliorants | ** | | ** | ** | ** | * | *** |

Level of significance, NS = P>0.05, * = P<0.05, ** = P<0.01, *** = P<0.001

These effects were more pronounced with sulphur and urea. PEG did not elicit similar effect on nutrient intake. However, with the *goetzei* treatment ameliorants generally increased nutrients intake though this was more dramatic on the basal intake when PEG was added. The increased intake of DM, OM and NDF were more pronounced with PEG than with sulphur or urea. However addition of both urea and sulphur significantly ($P<0.001$) increased N intake.

### 7.3.3 Effects of FLs and ameliorants on growth rate

Live-weight (LWG) changes are shown in Table 7.3. As expected, sheep fed sorghum stover alone lost weight. The loss in weight was more severe ($P<0.05$) for the BR sorghum stover (-27.0 g/day) than the NBR sorghum stover (-16.4) group. With the exception of sheep supplemented with *goetzei*, supplementation promoted higher ($P<0.001$) Lags. Differences in LWG of all supplemented treatments were significant ($P<0.05$). The highest LWG was observed for sheep fed on the leucaena (25.1 g/ day) followed by those on *lablab* (18.5 g/day), while sheep that fed on *goetzei* alone lost weight (-22.7 g/day).

Ameliorants significantly ($P<0.001$) improved LWG. There were significant ($P<0.001$) forage x ameliorant interactions effects on LWG. PEG promoted the highest ($P<0.05$) LWG, while urea and sulphur had the similar effect. Ameliorants did not have any significant ($P>0.05$) effect on LWG of the *desmodium* groups, while ameliorants significantly ($P<0.001$) improved LWG of sheep supplemented with *goetzei*. PEG promoted the highest weight gain within the *goetzei* group whereas sulphur tended to elicit the lowest LWG with the *desmodium* and *goetzei* groups.

### 7.3.4 Effects of FLs and ameliorants on intake during digestibility trial

Voluntary intake and digestibility data are presented in Table 7.4. There was a highly significant difference ($P<0.01$) in the DM and nutrients intake among treatments. Total DM, OM and NDF intakes were similar ($P>0.05$) between rams on BR and NBR sorghum stover control treatments. NBR sorghum stover however promoted higher ($P<0.001$) N intake than BR sorghum stover. Supplementation significantly influenced DM and OM ($P<0.01$) and N ($P<0.001$) intakes but did not affect NDF intake. *Leucaena* promoted the highest ($P<0.001$) nutrient intake followed by *lablab, desmodium* and *goetzei*.

Ameliorants significantly affected the intakes of DM, OM and NDF ($P<0.05$) and N ($P<0.001$). PEG tended to promoted ($P<0.05$) higher nutrient intake than urea or sulphur. Urea and sulphur had similar effects. There were significant ($P<0.05$) forage x ameliorant interaction effects of DM and OM intakes. Ameliorants did not affect nutrient intake with the *desmodium* treatments groups, however sulphur ($P<0.05$) lowered nutrient intake, while both urea and sulphur promoted ($P<0.001$) higher N intake than PEG. In contrast, PEG had greater influence on the groups supplemented with *goetzei,* since it promoted higher ($P<0.001$) DM, OM and NDF intakes, while urea and sulphur promoted ($P<0.001$) higher N intake.

### 7.3.5 Effects of FLs and ameliorants on digestibility and N utilization

Dry matter digestibility (Table 7.4) was affected by the treatment effects. Rams on NBR sorghum stover alone had similar DM, OM and NDF digestibility with those on BR sorghum stover alone. However, the NBR group had significantly ($P<0.001$) higher N digestibility and N retention ($P<0.05$) than the BR stover group. Supplementation with FLs significantly improved N retention, DM, N ($P<0.001$) and OM and NDF ($P<0.01$)

Table 7.4 Effect of ameliorants on intake (g/day), digestibility (g/kg DM) and N retention (g/day) by sheep fed BR sorghum stover supplemented with forage legumes (N=4)

| Treatments | Total intake | | | | | | Digestibility | | | | Retention |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | Basal | Supplement | DM | OM | N | NDF | DM | OM | N | NDF | N |
| 1. NBR sorghum stover alone | 513 | 0 | 513 | 484 | 4.3 | 355 | 471 | 525 | 229 | 456 | 0.4 |
| 2. BR sorghum stover alone | 500 | 0 | 500 | 472 | 3.1 | 360 | 475 | 500 | 68 | 447 | -0.4 |
| 3. BR sorghum stover + lablab alone | 401 | 178 | 579 | 547 | 9.1 | 313 | 544 | 591 | 694 | 407 | 4.7 |
| 4. BR sorghum stover + leucaena alone | 443 | 182 | 625 | 593 | 11.5 | 349 | 507 | 562 | 665 | 376 | 5.6 |
| 5. BR sorghum stover + desmodium alone | 421 | 162 | 583 | 545 | 8.3 | 361 | 484 | 536 | 573 | 411 | 3.2 |
| 6. BR sorghum stover + desmodium + PEG | 409 | 162 | 570 | 533 | 8.2 | 359 | 467 | 519 | 597 | 405 | 3.6 |
| 7. BR sorghum stover + desmodium + urea | 429 | 162 | 591 | 550 | 16.4 | 363 | 490 | 533 | 697 | 402 | 3.9 |
| 8. BR sorghum stover + desmodium + sulphur | 381 | 162 | 543 | 507 | 12.0 | 327 | 494 | 540 | 609 | 383 | 2.3 |
| 9. BR sorghum stover + goetzei alone | 360 | 180 | 540 | 513 | 8.4 | 314 | 378 | 449 | 487 | 208 | 2.5 |
| 10. BR sorghum stover + goetzei + PEG | 480 | 180 | 660 | 621 | 9.2 | 411 | 471 | 506 | 582 | 366 | 3.9 |
| 11. BR sorghum stover + goetzei + urea | 382 | 180 | 562 | 533 | 16.8 | 358 | 522 | 556 | 620 | 500 | 4.5 |
| 12. BR sorghum stover + goetzei + sulphur | 372 | 180 | 552 | 516 | 12.7 | 327 | 493 | 538 | 550 | 371 | 2.8 |
| Mean | 424 | 144 | 568 | 534 | 10.0 | 350 | 483 | 530 | 531 | 394 | 3.1 |
| SED | 33.8 | | 34 | 29.1 | 0.3 | 25.7 | 27.2 | 28.1 | 36.2 | 45.5 | 0.7 |
| Effects: | | | | | | | | | | | |
| Forage | *** | | ** | ** | *** | NS | *** | ** | *** | ** | *** |
| Ameliorants | * | | * | * | *** | * | ** | * | *** | ** | ** |
| Forage x ameliorants | * | | * | * | NS | NS | ** | NS | NS | *** | NS |

Level of significance, NS = P>0.05, * = P<0.05, ** = P<0.01, *** = P<0.001.

digestibility. Supplementation with *lablab* and *leucaena* promoted higher nutrient digestibility than *desmodium* and *goetzei* but did not have any effect (P>0.05) on NDF digestibility. *Leucaena* promoted higher N retention followed by *lablab, desmodium* and *goetzei.* There were forage x ameliorant (P<0.01) interaction effects on DM and NDF digestibility. Addition of ameliorants significantly affected digestibility of DM and NDF (P<0.05), OM (P<0.05), N (P<0.001) and N retention (P<0.01). Addition of urea promoted (P<0.05) higher N digestibility and N retention, while sulphur significantly (P<0.05) lowered N retention.

### 7.3.6 Rumen pH and ammonia nitrogen

The daily mean pH values are given in Tables 7.5. Rumen pH values for rams fed NBR and BR sorghum stover alone were similar. Forage supplements did not influence pH at 0 and 12 h, but significantly affected it at 3 and 6 h (P<0.01) and 9 and 24 (P<0.001) h post feeding. The pH ranged between 6.5 and 7.6. A decreased rumen pH was observed for sheep supplemented with *lablab, leucaena, desmodium* and *goetzei,* and the two control treatments between 0 and 9 h (Table 7.5). Between 9 and 12 h, pH dropped for all the treatments, and thereafter increased. pH measured at 24 h for all treatments was significantly increased (P<0.001). Effects of ameliorants on pH followed the same pattern as FL. Urea tended to increase the pH values. There was a slight increase in pH between 0 and 3 h urea treatments, but dropped between 6 and 9 h and increased between 9 to 12 h.

Treatment effects on rumen ammonia concentrations (RACs) are shown in Table 7.6. Rumen ammonia concentration was significantly (P<0.05) lower for BR and NBR sorghum stover groups throughout the sampling times than for the supplemented groups. Forage legume supplementation significantly (P<0.001) increased RACs across all sampling times.

Table 7.5 Effect of forage legumes and ameliorants on rumen pH

| Treatments | Sampling times post parandium (h) | | | | | |
|---|---|---|---|---|---|---|
| | 0 | 3 | 6 | ....9 | 12 | 24 |
| 1. NBR sorghum stover alone | 6.91 | 6.53 | 6.65 | 6.83 | 6.67 | 6.98 |
| 2. BR sorghum stover alone | 7.03 | 6.96 | 7.01 | 7.07 | 6.85 | 6.98 |
| 3. BR sorghum stover + lablab alone | 7.09 | 6.74 | 6.75 | 6.80 | 6.69 | 7.30 |
| 4. BR sorghum stover + leucaena alone | 7.11 | 6.73 | 6.74 | 6.97 | 6.89 | 7.29 |
| 5. BR sorghum stover + desmodium alone | 7.17 | 6.84 | 6.86 | 6.96 | 6.87 | 7.15 |
| 6. BR sorghum stover + desmodium + PEG | 7.12 | 6.78 | 6.77 | 6.83 | 6.82 | 7.21 |
| 7. BR sorghum stover + desmodium + urea | 6.88 | 7.11 | 7.00 | 7.20 | 6.56 | 6.90 |
| 8. BR sorghum stover + desmodium + sulphur | 7.05 | 6.99 | 6.81 | 7.06 | 6.88 | 7.29 |
| 9. BR sorghum stover + goetzei alone | 7.08 | 6.74 | 6.67 | 6.88 | 6.63 | 7.40 |
| 10. BR sorghum stover + goetzei + PEG | 6.93 | 6.66 | 6.56 | 6.78 | 6.69 | 7.27 |
| 11. BR sorghum stover + goetzei + urea | 7.11 | 7.17 | 6.78 | 6.85 | 6.81 | 7.32 |
| 12. BR sorghum stover + goetzei + sulphur | 6.86 | 6.87 | 6.81 | 6.66 | 6.71 | 7.64 |
| Mean | 7.0 | 6.8 | 6.8 | 6.9 | 6.8 | 7.2 |
| SED | 0.08 | 0.10 | 0.11 | 0.10 | 0.10 | 0.11 |
| Effects | | | | | | |
| Forage | NS | ** | ** | *** | NS | *** |
| Ameliorants | NS | ** | * | * | NS | ** |
| Forage x ameliorants | ** | NS | NS | * | ** | NS |

Level of significance, NS = P>0.05, * = P<0.05, ** = P<0.01, *** = P<0.001.

Table 7.6 Effect of forage legumes and ameliorants on rumen ammonia concentration (mg/100ml) in rumen fluid

| Treatments | Sampling times post parandium (h) | | | | | |
|---|---|---|---|---|---|---|
| | 0 | 3 | 6 | 9 | 12 | 24 |
| 1. NBR sorghum stover alone | 5.1 | 9.36 | 5.10 | 5.10 | 6.38 | 5.74 |
| 2. BR sorghum stover alone | 6.6 | 12.8 | 8.29 | 5.95 | 6.17 | 6.80 |
| 3. BR sorghum stover + lablab alone | 14.3 | 31.6 | 16.6 | 13.4 | 13.0 | 13.8 |
| 4. BR sorghum stover + leucaena alone | 12.5 | 25.7 | 16.6 | 11.1 | 10.6 | 13.6 |
| 5. BR sorghum stover + desmodium alone | 9.6 | 17.2 | 9.67 | 9.57 | 7.87 | 10.8 |
| 6. BR sorghum stover + desmodium + PEG | 11.1 | 20.8 | 15.1 | 11.5 | 8.08 | 13.0 |
| 7. BR sorghum stover + desmodium + urea | 13.8 | 58.3 | 53.2 | 35.9 | 28.3 | 14.0 |
| 8. BR sorghum stover + desmodium + sulphur | 14.0 | 37.6 | 23.4 | 12.8 | 11.5 | 11.5 |
| 9. BR sorghum stover + goetzei alone | 12.5 | 20.6 | 17.4 | 9.78 | 8.7 | 11.3 |
| 10. BR sorghum stover + goetzei + PEG | 13.4 | 38.7 | 22.3 | 14.7 | 11.9 | 11.1 |
| 11. BR sorghum stover + goetzei + urea | 21.1 | 38.5 | 35.1 | 25.7 | 17.7 | 14.0 |
| 12. BR sorghum stover + goetzei + sulphur | 14.7 | 27.2 | 24.0 | 17.0 | 12.8 | 9.4 |
| Mean | 12.4 | 28.0 | 20.5 | 14.4 | 11.9 | 11.3 |
| SED | 1.6 | 3.7 | 2.8 | 3.3 | 1.4 | 1.4 |
| Effects: | | | | | | |
| Forage | *** | *** | *** | NS | *** | *** |
| Ameliorants | *** | *** | *** | *** | *** | ** |
| Forage x ameliorants | * | *** | *** | * | *** | NS |

level of significance, NS = P>0.05, * = P<0.05, ** = P<0.01, *** = P<0.001.

There was a steady increase in RAC for all treatments from 0 to 3 h, which decreased between 3 to 6 h. The highest ammonia N at this time period (0-3h) was for sheep supplemented with *lablab* alone. This value was, however, not significant compared to treatments with *leucaena, desmodium* and *goetzei* alone. *Desmodium* promoted ($P<0.05$) lower RACs throughout the sampling times. Ameliorants significantly ($P<0.01$) increased RACs. The ammonia level for the groups receiving urea as ameliorant were significantly ($P<0.01$) higher than for those receiving PEG and sulphur. However, the high RAC steadily decreased after 3 h sampling time. There were significant ($P<0.05$) forage x ameliorant interaction effects on RACs for all the sampling times except at 24 h.

## 7.4 DISCUSSION

Basically, intake of nutrients (N, OM and NDF) among the experimental treatments appeared to be a function of their dietary levels. It was therefore not surprising that the control treatments had lower nutrient intakes and digestibility leading to depressed performance. BR sorghum stover had higher level of CTs as compared to NBR sorghum stover and as such the nutrient intakes and digestibility were lowered. A decrease in DM and OM digestibility of diets supplemented with *goetzei* compared with *lablab, leucaena* and *desmodium* could be due to the higher level of CTs in the former diet. The binding of proteins with CTs inhibits fermentation of structural carbohydrates (D'Mello, 1992) in the rumen and reduces N availability to rumen microbes. Since *lablab* and *leucaena* contained relatively low levels of CTs and higher levels of N compared to *goetzei* and *desmodium*, both provided adequate N for both the host and the ruminal microbes that in turn promoted higher digestibility and superior LWG. Since *lablab* and *leucaena* contained relatively low levels of CTs, the addition of ameliorants would be expected to have no added advantage (Chapter

four). Higher LWG by rams fed *leucaena, lablab* and *desmodium* may have been due to the low to moderate levels of CT, whereas the loss in weight by sheep supplemented with *goetzei* seem to suggest the anti-nutritional effect seen with high content of CTs. Barry (1989) and McNabb *et al.* (1996) had reported the beneficial effects of low to moderate tannins as increased amino acid N supply for post-ruminal digestion and increased N retention and LWG.

Urea and sulphur (S) were effective ameliorants although not to the same degree as PEG in improving nutrient digestibility and N retention. Whereas both were effective on sheep supplemented with *goetzei*, they had no effect on sheep supplemented with *desmodium*. The major chemical differences among the FL species was the presence of relatively large amounts of CTs and NDF in *desmodium* and *goetzei* and large amounts of N and low levels of NDF in both *lablab* and *leucaena*. Both *desmodium* and *goetzei* had similar N and NDF contents but did not elicit the same response. *Goetzei* that had higher (224.5 g/kg) content of CTs responded well to the addition of ameliorants but *desmodium*, which had a lower (100.7 g/kg) content, did not. However, addition of urea promoted higher DM, OM, N and NDF digestibility than the PEG and S groups in both *desmodium* and *goetzei* treatments.

The fact that addition of urea promoted higher N intake and higher DM, OM and N digestibility, may suggest that CTs in *desmodium* and *goetzei* interfered with digestion and as such affected intake of DM and OM. Therefore provision of extra N improved the rumen environment for microbes to be able to digest the fibre and in turn increased intake. Addition of S also released extra N and as such digestibility was similar to that seen with PEG although S depressed nutrient intake. The reasons for the suppression of nutrient intake

observed with S are not clear, but may suggest the negative effect of poor N:S ratio. Nitrogen:sulphur ratio is important for ruminal microbial populations (ARC, 1980). Sheep require a N:S ratio of at least 10:1 to maximize digestibility (ARC, 1980) and responses are greater in sheep than in cattle (1980). The calculated N:S ratio was 2:1 which is quite unfavourable to ruminal microbial populations because the calculated S intake was about 6 g, this well above the recommended level, 4 g (Umunna *et al.*, 1986)

The results of the growth trial did not agree with the earlier results from the *in vitro* trial. In the *in vitro* trial, the *goetzei* treatment did not respond to the addition of ameliorants while *desmodium* responded. In this experiment, *goetzei* responded more than *desmodium*. The possible explanation will be provided later. The lack of conformity between the *in vitro* and *in vivo* trial result may mean that the screening of FLs for nutritive value by qualitative methods may lead to some erroneous conclusions if not supported by feeding trials. The results, however, corroborate the results from the other feeding trial where similar diets were used (reported in chapter 6 of this thesis). In general, ameliorants annulled the negative effects of CTs, which were responsible for the lowered digestibility of diets supplemented with both *desmodium* and *goetzei*. There are two possible explanations. First the level of PEG may have been optimum to inactivate the particular CTs present. The calculated PEG:CT ratios were 1:1 and 1:2 for *desmodium* and *goetzei*, respectively. In a related *in vitro* experiment (chapter 5 of this thesis), it was determined that PEG:CT ratios of about 3:1 was adequate to alleviate the anti-nutritional attributes of CTs in *desmodium* and *goetzei*. Whereas the ratio determined *in vitro* for *desmodium* is higher than that used in this trial, it may provide an explanation as to why there was no effect on nutrient use and animal growth. The ratio determined *in vitro* for *goetzei* also differed from that used in the present study (1:3

vs 1:2) yet, it elicited a positive response, however. Although, the ratios were different, they were closer to the determined values, this may have provided the ameliorative effect better than the lower values for *desmodium*. This further points to the care needed in using *in vitro* results to predict *in vivo* response especially in situations involving anti-nutritional factors.

Similar improvement in performance with PEG-4000 treatment had been reported earlier (Pritchard *et al.*, 1988; Silanikove *et al.*, 1994; Silanikove *et al.*, 1996; Prasad *et al.*, 1997). Secondly, the provision of extra N (Russell and Lolley, 1989) and S (Gartner and Niven, 1978) in the form of ameliorants might have improved the rumen environment for efficient microbial action (Kumar and Singh, 1984). For example, higher protein levels or inclusion of amino acids for favourable tannin:protein ratios in the diet has been reported to alleviate some of the anti-nutritional effects of tannins (Mueller-Harvey and McAllan, 1992). On the other hand, Russell and Lolley (1989) observed that tannins in high tannin sorghum can be deactivated rapidly and completely by reconstitution with aqueous urea. The same authors have also reported the deactivation of tannins by urea used as a preservative for high moisture sorghum. Urea supplementation with tannin-rich feeds thus can improve the quality of feeds by providing an extra N source and by its chemical activity. Urea destabilizes the hydrogen bonds and hydrophobic interactions (Nozaki and Tanford, 1963, cited by Kumar and Singh, 1984), which participate in the formation of the protein-tannin complex. Therefore the use of urea may render protein free from the complex, for its further utilization by animals.

Sulphur is of similar importance to ammonia in the rumen, and bacteria derive at least half of their S from the rumen sulphide pool (McMeniman *et al.*, 1976). Sulphur is of special significance in the digestion of low-quality roughages, as loss in faeces is inversely

related to roughage digestibility (Langlands *et al.*, 1973), and rumen anaerobic fungal colonization responds to the S content of roughages (Akin *et al.*, 1983). It has been suggested that a considerable portion of the S (as amino acids) in high tannin feeds was unavailable for digestion because of the complexing nature of tannins and protein (Gartner and Niven, 1978). Thus the use of S as ameliorant could have improved the S pool available to ruminal microbes. On the other hand, S (glutathione, cysteine) and glucose are critical conjugates with tannins in the process of detoxification in the liver (Cheeke and Palo, 1995). The extra S provided as ameliorant could have improved this toxin elimination function reducing its stressful effects on both the microbes and the host. Beneficial effects to S supplementation has been demonstrated (Gartner and Niven, 1978; Pritchard *et al.*, 1992) in *A. aneura* (mulga), which contains high levels of phenolics. Goodchild and McMeniman (1994) demonstrated the importance of minerals in maximizing the intake and digestibility of sorghum stover and in maximizing the response to urea where the ration was deficient in N.

The results of this study corroborate the common view that the major anti-nutritional effect of tannins is reduction of N availability and depression of digestive tract enzymes activities (Kumar and Singh, 1984; Kumar and Vaithiyanathan, 1990), even though direct measurements of enzyme activities were not made but assessed by digestibility measurements. The results also confirmed the findings that tannins may reduce cell-wall digestibility by binding bacterial enzymes and/or forming indigestible complexes with cell-wall carbohydrates (Barry and Manley, 1984; Reed, 1986). This was demonstrated by the improvement in NDF digestibility of the *goetzei* supplemented diets with ameliorants. The improvement in nutrient digestibility of *goetzei* treatment with ameliorants reflects the N-sparing effect of PEG (Kumar, 1992) or the provision of extra N and S.

In this experiment, PEG was used as a standard phenolic binding compound. Urea and sulphur were compared to it to determine whether they could effectively alleviate phenolics-related antinutritive effects. Overall, the effectiveness of urea and S as ameliorants even though lower than that of PEG, were still significant. Thus suggesting that both urea and sulphur can be effective in alleviating anti-nutritional attributes of tanniferous feeds.

The overall performance of sheep in this experiment was, however, lower than what has been reported from similar sheep in other animal experiments from this laboratory (Kaitho *et al.*, 1998; Nsahlai *et al.*, 1998). The low performance is attributed to the imbalance of nutrients made available from fermentative digestion. It is possible that the energy:protein ratio from these feeds was lower than optimum, because of the lack of synchrony of ammonia release and energy, due to different fermentation rates of the feed components. Benefits due to supplying extra nutrients through provision of concentrate supplements of N and energy have been demonstrated. Recently, Silanikove *et al.* (1997) provided 10g/day PEG to goats given *Quercus calliprinos* (which is very high in CTs) leaves *ad libitum* and supplemented with 300 g/day concentrates containing 160 g crude protein per kg DM. Digestible crude protein intake was increased by 50 g/day which drastically improved animal performance. In this situation, supplementing tannin-rich leaves with concentrate was only beneficial when used in conjunction with tannin binding agent. This may explain why Nsahlai *et al.* (1998) failed to get benefits by supplementing BR stover with cotton seed cake and noug cake.

## 7.5 CONCLUSION

Generally these results indicate that supplementing BR sorghum stover with FLs improved sheep performance. However, FL with high contents of CTs (*goetzei*) gave less improvement in sheep performance. PEG, urea and sulphur proved effective ameliorants in alleviating phenolics-related antinutritive effects, although ameliorants did not have the same effect on utilization of high tannin feeds. This may be attributed to the different biological activity of the tannins from different sources. The use of PEG in routine feeding may not be as economical to use as urea and sulphur that are available in the farming systems in which case urea should be the first choice since it was more effective than sulphur.

# CHAPTER EIGHT

## 8.0 *IN VITRO* GAS AND AMMONIA PRODUCTION AS INDICES OF NUTRITIONAL EVALUATION OF FORAGE LEGUMES OR THEIR MIXTURES VARYING IN CONDENSED TANNIN CONCENTRATION


### ABSTRACT

The *in vitro* gas production (GP) technique of Menke *et al.* (1979) was used to assess the nutritive value of mixtures of forage legumes (FLs) varying in condensed tannins (CTs) concentration. The legumes studied were *lablab, tagasaste, goetzei, sesbania, desmodium* and *acacia.* They were all harvested from the Debre Zeit Research Station farm. Macerated leaves were mixed at different proportions and subjected to GP with sole samples as controls. Combinations of up to three FLs were mixed together in ratios of 1:2, 2:1, 1:1 and 1:1:1. Condensed tannin was related to NDF content ($r = 0.69$, $P<0.05$), while N content was negatively related to hydrolysable tannins (HTs) ($r = -0.69$, $P<0.05$). Gas production differed widely ($P<0.001$) among the forages incubated individually (sole). *Tagasaste* and *sesbania* promoted significantly ($P<0.001$) higher volumes of gas than the other FLs, while *acacia* produced the least ($P<0.001$) gas. Based on total GP, the FLs showed the following ranking order: *tagasaste, sesbania* > *goetzei, lablab, desmodium* > *acacia.* Mixing FLs at all ratios (sole vs rest) significantly ($P<0.05$) induced higher volumes of GP but not ($P>0.05$) the rate of GP. Mixing *acacia* with other forages at all ratios yielded the biggest improvement ($P<0.05$) in GP. When ammonia production was used to index the value of the forages, the ranking order with forages incubated individually was *goetzei, lablab* > *sesbania* > *tagasaste, desmodium* and *acacia.* Mixing the FLs improved ammonia production quite significantly ($P<0.01$). Five clusters were formed when both gas and ammonia productions were used as classification criteria. Results showed that mixtures of 1:2 or 2:1 ratios yielded the highest gas volumes and ammonia concentrations. Thus there is potential for improving the utilization of some forages which would normally pose problems if used sole by using

them in mixtures, preferably at 1:2 or 2:1 ratios. There is however, a need to verify these in vitro results in *in vivo* feeding trials.

## 8.1 INTRODUCTION

Animal production in the tropics and sub-tropics could be improved through the incorporation of tropical forage legume (FL) species into the diets of livestock. Animal productivity from forage plants may be low because of the presence of certain plant constituents (allelochemicals) that reduce the quantity eaten and the availability of nutrients. One important group of such compounds is tannins. Tannins are widespread, abundant and appear to be the major constraint of FLs because of their effect on intake, digestibility and animal metabolism (Kumar and Singh, 1984; D'Mello, 1992). On rangeland animals may have the advantage of selecting from a wide choice of browses obtaining a high quality diet and avoiding toxicity. With confined animals, the choice is limited by the available feeds provided by the owner; in such cases, the risk of toxicity becomes even greater. Free condensed tannins (CTs) may bind protein and make it less available to both the ruminal microbes and the host animal (Reed *et al.*, 1988). Since tannins bind with proteins, they can be useful in "protecting" proteins of high solubility from rapid degradation in the rumen by allowing their digestion post ruminally for more efficient utilization. This attribute can thus be harnessed to improve the efficient utilization of highly degradable protein forages.

One of the major concerns regarding the utilization of FLs is how to overcome allelochemical effects in order to increase the effective use of these forages. Consumption of various types of forages seems to reduce chances of poisoning (Dicko and Sikena, 1992). Sometimes animals show a low preference for plants that are nontoxic but of high feed quality. Le Houerou (1991) reported that consumption of mixed shrubs was higher than that of a single species. This observation suggests that there may be scope in alleviating the anti-nutritional problems through feeding mixtures of various forages. Strategies to reduce

allelopathy, if it exists, are of prime concern if productivity is to be maximized. One route is to exploit the vast plant resource base through feeding of mixed forage diets.

The *in vitro* gas production (GP) technique of Menke *et al.* (1979) has been proposed as a potential method for the initial assessment of multi-purpose trees (MPTs) (Khazaal *et al* 1993; Siaw *et al.*, 1993; Nsahlai *et al.*, 1994). Several studies (Khazaal *et al.*, 1994; Nsahlai *et al.*, 1994; Makkar *et al.*, 1995) have shown that the increase in the amount of gas produced when a material is incubated is due to fermentation in the system. Feed degradation is accompanied by the release of gases such as methane and carbon dioxide, and the amount of ammonia released during fermentation is an indication of protein degradation. Thus the amount of gas produced and ammonia released may be used to index the nutritive value of feeds. This method was therefore used to assess the nutritive value of mixtures of FLs varying in CTs concentrations.

## 8.2 MATERIALS AND METHODS

### 8.2.1 Forage legume samples

Samples were hand harvested from a number of plants (5-10 trees or herbaceous legumes) in single rows, the same day from the Debre Zeit Research station farm. Leaves were left to wilt for five hours under a shed, mixed at different proportions and combinations and then macerated in a mixer. Mixed macerated leaves of FLs were subjected to gas production (Menke *et al.*, 1979) with sole samples as controls. The legumes studied were *Dolichos lablab* (lablab), *Chamaecytisus palmensis* (tagasaste), *Sesbania goetzei 15007* (goetzei), *Sesbania sesban 15019* (sesbania), *Desmodium intortum* (desmodium) and *Acacia angustissima* (acacia). These forages were chosen because they are widely available in the farming systems of the tropics and are also known to contain some antinutritional factors

(ANFs), including CTs in varying concentrations. Furthermore, some are highly rumen degradable while others are not. Combinations of up to three FLs were mixed together and were mixed in ratios of 1:1, 1:2, 2:1 and 1:1:1. The combinations were designed to match the highly degradable (numbered 1 to 3) with the low degradable (numbered 4 to 6) FLs (Table 8.1). The numbers of combinations depended on the number of FLs chosen as given below.

1 & 4, 1 & 5, 1 & 6

2 & 4, 2 & 5, 2 & 6

3 & 4, 3 & 5, 3 & 6

1 & 4 & 5, 1 & 5 & 6, 1 & 4 & 6

2 & 4 & 5, 2 & 5 & 6, 2 & 4 & 6

3 & 4 & 5, 3 & 5 & 6, 3 & 4 & 6

4 & 1 & 2, 4 & 2 & 3, 4 & 1 & 3

5 & 1 & 2, 5 & 2 & 3, 5 & 1 & 3

6 & 1 & 2, 6 & 2 & 3, 6 & 1 & 3, 1&2&3, 4&5&6

### *8.2.3 In vitro* gas production procedure

Fermentation was carried out in graduated glass syringes (100-ml capacity) following the method described by Menke *et al.* (1979). Rumen liquor was collected before morning feeding from two fistulated steers fed bird resistant (BR) sorghum stover (890g organic matter (OM), 7 g nitrogen (N) and 740 g neutral detergent fibre (NDF) per kg DM) *ad libitum*, supplemented with 2 kg per day cottonseed cake (910 g OM, 86 g N and 380 g NDF per kg DM) and minerals. Approximately 500 mg of wilted plant material of each sample was incubated in triplicate in glass syringes into which was added 40 ml of the incubation medium prepared as described by Menke *et al.* (1979). The sample size was greater than that

Table 8.1 Protein rumen degradability characteristics of forage legumes used in the study

| Highly rumen degradable | Source | Low rumen degradable | Source |
|---|---|---|---|
| 1.*Dolichos lablab* | Umunna *et al* 1995 | 4.*Sesbania goetzei 15007* | Wiegand *et al* 1995 |
| 2.*Chamaecytisus palmensis* | " " | 5.*Desmodium intortum* | Getachew *et al* 1994 |
| 3.*Sesbania sesban 15019* | " " | 6.*Acacia angustissima* | Odenyo *et al* 1997 |

recommended by Menke *et al.* (1979) because the interest was to get enough fermented material for various analyses. Therefore the incubation media quantity was the same as that recommended by Tilley and Terry (1963). The syringes and their contents were maintained at 38.5-39° C in a thermostatic circulating water bath (260, Precision Scientific, Fisher Scientific, Springfield, USA). Six other syringes containing only the incubation medium were placed in the water bath to correct for gas production due to the activity of rumen fluid alone. Gas production readings were taken at 3, 6, 12, 24 and 48 h of incubation. After each reading, the piston of the syringe was re-set to 40 ml whenever it had gone beyond the 60 ml mark. At the end of incubation, sub-samples of substrate residue were collected into 5 ml test tubes and stored in the freezer at -20° C until needed for ammonia concentration analysis.

**8.2.4 Chemical analysis**

The dry matter (DM) and N contents of samples were determined using the method of the Association of Official Analytical Chemists (1980). Neutral detergent fibre was estimated by the method of Goering and Van Soest (1970). Soluble, insoluble and fiber-bound CTs contents of plant samples were determined according to methods used by Giner-Chavez *et al.* (1997) as described in chapter 4 of this thesis. Hydrolyzable tannins were assayed as total water-soluble phenolics after water extraction following the method described by Kaitho *et al.* (1993). After filtration the tannins were transformed to a blue-coloured product by Folin-Denis reagent and sodium carbonate. The intensity of the colour was measured at 760 nm. The content of total phenolics was calculated using a calibration line prepared using tannic acid (Sigma T0125) as the standard compound (substrate).

Ammonia concentration was determined from residues after 48 h incubation. Ammonia in the samples was assayed to estimate protein degradation. A modified method

of Chaney and March (1962) that uses phenol and hypochloride reagents was employed. At the end of incubation, a 50 ml of sample was added to a test tube. Approximately 3 ml of hypochloride reagent (25 g NaOH; 16.6 ml household bleach; 1 litre double distilled $H_2O$) was added and mixed, followed by addition of 3 ml phenol reagent. The standard was prepared with various concentrations of ammonium chloride. The samples were allowed to stand for 30 minutes at room temperature and the absorbance at 630 nm was read in a spectrophotometer 21D (Milton Roy, Brussels, Belgium). The standards were treated the same way.

**8.2.5 Statistical analysis**

The volume of gas produced (ml per 0.5 g wilted plant material) by the FLs being studied were calculated and gas production constants derived by fitting the non-linear model:

$V\ (ml/500\ mg\ DM) = b(1-e^{-ct})$ based on the argument that no gas is produced from feed that is not fermented (Nsahlai et al., 1995). V is the gas produced at time t, b cumulative extent of volume of gas produced, c the rate of gas produced ($h^{-1}$). Five groups were formed based on the proportions of FLs mixed together (i.e. 1:1, 1:1:1, 1:2, 2:1). Analysis of variance was carried out on *in vitro* gas production and ammonia production comparing the groups and samples within groups. The statistical significance of the differences between means was tested using least significance difference. Correlation analysis was performed to test the relationship between the chemical attributes. This was performed in order to relate the effects of the forages to their chemical attributes. Contrasts were performed to test the effects of group of forages and forage combinations on gas produced and ammonia released. Cluster analysis was performed to group forages and forage combinations based on gas production and ammonia production using Ward's minimum

variance analysis (SAS 1987). A five-cluster solution was selected based on the pseudo-T statistics.

## 8.3 RESULTS

### 8.3.1 Chemical composition characteristics

The N, NDF and CTs contents of the FLs are shown in Table 8.2. *Sesbania* had the highest N content followed in order by *acacia, desmodium, goetzei, lablab and tagasaste.* Condensed tannins content was in the order *goetzei* > *desmodium* > *acacia* > *sesbania* > *tagasaste* > *lablab.* Water soluble phenolics (HTs) followed this order *acacia, tagasaste* > *desmodium* > *sesbania, goetzei* > *tagasaste* > *lablab.* Neutral detergent fibre was high in *goetzei* followed in order by *desmodium, acacia, tagasaste, sesbania* and *lablab.* The correlation analyses revealed that CTs are related to NDF ($r=0.69$, $P<0.05$), while N content was negatively related to HTs ($r=-0.69$, $P<0.05$) content (Table 8.3).

### 8.3.2 Effect of forage type on gas and ammonia production

The volume of gas was significantly ($P<0.001$) different among forages when incubated alone (sole) (Tables 8.4). *Tagasaste* and *sesbania* promoted significantly ($P<0.001$) higher volumes of gas than *goetzei, desmodium* and *lablab*, while *acacia* produced ($P<0.001$) the smallest volume of gas.

Ammonia production was also significantly ($P<0.01$) different among the individual forages. *Goetzei* and *lablab* promoted higher ($P<0.05$) ammonia production than the other forages.

### 8.3.3 Effect of sole and mixtures on gas and ammonia productions

The volume of gas produced (ml/500mg wilted plant material) was significantly ($P<0.001$) affected by forage combination groups (Table 8.5), but rate of gas production (c) was not ($P>0.05$) affected by forage combination groups. Mixing FLs significantly ($P<0.01$)

**Table 8.2 Dry matter (g/kg) and chemical composition (g/kgDM) of forage legumes used in the study**

| Forage legumes | Dry matter | Nitrogen | Neutral detergent fibre | Hydrolyzable tannins | Total condensed tannins |
|---|---|---|---|---|---|
| *Acacia angustissima* | 255 | 47 | 240 | 74.6 | 30.6 |
| *Desmodium intortum* | 275 | 45 | 330 | 34.5 | 100.7 |
| *Lablab purpureus* | 358 | 38 | 135 | 16.4 | 1.9 |
| *Sesbania goetzei 15007* | 285 | 40 | 325 | 29.4 | 224.5 |
| *Sesbania sesban 15019* | 321 | 53 | 141 | 28.5 | 12.6 |
| *Chamaecytisus palmensis* | 355 | 37 | 230 | 59.5 | 2.1 |

Table 8.3 The correlation coefficients between chemical properties

| | DM | OM | N | NDF | CTs | HTs |
|---|---|---|---|---|---|---|
| Dry matter (DM) | - | -0.00$^{NS}$ | -0.24$^{NS}$ | -0.21$^{NS}$ | -0.22$^{NS}$ | 0.75$^{*}$ |
| Organic matter (OM) | | - | -0.33$^{NS}$ | -0.03$^{NS}$ | -0.04$^{NS}$ | 0.17$^{NS}$ |
| Nitrogen (N) | | | - | -0.16$^{NS}$ | -0.02$^{NS}$ | -0.69$^{*}$ |
| Neutral detergent fibre (NDF) | | | | - | 0.69$^{*}$ | -0.07$^{NS}$ |
| Condensed tannins (CTs) | | | | | - | -0.36$^{NS}$ |
| Hydrolyzable tannins (HTs) | | | | | | - |

Level of significance, NS = P>0.05, * = P<0.05

Table 8.4 Effect of sole forage legumes varying in condensed tannins concentration on volume of gas (b) (ml/500mg of wilted plant material), rate of gas production (c) ($h^{-1}$) and ammonia production (ppm) after 48 h incubation.

| **Group = sole** | | | |
|---|---|---|---|
| Sample | b | c | Ammonia |
| *Acacia* | 205.3 | 1.1 | 236.2 |
| *Desmodium* | 394.9 | 19.9 | 240.1 |
| *Lablab* | 435.0 | 8.7 | 306.7 |
| *Goetzei* | 447.2 | 20.3 | 324.6 |
| *Sesbania* | 566.6 | 24.3 | 268.9 |
| *Tagasaste* | 638.8 | 21.2 | 255.4 |
| Mean | 448.0 | 15.9 | 272.0 |
| SED | 33.7 | 9.5 | 18.0 |
| Level of significance | *** | NS | ** |

Acacia = *Acacia angustissima*, Desmodium = *Desmodium intortum*, Goetzei = *Sesbania goetzei 15007*, Sesbania = *Sesbania sesban 15019*, Tagasaste = *Chamaecytisus palmensis*.

Level of significance, NS = P>0.05, * = P<0.05, ** = P<0.01 and *** = P<0.001.

Table 8.5 Effect of sole or mixtures of forage legumes varying in condensed tannins concentration on volume of gas (b) (ml/500mg of wilted plant material), rate of gas production (c) ($h^{-1}$) and ammonia production (ppm) after 48 h incubation.

| Group | b | c | Ammonia |
|---|---|---|---|
| 1:1 | $410.7^{b}$ | 10.3 | $275.7^{b}$ |
| 1:1:1 | $449.5^{b}$ | 11.2 | $275.9^{b}$ |
| 1:2 | $618.2^{a}$ | 15.9 | $388.9^{a}$ |
| 2:1 | $650.2^{a}$ | 15.3 | $415.9^{a}$ |
| Sole | $448.0^{b}$ | 15.9 | $272.0^{b}$ |
| Mean | 529.8 | 13.6 | 334.4 |
| SED | 29.2 | 3.3 | 17.0 |
| Level of significance | *** | NS | *** |
| **Contrasts** | | | |
| Sole vs rest | ** | NS | *** |
| 1:1 & 1:1:1 vs 1:2 & 2:1 | *** | * | *** |

1:1 = combinations of two forages at ½ + ½ proportions
1:1:1 = combinations of three forages at 1/3 + 1/3 + 1/3 proportions
1:2 = combinations of two forages at 1/3 + 2/3 proportions
2:1 = combinations of two forages at 2/3 + 1/3 proportions
Sole = one forage legume alone.
Level of significance, NS = P>0.05, * = P<0.05, ** = P<0.01 and *** = P<0.001.

increased the volume of GP when compared to sole forages (Sole v rest). When forages were mixed at 1:2 or 2:1 ratio, they produced significantly ($P<0.001$) higher volume of gas than when mixed at 1:1 or 1:1:1 ratios. The volume of gas was significantly ($P<0.001$) different among 1:2 or 2:1 combination groups (Table 8.6), while among the 1:1 and 1:1:1 groups there was no significant ($P>0.05$) difference (Tables 8.7 and 8.8). In both the 1:2 and 2:1 groups, mixing *acacia* and *goetzei* consistently depressed the volumes of gas produced and mixtures which included *tagasaste, lablab* and *sesbania* increased volume of gas produced (Table 8.6). *Tagasaste* that produced the highest gas sole, when mixed with *acacia* resulted in a drastically depressed gas production even though for acacia reflected an improvement.

Ammonia production was significantly ($P<0.001$) affected by the forage combination groups (Table 8.5). Forage mixtures promoted higher ($P<0.001$) amounts of ammonia than individual forage (sole v rest), and among the combination groups, the 1:2 or 2:1 mixtures promoted significantly ($P<0.001$) higher ammonia production than the combination groups of 1:1 or 1:1:1. Ammonia production was significantly different among 1:1 ($P<0.001$) (Table 8.7) and1:1:1 ($P<0.05$) (Table 8.8), but not ($P>0.05$) within the 1:2 or 2:1 combination groups (Table 8.6). Within the 1:1 and 1:1:1 combination groups, the presence of *desmodium*, *lablab* and *goetzei* induced higher amounts of ammonia, while combinations which included *tagasaste* and *acacia* depressed ammonia production.

### 8.3.7 Cluster analysis

Using Ward's method, the pseudo-T clustering criterion, five clusters were formed for both gas and ammonia production. In Table 8.9 the classification of the forages and their combinations by both gas and ammonia production are given. Cluster 1 was characterised by high gas and ammonia production (b = 714 SD = 76.4, c = 12.5 SD = 5.6, Amon_PPM = 429 SD = 41.3) followed by cluster 3 (b = 611 SD = 41.6, c = 26.6 SD = 9.9, Amon_PPM

Table 8.6 Effect of mixtures of forage legumes varying in condensed tannins concentration on volume of gas (b) (ml/500mg of wilted plant material), rate of gas production (c) ($h^{-1}$) and ammonia production (ppm) after 48 h incubation.

| Group (combinations) = | 1:2 | | | 2:1 | | |
|---|---|---|---|---|---|---|
| Sample | b | c | Ammonia | b | c | Ammonia |
| *Acacia + Desmodium* | 444.6 | 4.7 | 443.5 | 511.5 | 10.1 | 459.7 |
| *Acacia +Goetzei* | 419.2 | 10.8 | 472.0 | 459.5 | 6.0 | 357.7 |
| *Goetzei+Desmodium* | 600.1 | 15.4 | 442.3 | 601.8 | 34.2 | 473.0 |
| *Lablab+Acacia* | 620.8 | 46.1 | 418.6 | 671.7 | 17.4 | 446.0 |
| *Lablab+Desmodium* | 576.1 | 15.5 | 324.4 | 599.8 | 19.1 | 463.4 |
| *Lablab+Sesbania* | 741.4 | 9.8 | 466.1 | 706.3 | 21.3 | 471.1 |
| *Lablab+Goetzei* | 587.2 | 27.3 | 267.5 | 688.8 | 16.9 | 451.6 |
| *Lablab+Tagasaste* | 761.0 | 5.7 | 360.8 | 686.4 | 4.8 | 453.3 |
| *Sesbania+Acacia* | 549.4 | 5.9 | 322.0 | 704.8 | 6.2 | 332.8 |
| *Sesbania+Desmodium* | 632.4 | 8.6 | 266.9 | 639.1 | 34.3 | 265.3 |
| *Sesbania+Goetzei* | 613.8 | 14.5 | 293.0 | 482.4 | 9.7 | 395.8 |
| *Tagasaste+Acacia* | 566.3 | 4.6 | 427.0 | 656.1 | 12.7 | 385.9 |
| *Tagasaste+Desmodium* | 654.6 | 25.1 | 443.5 | 756.2 | 3.9 | 417.9 |
| *Tagasaste+Goetzei* | 677.0 | 32.6 | 443.5 | 728.5 | 16.5 | 432.2 |
| *Tagasaste+Sesbania* | 829.1 | 12.5 | 442.3 | 871.8 | 12.6 | 445.0 |
| Mean | 618.2 | 15.9 | 388.9 | 650.2 | 15.3 | 415.9 |
| SED | 60.7 | 10.3 | 76.8 | 61.4 | 13.3 | 67.6 |
| Level of significance | *** | * | NS | *** | NS | NS |

Acacia = *Acacia angustissima*, Desmodium = *Desmodium intortum*, Goetzei = *Sesbania goetzei 15007*, Sesbania = *Sesbania sesban 15019*, Tagasaste = *Chamaecytisus palmensis*.

Level of significance, NS = P>0.05, * = P<0.05, ** = P<0.01 and *** = P<0.001.

Table 8.7 Effect of mixtures of forage legumes varying in condensed tannins concentration on volume of gas (b) (ml/500mg of wilted plant material), rate of gas production (c) ($h^{-1}$) and ammonia production (ppm) after 48 h incubation.

| Group (combinations) = 1:1 | | | |
|---|---|---|---|
| Sample | b | c | Ammonia |
| *Lablab+Acacia* | 362.7 | 4.8 | 246.5 |
| *Lablab+Desmodium* | 387.4 | 4.1 | 282.0 |
| *Goetzei+Sesbania* | 539.1 | 20.3 | 289.0 |
| *Goetzei+Tagasaste* | 519.4 | 14.8 | 309.7 |
| *Goetzei+Lablab* | 439.5 | 10.0 | 314.8 |
| *Sesbania+Acacia* | 307.0 | 23.5 | 258.3 |
| *Sesbania+Desmodium* | 410.5 | 4.4 | 293.1 |
| *Tagasaste+Acacia* | 322.3 | 6.0 | 232.2 |
| *Tagasaste+Desmodium* | 408.3 | 4.8 | 256.2 |
| Mean | 410.7 | 10.3 | 275.8 |
| SED | 97.4 | 7.4 | 15.7 |
| Level of significance | NS | NS | *** |

Acacia = *Acacia angustissima*, Desmodium = *Desmodium intortum*, Goetzei = *Sesbania goetzei 15007*, Sesbania = *Sesbania sesban 15019*, Tagasaste = *Chamaecytisus palmensis*.
Level of significance, NS = P>0.05, * = P<0.05, ** = P<0.01 and *** = P<0.001.

Table 8.8 Effect of mixtures of forage legumes varying in condensed tannins concentration on volume of gas (b) (ml/500mg of wilted plant material), rate of gas production (c) ($h^{-1}$) and ammonia production (ppm) after 48 h incubation.

| **Group (combinations) = 1:1:1** | | | |
|---|---|---|---|
| Sample | b | c | Ammonia |
| *Lablab+Desmodium +Acacia* | 326.6 | 7.4 | 282.7 |
| *Goetzei+Desmodium +Lablab* | 459.1 | 7.0 | 299.7 |
| *Goetzei+Desmodium +Sesbania* | 456.4 | 8.1 | 315.9 |
| *Goetzei+Desmodium +Tagasaste* | 336.9 | 4.1 | 261.7 |
| *Goetzei+Lablab+Acacia* | 473.1 | 11.8 | 281.2 |
| *Goetzei+Lablab+Tagasaste* | 556.4 | 21.0 | 269.1 |
| *Goetzei+Sesbania+Acacia* | 432.2 | 3.3 | 259.6 |
| *Goetzei+Sesbania+Lablab* | 511.7 | 19.5 | 276.4 |
| *Goetzei+Sesbania+Tagasaste* | 527.9 | 13.7 | 280.2 |
| *Goetzei+Tagasaste+Acacia* | 424.3 | 11.6 | 265.7 |
| *Sesbania+Desmodium +Acacia* | 398.0 | 18.3 | 260.3 |
| *Sesbania+Desmodium +Lablab* | 448.1 | 12.6 | 294.7 |
| *Sesbania+Desmodium +Tagasaste* | 516.6 | 10.8 | 273.7 |
| *Sesbania+Lablab+Acacia* | 409.0 | 5.7 | 270.6 |
| *Sesbania+Lablab+Tagasaste* | 540.1 | 30.0 | 281.1 |
| *Sesbania+Tagasaste+Acacia* | 466.5 | 6.3 | 248.2 |
| *Tagasaste+Desmodium +Acacia* | 400.8 | 4.6 | 268.6 |
| *Tagasaste+Desmodium +Lablab* | 473.8 | 11.1 | 298.7 |
| *Tagasaste+Lablab+Acacia* | 382.9 | 5.8 | 253.2 |
| Mean | 449.5 | 11.2 | 275.9 |
| SED | 81.3 | 8.5 | 17.5 |
| Level of significance | NS | NS | * |

Acacia = *Acacia angustissima*, Desmodium = *Desmodium intortum*, Goetzei = *Sesbania goetzei 15007*, Sesbania = *Sesbania sesban 15019*, Tagasaste = *Chamaecytisus palmensis*.

Level of significance, NS = P>0.05, * = P<0.05, ** = P<0.01 and *** = P<0.001.

Table 8.9 Classification of forage legume combinations using cluster analysis on gas and ammonia produced.

| Cluster 1 | | Cluster 2 | | Cluster 3 | | Cluster 4 | | Cluster 5 | |
|---|---|---|---|---|---|---|---|---|---|
| | Gas production Range = 599 – 872 Ammonia production Range = 333 - 471 | | Gas production Range = 327 – 549 Ammonia production Range = 240 – 358 | | Gas production Range = 540 – 677 Ammonia production Range = 255 – 473 | | Gas production Range = 205 – 383 Ammonia production Range = 232 – 258 | | Gas production Range = 419 – 566 Ammonia production Range = 396 – 472 |
| 1:2 | *Goetzei + Desm* | 1:1 | *Lab + Desm* | 1:1:1 | *Goetzei + Lab + Taga* | 1:1 | *Lab + Acacia* | 1:2 | *Acacia + Desm* |
| 1:2 | *Lab + Sesbania* | 1:1 | *Goetzei + Sesbania* | 1:1:1 | *Sesbania + Lab + Taga* | 1:1 | *Sesbania + Acacia* | 1:2 | *Acacia + Goetzei* |
| 1:2 | *Lab + Taga* | 1:1 | *Goetzei + Taga* | 1:2 | *Lab + Acacia* | 1:1 | *Taga + Acacia* | 1:2 | *Taga + Acacia* |
| 1:2 | *Taga + Sesbania* | 1:1 | *Goetzei + Lab* | 1:2 | *Lab + Goetzei* | 1:1:1 | *Taga + Lab + Acacia* | 2:1 | *Acacia + Desm* |
| 2:1 | *Lab + Sesbania* | 1:1 | *Sesbania + Desm* | 1:2 | *Sesbania + Desm* | SOLE | *Acacia* | 2:1 | *Sesbania + Goetzei* |
| 2:1 | *Lab + Acacia* | 1:1 | *Taga + Desm* | 1:2 | *Sesbania + Goetzei* | | | | |
| 2:1 | *Lab + Desm* | 1:1:1 | *Lab + Desm + Acacia* | 1:2 | *Taga + Desm* | | | | |
| 2:1 | *Lab + Goetzei* | 1:1:1 | *Goetzei + Desm + Lab* | 1:2 | *Taga + Goetzei* | | | | |
| 2:1 | *Lab + Taga* | 1:1:1 | *Goetzei + Desm + Sesbania* | 2:1 | *Goetzei + Desm* | | | | |
| 2:1 | *Sesbania + Acacia* | 1:1:1 | *Goetzei + Desm + Taga* | 2:1 | *Sesbania + Desm* | | | | |
| 2:1 | *Taga + Acacia* | 1:1:1 | *Goetzei + Lab + Acacia* | SOLE | *Sesbania* | | | | |
| 2:1 | *Taga + Desm* | 1:1:1 | *Goetzei + Sesbania + Acacia* | SOLE | *Tagasaste* | | | | |
| 2:1 | *Taga + Goetzei* | 1:1:1 | *Goetzei + Sesbania + Lab* | | | | | | |
| 2:1 | *Taga + Sesbania* | 1:1:1 | *Goetzei + Sesbania + Taga* | | | | | | |
| | | 1:1:1 | *Goetzei + Taga + Acacia* | | | | | | |
| | | 1:1:1 | *Sesbania + Desm + Acacia* | | | | | | |
| | | 1:1:1 | *Sesbania + Desm + Lab* | | | | | | |
| | | 1:1:1 | *Sesbania + Desm + Taga* | | | | | | |
| | | 1:1:1 | *Sesbania + Lab + Acacia* | | | | | | |
| | | 1:1:1 | *Sesbania + Taga + Acacia* | | | | | | |
| | | 1:1:1 | *Taga + Desm + Acacia* | | | | | | |
| | | 1:1:1 | *Taga + Desm + Lab* | | | | | | |
| | | 1:2 | *Lab + Desm* | | | | | | |
| | | 1:2 | *Sesbania + Acacia* | | | | | | |
| | | 2:1 | *Acacia + Goetzei* | | | | | | |
| | | SOLE | *Desmodium* | | | | | | |
| | | SOLE | *Lablab* | | | | | | |
| | | SOLE | *Goetzei* | | | | | | |

1:1 = combinations of two forages at ½ + ½ proportions, 1:1:1 = combinations of three forages at 1/3 + 1/3 + 1/3 proportions, 1:2 = combinations of two forages at 1/3 + 2/3 proportions, 2:1 = combinations of two forages at 2/3 + 1/3 proportions, Sole = one forage legume alone.
Acacia = *Acacia angustissima*, Desmodium = *Desmodium intortum*, Goetzei = *Sesbania goetzei 15007*, Sesbania = *Sesbania sesban 15019*, Tagasaste = *Chamaecytisus palmensis*.

= 329 SD = 86.8) followed by cluster 5 (b = 485 SD = 57.6, c = 8.0 SD = 3.1, Amon_PPM = 439.6 SD = 29.8) followed by cluster 2 (b = 451 SD = 61.1, c = 10.4 SD = 5.6, Amon_PPM = 287.8 SD = 27.3) and then cluster 4 which had the lowest gas and rate of gas production and ammonia production (b = 316 SD = 69.0, c= 8.2 SD = 8.7, Amon_PPM = 245.3 SD = 11.0). Cluster 1 which were characterised by high gas and ammonia productions was made up of members from the 1:2 and 2:1 combination groups only. These two groups, as was stated earlier, produced the highest volumes of gas and ammonia.

## 8.4 DISCUSSION

These results showed that when the FLs were incubated sole, *tagasaste* and *sesbania* promoted high volumes of gas. This observation is in agreement with findings of Bonsi *et al.* (1994) who reported that *tagasaste* produced highest amounts of GP among the FLs studied. *Acacia* on the other hand produced the lowest volume of gas. Recently Odenyo *et al.* (1997) reported that *tagasaste* produced the highest amount of (60.00 ml/200mg DM) gas at 12 h while *acacia* produced the lowest (21.93 m/200mg DMl). The main chemical differences between *tagasaste* and *acacia* were their contents of N and CTs; *tagasaste* had lower N and CT contents than *acacia* (37 v 47 g/kg DM, 2 v 31 g/kg DM, respectively). These results suggest that *acacia* may contain some compounds, that suppressed fermentation by the rumen microbes, which are not present in *tagasaste*. Thus *tagasaste* and *sesbania* could be better fodder than *acacia*. The results also indicate that the OM in both *tagasaste* and *sesbania* were easily degraded to release gases. Earlier, El Hassan (1994) had reported that *acacia* suppressed fermentation *in vitro* and that the extent of suppression depended on the amount included in the medium. Recently, Odenyo *et al.* (1997) showed that sheep fed *acacia* supplemented diet had low protozoa count, and eventually died after

9 and 21 days. *Tagasaste* had also been reported to be less acceptable to both sheep and cattle (Varvikko *et al.*, 1992; Varvikko and Khalili, 1993; Umunna *et al.*, 1995), while in one experiment reported in chapter 6 of this thesis, sheep readily accepted it.

In general, mixtures of FLs induced more gas and ammonia production and were profoundly so with the 1:2 or 2:1 combinations involving *tagasaste, sesbania* or *lablab*. The three FLs are known to be highly rumen degradable (Umunna *et al.*, 1995) and as such could have provided sufficient N and readily fermentable OM to the microbes thus inducing increased microbial activity. The greatest benefit of mixing was observed with *acacia* mixtures, probably because in mixtures, the other FLs effectively diluted the antinutrients in *acacia* and/or provided extra N and fermentable OM to the microbes. Particular compounds have discrete distributions; some are found only in a few related taxa (e. g. mimosine), others occur rather widely (e. g. condensed tannins) (NAS, 1979; Lowry, 1990; Norton, 1994). Secondary compounds have a diverse chemical nature and, in general, may interact in their effects. It may therefore be possible to reduce the antinutritive effects of some plants by mixing several plants, thus diluting the effective level of each compound (Kumar and Singh, 1984).

Initially when the forage combinations were decided, the intent was to harness the benefits of those that are usually fast rumen degradable in order to protect the proteins from extensive rumen degradability. Extensive rumen degradability is usually associated with the loss of valuable N, while very low rumen degradability is associated with a suboptimal rumen ammonia, which in turn depresses roughage digestibility. These attributes are both undesirable when appreciable amounts of FLs are included in the diet because the overall performance of animals can be affected negatively. The protein from fodder of leguminous

forages that contain low levels of tannins is rapidly degraded in the rumen (Norton, 1994). Furthermore, tropical legumes are low in soluble carbohydrates and high in non-protein N (Norton, 1994). These factors therefore contribute to low rumen efficiency by generating high levels of rumen ammonia, most of which is excreted in the urine and wasted. On the other hand, species that contain some tannins supply both degradable and undegradable proteins, and are better supplemental sources of N for ruminants (Reed, 1995).

However, a reduction in dietary N digestibility resulting from the use of FLs high in tannins has been reported (Reed *et al.*, 1990; Tanner *et al.*, 1990; Waghorn *et al.*, 1994; Wiegand *et al.*, 1995; Kaitho *et al.*, 1998). Protein digestibility was greatly reduced with increasing levels of tannins in *in vivo* studies (Reed *et al.*, 1990; Kaitho *et al.*, 1998). This could lead to a shortage of ruminal degradable N and it is this insufficiency which partly impairs the digestibility of the fiber fraction. In this study, when FLs that are low in tannins were mixed with those high in tannins, there was general improvement in gas and ammonia production compared to the high tannin component.

The fact that FLs low in tannins and others high in tannins, responded only at certain inclusion proportions (1:2 or 2:1) in gas and ammonia production may suggest that these are the acceptable levels that will harness the benefit of mixtures. At these levels, one forage could have provided the balance required in diluting the antinutritive principles of the other and/or provided extra N or fermentable OM to rumen microbes to promote digestion. Most toxic compounds have an acceptable level below which no adverse effects are apparent suggesting that some plants can be safely fed at a certain proportion of the diet (Lowry, 1990). Phenolic compounds affect enzymes by reducing the solubility of the enzyme protein and forming insoluble protein-phenolic complexes or inhibiting the enzyme activity forming

a soluble but inactive enzyme-inhibitor complex (Kumar and Singh, 1984). If microbial enzyme activity is impaired, this can result in lowered gas or ammonia production as was observed with *acacia*. The response from *acacia, desmodium* and *goetzei* demonstrate the benefit of mixtures of FLs with varying phenolic concentrations and/or types. Thus it should be possible to select a set of species, each containing a different antinutrient factor, none of which can be fed as the sole diet but make an acceptable feed in mixture.

The nutritional differences which usually result when FLs are fed have been attributed to maturity, concentration of antinutritional factors and form of presentation (fresh or dried) (Terrill *et al.*, 1989; Palmer and Schlink, 1992; Waghorn *et al.*, 1994). These factors determine their chemical composition, palatability, the extent and rates of degradation and passage out of the rumen (Terrill *et al.*, 1989; Bonsi *et al.*, 1994; Umunna *et al.*, 1995; Kaitho *et al.*, 1997). Thus mixing forages of different chemical composition, palatability and degradability would provide a mechanism by which antinutritive effects can be circumvented. Le Houerou (1980), for example, inferred that the increased consumption of mixed shrubs compared to a single species, may be a strategy to avert toxicity.

An attempt was made to use cluster analysis for both gas produced and ammonia released to classify the FLs and their combinations. However, no attempts were made to analyze for chemical constituents of the forage combinations (mixtures). Mixing different forages in varying proportions may influence the chemical interaction of the anti-nutritional factors. Both gross chemical differences, such as those distinguishing between phenolic compounds and subtle differences, such as molecular weight or stereochemical configuration (Clauesen *et al.*, 1990) can influence the biological activities of phenolic compounds. It is, therefore, possible that mixing different forages with different compounds and levels could

have a dilution effect. In this study these beneficial effects have been demonstrated and warrant further investigation *in vivo*.

## 8.5 CONCLUSION

Results from this experiment, suggest that there is a potential for improving the utilization of some forages which would normally pose problems if used sole by using them in mixtures to dilute antinutritive principles. In general, it is recommended to feed these mixtures in proportions of 1:2 or 2:1. This conclusion is, however, based on *in vitro* results; there is therefore, a need to test these findings in *in vivo* feeding trials. The *in vitro* methods such as gas production may have potential in terms of ranking forage legumes, especially when other indices of fermentation can be included in the model. In this experiment, both gas produced and ammonia released were used to rank the forages and it seems that the combination of both gives a better insight.

# CHAPTER NINE

## 9.0 ESTIMATION OF THE REACTIVITY OF CONDENSED TANNINS IN FORAGE LEGUMES AS MEASURED BY THE BINDING CAPACITY OF POLYETHYLENE GLYCOL (PEG) TO TANNINS


### ABSTRACT

A procedure to measure the condensed tannin reactivity by reacting polyethylene glycol-4000 (PEG) with tannins is described. Condensed tannins (CTs) were reacted with PEG in a buffer solution overnight and then the quantity of PEG that bound with CTs was determined in the supernatant. The procedure was compared with the radial diffusion assay (RDA) (protein-precipitation assay) on its ability to predict the biological activity of CTs in forages. The two methods were compared with *in vitro* and *in sacco* techniques as a means of indexing the influence of CT reactivity on forage utilization. The reactivity, as measured by the amount of PEG reacted with forage samples was significantly ($P<0.001$) influenced by forage tannin level. PEG reactivity was highly correlated ($r = 0.92$, $P<0.001$) with CTs and was also correlated with NDF ($r = 0.78$, $P<0.05$). Forage legumes that are high in CT concentration and NDF reacted with high amounts of PEG. There were, however, some differences in FLs. For example *calliandra* that had similar CT levels with *leucaena* and *acacia* reacted with a higher amount of PEG than *leucaena* or *acacia*. In the case of *desmodium* and *S. sesban 2024*, both precipitated similar amounts of PEG even though *S. sesban 2024* had a higher (152.3 g/kg DM) concentration of CTs than *desmodium* (100.7 g/kg DM). There was a significant ($P<0.001$) influence of forage type on the amount of protein precipitated by tannins. The RDA was correlated to NDF ($r = 0.66$, $P<0.05$) and CTs ($r = 0.67$, $P<0.05$). The results showed a high correlation ($r = 0.87$, $P<0.01$) between PEG-reaction and RDA method. This indicates that PEG-reaction may be an effective method of indexing tannin reactivity. There was however, poor correlation ($r = 0.47$, $P>0.05$) between PEG-reaction and *in vitro* gas production but good correlation ($r = 0.83$, $P<0.01$) with *in sacco* degradation. The high correlation with the DM degradation suggests that this method can be effectively used to predict the possible antinutritional effects of the forages.

## 9.1 INTRODUCTION

The cost of conventional N supplements such as oilseed cakes and other agro-industrial by-products prohibits the wide use of this technology, especially by smallholder farmers in tropical countries. Increased attention has been given to the use of legume fodder (multi-purpose trees (MPTs), browses and herbaceous forages) as sustainable sources of limiting nutrients in roughage-based feeding systems (Mosi and Butterworth, 1985; Borens and Poppi, 1990; Varvikko and Khalili, 1993; Devendra, 1993; Umunna *et al.*, 1995). Energy, nitrogen and mineral contents of legume fodder crops and browses make them attractive alternatives to expensive concentrates as supplements for poor quality roughages.

Large differences in animal response have been reported when forage legumes are fed as protein supplements (Reed *et al.*, 1990; Wiegand *et al.*, 1995; Boitumelo *et al.* Chapter). The causes of these differences are not very clear, but some researchers have attributed these differences to the levels (concentrations) of phenolic compounds in forage legumes (Kaitho *et al.*, 1998). Some research reports (Jackson *et al.*, 1996; Khazaal *et al.*, 1996; Kaitho *et al.*, 1998) suggest that the concentration of condensed tannins *per se* may not be the only factor impacting on the nutritive value of tropical forages. The biological activity (reactivity) of CTs, which depends on other criteria such as chemical structure and degree of polymerization as well as CTs concentration, could be factors. The reactivity (biological response) of polyphenolic compounds seems to depend on their nature and this varies between plant species. According to Bate-Smith (1973), reactivity can be defined as the ability of CTs to precipitate protein per unit weight. The ability of tannins to form strong complexes with proteins is the most important aspect of their nutritional and toxicological effects. The strength of these complexes depends on characteristics (molecular weight,

tertiary structure, isoelectric point, and compatibility of binding sites) of both the tannins and protein (Hagerman and Butler, 1980). Although it is recognized that other plant characteristics (such as NDF and ADF concentrations) could mediate the response, it is possible that the quantity and reactivity of CTs could interact to determine the degree of animal response. There is therefore need for a better understanding of the differences between CTs of various FLs, especially their possible antinutritional effects on livestock.

Physiological activities of tannins are attributed to their capacity to bind and precipitate proteins. Therefore, protein precipitation methods are considered to correlate better with the biological value of tannin-rich feeds and foods (Makkar, 1989). If tannins can precipitate protein, and PEG can preferentially bind to tannins (Kumar and Singh, 1984), then it is possible to assess the biological properties of tannins by reacting them with PEG. There is a considerable analogy between PEG-tannin and protein-tannin complexation and information obtained from the amount of PEG bound to a plant sample might be analogous to that obtained from protein precipitation capacity (Silanikove *et al.*, 1996). Because of the solubility of PEG in water and most organic solutions, bound PEG can be measured *in situ* without the need to pre-extract the tannins from the sample. PEG may react *in situ* with tannins that can not be extracted with conventional organic solvents because these tannins are bound to proteins and cell-wall components. Some workers have used tannin complexing agents to quantify tannins in forages (Makkar *et al.*, 1995; Silanikove *et al.*, 1996). However these entail laborious methods and often hazardous chemicals which may not be appropriate in most of the laboratories in the developing world with limited equipment. There is, therefore, a need for simple and practical methods that can be used to predict the antinutritive effects of tannins in legume forages.

Two types of protein precipitation assays to determine the biological activities of tannins have been recommended (Hagerman and Butler, 1978; Makkar, 1989). One group of methods estimates the amount of tannins precipitated by a standard protein such as bovine serum albumin (BSA) and the other measures the amount of protein in a tannin-protein complex. The radial diffusion method developed by Hagerman (1987) indirectly measures the ability of tannins to precipitate BSA by radial diffusion (RDA) properties. The tannin diffuses through a protein-containing gel resulting in the formation of a disk-shaped tannin-protein precipitate. The area of the precipitate is proportional to the amount of tannin and possibly the size of the molecule in the extract. A large number of samples can be handled in laboratories with limited facilities using this method. The main disadvantage is the need for extraction of tannins with conventional organic solvents as the tannins are bound to proteins and cell-wall components. In this study therefore, the biological activity of CTs were evaluated by determining their ability to bind to polyethylene glycol (PEG). The ability to bind to PEG is analogous to the ability to precipitate protein, therefore CT reactivity was determined by their ability to bind to PEG. The study further evaluated the developed method as a quick *in vitro* procedure that could be used to screen the reactivity of CTs of forages and compared it with the protein-precipitation assay (RDA) on their ability to predict the biological activity of CTs in forages. Furthermore, the two methods were compared with *in vitro* and *in sacco* techniques in assessing the reactivity of CTs on forage utilization.

## 9.2 MATERIALS AND METHODS

### 9.2.1 Plant materials

Ten forage legumes and stover from two sorghum varieties (bird-resistant (BR) and non-bird-resistant (NBR)) were used in this study. Specifically the samples included two

herbaceous legumes (*Dolichos lablab (lablab)* and *Desmodium intortum (desmodium)*), eight multipurpose trees (*Acacia angustissima (acacia), Chamaecytisus palmensis (tagasaste), Leucaena leucocephala (leucaena), Sesbania sesban 15019 (S. sesban 15019), S. sesban 15036, S. sesban 2024, S. goetzei 15007 (goetzei) and Calliandra calothyrsus (calliandra)*) and two cereals, NBR and BR sorghum stover. Non-bird-resistant sorghum stover was used as a reference for low tannin forages. Four hundred grams (fresh weight basis) of each of the above ten forage legumes was harvested on the same day at the Debre Zeit research station farm, dried at 40° C to constant weight, and then ground to pass through 1-mm screen. These forages were chosen since earlier reports had indicated that they had good agronomic performance and that their tannin levels were variable.

### 9.2.2 PEG reaction with tannins in plant samples

In order for PEG to react with the plant sample, an environment where the reaction took place was created. A stock solution containing 100 g/L PEG-4000 (MW 4000) in a 0.5 M buffer Tris-BASE, pH 7.1 (Silanikove *et al.*, 1996) was prepared. Then a working solution was prepared by mixing 1 part of stock solution and two parts of distilled water. The ratio between the plant sample weight to working solution was 1:30. One gram of plant sample was used. The reaction was carried out in 50 mL centrifuge tubes. After the samples have been mixed with the working solution or distilled water (in the case of those untreated or control), the tubes were left for 24 hours, with occasional mixing. The tubes were then centrifuged for 30 minutes at 2500*g*, and the supernatant was collected. Two porcelain weighing dishes, one for PEG-treated and the other one for untreated feed sample were weighed. Aliquots (10 ml untreated and 20 ml PEG-treated) were pipetted into the respective dishes and dried to a constant weight (about 30 minutes for untreated and 60 minutes for

PEG-treated) in an oven at 100° C. The dishes were then transferred into a dessicator to cool down before weighing. The procedure of drying and weighing was repeated three times to obtain four values for each treated and untreated feed sample. The PEG reaction with plant samples was developed to estimate the amount of biologically active tannins present in a forage sample by reacting tannins with PEG and estimating the amount of PEG precipitated. The difference in weight between the treated and untreated was the weight of PEG in 10 ml of supernatant. The difference between the resultant and the original PEG in working solution was the PEG, which reacted with tannins.

**9.2.3 Radial diffusion assay (RDA)**

The procedure used was that of Hagerman (1987). The radial diffusion assay (RDA) with agar containing bovine serum albumin (BSA) was utilized. The original idea was to use leaf protein extracted from the plants of interest but because of difficulties in purification, BSA was used for this purpose. Plastic petri plates were filled with 9.5 ml of a solution of 1% (w/v) agarose (type I) and 0.1% BSA (w/v) in 50mM acetic acid and 60 mM ascorbic acid solution pH 5.0. Plates were allowed to dry on a level surface, sealed with a strip of parafilm and stored at 4° C for four days before use. Wells were made 1.5 cm apart in the gel with a punch 4 mm in diameter. Samples (tannin-containing solutions) in 8 ml aliquots were dispensed into individual wells with a 10 ml Hamilton syringe and plates were sealed and incubated at 39° C for 96 hours. The amount of protein precipitated was determined by multiplying the volume of the ring formed by the amount of protein per ml of the protein-containing gel.

### 9.2.4 *In vitro* gas production

The feed samples (residues) obtained after centrifuging from the PEG reaction with tannin were dried at 40°C until no weight change. Then a subsample of 500 mg was incubated in a 100 ml calibrated glass syringe according to Menke *et al.* (1979). The incubation was carried out with rumen fluid from steers fed BR sorghum stover offered *ad libitum* and supplemented with cotton seed cake at 2 kg per head per day. The feed samples with or without PEG were incubated in triplicate for 48 h. Also rumen fluid with or without addition of 1g PEG was incubated alongside the feed samples. The sample size of 500 mg was greater than that used by Menke *et al.* (1979) because the goal was to get enough fermented material for various analyses and therefore the quantity of rumen fluid inoculum was the same as that used by Tilley and Terry (1963). Cumulative gas production at 0, 3, 6, 9, 12, 24 and 48 h were recorded..

### 9.2.5 *In situ* degradability

I*n situ* DM degradability was determined by weighing 2.5 g of DM of each plant material into nylon bags. Samples were suspended for 24 h, either in the working solution (prepared as above) or distilled water and then suspended in the rumen of fistulated steers for 48 h. Steers were fed grass hay offered *ad libitum* and supplemented with cotton seed cake at 2 kg per head per day. After incubation, samples were air-dried at 105° C and analyzed for DM disappearance. The weight loss in DM after incubation in the rumen was assumed as material degraded *in situ*.

### 9.2.6 Chemical analyses

Dry matter and nitrogen (N) were determined by the methods of the Association of Official Analytical Chemists (1980). Neutral detergent fibre (NDF) was assayed according

to Goering and Van Soest (1970). Soluble, insoluble and fiber-bound CTs of plant samples were determined according to methods used by Giner-Chavez *et al.* (1997) as described in chapter 4 of this thesis. Hydrolyzable tannins (HTs) were assayed as total water-soluble phenolics after water extraction following methods described by Kaitho *et al.* (1993). After filtration, the tannins were transformed to a blue-coloured product by Folin-Denis reagent and sodium carbonate. The intensity of the colour was measured at 760 nm. The content of total phenolics was calculated using a calibration line prepared using tannic acid (Sigma T0125) as the standard compound (substrate).

**9.2.7 Statistical analysis**

The results are presented as the mean of replicates. Data analyzed was the difference between the PEG treated and untreated samples for PEG-reaction, gas production and in situ degradation. Differences between the samples in the PEG reaction, radial diffusion assay (protein precipitation), gas production and *in situ* degradation were analyzed using analysis of variance. A correlation matrix of the relationship between chemical attributes, PEG-reaction, radial diffusion assay, gas production and *in situ* degradability was done by simple regression to determine whether was any relationship between these variables.

## 9.3 RESULTS

### 9.3.1 Chemical attributes of the samples under investigation

The DM, N, NDF, HT and CT concentrations of the FLs are shown in Table 9.1. *S. sesban 15019, S. sesban 2024 and lablab* had the highest N content followed in order by *acacia, desmodium, S. sesban 15036, leucaena, goetzei, tagasaste* and *calliandra*. Condensed tannins (CTs) content were in the order *goetzei* > *S. sesban 2024* > *desmodium* > *S. sesban 15036* > *calliandra* > *leucaena* > *acacia* > *S. sesban 15019* > *tagasaste* > *lablab*. Water soluble phenolics (HTs) followed the order *calliandra , acacia* > *leucaena* > *tagasaste* > *desmodium* > *S. sesban 15036* > *goetzei* > *S. sesban 15019* > *S. sesban 2024* and *lablab*. Neutral detergent fibre followed the order *desmodium, goetzei, S.sesban 2024, acacia, tagasaste, calliandra, leucaena, S. sesban 15036, S. sesban 15019* and *lablab*. Condensed tannins content related positively with high NDF content ($r = 0.69$, $P<0.05$), while N content was related negatively to HTs ($r=-0.69$, $P<0.05$); DM concentration also related positively to HTs ($r=0.75$, $P<0.05$).

### 9.3.2 PEG reaction with tannins

Table 9.2 shows the amount of PEG that reacted with tannins. Tannin reactivity, as measured by the amount of PEG that reacted with tannins in forage samples was significantly ($P<0.001$) influenced by forage tannin level. PEG reacted with tannins correlated highly ($r = 0.92$, $P<0.001$) with CTs and with NDF ($r = 0.78$, $P<0.05$) concentrations (Table 9.3). Forage legumes with high concentrations of CTs and of NDF generally reacted with high amounts of PEG. There were, however, some differences within FLs. For example *calliandra* that had similar CTs levels to *leucaena* and *acacia* reacted with a higher amount of PEG than *leucaena* or *acacia*. In the case of *desmodium* and *S. sesban 2024* both precipitated similar amounts of PEG even though *S. sesban 2024* had a higher (152.3 g/kg DM) concentration of CTs than *desmodium* (100.7 g/kg DM)

Table 9.1 Dry matter (g/kg) and chemical composition (g/kg DM) of forage and sorghum stover the samples

| Sample | Dry matter | Organic matter | Nitrogen | Neutral detergent fibre | Condensed tannins | Hydrolyzable tannins |
|---|---|---|---|---|---|---|
| *Acacia angustissima* | 90.2 | 87.8 | 46.2 | 303 | 30.6 | 74.6 |
| *Calliandra calothyrsus* | 89.2 | 89.9 | 30.9 | 242 | 39.1 | 88.2 |
| *Desmodium intortum* | 88.1 | 89.2 | 46.0 | 426 | 101 | 34.5 |
| *Lablab purpureus* | 88.0 | 89.1 | 50.5 | 180 | 1.90 | 16.4 |
| *Leucaena leucocephala* | 90.2 | 92.2 | 42.0 | 201 | 33.3 | 73.6 |
| *Sesbania goetzei 15007* | 88.0 | 89.6 | 40.2 | 421 | 225 | 29.4 |
| *S.sesban 15019* | 88.0 | 88.8 | 53.1 | 183 | 12.6 | 28.5 |
| *S.sesban 15036* | 88.8 | 90.1 | 42.4 | 201 | 77.4 | 31.5 |
| *S.sesban 2024* | 88.7 | 91.9 | 51.1 | 307 | 152 | 26.0 |
| *Chamaecytisus palmensis* | 88.2 | 94.0 | 37.3 | 302 | 2.10 | 59.5 |

Table 9.2 Assessment of PEG reactivity through PEG-CT reaction, RDA, nylon bag, DM degradability and gas production technique.

| Sample | PEG reaction (% PEG reacted) | | | DM degradability | | | Gas production | | | RDA |
|---|---|---|---|---|---|---|---|---|---|---|
| | Treated | Untreated | Difference | Treated | Untreated | %difference | Treated | Untreated | %difference | mg/g |
| *Acacia angustissima* | 7.7 | 0.1 | 7.6 | 747.6 | 648.9 | 13.2 | 399.7 | 314.7 | 21.3 | 96.3 |
| Bird-resistant sorghum stover | 4.0 | 1.5 | 2.5 | 448.5 | 424.9 | 5.1 | 445.0 | 320.6 | 27.6 | - |
| *Calliandra calothyrsus* | 18.8 | 2.5 | 16.3 | 695.2 | 604.7 | 13.5 | 467.7 | 343.8 | 26.6 | 178.8 |
| *Desmodium intortum* | 17.6 | 0.4 | 17.2 | 866.9 | 769.4 | 11.2 | 618.1 | 463.3 | 25.2 | 236.5 |
| *Lablab purpureus* | 1.7 | 0.2 | 1.5 | 845.3 | 829.5 | 1.9 | 697.7 | 544.6 | 21.9 | 14.5 |
| *Leucaena leucocephala* | 7.1 | 0.2 | 6.9 | 852.3 | 803.6 | 5.6 | 616.7 | 550.9 | 10.1 | 139.7 |
| Non-bird-resistant sorghum | 0.4 | 0.3 | 0.0 | 605.4 | 589.7 | 1.2 | 571.3 | 322.2 | 43.6 | - |
| *Sesbania goetzei 15007* | 34.1 | 8.6 | 25.5 | 896.0 | 718.5 | 19.8 | 628.3 | 486.7 | 21.3 | 184.9 |
| *Sesbania sesban 15019* | 5.0 | 0.3 | 4.7 | 825.3 | 729.1 | 11.6 | 685.0 | 530.0 | 22.8 | 39.3 |
| *Sesbania sesban 15036* | 14.7 | 0.0 | 14.6 | 934.9 | 808.3 | 13.5 | 845.6 | 557.6 | 33.3 | 168.9 |
| *Sesbania sesban 2024* | 19.8 | 0.0 | 19.7 | 957.8 | 801.5 | 16.3 | 1189.5 | 881.7 | 25.7 | 184.9 |
| *Chamaecytisus palmensis* | ND | ND | ND | 748.9 | 721.1 | 3.5 | 1000.2 | 664.3 | 33.2 | 109.6 |
| Mean | 11.9 | 1.3 | 10.6 | 759.9 | 684.6 | 9.0 | 692.8 | 507.7 | 25.9 | 135.3 |
| SED | | 1.7 | 3.2 | 33.5 | 39.9 | 6.1 | 57.8 | 36.1 | 8.6 | 15.3 |
| Level of significance | *** | NS | *** | *** | *** | * | *** | *** | NS | *** |

PEG-CT = Polyethylene glycol- condensed tannin
RDA = Radial diffusion assay
PEG = Polyethylene glycol
Level of significance, NS = P>0.05, * = P<0.05, ** = P<0.01, *** = P<0.001.

Table 9.3 Linear correlation among the reactivity variables and chemical attributes

| | DM | OM | N | NDF | CT | HT | DEG | PPC | PEG-T | GAS |
|---|---|---|---|---|---|---|---|---|---|---|
| Dry matter (DM) | | -0.0$^{NS}$ | -0.23$^{NS}$ | -0.21$^{NS}$ | -0.22$^{NS}$ | 0.75$^{*}$ | 0.0$^{NS}$ | 0.10$^{NS}$ | -0.22$^{NS}$ | -0.63$^{*}$ |
| Organic matter (OM) | - | | -0.33$^{NS}$ | -0.03$^{NS}$ | -0.04$^{NS}$ | 0.17$^{NS}$ | -0.39$^{NS}$ | 0.18$^{NS}$ | 0.24$^{NS}$ | 0.01$^{NS}$ |
| Nitrogen (N) | | - | | -0.16$^{NS}$ | -0.02$^{NS}$ | -0.69$^{*}$ | -0.04$^{NS}$ | -0.43$^{NS}$ | -0.37$^{NS}$ | -0.14$^{NS}$ |
| Neutral detergent Fibre (NDF) | | | - | | 0.69$^{*}$ | -0.07$^{NS}$ | 0.44$^{NS}$ | 0.66$^{*}$ | 0.78$^{*}$ | 0.17$^{NS}$ |
| Condensed tannins (CT) | | | | - | | -0.36$^{NS}$ | 0.81$^{**}$ | 0.67$^{*}$ | 0.92$^{***}$ | 0.11$^{NS}$ |
| Hydrolyzable tannins (HT) | | | | - | | | -0.17$^{NS}$ | 0.14$^{NS}$ | -0.12$^{NS}$ | -0.63$^{NS}$ |
| Degradability (DEG) | | | | | - | | | 0.57$^{NS}$ | 0.83$^{**}$ | 0.20$^{NS}$ |
| Protein precipitation (RDA) | | | | | | - | | | 0.87$^{**}$ | 0.19$^{NS}$ |
| PEG-REACTION (PEG-REACT) | | | | | | | | - | | 0.41$^{NS}$ |
| Gas production (GAS) | | | | | | | | | | |

PEG-CT = Polyethylene glycol- condensed tannin
RDA = Radial diffusion assay
PEG = Polyethylene glycol

Level of significance, NS = P>0.05, * = P<0.05, ** = P<0.01, *** = P<0.001.

### 9.3.3 Radial diffusion assay (RDA)

The mean amounts of protein precipitated by tannins with the radial diffusion assay (RDA) are shown in Table 9.2. There was a significant ($P<0.001$) influence of forage type on the amount of protein precipitated by tannins. The RDA values correlated positively to NDF ($r = 0.66$, $P<0.05$) and CTs ($r = 0.67$, $P<0.05$). Thus FLs with high NDF and CTs concentrations tended to precipitate higher amounts of protein. For example, *desmodium* that had the highest NDF content (426 g/kg DM) and medium to high CTs (100.7 g/kg DM) precipitated the highest amount of protein (236.5 mg/g). This was greater than either *S. sesban 2024* or *goetzei* that had higher CTs (224.5 and 152.3 g/kg DM, respectively) and relatively high NDF contents (306.6 and 420.7 g/kg DM, respectively).

### 9.3.4 Gas production

Table 9.2 shows the effect of incubating forage samples with or without PEG on gas production (GP). Addition of PEG significantly ($P<0.001$) increased GP. The increase in GP with PEG was, however, not related ($r = 0.17$, $P>0.05$) to CTs content of forages but was negatively correlated ($r = -0.63$, $P<0.05$) to DM content of the forages.

### 9.3.5 DM degradability

Table 9.2 shows mean DM degradability values as influenced by PEG. Addition of PEG tended ($P=0.051$) to influence DM degradability. The increase in DM degradability with PEG was significantly ($r = 0.81$, $P<0.01$) correlated to CTs level of the forage samples. Forages with high CTs content (e.g. *goetzei* and *S. sesban 2024*) had higher percentage increase in DM degradability, while forages with low CTs content (e. g *lablab* and *tagasaste*) had lower percentage increase in DM degradability.

### 9.3.6 Correlation between PEG reaction and the other methods

PEG-reaction correlated very well with DM degradability ($r = 0.83$, $P<0.01$) and RDA ($r = 0.87$, $P<0.01$) (Table 9.3). There was little correlation ($r = 0.41$, $P>0.05$) between PEG-reaction and GP.

## 9.4 DISCUSSION

Condensed tannin reactivity was measured by the binding capacity of PEG to plant CTs. The precipitation of CTs by PEG is through hydrogen bonding between oxygen (ether) of the PEG chain and the phenolic hydroxyl group on the tannin moiety. This reaction therefore suggests that there is a considerable analogy between PEG-tannin and protein-tannin complexation (Jones, 1965; Hagerman and Butler, 1981). Therefore, the amount of PEG that reacts with CTs is analogous to that obtained from protein precipitation assays. The ecological and biological roles of CTs are attributed to the complexation of tannins with proteins (Bate-Smith, 1973; Hagerman and Butler, 1978). Moreover, protein precipitation assays for tannins are highly correlated with the biological value of tannin-rich foods and feeds (Makkar, 1989). The biological activity of CTs as measured by the amount of PEG that reacted with CTs showed that it depended on the CT concentration to some extent. But in some cases, it did not depend on the concentration. For example *calliandra* that had similar CTs concentration with *leucaena* and *acacia* reacted with higher amounts of PEG. This observation clearly shows that the reactivity of plant CTs depends on other criteria such as chemical structure degree of polymerization as well as concentration as suggested by Horigome *et al.* (1988). The different PEG binding capacities suggest different biological responses of different CTs even at the same level in different forages. In this study, reactivity also correlated significantly with NDF agreeing with the assertion by Jackson *et al.* (1996)

that other plant characteristics (such as NDF concentration) could mediate response to CTs. Khazaal *et al.* (1996) similarly observed that biological response to polyphenolic compounds depend on their nature which varies between plant species. The content and types of CTs (mixtures) in a diet jointly determine the chemical reactivity of the tannins and also influence the direction of the effects (Mcleod, 1974). Therefore CTs with different structural and biological characteristics may elicit different responses which are not necessarily based on their concentration. Results of the PEG reaction, RDA, DM degradability and to some extent GP seem to point to the fact that tannin concentration was not the only factor impacting on the use of the forages. The results clearly showed that CTs reactivity depended on the CTs concentration, NDF content and probably other chemical attributes. In confirmation of this, McNabb *et al.* (1998) have recently shown that the reactivity of CTs also depended on the molecular weight of tannins. Therefore, based on current knowledge, it is safe to assume that the reactivity of CTs is influenced by factors such as concentration, molecular weight, and NDF content and probably other chemical attributes. According to Jones *et al.* (1976) and Field and Lettinga (1992), the reactivity of tannins in relation to protein precipitation decreases as their molecular weight exceeds 20,000 g per mole. Since the binding of tannins with PEG is analogous to the binding of tannins with proteins, it would be expected that the molecular weight of CTs in the forages under study will influence their reactivity with PEG.

All the FLs with high CTs concentration and high NDF content precipitated higher amounts of protein in the BSA procedure and this is similar with the results from the PEG-reaction method. The relationship between the PEG-reaction method as a measure of tannin reactivity with the RDA which measures the biological activity of tannins was high ($r = 0.87$, $P < 0.01$). This observation suggests that PEG-reaction method may be an effective method

to estimate the biological activity (reactivity) of CTs present in forages. The high correlation between the RDA and PEG-reaction, supports the assertion by Giner-Chavez (1997) that in order to have a better understanding of the biological activity of tannins, protein precipitation assays also should be pursued. Because of the simplicity of the method, it offers an advantage over the standard protein precipitation methods.

The PEG-reaction method also correlated positively with DM degradability. When CTs are not rendered inert with PEG, DM degradability is usually negatively correlated with CTs concentration (Nsahlai *et al.*, 1995). This is so because CTs protect proteins from rumen fermentation and depresses the fermentation of structural carbohydrates (Barry, 1989). In this study, the increase in DM degradability was correlated with the CTs concentration ($r = 0.81$, $P<0.01$). The increase in DM degradability of samples treated with PEG suggests the removal of adverse effects of CTs by PEG leading to the increased DM degradation, suggesting that this method may be effective in predicting the possible antinutritional effects of CTs in forages.

The biological value of tannins was also measured using the gas production approach (Makkar *et al.*, 1995) in which the tannin containing forages were incubated in an *in vitro* gas procedure (Menke *et al.*, 1979) in the presence or absence of PEG. It was assumed that PEG would bind with CTs making them inert, which was expected to increase the gas production. In other studies, it was found that percent increase in gas production was positively correlated with the potential adverse effects of tannins on digestion in the rumen (Makkar *et al.*, 1996; Khazaal *et al.*, 1996). There was, however, an increase in gas production with PEG that was not related to the CTs concentration of the forages. The poor relationship between gas production and PEG-reaction, RDA and DM degradability suggests

that GP alone may not be adequate in predicting the antinutritive effects of CTs in forages. This assertion is in line with observations made in the earlier experiments (chapter 4 and chapter 5), where it was found that the increase in GP with PEG treated samples was not related to the concentration of CTs. It has been suggested that other factors like protein content of the forages may mimic the effect of the PEG (Makkar *et al.*, 1996). Increasing the protein concentration may diminish the efficacy of tannins to bind protein. Therefore the increase in gas production with PEG from feeds containing drastically different protein contents would not be expected to give a good correlation with protein precipitation assays. On the other hand, individual forages are likely to contain a mixture of phenolics which together produce antinutritive effects when tested by *in vitro* techniques (Khazaal *et al.*, 1994).

Techniques have been developed to quantify CTs using tannin binding agents (Makkar *et al.*, 1993; Silanikove *et al.*, 1996), but these methods require sophisticated laboratory equipments and hazardous chemicals. A simple and more practical method of estimating the CTs of forages using PEG has been described. This method was highly correlated with CTs concentration and RDA, which measures the biological activity of tannins. This observation suggests that this method can be effectively used to predict the possible antinutritional effects of CTs in forages.

## 9.5 CONCLUSIONS

It was possible to assess the biological activity (reactivity) of tannins by reacting them with PEG and relating the results with those obtained with RDA technique. The information obtained from the PEG-reaction method was equivalent to that obtained from the RDA method, thus indicating that PEG-CT reaction may be an effective method of indexing tannin

reactivity. The PEG-reaction method has several advantages. For example, there is no need for the use of sophisticated laboratory equipment and hazardous chemicals and large number of samples can be handled in a short space of time.

# 10.0 GENERAL DISCUSSION

**Stage at harvest and post - grain - harvest management practices on the nutritive value of cereal stover.**

There is wide variation in the nutritional attributes of roughages available to ruminant livestock in the sub-tropics and tropics, especially in the dry season. Variations in the feeding value of roughages or crop residues is influenced in part by plant and management factors. For example, the management of stover after grain harvest can influence stover quality. A study on management factors which could affect the nutritive quality of maize and sorghum stover was therefore carried out with the objective of developing strategies for preserving and maintaining high quality stover (chapter 3). Delay in harvesting stover after the grain has reached physiological dead ripe stage resulted in loss of nutritive quality. And an increase in NDF and phenolic compound concentrations causes the decline in nutritive value beyond the stage of physiological maturity. Nitrogen decreased with maturity both because of decreases of N in leaves and stems, and because stems, which have lower N concentrations than leaves, make up a significant portion of the forage. The increase in phenolic compounds content may be due to moisture stress and the continued translocation of soluble cell contents from leaves and stems to grain. Stover left rooted in the field for extended periods (six weeks) after grain harvest had lower nutritive value because of changes in the botanical composition due to plant shedding and shattered off dried leaves and environmental stresses. However, protecting stover from environmental stresses by cutting immediately after grain harvest and storing either in the open or under shade prevented nutrient loss compared to leaving it rooted. Changes in the concentrations of CTs with stage of growth and storage

methods could allow for harvesting programs that take advantage of plants when concentration of CTs in the plants are low.

The nutritive value of the three crops studied progressively declined with delay in harvesting time; however, the rate of decline was higher for the BR sorghum variety than for maize and the NBR variety. The differences may be due to differences in biochemical pathways among the crops and their ability to respond differently to environmental stresses. Bird-resistant sorghum variety is known to synthesize CTs in large quantities (Butler, 1982) and this may indicate its genetic make up to respond to environmental stresses. When the stover was left rooted for extended periods of time, BR sorghum stover remained green and some new green shoots and leaves (sprouts) were present at harvesting time while maize stover was already dry with evidence of leaf loss. That is why sorghum stover had better nutritive quality than maize. Therefore, different harvesting and storage strategies are required for maize and sorghum crops. It is concluded from this study that, improved utilization of otherwise tannin-rich BR sorghum stover may be possible through post-grain-harvest management practices.

**Ameliorants to tanniferous feed by the *in vitro* digestibility and gas production techniques.**

Generally, animals in smallholdings in the sub-tropics and tropics gain weight during the rainy season, part of that is lost during the harsh periods of the dry season. This results from the fact that ruminants subsist principally on poor quality roughage diets that are deficient in energy and essential nutrients such as nitrogen, sulphur, minerals and vitamins (Nsahlai *et al.*, 1998). Consequently, supplementation with nutrient-rich concentrates is one

strategy aimed at alleviating the problem. However, the strategy that may win acceptance of this class of producers is that which requires/entails minimum external input. Hence supplementation with on-farm generated forage legumes (FLs) as a sustainable means of overcoming nutritional deficiencies is gaining popularity (Nsahlai *et al.*, 1998). However, FLs contain various levels and types of secondary plant factors (or anti-nutritional factors) among which are tannins, which influence nutrient utilization to the level those animal responses, have not always matched expectation. If resource poor farmers have to improve the productivity of their animals, it is necessary to develop practical and simple strategies to overcome the anti-nutritional effects of FLs supplements (chapter 4).

*In vitro* digestion techniques provide comparative estimates of dry matter digestibility among feeds. The values are used to rank the quality of feeds although there is a tendency of underestimating values obtained from *in vivo* digestibility. In a study that examined the ameliorative effects of PEG, sulphur and urea on IVDMD and gas production of tanniferous feeds, rumen fluid inoculum from steers fed high tannin basal diet (bird-resistant sorghum stover) depressed DMD when *goetzei* that had the highest CTs concentration was used as a supplement. Can this mean that there was accumulation of CTs (from the free CTs in the rumen fluid inoculum and that from *goetzei*) which affected the digestibility of the forage? If this were the case, it would be detrimental to supplement high tannin basal feeds with high tannin FL supplements without ameliorative additives. One of many reasons for supplementing the basal substrate with FLs is to alleviate nutritional deficiencies of N (McMeniman *et al.*, 1988) in order to stimulate microbial activity. In this study, ruminal microbial activity was indexed by DMD, which showed about 6 %

improvement. Thus the benefit of supplementing low quality roughages with FLs was demonstrated. This improvement was, however, higher with FLs that are low in CTs content (*lablab* and *leucaena*), than with those high in CTs content (*desmodium* and *goetzei*). Ameliorants (polyethylene glycol (PEG), urea or sulphur) greatly improved ($P<0.001$) the digestibility of treatments supplemented with high tannin FLs suggesting that ameliorants counteracted the antinutritive effects of the CTs (Barry and Manley, 1984; Pritchard *et al.*, 1992). Urea and sulphur had similar effects on IVDMD as PEG in alleviating antinutritive effects. The improvement in *IVDMD* with PEG was attributed to the binding of dietary tannins with PEG. This accords with previous reports of Pritchard *et al.* (1992) and Silanikove *et al.* (1996) which showed that PEG can complex tannins rendering them inert and/or displace protein from tannin-protein complex. On the other hand, the improved IVDMD recorded with urea or sulphur could be through the provision of extra nutrients (Kumar and Singh, 1984) which may have been limiting due to the binding of protein with CTs. Nonetheless, urea has been reported as having destabilizing effects on CTs (Russell and Lolley, 1989). Urea destabilizes the hydrogen bonds and hydrophobic interactions, which participate in the formation of the protein-tannin complex. Therefore urea may render protein free from the complex, for its further utilization. Sulphur, on the other hand, is of special significance in the digestion of low-quality roughages, since rumen anaerobic fungal colonization responds to the sulphur concentration of roughages (Akin *et al.*, 1983). A considerable portion of the S (as amino acids) in high tannin feeds is unavailable for digestion because of the complexing nature of tannins with protein (Gartner and Niven, 1978) and therefore provision of extra S will annul this problem.

**Determination of optimal level of PEG to overcome the antinutritional effects of CTs in FLs.**

Since researchers have divergent views on the quantity/level of PEG per unit of tannin to counteract phenolics-related antinutritive effects in feeding trials, it was necessary to document the appropriate levels needed for various FLs (chapter 5). The optimum levels of PEG needed to alleviate the anti-nutritive effects of CTs varied with forages. For example, with *goetzei* and *S. sesban 2024* that are high in CTs, a PEG:CTs ratio of 1:1 was found adequate, whereas *acacia* and *calliandra* required a PEG:CTs ratio of 3:1. Interestingly, *acacia* is low in CTs compared to *calliandra* indicating that CTs concentration *per se* may not be the only factor impacting on forage utilization. The biological activity (reactivity) of CTs could be a factor and seems to depend on other criteria such as chemical structure and degree of polymerization as well as CTs concentrations and these vary between plant species. It is, however, possible that the quantity and reactivity of CTs could interact to determine the degree of antinutritive effect. There is therefore, need for a better understanding of the differences between CTs of various FLs, especially their possible antinutritional effects when FLs are used as livestock feed. Nevertheless, a PEG:CT ratio of 2:1 was found optimum for the forages studied.

**Effect of supplementing high tannin basal feed with FLs varying in CTs concentrations on the performance of sheep.**

In an earlier *in vitro* experiment (chapter 4), it was shown that the use of high tannin FLs as supplement for high tannin basal substrate (BR sorghum stover) may accentuate phenolics-related anti-nutritional toxicity. Thus, it was postulated that a diet comprising high

tannin basal feed and a tannin-rich forage supplement will accentuate anti-nutritional or toxicity problems because CTs may accumulate and possibly exceed the threshold level that animals can tolerate (chapter 6). Increases in total feed intake and digestibility of total diet due to supplementation with FLs should be manifested in a significant increase in animal performance. The use of unsupplemented diets depressed the performance (intake, digestibility and growth) of the animals, while supplementation with FLs resulted in higher nutrient intake and digestibility and, consequently, liveweight gains.

The FLs with high N content elicited higher nutrient intake and digestibility because legumes high in N seemed to contain less fibre leading to an increased OM fermentation. It may also be that energy available to the microorganisms is also a factor equal in significance to the supply of degradable N. A decrease in nutrient digestibility of *goetzei* compared to other supplements, *lablab*, *tagasaste*, and *desmodium* was caused by its high content of CTs. The binding of CTs with proteins inhibits fermentation of structural carbohydrates (D'Mello, 1992) in the rumen and reduces protein availability to rumen microbes. Condensed tannins also bind with other nutrients in the rumen, which together with protein are critical in enhancing the rumen ecosystem so as to increase microbial growth, rate of fibre digestion, and escape of dietary protein. However, moderate levels of tannins have a beneficial effect. Increasing levels of CTs in the forage supplements improved intake and digestibility up to a maximum of medium-high (about 100-150 g/kg DM) concentration, beyond which performance was depressed, suggesting a threshold point beyond which response decreased. Low to moderate concentrations of CTs may have provided protection to proteins from extensive rumen degradation (Kaitho *et al.*, 1998) and as such resulted in higher weight gain.

The depressed weight changes by rams supplemented with high CTs forage (*goetzei*) suggest the anti-nutritional effect of a high content of CTs. However, rams supplemented with *S. sesban 2024* that had the next highest CTs level to *goetzei*, had a higher rate of live weight gain than *desmodium* that had lower CTs content. This observation reinforces the view that tannin (polyphenols) concentration *per se* may not be the only factor impacting on the nutritive value/utilization of tropical forages (Kaitho *et al.*, 1998) but also how different tannins from different sources react with other molecules is important (Hagerman *et al* 1992). Generally, concentrations of high tannins in forages lower voluntary feed intake, diminish the utilization of nutrients and cause toxicity and cumulatively, have a negative influence upon the animal productivity.

**Effects of ameliorants (PEG, urea or sulphur) on intake, digestibility and growth of sheep fed tanniferous diets.**

Since sorghum stover from a bird-resistant variety contains ample quantities of condensed tannins and together with forage legumes constitute the major feed resources in the semi-arid zones, it was demonstrated *in vitro* (chapter 4) and *in vivo* (chapter 6) that the use of both in diets may accentuate phenolics-related anti-nutritional toxicity problems. Unfortunately, given the low resource base of smallholders in the sub-tropics and tropics, they depend more on the leguminous crops as protein supplements. Practical strategies to overcome the antinutritive effects of polyphenolic compounds are required in order to fully utilize these feed resources (chapter 7). The intake of nutrients by sheep on the control diets appeared to be a function of their dietary levels, therefore lower nutrient intake and digestibility resulted in poor performance of the sheep. The decrease in nutrient digestibility

of diets supplemented with *goetzei* compared to *lablab, leucaena,* and *desmodium* was due to the high content of CTs. *Lablab* and *leucaena* that are low in CTs concentration provided adequate N for microbes that in turn promoted higher nutrient intake, digestibility and superior live weight gain. Therefore ameliorants would be expected to have no added advantage (Chapter 4). The losses in weight by sheep supplemented with *goetzei* suggests the anti-nutritional effect of high CTs. Urea and sulphur were effective, though not to the same degree as PEG in improving nutrient digestibility and N retention of sheep supplemented with *goetzei.* Sulphur and urea could have mediated their action through the provision of extra nutrients (in the form of N and sulphur) which improved the rumen environment for microbes to digest fibre and in turn increase intake. Even though sulphur was effective as an ameliorant in counteracting the effects of high CTs, it suppressed nutrient intake, which could have been caused by unfavourable nitrogen:sulphur ratio (N:S). The ratio of N:S was calculated to be 2:1, which is highly unfavourable because the recommended N:S ratio is between 10-14:1 (ARC, 1980). Ameliorants annulled the negative effects of the CTs, which were responsible for the lowered performance in diets supplemented with *desmodium* or *goetzei*. Ameliorants may have deactivated the particular CTs present in these forages and/or provided extra N (Russell and Lolley, 1989) and S (Gartner and Niven, 1978) and as such improved the rumen environment for efficient microbial action (Kumar and Singh, 1984). Since most toxic compounds after metabolism in the liver or other tissues are excreted as conjugates with glycine, glucuronic acid or sulphate (Lowry, 1990), S could have been useful in eliminating the toxins associated with high tannin diets and improved the efficiency. Also S is of special significance in the digestion of low-quality roughages. It has

been suggested that a considerable portion of the S (as amino acids) in high tannin feeds was unavailable for digestion because of the complexing nature of tannins and protein (Gartner and Niven, 1978) and therefore provision of extra S will annul the problem. Urea could also have provided extra N to improve both the rumen environment and N availability. On the other hand, urea is known to deactivate CTs (Russell and Lolley, 1989). Urea destabilizes the hydrogen bonds and hydrophobic interactions, which participate in the formation of the protein-tannin complex (Kumar and Singh, 1984). The improvement in the nutrient digestibility of *goetzei* with the addition of ameliorants reflects the N-sparing effect of PEG (Kumar, 1992) and/or the provision of extra N and S. Although the overall effectiveness of S and urea was lower than that of PEG, still the improvement was significant. It thus suggests that both urea and sulphur can be used to alleviate the anti-nutritional effects of high tanniferous forages.

**Nutritional evaluation of FLs or their mixtures.**

To further develop farmer-friendly practical strategies for overcoming the antinutritional effects of polyphenolics in FLs, effects of mixtures of FLs with different nutritional attributes were assessed (Chapter 8). Since FLs have different nutritional attributes, these attributes may have a synergistic effect and as such improve the utilization of forages. FLs known to have low concentration of CTs *(tagasaste* and *sesbania)* were easily degraded while those with high CTs concentrations (and which have been shown to be problematic in feeding trials) (*acacia* and *desmodium*) had depressed degradation and OM fermentation. It can therefore be safely inferred that FLs like *tagasaste* and *sesbania* may be used in diets as sole feed, while those like *acacia* may be detrimental to the animals. When

FLs were mixed, their performance as measured by both gas and ammonia production drastically increased. The main benefit was when the forages were mixed at 1:2 or 2:1 ratio. It was particularly true when either *tagasaste, sesbania* or *lablab* that are known to be highly rumen degradable (Umunna *et al.*, 1995) were used in the combinations, and as such provided enough N for the microbes and promoted higher OM digestibility. The greatest benefit was when *acacia* was mixed with these legumes at all proportions. It seems that the other FLs in the mixtures effectively diluted the antinutrients in *acacia* or provided extra N for the microbes. In this case it may be possible to reduce the antinutritive effects of some plants by mixing several plants, thus diluting the effective level of each compound (Kumar and Singh, 1984). The use of FLs high in tannins may reduce N digestibility (Reed *et al.*, 1990) because of shortage of ruminal degradable N and this insufficiency can impair the digestibility of fiber fractions (chapters 6 and 7). Most compounds have an acceptable level below which no adverse effects are apparent and this suggests that some plants can be safely fed at a certain proportion of the diet (Lowry, 1990). Thus it should be possible to select a set of species, each containing a different antinutrient factor, none of which can be fed as the sole diet but make an acceptable feed in mixture. Animals in the wild consume various types of forages to reduce the chances of poisoning. There is scope in alleviating the anti-nutritional problems of some forages which would normally pose problems if used sole by using them in mixtures, preferably at 1:2 or 2:1 ratios. There is however, a need to verify these *in vitro* results in *in vivo* feeding trials.

**Estimation of the reactivity of CTs in FLs as measured by the binding capacity of PEG**

Large differences in animal response have been reported when FLs are fed as protein supplements (Wiegand *et al.*, 1995). The causes of these differences are not very clear, but some researchers have attributed these differences to the level of phenolic compounds in FLs (Kaitho *et al.*, 1998). Some research reports (Kaitho *et al.*, 1998) however suggest that the concentration of CTs *per se* may not be the only factor impacting on the nutritive values of tropical forages. The biological activity (reactivity) of CTs which depends on the chemical structure, degree of polymerization and concentration could be a factor (Hagerman *et al.*, 1998; McNabb *et al.*, 1998). The reactivity (biological activity) of CTs were therefore evaluated by determining their ability to bind with PEG (chapter 9). The ability of PEG to bind with CTs is analogous to the ability to precipitate protein, therefore suggests that there is a considerable analogy between PEG-tannin and protein-tannin complexation (Hagerman and Butler, 1981). The reactivity of the CTs as measured by the amount of PEG reacted with CTs showed that it depended on the CTs concentration to a limited extent. This observation clearly shows that the reactivity of the plant CTs depend on other criteria such as chemical structure and may be degree of polymerization (Horigome *et al.*, 1988) as well as concentration. The different PEG-CTs reaction capacities suggest different biological responses of different CTs even at the same level in the forage samples. The CTs reactivity was significantly correlated with NDF, which is in agreement with assertion by Jackson *et al.* (1996) that other plant characteristics could mediate the same response like CTs concentration. The content and type of CTs mixtures in the diet can jointly determine the chemical reactivity of the tannins and that can also influence the direction of the effects

(Mcleod, 1974). Therefore CTs with different structural and biological characteristics may exhibit different responses which are not necessarily based on concentration. For example, there are suggestions that the reactivity of CTs depends on the molecular weight of the tannins (McNabb *et al.*, 1998) and that reactivity relating to protein precipitation decreases as the molecular weight exceeds 20,000 g per mol. Therefore it may be safe to assume that the reactivity of CTs will be influenced by CTs concentration, molecular weight and other chemical attributes. Since the binding of tannins with PEG is analogous to the binding of tannins with proteins, it is expected that the molecular weight of CTs in the forages under study will influence the reactivity with PEG. Condensed tannin reactivity also depends on the degree of polymerization, as the degree of polymerization increases, fewer active sites are available for interaction with proteins, and the molecule becomes less open and flexible (Hagerman and Butler, 1991). Because the relationship between the PEG-reaction method as a measure of tannin reactivity with the radial diffusion assay (RDA) which measures the biological activity of tannins showed a high correlation ($r = 0.87$, $P < 0.01$), it suggests that PEG-reaction method may be an effective method of measuring the reactivity (biological activity) of the CTs present in forages. The poor relationship between gas production and PEG-reaction, RDA and DM degradability suggests that gas production alone may not be adequate to predict the antinutritive effects of CTs in forages. This assertion is in line with observations made in the earlier experiments (chapter 4 and chapter 5), where it was found that the increase in gas production in PEG treated samples was not related to the concentration of CTs.

# 11 GENERAL CONCLUSIONS

It was concluded that timely harvesting of sorghum and maize and proper storage (whole crop cut with heads and cobs removed) either in the open like the farmers usually do or under shade, can provide stover of considerable nutritive value. Therefore, if farmers can harvest the stover immediately after grain harvest at dead ripe stage and store it in stacks in the open or under shade, higher nutritive quality stover can be achieved. The use of FLs increased the IVDMD of high tannin basal substrates and ameliorants such as PEG, urea and S further improved the increased IVDMD. It is therefore possible to use urea or sulphur as ameliorants to high tannin feeds. It is, however, recognised that the lack of response by some FLs to the ameliorants may indicate the fundamental differences in the chemical constituents of the forages. The optimal levels of PEG required to alleviate the antinutritive effects of CTs varied with forages and did not relate with CTs concentrations. The findings reinforce the idea that the biological activity of tannins may be more important than the concentration. Nevertheless, a PEG:CTs ratio of 2:1 was found optimum for the forages studied. Supplementation with FLs improved the utilization of the sorghum stover and overall performance of animals. However, those with high concentrations of CTs, when used as supplements to BR sorghum stover, depressed animal performance (feed intake, growth and feed efficiency). The response both assumed a linear and quadratic pattern suggesting a threshold point beyond which response decreased. The results seem to reinforce the view that tannin (polyphenols) concentration *per se* may not be the only factor impacting on the nutritive value/utilization of forages. How different tannins from different sources react with other molecules is also important. Supplementing BR sorghum stover with FLs improved

sheep performance. However FLs with high contents of CTs depressed sheep performance. PEG, urea or S proved effective ameliorants in alleviating phenolics-related antinutritive effects. The provision of urea and S as ameliorants on high tannin diets offers a practical opportunity to smallholder farmers to overcome the anti-nutritional problems in these feed resources. The use of PEG in routine feeding may not be economical as urea and S that are available in the farming systems in which case urea should be the first choice given that it was more effective than S. Mixing FLs with different nutritional attributes at 1:2 or 2:1 ratio drastically improved their performance as measured by both gas and ammonia production. Therefore potential of improving the utilization of some legumes, which will normally pose problems as sole legumes by mixing them with other forages was demonstrated. More work is, however required, especially detailed animal feeding studies to ascertain this preliminary observation. A simple and more practical method of estimating the CTs reactivity of forages using PEG has been described. The method was highly correlated to CTs concentration and RDA, which measures the biological activity of the tannins. This suggests that this method can be effectively used to predict the possible antinutritional effects of forages.

## 12. FUTURE RESEARCH

In order to further understand the factors that affect the quality of basal diet, it is important to further study the storage methods effects. In this experiment, the two storage methods where the whole crop was cut and followed by removal of heads tended to have higher tannins than the other two where the heads were not removed. Can this mean that the grain provided sink for tannins during the drying period? It would be interesting to analyze the tannins content of the grains from the different storage methods, to see if there are any

differences in the tannin content due to storage methods. If this were found true, it would offer another strategy for better stover management.

The potential of improving the utilization of some legumes, which will normally pose problems as sole fodder, was demonstrated. This potential was however demonstrated through *in vitro* experiments; more detailed animal feeding studies to ascertain this preliminary observation is required.

The results from the animal experiments suggest that the accumulation of tannins from the diets (basal diet and forage legumes) was detrimental to animal performance. The potential of alleviating the antinutritive effects of these forages using PEG, urea or sulphur was demonstrated. However, the general performance was lower than expected. Nutrient imbalances in these feeds could be responsible. Can there be any benefits if additional supplements are provided, for example high energy concentrates. This could be an area to be further explored.

## 12.0 REFERENCE:


Abule, E. 1994. Performance of crossbred calves fed teff (*Eragrostis tef*) straw supplemented with graded levels of cowpea (*Vigna unguiculata*) and dolichos lablab (*Lablab purpureus*) hay. M. Sc. Thesis. Alemaya University of Agriculture, Ethiopia, 141 p.

Abule, E., Umunna, N. N., Nsahlai, I. V. Osuji, P. O. and Alemu, Y. 1995. The effect of supplementing teff (*Eragrostis tef*) straw with graded levels of cowpea (*Vigna unguiculata*) and dolichos lablab (*Lablab purpureus*) hays on degradation, rumen particulate passage and intake by crossbred (Friesian x Boran (Zebu)) calves. Livestock Production Science, 44: 221-228.

Aboud, A. A. O. 1991. Strategies for utilization of sorghum stover as feed for cattle, sheep and goats. PhD Thesis, University of Reading, UK.

Aboud, A. A. O., Owen, E., Reed, J. D., Said, A. N. and McAllan, A. B. 1991. Feeding sorghum stover to Ethiopian goats and sheep: Effect of amount offered and growth, intake and selection. Summary. Winter meeting of British Society of Animal Production, 18-20 March. 1991.

Ademosum, A. A., Bosman, H. G. and Jansen, H. J. 1988. Nutritional studies with West African dwarf goats in the humid tropics. In: Goat production in the Humid Tropics. Smith, O. B. and Bosman, H.G. (eds.). Wageningen. Netherlands: Pudoc. pp. 51-61.

Adu, I. F., Fajemisin, B. A. and Adamu, A, M. 1992. The utilization of sorghum stover fed to sheep as influenced by urea or graded levels of lablab supplementation. In: Rey, B., Lebbie, S. H. and Reynolds, L. (eds.). Small Ruminant Research and Development in Africa. International Livestock Centre for Africa, Addis Ababa, Ethiopia. pp 367-374.

Aganga, A. A., Tsopito, C.M. and Mwandamena, M. 1996. Growth and nutritive value of some varieties of sorghum and millet as forage crops in Botswana. Tropical Science, 36: 86-91

Agricultural Research Council. 1980. The Nutrient Requirements of Ruminant Livestock. Slough: Commonwealth Agricultural Bureau.

Ahn, J. H.., Robertson, B. M., Elliott, R., Gutteridge, R. C. and Ford, C. W. 1989. Quality assessment of tropical browse legumes: tannin content and protein degradation. Animal Feed Science and Technology, 27: 147-56.

Ahn, J. H.., Elliott, R. and Norton, B. W. 1997. Oven drying improves the nutritional value of *Calliandra calothyrsus* and *Gliricidia sepium* as supplements for sheep given low-quality straw. Journal of the Science of Food and Agriculture, 75: 503-510.

Akin, D. E, Gordon, G. L. R. and Hogan, J. P. 1983. Rumen bacterial and fungal degradation of *Digitiria pentzii* grown with or without sulphur. Applied and Environmental Microbiology, 46: 738-748.

Akin, D. E. 1980. Evaluation by electron microscopy and anaerobic culture of type of rumen bacteria associated with digestion of forage cell walls. Applied and Environmental Microbiology, 39: 242-252.

Akin, D. E. 1982. Forage cell wall degradation and p-coumaric, ferulic and cinnamic acids. Agronomy Journal, 74: 424-428.

Akin, D. E. 1988. Biological structure of lignocellulose and its degradation in the rumen. Animal Feed Science and Technology, 21: 295-310.

Alhassan, W. S. 1985. Senescent forages. general overview. National Animal Production Research Institute (NAPRI), Bulletin No. 11: 31-35.

Alhassan, W. S., Ehoche, W. O. and Adu, I. F. 1986. Influence of graded levels of cotton seed cake supplementation on nutritive value of cereal straws fed to sheep and goats. Journal of Animal Production Research, 6 (1): 39 –53.

Alhassan, W. S., Kallah, M. S. and Bello, S. A. 1987. Influence of duration of stay on the field on the chemical composition and nutritive value of crop residues. Tropical Agriculture (Trinidad), 64 (1): 61-64.

Alimon, A. R. 1989. An examination of strategies to maximise dietary selection for improving the intake and utilization of barley straw by goats. Ph. D thesis, University of Reading.

Anderson, D. C. 1978. Use of cereal residue in beef cattle production systems. Journal of Agricultural Science (Cambridge), 46: 849-861.

Ash, A. J. 1990. The effect of supplementation with leaves from the leguminous trees *Sesbania grandiflora, Albizia chinensis* and *Gliricidia* on the intake and digestibility of Guinea grass hay by goats. Animal Feed Science and Technology, 28: 225-232.

Association of Official Analytical Chemists (AOAC) 1980. Official Methods of Analysis. 14th Edition. AOAC. Washington, D.C.

Atta-Krah, A. N. and Okali, D. U. U. 1986. Woody plants environmental stability and agricultural productivity in tropical Africa. Paper presented at the advisory Group Meeting on use of Nuclear Techniques in studying the roles of trees in restoring and maintaining soil fertility. International Atomic Energy Agency, Vienna, Austria.

Badve, V. C., Nisal, P. R., Joshi, A. L. and Rangnekar, D. V. 1994. Genotype and environmental effects on sorghum stover production and quality. In: Joshi, A. L., Doyle, P. T. and Oosting, S. J. (eds.) Variation in the quantity and quality of Fibrous crop residues. Indian Council of Agricultural Research, New Dehli and Agricultural University, Wageningen. pp 9-19.

Barry, T. N. 1985. The role of condensed tannins in the nutritional value of *Lotus pedunculatus* for sheep. 3. Rates of body and wool growth. British Journal of Nutrition, 54: 211-217.

Barry, T. N. 1989. Condensed tannins: their role in ruminant protein and carbohydrate digestion and possible effects upon the rumen ecosystem. In: Nolan, J. V., Leng, R. A. and Demeyer, D. I. (eds.). The roles of Protozoa and Fungi in Ruminant Digestion. Penambul Books, Armidale, Australia. pp. 153-169.

Barry, T. N. and Duncan, S. J. 1984. The role of condensed tannins in the nutritional value of *Lotus pedunculatus* for sheep 1. Voluntary intake. British Journal of Nutrition, 54:485-491.

Barry, T. N. and Manley, T. R. 1984. The role of condensed tannins in the nutritional value of *Lotus pedunculatus* for sheep. 2. Quantitative digestion of carbohydrates and proteins. British Journal of Nutrition, 51:493-504.

Barry, T. N., Manley, T. R. and Duncan, S. J. 1986. The role of condensed tannins in the nutritional value of *Lotus pedunculatus* for sheep. 4. Sites of carbohydrate and protein digestion as influenced by dietary reactive tannin concentration. British Journal of Nutrition, 55: 123-137.

Bate-Smith, E. C. 1973. Haemanolysis of tannins: The concept of relative astringency. Phytochemistry, 12:907-912.

Baumer, M. 1992. Trees as browse and to support animal production. In: Speedy, A. and Pugliese, P. L. (eds.). Legume trees and other fodder trees as protein sources for livestock. Proceedings of the FAO Expert Consultation held at the Malaysian Agricultural Research and Development Institute (MARDI) in Kuala Lumpur, Malaysia 14-18 October 1991 pp 1-10.

Beever, D. E., Thompson, D. J. and Cammell, S. B. 1976. The digestion of frozen and dried grass by sheep. Journal of Agricultural Science (Cambridge), 86: 443-452.

Beever, D. E. 1996. Meeting the protein requirement of ruminant livestock. South African Journal of Animal Science, 26: 20-27.

Ben-Ghedalia, D. and Miron, J. 1981. Effect of sodium hydroxide, ozone and sulphur dioxide on the composition and in vitro digestibility of wheat straw. Journal of the Science of Food and Agriculture, 32: 224-228.

Bindon, B. M. and Lambond, D. R. 1966. Examination of tropical legumes for deleterious effects of animal reproduction. Proceedings of the Australian Society of Animal Production, 6:109-116.

Blytt, H.J., Guscar, T. K and Butler, L. G. 1988. Antinutritional effects and ecological significance of dietary condensed tannins may not be due to binding and inhibiting digestive enzymes. Journal of Chemical ecology, 14: 1455-1465.

Boitumelo, W. S. and Mahabile, W. 1992. Improving milk production in Small-scale farms in Botswana: Incorporating legume fodder in the farming systems. In: Stares, J. E., Said, A. N. and Kategile, J. A. (eds.). Complimentarity of feed resources for animal production in Africa. African Feeds Research Network, ILCA, Addis Ababa, Ethiopia. pp 353-362.

Bonsi, M. L. K., Osuji, P. O. and Tuah, A. K. 1995. Effects of supplementing teff straw with different levels of *leucaena* or *sesbania* leaves on the degradabilities of teff straw, *sesbania, leucaena, tagasaste* and *vernonia* and on certain rumen and blood metabolites in Ethiopian Menz sheep. Animal Feed Science and Technology, 52:101-129.

Bonsi, M. L. K., Osuji, P. O., Nsahlai, I. V. and Tuah, A. K. 1994. Graded levels of *Sesbania sesban* and *Leucaena leucocephala* as supplements to teff straw fed to Ethiopian Menz sheep. Animal Production, 59: 235-244.

Borens, F. M. B., and Poppi, D. P. 1990. The nutritive value for ruminants of tagasaste (*Chamaecytisus palmensis*), a leguminous tree. Animal Feed Science and Technology, 28:275-292.

Brewbaker, J. L. 1986. Leguminous trees and shrubs for Southeast and South Pacific. In "Forages in Southeast Asian and South Pacific Agriculture". Blair, G. J., Ivory, D. A. and Evans, T. R. (eds.). ACIAR Proceedings NO. 12. ACIAR Canberra. pp. 43-50.

Brumby, P. J. 1987. Livestock: A sector policy paper. World Bank, Washington, D. C. U.S.A.

Bullard, R. W. and Elias, D. J. 1980. Sorghum polyphenolics and bird resistance. In: J. H. Hulse (ed.), Polyphenols in cereals and legumes. International Development Research Centre, Ottawa, Canada.

Butler, G. W. and Bailey, R. W. 1973. Chemistry and biochemistry of herbage, pp. 294-296. Academic press, London.

Butler, L. G. 1982. Polyphenolics and their effects on sorghum quality. Proceedings of the International Symposium on sorghum grain quality. International Crops Research Institute for the Semi-Arid tropics, Patancheru, India. pp. 294-311.

Butterworth, M. H. and Mosi, A. 1986. The intake and digestibility by sheep of oat straw and maize stover offered with different levels of noug (*Guizota abyssinica*) meal. Animal Feed Science and Technology, 16:99-107.

Buxton, D. R. 1996. Quality-related characteristic of forages as influenced by plant environment and agronomic factors. Animal Feed Science and Technology, 59: 37-49.

Capper, B. S., Thomson, E. F. and Herbert, F. 1988. Genetic variation in feeding value of crop residues. In: Reed, J. D., Capper, B. S and Neate, P. J. H. (Eds). Plant breeding and nutritive value of crop residues. Proceedings of a workshop held at ILCA Addis Ababa, Ethiopia, 7-10 December 1987. ILCA. Addis Ababa. pp. 177-194.

Chabala, C. 1984. Aspects of the draught power constraint on maize production in Recommendation Domain No. 5, Kabwe Rural District, Zambia. In: Draught power and animal feeding in Eastern and Southern Africa. Report of a network workshop in Ezulwini, Swaziland, 4-6 October, 1983. CIMMYT (Centro Internacional de Mejoramiento de Maiz y Trigo) Eastern and Southern Africa Economics Programme, Mbabane, Swaziland. pp. 21-23.

Chadhokar, P. A. 1982. *Gliciridia maculata*: a promising legume fodder plant. World Animal Review, 44: 36-43.

Chandler, D. R. 1984. The grazing animal and land pressures in small farm systems: Must ruminant nutrition create inevitably a confrontation between arable lands and grazing requirements? In: Draught power and animal feeding in Eastern and Southern Africa. Report of a network workshop in Ezulwini, Swaziland, 4-6 October, 1983. CIMMYT (Centro Internacional de Mejoramiento de Maiz y Trigo) Eastern and Southern Africa Economics Programme, Mbabane, Swaziland. pp. 56-57.

Chaney, A. L. and Marback, E. P. 1962. Modified reagents for determination of urea and ammonia. Clinical Chemistry, 8: 130-132.

Cheeke, P. R. and Hull, I. R. 1985. Natural Toxicants in Feeds and Poisonous plants. Westport, CT, USA; AVI Publishing Co. Inc.

Cheeke, P. R. and Palo, R. T. 1995. Plant toxins and mammalian herbivores: co-evolutionary relationships and antinutritional effects. In: Journet, M., Grenet, E., Farce, M-H., Theriez, M. and Demarquilly, C. (eds.). Recent developments in the Nutrition of Herbivores. Proceedings of the IVth International Symposium on the Nutrition of Herbivores, INRA Editions, Paris. Pp 437-456.

Cherney, D. J. R., Cherney, J. H., Patterson, J. A. and Axtell, J. D. 1992. *In vitro* ruminal fiber digestion as influenced by phenolic-carbohydrate complexes released from sorghum cell walls. Animal Feed Science and Technology, 39: 79-93.

Chesson, A., Stewart, C. S. and Wallace, R. J. 1982. Influence of plant phenolic acids in growth and cellulolytic activity of rumen bacteria. Applied and Environmental Microbiology, 44: 597-603.

Chesson, A. 1988. Lignin-polysaccharide complexes of plant cell wall and their effect on microbial degradation in the rumen. Animal Feed Science and Technology, 21: 219-228.

Chesworth, J. M., McKillop, D. F. and Spriggs, D. J. 1989. Combinations of agro-industrial by-products for use in dairy diets formulations on-farm use of least cost ration system. In: Said, A. N. and Dzowela, B. H. (eds.). Overcoming constraints to the efficient utilization of agricultural by-products as animal feed. pp. 61-79.

Clausen, T. P., Provenza, F. D., Burritt, E. A., Reichardt, P. B. and Bryant, J. P. 1990. Ecological implication of condensed tannin structure: A case study. Journal Chemical Ecology, 16: 2381-2391.

Dann, P. and Trimmer, B. 1986. *Tagasaste*: a tree legume for fodder and other uses. New Zealand Journal of Agricultural Science, 20: 142-145.

Davies, J. C. 1982. Pest losses and control of damage on sorghum in developing countries – The realities and the myths. In: House, L. R., Mughogho, L. K. and Peacock, J. M. (eds.). Sorghum in the Eighties. pp 215-224.

Devendra, C. 1983. Physical treatment of rice straws for goats and sheep, and response to substitution with variable levels of cassava (*Manihot esculenta* Cranz), Leucaena (*leucaena leucocephala*) and Gliricidia (*Gliricidia maculata*) forages. MARDI Research Bulletin II:272-290.

Devendra, C. 1993. Trees and shrubs as sustainable feed resources. Proceedings VII World Conference on Animal Production, Edmonton, Canada, Vol. 1, pp. 119-138.

Dicko, M. S. and Sikena, L. K. 1992. Feeding behavior, quantitative and qualitative intake of browse by domestic ruminants. In: Speedy, A. and Pugliese, P. (eds.). Legume trees and other fodder trees as protein sources for livestock. Food and Agricultural Organization Animal production and health paper No. 102. Rome.

D'Mello, J. F. P. 1992. Chemical constraints to the use of tropical legumes in animal nutrition. Animal Feed Science Technology, 38:237-261.

D'Mello, J. P. F. 1982. Toxic factors in some tropical legumes. World Review of Animal Production. Vol. XVII, (No.4): 41-46.

Doggett, H. and Jowett, D. 1966. Yields of maize, sorghum varieties and sorghum hybrids in East African lowlands. Journal of Agricultural Science (Cambridge), 67 (1) : 31 – 39.

Doggett, H. 1988. Sorghum. London and Harlow, UK; Longmans Green Company Limited. 403 pp.

Ebong, C. 1989. The nutritional effects of tannins and related polyphenols in bird resistant and non-bird resistant sorghum varieties and in legume browses. Ph.D. thesis, University of Aberdeen.

Egan, A. R. and Ulyatt, M. J. 1980. Quantitative digestion of fresh herbage by sheep. VI. Utilization of nitrogen in five herbages. Journal of Agricultural Science (Cambridge), 94: 47-56.

El Hassan, S. M. 1994. Yeast culture and multipurpose fodder trees as feed supplements for ruminants. Ph.D. Thesis, University of Aberdeen.

Erickson, D. O., Meyer, D. W. and Foster, A. E. 1982. The effect of genotype on the feed value of barley straw. Journal of Animal Science, 55: 1015-1026.

F. A. O. Production Yearbook – 1981. Food and Agriculture Organization of the United Nations, Vol. 42, Rome.

F. A. O. Production Yearbook. 1991. Food and Agriculture Organization of the United Nations, Vol. 44, Rome.

Fan, M. Z., Adeola, O. and Asem, E. K. 1997. Effects of tannin on porcine jejunal brush border membrane-bound enzyme activity. Journal of Animal Science (Suppl.) 75: 199.

Farrington, I and Mayer, L. 1987. Technical and economic implications of feeding sorghum stover with or without maize and sorghum bran supplements to beef cattle in Botswana. In ARNAB Newsletter, Quarterly Publication of African Research Network for Agricultural by products ILCA. Vol. 7 No.2

Feeny, P. 1976. Plant apparency and chemical defense. In: Wallace, J. W. and Mansell, R. L (eds.). Biochemical interactions between plants and insects. Recent Advances in Phytochemistry. Volume 10. Plenum, New York, USA.

Feng Yu and Leng, R. A. 1991. Effect on wool growth of supplementation of roughage fed sheep with condensed tannin treated lupins. In: Romney, D. L., Orskov, E. R. and Gill, M. (eds.). Utilization of straw in ruminant production systems. Proceedings of a workshop held at the Malaysian Agricultural research and development Institute, 7-11th, October. NRI, ODA, UK

Field, J. A and Lettinga, J. 1992. Biodegradation of tannins. In: Sigel, H. and Sigel, A. (eds.). Degradation of environmental pollution by microorganisms and their metalloenzymes. Metal ions in biological systems, 28: 61-97. Marcel Dekker, New York, USA.

Garrido, A., Gomez-Cabrera, A., Guerrero, J. E. and Van der Meer, J. M. 1991. Effects of treatment with polyvinylpyrrolidone and polyethylene glycol on faba bean tannins. Animal Feed Science and Technology, 35: 199-203.

Gartner, R. J. W. and Hurwood, I. S. 1976. The tannin and oxalic acid content of *Acacia aneura* (mulga) and their possible effects of S and Ca availability. Australian Veterinary Journal, 52: 194-196.

Gartner, R. J. W. and Niven, D. R. 1978. Studies on the supplementary feeding of sheep consuming mulga (*Acacia aneura*). 4. Effect of sulphur on intake and digestibility and growth and sulphur content of wool. Australian Journal of Experimental Agriculture and Animal Husbandry, 18: 768-772.

Getachew, G., Said, A.N. and Sundstol, F. 1994. The effect of forage legume supplementation on digestibility and body weight gain by sheep fed a basal diet of maize stover. Animal Feed Science and Technology, 46: 97-108.

Gibb, M. J. and Treacher, T. T. 1976. The effect of herbage allowance on herbage intake and performance of lambs grazing perennial ryegrass and red clover swards. Journal of Agricultural Science (Cambridge), 86: 355-365.

Giner-Chavez, B. I., Van Soest, P. J., Robertson, J. B., Lascano, C., Reed, J. D. and Pell, A. N. 1997. A method for isolating condensed tannins from crude plant extracts with trivalent ytterbium. Journal of the Science of Food and Agriculture, 74: 359-368.

Goering H. K and Van Soest, P. J. 1970. Forage fibre analyses (apparatus, reagents, procedures and some applications). Agriculture Handbook No. 379. United States Department of Agriculture, Washington, D. C., USA. 20 pp.

Goodchild, A. V. and McMeniman, N. P. 1994. Intake and digestibility of low quality roughages when supplemented with leguminous browse. Journal of Agricultural Science (Cambridge), 122: 151-160.

Grovum, W. L. 1987. A new look at what is controlling food intake. Feed intake by beef cattle. In: Symposium proceedings: feed intake by beef cattle. November 20-22, 1986. pp.1-40.

Gupta, R. K and Haslam, E. 1980. Vegetable tannins - their structure and biosynthesis. In: J. H. Hulse (ed.), Polyphenols in cereals and legumes. Proceedings of a symposium held during the 36th annual meeting of the Institute of Food Technologists, St Louis, Missouri, 10-13 June 1979. International Development Research Centre, Ottawa, Canada. 72 pp.

Hagerman, A. E and Butler, L. G. 1981. The specificity of proanthocyanidin-protein interactions. Journal of Biological Chemistry, 256: 4494-4497.

Hagerman, A. E and Butler, L. G. 1978. Protein precipitation method for the quantitative determination of tannins. Journal of Agricultural Food Chemistry, 26: 809-812.

Hagerman, A. E. and Butler, L. G. 1980. Determination of protein in tannin-protein precipitates. Journal of Agriculture and Food Chemistry, 28: 944-947.

Hagerman, A. E., 1987. Radial diffusion method for determining tannin in plant extract. Journal of Chemical Ecology, 13:437-449.

Hagerman, A. E., Robbins, C. T., Weerasuriya, Y., Wilson, T. C. and McArthur, C. 1992. Tannin chemistry in relation to digestion. Journal of Range Management, 45: 57-62.

Hagerman, A. E., Rice, M. E. and Richard, N. T. 1998. Mechanisms of protein precipitation for two tannins, pentagalloyl glucose and epicatechin$_{16}$ (4®8) catechin (procyanidin). Journal of Agriculture and food chemistry, 46: 2590-2595.

Hamilton, R. T., Donaldson, L. E. and Lam-Bourne, L. J. 1971. *Leucaena leucocephala* as a feed for dairy cows: direct effect on reproduction and residual effect on the calf and lactation. Australian Journal of Agricultural Research, 22:681.

Harika, A. S. and Sharma, D. D. 1994. Quality and yield differences in maize stover due to varieties and stage of harvesting. In: Joshi, A. L., Doyle, P. T. and Oosting, S. J. (Eds.) Variation in the quantity and quality of fibrous crop residues. Indian Council of Agricultural Research, New Dehli and Agricultural University, Wageningen. pp 20-28.

Harris, H. B. and Burns, R. E. 1970. Influences of tannin content on pre-harvest seed germination in sorghum. Agronomy Journal, 62: 835-836.

Haslam, E. 1982. Proanthocyanidins. In: J. B. Harborne and T. J. Mabrey (eds.), The flavonoids: Advances in research. Chapman and Hall, London, UK.

Haslam, E. 1989. Plant Polyphenols. Cambridge University Press, Cambridge, UK.

Horigome, T., Kumar, R. and Okamoto, K. 1988. Effects of condensed tannins prepared from leaves of fodder plants on digestive enzymes *in vitro* and in the intestine of rats. British Journal of Nutrition, 60:275-285.

Horigome, T., Okhuma, T and Muta, M. 1984. Effect of condensed tannins of false acacia leaves on protein digestibility as measured with rats. Japanese Journal of Zootechnical Science, 55: 209-306.

Hulse, J. H., Laing, E. M and Pearson, O. E. 1980. Sorghum and Millets: Their composition and nutritive value. Academic Press, London, UK.

Hunter, R. A. 1988. Some aspects of the role of concentrates in increasing feed intake and productivity of cattle fed fibrous diets. In: Dixon, R. M. (ed.). Ruminant feeding systems utilizing fibrous agricultural residues. Australian International Development Programme, Canberra, Australia. pp. 37-48.

Ivory, D. A. 1990. Major characteristics, agronomic features, and nutritional value of shrubs and tree fodders. In: Devendra, C. (ed.). Shrubs and tree fodders for farm animals, Ottawa, Ontario, Canada: IDRC. pp. 22-28.

Jackson, M. G. 1977. Review article: The alkali treatment of straw. Animal Feed Science and Technology, 2: 105-130.

Jackson, F. S., Barry, T. N., Lascano, C. and Palmer, B. 1996. The extractable and bound condensed tannin content of leaves from tropical tree, shrub and forage legumes. Journal of the Science of Food and Agriculture, 71:103-110.

Jones, D. E. 1965. Banana tannins and its reaction with polyethylene glycol. Nature (London), 206: 299-300.

Jones, R. J. 1979. The value of Leucaena leucocephala as a feed for ruminants in the tropics. World Animal Review, 31: 1-11.

Jones, R. J. and Megarrity, R. G. 1986. Successful transfer of DHP-degrading bacteria from Hawaiian goats to Australian ruminants to overcome the toxicity of Leucaena. Australian Veterinary Journal, 63: 259-262.

Jones, W.T., Broadhurst, R. B. and Lyttleton, J. W. 1976. The condensed tannins of pasture legume species. Phytochemistry, 15: 1407-1409.

Jones, R. J., McMeniman, M. W. and Dowsett, K. F. 1989. The effect of *Leucaena leucocephala* on the reproduction of beef cattle grazing *leucaena*/grass pastures. Tropical Grasslands, 23:108-114.

Jones, W. T. and Mangan, J. L. 1977. Complexes of the condensed tannins of sainfoin (*Onobrychis viciifalia Scop.*) with fraction 1 leaf protein and with submaxillary mucoprotein and their reversal by PEG and pH. Journal of the Science of Food and Agriculture, 28: 126-136.

Jordan, W. R. and Sullivan, C. Y. 1981. Reaction and resistance of grain sorghum to heat and drought. In: House, L. R., Mughogho, L. K. and Peacock, J. M. (eds.). Proceeding of International Symposium on sorghum. ICRISAT. Patancheru, Animal Production, India; 2: 131-142.

Kaitho, R. J., Tamminga, S. and van Bruchem, J. 1993. Chemical composition and nylon bag degradation characteristics of dried *Calliandra calothyrsus* leaves. Animal Feed Science and Technology, 43: 23-45.

Kaitho, R. J., Umunna, N. N., Nsahlai, I. V., Tamminga, S., Van Bruchem, J., Hanson, J. and Van Der Wouw, M. 1996. Palatability of 40 multipurpose tree species to sheep. Agroforestry Systems, 33: 249-261.

Kaitho, R. J., Umunna, N. N., Nsahlai, I. V., Tamminga, S. and van Bruchem, J. 1998. Utilization of browse supplements with varying tannin levels by Ethiopian Menz sheep: 1. intake, digestibility and live weight changes. Agroforestry Systems, 39: 145-159.

Kalyankar, S. P., Yaqub, S., Choudhari, S. D. and Rajmane, K. D. 1989. Relationship of rainfall and Khafir sorghum grain yield in scarcity and assured rainfall zones. Journal of Maharashtra Agricultural Universities, 14 (1): 60-62.

Kearl, L. C. 1982. Nutrient Requirements of Ruminants in Developing Countries. International Feedstuffs Institute, Utah Agricultural Experiment Station, Utah State University, Logan, Utah. USA.

Kebede, Y., Mulatu, T. and Degu, E. 1990. Assessing the performance of sorghum genotypes under various abiotic stresses. In: Proceeding of Eastern African Regional Sorghum and Millet Regional workshop on sorghum and millet improvement in Eastern Africa held in Nairobi, Kenya. pp. 25-28.

Kempton, T. J. and Leng, R. A. 1979. Protein nutrition of growing lambs. 1. Responses in growth and rumen function to supplementation of a low-protein-cellulosic diet with either urea, casein or formaldehyde-treated casein. British Journal of Nutrition, 42: 289-302.

Kennedy, P. M and Milligan, L. P. 1980. The degradation and utilization of endogenous urea in the gastrointestinal tract of ruminants: A review. Canadian Journal of Animal Science, 60:

Khazaal, K., Mueller-Harvey, I., McAllan, A. B., Osafo, E., Owen, E. and Said, A. N. 1993. Effect of harvesting at different stages of growth and long-term storage on phenolics in sorghum stover. In: Gill, M., Owen, E., Pollot, G. E. and Lawrence, T. L. J., (eds.). Animal Production in Developing Countries. Occasional Publication No. 16, British Society of Animal Production, pp. 210-211.

Khalili, H. and Varvikko, T., 1992. Effect of replacement of concentrate mix by wilted Sesbania (*Sesbania sesban)* forage on diet digestibility, rumen fermentation and milk production in Friesian x Zebu (Boran) crossbred cows fed low quality nature hay. Animal Feed Science and Technology, 36:275-286.

Khalili, H., Osuji, P. O., Umunna, N. N and Crosse, S. 1994. The effect of forage type (maize-lablab or oat-vetch) and level of supplementation (wheat-middlings) on food intake, diet apparent digestibility, purine excretion and milk production of crossbred (Bos taurus x Bos indicus) cows. Animal Production, 58: 321-328.

Khazaal, K. A., Dentinho, M. T., Ribeiro, R. C. and Orskov, E. R. 1993. A comparison of gas production during incubation with rumen contents *in vitro* and nylon bag degradability as predictors of apparent digestibility *in vivo* and voluntary intake of hays. Animal Production, 57: 105-112.

Khazaal, K., Mueller-Harvey, I., McAllan, A. B., Osafo, E., Owen, E. and Said, A. N. 1993. Effect of harvesting at different stages of growth and long-term storage on phenolics in sorghum stover. In: Gill, M., Owen, E., Pollot, G. E. and Lawrence, T. L. J., (eds.). Animal Production in Developing Countries. Occasional Publication No. 16, British Society of Animal Production. pp. 210-211.

Khazaal, K., Boza, J. and Orskov, E. R. 1994. Assessment of phenolics-related antinutritive effects in Mediterranean browse: a comparison between the use of the *in vitro* gas production technique with or without insoluble polyvinylpolypyrrolidone or nylon bag. Animal Feed Science and Technology, 49: 133-149.

Khazaal, K. A., Parissi, Z., Tsiouvaras, C., Nastis, C. and Orskov, E. R. 1996. Assessment of phenolics-related antinutritive levels using the *in vitro* gas production technique: a comparison between different types of polyvinylpyrrolidone or polyethylene glycol. Journal of the Science of Food and Agriculture, 71:405-414.

Kiflewahid, B and Mosimanyana, B. M. 1989. Dolichos lablab (*Lablab purpureus*.) in by-product-based diets for lactating cows in Botswana. In: Said, A. N. and Dzowela, B. H. (eds.). Overcoming constraints to the efficient utilization of agricultural by-products as animal feed. International Livestock Centre for Africa. Addis Ababa, Ethiopia. pp 155-172.

Kossila, V. 1988. The availability of crop residues in developing countries in relation to livestock populations. In: Reed, J. D., Capper, B. S and Neate, P. J. H. (eds.). Plant breeding and nutritive value of crop residues. Proceedings of a workshop held at ILCA Addis Ababa, Ethiopia, 7-10 December 1987. ILCA. Addis Ababa. pp. 29-39.

Kossila, V. L. 1985. Global review of the potential use of crop residues as animal feed. Production and Health paper, No. 50. FAO, Rome.

Krebs, G. and Leng, R. A. 1984. The effect of supplementation with molasses/urea block on ruminal digestion. Proceedings of the Australian Society of Animal Production, 15:704

Kumar, R. 1992. Anti-nutritional factors. The potential risks of toxicity and methods to alleviate them. In: Speedy, A and Pugliese, P (eds.). Legume trees and other fodder trees as protein sources for livestock. Proceedings of the FAO Expert consultation. FAO Animal Production Health paper No. 102. pp. 145-160.

Kumar, R. and Singh, M. 1984. Tannins: their adverse role in ruminant nutrition. Journal of Agricultural and Food Chemistry, 32: 447-453.

Kumar, R. and Vaithiyanathan, S. 1990. Occurrence, nutritional significance and effect on animal productivity of tannins in tree leaves. Animal Feed Science and Technology, 30, 21-38.

Kumar, R. and D'Mello, J. P. F. 1995. Anti-nutritional factors in forage legumes. In: D'Mello, J. P. F. and Devendra, C. (eds.). Tropical legumes in animal nutrition. CAB International, Wallingford. pp. 95-133.

Labuda, A., Helubec, V. and Sadecky, J. 1979. Effect of pelleting feeds on digestibility of nutrients, feeding value and live weight gains in bullocks. Nutrition Abstracts Reviews, 42: 2591.

Lambert, M. G., Jung, G. A., Fletcher, R. H., Budding, P. J. and Costall, D. A. 1989. Forage shrubs in north island country 2. Sheep and goat preferences. New Zealand Journal of Agricultural Research, 32: 485-490.

Langlands, J. P., Sutherland, H. A. M. and Payne, M. J. 1973. Sulphur as a nutrient for merino sheep. 2. The utilization of sulphur in forage diets. British Journal of Nutrition, 30: 537-543.

Le Houerou, H. N. 1980. Browse in northern Africa: In: H. N. Le Houerou (ed.), Browse in Africa: The current state of knowledge papers presented at the International Symposium on Browse in Africa, Addis Ababa, 8-12 April 1980. ILCA (International Livestock Center for Africa), Addis Ababa, Ethiopia, pp.55-82.

Leng, R. A. 1990. Factors affecting the utilization of poor quality forages by ruminants particularly under tropical conditions. Nutrition Research Review, 3: 277-303.

Lowry, J. B. 1983. Detoxification of *leucaena* by enzymatic or microbial processes. In: *Leucaena* research in the Asian-Pacific region. Proceedings of a workshop held in Singapore, 23-26 November 1982. International Development Research Centre. Ottawa, Ontario, Canada. IDRC-2IIe. pp. 49-54.

Lowry, J. B. 1990. Toxic factors and problems: methods of alleviating them in animals. In Shrubs and Tree Fodders for Farm Animals. Devendra, C. (ed.). Ottawa, Ontario, Canada; IDRC. 76-88.

Macala, J., Sebolai, B and Majinda, R. R. 1992. Colophspermum mopane browse plant and sorghum stover as feed resources for ruminants during dry season in Botswana. In: Stares, J. E., Said, A. N. and Kategile, J. A. (eds.). Complimentarity of feed resources for animal production in Africa. African Feeds Research Network, ILCA, Addis Ababa, Ethiopia. pp. 151-162.

Mahabile, W., Masilo, B. and Kiflewahid, B. 1990. Use of sorghum bran and groundnut haulms in sorghum stover based diets for crossbred cows. In: Dzowela, B.H., Said, A.N., Asrat Wendem-Agenehu and Kategile, J. A. (Eds). Utilization of research results on forage and agricultural by-product materials as animal feed resources in Africa. ILCA, Addis Ababa. pp. 436-451.

Makkar, H. P. S., 1989 Protein precipitation methods for quantitation of tannins: a review. Journal of Agriculture and Food Chemistry, 37: 1197-1202.

Makkar, H. P. S., 1993. Antinutritional factors in foods for livestock. In: Gill, M., Owen, E., Pollott, G. E., and Lawrence, T.L.J. (eds.) Animal production in developing countries, occasional publication no. 16. British Society of Animal Production: pp 69-85.

Makkar, H. P. S., and Becker, K., 1993. Vanillin-HCl method for condensed tannins: effect of organic solvents used for extraction of tannins. Journal of Chemical Ecology, 19: 613-621.

Makkar, H. P. S., and Becker, K., 1993. Behavior of tannic acid from various commercial sources towards redox, metal complexing and protein precipitation assays of tannins. Journal of the Science and Food Agriculture, 62: 295-299.

Makkar, H. P. S., Blummel, M. and Becker, K. 1995. Formation of complexes between polyvinyl pyrrolidones or polyethylene glycols and tannins, and their implication in gas production and true digestibility in *in vitro* techniques. British Journal of Nutrition, 73: 897-913.

Makkar, H. P. S., Blummel, M., Borowy, N., and Becker, K., 1993. Gravimetric determination of tannins and their correlation with chemical and protein precipitation methods. Journal of the Science and Food Agriculture, 61: 161-165.

Makkar, H. P. S., Dawra, R. K., and Singh, B., 1988. Determination of both tannin and protein in a tannin-protein complex. Journal of Agriculture and Food Chemistry. 36:523-525.

Males, J. R. 1987. Optimizing the utilization of cereal crop residues for beef cattle. Journal of Animal Science, 65: 1124-1130.

Malton, P. J. 1990. Improving productivity in sorghum and pearl millet in Semi-arid Africa. Food Research Institute Studies. XXII (1): 1-44.

Mangan, J. L. 1988. Nutritional effects of tannins in animal feeds. Nutrition Research Reviews. 1: 201-231.

Mayer, L. 1983. Winter feeding of cereal and legume stover to draught cattle in a communal area in Botswana. Paper presented at the Southern African Regional Committee for Conservation and Utilization of Soil Meeting, Maun, Botswana, October, 1983. Animal production Research Unit, Sebele Agricultural Research Station, Gaborone. 12pp.

McDonald, P., Edwards, R. H. and Greenhalgh, J. F. D., 1981. Animal Nutrition 3rd Edition. Longmans, London.

McDowell, R. E. 1988. Importance of crop residues for feeding livestock in smallholders farming systems. In: Reed, J. D., Capper, B. S. and Neate, P. J. H. (eds.). Plant Breeding and the nutritive value of crop residues. International Livestock Centre for Africa, Addis Ababa, Ethiopia. pp. 3-27.

McDowell, R. E. and Hildebrand, P. E. 1980. Integrated crop and animal production: Making the most of resources available to small farmers in developing countries. Working papers: The Rockefeller Foundation, New York, NY. USA.

McGowan, A. A., Robinson, I. And Moate, P. 1988. Comparison of liveweight gain and mineral metabolism of sheep fed pasture or *tagasaste*. Proceedings of the Australian Society of Animal Production. 17: 230-233.

McLeod, M. N. 1974. Plant tannins-their role in forage quality, Nutrition Abstracts and Reviews 44:803-815.

McMeniman, N. P. and Little, D. A. 1974. Studies on the supplementary feeding of sheep consumption mulga (*Acacia aneura*). 1. The provision of phosphorus and molasses supplements under grazing conditions. Australian Journal of Experimental Agriculture and Animal Health, 14: 316-321.

McMeniman, N. P., Ben-Ghedalia, D and Elliot, R. 1976. Sulphur and cysteine incorporation into rumen microbial protein. British Journal of Nutrition, 36: 571-574.

McMeniman, N. P., Elliot, R. and Ash, A. J.1988. Supplementation of rice straw with crop by-products. 1. Legume straw supplementation. Animal Feed Science and Technology, 19: 43-53.

McNabb, W. C., Waghorn, G. C., Peters, J. S. and Barry, T. N. 1996.The effect of condensed tannins in *Lotus pedunculatus* on the solubilization and degradation of *ribulose-1,5-bisphosphate carboxylase* (EC 4.1.1.139; Rubisco) protein in the rumen and the sites of Rubisco digestion. British Journal of Nutrition, 76: 535-549.

McNabb, W. C., Peters, J. S., Foo, L.Y., Waghorn, G. C. and Jackson, F. S. 1998. Effect of condensed tannins prepared from several forages on the *in vitro* precipitation of Ribulose-1,5-bisphosphate Carboxylase (Rubisco) protein and its digestion by trypsin (EC 2.4.21.4) and chymotrypsin (EC 2.4.21.1). Journal of the Science of Food and Agriculture, 77: 201-212.

Meggison, P. A., McMeniman, N. P. and Armstrong, D. G. 1979. Efficiency of utilization of non-protein nitrogen in cattle. Proceedings of Nutrition Society, 38: 147A.

Mehrez, A. Z., Orskov, E. R. and McDonald, I. 1977. Rates of rumen fermentation in relation to ammonia concentration. British Journal of Nutrition, 38: 437-442.

Mehrez, N. P. and Orskov, E. R. 1977. A study of artificial fibre bag technique for determining the digestibility of feeds in the rumen. Journal of Agricultural Science (Cambridge), 88: 645-650.

Menke, H., Raab, L., Salewski, A., Steingass, H., Fritz, D. and Schneider, W., 1979. The estimation of the digestibility and metabolizable energy content of ruminant feedingstuffs from the gas production when they are incubated with rumen liquor *in vitro*. Journal of Agriculture Science (Cambridge), 93: 217-222.

Mitjavila, S., Lacombe, C., Carrera, G and Derache, R. 1977. Tannic acid and oxidized tannic acid on functional state of rat functional epithelium. Journal of Nutrition, 107: 2113-2121.

Mlay, G. I. 1986. The economics of by-products transportation. In: Little, D. A. and Said, A. N. (eds.). Utilization of Agricultural By-products as Feeds in Africa. ARNAB, ILCA, Addis Ababa, Ethiopia. pp. 111-117.

Molapo, M., Motjope, L and Klosterman, E. W. 1984. Value of winter lick to cattle under Lesotho farmer conditions. In: Draught power and animal feeding in Eastern and Southern Africa. Report of a network workshop in Ezulwini, Swaziland, 4-6 October, 1983. CIMMYT (Centro Internacional de Mejoramiento de Maiz y Trigo) Eastern and Southern Africa Economics Programme, Mbabane, Swaziland. pp. 42-44.

Mosi, A. K and Butterworth, M. H. 1985. The voluntary intake and digestibility of combinations of cereal crop residues and legume hay for sheep. Animal Feed Science and Technology, 12:241-251.

Mosienyane, B. 1983. Crop residues for Animal Feeding. The Bulletin of Agricultural Research in Botswana, 1: 3.

Mosimanyana, B. and Kiflewahid, B. 1987. Feeding crop residues to milking cows in small-scale farms in Botswana. In Utilization of Agricultural By-products as Livestock Feeds in Africa; D. A. Little and A. N. Said (eds.). ARNAB, ILCA Addis Ababa, Ethiopia; pp 127 – 135.

Mueller-Harvey, I. and Dhanoa, M. S. 1991. Varietal differences among sorghum crop residues in relation to their phenolic HPLC Finger prints and response to different environments. Journal of the Science of Food and Agriculture, 57: 199-216.

Mueller-Harvey, I. And McAllan, A. B. 1992. Tannins: Their biochemistry and nutritional properties. Advances in Plant Cell Biochemistry and Biotechnology, 1: 151-217.

Mueller-Harvey, I. and Reed, J. D. 1992. Identification of phenolic compounds and their relationships to in vitro digestibility of sorghum leaves from bird-resistant and non-bird-resistant varieties. Journal of the Science of Food and Agriculture, 62: 179-196.

Mueller-Harvey, I., McAllan, A. B., Theodorou, M. K. and Beever, D. E. 1988. Phenolics in fibrous crop residues and plants and their effects on the digestion and utilization of carbohydrates and proteins in ruminants. In: Reed, J. D., Capper, B. S and Neate, P. J. H. (eds.). Plant breeding and nutritive value of crop residues. International Livestock Centre for Africa. Addis Ababa, Ethiopia. pp. 97-132.

Mueller-Harvey, I., Reed, J. D. and Hartley, R. D. 1987. Characteristics of phenolic compounds, including flavonoids and tannins, of ten Ethiopian browse species by high performance liquid chromatography. Journal of the Science of Food and Agriculture, 39: 1- 14.

Murdiati, T. B. and Mahyudin, P. 1985. The residual tannin and crude protein of *Calliandra calothyrsus* and *Albizia falcataria*, following incubation in heated and unheated rumen fluid. Proceedings of the third Australian Association for Animal production , Animal Science Congress, Seoul, Korea Republic, 2: 814-816.

Murdiati, T. B., McSweeney, C. S. and Lowry, J. B. 1987. Hydrolyzable tannins in forages: Metabolism in sheep. Proceedings of the II International Symposium on the nutrition of herbivores, University of Queensland, Brisbane, Australia. pp. 45-46.

NAS (National Academy of Sciences). 1979. Tropical legumes: resources for the future. NAS, Washington, D. C., USA. Pp 332.

Ncube, S. and Smith, T. 1992. The feeding value of crop residues from sorghum, pearl and finger millet cultivars. In: Stares, J. E., Said, A. N. and Kategile, J. A. (eds.). Complimentarity of feed resources for animal production in Africa. African Feeds Research Network, ILCA, Addis Ababa, Ethiopia. pp. 189-194.

Ndlovu, L. R and Buchanan-Smith, J. G. 1985. Utilization of poor quality roughage by sheep: effects of alfalfa supplementation on ruminal parameters, fibre digestion and rate of passage from the rumen. Canadian Journal of Animal Science, 65: 693-703.

Nelson, K. E., Pell, A. N., Schofield, P and Zinder, S. 1995. Isolation and characterization of an anaerobic hydrolyzable tannin degrading bacterium. Applied Environmental Microbiology, 61: 3293-3298.

Norton, B. W. 1994. Anti-nutritive and toxic effects in forage tree legumes. In: Gutteridge, R. C. and Shelton, H. M. (eds.). Forage tree legumes in tropical agriculture. CAB International. Pp. 202-215.

Norton, B. W., Kamau, F. K. and Rosevear, R. 1992. The nutritive value of some tree legumes as supplements and sole feed for goats. In: Reodecha, C., Sangid, S. and Bunyavetchewin, P. (eds.). recent advances in animal production, Proceedings of the 6th AAAP Animal Science Congress, 23-28 November, 1992. Sukothai Thammathirat Open University, Nonthaburi, Thailand, Volume III: 151.

Nsahlai, I. V., Seleshi, Z., Seyoum, B. and Umunna, N. N. 1996. Nutritional characteristics and strategies to enhance the utilization of feeds for low-resource livestock producers. In: ESAP Proceedings. Fourth National Conference of the Ethiopian Society of Animal Production (ESAP), 18-19 April 1996, pp. 40-56. Addis Ababa, Ethiopia: ESAP.

Nsahlai, I. V., Siaw, D. E. and Osuji, P. O. 1994. The relationships between gas production and chemical composition of 23 browse of the genus *Sesbania*. Journal of the Science of Food and Agriculture. 65: 13-20.

Nsahlai, I. V., Siaw, D. E. and Umunna, N. N. 1995. Inter-relationships between chemical constituents, rumen dry matter and nitrogen degradability in fresh leaves of multipurpose trees. Journal of the Science of Food and Agriculture, 69: 235-246.

Nsahlai, I. V. and Umunna, N. N. 1996. *Sesbania* and *Lablab* supplementation of oat hay basal diet with or without maize grain. Animal Feed Science and Technology, 61: 275-289.

Nsahlai, I. V. and Umunna, N. N. 1996. Comparison between reconstituted sheep faeces and rumen fluid inocula and between *in vitro* and *in sacco* digestibility methods as predictors of intake and *in vivo* digestibility. Journal of Agricultural Science (Cambridge), 126: 235-248.

Nsahlai, I. V., Umunna, N. N. and Osuji, P. O. 1998. Complimentarity of bird-resistant and non-bird-resistant varieties of sorghum stover with cottonseed cake and noug (*Guizotia abyssinica*) cake when fed to sheep. Journal of Agricultural Science (Cambridge) 130: 229-239.

Odenyo, A. A., Osuji, P. O., Karanfil, O. and Adinew, K. 1997. Microbiological evaluation of *acacia angustissima* as a protein supplement for sheep. Animal Feed Science and Technology. 65: 99-112.

Oh, H. I., Hoff, J. E., Armstrong, G. S and Haff, L. A. 1980. Hydrophobic interaction in tannin - protein complexes. Journal of Agriculture and Food Chemistry 28: 394 - 398.

Olayiwale, M. B. and Olorunju, S. A. S. 1986. Effect of ensilage added N an energy on the utilization of grain sorghum stover by beef cattle. In: Preston, T. R. and Nuwanyankpa, M. Y. (eds.). Towards optimal feeding of agriculture by-products to Livestock in Africa. Pp. 102-108.

Olayiwale, M. B. and Olorunju, S. A. S. 1987. Feedlot performance of yearling steers previously maintained on different crop residue/supplementation regimes. In Utilization of Agricultural By-products as Livestock Feeds in Africa. D. A. Little and A. N. Said (eds.). ARNAB, ILCA Addis Ababa, Ethiopia; pp 72 – 82.

Orskov, E. R. 1988. Consistency of differences in nutritive value of straw from different varieties in different seasons. In: Reed, J. D., Capper, B. S and Neate, P. J. H. (eds.). Plant breeding and nutritive value of crop residues. International Livestock Centre for Africa. Addis Ababa, Ethiopia. pp. 163-176.

Orskov, E. R. 1991. Manipulation of fibre digestion in the rumen. Proceedings of nutrition Society. 50: 187-196.

Orskov, E. R. and McDonald, I. 1979. The estimation of protein degradability in the rumen from incubation-measurements weighted according to rate of passage. Journal of Agricultural Science. (Cambridge). 92: 499-503.

Osafo, E. L. K. 1993. Sorghum stover as a Forage: Cultivar Effects on Yield and Effects of Chopping, Amount Offered, Supplementation and Variety on Intake, Selection and Liveweight gain in Ethiopian Sheep and Cattle. Ph.D. thesis, University of Reading.

Owen, E and Aboud, A. A. O. 1988. Practical problems of feeding crop residues. In: Reed, J. D., Capper, B. S and Neate, P. J. H. (eds.). Plant breeding and nutritive value of crop residues. International Livestock Centre for Africa. Addis Ababa, Ethiopia. pp. 133-156.

Owen, E. and Jayasuriya, M. C. N. 1989. Use of crop residues as animal feeds in developing countries. Research and Development in Agriculture. 6 (3): 129-138.

Owen, E., Wahed, R. A., Alimon, R. and EL-Naimu. 1989. Strategies of feeding straw to small ruminants: Upgrading or generous feeding to allow selective feeding. In: Utilization of Research Results on Forage and Agriculture by-products Materials as Animal Feed Resources in Africa. Dzowela, B. H., Said, A. N., Wendem-Agenehu, A and Kategile, J. A. (eds.). PANESA/ARNAB; International Livestock Centre for Africa, Addis Ababa, Ethiopia. pp 1-21.

Palmer, B. and Schlink, A. C. 1992. The effect of drying on the intake and rate of digestion of the shrub legume *Calliandra calothyrsus*. Tropical Grasslands 26: 89-93.

Pearce, G. R. 1983. Variability in the composition and in vitro digestibility of cereal straws. In: Feed Information and Animal Production Proceedings. 2nd Symposium of International Network of Feed Information Centres, Australia. Pp. 417-420.

Pearce, G. R., Lee, J. A., Simpson, R. J. and Doyle, P. T. 1988. Sources of variation in the nutritive value of wheat and rice straws. In: Reed, J. D., Capper, B. S and Neate, P. J. H. (eds.). 1988. Plant breeding and nutritive value of crop residues. International Livestock Centre for Africa. Addis Ababa, Ethiopia. pp. 195-231.

Petersen, P. B. 1988. Separation and characteristics of botanical components of straw. Agricultural progress. The Journal of the Agricultural Education Association. Pp. 8-23.

Perez-Maldonado, R. A. and Norton, B. W. 1996. The effects of condensed tannins from *Desmodium intortum* and *Calliandra calothyrsus* on protein and carbohydrate digestion in sheep and goats. British Journal of Nutrition. 76: 515-533.

Porter, L. J. 1989. Tannins. Meth Plant Biochemistry. 1: 389-419.

Porter, L.J., Hirstich, L.N., and Chan, B. C., 1986. The conversion of procyanidins and prodelphinidins to cyanidin and delphinidin. Phytochemistry 25:223-230.

Powell, J. M. 1984. Sorghum and millet yields and consumption by livestock in the subhumid zone of Nigeria. Tropical Agriculture (Trinidad) 62:77-81.

Prasad, R., Kumar, R., Vaithiyanathan, S and Patnayak, B. C. 1997. Effect of polyethylene glycol-4000 treatment up on nutrient utilization from khejri (*Prosopis cineraria*) leaves in sheep. Indian journal of Animal Science 67 (8): 712-715.

Preston, T. R. 1986. Strategic supplementation to optimize rumen function and the balance of absorbed nutrients in ruminants fed largely on crop residues. In: Preston, T. R. and Nuwanyakpa, M. Y. (eds.). Towards optimal feeding of agricultural by-products to livestock in Africa. Proceedings of a workshop held at the University of Alexandra, Alexandra, Egypt, October 1985. African research Network for Agricultural by-products. pp. 168-180.

Preston, T.R. and Leng, R. A. 1984. Supplementation of diets based on fibrous residues and by-products. In: Sundstol, F. and Owen, E. (eds.). Straw and other fibrous by-products as feeds. Developments in Animal and Veterinary Sciences, 14: 373-409.

Preston, T.R. and Leng, R. A. 1987. Matching livestock systems to available feed resources in developing countries. University of Armidale press, Armidale, NSW, Australia.

Pritchard, D. A., Stocks, D. C., O'Sullivan, B. M., Martin, P. R., Hurwood, I. S and O'Rourke, P. K. 1988. The effect of polyethylene glycol (PEG) on wool growth and liveweight of sheep consuming Mulga (*Acacia aneura*) diet. Proceedings of Australian Society of Animal Production. 17: 290-293.

Pritchard, P. H., Martin, P. R. and O'Rourke, P. K. 1992. The role of condensed tannins in the nutritional value of mulga (*Acacia aneura*) for sheep. Australian Journal of Agricultural Research. 43: 1739-1756.

Ramazin, M., Orskov, E. R. and Tauh, A. K. 1986. Rumen degradation of straw. 2. Botanical fractions of straw from 2 barley cultivars. Animal Production. 5: 195-213.

Reddy, S. K. and Reddy, M.R. 1989. Utilization of wheat straw/bean hay and poultry droppings in the development of complete feeds for crossbred bull. Indian Journal of Animal Science. 59:981-985.

Reddy, C. N., Reddy, M. R. and Reddy, G. V. N. 1989. Ammoniation of sorghum straw with urea/anhydrous ammonia for improved utilization among crossbred cattle. Indian Journal of Animal Science. 59 (8): 986-990.

Reed, J. D and Hoefs, S. 1987. Differences among sorghum varieties in intake and digestibility of crop residues by oxen. ILCA – (unpublished). Pp. 17.

Reed, J. D and Soller, H. 1987. Phenolics and nitrogen utilization in sheep fed browse. In: M. Rose (ed.), Herbivore nutrition research. Proceedings of the Second International Symposium on the Nutrition of Herbivores, University of Queensland, Brisbane, 6 - 10 July 1987. An occasional publication. Australian Society of Animal Production, Australia. 236p.

Reed, J. D. 1986. Relationships among soluble phenolics, insoluble proanthocyanidins and fibre in East African browse species. Journal of Range Management 39: 5-7.

Reed, J. D. 1987. Phenolics, Fiber, and Fiber Digestibility in Bird Resistant and Non Bird Resistant Sorghum Grain. Journal of Agricultural and Food Chemistry. 35: 461-464

Reed, J. D. 1995. Nutritional Toxicology of Tannins and related polyphenols in forage legumes. Journal of Animal Science. 73: 1516-1528.

Reed, J. D., Horvath, P. J., Allen, M. S and Van Soest, P. J. 1985. Gravimetric determination of soluble phenolics including tannins from leaves by precipitation with trivalent ytterbium. Journal of the Science of Food and Agriculture. 36: 255 - 261.

Reed, J. D., Kebede, Y. and Fussel, L. K. 1988. Factors affecting the nutritive value of sorghum and millet crop residues. . In: Reed, J. D., Capper, B. S and Neate, P. J. H. (eds.). Plant breeding and nutritive value of crop residues. International Livestock Centre for Africa. Addis Ababa, Ethiopia. pp. 233-249.

Reed, J. D., McDowell, R. E., Van Soest, P.J. and Horvath. P.J., 1982. Condensed tannins: A factor limiting the use of cassava forage. Journal of Science, Food and Agriculture. 33:213-220.

Reed, J. D., Soller, H and Woodward, A. 1990. Fodder tree and straw diets for sheep: Intake, growth, digestibility and nitrogen utilization. Animal Feed Science and Technology 30: 39 - 50.

Reed, J. D., Tedla, A. and Kebede, Y. 1987. Phenolics, Fibre and Fibre Digestibility in the Crop Residue from Bird Resistant and Non-Bird Resistant Sorghum varieties. Journal of the Science of Food and Agriculture, 39: 113-121.

Reid, C. S. W., Ulyatt, M. J. and Wilson, M. J. 1974. Plant tannins, bloat and nutritive. Proceedings of the New Zealand Society of Animal Production, 34:82-93.

Robbins, C. T., Hanley, T. A., Hagerman, A. E., Hjeljord, O., Baker, D. L., Schwartz, C.C., and Mautz, W.W., 1987. Role of tannins in defending plants against ruminants reduction in protein availability. Ecology 68:98-107.

Robinson, P. H., Fadel, J. G. and Tamminga, S. 1986. Evaluation of mathematical models to describe neutral detergent residue in terms of its susceptibility to degradation in the rumen. Animal Feed Science and Technology. 15: 249-271.

Russell, J. B., O'Connor, J. D., Fox, D. G., Van Soest, P. J. and Sniffen, C. J. 1992. A net carbohydrate and protein system for evaluating cattle diets: I. Ruminal fermentation. Journal of Animal Science. 70: 3551-3561.

Russell, R. W. and Lolley, J. R. 1989. Deactivation of Tannin in High Tannin Milo by Treatment with Urea. Journal of Dairy Science. 72: 2427-2730.

Russel-Smith, A. 1987. The psyllid threat to *Leucaena* in Africa. The PANESA Newsletter No. 4. June 1987. ILCA, Addis Ababa, Ethiopia.

Said, A. N. and Tolera, A. 1993. The supplementary value of forage legume hays in sheep feeding: feed intake, nitrogen retention and body weight change. Livestock Production Science. 33:229-237.

Said, A. N. and Wanyoike, M. M. 1987. The prospects of utilising urea-treated maize stover by smallholders in Kenya. In: Little, D. A. and Said, A. N. (eds.). Utilization of agricultural by-products as livestock feeds in Africa. International Livestock Centre for Africa, Addis Ababa, Ethiopia. pp. 15-26.

Saini, M. L., Paroda, R. S. and Goyal, K. C. 1977. Path analysis for quality characteristics in forage sorghum. Forage Research. 3: 131-136.

Satter, L. D. and Roffler, R. E. 1981. Influence of nitrogen and carbohydrate inputs on rumen fermentation. In: Haresign, W. and Cola, D. J. A. (eds.). Recent Developments in ruminant nutrition. Butterworths, London. Pp. 115-139

Satter, L. D. and Slyter, L. L. 1974. Effects of ammonia concentration on rumen microbial protein production *in-vitro*. British Journal of Nutrition. 32: 119-208.

Senani, S., Joshi, D. C., Singh, R. and Chattopadhyay, S. K. 1996. Effect of long-term feeding of subabul (*Leucaena leucocephala*) on Barbari goats. Indian Journal of Animal Sciences, 66(5): 494-498.

Sharma, D. D., Rangnekar, D. V. and Singh, M. 1993. Physical and Chemical treatment of fibrous crop residues to improve nutritive value - A review. In: Singh, K and Schiere, J. B. (eds.). Feeding of ruminants on fibrous crop residues. ICAR, New Dehli and Agricultural university, Wageningen. Pp. 263-276.

Shumba, E. M. 1984. On-farm minimum tillage experiments: A possible solution to draught power problems gripping Zimbabwe's communal areas. In: Draught power and animal feeding in Eastern and Southern Africa. Report of a network workshop in Ezulwini, Swaziland, 4-6 October, 1983. CIMMYT (Centro Internacional de Mejoramiento de Maiz y Trigo) Eastern and Southern Africa Economics Programme, Mbabane, Swaziland. pp. 24-27

Siaw, D. E. K. A., Osuji, P. O. and Nsahlai, I. V. 1993. Evaluation of multipurpose tree germplasm: the use of gas production and rumen degradation characteristics. Journal of Agricultural Science, (*Cambridge*) 120: 319 – 330.

Silanikove, N., Gilboa, N. and Nitsan, Z. 1997. Interactions among tannins, supplementation and polyethylene glycol in goats given oak leaves: effects on digestion and food intake. Animal Science. 64: 479-483.

Silanikove, N., Gilboa, N., Nir, I., Perevolotsky, A. and Nitsan, Z. 1996. Effect of a daily supplementation of polyethylene glycol on intake and digestion of tannin containing leaves (*Quercus calliprinos, Pistacia lentiscus* and *Ceratonia siliqua*) by goats.

Journal of Agricultural and Food Chemistry. 44: 199-205.

Silanikove, N., Nitsan, Z. and Perevolotsky, A. 1994. Effect of a daily supplementation of polyethylene glycol on intake and digestion of tannin containing leaves (*Ceratonia siliqua*) by sheep. Journal of Agriculture and Food Chemistry. 42: 2844-2847.

Silva, A. T. and Orskov, E. R. 1988. The effect of five different supplements on degradation of straw in sheep given untreated barley straw. Animal Feed Science and Technology. 19: 277-287.

Singh, K. and Arora, S. P. 1978. Studies on detanning and determining the nutritive value of alkali-treated salseed meal (*Shorea robusta*). Indian journal of Animal Science, 48: 84-89.

Singhal, K. K., Kundu, S. S. and Sharma, D. D. 1991. Effect of chopping of oat straw on nutrient utilization and rate of passage of liquid digesta through G. I. Tract in crossbred calves. Indian Journal of Dairy Science.

Skerman, P.J. 1977. Tropical Forage Legumes. FAO Plant Production and Protection Series No. 2, FAO, Rome, 609 pp.

Statistical Analysis Systems 1987. SAS/STAT *User's Guide*, release 6.04. . SAS Institute Inc. Cary, North Carolina

Sundstol, F. and Owen, E. 1984. Ammonia treatment. In: Sundstol, F and Owen, E (eds.). Straw and other fibrous by-products as feeds. Development in Animal and Veterinary Sciences. 14: 196-247.

Tanner, J.C., Reed, J. D. and Owen, E., 1990. The nutritive value of fruits (pods with seeds) from four acacia species compared with extracted noug (*Guizotia abyssinica*) meal as supplements to maize stover for Ethiopian highland sheep. Animal Production. 51:127.

Terrill, T. H., Windham, W. R., Hoveland, C. S. and Amos, H. E. 1989. Influence of forage preservation method on tannin concentration, intake and digestibility of *Sericea lespediza* by sheep. Agronomy Journal. 81: 435-439.

Terrill, T. H., Rowan, A. M., Douglas, G. B. and Barry, T. N. 1992. Determination of extractable and bound condensed tannin concentrations in forage plants, protein concentrate meals and cereal grains. Journal of the science of food and Agriculture, 58: 321-329.

Tessema, S. 1984. Animal feeding in small farm systems. In: Draught power and animal feeding in Eastern and Southern Africa. Report of a network workshop in Ezulwini, Swaziland, 4-6 October, 1983. CIMMYT (Centro Internacional de Mejoramiento de Maiz y Trigo) Eastern and Southern Africa Economics Programme, Mbabane, Swaziland. pp 61-68.

Tessema, S. and Emojong, E. E. 1984. Utilization of maize and sorghum stover by sheep and goats after feed quality improvement by various treatment and supplements. East African Agriculture and Forestry Journal. 44 (suppl) 408-415.

Thiago, L. R. L. and Kellanway, R. C. 1982. Botanical composition and extent of lignification affecting digestibility of wheat and oat straw and paspalum hay. Animal Feed Science and Technology, 7: 71 – 81.

Tilley, J. M. A. and Terry, R. A. 1963. A two-stage technique for *in vitro* digestion of forage crops. Journal of British Grassland Science. 18:104-111.

Topps, J. H. 1992. Potential, composition and use of legume shrubs and trees as fodder for livestock in the tropics. Journal of Agricultural Science (Cambridge). 118: 1-8.

Tsai, C. G. and Jones, G. A. 1975. Isolation and identification of rumen bacteria capable of anaerobic phloroglucinol degradation. Canadian Journal of Microbiology, 21: 94-801.

Tuah, A. K., Lufadeju, E., Orskov, E. R. and Blacket, G. A. 1986. Rumen degradation of straw. 1. Untreated and ammonia treated barley, oat and wheat straw varieties and triticale straw. Animal Production 43: 261-269.

Turner, N. C. 1981. Designing crops for dryland Australian: can the desert help us? The Journal of Australian Institute of Agricultural Science, 47: 29-34.

Umunna, N. N., Bartling, R. R. and Klopfenstein, T. J. 1986. High temperature and pressure processing of maize cobs. II. Animal response and effect of sodium metabisulphite. Animal Feed Science and Technology, 14: 161-169.

Umunna, N. N. and Agishi, E. C. 1988. Nutritional value of savannah grasses and harvest residues for use in dry season animal feeding. A paper presented at a Symposium on Scrub Savannah Studies, Buchi, Nigeria. February 7-8, 1988.

Umunna, N. N., Nsahlai, I. V. and Osuji, P. O. 1995. Degradability of forage protein supplements and their effects on the kinetics of digestion and passage. Small Ruminant Research. 17: 145-152.

Umunna, N. N., Osuji, P. O., Khalili, H., Nsahlai, I. V. and Crosse, S. 1995. Comparative feeding value of forages from two cereal-legume based cropping system for beef production from crossbred (*Bos taurus* x *Bos indicus*) steers and subsequent performance of underfed and realimented steers. Animal Production. 61:35-42.

Umunna, N. N., Osuji, P. O., Nsahlai, I. V., Khalili, H. and Saleem, M. A. 1995. The effect of supplementing oats hay with either *lablab, sesbania, tagasaste* or wheat middlings on the voluntary intake, nitrogen utilization and liveweight gain of

Ethiopian Menz sheep. Small Ruminant Research. 18: 113-120.

Urio, N. A. 1985. Inventory of some important crop residues and by-products in Tanzania. In: Preston, T. R., Kosilla, V. L., Goodwin, J. and Reed. S. B. (eds.). Better utilization of crop residues and by products in animal feeding: Research guidelines. 1. State of knowledge. Proceedings of the FAO/ILCA Expert Consultation held at ILCA, Addis Ababa, Ethiopia, 5-9 March, 1985. FAO Animal Production and Health Paper 50. Food and Agriculture Organization of the United Nations, Rome, Italy. Pp. 115-116.

Van Hoven 1984. Tannins and digestibility in the greater Kuddu. Canadian Journal of Animal Science. 64: Suppl. 177-178.

Van Soest, P. J., 1982. Nutritional Ecology of Ruminants. O and B Books Inc., Corvallis, Oregon 97390, USA.

Van Soest, P. J. 1988. Effect of environment and quality of fibre on the nutritive value of crop residues. In: Plant breeding and the nutritive value of crop residues. Reed, J. D., Capper, B. S. and Neate, P. J. H. (eds.). International Livestock Centre for Africa. Pp 71 – 96.

Van Soest, P. J., 1994. Nutritional Ecology of Ruminant (2nd edition). Cornell University, Ithaca, NY, USA.

Varvikko, T. and Khalili, H., 1993. Wilted tagasaste (*Chamaecytisus palmensis*) forage as a replacement for a concentrate supplement for lactating crossbred Friesian x Zebu (Boran) dairy cows fed low quality native hay. Animal Feed Science and Technology. 40: 239-250.

Varvikko, T., Khalili, H. and Crosse, S. 1992. Supplementation of native grass hay with cowpea (*Vigna unguiculata*) hay, wilted leucaena (*Leucaena leucocephala*) forage, wilted tagasaste (*Chamaecytisus palmensis*) forage or wheat middling for young Friesian x Zebu (Boran) crossbred steers. Agricultural Science of Finland. 1: 247-254.

Waghorn, G. C., Jones, W. T., Shelton, I. D. and McNabb, W. C. 1990. Condensed tannins and nutritive value of herbage. Proceedings of New Zealand Grassland Association. 51: 171-176.

Waghorn, G. C., Shelton, I. D. and McNabb, W. C. 1994. Effects of condensed tannins in *Lotus pedunculatus* on its nutritive value for sheep. 1. Non-nitrogenous aspects. Journal of Agriculturai Science, (*Cambridge*). 123: 99-107.

Waghorn, G. C., Ulyatt, M.J., John, A., Fisher, M.J. 1987. The effect of condensed tannins on the site of digestion of amino acids and other nutrients in sheep fed on *Lotus corniculatus*. British Journal of Nutrition. 57:115-126.

Wahed, R. A. and Owen, E. 1986. The effect of amount offered on selection and intake of barley straw by goats. Animal Production. 42: 473 (abstr).

Wahed, R. A., Owen, E., Naate, M and Hosking, B. J. 1990. Feeding straw to small ruminants: effect of amount offered on intake and selection of barley straw by goats and sheep. Animal Production. 51: 283-289.

Wanapat, M and Devendra, C. 1985. (eds.). Relevance of crop residues as animal feeds in developing countries. Proceedings of International workshop, Department of Animal Science, Faculty of Agriculture, Khon Kaen University, Khon Kaen, Thailand, November, 29th December, 1984. Funny Press, Bangkok.

Ward, J. K., Perry, L. T., Smith, D. H. and Schmitz, J. T. 1979. Forage composition and utilization of grain sorghum residue by beef cows. Journal of Animal Science. 48: 919-925.

Waterman, P. G. and Mole, S. 1994. Analysis of Phenolic Plant Metabolites. Blackwell Science Publishing, London, UK.

Waterman, P. G., Mbi, C. N., Mckey, D. B. and Gartlan, J. S. 1980. African rainforest vegetation and rumen microbes: phenolic compounds and nutrients as correlates of digestibility. Oceologia. 747: 22-33.

Watson, V., Mamba, Z. and Mamba, S. L. 1984. Draft animal feeding and management on Swazi Nation land: Trends, prospects and recommendations for research. . In: Draught power and animal feeding in Eastern and Southern Africa. Report of a network workshop in Ezulwini, Swaziland, 4-6 October, 1983. CIMMYT (Centro Internacional de Mejoramiento de Maiz y Trigo) Eastern and Southern Africa Economics Programme, Mbabane, Swaziland. pp. 18-20.

White, L. M., Hartman, G. P and Bergman, J. W. 1981. Straw digestibility of winter wheat, spring wheat, barley and oat straws in Montana. Agronomy Journal, 73: 117-120.

Wiegand, R. O, Reed, J. D., Said, A. N. and Umunna, N. N. 1995. Proanthocyanidins (condensed tannins) and the use of leaves from *Sesbania sesban* and *Sesbania goetzei* as protein supplements. Animal feed Science and Technology. 54: 175-192.

Wiegand, R. O., 1991. Tree leaves in the diets of small ruminants. M.Sc. Thesis, University of Wisconsin. Madison, Wisconsin, U. S. A.

Wilkinson, J. M. 1984. Farm-scale treatment with sodium hydroxide. In: Sundstol, F. and Owen, E. (eds.). Straw and other fibrous by-products as feeds. Developments in Animal and Veterinary Sciences, 14: 162-180.

Wilson, G. L. and Eastin, J. D. 1982. The plant and its environment. . In: House, L. R., Mughogho, L. K. and Peacock, J. M. (eds.). Sorghum in the Eighties. Patancheru,

Andhra Pradesh, India. Pp 101-119.

Woodward, A and Reed, J. D. 1990. Intake and digestibility for sheep and goats consuming *Acacia brevispica* and *Sesbania sesban*. Animal Feed Science and Technology 30:

Woodward, A. and Reed, J. D., 1989. The influence of polyphenolics on the nutritive value of browses. A summary of research conducted at ILCA. ILCA Bulletin No. 35:2-11.

World Bank, 1987. West Africa agricultural research review. World Bank Western African Projects Department, Washington, D. C. U.S.A.

Yilala, K. 1990. The effect of supplements of oil seed by-products on the utilization of low-nitrogen fibrous diets by sheep. In: Dzowela, B. H., Said, A. N., Asrat Wendem-Agenehu and Kategile, J. A. (eds.). Utilization of research results on forage and agricultural by-product materials as animal feed resources in Africa. International Livestock Centre for Africa. Addis Ababa, Ethiopia.

Zemmelink, G. 1980. Effect of selective consumption on voluntary intake and digestibility of Tropical forages. Agricultural Research Reports 896. Centre for Agricultural Publishing and Documentation. Wageningen, The Netherlands.

# APPENDIX TABLES

Appendix Table 3.1 Effects of period of harvest and storage methods on cereal stover yield (tonnesDM/ha).

| Crop | Storage methods[1] | | Period of harvest (in weeks)[2] | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | -3 | -2 | 0 | 2 | 4 | 6 | Mean |
| Maize | | A | 8.8 | 9.2 | 9.0 | 8.3 | 7.1 | 7.1 | 8.2 |
| | | B | 8.3 | 8.3 | 8.3 | 7.7 | 7.7 | 6.4 | 7.8 |
| | | C | 8.3 | 8.8 | 7.9 | 8.2 | 7.6 | 5.7 | 7.8 |
| | | D | 8.5 | 8.5 | 8.1 | 7.6 | 7.4 | 6.9 | 7.8 |
| | | E | 7.3 | 8.1 | 7.0 | 8.1 | 7.7 | 5.1 | 7.2 |
| | Mean | | 8.2 | 8.6 | 8.1 | 8.0 | 7.5 | 6.2 | 7.8 |
| Sorghum (BR[4]) | | A | 9.0 | 10.6 | 10.2 | 8.6 | 8.3 | 8.1 | 9.1 |
| | | B | 7.5 | 7.5 | 7.9 | 7.6 | 6.2 | 6.7 | 7.3 |
| | | C | 7.7 | 8.8 | 8.1 | 7.4 | 6.9 | 7.1 | 7.7 |
| | | D | 7.4 | 7.3 | 7.8 | 7.1 | 6.1 | 5.9 | 6.9 |
| | | E | 7.0 | 7.5 | 7.9 | 6.9 | 6.1 | 6.1 | 6.9 |
| | Mean | | 7.7 | 8.3 | 8.4 | 7.5 | 6.7 | 6.8 | 7.6 |
| Sorghum (NBR[4]) | | A | 9.0 | 9.6 | 9.4 | 8.6 | 8.6 | 7.7 | 8.8 |
| | | B | 7.7 | 7.3 | 6.7 | 7.8 | 6.3 | 6.9 | 7.1 |
| | | C | 7.7 | 7.7 | 6.9 | 7.8 | 6.4 | 7.5 | 7.4 |
| | | D | 7.4 | 6.8 | 6.5 | 6.9 | 6.1 | 5.6 | 6.6 |
| | | E | 7.5 | 6.8 | 6.3 | 7.8 | 7.2 | 6.3 | 7.0 |
| | Mean | | 7.9 | 7.7 | 7.2 | 7.8 | 6.9 | 6.8 | 7.4 |
| **Overall mean** | | | **7.9** | **8.2** | **7.9** | **7.8** | **7.1** | **6.6** | **7.6** |

[1]Storage methods: A = Stover left rooted in the field until about 85% dry.
B&C = Stover cut and dried with (B) or without (C) grain heads or cobs and stored in the open.
D&E = Stover cut and dried with (D) or without (E) grain heads or cobs and stored under shade.[2] Period of harvest: (-3 week physiological mature stage, 0 week = physiological dead ripe stage, i.e. normal harvesting time)

[3] Level of significance: NS P>0.05, * P<0.05, ** P<0.01, *** P<0.001

[4]BR = Bird-resistant sorghum stover, NBR =Non-bird-resistant sorghum stover

Appendix Table 3.2 Effects of period of harvest and storage methods on N content (g/kgDM) of cereal stover

| Crop | Storage methods[1] | Period of harvest (in weeks)[2] | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | -3 | -2 | 0 | 2 | 4 | 6 | Mean |
| Maize | A | 8.2 | 7.2 | 6.8 | 6.2 | 6.0 | 5.8 | 6.7 |
| | B | 8.9 | 8.6 | 7.4 | 7.2 | 6.9 | 6.2 | 7.5 |
| | C | 9.6 | 9.4 | 7.7 | 7.6 | 5.8 | 5.8 | 7.6 |
| | D | 8.1 | 9.2 | 7.7 | 7.4 | 6.1 | 5.8 | 7.4 |
| | E | 9.1 | 8.8 | 7.3 | 7.1 | 6.4 | 5.6 | 7.4 |
| | Mean | 8.8 | 8.6 | 7.4 | 7.1 | 6.2 | 5.8 | 7.3 |
| Sorghum (BR[4]) | A | 10.1 | 9.7 | 7.3 | 6.1 | 6.1 | 6.7 | 7.7 |
| | B | 12.2 | 11.3 | 8.5 | 6.2 | 7.9 | 7.8 | 9.0 |
| | C | 11.9 | 12.3 | 9.5 | 7.7 | 8.3 | 7.8 | 9.6 |
| | D | 12.5 | 12.9 | 8.6 | 8.2 | 8.6 | 7.6 | 9.8 |
| | E | 12.7 | 12.0 | 9.4 | 9.2 | 8.3 | 7.4 | 9.8 |
| | Mean | 11.9 | 11.6 | 8.7 | 7.5 | 7.9 | 7.5 | 9.2 |
| Sorghum (NBR[4]) | A | 10.3 | 9.2 | 7.8 | 7.1 | 6.1 | 6.0 | 7.7 |
| | B | 11.5 | 11.3 | 10.3 | 9.3 | 7.3 | 6.5 | 9.3 |
| | C | 12.9 | 9.3 | 10.4 | 9.8 | 8.1 | 6.8 | 9.6 |
| | D | 12.2 | 11.2 | 11.6 | 8.0 | 7.4 | 6.1 | 9.4 |
| | E | 11.9 | 11.2 | 8.8 | 8.4 | 7.6 | 6.0 | 9.0 |
| | Mean | 11.7 | 10.4 | 9.8 | 8.5 | 7.3 | 6.3 | 9.0 |
| **Overall mean** | | **10.8** | **10.2** | **8.6** | 7.7 | **7.1** | **6.5** | **8.5** |

[1]Storage methods: A = Stover left rooted in the field until about 85% dry.
B&C = Stover cut and dried with (B) or without (C) grain heads or cobs and stored in the open.
D&E = Stover cut and dried with (D) or without (E) grain heads or cobs and stored under shade.

[2] Period of harvest: (-3 week physiological mature stage, 0 week = physiological dead ripe stage , i.e. normal harvesting time)

[3] Level of significance: NS P>0.05, * P<0.05, ** P<0.01, *** P<0.001

[4]BR = Bird-resistant sorghum stover, NBR =Non-bird-resistant sorghum stover

Appendix Table 3.3 Effects of period of harvest and storage methods on NDF content (g/kgDM) of cereal stover

| Crop | Storage methods[1] | Period of harvest (in weeks)[2] | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | -3 | -2 | 0 | 2 | 4 | 6 | Mean |
| Maize | A | 551.7 | 546.3 | 597.4 | 584.0 | 582.3 | 604.2 | 577.7 |
| | B | 534.3 | 526.0 | 545.2 | 589.8 | 576.7 | 597.4 | 561.6 |
| | C | 507.1 | 525.6 | 553.1 | 585.9 | 592.3 | 583.3 | 557.9 |
| | D | 532.1 | 498.4 | 571.7 | 583.3 | 596.1 | 581.6 | 560.5 |
| | E | 541.7 | 548.3 | 576.5 | 591.9 | 570.0 | 573.6 | 567.0 |
| | Mean | 533.4 | 528.9 | 568.8 | 587.0 | 583.5 | 588.0 | 564.9 |
| Sorghum (BR[4]) | A | 459.5 | 456.1 | 540.7 | 529.3 | 546.7 | 566.8 | 516.5 |
| | B | 437.1 | 446.2 | 547.6 | 523.6 | 519.6 | 545.9 | 503.3 |
| | C | 439.4 | 447.3 | 488.7 | 537.0 | 515.5 | 536.4 | 494.1 |
| | D | 437.1 | 424.0 | 490.2 | 484.6 | 514.5 | 525.3 | 479.3 |
| | E | 445.8 | 442.6 | 468.0 | 498.8 | 516.0 | 528.3 | 483.3 |
| | Mean | 443.8 | 443.2 | 507.1 | 514.7 | 522.5 | 540.6 | 495.3 |
| Sorghum (NBR[4]) | A | 460.8 | 499.9 | 500.7 | 522.1 | 535.3 | 527.8 | 507.7 |
| | B | 430.0 | 454.3 | 481.5 | 481.5 | 514.3 | 526.4 | 481.3 |
| | C | 432.4 | 453.4 | 499.2 | 518.4 | 524.9 | 529.4 | 492.9 |
| | D | 433.9 | 483.7 | 465.7 | 517.2 | 506.4 | 529.1 | 489.3 |
| | E | 420.1 | 477.8 | 474.7 | 467.0 | 506.8 | 532.8 | 479.9 |
| | Mean | 435.4 | 473.8 | 484.4 | 501.2 | 517.5 | 529.1 | 490.2 |
| **Overall mean** | | **470.9** | **482.0** | **520.1** | **534.3** | **541.2** | **552.6** | **516.8** |

[1]Storage methods: A = Stover left rooted in the field until about 85% dry.
B&C = Stover cut and dried with (B) or without (C) grain heads or cobs and stored in the open.
D&E = Stover cut and dried with (D) or without (E) grain heads or cobs and stored under shade.

[2] Period of harvest: (-3 week physiological mature stage, 0 week=physiological dead ripe stage, i.e. normal harvesting time)

[3] Level of significance: NS P>0.05, * P<0.05, ** P<0.01, *** P<0.001

[4]BR = Bird-resistant sorghum stover, NBR =Non-bird-resistant sorghum stover

Appendix Table 3.4 Effects of period of harvest and storage methods on condensed tannins (CTs) content (g/kgDM) of sorghum stover

| Crop | Storage methods[1] | Period of harvest (in weeks)[3] | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | -3 | -2 | 0 | 2 | 4 | 6 | Mean |
| Sorghum (BR[4]) | A | 18.8 | 23.7 | 22.8 | 22.0 | 28.5 | 33.9 | 25.0 |
| | B | 20.6 | 20.1 | 18.6 | 12.9 | 22.2 | 29.0 | 20.6 |
| | C | 23.1 | 25.5 | 20.8 | 24.5 | 28.7 | 31.4 | 25.7 |
| | D | 13.4 | 18.6 | 21.4 | 25.1 | 35.5 | 31.6 | 24.2 |
| | E | 16.5 | 20.7 | 23.9 | 18.9 | 31.0 | 32.4 | 23.9 |
| | Mean | 18.5 | 21.7 | 21.5 | 20.7 | 29.2 | 31.7 | 23.9 |
| Sorghum (NBR[4]) | A | 1.8 | 1.3 | 1.7 | 4.6 | 4.1 | 4.7 | 3.0 |
| | B | 2.6 | 1.9 | 1.8 | 2.9 | 2.8 | 3.0 | 2.5 |
| | C | 0.1 | 2.1 | 2.1 | 2.5 | 2.0 | 3.8 | 2.1 |
| | D | 3.0 | 3.0 | 1.8 | 1.5 | 2.3 | 2.9 | 2.4 |
| | E | 3.7 | 2.5 | 2.3 | 5.1 | 2.6 | 3.6 | 3.3 |
| | Mean | 2.3 | 2.2 | 1.9 | 3.3 | 2.7 | 3.6 | 2.7 |
| **Overall mean** | | **10.4** | **11.9** | **11.7** | **12.0** | **16.0** | **17.6** | **13.3** |

[1]Storage methods: A = Stover left rooted in the field until about 85% dry.
B&C = Stover cut and dried with (B) or without (C) grain heads or cobs and stored in the open.
D&E = Stover cut and dried with (D) or without (E) grain heads or cobs and stored under shade.

[2] Period of harvest: (-3 week physiological mature stage, 0 week = physiological dead ripe, normal harvesting time)

[3] Level of significance: NS P>0.05, * P<0.05, ** P<0.01, *** P<0.001

[4]BR = Bird-resistant sorghum stover, NBR =Non-bird-resistant sorghum stover

Appendix Table 3.5 Effects of period of harvest and storage methods on DM degradability (g/kgDM) of cereal stover

| Crop | Storage methods[1] | Period of harvest (in weeks)[2] | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | -3 | -2 | 0 | 2 | 4 | 6 | Mean |
| Maize | A | 456.5 | 447.9 | 441.9 | 457.7 | 442.7 | 433.7 | 447.0 |
| | B | 476.9 | 492.3 | 456.2 | 459.0 | 479.3 | 436.9 | 467.0 |
| | C | 444.9 | 459.6 | 451.1 | 455.2 | 452.8 | 427.3 | 448.9 |
| | D | 492.9 | 496.8 | 467.3 | 432.7 | 464.9 | 413.7 | 461.4 |
| | E | 445.6 | 439.8 | 470.5 | 428.4 | 445.0 | 436.7 | 444.4 |
| Mean | | 461.7 | 465.4 | 457.3 | 446.7 | 456.7 | 429.6 | 453.4 |
| Sorghum (BR[4]) | A | 386.9 | 384.4 | 375.0 | 366.9 | 364.2 | 351.9 | 372.1 |
| | B | 400.0 | 397.3 | 374.2 | 401.0 | 381.5 | 357.5 | 385.9 |
| | C | 410.1 | 407.8 | 391.0 | 406.2 | 373.4 | 357.5 | 392.0 |
| | D | 418.3 | 353.2 | 410.2 | 390.1 | 371.3 | 362.5 | 385.1 |
| | E | 401.8 | 393.5 | 387.3 | 376.1 | 374.4 | 393.9 | 388.0 |
| Mean | | 403.6 | 387.3 | 387.9 | 388.4 | 372.9 | 365.3 | 384.8 |
| Sorghum (NBR[4]) | A | 390.5 | 391.6 | 400.5 | 401.9 | 382.7 | 393.2 | 393.3 |
| | B | 351.4 | 414.2 | 398.4 | 393.7 | 408.2 | 380.1 | 391.4 |
| | C | 424.2 | 392.5 | 410.0 | 394.3 | 407.9 | 409.0 | 406.4 |
| | D | 431.7 | 392.2 | 400.1 | 395.7 | 425.5 | 402.9 | 408.0 |
| | E | 413.9 | 396.3 | 393.3 | 402.6 | 403.4 | 407.9 | 403.0 |
| Mean | | 403.7 | 397.5 | 400.4 | 397.6 | 405.6 | 398.4 | 400.5 |
| **Overall mean** | | **422.2** | **415.9** | **413.4** | **409.5** | **410.9** | **397.2** | 411.9 |

[1]Storage methods: A = Stover left without heads or cobs in the field until 85% dry.

B&C = Stover cut and dried with (B) or without (C) grain heads or cobs in the open.

D&E = Stover cut and dried with (D) or without (E) grain heads or cobs under shade.

[2] Period of harvest: (-3 week physiological mature stage, 0 week = physiological dead ripe, normal harvesting time)

[3] Level of significance: NS P>0.05, * P<0.05, ** P<0.01, *** P<0.001

[4]BR = Bird-resistant sorghum stover, NBR =Non-bird-resistant sorghum stover