EFFECT OF INCLUSION OF PROTEIN-RICH FORAGES ON QUALITY OF NAPIER GRASS SILAGE

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DECLARATION

This thesis is my original work and has not been presented for a degree in any other University.

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DEDICATION

To the three girls in my life:

Naomi – my wife,

Eva – my daughter,

Winnie (Dee Dee) – my daughter,

for being there for me during the difficult times and for inspiring me all the way.

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ABBREVIATIONS

ADF	Acid detergent fibre
ADL	Acid detergent lignin
CF	Crude fibre
CP	Crude protein
DCF	Digestible crude fibre
DEE	Digestible ether extract
DM	Dry matter
DMD	Dry matter digestibility
DMI	Dry matter Intake
DOM	Digestible organic matter
EE	Ether extract
FCM	Fat-corrected milk
GP	Gas production
GP24	% potential gas production achieved after 24 hours
HFT	Hohenheimer Futterwertstest (Menke's gas production technique)
IVDN	In vitro degradability of nitrogen
IVOMD	In vitro organic matter degradability
LAB	Lactic acid bacteria
ME	Metabolizable energy
NAN	Non-ammonia nitrogen
NE	Net energy
NEL	Net energy for lactation
MPT	Multipurpose trees and shrubs
NDF	Neutral Detergent Fibre
NG4	Napier grass of 4 weeks maturity
NG8	Napier grass of 8 weeks maturity
NG12	Napier grass of 12 weeks maturity
NH3-N	Ammonia nitrogen
OMD	Organic matter digestibility

PEG	Polyethylene glycol
PEG-6000	Polyethylene glycol of 6000 Molecular weight
PRF	Protein-rich forage
RUSITEC	Rumen Simulation Technique
T^{70}	Time required for production of 70% of potential gas volume
VFA	Volatile fatty acid
WSC	Water soluble carbohydrates

ABSTRACT

In order to study the effect of inclusion of protein rich forages (PRF) and molasses on chemical composition, metabolisable energy (ME) content, voluntary intake, *in vivo* and *in vitro* digestibility of Napier grass silage, four experiments were performed.

In experiment one, Napier grass harvested at three stages of growth (4, 8 and 12 weeks from the last cutting) was ensiled singly, or mixed with protein rich forage (PRF) *Lablab purpureus, Mucuna pruriens, Crotalaria ochroleuca and Leucaena leucocephala* (20%) for 30, 60 and 90 days in miniature polythene silos. Each of the silages was made with and without 3% molasses. Inclusion of molasses, with or without PRF, significantly ($p<0.01$) lowered silage pH over the storage period for all Napier grass ages. Inclusion of PRF except *Leucaena* increased the ammonia nitrogen of the silage ($P<0.05$). Addition of molasses reduced ammonia nitrogen content of both non-supplemented and PRF-Napier grass silages.

In experiment two, the in vitro fermentation kinetics of the silages and pre-ensiled forage were determined using a gas production technique. The rate of gas production for the pre-ensiled PRFs and PRF-Napier grass silages were higher ($P<0.05$) than for pre-ensiled Napier grass and non-supplemented Napier grass silage. The stage of growth (8 and 12 weeks) of Napier grass did not affect the rate of gas production or the cumulative gas volume of the silages. Within the PRFs, *Lablab* had the highest (10.4%/hour) and *Leucaena* the lowest (7.3%/hour) rate of gas production. Inclusion of molasses increased ($P<0.001$) the rate of gas production and ME for both Napier and Napier-PRF silages irrespective of age of Napier grass.

In experiment three, Napier grass was ensiled singly (NG) or in combination with *Mucuna pruriens* (NGM) and *Dolichos lablab (NGL)* in pit silos. Molasses was included at 3% in all the silages. In sheep, the voluntary dry matter intakes were 74, 81 and 83 $g/kgLW^{0.75}$ for the NG, NGM and NGL silages respectively. Inclusion of legumes did not affect the voluntary intakes of DM and OM but increased ($P<0.05$) the CP intake (145, 183 and 183 g/animal/day for NG, NGL and NGM respectively. PRF inclusion did not

affect the digestibility of dry matter but increased the digestible crude protein (g/day). The ME calculated both from *in vivo* digestible nutrients and *in vitro* gas production was higher ($P<0.05$) for NGL (8.0 and 7.7) and lower for NGM (7.6 and 7.2) than the NG (7.8 and 7.4 $MJkg^{-1}$ DM). Inclusion of PRF increased the nitrogen retained from 4.46 g/day for NG to 7.74 and 6.30 for NGL and NGM respectively. The ratio of nitrogen to ME was higher in the supplemented silage (2.3 and 2.4 for NGL and NGM respectively) compared to 2.0 gMJ^{-1} for NG.

In experiment four, the effects of tannins in PRFs (*Mucuna pruriens, Dolichos lablab, Crotalaria ochroleuca and Leucaena leucocephala, Acacia tortilis, Acacia angustissima, Morus alba, Calliandra calothyrsus)* on *in vitro* fermentation were investigated. Polyethylene glycol (PEG, MW 6000) was used as a tannin binder. Inclusion of PEG increased ($P<0.001$) the gas production by 23, 54, 112 and 475% for *Leucaena leucocephala, Acacia tortilis, Calliandra calothyrsus* and *Acacia angustissima* respectively. Addition of PEG increased ($P<0.001$) the total volatile fatty acids, acetic, propionic, butyric, isobutyric and isovaleric acids for *Leucaena leucocephala, Calliandra calothyrsus and Acacia angustissima.* There were minimal gas production increases for *Mucuna pruriens, Lablab purpureus, Crotalaria ochroleuca* and *Morus alba* on incubation with PEG. Gas production from Napier grass was reduced ($P<0.05$) by inclusion of *Acacia angustissima, Calliandra calothyrsus and Leucaena leucocephala.* This effect was dose dependent and was reversed by inclusion of PEG. *In vitro* nitrogen degradability and microbial nitrogen uptake were increased ($P<0.001$) by the inclusion of PEG.

1. INTRODUCTION

The shortage of acceptable quality feed for ruminants in the tropics is well documented (Preston, 1995). The pattern of forage growth, quality and availability closely follows the seasonal distribution of rainfall with shortages being most severe in the dry season. Due to high population density in the high potential areas in Kenya, there is stiff competition for land use between pasture and fodder production on one hand and food crop production on the other.

Due to its high biomass productivity (10-40 t/ha), Napier grass (*Pennisetum purpureum*) is the main forage feed in the smallholder dairy farming sector in Kenya (Anindo and Potter, 1986; Kariuki *et al.*, 1998; Muia *et al.*, 1999). The other reason for the adoption of Napier grass is the intensive agricultural extension services offered by the civil service and the support from special projects such as the National Dairy Development Project (MALDM, 1997). At the optimal stage of growth recommended for feeding, Napier grass has an average of 100gkg^{-1} DM crude protein (Kariuki *et al.*, 1998, Muia *et al.*, 1999) and a metabolisable energy content of 8.6 MJ/kg DM (NRC, 1988). However, due to seasonal effects, Napier grass is offered to animals either too young or at later stages of maturity. It is, therefore, expected that dairy cows subsisting on Napier grass are not able to meet their nutrient requirements for maintenance (0.488 MJ ME/kg LW$^{0.75}$) and milk production (4.83 MJ ME/kg FCM) (GfE, 1995). According to NRC (1988), crude protein content of diets offered to dairy cows weighing up to 400kg and producing 8 to 13 kg milk should be 140g/kg DM, a level that is rarely achieved in Napier grass on smallholder dairy farms.

In the rainy season, Napier grass grows rapidly and its quality deteriorates (increase in cell wall contents and reduction in fermentable crude protein and carbohydrates) as the dry season approaches (Muia *et al.*, 1999). Most farmers leave standing Napier grass as a reserve for the dry season while others purchase equally low quality; overgrown Napier from commercial producers at a high cost. In circumstances of excessive rain (e.g. the *El Nino* phenomenon), excess Napier grass is cut and used for compost rather than preserved.

The availability of good quality forage to maintain animal production in the dry season is a critical problem faced by almost all smallholder livestock farmers. The abundant forage in

and forage is in short supply. There are very few reported cases of Napier grass preservation as silage (Brown and Chevalimu, 1985; Panditharatne *et al.*, 1986; Snijders and Wouters, 1990).

To maximise ruminant production from lignocellulose-rich forages, fermentation and microbial protein synthesis should be maximised ensuring that the end products are usable by the animal (Hobson, 1988). However, judging from its nutrient composition, Napier grass only diets fed to dairy cattle may not support conditions conducive to high microbial activity in the rumen. This leads to low whole-tract digestibility and wastage of ingested nutrients and recycled urea (Preston and Leng, 1987). Such diets also lead to poor nitrogen:energy ratios; 1.56gN/MJ ME for good quality Napier grass as opposed to 1.92gN/MJ ME recommended by NRC (1988).

The situation may be improved by the supplementation of the Napier grass with grain- and oilseed-based high energy/protein concentrate feed. However, these are beyond the reach of most smallholder dairy farmers due to high costs. The high cost of grain-based concentrates is due to competition between ruminant animals on one hand, and human beings and non-ruminant animals on the other. Crude protein supplements are also in short supply due to the competition from non-ruminant animals and the fact that not much oil seed production is practised in Kenya. Almost all the fishmeal produced goes into the formulation of poultry diets. It is therefore necessary to continue searching for alternative and affordable sources of soluble nutrients (protein and energy) to meet the requirements of the rumen microorganisms.

Forage legumes are less expensive sources of fermentable nitrogen in ruminant diets. Several studies have been done on different types of forage legumes as a source of crude protein in ruminant diets in the tropics (Kariuki *et al*, 1998; Kariuki *et al*, 1999; Muinga *et al*, 1992; Muinga *et al*, 1995; Sarwatt, 1992; Hindrichsen *et al.*, 2002). However, the adoption of forage legumes in smallholder mixed crop-livestock enterprises is still low. This study, therefore, aimed at evaluating the suitability of some forage legumes, already being used as cover crops and green manure for crop production and in agroforestry, as protein supplements in Napier grass-based diets. Their possible use as animal feed will enhance their value to the smallholder farmer. In this study, the use of leguminous protein-rich forages to improve the quality of Napier grass silage was investigated.

The presence of high crude protein level in a leguminous forage species is, in itself, not an adequate criterion for its inclusion in animal feeding programmes. Other factors that should be considered are the degradability of the crude protein fraction and the presence and activity of secondary plant metabolites that may negatively affect rumen microbial activity (Ørskov and McDonald, 1979). These aspects were investigated for the legumes used in soil conservation and agroforestry (*Mucuna pruriens*, *Crotalaria ochroleuca*, *Leucaena leucocephala* and *Lablab purpureus*) and others (*Acacia angustissima*, *Calliandra calothyrsus*, *Morus alba* and *Acacia tortilis*) that have potential for use as protein supplements in dairy cattle diets in the smallholder dairy farms in Kenya.

There is a dearth of information on the effects of including legume forage, during ensiling, on the quality and feeding value of tropical grass silage. The tropics are endowed with a diversity of both leguminous and non-leguminous protein-rich forages. However, it is important to screen them for antinutritive factors. In this study, the effect of tannin activity in PRFs on digestibility was investigated by *in vitro* gas production techniques using PEG as a tannin binder.

This study sets out to demonstrate that under the prevailing circumstances, making silage from PRF-supplemented Napier grass has practical and nutritive advantages over other options of dry season animal feed supply.

2 OBJECTIVES

2.1. General objective

To study the effect of supplementation of Napier grass with protein-rich forage and molasses on silage characteristics, voluntary intake, *in vivo* digestibility in sheep and *in vitro* fermentation kinetics.

2.2. Specific objectives

2.2.1 To study the fermentation characteristics of Napier grass cut at different stages of growth when ensiled singly or in combination with leguminous forages and molasses.

2.2.2 To investigate *in vitro* fermentation kinetics and metabolisable energy content of Napier grass, legume forage, and Napier grass/legume silages using *in vitro* gas production technique.

2.2.3 To determine the voluntary intake, *in vivo* digestibility and nitrogen balance in sheep fed legume-supplemented and non-supplemented Napier grass silage.

2.2.4 To evaluate the effect of tannin activity in leguminous browse plant species on *in vitro* fermentation, metabolisable energy content, and nitrogen degradability.

3 LITERATURE REVIEW

3.1 An overview of the small holder dairy sector in Kenya

The dairy cattle population in Kenya is estimated at 3.5 million head, 80% of which are owned by smallholder dairy farmers who generally practise mixed crop-livestock production activities (MALDM, 1997). The smallholder dairy farms are mainly located in the highlands, where, due to favourable climatic conditions for arable agriculture, the population pressure is very high resulting in stiff competition for land use between crop production and forage production (Mureithi *et al*, 1998). Individual land parcels are small and average below 2 hectares (Nyangito, 1992) with a herd size rarely exceeding 2-3 lactating cows and their followers. The cut-and-carry method or semi-zero grazing is practised due to the shortage of grazing land.

The Kenyan climate is characterised by two main seasons (wet and dry). During the wet season, pasture growth is fast and there is enough feed for the animals. Due to the rapid growth, there is rapid increase in dry matter, fibre and lignin resulting in concomitant decrease in nutritive value (Muia *et al.*, 1999). In the dry season pasture growth is limited and most of the available animal feed is in form of low quality crop residues and standing hay (Preston and Leng, 1987). This fluctuation in feed quality and supply leads to fluctuations in animal production (growth and milk yield) (Muia, 2000). The need for feed conservation as a means of levelling the nutrient supply to ruminants throughout the year cannot be over-emphasized.

The smallholder dairy farming in Kenya evolved from the pre-independence large-scale white settler farming (Mosi, 1984) where mechanized feed conservation mainly as silage in large trench and tower silos was a key component. However after independence and subsequent sub-division of land into small units, feed conservation as practiced by the pre-independence farmers was not feasible under the new 'zero grazing' systems that emerged, although the genetic potential of the animals did not change much. Prices of milk also remained fairly constant throughout the year, as they were government controlled (Abate, 1992), which implied that farmers could still make money from milk even during the 'flush periods'. However, since liberalization of the economy in the early 1990s, milk prices are subject to market forces (Owango *et al.*, 1996) and, therefore, tend to be very

low during the wet seasons and very high during the dry seasons (MoALDM, 1993). There is need to even out the milk supply pattern and hence prices through feed conservation using techniques that are adoptable by the smallholder farmers.

3.2 General characteristics of tropical forages

Most tropical grasses are C4 plants (Van Soest, 1994). These distinguish themselves from the temperate grasses (usually C3 plants) in that their first stable products of photosynthesis are four carbon compounds. In addition, their vascular bundles are more closely spaced and they have fewer mesophyll cells (Wilson *et al.*, 1989). They are photosynthetically more efficient and accumulate biomass more rapidly, often low in nutritive value and store starch unlike temperate plants, which store the water-soluble fructans (Barden *et al.*, 1987; Van Soest, 1994). Tropical grasses generally produce more biomass per gram of absorbed nutrients than temperate grasses (Bayer and Waters-Bayer, 1998). This means that the nutrient concentration in the biomass of tropical grasses is lower. Tropical grasses and legumes are characterised by relatively higher cell wall contents (lignin, cellulose and hemicellulose) at the same stage of growth than temperate forage plants (Cowan and Lowe, 1998). This is reflected by their higher content of crude fibre and detergent fibres than temperate grass species at the same stage of growth (Catchpoole and Henzell, 1971; Jarrige *et al.*, 1982; Bayer and Waters-Bayer, 1998). Consequently, they have been reported to have, on average, 15 units of digestibility lower than temperate grasses and this is greatly influenced by stage of growth (Van Soest, 1994).

Digestion of polysaccharides from intact cell walls is limited by the presence of phenolic compounds within the cell matrix (Hartley and Akin, 1989; Akin, 1989). The phenolics consist mainly of lignin and phenolic acids chemically bound to lignin or directly to cell wall polysaccharides. Lignin is virtually indigestible and is the major non-polysaccharide component of plant cell wall (Van Soest, 1994). It is implicated in limiting the digestion of cell wall polysaccharides by its close association and the formation of covalent bonds with cellulose and hemicellulose (Van Soest, 1994). Tropical forages exhibit higher rates of lignification than temperate ones and this partly explains the lower degradability. The lignin in grasses is 61% more inhibitory to microbial enzymes than that of legumes (Buxton and Russel, 1988). Therefore at similar lignin concentrations, legumes are more digestible than grasses.

Compared to temperate forage plant species, tropical forages have lower levels of soluble fermentable cell-contents (Cowan and Lowe, 1998). Water-soluble carbohydrates (WSC) are a group of non structural carbohydrates comprised of glucose, sucrose, fructose and fructans, the latter only occurring in temperate plant species (Van Soest, 1994). Fructans are levans consisting of β (2-6) linked fructofuranose units terminating in sucrose residues. They are the most abundant soluble carbohydrates in temperate grasses. Tropical grasses on the other hand accumulate starch in their vegetative tissues (Smith 1973). Starch is only sparingly soluble in cold water and is not fermented by lactic acid bacteria directly unless it is broken down to simpler sugars by other microorganisms in silage (McDonald et al., 1991). The sugar content of plants is greatly affected by the prevailing ambient conditions (Thomas and Morrison, 1982). For example, high light intensity and photosynthetic rate increase whereas high temperatures lower the sugar content by increasing the metabolic rate (O'Kiely and Muck, 1998). In darkness, plant respiration continues to break down the sugars made by photosynthesis (Barden *et al.*, 1987). Therefore, in tropical conditions where temperatures are usually high and the nights long and warm, the grasses would be expected to have lower sugar content than temperate grasses in summer when the daylight may be as long as 16 hours.

Immediately after harvesting of forage, both sucrose and fructans are rapidly hydrolysed to glucose and fructose (McDonald *et al.*, 1991; Elferink *et al.*, 2000)). Since WSC's are what is utilized by fermentation bacteria during the silage making process, it follows that the levels present in tropical forage plants are inadequate to produce enough lactic acid to prevent spoilage during ensilation. Figuerido and Marais (1994) reported a range of 23-41 g/kg DM soluble carbohydrates in Kikuyu grass. McDonald *et al* (1991) summarised the reported water-soluble carbohydrate levels in various forage species (Table 3.1).

The problem of low concentration of WSC is aggravated by the higher buffering capacity of tropical forage material (Weissbach, 1996). This refers to the ability of plant extracts to resist pH change when an acid is added to them and is defined as the equivalents of acid per unit DM required to lower crop pH from 6 to 4 (O'Kiely and Muck, 1998). Buffering capacity is highest in legume plant species and is thought to be due to the presence of high levels of organic acids as well as high protein levels (McDonald et al., 1991). As expected, this leads to higher pH values in silage with resultant proliferation of spoilage microorganisms such as Clostridia bacterial species. This problem may be solved by use

of additives, which increase the soluble sugar content of the ensiled material (Preston and Leng 1987; Snijders and Wouters, 1990; McDonald, 1991; Elferink *et al.*, 2000). Research in this area has come up with additives that lower the silage pH. Organic acids such as formic acid or mixtures of various acids have been tried with promising results (Elferink *et al.*, 2000; McDonald *et al.*, 1991; Bolsen *et al.*, 1995).

Table 3.1: Water-soluble carbohydrate (WSC) contents of some forage plant species

Group	Species	WSC (gkg^{-1} DM)
Temperate grass Spp.	Rye grass	170-241
	Timothy grass	110
Tropical grass Spp	Elephant/Napier grass	46[1]
	Digitaria eriantha	27[2]
	Setaria sphacelata	45-61
	Rhodes grass	30-35
	Paspalum dilatum	27-34
Cereal crops	Barley	140-318
	Maize	100-300

Source: McDonald *et al.* (1991), [1]Mühlbach, (2000),
[2]Meeske *et al.* (1999)

3.3 Common forages for silage making in the tropics

Maize has been one of the most important silage crops in temperate countries as well as some tropical countries such as South Africa, Zimbabwe and Kenya (Titterton and Bareeba, 2000). Its popularity is due to the high yield obtained in a single harvest, high water-soluble carbohydrate content and its high energy value as a feed (Ashbell and Weinberg, 2000). However, deteriorating weather conditions, population pressure on land and competition with use for human nutrition has made maize less and less available for silage making (Titterton and Bareeba, 2000). In the smallholder mixed crop-livestock production systems, maize forage is usually only available for ruminant production after the harvesting of green maize or after complete maturity and drying (Methu *et al.*, 1996;

Kaguongo *et al.*, 1996). Such material is of low quality and efforts to conserve it as silage may not be justified. This has led to the search for other alternative forages. The alternative crops investigated include grain sorghum (Havilah and Kaiser 1992; Nichols *et al.*, 1998; Ashbell and Weinberg, 2000), and millet (Tielkes and Gall, 1998; Ward *et al.*, 2001) among others. These, however, also have low crude protein contents 7-9.5% on DM basis (Kariuki *et al.*, 1998) and none can match the WSC content of maize forage.

The crude protein content of maize silage and probably that of Napier grass silage may be improved by intercropping with a leguminous crop such as *Dolichos lablab* or Desmodium species. Kariuki *et al.* (1999) reported data on the intercropping of Napier grass with *Desmodium intortum*. In their study, intercropping with legume increased the crude protein content from 117 to 142 gkg^{-1} DM for Napier grass alone and with legume respectively. These results were obtained despite the fact that Napier grass received nitrogen fertilizer whereas none was applied to the intercrop. The higher CP content is attributable to nitrogen fixation by the legumes and the actual presence of legume forage, which is higher in protein than the grass. Ensilage of such intercrops would, therefore, be expected to result in silage of higher crude protein content. Even in temperate conditions, there are recent research reports on the bi-cropping of wheat and legumes and the ensilage of the same with resultant better quality silage (Adesogan *et al.*, 2002; Salawu *et al.*, 2001).

Sole crops of maize and protein-rich forage may be mixed just before ensiling. Many alternatives are available for this type of application and their use will depend on local availability. In the Kenyan highlands, *Leucaena*, *Desmodium*, *Calliandra*, *Lablab*, and *Morus alba* are available to differing extents. After mixing maize forage and legumes (50:50) before ensilage, Titterton and Maarsdorp (1997) reported an average pH of 3.7-4.5 and an NH_3:N ratio of less than 12% in the resultant silage. The crude protein increased from 7% for pure maize silage to 9.3% (maize and yellow lupin) to 15.3% (maize and forage soybean). Maize and *Dolichos* bean silage had 12.8% crude protein. This silage was stored in plastic bags and the results were replicable in conventional pit silos (Taruona and Titterton, 1996). Regan, (1997) ensiled a mixture of the legume *Calopogonium mucunoides* and Napier grass in plastic drums without additive and reported fair silage quality though this could probably have been improved by addition of a source of soluble carbohydrates.

3.4 Potential forages for tropical silage making

3.4.1 Napier grass (*Pennisetum purpureum*)

Napier grass (*Pennisetum purpureum*) is the main forage feed for dairy cattle in the smallholder mixed crop-livestock production system in Kenya (Anindo and Potter 1986). The crude protein content is generally low, ranging from 12% in early vegetative stage to below 5% in late maturity (Kariuki *et al*, 1998, Muia *et al.*, 1999, Abdulrazak *et al.*, 1996). The CP content also depends on the agronomic practises such as fertilizer application or manuring (Snijders *et al.*, 1992). Since most smallholder farmers do not use fertilizer on Napier fields, the CP figures are likely to be on the lower levels at the farm. Table 3.2 shows Napier grass composition reported from previous investigations.

Table 3.2: Chemical composition and dry matter productivity of Napier grass

Nutrient/parameter	Young	Mature	Source
DM g/kg	170	260	Anindo and Potter 1986
CP g/kg DM	85-118	45-63	Kariuki *et al.*, 1998, Muia *et al.*, 1999
OM g/kg	788	797	Kariuki *et al.*, 1999
ME for cattle (MJ/kg DM)	8.6	8.6	NRC, 1988, Kariuki *et al.*, 1998
ADF g/kg DM	204-236	384	Kariuki *et al.*, 1999
NDF g/kg DM	593	611	Kariuki *et al.*, 1999
CF g/kg DM	230	390	Kariuki *et al.*, 1993
WSC g/kg DM	Not reported	46	Mühlbach (2000)
DM productivity	10-40 tonnes/ha/yr		Anindo and Potter 1986

The foregoing shows that Napier grass is best used for ruminant feeding at the early stages of growth since the nutrient levels at the mature stage are low. However, the actual situation at the farm level is the opposite in that the farmers feed the animals in most cases on overgrown Napier grass (Kariuki, 1998). This happens in the rainy season when Napier grass growth is fast and the farmer is not able to cope with the fast onset of maturity and lignification. Some farmers usually leave a stand of Napier grass as storage feed for the long dry seasons (Anindo and Potter, 1994). Feeding animals with such low quality

overgrown Napier grass hardly meets the animals' maintenance requirements and leads to losses of production. This may be prevented by preserving the Napier grass at the optimum stage of growth where the nutrient concentration is optimum e.g. at 6-8 weeks of growth (Muia *et al.*, 1999).

Due to its thick succulent stems, Napier grass does not preserve well as hay due to the long time needed to dry the cut forage, resulting in great losses via aerobic respiration and molding of inadequately dried hay (Brown and Chavalimu, 1985). The other problem is that dried Napier grass leaves are hard and brittle and the palatability is low. Therefore, the best option for Napier grass preservation is silage making.

Reports on nutritive value of ensiled Napier grass, alone or with additives, are scanty. Brown and Chavalimu (1985) reported tests on small-scale preservation of Bana and Pakistan varieties of Napier grass using nested polyethylene bags. Though they did not measure fermentation parameters or carry out a feeding trial, the evidence of molding of the ensiled material was a good indicator of poor fermentation. Snijders and Wouters (1990) ensiled Napier grass with or without addition of molasses (3-6%) and reported good butyric acid (0.01-0.5%) and ammonia nitrogen (less than 11% of total N) concentrations. Panditharatne *et al.* (1986) reported on the ensilation of NB-12 (*Pennisetum purpureum* x *Pennisetum americanum*) harvested at 1, 2 and 3 weeks of age and ensiled singly or in combination with cassava tuber meal, coconut oil meal or formic acid. They reported that acetic acid concentration was lowest when the grass was harvested after 1 week of growth and that addition of cassava tuber meal or coconut oil meal decreased pH and increased lactic acid concentration of the resultant silage. Neiva *et al.* (2000) ensiled 10 week-old Napier grass with dehydrated sugar cane as an additive. They reported linear increase in DM content with increasing sugar cane inclusion but a reduction in crude protein due to dilution (low CP in sugar cane). The fermentation parameters were not affected by the treatments.

3.4.2 Herbaceous forage legumes

3.4.2.1 Velvet bean (*Mucuna pruriens*)

Velvet bean is a leguminous herbaceous that has, in recent years, been tried as a cover crop in rubber plantations in West Africa and in smallholder mixed farming systems in

East Africa (Maobe *et al.*, 1998). While screening for suitable legumes for green manure, Maobe *et al.*, 1998 identified *Mucuna pruriens, Crotalaria ochroleuca, C. juncea* and C. *Vicia* species as the most suitable, based on biomass production, nodulation and nitrogen fixation. Annual dry matter yields of over 4T/ha have been reported in West Africa for *Mucuna*, *Crotalaria* and *Cajanus* (Carsky and Ndikawa, 1998). Despite its high crude protein content (250 g/kg DM (Ravindran, 1988)), few investigations have been carried out on the use of this forage as a supplement for poor quality ruminant feeds. Ravindran (1988) reported the chemical composition of velvet bean forage at different stages of growth of (Table 3.3).

Table 3.3: Chemical composition of velvet bean (*Mucuna pruriens*) forage at different stages of growth (adapted from Ravindran (1988)

Parameter	Plant age (days)			
	60	90	120	150
DM g/kg	182	211	237	204
CP g/kg DM	248	206	148	128
EE g/kg DM	28	27	25	24
Ash g/kg DM	55	58	46	64
ADF g/kg DM	332	401	458	479
Cellwall g/kg DM	402	514	568	592
Cell contents g/kg DM	597	487	432	409
Hemicellulose g/kg DM	71	112	110	112
Cellulose g/kg DM	279	311	362	375
IVOMD	66.4	55.4	50.6	42.2

The above findings suggest that Mucuna forage has comparable composition to other frequently used high protein forage such as *Lucerne* and *Desmodium* (Makoni, 1993; Kariuki, 1998, Tolera *et al.*, 2000). It has, however, the advantage of being adapted to tropical environments and therefore able to tolerate higher environmental temperatures and other abiotic stresses (Buckles *et al.*, 1998). It exhibits aggressive growth and is not suppressed by weeds. Therefore, if it could be used as a ruminant feed supplement in the Kenyan smallholder dairy sector, it would solve two problems at the same time, namely; that of low soil fertility (via soil nitrification) and poor quality feeds.

There are few reports in the literature on the voluntary intake or palatability of Mucuna forage by ruminants (Muinga *et al.*, 2000). *Mucuna pruriens* has been recommended for use as a pasture species in Sri Lanka (Ravindran, 1988). There were no reports on ensilage of *Mucuna Pruriens* in the literature reviewed.

3.4.2.2 Crotalaria ochroleuca

This is a leguminous plant introduced in southern Tanzania for improving soil fertility and combating weeds (Kullaya *et al.*, 1998). It is used for feeding dairy cattle and goats and initial investigations (Mkiwa *et al.*, 1990a and 1990b) showed that it has a high potential as a protein-rich forage. It has recently been introduced in Kenya for the purpose of green manuring in the high potential areas where the soils are deteriorating due to over-cultivation (Maobe *et al.*, 1998). Reported values of various parameters as pertains to *Crotalaria ochroleuca* are shown in table 3.4.

Table 3.4: Some important nutritional parameters of *Crotalaria ochroleuca*[1]

Parameter	Stage of Growth (weeks)			
	2	6	10	16
Yield kgDM/ha	60	1800	4515	4670
DM g/kg	144	155	243	334
CP g/kgDM	390	302	269	99
CF g/kgDM	184	253	365	427
Ca g/kgDM	12	7.2	8.0	7.7
P g/kgDM	3.8	3.7	3.5	3.2

[1] *adapted from Mkiwa et al., 1990a*

Sarwatt (1990) reported that growth rates, DM and organic matter digestibilities increased for sheep on a basal diet of grass hay supplemented with *Crotalaria ochroleuca*. However, the use of dried Crotalaria to replace sunflower seed cake for growing sheep resulted in reduced growth, digestibility, N intake and N retention (Sarwatt, 1992). Further, Mkiwa *et al.* (1994) tested the effect of feeding 10, 30 and 50% of Crotalaria seeds and leaves to growing rats and reported depressed growths when the inclusion rate went above 10%, which they attributed to anti-nutritive factors. This, however, is unlikely to be the case in

ruminants where the anti-nutritive factors are modified by rumen microbial activity before they reach the absorption sites in the lower gut.

3.4.3 Multipurpose trees (MPTs) and shrubs

3.4.3.1 White mulberry (*Morus alba*)

Morus alba is a fast growing shrub of the family *Moraceae* originating from south east Asia and introduced as an exotic MPT in parts of east African highlands especially in Kenya and Tanzania (ICRAF online, 2003). In Asia, the leaves are used to feed silkworms. Mulberry leaves are well accepted by livestock and up to 6 kg/day can be consumed by cattle (ICRAF online, 2003; Shayo, 2000; Liu *et al.*, 2000).

Though suited for temperate and sub-tropical climates, mulberry has been shown to produce substantial amounts of forage in semi-arid areas of central Tanzania (Shayo, 2000). Mulberry leaves have relatively high CP content ranging from 180 to 270 gkg^{-1} DM (Liu *et al.*, 2000; Ojeda *et al.*, 2000) and may be used as a protein supplement in ruminant diets thereby reducing competition for oilseed cakes and meals. Unlike the common tropical MPTs, mulberry is not leguminous and cannot fix atmospheric nitrogen and therefore its culture requires to be fully linked with farm nitrogen recycling via animal manure (ICRAF online, 2003).

There are few reports on the preservation of mulberry forage as silage alone or in combination with other forages. Ojeda *et al.* (2001) reported on the ensilage of mulberry forage in laboratory silos (3 kg fresh forage capacity) with and without addition of molasses (2, 4 and 6 %) and formic acid (0.1, 0.2 and 0.3%). They reported lower pH values (4.8 and 4.6) for silages treated with 4 and 6% molasses than the control and acidified silages. The ammonia nitrogen levels were generally low across all treatments and ranged from 6.2 for untreated, wilted silage to 14.0 for silage treated with 0.3% formic acid. Their results therefore show that satisfactory silage can be made from *Morus alba* using molasses as an additive.

3.4.3.2 *Acacia angustissima*

Acacia angustissima is a member of the *Mimosaceae* family originating from central America (Dzowela, 1994). Unlike most other Acacias, it is thornless and has a high potential growth rate and nitrogen fixing capabilities (Factnet, 1999). It is still under investigation to determine its potential as a multipurpose tree for use in agroforestry farming systems (Factnet, 1999). Its biomass production ranges from 10.3 to 11.4 $tDMha^{-1}$ (Brook et al., 1992) and can withstand frequent cutting. It is reported to have high tannin activity (McSweeney *et al.*, 1999). However, there are conflicting reports on its effect on animal performance and digestive mechanisms (Odenyo *et al.*, 1997; Larbi *et al.*, 1998; Smith et al., 2001; Hove *et al.*, 2001). Whereas, Odenyo (1997) reported high toxicity of *A. angustissima* forage in Ethiopian highland sheep, Hove *et al.* (2001) reported satisfactory performance in sheep fed on Native pasture hay supplemented with *A. angustissima* leaves. It was postulated that the tannin activity in different accessions may be different. Based on the literature reviewed, there were no reports on ensilage of *Acacia angustissima*.

3.4.3.3 *Leucaena leucocephala*

Leucaena leucocephala is a fast-growing, multipurpose, nitrogen-fixing tree of the tropics with a high potential as a forage legume (Jones, 1979). It is native in Central America and Mexico (Brewbaker, 1987; NFTA, 1990) but can now be found in most tropical regions. It may be used for wood production but is best known as a fodder plant for ruminants. The forage and wood production from *Leucaena* and other multipurpose trees and shrubs depend on climatic considerations, soil fertility, and method and frequency of harvesting. Akkasaeng *et al.* (1989) reported an accumulated dry matter production of 2 kg/tree for *Leucaena leucocephala* when cut at 1 metre for five times over a 30 month period. Sampet and Pattaro (1987) evaluated the productivity of *Sesbania grandiflora*, *Gliricidia maculata* and *Leucaena leucocephala*. The researchers reported that *Leucaena* gave higher 2 year total edible dry matter (EDM) yields (17.39 t/ha) than both *Sesbania grandiflora* (9.96 t/ha) and *Gliricidia maculata* (9.85 t/ha). They further observed that the forage production of all legumes dropped markedly during the dry season but *Leucaena* was still a better producer even under such conditions.

Leucaena leucocephala is generally well accepted by animals and has relatively high crude protein and dry matter digestibility (NFTA, 1990). Akkasaeng et al. (1989) reported crude protein level of 200 g/kg DM and an in vitro dry matter digestibility (IVDMD) of 62%. Sampet and Pattaro (1987) reported 252 g/kg DM CP and organic matter digestibility of 65%. Larbi *et al.* (1998) reported CP of 280 and 260 g/kg DM for *Leucaena leucocephala* edible forage harvested in the wet and dry season respectively. These values were higher than for *Leucaena diversifolia* in the same study (237 and 226 g/kg DM). The rates and extent of nitrogen degradation for *L. leucocephala* and *L. diversifolia* were reported to be 0.04 and 0.02/hour and 706 and 685 g/kg DM respectively (Larbi *et al.*, 1998).

There are several reports on the use of *Leucaena leucocephala* as a supplement to basal forage diets. Hove et al. (2001) reported on the supplementation of native pasture hay with *L. leucocephala* in sheep diets. In their study, *Leucaena* had a crude protein content of 200g/kg DM. The inclusion of *L. leucocephala* in the diet at 80, 160 and 320 g/day increased the digestible DM intake from 228 for the control to 298, 328 and 496 g/day respectively.

Despite high intake and digestibility, *Leucaena* may have a negative effect on animal health owing to the presence of the non protein amino acid mimosine which comprises 3-5% of the dry weight of the protein (Liener, 1980). Rumen bacteria convert mimosine to 3,4-dihydroxypyridine (DHP). DHP affects the thyroid gland and can lower the level of thyroid hormone in the blood thus depressing growth and liveweight gain (Jones and Jones, 1984). In a study involving beef cattle grazed on leucaena-grass pastures for 4 years, Jones *et al.* (1989) reported that the calving percentage of cows grazing leucaena (66%) was significantly lower than the herd average (92%). In recent years, the problem of mimosine toxicity has been overcome by the discovery in Hawaian goats of a rumen bacteria type capable of degrading DHP (Jones and Megarrity, 1986).

An even greater challenge for the utilisation of *Leucaena leucocephala* in farming systems is its susceptibility to parasitic infestation of by the psyllid bug (*Heteropsylla cubana*) (Reynolds and Bimbuzi 1993). This has led to increased research into alternative, psyllid resistant Leucaena species such as *L. diversifolia* (Nherera *et al.*, 1998; McSweeney *et al.*, 1999; Hindrichsen *et al.*, 2002), *L. pallida* (Kaitho *et al.*, 1998; Nherera *et al.*, 1998; McSweeney *et al.*, 1999) and *L. esculenta* (Nherera *et al.*, 1998). McSweeney *et al.* (1999)

reported higher *in vitro* apparent digestibility of DM for *L. leucocephala* in comparison to *L. diversifolia* and *L. pallida*, which they attributed to the higher total condensed tannin content in *L. diversifolia* and *L. pallida* (7.7 and 4.7% DM) compared to *L. leucocephala* (3.8% DM). Kaitho *et al.*, 1998 reported that supplementation of teff straw with *L. leucocephala* in sheep diets resulted in higher digestible organic matter intake (27 to 33 g $kg^{-0.75}$) compared to *L. pallida* (28 to 29 g $kg^{-0.75}$).

Studies on the preservation of *Leucaena leucocephala* as silage singly or in combination with other forage are few. Kato *et al.* (2001) reported on the ensilage of tropical leguminous multipurpose trees and shrubs and maize fodder. In their study, *Leucaena* silage had a pH of 5.3 and an ammonia nitrogen content of 73.3 g/kg total nitrogen. *Leucaena* fermented poorer than *Gliricidia sepium* and maize fodder which had lower pH (5.08 and 3.86 respectively).

3.4.3.4. *Calliandra calothyrsus*

Calliandra calothyrsus is a small leguminous tree originating from central America and introduced to other parts of the tropical and subtropical world (Dommergues, 1987). Due to its high nitrogen fixation and production of abundant litter with rapid decay, it is used for improvement of degraded soils (Young, 1989). It usually has high crude protein content and is used as a protein supplement for ruminants subsisting on poor quality forages and crop residues. A wide range of CP content in *Calliandra* has been reported; Hove *et al.* (2001) reported a CP content of 119 g/kg DM lower than 180 g/kg (Seresinhe and Iben, 2003) and over 200g/kg reported by Ahn *et al.* (1989), Balogun *et al.* (1998) and McSweeney *et al.* (1999).

Reports in the literature are in agreement that *Caliandra calothyrsus* is a high tannin browse, however, the reported condensed tannin contents vary widely between authors. Balogun *et al.* (1998) and Seresinhe and Iben (2003) reported low levels (6% DM). Higher levels (11% DM) were reported by Ahn *et al.* (1989), Hove *et al.* (2001) 19.6% and Jackson *et al.* (1996) 19-21% DM. These differences are likely to be due to the differences in the physiological state of the plant at the time of harvest and post harvest processing such as drying and grinding (Balogun *et al.*, 1998).

The high condensed tannin content in *Calliandra calothyrsus* has been reported to affect nitrogen and dry matter digestibility to different extents. Hove *et al.* (2001) reported that goats native pasture hay supplemented with *C. calothyrsus* excreted higher amounts of faecal nitrogen (95.8% N intake) than those supplemented with *Acacia angustissima* (63%) and *Leucaena leucocephala* (62%) suggesting lower nitrogen digestibility. In *in vitro* studies, Palmer and Jones (2000) reported higher increases in *in vitro* dry matter and nitrogen digestibility on inclusion of PEG (tannin binder) for *C. calothyrsus* than for *L. leucocephala*, an indication of higher tannin activity in *C. calothyrsus*. Using the rumen simulation technique (RUSITEC), Seresinhe and Iben (2003) reported lower in vitro dry matter digestibility for *C. calothyrsus* (36%) compared to *Gliricidia sepium* (62%). The IVDMD for *C. calothyrsus* increased to 54% on addition of PEG at a rate of 10% whereas that of *G. sepium* did not change.

Ensiling of *Calliandra calothyrsus* resulted in poor quality silage characterised by high pH (5.38) and low lactic acid content (9.4 g/kg DM) (Kato et al., 2001). In the same study, *Calliandra* had lower ammonia nitrogen content (12.7 g/kg total N) compared to maize forage, *Gliricidia* and *Leucaena* (102.8, 55.7 and 73.3 g/kg total N respectively). This was attributed to the higher tannin activity in *Calliandra* that may have inhibited the lactic acid bacteria and prevented breakdown of carbohydrates as well as protein.

3.5 The ensiling process

3.5.1 The concept of silage

Silage is the material produced by the controlled fermentation of fresh forage of relatively high moisture content (McDonald *et al.*, 1991). Forage preservation by ensiling involves a spontaneous anaerobic fermentation of forage water-soluble carbohydrates by lactic acid bacteria to lactic acid and, to a lesser extent to acetic acid. The acid production lowers the pH and consequently inhibits the activity of undesirable microorganisms such as clostridia species which cause spoilage (McDonald *et al.* 1991). To ensure ideal preservation conditions, the forage material requires to be tightly compressed to expel air. Anaerobic conditions should thereafter be maintained by effective sealing. Several reviews of the silage making process have been written (Elferink *et al.*, 2000, McDonald *et al.*, 1991, Titterton and Bareeba, 2000).

3.5.2 Raw material for silage making

The raw material for silage production should be good quality forage which may be either natural or improved pasture grasses and legumes or cultivated fodder crops such as maize, sorghum and wheat (Reid, 1982; Snijders and Wouters, 1990; McDonald *et al.*, 1991; O'Kiely and Muck, 1998; Titterton and Bareeba, 2000 and Ashbell and Weinberg, 2000). The material is harvested when it has the best compromise between dry matter yield and nutritive value. This is usually just before flowering for most plant species (O'Kiely and Muck, 1998). For Napier grass, the best time would be between 7 and 8 weeks regrowth (Kariuki *et al.*, 1998). If the crop is harvested too early, the nutritive value and the dry matter content will be low due to high water content in comparison to harvesting at a more mature stage (McDonald et al., 1991). In addition, the buffering capacity is highest in immature forage plants and decreases with maturity and, therefore, poor fermentation is expected (O'Kiely and Muck, 1998). High water content in ensiled forage leads to nutrient losses due to high effluent production. Field wilting of the cut forage can reduce the moisture content but this may be risky in the rainy season since the material could be rained on leading to losses of soluble components through leaching (Castle, 1982; Wilkins, 1988). On the other hand, over-mature forage will have relatively low water-soluble carbohydrate and crude protein and high fibre content leading to poor fermentation and low nutritional value.

3.5.3 Harvesting and ensiling

Harvesting is usually mechanised in large enterprises (Castle, 1982) but can be done manually in smaller enterprises depending on the size of the enterprise and labour availability (Snijders and Wouters, 1990; Regan, 1997). It is advisable to harvest on a dry, sunny day in order to allow the wilting of the forage to a dry matter content of at least 250-300g/kg (Castle, 1982). This increases the soluble sugar concentration in the silage and reduces the amount of effluent produced. However, it is also important to limit the duration of wilting to a few hours since aerobic respiration occurs and leads to breakdown of plant nutrients and loss of dry matter. Field losses of DM associated with wilting have been reported to average 20-30 $g.kg.^{-1}d^{-1}$ (Castle 1982).

After wilting it is advisable to chop the material to sizes below 1 cm to enables adequate compaction and to release the soluble sugars for microbial fermentation (O'Kiely and Muck, 1998). Grasses and legumes may be ensiled unchopped (Snijders and Wouters, 1990, Panditharatne *et al.*, 1986; Regan, 1997). The filling and compaction of silos may be done mechanically or manually depending on the enterprise and the resources available. Of importance is good degree of compaction to expel as much air as possible. For smallholder farmers, only the manual options are available although some farmers may possess a manual or a motorised chaff cutter. Compaction is done by feet trampling in plastic tube silos or by rolling a water-filled drum on forage in pit/trench and bunker silos (Snijiders and Wouters, 1990).

3.5.4 The fermentation process

Once the fresh material has been compacted into the silo and sealed, the ensiling process may be divided into 4 stages (Elferink *et al.*, 2000).

Stage 1. Aerobic phase: This lasts a few hours after the sealing of the silo. It is the time when the atmospheric oxygen trapped within the forage material is used up by aerobic respiration of plant cells and aerobic micro-organisms. Plant enzymes such as proteases and carbohydrases continue their activity and contribute to the breakdown of complex structural carbohydrates such as hemicellulose and storage carbohydrates. This phase is greatly dependent on the effectiveness of the compaction and sealing (Elferink *et al.*, 2000).

Stage 2. Fermentation stage: This is the most important phase in silage making and determines whether the forage will be preserved or become compost. It starts when the conditions in the silo become anaerobic and continues for several days to several weeks depending on the properties of the ensiled forage crop and the ensiling conditions. Bacteria present in the silo compete for the available nutrients and their relative success determines the quality of the silage produced. If the fermentation process is successful, homofermentative lactic acid bacteria predominate and ferment the water-soluble sugars to lactic acid. They are active in the pH range 4.0-6.8 (McDonald *et al.*, 1991).

Lactic acid bacteria (LAB) are divided into homofermentative and heterofermentative types (McDonald *et al.*, 1991, Elferink *et al.*, 2000). Homofermentative LAB are facultative anaerobes. In anaerobic conditions, they ferment hexose sugars to predominantly lactic acid. In the presence of oxygen, the end product may be acetic acid (Thomas and Morrison, 1982).

A rapid decrease in pH to a satisfactory level is essential to prevent undesirable microbial activity and butyric acid fermentation. If the acidification is inadequate, lactic acid is subjected to secondary fermentation to acetic acid, a weaker acid, and the silage deteriorates. The amount of lactic acid required to avoid this depends on the buffering capacity of the herbage (Weissbach, 1996) referring to the requirement of lactic acid for bringing the pH down to 4.0. The extent of acidification required for stable silage is also dependent on the dry matter content of the silage (Table 3.5).

Stage 3: Stable phase: If the anaerobic conditions are maintained, little changes occur and the silage remains preserved for a long time. The pH remains low and the microbial number decreases. Acid tolerant microbes survive this phase in an inactive state and Clostridia and Bacilli exist as spores.

Stage 4: Feed-out phase/ aerobic spoilage phase

This commences as soon as the silo is opened for purposes of feeding the silage to animals. It can also start earlier if the anaerobic seal is damaged e.g. by rodents. Spoilage sets in when the preserving organic acids (mainly lactic acid) and residual water-soluble carbohydrates are digested by acetic acid bacteria and yeasts (Elferink *et al.*, 2000). The oxidation of these nutrients results in the production of weaker acids, carbon dioxide and water, and the evolution of heat. This leads to an increase in pH and a proliferation of spoilage microorganisms such as Bacilli, Clostridia, Enterobacteria and moulds (O'Kiely and Muck, 1982). Losses of 1.5-4.5% DM per day may occur in all areas exposed to air and these losses can be minimised by ensuring a high feed-out rate (Elferink *et al.*, 2000). This is usually not possible in small enterprises due to the small number of animals. However, this could be circumvented by having smaller silos having a small cross-sectional area or treatment with organic acids such as formic acid and propionic acid (Bolsen *et al.*,1995). In all cases, the silage should be consumed by animals the same day as it is removed from the silo.

Table 3.5: Critical values of silage pH[1]

Dry matter (g/kg DM)	Critical pH
150	4.10
200	4.20
250	4.35
300	4.45
350	4.60
400	4.75
450	4.85
500	5.00

[1] *adapted from Weissbach (1996)*

3.6 Silage additives

There are over 150 different silage additives (Bolsen *et al.*, 1995). The main reasons for use of additives during silage making are to prevent secondary fermentation, breakdown of protein and the formation of butyric acid. Other reasons include the prevention of excessive effluent production in low DM silages, improvement of the crude protein content of the silage and the provision of readily fermentable/soluble carbohydrates especially in silages prepared from tropical forages. The efficacy of the additive is generally judged by its effect on fermentation parameters e.g. pH, concentrations of NH_3-N, lactic, acetic and butyric acids. Silage additives may be classified into the following categories:

3.6.1 Fermentation Stimulants

The success of ensiling forage depends on the water-soluble carbohydrates and dry matter contents at the time of ensiling (McDonald *et al.*, 1991). This means that forages having low WSC content (below 10% DM) do not ferment well. A source of WSC may, however, be added to the forage at the time of ensiling to provide substrate for the lactic acid bacteria. Such additives are referred to as fermentation stimulants. In the context of the smallholder dairy farmer in the tropics, sugarcane molasses is the most appropriate and affordable. Several studies have been reported on the use of molasses in tropical silage

making (Piltz *et al.*, 1999; Snijders and Wouters, 1990). Piltz *et al.* (1999) reported a reduction in pH and NH_3-N content of Kikuyu grass silage ensiled with molasses. Attempts to use dehydrated sugar cane as an additive in Napier grass silage did not lead to any gains in terms of reduction of pH or ammonia content (Neiva *et al.*, 2000). Though it is known that most naturally occurring LAB do not ferment starch directly (McDonald et al., 1991), some workers have used starch-rich additives during grass ensilage. Panditharatne *et al.* (1986) reported lower pH values and higher lactic acid content in Guinea grass and *Pennisetum purpureum x Pennisetum americanum* (NB-21) silage made with cassava tuber meal as an additive.

Lactic acid bacteria may also be added to forage material as fermentation stimulants. *Lactobacillus plantarum* is one of the most suitable bacterial for silage inoculation (McDonald et al., 1991). Others include *Lactobacillus brevis, Lactobacillus buchneri, Leuconostoc dextranicum* and *Leuconostoc messenteroides.*

3.6.2. Substrate sources

Tropical grass silages are generally of lower total nitrogen than temperate ones (usually less than 10g/kg DM), which is comparable to that of maize silage. It may, therefore, be necessary to supplement such forage with sources of nitrogen. Studies on the utilisation of maize silage by growing and lactating cattle have shown the necessity of supplying the diet with extra nitrogen to achieve adequate rates of live weight gain (Thomas and Wilkinson (quoted by McDonald *et al.*, 1991). These nitrogen sources may be in the form of urea, ammonia, biuret or poultry waste (Mühlbach, 2000). There are reports on the addition of urea, ammonia or poultry waste to maize forage, sugarcane tops or Napier grass at the time of ensiling and also to maize silage at the time of feeding (Snijders and Wouters, 1990; Bolsen *et al.*, 1995; Mthiyane *et al.*, 2001; Sarwatt *et al.*, 2001)). Urea may be included in silage in form of molasses-urea-mineral mixtures (Snijders and Wouters, 1990) or in solution with molasses (Sarwatt *et al.*, 2001).

Addition of urea or Poultry waste to forage before ensiling leads to the production of higher levels of fermentation acids. However, the pH values of the treated silages tend to be higher than for untreated silages due to the increased buffering capacity resulting from the NH_3 released from the urea (Snijders and Wouters, 1990; Bolsen *et al.*, 1995). Treated

silages exhibit higher crude protein, true protein, free amino acids and ammonia than untreated silages (Mthiyane et al., 2001). Urea treatment therefore has a sparing effect on true protein.

3.7 Secondary plant metabolites as antinutritive factors in tropical PRFs

The value of forages as feed supplements depends on their capacity to provide the nutrients deficient in the basal diet (Preston and Leng, 1987). For ruminants subsisting on high fibre roughage such as mature tropical grasses and crop residues, the first limiting nutrient for microbial activity is nitrogen which must not only be present in adequate quantities in the feed but must also be available to the micro-organisms (Tamminga, 1989). Tropical PRF's generally have good nutrient profiles (high CP, low NDF and ADF) compared to tropical grasses (Smith, 1992). However, they usually have high levels of secondary plant metabolites or anti-nutritive factors (Kumar and Vaithiyanathan, 1990; Reed *et al.*, 1990; Khazaal et al., 1994; Schofield et al., 2001; Mueller-Harvey, 2001). Antinutritive factors (ANFs) are defined as chemical features of plants, which result in lower levels of animal productivity than would be expected from proximate and mineral analysis of the forage. They also decrease the digestibility of organic matter and protein and adversely affect growth and milk production (Jones *et al.*, 1989; Balogun *et al.*, 1998; Seresinhe and Iben, 2003). Antinutritive factors include polyphenolic compounds such as tannins (Gupta and Haslam, 1989; Woodward and Reed, 1989; Schofield *et al.*, 2001), alkaloids, saponins, toxic amino acids such as mimosine and various other toxic agents (Liener, 1980). Many of the ruminant feed resources in tropical countries, especially trees and shrubs, contain tannins which, when present in sufficient amounts, decrease palatability and feed intake by ruminants (Woodward and Reed 1989).

3.7.1 Tannins and other polyphenolic compounds

Tannins are the most common secondary compounds in tropical fodder trees and shrubs. They are defined as water-soluble polyphenolic compounds having molecular weights between 500 and 3000 and having the ability to precipitate alkaloids, gelatine and other proteins (Gupta and Haslam, 1979). They may be classified as hydrolysable (gallo- and ellagitannins) and condensed tannins (proanthocyanidins). Hydrolysable and condensed tannins may be differentiated by their structure and reactivity towards hydrolytic reagents.

Hydrolysable tannins usually have a central core of glucose or other polyhydric alcohol esterified with gallic acid or hexahydrodiphenic acid. They are readily hydrolysed by acids, bases and certain enzymes (Kumar and Vaithiyanathan, 1990). Condensed tannins are either flavans, polymers of flavon-3-ols or flavon-3-4--diols. Heating in air converts condensed tannins to the corresponding anthocyanidins and phlobaphene-like polymers (Gupta and Haslam, 1979).

Both the condensed and hydrolysable tannins are generally present in tropical trees and shrub leaves bound to the neutral detergent fibre (Reed *et al.*, 1990). They are among the mechanisms developed by woody plants to defend themselves against herbivores (Goodchild and McMeniman, 1987). The presence of tannins in some unripe fruits and some immature grains gives them an astringent property, which acts as a defence mechanism against destruction by animals and birds (Gupta and Haslam, 1979). Leaves and apices from browse can contain up to 50% of their organic matter as phenolics including tannins that are soluble in aqueous acetone (Reed, 1995). Due to their protein binding capacity, tannins inhibit many enzyme systems and thus protect the plant cells from attack by fungi and other pathogens (Kumar and Vaithiyanathan, 1990; McSweeney *et al.*, 2001).

In ruminants, dietary tannins adversely affect rumen metabolism by bactericidal and bacteristatic activities and by inactivating several bacterial enzymes such as carboxymethyl cellulase, proteases, glutamate dehydrogenase and chymotrypsin. In addition, the availability of sulphur and iron becomes limited to animals consuming tannin rich diets (Kumar and Vaithiyanathan, 1990). Animal productivity may be lowered for animals consuming browse having a tannin content of up to 5% (Barry and Manley, 1984). This may be due to both a depression in feed intake as well as negative influence on digestibility of protein and carbohydrates (Woodward and Reed, 1989; Reed *et al.*, 1990; Leng, 1997).

Mueller-Harvey and Reed (1992) reported that the polyphenolic compounds; luteolin, apigenin and flavone derivatives, butin and dihyroflavonols in sorghum crop residues were highly negatively correlated with in vitro digestibilities. They suggested that selecting sorghum varieties for lower concentrations of the above mentioned polyphenolic compounds would increase the digestibility of sorghum crop residues. The same could be suggested for fodder trees and shrubs as concerns tannins and other phenolic compounds.

Tannins are protein binders (Makkar *et al.*, 1995). In the rumen milieu, protein may be in the form of feed protein, microbial cells and microbial extracellular enzymes. Binding by tannins renders feed protein undegradable (McSweeney *et al.*, 1999). It also inhibits the activity of microbial enzymes. At certain levels, tannins may also be directly toxic when absorbed by the host animal (Smith *et al.*, 2001).

3.7.2 Tannin binders

Due to the high content of tannins associated with tropical protein-rich forage, there is need to use tannin inhibitors to determine the effect of the tannins on true digestibility, nitrogen degradability, microbial yield and microbial nitrogen uptake and metabolisable energy.

Makkar *et al.* (1995) tested various agents having tannin-complexing properties including polyvinyl polypyrrolidone (PVPP, MW 2000-35000), polyvinyl pyrrolidone (PVP, MW 10000-360000) and polyethylene glycol (PEG, MW 2000-35000) on their ability to bind tannin extracts from trees and shrubs. They reported that PEG was the most effective followed by PVP and PVPP in that order. PEG 6000 was preferred to PEG 2000 and 4000 as its binding to tannins was higher at near neutral pH values that prevail in the rumen ecosystem. Since then PEG has been extensively used in *in vitro* fermentation studies of tropical tanniniferous forage. Getachew *et al.* (2000) used PEG 6000 to study the effect of tannins on the in vitro degradability of nitrogen and microbial protein synthesis from tropical tannin-rich browse (*Acacias*) and herbaceous legumes (*Desmodium* species). Jones and Palmer (2000) used ^{14}C-labelled PEG-4000 to study the effect of condensed tannins on *in vitro* digestibility of dry matter and of nitrogen while Baba *et al.* (2002) used PEG-8000 to study the effect of tannins on *in vitro* degradation of tropical browse plant species.

In recent years, tannin binders (mainly PEG) have been used in *in vivo* studies of the effect of condensed tannins on ruminant animal feeding behaviour, nutrient intake, digestion, and growth performance. Bhatta *et al.* (2002) reported that feeding of PEG-6000 at a rate of 5 g/day alleviated the effects of tannins of *Prosopis cineraria* on protein digestion in goat kids. Voluntary intake of *Prosopis* forage was increased and so was the growth performance. Provision of PEG-3350 at specific sites during a feeding experiment using

sheep fed with meals high in Quebracho tannin increased intake and foraging time in sites containing PEG than those not having PEG (Villalba and Provenza, 2002).

3.7.3 Methods for evaluating the effects of tannin on digestibility of browse

Like in evaluation of other types of ruminant feed, *in vivo* digestibility/feeding trials are the best suited. However, these are very expensive and labour intensive; requiring many experimental animals and long experimental periods. *In vitro* methods have, therefore, been developed to circumvent the shortcomings of the *in vivo* trials in evaluating large numbers of different feeds. Among the early techniques is the Tilley and Terry method (Tilley and Terry, 1963), which determines the disappearance of feed components after a 24-hour incubation in rumen fluid. The *in sacco* nylon bag method (Ørskov and McDonald, 1979) determines the dry matter disappearance from nylon bags incubated in the rumen of a rumen-fistulated animal. The *in vitro* gas production techniques (Menke *et al.*, 1979) measures the amount of gas produced by a feed sample incubated with buffered rumen fluid. To determine the amount of substrate truly degraded, the fermentation residue may be collected, refluxed with neutral detergent solution and weighed (Blümmel and Becker, 1997).

Ahn *et al.* (1989) used the *in sacco* method to study the effect of tannin content in tropical browse legumes (*Acacia aneura, Acacia angustissima, Albizia chinensis, Calliandra calothyrsus, Sesbania sesban, Albizia lebbek, Enterolobium cyclocarpum* and *Samanea saman*) on the DM and nitrogen degradability. They reported a poor correlation between digestibility of nitrogen and the total condensed tannin content measured by vanillin-HCl and butanol-HCl assays. Browse species, which had low condensed tannin content e.g. *Acacia aneura* and *angustissima* had lower nitrogen degradabilities than some species with higher tannin content. This has been attributed to differences in biological activities of tannins from different plant species or accession of the same species. This is best illustrated by *in vivo* experiments using *Acacia angustissima* in sheep; Odenyo *et al.* (1997) observed very low intakes and the sheep on this legume all died before completion of the experiment whereas Hove *et al.* (2001) reported satisfactory intake and performance of sheep fed the same legume but probably a different accession. Abdulrazak *et al.* (2000) investigated the correlation between the total extractable phenolics, total extractable tannins and condensed tannins and *in vitro* gas production in 6 *Acacia* species. They

reported a consistent weak correlation (-0.11 to -0.46). Some authors, however, have reported significant correlations between condensed tannins and gas production (Khazaal and Ørskov, 1994; Tolera *et al.*, 1997). There is however agreement that the 'nature' or biological activity of tannins is more important than the actual amount.

Since methods for correctly quantifying biologically active tannins in browse plants are yet to be developed, the best method for evaluation and ranking of tanniniferous forage is by incubation in rumen liquor and the measurement of gas production and *in vitro* degradability of nitrogen in the presence and absence of a tannin binder. For the purposes of assessing the antinutrive effects in browse plants the *in vitro* gas production method was found to be more efficient than the nylon bag technique (Khazaal *et al.*, 1994).

3.8 *In vitro* techniques for evaluation of ruminant feeds

In vitro methods of feed evaluation offer several advantages over *in vivo* methods; they are less expensive, more rapid and allow more precise control of incubation conditions than *in vivo*. Further to this, *in vitro* methods utilize very small amounts of test feeds and therefore suited to the screening of large numbers of different feeds. Most *in vitro* methods are based on gravimetric measurements that quantify the disappearance of the substrate.

The most common *in vitro* method is the two-stage Tilley and Terry method (Tilley and Terry, 1963). It is based on the incubation of the sample material in buffered rumen liquor for 48 hours followed by treatment with pepsin for a further 48 hours. The residue is taken to be the indigestible fraction of the feed incubated from which apparent digestibility can be calculated. This method has been validated using *in vivo* values. However, the main disadvantage is that it is an end-point measurement technique, i.e. gives only one observation per sample. It can be adapted for the measurement of the time course of the digestibility process but this involves lengthy and tedious undertakings. It does not normally provide information on the kinetics of forage fermentation since the residue determination destroys the sample. In studies on the *in vitro* digestibility of tanniniferous plants using PEG as a tannin binder, Getachew *et al.* (1998b) and Palmer and Jones (2000) reported formation of insoluble PEG-tannin complexes caused the underestimation of digestibility.

3.8.1 Menke's *in vitro* gas measuring techniques

An overview of the existing in vitro gas production techniques is presented in a recent review (Getachew *et al.*, 1998a). Though the fact that rumen fermentation produces gas has been known for a long time, the work of Menke *et al* (1979) led to the consideration of the gas measuring technique as a routine method for feed evaluation. In their study, they reported a high correlation between *in vivo* apparent digestibility and *in vitro* gas production. This method has since been standardised for the routine estimation of metabolisable energy using the gas production values, crude protein and fat (Menke and Steingass, 1987). Since the *in vitro* techniques attempt to simulate the activities that occur in the rumen *in vivo*, the conditions *in vitro* should be as close as possible to those *in vivo*. There are several factors that affect *in vitro* gas production. These are mainly factors that affect rumen bacteria such as provision of anaerobic conditions, control of temperature and pH and proper mixing of the incubation mixture (Menke *et al.*, 1979). In the *in vitro* gas technique of Menke *et al.* (1979), the rumen liquor is collected in a pre-warmed thermos flask and flushed with carbon dioxide. After mixing the rumen fluid with buffer, trace and major mineral solutions, the mixture is incubated with 200 mg of the test sample at 39°C.

In this system, gas volume produced after 24 hours correlated well with the *in vivo* organic matter digestibility determined in sheep (Menke et al., 1979). Steingass and Menke, 1986) used the 24-hour gas volume together with the concentration of other chemical constituents to predict the metabolisable energy.

The method of Menke *et al.* (1979) was modified by Blümmel and Ørskov (1993) in that the syringes were incubated in a thermostatically controlled water bath instead of the usual rotor-equipped incubator. This had the advantage that minimal drop in temperature occurs during the period of taking gas volume readings. This is particularly suited for use in studies of fermentation kinetics where the frequent recording of gas volumes is practised.

Though the method for ME determination from gas production has been validated and standardised using *in vivo* data (Steingass and Menke, 1986), there is still significant inter-laboratory variation (Getachew *et al*, 2001) which is ascribed to differences in rumen

liquor sources (animal species, physiological status), feeding regime of donor animals and the time of rumen liquor collection.

Other modifications to the method of Menke have been reported by Blümmel and Bullerdieck (1997). They increased the amount of sample incubated from 200 mg to 500 mg and determined *in vitro* apparent and true degradability concomitant to gas production. They came up with the concept of partitioning factor (PF), which relates the amount of organic matter truly digested to the volume of gas produced and describes the efficiency of microbial biomass yield from a particular feedstuff. This adaptation has since been used in studies to determine the effect of tannins in tropical browse plant species on microbial biomass yield (Baba *et al.*, 2002).

3.8.2 *In vitro* degradability of nitrogen (IVDN)

The degradability of intake crude protein in the rumen is one of the problematic issues in ruminant nutrition (Ørskov, 1982). The main difficulty lies in the fact that protein degradation in the ruminant is effected by microbial rather than host enzymes hence the residue is contaminated by microbial crude protein (Blümmel *et al.*, 1999a). Furthermore, there is continuous incorporation of feed nitrogen into microbial protein (Raab *et al.*, 1983). Protein of microbial origin must be separated from undegraded feed crude protein in the residue in order to determine the true degradability. The continuous influx of recycled nitrogen in form of urea into the rumen and the absorption of ammonia into the blood stream through the rumen wall make the situation even more complicated.

A number of methods are available for the estimation of protein degradability. The sampling of duodenal digesta from duodenally canulated animals followed by the analyses for ammonia nitrogen, microbial nitrogen and undegraded intake crude protein (non ammonia, non-microbial nitrogen) (Lebzien *et al* 1996) gives a close estimation of the degradability as well as the flow of metabolisable crude protein and the rumen nitrogen balance (RNB). However, the method is highly invasive (canulation at the rumen and abomasums or duodenum) and tedious and the animal may not reflect a normal physiological status due to the associated discomfort. Furthermore, the use of invasive surgical procedures for nutritional research is becoming less acceptable to the public due to animal welfare considerations. In addition, suitable markers are required for calculating

flow rate of digesta and for differentiation between microbial and dietary nutrients flowing to the duodenum (Lebzien et al., 1996).

Another method is the nylon bag or *in sacco* technique (Ørskov and McDonald, 1979) which measures the loss of crude protein from nylon bags suspended in the rumen in animals fitted with a rumen canula. This technique has to contend with contamination of the residue with bacterial protein (Ørskov, 1982). The disappearance of protein from the nylon bags may not necessarily mean that the said protein was degraded. It gets even more complicated when tanniniferous feedstuffs are being assessed since the amount of feed in the bags is minute compared to the rumen volume and therefore, may not have an effect on microbial activity as would occur in a practical feeding situation (Balogun *et al.*, 1998).

The two-stage *in vitro* method of Tilley and Terry (1963) may also be used for this purpose (Palmer and Jones, 2000) but similar problems are encountered as in the nylon bag techniques since the fermentation residue has to be washed, filtered, dried and analysed for crude protein therefore introducing errors associated with weighing minute quantities as well as contamination with microbial protein.

Raab *et al.* (1983) modified the *in vitro* gas production method of Menke *et al.* (1979) to measure the *in vitro* degradability of nitrogen by including the measurement of ammonia in the incubation medium at the end of incubation, in addition to the usual gas volume measurements. In this set-up, the sample is incubated alone or with starch. Incubation with starch increases gas production and reduces ammonia concentration in the incubation medium. The reduction in ammonia is rightly assumed to represent incorporation by rumen microbes since the system is closed and there is no absorption of ammonia as would occur in the rumen *in vivo*. A plot of ammonia concentration against gas production and linear regression curve-fitting yields a y-intercept value, which after subtraction of the ammonia concentration of blank incubation gives a theoretical value of ammonia produced by microbial breakdown of sample protein in the absence of substrate energy. Under such circumstances, microbial incorporation of substrate nitrogen is assumed to be negligible and therefore, to have no effect on ammonia released from the test feed. Division of the net ammonia produced from the test feed by the amount of nitrogen incubated gives the *in vitro* degradability of nitrogen (IVDN) as shown in the following equation:

$$\text{IVDN}(\%) = \frac{\text{NH}_3\text{-N at zero gas production-NH}_3\text{-N in blank incubation x 100}}{\text{Total N of feed incubated}}$$

This modification has been used for the determination of IVDN of poor quality forages (Getachew *et al.*, 1998b). By incubating different types of tropical leguminous browse and herbaceous plant species with polyethylene glycol (PEG), Getachew *et al.* (2000) reported several-fold increases in IVDN of some *Acacia*, *Desmodium* and *Quercus* species. In their study, the IVDN ranges were 19-40% in tannin-containing feed alone and 40-80% in tannin-containing feed with added PEG. The method is currently being used to determine the *in vitro* nitrogen degradability of various oilseed meals including Soya- and Rapeseed meals in Germany (Hovenjürgen, M., pers. Comm.).

3.8.3 Importance of protein and energy interrelationships in ruminant diets

One of the most important considerations in rumen digestion and transfer of dietary protein to the lower gut is the availability of fermentable energy (Poppi and McLennan, 1995). The major source of amino acids at absorption sites in ruminants is microbial protein. Microbial protein synthesis uses nitrogen from dietary protein, non-protein N and recycled N and requires energy for both the synthetic process as well as for supplying the carbon skeletons for the *de novo* synthesis of amino acids (Asplund, 1994). The crude protein and energy relationship in the diet may be expressed in various ways e.g. the ratio of CP:digestible organic matter (Poppi and McLennan 1995; ARC, 1984) or simply N:ME (GfE, 1997; NRC, 1988,).

Different researchers have come up with ideal/optimum ratios for various scenarios in the rumen. Synchronisation of the rate of rumen degradation of protein and energy has been shown to be beneficial in terms of microbial cell growth, rumen digestibility, efficiency of protein and energy utilization, and milk production (Huber and Herrera-Saldana, 1994). When nitrogen is degraded at a faster rate than the energy sources available for utilization, excess ammonia will be absorbed and transported via portal blood to the liver. Of this, some is recycled to the digestive tract but a large portion is lost through excretion in the urine. It should be the aim of ruminant nutritionists to provide rations with optimum N:ME ratios (approximately 1.97 g/MJ, GfE, 1997) which ensure adequate nitrogen and energy for rumen microorganisms.

4 MATERIALS AND METHODS

4.1 Experiment I: Evaluation of the effects of stage of growth, addition of molasses and leguminous protein-rich forage on the fermentation characteristics of Napier grass silage

The objective of this experiment was to study the fermentation characteristics of Napier grass silage when ensiled singly or in combination with leguminous forages and molasses. Napier grass (*Pennisetum purpureum*, variety Bana) at 3 stages of maturity was ensiled in miniature polythene tubing silos either alone or in combination with legume forages (*Mucuna pruriens*, *Crotalaria ochroleuca*, *Leucaena leucocephala or Lablab purpureus*). Each silo contained approximately 5kg of wilted material.

4.1.1 Experimental design

The experimental design was a completely randomised design (Neter, *et al.*, 1996) and a 3x2x2 factorial arrangement of treatments (Snedecor and Cochran, 1980). The factors were: three stages of Napier grass growth (4, 8, and 12 weeks old regrowth), supplementation with *Mucuna pruriens*, *Lablab purpureus*, *Crotalaria ochroleuca or Leucaena leucocephala* (0 or 20% on wilted weight basis) and molasses (0 and 3%) at the time of ensiling.

4.1.2 Forage production and ensilage

Napier grass (variety Bana) was established under irrigation at the Animal Husbandry Research Institute of the Kenya Agricultural Research Institute (KARI) at Naivasha in the Rift valley, latitude 00° 40' south, longitude 36° 26' east and 1900m above sea level. The Napier grass was clear-cut, weeded and fertilized with 100 kg/ha. of nitrogen, phosphorous and potassium (N:P:K, 20:10:10). To obtain uniform growth, the Napier grass was cleared and weeded again after a month. Subsequently, 2 respective sections measuring 2800m^2 were clear-cut after 4 and 8 weeks of re-growth. This ensured that the Napier grass was 4, 8 and 12 weeks old by the start of the experiment. The herbaceous legume forages were planted 12 weeks before the start of the experiment and were therefore 12 weeks old at the time of harvesting. However, *Mucuna pruriens* was

harvested at 16 weeks of age because the first planting failed and the Napier grass was too young to ensile when Mucuna was 12 weeks old. *Leucaena leucocephala* was obtained from established *Leucaena* hedges of 1m height.

The Napier grass was harvested manually using machetes and left to wilt for a period of about 24 hours to a DM content of above 200g/kg. Herbage legumes (*Lablab, Crotalaria* and *Mucuna*) were similarly harvested but *Leucaena* was harvested using hands to break the leafy terminal twigs. Wilting for legumes was done for only 6 hours to prevent losses associated with leaf shattering. Chopping was done using a motorised chaff cutter set to cut at 2.5 cm length. The chopped Napier grass was weighed and manually mixed with the appropriate amount of legume forage and molasses where necessary on a plastic sheet spread on the ground.

The mixtures were then immediately packed in quantities of 5 kg (wilted weight) in duplicate in polythene bags measuring (18 by 27 inches and a thickness of 500 gauge). Compaction was done manually by feet trampling and the bags were sealed using cello-tape after expelling out as much air as was physically possible. To avoid interference with the bags during storage, all the sealed bags were buried in 1x1x 0.5m pits lined with plastic sheets. Since opening of the silos for sampling would lead to air ingress into the silage material, silos were sampled only once. Therefore, to cater for the 3 sampling times (30, 60 and 90 days), triplicate silos were made for each treatment, so that once a bag was sampled, the remaining silage was discarded.

4.1.3 Sample collection and preparation

Samples of silage raw materials (chopped Napier grass and legumes) were collected before ensiling and before addition of molasses. These were referred to as the day zero samples or pre-ensiled material. The first set of silos was opened and sampled after 30 days of storage and the other two sets were opened after 60 and 90 days respectively. On opening the laboratory silos, the material found to be visibly spoiled (mouldy or clamped together) was separated and weighed separately.

To obtain a representative sample, the pre-ensiled forage or silage was thoroughly mixed on a plastic sheet spread on the ground then worked into a ridge from which fistfuls of

material were randomly taken to make up a sample of about 500g. About 100 g of the sample was immediately extracted with water for pH, ammonia and volatile fatty acid determination as described below. The rest was frozen in airtight plastic bags and later dried for chemical analyses.

4.1.4 Chemical analyses

Dry matter and Ash

Dry matter of forage and silage samples was determined by drying in an oven at 105°C overnight. Dry matter was calculated as the residue expressed as a percentage of the initial weight whereas the ash was obtained by ashing of dry matter residue in a muffle furnace at 550 °C overnight (AOAC, 1990 method 934.01). The organic matter was calculated as the difference between the dry matter and the ash.

Crude protein

The crude protein in forage and silage samples was determined by the nitrogen combustion method (Dumas method, AOAC 1990 method 990.03) using a Leco FP-328 analyser.

Detergent fibres

Dried forage and silage samples were ground in a Wiley mill to pass through a 1 mm screen. Serial NDF (neutral detergent fibre) and ADF (acid detergent fibre) determinations were carried out according to Van Soest and Robertson (1985) with the modification that the fibre bags method was used in place of the normally used Fibertec (Tecator) method. The samples (1 g) were weighed into pre-tarred dacron bags measuring 4 by 12 cm and pore size of 40 µm (Gerhadt, Germany). The bags were held upright by glass holders and put into a 1-liter spoutless beaker containing the appropriate detergent solution standing on a hot plate and held in place by a tripod stand. Each beaker could hold six bags or three samples in duplicate. The cooling system was a glass ball put on top of the beaker, through which a continuous flow of tap water was maintained. The boiling durations were 1 hour, similar to the standard procedure. The samples were then washed with about 1 litre of hot

distilled water and dried overnight at 105° C. After re-weighing of NDF residue in bags, the procedure was repeated for ADF and finally the ADF residue was ashed together with the bags overnight at 550° C. The fibre fractions were then calculated by subtracting the weight of the bags and ash. The ash originating from the fibre-bag fabric was assumed to be negligible and therefore having no effect on the calculations.

Water-Soluble Carbohydrates (WSC)

Water-soluble carbohydrates content was determined on oven-dried samples using the Luff-Schoor method according to VDLUFA (1997). This was carried out in the LUFA laboratory at Roleber, Germany. Water-soluble sugars include both easily soluble and easily hydrolysable substances e.g. glucose, fructose, disaccharides such as sucrose and fructans. The principle behind the procedure is the reduction of copper^{2+} salts by invert sugar to copper^{+}. The excess copper^{2+} is thereafter iodometrically determined. The analysis results were reported as total sacharrose units.

In addition to the above-mentioned analyses, the silage samples were analysed for the following:

Ammonia nitrogen

Sample extraction was done according to Meeske *et al* (1999), Nadeau *et al* (2000) and Fellner *et al* (2000). A 100 g sample of silage was mixed with 1litre of distilled water and blended for 45 seconds using a kitchen blender. The mixture was then left to stand in 1.5 litre glass jars covered with aluminium foil for two hours. The pH of the silage extracts was then measured using a glass electrode pH meter, which had been standardised with buffers of pH 4 and pH 7 before taking readings. The blended material was thereafter squeezed through two layers of cheese cloth and centrifuged at 2500 rpm for 30 minutes.
A 20 ml aliquot of the supernatant was put into a glass bottle and 4 ml of 20% sulphuric acid was added to lower the pH to below 3 to prevent loss of ammonia. The mixture was immediately frozen pending analysis.

Ammonia nitrogen was determined on a 5 ml sample aliquot alkalinized with 40% sodium hydroxide by Kjeldahl steam distillation and titration (AOAC, 1990). The results were

given as mg ammonia-N/100ml acidified silage extract and then converted to grams of ammonia-N /kg total N in the original sample dry matter.

4.1.5 Statistical analyses

The data were analysed by multivariate ANOVA (SPSS statistical package (SPSS inc. Release 11.0.1, 2001) using the following model:

$Y_{ijkl}=\mu+\alpha_i+\beta_j+\gamma_k+(\alpha\beta)_{ij}+(\alpha\gamma)_{ik}+(\beta\gamma)_{jk}+(\alpha\beta\gamma)_{ijk}+\varepsilon_{ijkl}$

Where: μ is the parametric population mean and α_i, β_j and γ_k are the fixed treatment effects for the ith, jth and kth levels of molasses, legume and age of Napier grass respectively; $(\alpha\beta)_{ij}$,$(\alpha\gamma)_{ik}$ and $(\beta\gamma)_{jk}$ are the first order interaction effects and $(\alpha\beta\gamma)_{ijk}$ the second order interaction effect. *Post hoc* tests (Duncan multiple range test) were performed for main effects found to be significant by ANOVA. In respect to pH, an extra treatment (effect of length of ensilage; 30, 60 and 90 days) was superimposed on the original design and was included in the analyses.

4.2 Experiment 2: *In vitro* gas production kinetics and estimation of metabolisable energy of pre-ensiled Napier grass, Protein-rich forages and Napier grass silage

The objective of this experiment was to determine the effect of molasses and protein-rich forage inclusion on *in vitro* fermentation kinetics and metabolisable energy of Napier grass silage. The Napier grass silage combinations outlined in experiment l were tested for the effects of inclusion of legume forage and molasses on the *in vitro* fermentation kinetics and the estimated metabolisable energy content. The Hohenheimer feeding value test (HFT, Menke and Steingass, 1987) was used to estimate the metabolisable energy content of the silage raw materials (Napier grass and legumes) and of the resultant silages.

4.2.1 Experimental design

The experimental design was completely randomized with a 3 factorial arrangement of treatments. The fixed factors were age of Napier grass (8 and 12 weeks), molasses inclusion (control and 3% on wilted weight basis) and legume inclusion (4 types of legumes each included at 20% wilted weight basis). The legumes used were, *Lablab*

purpureus, *Crotalaria ochroleuca*, *Mucuna pruriens* and *Leucaena leucocephala*. Details on silage preparation are presented under experiment 1.

4.2.2 Sample preparation

The test materials were ground to pass through 1 mm screen using a centrifugal mill. The dry matter, crude protein and ether extract were determined by proximate analysis (AOAC, 1990).

4.2.3 Incubation Syringes

The incubation syringes (model Fortuna, Häberle Labortechnik, Germany; Figure 4.1) were made of glass and had 32 mm internal diameter and 100ml volume graduated in 1 ml intervals and could be read with a precision of 0.5 ml. The needle end of the syringe was extended by a 6 cm long silicon tube on which a plastic clip was attached. The syringe plungers/pistons were also made of glass and were designed to fit snugly into the syringes.

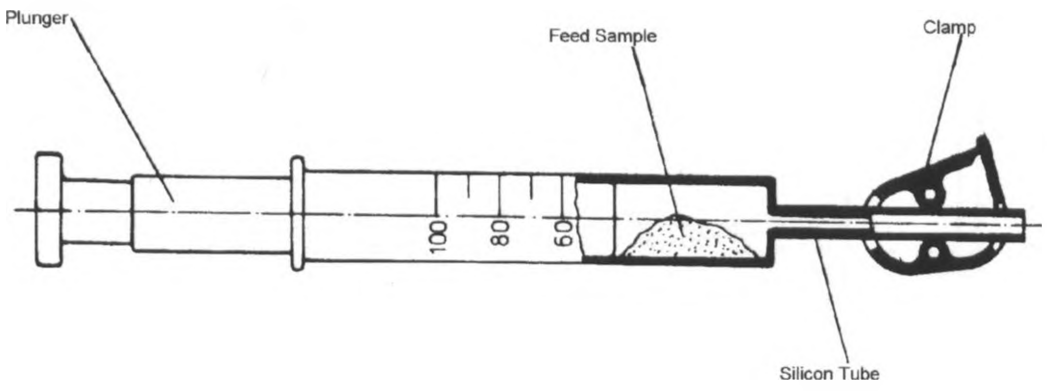

Figure 4:1: Sketch of incubation syringe showing the position of feed sample

4.2.4 Weighing of samples and incubation

Weighing of samples was done a day before the start of the incubation. Exactly 200 mg of the test material was weighed in triplicate using an analytical balance (Sartorius, Germany) with a precision of 1 mg. The weighing vessel was an aluminium 'boat' screwed onto an aluminium rod, 20 cm long. The rod was unscrewed before placing the

'boat' onto the balance. After the sample was weighed, the rod was screwed back in and helped to place the sample at the bottom of the syringe without touching the sides above the 30 ml mark. To ensure smooth movement, water- and air-tightness during incubation, the syringe plungers were lubricated with Vaseline. The assemblage was placed in an incubator set at 39° C overnight (Fig. 2) to equilibrate to the temperature of the rumen fluid at the start of the incubation.

The incubation apparatus was as described by Menke *et al.*, 1979. It comprised of an incubator in which an electric motor-driven rotor (Figure 4.2) was mounted. The rotor comprised of two plastic pieces mounted 13 cm parallel to each other. Both had 57 circular holes drilled to them each having a diameter slightly bigger than the external diameter of the incubation syringes above described. The system could therefore hold 57 syringes representing 16 samples, hay and concentrate standard and blanks all in triplicate.

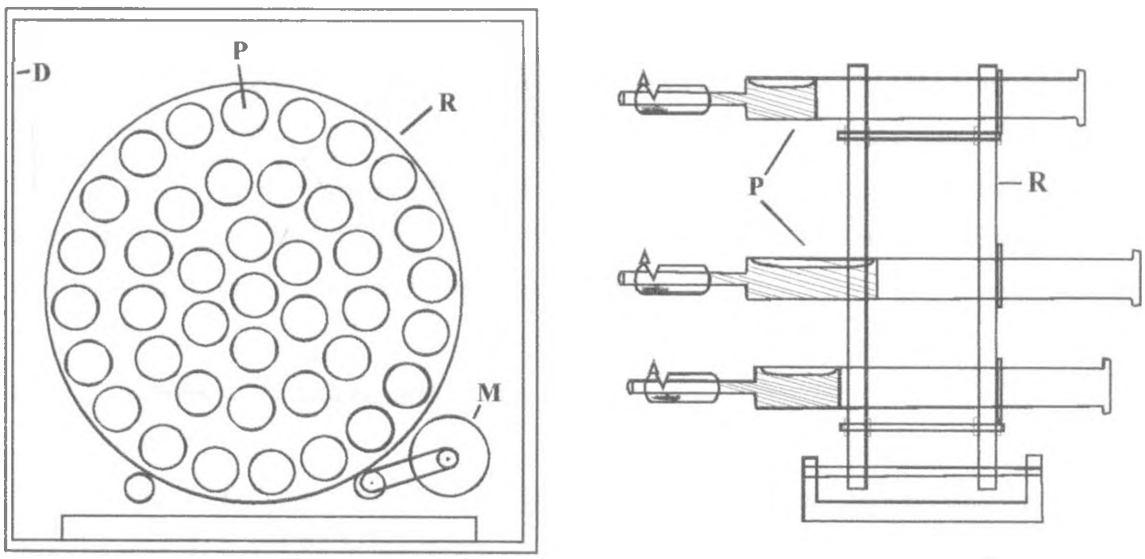

Figure 4.2: Left:Front view of the incubator showing, D-door, P- Plunger, R- Rotor and M- motor. Right: Cross-sectional view of the rotor showing syringes

4.2.5 Incubation medium

The incubation medium was prepared from a mixture of rumen liquor and buffer medium (Menke *et al.*, 1979). Rumen liquor was collected before morning feeding from 3 adult ewes fitted with permanent rumen canulae. The diet of the sheep consisted of 640 g

chaffed hay and 360 g concentrate. The rumen fluid was put into a pre-warmed thermos flask and immediately taken to the laboratory where it was filtered using two layers of cheese cloth. Under CO_2 gas and continuous stirring, 610 ml of the rumen liquor was mixed with a pre-warmed mixture of the following solutions:

1. 0.5 ml trace elements solution: 3.86g $CaCl_2$, 2.93g $MnCl_2$, 0.29g $CoCl_2$, 2.34g $FeCl_3$ in 100 ml distilled water
2. 290 ml buffer solution: 35g $NaHCO_3$ and 4g NH_4PO_4 in 1 liter distilled water
3. 290 ml Major elements solution: 7.14g $Na_2HPO_4x2H_2O$, 6.2g KH_2PO_4 and 0.6g $MgSO_4$ dissolved in 1 litre distilled water
4. 5 ml Resazurin indicator: 100mg Resazurin in 100ml distilled water
5. 60.5 ml Redox solution: 2.5ml 1N NaOH and 380mg Na_2S in 58 ml distilled water

The mixing was done in a water bath maintained at 39° C. After mixing for about 15 minutes, 30 ml of the rumen liquor was pumped into each of the pre-warmed sample-containing syringes through the silicon tube. The plunger was rapidly pushed in to expel air and gas bubbles and the plastic clip was closed when the fluid reached the silicon tube. The initial volume was recorded and the syringes returned to the incubator after which the slow rotating rotor was switched on. Three blank incubations (rumen liquor in incubation medium) were included in every run. Two incubation runs were made for each test material to make a total of six replicates per sample.

4.2.6 Gas volume measurements

The volume of the space occupied by the incubation medium, sample and gas was recorded after 4, 8 and 24 hours. If the reading was equal to or more than 50 ml after 8 hours of incubation, the clip was opened and the plunger was pushed back to 30 ml. This was done to avoid the plunger being pushed too far out of the syringe with the risk of touching the incubator wall. The cumulative gas volume after 4, 8, and 24 hours of incubation was calculated as described by Menke et al. (1979) and Steingass and Menke (1986) by taking into account the initial volume, and subtracting the gas volume obtained in blank incubations. A correction factor was calculated by dividing the expected 24-hour gas production from 200mg of standard hay (44.43 ml) and concentrate feed (65.18 ml) by the actual gas volume obtained from the same standards in each incubation run. The correction factor was used to adjust the gas production of the test materials for changes in

rumen fluid between runs. For the purpose of describing the fermentation kinetics by regression curve fitting, additional readings were done at 28, 32 and 36 hours.

4.2.7 Estimation of metabolisable energy content

The corrected 24-hour gas production was used to calculate the ME content of the test materials using the equation:

ME $=0.136GP+0.0057CP+0.000286EE^2+2.2$ (MJ; ml/0.2g DM; gkg^{-1} DM;) r=0.97 (Menke and Steingass, 1987). Where GP is the gas volume after 24 hours of incubation, CP the crude protein and EE the ether extract.

4.2.8 Fermentation kinetics

The cumulative gas volumes recorded after 4, 8, 12, 24, 28, 32 and 36 hours of incubation (3 replicates in two runs) were used to determine the fermentation pattern of the silages and the silage raw materials. The gas volume readings (42 values for each test material) were fitted to non-linear regression curves (Graphpad prism) using the exponential relationship of Ørskov and McDonald (1979):

$Y=A+B\ (1-e^{-ct})$ where:

Y is the gas volume in ml/200mg substrate dry matter at time t;

A is the y intercept

B is the plateau gas volume value

C is the rate of gas production

t is the time (hours) taken to produce gas volume Y.

Values of the constants in the equation were used to calculate the percentage of the potential gas volume (A+B) from a 200mg sample dry matter achieved after 24 hours of incubation (GP24). This value was used to compare the different classes of silages and Napier grass.

4.2.9. Statistical analysis

Measures of central tendency and analyses of variance were performed as described in experiment 1. The following comparisons were made:

• Napier age: 8 versus 12 weeks

- Forage type: Napier grass versus legumes
- Molasses: molassed versus non-molassed silage
- Legume supplementation: legume-supplemented versus non-supplemented silage

Bivariate correlation analyses were done to establish the relationship between gas production at different stages of incubation, rate of gas production and the content of CP, NDF and ADF in the silages. Pearson's correlation coefficient (SPSS, 2001) was used to quantify the relationships.

4.3 Experiment 3: Effect of inclusion of leguminous protein-rich forages in Napier grass silage at the time of ensiling on voluntary intake, *in vivo* digestibility and nitrogen balance in sheep

Three of the silage combinations from experiment 1 were prepared in large quantities for use in a feeding trial. These were: Napier grass/molasses (NG), Napier grass/molasses/Lablab (NGL) and Napier grass/molasses/Mucuna (NGM). The Napier grass used was 8 weeks old from the last cut. It was irrigated twice a week and was fertilized once using NPK (ratio, 20:10:10) mineral fertilizer at a rate of 100 kg/ha.

4.3.1 Experimental design

The experiment had a 3x3 Latin square design replicated twice (6 mature Dorper rams, 3 treatments and 3 periods). The three dietary treatments were: Napier/molasses, Napier/molasses/Lablab, and Napier/molasses/mucuna silages each allocated randomly to 6 adult rams in three trial periods each lasting 21 days to test their effects on voluntary feed intake and *in vivo* digestibility.

4.3.2 Silage Preparation

Napier grass was harvested by hand using machetes in the morning and left to wilt in the field for 24 hours i.e. till noon of the following day. It was then loaded on to a tractor trailer and transported to the forage chopper. Chopping was done to lengths smaller than 2.5 cm for stems and much longer for leaves. The legumes were harvested in the morning, allowed to wilt for 6 hours and similarly chopped. The chopped Napier grass was put into

gunny bags, weighed and directly emptied into polythene-lined (500 gauge in thickness) silo pits measuring 3 x 2 x 1 m. Where necessary, an appropriate amount (20% of wilted Napier grass weight) of chopped wilted legume forage was weighed and mixed manually with the Napier grass. An appropriate amount of molasses corresponding to 3% of the weight of forage was weighed, diluted at a rate of 3:1 with water and sprinkled on the forage using a garden bucket sprinkler. The molasses and the forage were then thoroughly mixed manually, raked flat and trampled on until compact. This was repeated layer by layer until the silo was full.

The silos were sealed with a sheet of polythene (500 gauge) as above. Soil was heaped on top of the polythene to form a convex mound. A trench was dug around the silos to prevent entry of run-off water. The sealing was maintained until the first day of feeding out.

4.3.3 Experimental animals

Six adult male Dorper sheep (body weight 60.3±4.6 kg) were selected from the sheep herd of the Sheep and Goat Research Station at Olmagogo and transported to the Animal Husbandry Research Centre, Naivasha, Kenya. The animals were randomly given numbers 1 to 6 and then they were allocated to each of the 3 dietary treatments (Table 4.1).

Table 4.1: Allocation of animals (numbers) to dietary treatments and periods

Period/diet	NG	NGL	NGM
1	1&2	3&4	5&6
2	3&4	5&6	1&2
3	5&6	1&2	3&4

4.3.4 Feeding

At the beginning of the experiment, the sheep were dosed with antihelminthics (Nilzan^{R}), sprayed with acaricide (Triatix^{R}) and weighed. They were then put into individual metabolic crates having facilities for feeding, watering and separate collection of urine and faecal material. The feeding was done in the morning and in the evening. The amount of feed offered was adjusted every morning to ensure that the amount refused was about 10%

of that offered. Water and mineral supplement were available *ad libitum*. Each feeding trial period comprised of 21 days, being 14 days adaptation period and 7 days sampling period.

4.3.5 Sample collection

Samples (200g) of the feed offered and the refusals were collected daily and stored frozen in plastic bags. The frozen samples were thawed, pooled and thoroughly mixed for individual animals on a weekly basis and their dry matter determined. From the pooled feed, a sample weighing about 600 g was collected, dried at 60° C for 72 hours in an oven, milled to pass through 1mm sieve and stored in airtight polythene bags. Apart from DM determination, the refusals were not analysed chemically.

The total amount of faecal material and urine produced over a 24-hour period were quantitatively collected and weighed. To avoid moisture loss from faeces in the faecal containers, faeces were removed twice a day and put into airtight plastic bags. The total faecal weight for each 24-hour period was taken in the morning and the faecal containers cleaned. A faecal sample (20% of total 24-hour faecal weight) for each animal was stored frozen and pooled at the end of each trial period. Samples of the pooled faecal material (500 g) were collected, dried at 60° C for 72 hours and milled to pass through 1mm screen. The samples were then stored in sealed, airtight polythene bags pending chemical analyses.

Urine was collected in closed plastic buckets and acidified with 20% sulphuric acid at a ratio of 1:5 (acid:urine). Urine sampling was done twice a day but the total production was expressed in grams per 24 hours. The urine samples were stored frozen and pooled for individual animals at the end of each trial period. A 200ml sample of the pooled urine was collected and stored frozen pending chemical analyses.

4.3.6 Chemical analyses

Dry matter, ash, crude protein, neutral detergent fibre and acid detergent fibre of the feed offered and of the faecal material were determined as described in experiment 1. Crude fibre and ether extract in the feed and faecal material were determined according to AOAC

(1990) and the gross energy was determined by bomb calorimetry using an adiabatic bomb calorimeter (IKA, Germany).

4.3.7 Dry and organic matter intake

The daily dry matter intake per animal was calculated as the difference between the daily feed dry matter offered and the weight of the dry matter refused. This was done on seven consecutive days after the adaptation period of 14 days.

4.3.8 Dry matter and organic matter digestibility

This was determined by total faecal collection as described by Osuji *et al*, 1993. Digestibility was calculated as the difference between the dry matter intake and the dry matter appearing in faeces. Ashing of samples of the feed offered and the faeces was done to calculate the organic matter digestibility. Digestibilities of NDF, ADF and apparent digestibility of nitrogen were similarly calculated after nutrient analyses of feed and faecal material.

4.3.9 Estimation of Metabolisable energy

Metabolisable energy of the three silage diets were calculated from the digestible crude nutrients according to GfE (2001) using the formula:
ME (MJ/kg DM) =0.0312*gDEE+0.0136*gDCF+0.0147*g(DOM-DEE-DCF)+0.0234g CP where, DEE is the digestible ether extract; DCF, digestible crude fibre; DOM, digestible organic matter and CP the crude protein (g/kg DM).

4.3.10 Determination of nitrogen balance

Nitrogen balance was estimated according to the method of Osuji *et al* . (1993). This involved measuring the daily urine and faecal production by total collection as described above. Pooled faecal and urine samples were analysed for total nitrogen by the Dumas method (AOAC 1990) as mentioned in experiment 1. Total nitrogen output was calculated as the sum of faecal and urinary nitrogen. Nitrogen balance was calculated as the difference between nitrogen intake and nitrogen output in grams per animal per day.

4.3.11 Statistical analysis

The statistical model (2) was used to test the effects of period, animal and silage type on the variables investigated.

$Y_{ijk} = \mu + \alpha_i + \beta_j + \gamma_k + e_{ijk}$ ..... (2)

Where: Y_{ijk} is the independent variable

α_i (I = 1,2,3) is the fixed effect of silage types

β_j (j = 1,2,3) is the effect of period

γ_k (k = 1,2,3) is the effect of the animal

e_{ijk} is the random error

4.4 Experiment 4: Effect of tannins in leguminous browse plant species on *in vitro* gas production kinetics, estimated metabolisable energy and fermentation of other substrates

The gas production method (Menke *et al.*, 1979) was modified to determine the effect of anti-nutritive factors on rumen fermentation parameters and to calculate in vitro degradability of nitrogen (IVDN) of different tropical Protein-rich forages. The PRFs included two herbaceous legumes; *Mucuna pruriens* and *Lablab purpureus*, three multipurpose trees and shrub legumes; *Leucaena leucocephala*, *Crotalaria ochroleuca*, *Calliandra callothyrsus*, *Acacia tortilis* and *Acacia angustissima* and a non-legume high protein shrub; *Morus alba* (white mulberry). The samples were collected at different times during the rainy season in late 2001 and early 2002 in the central highlands of Kenya and sun-dried. *Acacia angustissima* and *Morus alba* were collected from the Regional Research Centre of the Kenya Agricultural Research Institute (KARI) in Embu. The research centre is situated 1490 m above sea level, 00°30' south and 37°27' east. The others were obtained from Naivasha (same location as for experiments 1 and 3).

4.4.1 Pre-screening for tannin activity

Approximately 200 mg of the test material was weighed in 2 sets of triplicates into incubation syringes (model Fortuna, Häberle Labortechnik, Germany) of 32 mm internal diameter and 100ml volume as described in experiment 2. To one set of sample triplicates

was added 200mg polyethylene glycol (molecular weight 6000, Merck, Germany). This amount of PEG had previously been tested in this study and found to adequately suppress the tannin effects in *Acacia angustissima* in terms of *in vitro* gas production, in that, gas production did not increase when PEG was increased from 200 to 400 mg. Through out this experiment, the level of PEG was kept at a ratio of 1:1 to the weight of legume common with previous studies (Getachew *et al.*, 2000; Baba *et al.*, 2002).

The incubation medium and the incubation procedures are as described in experiment 2. Gas production was measured after 4, 8 and 24 hours of incubation. The plants whose 24-hour gas production increased by more than 10% on addition of PEG were presumed to contain significant amounts of active tannins, which inhibit *in vitro* fermentation. *In vitro* fermentation kinetics of the PRFs in the presence and absence of PEG were determined as in experiment 2. Gas production data recorded at 4, 8, 12, 24, 28, 32 and 36 hours of incubation were fitted to a non linear regression model from which the rate of gas production and time taken to achieve 70% of the potential gas production (T^{70}) were calculated.

Acacia angustissima which exhibited the highest fermentation inhibition effect was further incubated with 200 mg Napier grass at a ratio of 1:1 with and without addition of PEG. The gas production of sole incubations of Napier grass and the combinations of Napier grass with *Acacia angustissima* and PEG were recorded. Similar incubations were done combining 200 mg of *Acacia angustissima* and 150 mg wheat starch with and without added PEG.

At the end of the incubation run, the syringe contents were emptied into chilled 100ml glass bottles (standing in ice) and shortly afterwards centrifuged at 33000 x g at 4° C for 15 minutes (Heraeus Christi model, Germany). The supernatant was collected in 1.5 ml plastic vials and frozen awaiting determination of short chain fatty acids.

4.4.2 Effect of graded levels of some tanniniferous legumes on *in vitro* fermentation of Napier grass-based rations

After determining the effect of tannins in *Acacia angustissima* on Napier grass fermentation at the 1:1 ratio, further incubation runs were done to evaluate the effect of

graded levels of *Acacia angustissima, Calliandra calothyrsus and Leucaena leucocephala* on the fermentation and the metabolisable energy of legume-supplemented Napier grass. *Morus alba* (a non-leguminous PRF) was similarly incubated and served as a low tannin-activity control. Ensuring that the total weight of substrate incubated remained 200mg, the level of the PRF was increased from 0 to 200mg. The ratios of PRF to Napier grass used were: 0:200, 50:150, 100:100, 150:50, 175:25 and 200:0 (0, 25, 50, 75, 88 and 100% PRF). The gas production was recorded as above mentioned and used to calculate expected ME values using crude protein and ether extract values calculated from the relative proportion of Napier grass and PRF in the incubation mixture.

The same set-up was repeated for *Acacia angustissima, Calliandra calothyrsus and Leucaena leucocephala*, this time with the addition of PEG at a ratio of 1:1 to tanniniferous legume. Gas production and ME values were plotted against the amount of PRF in the mixture.

4.4.3 *In vitro* degradability of nitrogen (IVDN) of Protein-rich forages

For the purpose of estimating IVDN, the gas production method was modified according to Raab *et al* (1983) and Getachew *et al* (2000). Samples (200 mg) of *Acacia angustissima, Calliandra calothyrsus, Acacia tortilis, Leucaena leucocephala, Morus alba, Lablab purpureus, Crotalaria ochroleuca and Mucuna pruriens* were incubated either alone or in combination with 75 and 150 mg potato starch in triplicate. The incubations were performed in two runs on different days. Therefore, 18 incubations were done for every PRF. For the tannin-containing samples (judged from gas production values in 4.4.1 above), additional incubations were done in which PEG (200mg) was added.

In each case, gas production was recorded as described above and fluid samples were collected and processed as in 4.4.1.1 above. After centrifugation, the supernatant was collected into chilled 20ml plastic bottles and frozen awaiting ammonia analysis which was carried out after a maximum of 2 months.

4.4.4 Chemical analyses

Volatile fatty acids

Volatile fatty acids (acetic, propionic, butyric, isobutyric and isovaleric acids) were determined using a gas liquid chromatograph (Perkin Elmer, Gaschromatograph Autosystem) fitted with a GV9 flame ionisation detector as outlined by Meeske *et al* (1999). The column type was Porapack number 100 of 1.8 m length. The carrier gas was Helium and the make-up gas nitrogen. Valeric acid was found to be present in trace amounts and was not considered. The internal standard used was formic acid. The results were expressed in micromols/30ml of incubation medium after subtraction of VFA values of blank incubations.

Ammonia nitrogen

Ammonia nitrogen was measured in 10 g of the liquid sample by Kjeldahl distillation (AOAC 1995, Official Method 984.13) using an automatic distillation and titration unit (Vapodest, Gerhadt Labortechnik, Germany). Distilled ammonia was collected into 3% boric acid and titration was done automatically using a 0.05M sulphuric acid solution. The results were recorded as percent N and were then converted to mg N/30 ml. Ammonia nitrogen values so obtained were plotted against gas production (Figure 4.1) and fitted to a linear regression curve. The Y-intercept of this curve theoretically denotes the ammonia produced at zero gas production, i.e. the protein degraded when no dietary nitrogen is incorporated into the rumen micro-organisms (Raab *et al.*, 1983). Subtraction from the Y-intercept value of the NH_3-N value obtained from blank incubation and subdivision by the total N in the original sample gives the *in vitro* degradability of nitrogen.

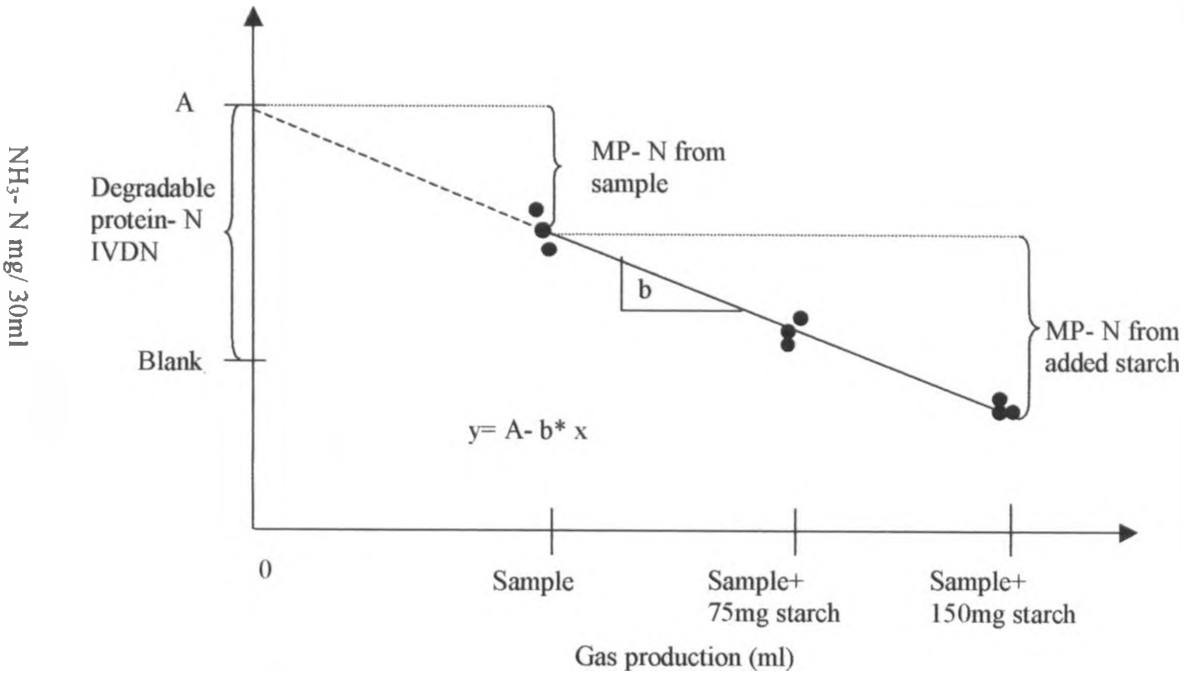

Figure 4.3 : Sketch of the main points used in the calculation of IVDN.

*MP-N: microbial protein nitrogen

$$\text{IVDN\%} = \frac{\text{NH}_3\text{-N at zero gas production (A)-NH}_3\text{-N of blank} * 100}{\text{Total N of feedstuff incubated}}$$

5 RESULTS

5.1 Experiment 1: Evaluation of the effects of stage of growth, addition of molasses and legume forage on the fermentation characteristics of Napier grass silage

5.1.1 Chemical composition of pre-ensiled material

The proximate and detergent fibre composition of Napier grass and legumes that were used to prepare laboratory silages are shown in Table 5.1. The legume forages had higher crude protein and lower NDF than Napier grass. The water-soluble carbohydrate content tended to be higher in *Lablab* and *Mucuna* than in Napier grass.

Table 5.1: Chemical composition (gkg^{-1} DM) of wilted pre-ensiled forage

	NG4[1]	NG8	NG12	*Leucaena*	*Crotalaria*	*Lablab*	*Mucuna*
DM (g/kg)	222	254	309	260	180	170	192
OM	796	808	817	908	865	865	870
CP	133	94.7	83.3	310	176	174	193
EE	19.4	19.3	12.8	34.0	22.5	21.7	27.3
NDF	547	597	608	397	445	390	497
ADF	283	309	323	162	322	253	389
WSC	32.1	39.2	40.4	27.3	26.1	55.3	49.6

[1]*NG4, NG8 and NG12 – Napier grass 4, 8 and 12 weeks old respectively*

5.1.2 pH of silages

The effects of treatment main effects on the pH of silage are presented in Tables 5.2, 5.3, 5.4 and 5.5. In the absence of molasses, there were no significant differences in the pH of silage at different stages of Napier grass maturity. In the presence of molasses, NG4 silage had significantly higher ($P<0.05$) pH than NG8 and NG12 (Table 5.2). In all cases, inclusion of molasses reduced ($P<0.05$) the pH of silage (Table 5.3).

Table 5.2: Effect of the stage of maturity of Napier grass on the pH of silage

Molasses (%)	Legume (%)	Stage of maturity of Napier grass (weeks) 4	8	12	SE
0	0	5.55	5.90	5.54	0.08
	20	5.48	5.35	5.33	0.08
3	0	5.13 [a]	5.01 [ab]	5.03 [a]	0.10
	20	5.0[a]	4.72[b]	4.56[c]	0.06

Row means within molasses level with different superscripts are significantly different (P ≤0.05)
SE- standard error of the mean

Table 5.3: Effect of inclusion of molasses on the pH of Napier grass silage

Age of Napier (weeks)	Legume (%)	Molasses inclusion 0	3%	SE
4	0	5.55 [a]	5.13 [b]	0.11
	20	5.48 [a]	4.99 [b]	0.05
8	0	5.90 [a]	5.01 [b]	0.15
	20	5.35 [a]	4.72 [b]	0.06
12	0	5.54 [a]	5.03 [b]	0.07
	20	5.33 [a]	4.56 [b]	0.06

Row means within molasses level with different superscripts are significantly different (P ≤0.05)
SE- standard error of the mean

Legume- supplemented silage (Table 5.4) tended to have lower pH than non-supplemented silage. However, the effects of legume inclusion were significant (P<0.05) in silage made with NG8 and NG12 but not NG4. Increasing storage duration from 30 to 90 days (Table 5.5) significantly reduced the pH in silage made in the absence of molasses and legumes (P<0.05). The trend of reducing pH with increasing storage time was also observed in the other silages though not statistically significant.

Table 5.4: Effect of inclusion of legume forage on the pH of silage

Age of Napier	Molasses (%)	Legume inclusion (%) 0	20	SE
4 weeks	0	5.55	5.48	0.05
	3	5.13	4.99	0.05
8 weeks	0	5.89[a]	5.35[b]	0.07
	3	5.01[a]	4.72[b]	0.04
12 weeks	0	5.54	5.33	0.05
	3	5.30[a]	4.56[b]	0.06

Row means with different superscripts are significantly different (P ≤0.05). SE- standard error of the mean

Table 5.5: Effect of duration of storage on the pH of silage

Age of Napier grass (weeks)	Silage type*	Storage time 30	60	90	SE
4	NG	6.0[a]	5.45[b]	5.20[b]	0.16
	NGL	5.64[a]	5.47[ab]	5.31[b]	0.04
	NGM	5.26[a]	5.25[a]	4.88[b]	0.08
	NGML	5.0	4.99	5.0	0.06
8	NG	6.25[a]	5.8[b]	5.65[b]	0.11
	NGL	5.51	5.30	5.24	0.07
	NGM	5.14	5.05	4.85	0.07
	NGML	4.79	4.75	4.64	0.04
12	NG	5.9[a]	5.45[b]	5.27[b]	0.12
	NGL	5.37	5.41	5.20	0.08
	NGM	5.4	5.25	5.24	0.04
	NGML	4.58	4.53	4.57	0.03

**NG-Napier grass alone, NGL-Napier grass with legumes, NGM-Napier grass with molasses, NGML-Napier grass with molasses and legumes. Row means with different superscripts are significantly different (P ≤0.05). SE-Standard error of the mean*

There were some significant ($P<0.05$) first and second order interactions of the main effects, which accounted for a minor proportion of the variation in silage pH. Further analyses of the Napier grass maturity*Molasses, storage time*molasses and legume inclusion*molasses interactions were performed. The effect of molasses was found to be less with silage made of Napier grass of 4 weeks maturity than with the older Napier grass (pH reductions from 5.49-5.02, 5.46-4.78 and 5.37-4.71 for NG4, NG8 and NG12 respectively). Inclusion of molasses was found to reduce the pH of silage to a greater extent at 30 days storage than at 60 and 90 days (5.61-4.88, 5.43-4.84 and 5.27-4.78 respectively). The reduction of pH by molasses inclusion was found to be greater in the presence of legumes (5.39 to 4.76) than in the absence of legumes (5.66 to 5.15). There was no interaction of the storage time with inclusion of molasses.

5.1.3 Crude protein and ammonia nitrogen of silages

The crude protein values of legume-supplemented and non-supplemented Napier grass silage after 90 days of storage are shown in Table 5.6. The crude protein content of silage made from NG8 increased from 105 to 155 g/kg DM on inclusion of *Leucaena* forage whereas that made from 12 week old Napier grass increased from 84 to 130 g/kg DM. The other legumes increased the crude protein to intermediary levels.

Addition of molasses during ensilage had no effect on the Crude protein content of the silage irrespective of the age of Napier grass used (Table 5.7). Ensiling of the younger Napier grass (NG8) resulted in silage having higher ($P<0.05$) crude protein than that made from NG12 with or without molasses addition (Table 5.8).

Ammonia nitrogen contents of Napier grass silage (Table 5.9) ranged from 76-to 280gkg^{-1} total nitrogen. *Leucaena*-supplemented silage had the lowest ($P\leq0.05$) ammonia nitrogen levels in all cases apart from NG8 preserved without molasses. Inclusion of molasses significantly decreased ($P\leq0.05$) the ammonia nitrogen levels of both NG8 and NG12 silages in the presence and absence of legume forage (Table 5.10). Age of Napier grass had no effect on ammonia nitrogen content of silage either with or without molasses (Table 5.11).

Table 5.6: Effect of inclusion of different legume forages on Crude protein content (g/kg DM) of Napier grass silage

	Without molasses		With molasses (3%)	
	NG8[1]	NG12	NG8	NG12
Control	105.0[b]	84.0[c]	106.5[c]	79.5[d]
NG+Crotalaria	114.5[b]	96.0[bc]	116.0[c]	105.5[c]
NG+Lablab	111.0[b]	107.0[b]	114.5[c]	108.0[bc]
NG+Mucuna	118.5[b]	98.5[bc]	128.5[b]	112.0[b]
NG+Leucaena	154.5[a]	129.0[a]	156.0[a]	118.0[a]
SE	7.4	6.0	3.0	1.9

[1]*NG8 and NG12 – Napier grass of 8 and 12 weeks old respectively*
Means having different superscripts within columns are significantly different (P≤0.05)
SE:-Standard error of the mean

Table 5.7: Effect of inclusion of molasses on the Crude ptotein (gkg^{-1}) of Napier grass silage

		Molasses inclusion		
Age of Napier (weeks)	Legume (%)	0	3%	SE
8	0	105.0	118.5	4.81
	20	124.6	128.8	4.54
12	0	84.0	79.5	1.38
	20	107.6	110.9	2.71

SE:-Standard error of the mean

Table 5.8: Effect of the stage of maturity of Napier grass on the crude protein (gkg^{-1}) of Napier grass silage

Molasses (%)	Legume (%)	Stage of maturity of Napier grass (weeks) 8	12	SE
0	0	105.0 [a]	84.0 [b]	6.55
	20	124.6 [a]	107.6 [b]	4.7
3	0	118.5 [a]	79.5 [b]	11.4
	20	128.8 [a]	110.9 [b]	3.9

Row means with different superscripts are significantly different (P ≤0.05). SE-Standard error of the mean

There was a significant (P<0.001) interaction between the main effects of age of Napier grass and inclusion of legume on silage crude protein. This was attributed to the higher DM of wilted NG12 (309 gkg-1 DM) in comparison to NG8 (254 gkg-1 DM). Since the legume inclusion was on wilted weight basis, it was likely that more legume forage was included in NG12 than NG8 silage.

Table 5.9: Effect of inclusion of different legume forages on ammonia nitrogen content (g/kg total nitrogen) of Napier grass silage

	Without molasses		With molasses (3%)	
	NG8[1]	NG12	NG8	NG12
Control	192	205[a]	156 [a]	161 [b]
NG+Crotalaria	172	280 [a]	160 [a]	220 [a]
NG+Lablab	185	235 [a]	164 [a]	106 [cd]
NG+Mucuna	242	253 [a]	173 [a]	130 [bc]
NG+Leucaena	175	98 [b]	118 [b]	76 [d]
SE	28.9	32.2	10.4	15.3

[1]*NG8 and NG12 – Napier grass 8 and 12 weeks old respectively*
Means having different superscripts within columns are significantly different (P≤ 0.05)
SE:-Standard error of the mean

Table 5.10: Effect of inclusion of molasses on the ammonia nitrogen content (g/kg total nitrogen) of Napier grass silage

		Molasses inclusion		
Age of Napier (weeks)	Legume (%)	0	3%	SE
8	0	192.0 [a]	157.5 [b]	10.36
	20	193.1 [a]	153.6 [b]	9.32
12	0	205.0 [a]	160.5 [b]	14.23
	20	216.3 [a]	132.8 [b]	20.03

Row means with different superscripts are significantly different (P ≤0.05). SE-Standard error of the mean

Table 5.11: Effect of the stage of maturity of Napier grass on the ammonia nitrogen content (g/kg total nitrogen) of Napier grass silage

		Stage of maturity of Napier grass (weeks)		
Molasses (%)	Legume (%)	8	12	SE
0	0	192.0	205.0	7.1
	20	193.1	216.3	15.4
3	0	157.5	160.5	3.2
	20	153.6	132.8	11.2

SE-Standard error of the mean

5.1.4. Fibre content of silages

The effects of age of Napier grass and molasses inclusion on the NDF and ADF content of silage are shown in Tables 5.12 and 5.13. In general, Napier alone silage and *Mucuna*-supplemented silage tended to have higher NDF and ADF than the other silage types irrespective of the age of Napier grass used and whether molasses was included or not. Silage made from older Napier grass (NG12) had higher NDF ($P<0.05$) and ADF

($P<0.05$). There was a tendency of decreases in ADF and NDF when molasses was included in silage.

Table 5.12: Effects of stage of maturity of Napier grass and molasses inclusion on NDF (gkg^{-1} DM) of Napier grass silages

	Age of Napier grass (weeks)		Molasses (%)		
Silage type	8	12	0	3	SE
Napier (NG) alone	518.0[b]	552.3[a]	540.3	530.0	7.53
NG+ Crotalaria	488.5	508.3	514.5[a]	482.3[b]	8.68
NG+ Lablab	476.3[b]	515.3[a]	500.3	491.3	8.72
NG + Leucaena	516.0	530.3	535.0	511.3	6.13
NG + Mucuna	516.3[b]	566.8[a]	564.3[a]	518.5[b]	12.96

Means are compared between age of Napier grass and within molasses treatment. Row means with different superscripts are significantly different ($P \leq 0.05$)
SE-Standard error of the mean

Table 5.13: Effects of stage of maturity of Napier grass and molasses inclusion on ADF (gkg^{-1} DM) of Napier grass silages

	Age of Napier grass (weeks)		Molasses (%)		
Silage type	8	12	0	3	SE
Napier (NG) alone	297.0[b]	321.0[a]	317.0	301.8	5.82
NG+ Crotalaria	295.5[b]	321.8[a]	326.5	290.8	10.16
NG+ Lablab	281.5[b]	325.3[a]	310.5	296.3	9.67
NG + Leucaena	283.3[b]	305.3[a]	307.5	281.0	7.72
NG + Mucuna	324.0[b]	348.0[a]	357.0[a]	315.0[b]	9.36

Means are compared between age of Napier grass and within molasses treatment. Row means with different superscripts are significantly different ($P \leq 0.05$)
SE-Standard error of the mean

5.2 Experiment 2: *In vitro* gas production kinetics and estimation of metabolisable energy of Napier grass, Napier grass silage and leguminous Protein-rich forage

5.2.1 Gas production from pre-ensiled forage

The *in vitro* gas production values of pre-ensiled Napier grass (8 and 12 weeks maturity stages) and legumes are shown on Table 5.14. The legumes generally had higher rates of gas production and a bigger percentage of the total potential gas production was achieved at 24 hours of incubation than Napier grass. The legumes had significantly higher estimated ME values ($P \leq 0.05$) than Napier grass.

Table 5.14: Gas production and estimated ME (Means±SD) of pre-ensiled forage

	Gas production				
Forage	24-hour (ml)	A+B[1] (ml)	GP[2] %	Rate (%/hr)	ME (MJ/kg DM)
Crotalaria	40.2 *±0.6*	45.5 *±0.3*	88.9±0.4	9.4 *±0.03*	8.8 *±0.1*
Lablab	46.4 *±0.1*	49.5 *±0.5*	91.8±1.1	10.4 *±0.04*	9.7 *±0.1*
Leucaena	34.4 *±0.5*	38.8 *±0.4*	80.9±1.1	7.3 *±0.03*	9.0 *±0.01*
Mucuna	36.8 *±0.4*	41.7 *±0.4*	88.2±1.4	10.5 *±0.05*	8.6 *±0.1*
[3]NG8	38.6 *±1.1*	53.2 *±1.7*	72.5±1.2	5.3 *±0.1*	8.1 *±0.1*
NG12	39.8 *±1.3*	51.6 *±2.0*	77.1±1.0	5.9 *±0.3*	8.2 *±1.2*

[1] A + B: effective gas production (y-intercept plus asymptotic gas volume)
[2] Gas produced after 24 hours of incubation expressed as a percentage of the calculated asymptotic gas production
[3] NG8 and NG12 – Napier grass 8 and 12 weeks old respectively

5.2.2 Gas production from silage

The effects of inclusion of legume in Napier grass silage on *in vitro* gas production are shown in Table 5.15. The cumulative gas volume produced after 24 hours of *in vitro* incubation of silage was not affected by legume forage inclusion. However, the rate of gas production was significantly ($P<0.05$) higher in Leucaena-supplemented silage than the control. The other legume-supplemented silages tended to have higher rates than the control silage. The supplemented silages achieved higher ($P<0.05$) percentage of their

potential gas production after 24 hours of incubation (60-65%) than the control Napier grass silage (53%).

Table 5.15: Effect of inclusion of legume forage on the in vitro gas production and fermentation kinetics of Napier grass silage

	Legume type[1]					
Variable	NG	NGC	NGL	NGLL	NGM	SE
GP24[2] (ml)	31.7	30.7	31.6	32.3	30.7	1.12
Rate[3] (%/hr)	2.96[b]	3.91[ab]	3.89[ab]	4.28[a]	3.90[ab]	0.55
GP[4] (%)	53.6[b]	61.9[a]	62.4[a]	64.7[a]	60.2[a]	5.46

[1]NG-Napier grass alone; NGC, NGL, NGLL, NGM- Napier grass supplemented with Crotalaria ochroleuca, Lablab purpureus, Leucaena leucocephala and Mucuna pruriens respectively
[2] Gas production after 24 hours of incubation
[3] Rate of gas production
[4] % of total gas production achieved in 24 hours of incubation
Row means with different superscripts are significantly different (P<0.05)

Molasses inclusion during ensilage led to a significant increase in the cumulative gas production in both NG8 (P<0.007) and NG12 (P<0.001) (Table 5.16). It also increased the rate of gas production (P<0.001) and caused an increase in the percentage of total gas produced after 24 hours of incubation from 52% in the absence to 69% in the presence of molasses.

Table 5.16: Effect of inclusion of molasses on the *in vitro* gas production and fermentation kinetics of Napier grass silage

	Molasses (%)		
Variable	0	3	SE
GP24[1] (ml)	30.0[b]	32.8[a]	0.35
Rate[2] (%/hr)	2.93[b]	4.64[a]	0.18
GP[3] (%)	52.1[b]	69.0[a]	1.74

[1] Gas production after 24 hours of incubation, SE-standard error of the mean
[2] Rate of gas production
[3] % of total gas production achieved in 24 hours of incubation
Row means with different superscripts are significantly different (P<0.05)

The age of Napier grass (8 and 12 weeks) conserved did not affect the cumulative gas production, the rate of fermentation or the proportion of the total gas volume achieved in 24 hours (Table 5.17). There was, however, a tendency for the rate of gas production to decrease with advancing age (4 to 3.6%/hr for NG8 and NG12 respectively). The percentage of total gas volume produced after 24 hours of incubation tended to be higher in NG8 than NG12.

Table 5.17: Effect of stage of maturity of Napier grass on the *in vitro* gas production and fermentation kinetics of Napier grass silage

	Stage of growth (weeks)		
Variable	8	12	SE
GP24[1] (ml)	31.2	31.5	0.35
Rate[2] (%/hr)	4.03	3.55	0.18
GP[3] (%)	62.9	58.2	1.74

[1] *Gas production after 24 hours of incubation*
[2] *Rate of gas production*
[3] *% of total gas production achieved in 24 hours of incubation*
SE-standard error of the mean

Table 5.18 shows the correlation between a few gas production variables and the level of CP, NDF and ADF in Napier grass silages. Crude protein levels were highly positively related to gas production in the early stages of fermentation (4 hours) but not in later stages (24 and 36 hours). Correlation between CP and the rate of gas production was positive and significant (P=0.026). The fibre fractions, NDF and ADF, were significantly negatively correlated with gas production more so at early stages of incubation. The rate of gas production was also significantly negatively correlated with NDF. Negative and significant correlations were also recorded between ADF and gas production as well as the rate of gas production.

Table 5.18: Correlation coefficients between Crude protein (CP), neutral detergent fibre (NDF), acid detergent fibre (ADF) and *in vitro* gas production (GP) parameters of silages

	CP		NDF		ADF	
Variable	r	P	r	P	r	P
[1]GP4 (ml)	0.504	0.001	-0.561	0.000	-0.615	0.000
GP24 (ml)	0.177	0.274	-0.373	0.018	-0.572	0.000
GP36 (ml)	0.07	0.667	-0.179	0.270	-0.426	0.006
Rate (%/hr)	0.351	0.026	-0.485	0.002	-0.550	0.000

[1] *GP4, GP24, GP36 = gas production at 4, 24 and 36 hours of incubation respectively*

r = Pearson's correlation coefficient (n=40); P= level of significance

5.2.3 Estimated metabolisable energy of silages

Values of metabolisable energy content of Napier grass alone and Napier grass-legume mixed silages were estimated from gas production (Menke and Steingass, 1987). Legume forage inclusion did not affect the ME content of silage for NG8 with and without molasses inclusion (Table 5.19). However, the ME content of NG12 silage was higher when *Leucaena* was used as a supplement ($P<0.05$) and lowest when *Mucuna* was included. For silage made using NG12 and molasses, *Leucaena* supplementation significantly increased ME above that of the control. The other legumes did not significantly affect this parameter.

Table 5.20 shows the ANOVA results for the effects of Napier grass age and molasses inclusion. The former did not affect ME of silage irrespective of whether molasses was added or not. Molasses inclusion increased ME content of both NG8 ($P<0.01$) and NG12 ($P<0.001$) silages.

Table 5.19: Effect of stage of growth of Napier grass, inclusion of legume forage and molasses on metabolisable energy content (MJ/kg DM) estimated from *in vitro* gas production

	Without molasses		3% molasses	
Age of Napier grass	8	12	8	12
Napier grass (NG)	7.1 ± *0.3*	6.9^{abc} ± *0.2*	7.5 ± *0.1*	7.3^{b} ± *0.1*
NG+ Crotalaria	7.2 ± *0.1*	6.8^{bc} ± *0.4*	7.3 ± *0.1*	7.5^{ab} ± *0.1*
NG+ Lablab	7.1 ± *0.1*	7.3^{ab} ± *0.2*	7.4 ± *0.1*	7.4^{ab} ± *0.4*
NG+Leucaena	7.5 ± *0.4*	7.4^{a} ± *0.01*	7.5 ± *0.4*	7.8^{a} ± *0.01*
NG+Mucuna	7.0 ± *0.1*	6.6^{c} ± *0.1*	7.7 ± *0.01*	7.5^{ab} ± *0.01*
wilted Napier grass	8.1 ±*0.1*	8.2 ±*1.2*	NA	NA

Means(± SD) with different superscripts within columns are significantly different (P<0.05)
NA: not applicable

Table 5.20: ANOVA of the effects of stage of growth of Napier grass and inclusion of molasses on metabolisable energy of silage

Effect	condition	Significance	P-value
Napier grass age	-molasses	NS	0.622
	+ molasses	NS	0.190
Molasses	NG8	**	0.009
	NG12	***	0.001

5.3 Experiment 3: Effect of inclusion of protein-rich forages at the time of ensiling on voluntary intake, *in vivo* digestibility and nitrogen balance in sheep fed Napier grass silage

5.3.1 Chemical composition of silage raw materials and silage

The chemical composition of fresh Napier grass and legumes used to prepare silage are shown in Table 5.21. Due to failure of Mucuna seeds to germinate after first sowing, Napier-Mucuna mixed silage was made after Napier-Lablab silage. This meant that different batches of Napier grass (NG1 and NG2) were used which, though of the same age, had slightly different chemical composition. Legume addition increased the crude protein content of the resultant silage (112 and 113 g/kg) compared to the Napier alone silage (97 g/kg). Napier grass had higher NDF content (579-613 g/kg) than the legumes (390-497 g/kg DM). Among the legumes, *Mucuna* had higher NDF than Lablab. The silages had lower NDF than expected from the relative levels of Napier grass and legumes. The water-soluble carbohydrate content of Napier grass (39-40 g/kg DM) was within the range expected for tropical grasses whereas that of the legumes was higher than for Napier grass.

Table 5.21: Average composition of silage raw materials and the individual silages (g/kg DM or as stated)

	Fresh-pre-ensiled forage				Silage (mean±*SD*)[2]		
	NG1	NG2	Lablab	Mucuna	NG1	NG1+Lablab	NG2+Mucuna
DM (g/kg)	160	160	170	170	278 ±*19*	264 ±*13*	261 ±*15*
OM[1]	809	791	865	870	785 ±*8*	799 ±*9*	798 ±*9*
CP	95	107	174	166	97 ±*1*	112 ±*8*	113 ±*2*
CF	290	278	211	273	281 ±*13*	271±*4*	291±*8*
EE	20.5	13.7	21.7	21.8	20.4 ±*1.9*	21.9 ±*0.4*	17.1 ±*1.0*
NDF	613	579	390	497	520 ±*7*	478 ±*20*	539 ±*8*
ADF	303	318	253	389	308 ±*4*	295 ±*4*	333 ±*3*
WSC	39.2	40.2	55.3	49.6	7.1	9.4	1.5
GE(MJ/kg DM)	15.4	14.8	17.5	17.1	15.6 ±*0.2*	15.5 ±*0.7*	15.1 ±*0.3*
pH	5.80	5.76	5.76	5.23	4.57 ±*0.03*	4.47 ±*0.17*	4.31 ±*0.04*

[1] *OM= organic matter, CP=crude protein, NDF= neutral detergent fibre, ADF=acid detergent fibre, GE=gross energy, NG= Napier grass, WSC = water soluble carbohydrates*

[2] *n=3 (3 trial periods) except for WSC where 1 sample was analysed*

5.3.2 Voluntary feed intake and digestibility

The dry matter, organic matter and crude protein intakes are shown in Table 5.22. There was a tendency of increased voluntary intake of dry and organic matter when sheep were fed legume-enriched Napier grass silage. Digestible dry matter and organic matter intakes also tended to be higher in animals fed legume-supplemented silage. The crude protein intake was significantly higher ($P \leq 0.05$) in sheep fed on legume-supplemented silage.

The digestibility coefficients of dry matter, organic matter, CP, GE and fibre components of the 3 silages are shown in Table 5.22. Digestibility of dry matter of Lablab-enriched Napier grass silage was significantly higher than that of *Mucuna*-supplemented silage but similar to the control. The digestibility of organic matter was significantly higher ($P \leq 0.05$) for Lablab-enriched silage and the control than *Mucuna* -supplemented silage. The apparent digestibility of crude protein was significantly higher for lablab-supplemented silage than the other two types and that of the control tended to be higher than *Mucuna*-supplemented silage. Crude fibre, neutral detergent and acid detergent fibres (NDF and ADF) digestibilities were lower ($P \leq 0.05$) for *Mucuna* -supplemented silage than the control and Lablab-enriched silage. There was, however, no effect of silage type on the digestibility of gross energy.

5.3.3 Estimation of metabolisable energy

Metabolisable energy values of the three silages calculated from digestible crude nutrients in the *in vivo* digestibility trial are shown in Table 5.23. The *Lablab*-supplemented silage had higher ME than the rest and the control silage had higher ME than *Mucuna*-supplemented silage. Incubation of the three silages with buffered rumen fluid in vitro resulted in significantly higher ($P<0.005$) 24-hour cumulative gas production from *Lablab*-supplemented silage than the control and the *Mucuna*-supplemented silage. This was reflected in the metabolisable energy values estimated from *in vitro* gas production and crude nutrients (crude protein and ether extract). The ME estimated from *in vitro* gas production was significantly higher ($P \leq 0.05$) in Lablab supplemented silage than the control and the Mucuna-supplemented silages. The latter two were not significantly different from each other. The ME values estimated from *in vitro* gas production (7.2-7.7

MJ/kg DM), though slightly lower, corresponded closely to the values calculated from the *in vivo* digestibility trial (7.6-8.0 MJ/kg DM). Furthermore, the ranking of the 3 silages remained the same.

Table 5.22: Voluntary intake of DM, OM, digestible dry matter, organic matter and CP

	Silage type		
Variable	Napier alone	Napier+Lablab	Napier+Mucuna
DMI* (g/kg$^{0.75}$/day)	74 ±5	83 ±5	81 ±4
OMI (g/kg$^{0.75}$/day)	59 ±4	68 ±4	66 ±3
DMI (% Lwt)	2.8 ±0.3	3.1 ±0.2	3.0 ±0.2
CPI (g/ animal /day)	145^{b} ±8	183^{a} ±11	183^{a} ±13
Digestibility of DM (%)	54.3^{ab} ±1.4	57.4^{a} ±1.6	52.4^{b} ±1.6
DDM* (g/animal/day)	802 ±43	960 ±94	851 ±61
Digestibility of OM (%)	64.4^{ab} ±1.1	65.8^{a} ±1.4	60.8^{b} ±1.4
DOM (g/ animal /day)	765 ±41	893 ±79	801 ±56
Digestibility of CP (%)	50.3^{ab} ±1.8	55.6^{a} ±2.0	49.7^{b} ±1.4
Digestibility of GE (%)	63.9 ±1.7	63.3 ±2.0	59.6 ±1.5
Digestibility of CF (%)	74.1^{a} ±1.3	70.7^{ab} ±1.2	68.4^{b} ±1.7
Digestibility of NDF (%)	68.2^{a} ±1.4	64.7^{ab} ±1.4	61.2^{b} ±2.0
Digestibility of ADF (%)	62.3^{a} ±1.0	60.7^{ab} ±1.6	60.2^{b} ±2.0

**DMI, OMI, CPI: Dry matte, organic matter and crude protein intake *DDM: Digestible dry matter, DOM: Digestible organic matter*
Row means (±SEM) having different superscripts are significantly different ($P \leq 0.05$)

Table 5.23: Calculated ME, *In-vitro* gas production, and ME values estimated from *in vitro* gas production of Napier grass based silages

	Silage type		
	Napier alone	Napier+Lablab	Napier+Mucuna
ME[1](MJ/kg DM)	7.80^{b} ±0.01	8.0^{a} ±0.02	7.6^{c} ±0.02
GP* (ml/200mg)	33.1^{ab} ±0.9	34.4^{a} ±0.3	31.2^{b} ±0.6
Rate (%/hour)	4.7^{c}	6.5^{a}	5.4^{b}
ME[2] (MJ/kg DM)	7.4^{b} ±0.2	7.7^{a} ±0.03	7.2^{b} ±0.1

[1] ME estimated from in vivo trial (GfE 2001),
[2]ME estimated from in vitro gas production (Menke and Steingass, 1987)
**GP: Gas production*
Row means (±SEM) having different superscripts are significantly different ($P \leq 0.05$)

5.3.4 Nitrogen balance

Nitrogen intake (Table 5.24) ranged from 23 to 29 g/animal/day. It was significantly higher ($P \leq 0.05$) in animals fed legume-supplemented Napier grass silage than the control group. The dietary treatment did not affect the urinary N losses though the animals fed legume-supplemented silage tended to have higher losses. However, the faecal N losses were higher ($P \leq 0.05$) in animals fed legume-supplemented silage, more so in animals fed *Mucuna*-supplemented than those fed Lablab-supplemented silage. Animals fed *Lablab*- and *Mucuna*-supplemented silage retained significantly higher ($P \leq 0.05$) nitrogen in their tissues than those fed non-supplemented silage and the N retained expressed as a percentage of the nitrogen intake was higher in the Lablab-supplemented than the control and the *Mucuna*-supplemented groups.

Table 5.24: Nitrogen intake, nitrogen losses and nitrogen balance of sheep fed legume-enriched or non-enriched Napier grass silage (mean±SEM)

	Silage type		
	Napier alone	Napier+Lablab	Napier+Mucuna
N intake (g/day)	23.1^{b} *±1.3*	29.4^{a} *±1.7*	29.3^{a} *±2.0*
N faecal (g/day)	11.5^{b} *±0.9*	12.9^{ab} *±0.7*	14.6^{a} *±1.2*
N faecal (% intake)	49.3 *±1.8*	44.1 *±2.0*	49.5 *±1.4*
N urinary (g/day)	7.21 *±0.40*	8.73 *±0.91*	8.43 *±0.66*
N urinary (% intake)	31.6 *±2.1*	29.7 *±2.5*	29.0 *±1.8*
Total N loss (g/day)	18.7^{b} *±1.1*	21.6^{ab} *±1.4*	23.0^{a} *±1.8*
Total N loss (% intake)	80.9 *±2.5*	73.8 *±3.5*	78.5 *±2.5*
N retained (g/day)	4.46^{b} *±0.66*	7.74^{a} *±1.22*	6.30^{ab} *±0.91*
N retained (% intake)	19.1^{b} *±2.5*	26.2^{a} *±3.5*	21.5^{ab} *±2.5*

Means are compared between treatments. Means having different letter superscripts within rows are significantly different ($P \leq 0.05$)

The ratios of CP to DOM and ME and of nitrogen to ME in the silages are shown in Table 5.25. The CP to DOM ratios ranged from 152 to 188 (g/kg); CP to ME ranged from 12.5 to 15 (g/MJ) and N to ME from 2 to 2.4 (g/MJ). All the ratios were significantly higher ($P<0.05$) in the legume-supplemented silages than the control.

Table 5.25: Relationship between protein and energy in Napier-based silage

Ratio	Silage type		
	Napier alone	Napier+Lablab	Napier+Mucuna
CP:DOM[1] (g/kg)	152^{c} *±2*	173^{b} *±4*	188^{a} *±5*
CP:ME (g/MJ)	12.5^{c} *±0.1*	14.0^{b} *±0.3*	15.0^{a} *±0.1*
N:ME (g/MJ)	2.0^{c} *±0.001*	2.3^{b} *±0.04*	2.4^{a} *±0.03*

[1] *DOM – digestible organic matter; Means having different letter superscripts within rows are significantly different ($P \leq 0.05$)*

5.4 Experiment 4: Effect of tannins in protein-rich forage on *in vitro* gas production kinetics, estimated metabolisable energy and fermentation of other substrates

5.4.1 Chemical composition of browse plants

The average chemical composition of the tropical protein-rich forages investigated (Table 5.26) shows generally higher CP values for multipurpose trees and shrubs (*Leucaena, Morus, Calliandra and Acacia*) than herbaceous species (*Mucuna* and *Lablab*).

Table 5.26: Chemical composition (g/kg DM) of protein-rich forage[1] used in current study

Species	CP g/kg DM	OM	NDF	ADF
Mucuna pruriens	166	870	497	389
Leucaena leucocephala	310	908	397	162
Morus alba	249	895	218	140
Lablab purpureus	174	870	390	253
Crotalaria ochroleuca	176	865	445	322
Calliandra calothyrsus	258	920	411	153
Acacia tortilis	170	899	409	308
Acacia angustissima	260	938	355	156

[1]*The PRF samples were mixed for individual species to make up one sample whose composition was determined*

5.4.2 *In vitro* fermentation kinetics

Figure 5.1 shows the gas production patterns of the multipurpose trees and shrubs (MPTS) over a 36-hour incubation period. *Morus alba* had the highest rate with 78% of the gas produced in 12 hours of fermentation compared to less than 58% for the others. Whereas all the MPTS exhibited an exponential gas production pattern, the net gas production (corrected for gas production of blank incubations) from *A. angustissima* increased slightly and then showed a reducing trend as the incubation time increased. The *in vitro* fermentation characteristics of tannin containing leguminous browse species in the presence or absence of polyethylene glycol (Table 5.27) showed significantly higher tannin effects in *Acacia angustissima* than *Calliandra* and *Leucaena* as suggested by 24-hour *in vitro* gas production and the calculated asymptotic gas production from regression equations obtained by curve fitting. *Acacia angustissima* and *Calliandra* produced lower gas volumes (3.6 and 14.6 ml respectively) than the other PRF (20 to 50 ml) on incubation

without PEG. When PRFs were incubated together with PEG, the volume of gas produced increased 6-fold for *Acacia angustissima* and twice for *Calliandra* (3.6 to 21 ml and 14 to 30 ml respectively). The change in gas production for *Leucaena* was 8 ml. There were minimal increases in gas production from other PRFs when incubated with added PEG. This was reflected in the metabolisable energy values estimated using *in vitro* gas production and crude nutrients (Menke and Steingass, 1987). *Acacia angustissima*, *Calliandra*, *Acacia tortilis* and *Mucuna* had the lowest ME values in that order (4.9-8 MJ/kg DM). On incubation with PEG, ME values increased most significantly for *Acacia angustissima*, *Calliandra*, *Acacia tortilis* and *Leucaena* (57, 37, 25 and 8% respectively).

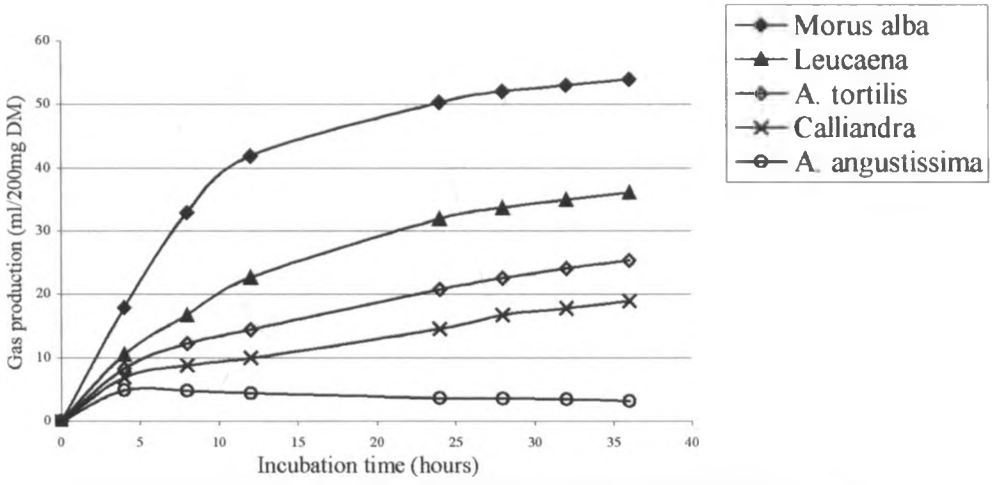

Figure 5.1 In vitro gas production from protein-rich tropical browse species

The potential (calculated asymptotic) gas production (A+B component of the equation $Y=A+B(1-e^{-ct})$) increased significantly when PEG was included in the incubation medium. The rate of gas production in tanniniferous forage increased significantly for *Calliandra* (1.2-3.5%/hour), *Leucaena* (7-11 %/hour) and *Acacia tortilis* (6.7-8.1%/hour). Due to the low amount and the decreasing tendency of the net gas produced by *Acacia angustissima* in the absence of PEG, the values over a 36-hour incubation period could not be fitted to an exponential regression curve. Therefore the rate of gas production of *Acacia angustissima* was not calculated. When PEG was added, the rate of gas production from *A. angustissima* was 12.8%/hour and therefore, the highest among the PRF investigated.

The time required to produce 70 % of the calculated asymptotic gas volume from a substrate (T^{70}) was calculated from the regression results and ranged from 9 hours in *A.*

angustissima treated with PEG to 97 hours for *Calliandra* incubated without PEG (Table 5.27). There was a decrease in T^{70} from 97 to 35, 11 to 16 and from 15.3 to 14.9 hours when *Calliandra*, *Leucaena* and *Acacia tortilis* respectively were incubated with PEG. In the low-tannin activity PRF group (Table 5.27), *Morus alba* and *lablab* had higher 24-hour gas production (49 and 46 ml respectively) than *Crotalaria* and *Mucuna* (40 and 35 ml respectively). The same relationship was observed for the potential gas production whereby *Morus alba* had a value of 55 ml against 36 ml of *Mucuna*. *Morus alba* had the highest estimated metabolisable energy content (11 MJ/kg DM) compared to 8 MJ/kg DM for *Mucuna*. It also took less time to produce 70% of the gas from *Morus alba* and *Lablab* than from *Crotalaria* and *Mucuna*.

The net total and individual volatile fatty acid production from incubation of PRFs with and without PEG-6000 are shown in Table 5.28. Only 3 of the legumes were incubated with PEG. *Acacia angustissima* produced the least total VFA when incubated without PEG. Addition of PEG increased ($P<0.001$) the production of total VFA by 23% in *L. leucocephala*, 54% in *Acacia tortilis*, 112% in *C. calothyrsus* and 475% in *A. angustissima*. Among the tannin-free species, *Morus alba* had significantly higher total volatile fatty acids than *Lablab*, *Crotalaria* and *Mucuna*.

Table 5.27: Gas production (ml), rate of gas production, time taken to produce 70% of the gas, and metabolisable energy estimated from *in vitro* gas production of tannin-containing and relatively tannin-free high protein forage plants

		Gas production					
Legume	PEG	24-hour	Potential (ml)	rate (%/h)	$^{4}T^{70}$ (hours)	ME (MJ/kg)	Sign.[3]
Acacia angustissima	-	3.6 ±0.1	4.0 ±0.4	NR	NR[1]	4.9 ±0.00	****
	+	20.7 ±0.5	21.7 ±0.8	12.8	9.4	7.7 ±0.1	
Calliandra	-	14.6 ±0.9	50.3 ±1.2	1.2	97.2	6.0 ±0.1	****
	+	31.0 ±0.1	52.5 ±2.9	3.5	34.5	8.2 ±0.02	
Acacia tortilis	-	20.8 ±0.4	27.0 ±0.5	6.7	15.3	6.4 ±0.1	****
	+	32.0 ±0.7	37.4 ±0.6	8.1	14.9	8.0 ±0.1	
Leucaena leucocephala	-	32.1 ±0.2	38.8 ±0.4	7.3	16.6	9.0 ±0.1	****
	+	39.6 ±0.8	44.8 ±1.6	10.8	11.2	9.7 ±0.1	
Morus alba	-	50.3 ±0.6	54.5 ±0.7	11.5	10.5	10.6 ±0.04	NS
	+	50.6 ±0.2	NA[2]	NA	NA	10.8 ±0.03	
Lablab	-	44.8 ±0.3	49.5 ±0.5	10.4	11.6	9.7 ±0.1	NS
	+	47.9 ±0.9	NA	NA	NA	9.9 ±0.1	
Crotalaria	-	39.9 ±0.1	45.5 ±0.3	9.4	12.9	8.8 ±0.1	*
	+	42.9 ±0.2	NA	NA	NA	9.2 ±0.03	
Mucuna	-	31.9 ±0.3	36.1 ±0.4	9.8	12.4	8.0 ±0.1	*
	+	37.2 ±0.03	NA	NA	NA	8.3 ±0.00	

[1] *NR- not recorded since data could not be fitted to an exponential curve*
[2] *NA- not analysed*
[3] *Significance level from ANOVA of gas production with and without PEG (NS; non significant; *, P<0.05; **, P<0.01, ***, P<0.005, ****, P<0.001)*
[4] *T^{70} Time taken for the production of 70% of the calculated asymptotic gas volume*

Table 5.28: Net total and individual volatile fatty acid production (micromol/30ml) in incubations of tannin-containing and tannin-free protein-rich forages (means ±SEM)

Legume	Total VFA	C2*	C3	C4	C4i	C5i
A. angustissima	116^{d} ±43	87^{e} ±27	52.6^{d} ±7.6	-2.7^{d} ±5.4	-7.2^{d} ±1.4	-12.9^{d} ±2.8
A. angustissima +PEG	623^{y} ±22	437^{y} ±18	134^{y} ±4.5	31.8^{y} ±3.3	6.7^{y} ±0.6	14.2^{y} ±1.6
Calliandra	218^{d} ±19	219^{d} ±33	19.9^{e} ±9.5	-4.8^{d} ±5.2	-5.2^{d} ±1.0	-10.9^{d} ±1.4
Calliandra +PEG	582^{y} ±68	447^{y} ±22	93.5^{z} ±4.8	18.1^{z} ±3.5	9.0^{xy} ±1.4	15.2^{y} ±0.4
Leucaena	620^{bc} ±31	$441b^{c}$ ±19	139^{b} ±5.1	27.2^{bc} ±5.8	5.2^{b} ±0.8	8.4^{b} ±1.2
Leucaena + PEG	778^{x} ±12	537^{x} ±10	166^{x} ±3.6	43.8^{x} ±2.0	11.2^{x} ±0.7	20.2^{x} ±0.7
ANOVA (effect of PEG)	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Morus alba	833^{a} ±49	595^{a} ±49	158^{ab} ±11.1	54.4^{a} ±8.6	9.8^{a} ±1.2	16.2^{a} ±2.1
Lablab	763^{ab} ±14	527^{ab} ±27	166^{a} ±3.8	52.2^{ab} ±6.5	7.7^{ab} ±0.7	10.6^{b} ±0.5
Crotalaria	694^{abc} ±55	482^{bc} ±40	156^{ab} ±6.4	42.3^{ab} ±6.4	5.4^{b} ±0.9	8.4^{b} ±1.4
Mucuna	543^{c} ±59	410^{c} ±41	108^{c} ±10.3	20.9^{c} ±6.7	2.1^{c} ±1.2	1.7^{c} ±1.7

Comparisons between legumes are done separately (with and without addition of PEG using xy and abc superscripts respectively). Means having different superscripts are significantly different (P<0.05)

**C2-acetic acid, C3-propionic acid, C4-Butyric acid, C4i-isobutyric acid and C5i-isovaleric acid*

Table 5.29 shows the ratios of propionic acid to acetic acid for legumes and *Morus alba* incubated with and without PEG. The effect of inclusion of PEG in the incubation medium on the C3:C2 ratio was varied: it doubled for *Calliandra* and halved for *Acacia* and there was no significant effect for *Leucaena*.

Table 5.29: Propionate:Acetate ratios after 24 hour incubation of legumes with and without PEG

	PEG-		PEG+	
Legume	Mean	SEM	Mean	SEM
A. angustissima	60.8	7.7	30.7	0.9
calliandra	9.1	1.5	21.1	1.0
Leucaena	31.6	0.4	31.0	0.5
Mucuna	26.5	0.7		
Lablab	31.7	1.1		
Morus	26.7	0.4		
Crotalaria	32.9	1.4		

5.4.3. Effect of tanniniferous forage on the fermentation of Napier grass

The negative effect of tannins of *A. angustissima* on in vitro fermentation of Napier grass (Table 5.30) was higher than on fermentation of wheat starch. There was a five-fold increase in gas production both after 12 and 24 hours of fermentation of a 50/50 mixture of *A. angustissima* and Napier grass when PEG was added into the fermentation medium. A similar mixture of *A. angustissima* and wheat starch did not double gas production after 12 or 24 hours of incubation with added PEG. The same observations were made in connection with production of total volatile fatty acids (Table 5.30).

The pattern of changes in gas production, metabolisable energy content and the ratio of nitrogen to metabolisable energy of Napier grass incubated with graded levels of *Acacia angustissima*, *Calliandra* and *Leucaena*, with or without PEG-6000, are shown in Figures 5.2, 5.3 and 5.4. The effect of increasing levels of *Morus alba* in the absence of PEG are shown in Figure 5.5. The reduction of gas production and metabolisable energy by increasing proportions of *Acacia angustissima* was not linear in the absence of PEG. The pattern changed to a linear relationship on inclusion of PEG and, despite a 42% reduction in gas production, the metabolisable energy content reduced by only 5 %. This is in contrast to the situation in the absence of PEG where the gas production reduced by 99.8% and ME by 36%. The pattern of reduction of both the gas production and ME by increasing the proportion of *Calliandra* in the diet was linear. The reduction of gas production with increasing level of *Leucaena* was less than for *Acacia angustissima and*

Calliandra and the ME increased with increasing *Leucaena* with and without PEG. A comparison of the effect of tannin activity in the three rations in terms of gas production and estimated ME are shown in Figures 5.6 and 5.7.

Table 5.30: Effect of tannins in *Acacia angustissima* on the gas and total volatile fatty acid production[1] from starch and Napier grass incubated for 12 and 24 hours *in vitro*

	Gas production (ml)		Total VFAs (micromol/30ml)	
	-PEG	+PEG	-PEG	+PEG
12 hours				
Napier grass[3]	23.0 *±0.5*	23.6 *±0.3*	nm[2]	nm
Starch	46.0 ±0.5	47.0 ±0.3	nm	nm
Acacia	0.8 *±0.1*	17.3 *±0.5*	148 *±24*	483 *±22*
Acacia+Napier grass	9.4 *±0.3*	40.4 *±0.0*	325 *±23*	946 *±28*
Acacia +starch	37.6 *±0.5*	63.1 *±0.4*	647 *±80*	1213 *±29*
24 hours incubation				
Napier grass	34.8 *±0.3*	35.2 *±0.4*	nm	nm
Starch	56.3 ±0.6	57.1 ±0.2	nm	nm
Acacia	0.5 *±0.05*	20.7 *±1.3*	116 *±43*	623 *±22*
Acacia+Napier grass	11.8 *±0.8*	56.9 *±0.3*	410 *±8*	1336 *±72*
Acacia +starch	46.4 *±1.0*	73.6 *±0.5*	866 *±155*	1548 *±39*

[1] *Least square means ± SEM*
[2] *nm: not measured*
[3] *Napier grass and Acacia incubated: 200mg, starch incubated: 150 mg)*

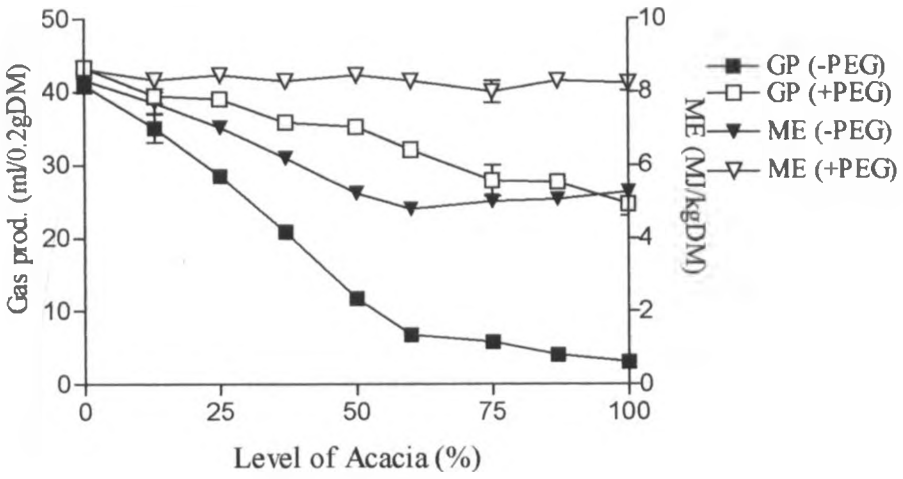

Figure 5.2 Effect of level of *A. angustissima* on gas production and ME of *Acacia*-Napier grass rations

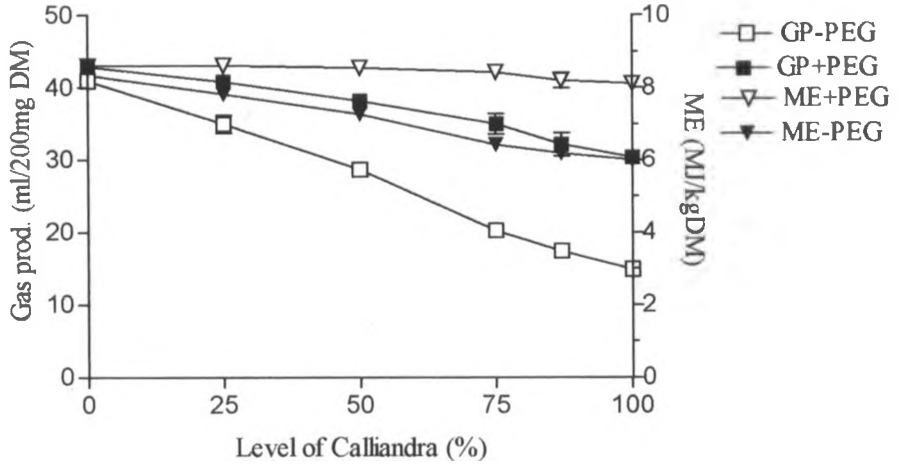

Figure 5.3 Effect of level of Calliandra on gas production and ME of Calliandra-Napier grass rations

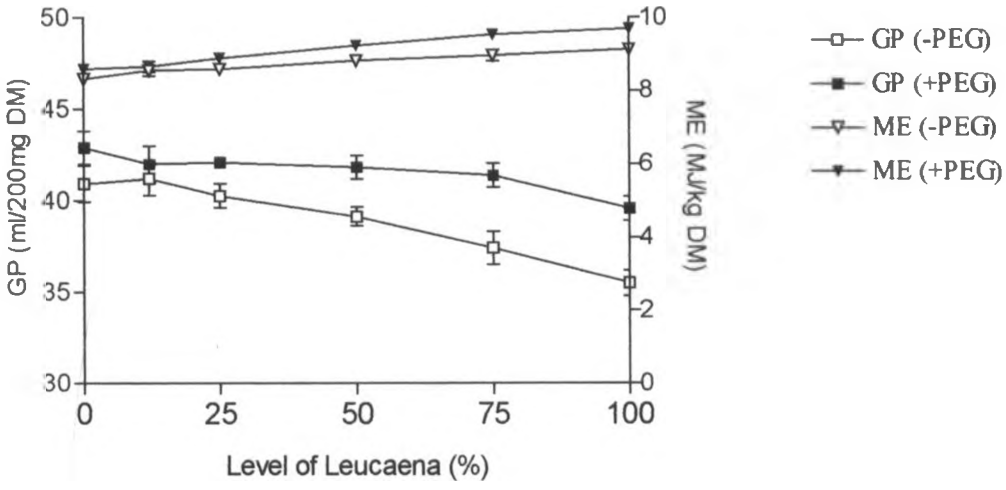

Figure 5.4 Effect of level of Leucaena on gas production and ME of Leucaena-Napier grass rations

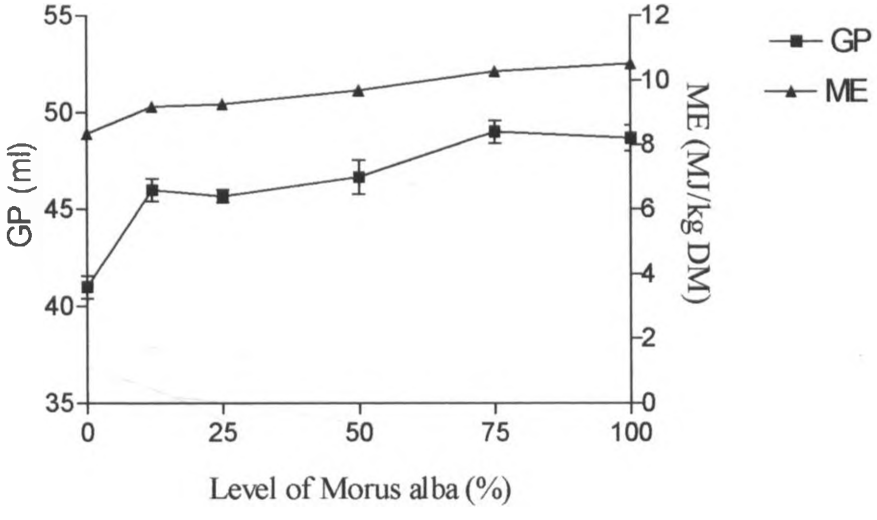

Figure 5.5 Effect of level of *Morus alba* on gas production and ME of *Morus alba*-Napier grass rations

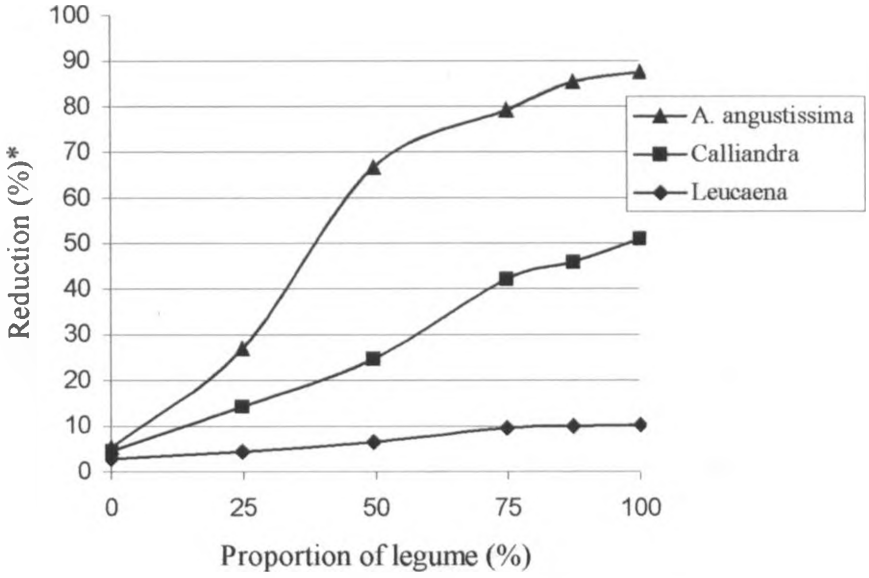

Figure 5.6 Effect of increasing proportion of legumes in Napier grass based rations on *in vitro* gas production
***Reduction in Gas production in the absence of PEG**

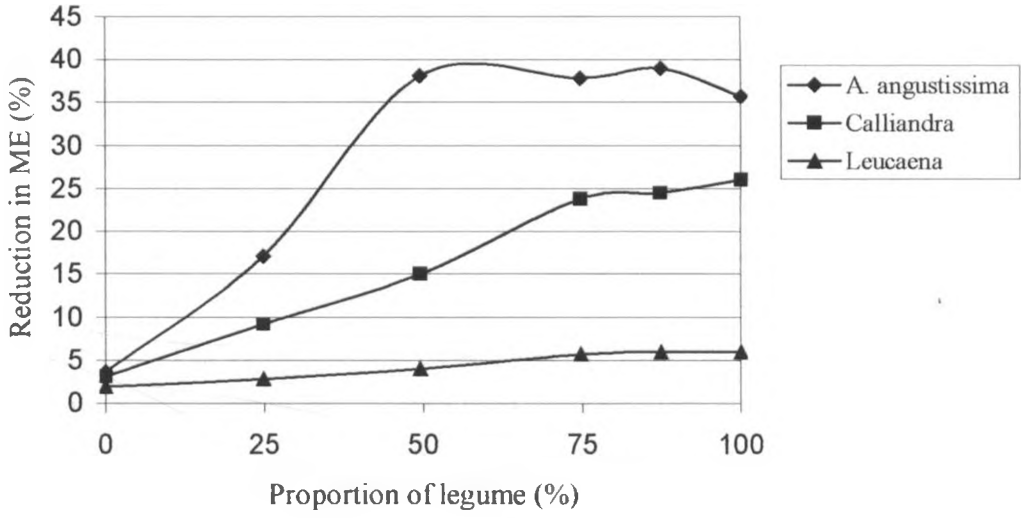

Figure 5.7 Effect of increasing the proportion of legume on ME content of Napier grass based rations

Figure 5.8 shows the effect of increasing the level of PRF on the ratio of nitrogen to ME of Napier grass based-rations. In all cases, the N:ME ratio increased more rapidly with proportion of legume in the diet in the absence than in the presence of PEG. The N:ME ratio of tanniniferous legumes (*Acacia angustissima, Calliandra calothyrsus* and *Leucaena leucocephala*) were higher than those of the low tannin activity *Morus alba* at all levels of inclusion.

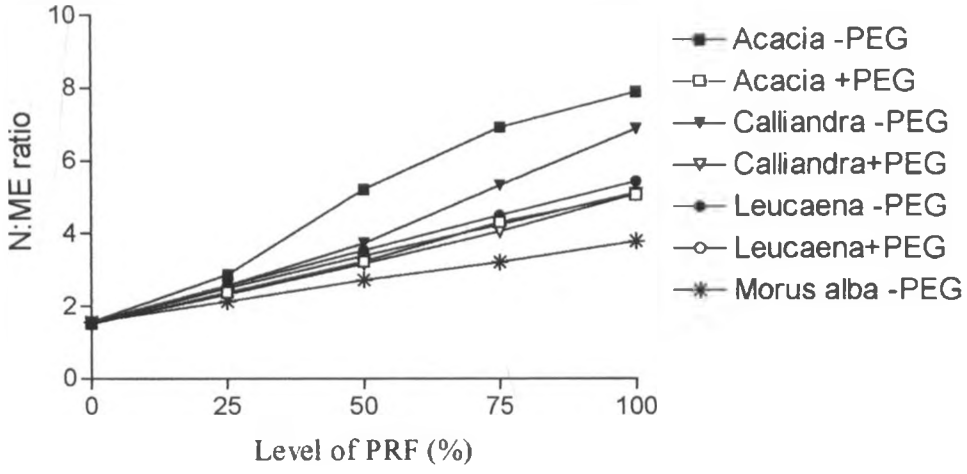

Figure 5.8 Effect of level of PRF on the ratio of N:ME of Napier grass-based rations

5.4.4 *In vitro* degradability of Nitrogen

The *in vitro* degradability of nitrogen in tanniniferous leguminous multipurpose trees and shrubs, herbaceous legumes and *Morus alba* is shown on Table 5.31. There was an increase in IVDN for all the tanniniferous forages from -13-33 without PEG to 33-50% on inclusion of PEG. The negative IVDN value for *A. angustissima* may be explained by the fact that rumen microorganisms may have been inhibited to the point that the blank incubations recorded higher NH_3-N values than incubations having *A. angustissima*. Inclusion of PEG did not affect the efficiency of nitrogen uptake (slope of regression curve) by rumen microorganisms. The ammonia level in the incubation medium increased significantly ($P<0.001$) when the tanniniferous PRF were incubated with PEG. In the absence of PEG, the non-leguminous *Morus alba* and the herbaceous legumes, *Lablab purpureus* and *Mucuna pruriens* had higher IVDN than the tanniniferous legumes; *Acacia*

angustissima, *Calliandra calothyrsus* and *Leucaena leucocephala*. *Crotalaria ochroleuca* had the lowest IVDN among the low tannin activity PRF.

Table 5.31: *In vitro* degradability of nitrogen and microbial uptake of nitrogen from incubations of tanniniferous and low tannin activity PRF

PRF	Gas volume	NH_3-N (mg/30ml)	Efficiency[4] (mg/ml)	IVDN (%)	Microbial N uptake (%)
Tanniniferous PRF					
Acacia a.[1]	0.9 ±0.06	4.08 ±0.46	0.06 ±0.01	-12.9 ±3.1	0.0 ±0.18
Acacia a. +PEG[2]	19.8 ±1.3	6.91 ±0.73	0.06 ±0.02	38.7 ±4.2	15.3 ±4.3
Calliandra	11.8 ±0.5	4.21 ±0.27	0.04 ±0.0	5.1 ±2.2	5.75 ±0.81
Calliandra+PEG	25.9 ±0.5	5.95 ±0.08	0.03 ±0.01	33.2 ±2.4	11.2 ±2.4
A. tortilis[3]	16.5 ±0.3	4.23 ±0.06	0.04 ±0.00	6.3 ±2.7	10.9 ±0.1
A. tortilis+PEG	24.1 ±0.7	5.46 ±0.22	0.05 ±0.01	46.3 ±2.7	26.2 ±0.2
Leucaena	28.8 ±0.9	6.07 ±0.11	0.04 ±0.01	33.4 ±2.5	12.2 ±2.1
Leucaena+PEG	33.3 ±1.0	6.9 ±0.36	0.05 ±0.02	50.8 ±7.9	20.4 ±4.1
Effect of PEG[5]	0.001	<0.001	0.93	<0.001	0.001
Low tannin PRF					
Morus alba	38.0^{a} ±3.3	7.35^{a} ±0.69	0.05 ±0.01	57.4^{a} ±5.2	25.1 ±4.4
Lablab	38.4^{a} ±0.9	5.06^{b} ±0.28	0.04 ±0.00	48.0^{ab} ±5.2	29.9 ±3.5
Crotalaria	32.9^{ab} ±2.4	5.22^{b} ±0.44	0.04 ±0.01	30.6^{b} ±6.9	19.9 ±4.9
Mucuna	28.4^{b} ±1.4	5.82^{ab} ±0.73	0.05 ±0.01	42.3^{ab} ±4.1	28.8 ±7.1

[1] Acacia angustissima, [2] Polyethylene glycol, [3]Acacia tortilis, [4]slope of regression of NH_3-N against gas production, [5]P value from ANOVA of the effect of PEG (tannin activity)in tanniniferousPRF

Means(±SD) of the low tannin PRF with different superscripts are significantly different ($P \leq 0.05$)

6 DISCUSSION

6.1 Experiment 1: Evaluation of the effects of stage of growth, addition of molasses and protein-rich forage on the fermentation characteristics of Napier grass silage

6.1.1 Chemical composition of Napier grass and legume forage

The chemical composition of Napier grass at 4, 8 and 12 weeks re-growth (Table 5.1) was comparable to values reported by Brown and Chavalimu (1985) for Napier grass of 5 weeks re-growth and by Muia *et al* (2001) for medium maturity and old Napier grass. The organic matter content of the NG (796, 808 and 817 g/kg DM for NG4, NG8 and NG12) was higher than 785 g/kg DM reported by Muia *et al.* (2001) for medium maturity Napier grass but the trend of increasing organic matter content with maturity was similar to that reported by Muia (2000). The crude protein and the detergent fibre contents were within the range expected for well- managed Napier grass at the particular stage of growth (Muia *et al.*, 2001, Kariuki *et al.*, 1999).

The legume forages had higher crude protein concentrations than the grasses and, among the legumes, *Leucaena* had the highest crude protein content (310 compared to 166, 174 and 176 gkg^{-1} for *Mucuna*, *Lablab* and *Crotalaria* respectively). It had been planned to harvest the annual legumes (*Crotalaria*, *Lablab* and *Mucuna*) at higher CP composition stage but shortage of seeds necessitated delaying of harvesting in order to attain the biomass required. Therefore, at 12 (*Crotalaria* and *Lablab*) and 16 (*Mucuna*) weeks of age, these legumes had CP values below 200 gkg^{-1} DM. In this study, the variation in *Mucuna* foliage with age was determined by sampling at 52, 84, 110 and 121 days after emergence. The CP content at these four times was 252, 233, 193 and 166 gkg^{-1} DM, respectively. *Mucuna* had the highest CP content (193 gkg^{-1}), which was close to 206 gkg^{-1} at 90 days of age reported by Ravindran (1988). The CP level of *Lablab* (174 gkg^{-1}) was comparable with 161 gkg^{-1} reported by Njarui *et al.* (2000) but lower than 221 gkg^{-1} reported by Muinga *et al.* (2000). The CP content of *Crotalaria* (176 gkg^{-1}) in this study was within those reported by Mkiwa *et al.* (1999a), who reported CP contents of 269 and 99 gkg^{-1} at 10 and 16 weeks of age respectively.

The legumes generally had lower NDF (397-497 gkg^{-1} DM) than Napier grass (547, 597 and 608 gkg^{-1} DM for NG4, NG8 and NG12 respectively). This is in agreement with previous

studies (Buxton and Russell, 1988) where it was reported that the proportion of cell wall components (structural tissue) is lower in legumes than grasses at similar stages of maturity. The water-soluble carbohydrate (WSC) content of Napier grass was low (32-40 gkg^{-1} DM). This was within levels expected for tropical grasses (McDonald *et al.*, 1991). Among the legumes, *Mucuna* and *Lablab* had higher WSC content compared to Crotalaria and *Leucaena*. This implies that the total WSC content of the forage mixtures used to prepare silage in this study were below 50 gkg^{-1} DM. This is much lower than the WSC content of the typical silage forages such as maize and Rye grass (>170 gkg^{-1} DM, McDonald *et al.*, 1991). Such material is not expected to achieve optimum fermentation for the production of enough fermentation acids for adequate preservation (Kellems and Church, 1998).

6.1.2 pH of silage

The average pH values recorded were above 4.5 (Table 5.2), which is the average maximum pH acceptable for low dry matter silage (McDonald *et al.*, 1991). The significant effect of molasses (Table 5.3) in reducing the pH of both Napier grass alone and legume-supplemented silage confirms that the WSC content of pre-ensiled material was too low. Pasture grass silages have been reported to have higher pH but usually not above 4.5. The pH values in the present study are, however, within range of values reported for tropical grass silages. Regan (1997) reported pH values of 5.8 and 6.1 for elephant grass and Calopo (*Calopogonium mucunoides*) silage made in 120L plastic drums. In the same study, he reported a pH value of 4.5 for medium-wilted Cavalcade legume (*Centrosema pascuorum*) and Pangola grass (*Digitaria eriantha*) silages made in bales. Fellner *et al.* (2000) achieved a pH of 4.8 in silage from a mixed sward of Timothy (*Phleum pratense*) and Brome (*Bromus inermis*) grass ensiled in a heap silo.

Typical pH values for Rye grass, sorghum and maize silage are 3.8 to 4.0 (McDonald *et al.*, 1991; Bolsen *et al.*, 1995). This is due to high WSC content in maize, sorghum and Rye grass. Surprisingly, legume-supplemented silage had significantly lower pH than non-supplemented silage in the present study (Table 5.4). This may partly be explained by the higher WSC content of some of the legumes (*Mucuna pruriens* and *Lablab*, Table 5.1). Legume forage is expected to have a higher buffering capacity due to the higher protein content and to lead to high pH in silage (McDonald *et al.*, 1991).

Cane molasses is an ideal substrate additive for tropical grass silages low in WSC. Being a by-product from sugar production, it is affordable especially in areas having a thriving sugar industry. It is mainly used for the production of industrial alcohol. Its use in animal production is limited to the dry season when farmers use it to induce cattle to consume low quality forage and straw. With an assumed WSC content of 650 g/kg in the DM and a DM content of 750 g/kg (NRC, 2001), cane molasses applied at 3% of the forage weight did not increase the WSC content to 100g/kg DM typical of good quality silage grasses and fodder crops (Kellems and Church, 1998).

In the absence of molasses, all the silages had high pH values in comparison to molassed silages (Table 5.2) and the effect of age of Napier grass was not apparent. When molasses was used as an additive, silage made from NG4 had higher pH than that made from NG8 and NG12. This may be attributed to the fact that NG4 had low WSC content compared to the other stages of growth.

The first order interactions of Napier grass maturity*Molasses, storage time*molasses and legume inclusion*molasses were significant ($P<0.05$) and accounted for a minor proportion of the variation in silage pH. The effect of molasses was found to be less with silage made of Napier grass of 4 weeks maturity than with the older Napier grass (pH reductions from 5.49-5.02, 5.46-4.78 and 5.37-4.71 for NG4, NG8 and NG12 respectively). This may be attributed to the higher buffering capacity expected for young grass (McDonald et al., 1991), which may have hindered a decrease in pH. However, it should also be borne in mind that NG4 had lower DM content than NG8 and NG12 meaning that the latter may have received slightly more molasses than the former. The interaction of storage time*molasses was found to be due to the fact that inclusion of molasses tended to reduce the pH of silage to a greater extent at 30 days storage than at 60 and 90 days (5.61-4.88, 5.43-4.84 and 5.27-4.78 respectively). The reduction of pH by molasses inclusion was found to be greater in the presence of legumes (5.39 to 4.76) than in the absence of legumes (5.66 to 5.15). This was attributed to the legumes having higher WSC content than Napier grass in this study (Table 5.1). The interaction of the storage time with inclusion of molasses was not significant..

6.1.3 Crude protein and ammonia-N content of silage

In general, legume supplementation increased the crude protein of resultant silage (Table 5.6). The level of CP increase depended on the CP content of the Napier grass and the legume

used. *Leucaena leucocephala*, therefore, led to the highest and significant ($P<0.05$) increases in CP. Silage made from NG8 had significantly higher CP than that made from NG12 in all cases (Table 5.8). Protein in silage is broken down by acid hydrolysis to peptides and amino acids or by proteolytic bacteria to ammonia (McDonald *et al.*, 1991). Therefore, ammonia levels in silage are indicative of the effectiveness of the silage preservation process and whether undesirable bacterial activity took place (Elferink *et al.*, 2000). In well-preserved temperate silages, NH_3-N content should not go above 110 gkg^{-1} total nitrogen (McDonald *et al.*, 1991). The values obtained in this study (Table 5.9) were much higher than 110 gkg^{-1} of the total nitrogen, which is indicative of inferior quality. Davies *et al.* (1998) reported even higher values (260 and 305 gkg^{-1} total N) in silage made from low WSC (66 g/kg DM) perennial Ryegrass and clover without additives.

The higher nitrogen content of legume-supplemented silage would lead one to expect higher levels of NH_3-N in such silages in comparison to non-supplemented ones. This was generally not the case in this study. Most legume-supplemented silages either did not differ, or had lower NH_3-N level than the non-supplemented silage (Table 5.9). Though the level of NH_3-N in silage depends on the effectiveness of the preservation process and particularly the activity of proteolytic micro-organisms such as Clostridial species (Davies *et al.*, 1998), the degree of degradability of nitrogen may play an important role especially when tanniniferous legumes are used. Leucaena-supplemented silage had significantly lower NH_3-N than the rest; a fact attributable to low N degradability due to the presence of tannins which protect protein from microbial degradation (Van Soest, 1994). This corroborates reports by *Kato et al.* (2001) who observed lower ammonia nitrogen levels in ensiled tanniniferous legumes (*Calliandra calothyrsus* and *Leucaena leucocephala*) compared to maize forage (12.7, 73.3 and 102.8 g/kg total nitrogen respectively).

Addition of molasses during ensilage led to significantly lower NH_3-N content in silage (Table 5.10). This was attributed to the increased WSC content, leading to a more rapid decrease in pH, which may have inhibited proliferation of proteolytic bacteria. The stage of maturity (8 and 12 weeks regrowth) did not affect the content of NH_3-N content of silage despite the fact that the crude protein content of the two silage types was significantly different (Table 5.11).

6.1.4 Fibre content of silages

Apart from the breakdown of WSC to carboxylic acids during ensilage, there is usually some breakdown of structural carbohydrates; cellulose, pectin and hemicellulose (McDonald *et al.*, 1991). These may be degraded by plant enzymes, microbial enzymes or hydrolysed by the fermentation acids. The net effect therefore is a reduction in the proportion of NDF and ADF in the silage compared to the fresh forage. This change in proportion can only be observed in the absence of losses of other components of the dry matter through effluent. In this study, there was a loss of 9 -15 % of NDF and 2-7% of ADF; the higher losses being registered in NG8 and the lower ones NG12. This agrees with findings by Yahaya *et al.* (2001) who reported losses of 20 and 17 % of hemicellulose, 17.3 and 17.7 of pectin and 3.3 and 0.5 of cellulose from Lucerne and Orchard grass respectively. Data reported by Filya (2003) also shows clear reduction in NDF content of whole-crop wheat silage made in laboratory silos. Kato *et al.* (2001) reported the reduction of ADF content of ensiled maize forage and *Gliricidia sepium* attributed to breakdown of cellulose. The breakdown of structural carbohydrates may be enhanced by the use of fungal enzymes such as cellulase, hemicellulase and pectinase. This may be of importance in ensilage of low WSC forages such as tropical grasses and legumes. Nadeau *et al.* (2000) reported significant increases of reducing sugars and lactic acid concentrations and reduction in NDF in silage treated with fungal cellulase.

The main reason for inadequate intake of dry matter by dairy cows fed on tropical forages is the 'bulkiness' of the feed (Tamminga, 1989). This alludes to the high NDF content; usually over 600g/kg in grasses (Kariuki *et al.*, 1998; Muia *et al.*, 2001) and up to 800g/kg DM in crop residues (Nherera *et al.*, 1998; Hove et al., 2001; Mthiyane *et al.*, 2001). Such forages have long rumen residence time and a large proportion of the NDF is not available for degradation in the rumen (Tamminga, 1989). Furthermore, digestive activities associated with such feed lead to large energy losses in form of heat, which worsens the heat stress in animals reared in hot tropical environments (Van Soest, 1994). One of the many options available for the reduction of the bulkiness of dairy cow rations is supplementation with legume forages which, as observed in the present study have lower NDF than grass. Therefore, apart from their main attribute of improving the supply of readily fermentable and rumen escape protein in ruminant diets, legume forage also reduce the bulkiness of the diet.

6.1.5 Conclusions

1. Silage made from non-molassed Napier grass or Napier grass/legume combinations has poor fermentation characteristics as evidenced by high pH.
2. Inclusion of molasses at 3% (w/w) improved the quality of silage
3. Inclusion of legume forage at 20% (w/w) did not affect the fermentation quality of the resultant silage
4. Inclusion of legume forage increased the crude protein content of Napier grass silage

6.2 Experiment 2: *In vitro* gas production kinetics and estimation of metabolisable energy of Napier grass, Napier grass silage and leguminous protein-rich forage

6.2.1 *In vitro* gas production

The *in vitro* gas production technique (Menke *et al.*, 1979) uses measure of gas production to estimate the organic matter degradability and metabolisable energy content of feedstuffs. Its main feature is the incubation of the test feed in buffered rumen fluid in a temperature-controlled system similar to the Tilley and Terry method. The major difference is that instead of measuring the disappearance of the DM components after stoppage of incubation, gas production is recorded at intervals during the course of the incubation. The gas produced is a direct result of organic matter breakdown to volatile fatty acids, CO_2 and methane. A big proportion of the CO_2 is produced by the buffering of VFAs by the bicarbonate buffer in the incubation medium (Blümmel *et al.*, 1999). Since ruminants obtain their energy requirements from volatile fatty acids (Van Soest, 1994), *in vitro* gas production is a good indicator of the energetic value of the feed. Using multiple regression analyses, Menke and Steingass (1987) came up with best fit equations relating *in vitro* gas production after 24 hours incubation, CP and crude fat content of a feed and the *in vivo* determined ME content. These equations are used to estimate the ME of test feeds using the gas production and crude nutrients. The main advantage of this method over other *in vitro* techniques is the ability to measure the rate of fermentation of a single feed sample without having to do serial incubations as would be required in the Tilley and Terry and the *in sacco* nylon bag techniques. Furthermore, unlike in other methods, only the sample is weighed and therefore, errors associated with washing, drying and re-weighing of the residue are eliminated.

In the present study, ensilage reduced the 24-hour gas volume from an average of 39 ml for fresh to 31ml for ensiled Napier grass; slightly higher for molassed silage and lower for non-molassed silage (Table 5.14-5.16). This is an expected result given that the main substrate of silo bacteria is the water-soluble carbohydrates present in the silage raw materials. The WSC and some structural carbohydrates are broken down to short chain fatty acids (SCFA); mainly lactic, acetic and propionic acids (McDonald *et al.*, 1991; Elferink *et al.*, 2000). Butyric acid is a rare occurrence in well-preserved silages and its appearance in more than trace quantities indicates poor preservation. The glycolytic process (oxidative decarboxylation) provides energy for the silo microorganisms but some is inevitably lost as heat. Apart from lactic acid,

the other SCFA cannot be utilized by rumen microorganisms since they are themselves end-products of rumen fermentation. This therefore means that silage has less WSC and generally lower energetic value than the fresh forage, the degree of reduction depending on the effectiveness of the preservation process.

For practical purposes and the need for repeatability, dried silage samples are used during the determination of metabolisable energy by the *in vitro* gas production method. The act of drying silage causes loss of a substantial proportion of the volatile components especially the SCFA and ammonia nitrogen (Weissbach and Kuhla, 1995). This, therefore, means that the energetic value is lower than would be obtained from *in vivo* digestibility trials where the animal ingests 'wet' silage with all the nitrogen and SCFA components intact. In the present experiment, silage was dried at 60° C for 72 hours common with previous reports (Regan, 1997; Meeske *et al.*, 1999; Fellner *et al.*, 2000; Filya, 2003) and therefore loss of some nutritionally useful components was expected.

Among the pre-ensiled forages (Table 5.14), *Lablab* and *Crotalaria* had higher gas production (46.4 and 40.2 ml) compared to Napier grass (38.6 and 39.8 for NG8 and NG12 respectively), *Leucaena* (34.4 ml) and *Mucuna* (36.8 ml). For the legumes, the 24-hour gas production accounted for a bigger percentage of the calculated asymptotic gas production (89, 92, 81, and 88% for *Crotalaria*, *Lablab*, *Leucaena* and *Mucuna* respectively) than for Napier grass (73 and 77% for NG8 and NG12). This implied that a bigger proportion of the energy in the legumes was released by microbial enzymes within 24 hours than for Napier grass. This is associated with the higher rates of *in vitro* degradation of legume forage in comparison to Napier grass as discussed below. The age of Napier grass (8 and 12 weeks) did not affect the 24-hour gas volume for silage (Table 5.17) with and without molasses. This was also observed in fresh Napier grass whereby the gas production from NG8 did not significantly differ from that of NG12 though the latter produced slightly more gas (38.6 and 39.8 ml/200mg sample respectively). Muia *et al.* (1999) studied the effects of stage of maturity (3-15 weeks) on CP, yields of DOM and DCP and the ratio of CP:DOM. They concluded that the optimal stage of maturity to feed Napier grass to dairy cattle should be 9-10 weeks in the high rainfall areas of Kenya. From the results obtained in the present study, it would be more advantageous to the farmer to make silage at 10-12 weeks of age due to the higher DM yields, since neither the silage quality nor the energy content are compromised.

6.2.2 Rate of gas production

The rate of gas production was derived from the constant c in the regression equation $Y=A+B(1-e^{-ct})$ and denotes the percentage of the asymptotic gas volume (B). Pre-ensiled legumes (*Mucuna*, *Crotalaria*, *Lablab* and *Leucaena*) had higher rates of gas production than pre-ensiled Napier grass (7-11 compared to 5-6 %/hour respectively) and a greater proportion of the asymptotic gas production was achieved after 24 hours of incubation than for Napier grass. Similar observations were made on Napier grass silage, whereby the rate of gas production was significantly lower for non-supplemented Napier grass silage at both maturity stages than legume-supplemented silage. Inclusion of molasses increased the rate of gas production from 2.1-3.9% in non-molassed to 3.2-5.5%/hour in molassed silage. The effect of molasses was observed across all silage treatments (Napier grass age and legume inclusion) and can be ascribed to the better preservation, which led to a sparing of easily fermentable carbohydrates. It is expected that WSC in non-molassed silage were broken down to a greater extent and converted mainly to ethanol, acetic and butyric acids which cannot be utilized by microorganisms and, since they are volatile, had been lost from the silage during the oven-drying process (Weissbach and Kuhla, 1995). This concept is supported by the observation in this study of higher rates of gas production in pre-ensiled forage (7-10 and 5-6%/hour for legumes and Napier grass) compared to silage (2-4 and 3-6%/hour for non-molassed and molassed silage respectively). These differences were mainly due to the low residual WSC in silages (2-9 gkg^{-1} DM) compared to the pre-ensiled material (39-50 gkg^{-1} DM, Table 5.21).

Since the ability of an animal to extract nutrients from a feed depends on the time available for digestibility and absorption, it follows that at a given duration of time, the animal is able to extract more nutrients from a feed with higher rate of digestibility than one with low digestibility. Therefore, the molassed and legume-supplemented silage in the present study was expected to be utilized to a greater extent than the non-supplemented, non-molassed silage. For medium producing, medium feed-intake dairy animals, the solid outflow rate from the rumen are estimated to be about 5% per hour (ARC, 1984; NRC, 1985; Shannak *et al.*, 2000), which translates to a mean retention time (MRT) of 20 hours. In the present study, gas production (for the purpose of ME calculations) was recorded at 24 hours of incubation, a duration which is close to the MRT. The amount of gas produced after 24 hours of incubation was expressed as a percentage of the asymptotic gas production from the non-linear regression model. The results agreed with those of rate of gas production in that only 56-69%

of the total gas was produced in 24 hours from non-molassed silage compared to 67-78% in molassed silage. Since most of the nutrient extraction from roughages by ruminants takes place in the rumen (Van Soest, 1994), the foregoing observations are an indication that supplementing of Napier grass (fresh or ensiled) with legumes and the use of molasses as a silage additive leads to better utilization of the feed offered.

Apart from high fibre content in tropical forages, slow rumen fermentation rates are a limitation to intake of adequate dry matter and therefore energy. Observations in the present study have shown the potential of legume forage as an option in improving the fermentation rate and, therefore, the voluntary intake of Napier grass-based diets. Even *Mucuna pruriens*, which did not reduce the 'bulkiness' of silage (by virtue of its high NDF content), increased the dry and organic matter intake in sheep (experiment 3, Table 5.22), a fact attributable to higher rumen fermentation rate, since the whole tract digestibility was not different from the control.

The correlations between CP and gas production were significant in the first 4 hours of fermentation but not in later stages (Table 5.18). The rate of gas production was significantly correlated with the CP content. This is due to the fact that the silages that had high CP content also contained legume forage, which as has been shown elsewhere in this study (Table 5.15), had a higher fermentation rate. Furthermore, there were significant negative and high correlations between NDF and ADF, and gas production and the rate of gas production. Again, the correlations at earlier stages of incubation (4 and 24 hour) were higher than at later stages (36 hours). The negative correlations were generally higher for ADF than NDF. These results may be attributed to the fact that gas production in the initial stages of fermentation originates from easily fermentable and soluble carbohydrates, which are in lower concentration in high fibre feedstuffs. At later stages of fermentation, the gas mainly originates from cell wall components (hemicellulose and cellulose) which are slow-fermenting hence the correlation is less negative. These findings corroborate those of Larbi *et al.* (1998) who reported similar relationships in tropical MPTs.

6.2.3 Estimated metabolisable energy of Napier grass and silages

Metabolisable energy of Napier grass (8.1 and 8.2 $MJkg^{-1}$ DM) estimated from *in vitro* gas production in this study (Table 5.19) were close to values reported by Kariuki *et al.* (1998), which were estimated from *in vivo* organic matter digestibility by the equation:

$ME(MJkg^{-1}\ DM) = DOM\ (gkg^{-1}\ DM) \times 18.5 \times 0.81.$

Metabolisable energy values for silages estimated from *in vitro* gas production ranged from 7.3-7.8 MJ/kg DM. These values are within the range observed in silage made in pit silos and ME values calculated from digestible nutrients (Experiment 3, Table 5.23). Though the effect of age of Napier grass on ME content was not significant, supplementation with legume forage increased ME for NG12 silage but did not affect ME content of NG8 silage. As expected, the use of molasses as an additive significantly increased the ME content of silage for both NG8 and NG12.

The increased rate of *in vitro* fermentation of legume-supplemented Napier grass silage observed in this experiment agrees with data obtained in experiment 3 (Table 5.23). This correlates with the tendency for higher digestible organic matter intake in animals offered legume supplemented Napier grass silage (Table 5.22). It should be noted that the *Mucuna* forage used in the two experiments were different in terms of maturity (121 versus 110 days for experiment 2 and 3 respectively) and chemical composition (Tables 5.1 and 5.21). However, there are cases where supplementation with high protein legume forage may not bring about the expected benefits. A case in point is when the legume has high NDF and ADF content as was observed in *Mucuna pruriens* (Table 5.21) in this study or when the content of biologically active tannins is high.

6.2.4 Conclusions

1. The use of molasses as an additive increased the rate and amount of gas produced as well as the metabolisable energy content of Napier grass silage.
2. The age of Napier grass (8 and 12 weeks) had no affect on rate of gas production or the fraction of the potential gas production achieved within 24 hours of incubation.
3. Inclusion of legumes in Napier grass silage increased the *in vitro* rate of gas production and the percentage of potential gas production achieved within 24 hours of incubation.

6.3 Experiment 3: Effect of inclusion of high protein forages at the time of ensiling on voluntary intake, *in vivo* digestibility and nitrogen balance in sheep fed Napier grass silage

6.3.1 Chemical composition of silages

The CP content of Napier averaged 95-107 gkg^{-1} DM at 8 weeks (Table 5.21). This was slightly lower than 117 gkg^{-1} reported by Kariuki *et al* (1999) at the same age but within range of values typical of Napier grass at 8 weeks of age (Muia *et al.*, 1999). The level of CP in Napier apart from age is also highly dependent on soil fertility, which may explain this difference. The NDF and ADF content for the Napier varied from 579-613 and 303-318 gkg^{-1} DM respectively and were within range reported by Kariuki *et al* (1999).

The nutrient content of Mucuna (CP: 166, NDF: 497, ADF: 389 gkg^{-1}) was within range reported by (Ravindran, 1988) but the CP was lower than 204 gkg^{-1} reported by Njarui *et al* (2000). The CP and fibre levels of the legume are highly dependent on age at harvesting. In the present study, the variation of CP content of Mucuna foliage with age was determined by sampling the forage at different stages of growth before harvesting. The values were 252, 233, 193 and 166 gkg^{-1} DM at 52, 84, 110 and 121 days post emergence. Due to failure of Mucuna to germinate after the first planting, it was not possible to use it at the stage of growth when CP level was optimum. The CP level of lablab (174 gkg^{-1}) compared with 161 gkg^{-1} reported by Njarui *et al* (2000) but lower than 221 gkg^{-1} reported by Muinga *et al* (2000). Both NDF and ADF content of lablab were lower than that of Mucuna probably due to the age difference at harvesting.

Inclusion of the legumes during ensilage increased the CP content of the silage. The aim was to improve the CP of the silage to 130g/kg DM but this was not achieved due to the lower CP content of legumes than expected. The fibre content of the silages reflected those of its constituents whereby inclusion of lablab resulted in silage with lower NDF and ADF (478 and 295 gkg^{-1} DM respectively) whereas inclusion of *Mucuna*, which had higher fibre content resulted in higher silage NDF and ADF (539 and 333 gkg^{-1} DM respectively). The NDF content of the silages was lower than would be expected from the relative proportions of Napier grass and legumes ensiled. This is indicative of breakdown of NDF either by plant

enzymes or acid hydrolysis of hemicellulose during ensilage. This corroborates the findings reported in experiment1 of the present study.

The water soluble carbohydrates (WSC) content (50-55 and 39-40 gkg^{-1} DM in legumes and Napier grass respectively) was lower than that of typical silage fodder crops such as forage maize and temperate grasses such as Rye grass, and Orchard grass (>170 gkg^{-1} DM) but within the range of values reported for tropical grasses such as *Setaria spp, Chloris gayana* and *Paspalum* spp (45-61, 30-35 and 27-34 gkg^{-1} respectively, McDonald *et al*, 1991). Higher values were reported in NB-21 (*Pennisetum purpureum*, Schumac X *Pennisetum americanum*, L.) by Panditharatne *et al* (1986) who found WSC levels between 82 and 114 gkg^{-1} for grass aged between 1 and 3 weeks. In the same trial and the same stage of maturity, *Panicum maximum* had WSC values between 67 and 76 gkg^{-1}. However, the grasses in their study were all below 4 weeks of age and unlikely to have acquired enough dry matter to justify investment in silage in a practical smallholder farm situation. The WSC content of the three forages used in the present study were below 100 gkg^{-1} level required for adequate silage fermentation (Kellems and Church, 1998). The addition of sugar cane molasses having a dry matter of 740 gkg^{-1} and a WSC content of 650 gkg^{-1} DM was estimated to have increased the sugar content of pre-ensiled forage to approximately 60 gkg^{-1}. The WSC content decreased on ensiling due to their breakdown by bacterial fermentation from the estimated 60 in the pre-ensiled forage-molasses mixture to below 10 gkg^{-1} in silage.

The pH of the silages (Table 5.21) were within range previously reported (4.5 to 5.4) for well fermented tropical grass silages with molasses as an additive (Regan, 1997; Snijders and Wouters, 1990). They were higher than 4.2 reported for tropical silages with cassava flour as an additive (Panditharatne *et al.*, 1986). The average pH of the silages made in pit silos (Table 5.21) was lower than those of the same forage ensiled in miniature polyethylene silos in experiment 1 (Table 5.2). This was attributed to the better compaction of the forage in the pit silos and probably better sealing. The pH of legume-supplemented silage in this experiment tended to be lower than that of the control, an observation seen in experiment 1 and which was likely due to the higher WSC content of the legumes in comparison to Napier grass.

6.3.2 Voluntary feed intake and digestibility

The voluntary DMI both in $gday^{-1}$ (1481-1661) and gkg^{-1} metabolic weight (74-83) were higher in the present study than those reported by Potkanski and Kujawa (1996) for perennial Ryegrass-clover silage (1.1-1.2 kg/day and 58-70 g/kg $W^{0.75}$/day) and Sarwatt *et al.* (2001) for maize, sorghum and Rhodes grass silages fed to sheep (52, 54.5 and 47.3 g/kg $W^{0.75}$/day). The voluntary DMI tended to be higher for legume-enriched Napier silage than the control (Table 5.22). Inclusion of legumes in Napier based diets have previously been shown to increase DMI (Abdulrazak *et al*, 1996) attributable to improved palatability and digestibility. This has also been demonstrated for wheat-pea bi-crop silage fed to sheep and cattle (Adesogan *et al.*, 2002, Salawu *et al.*, 2001), maize stover supplemented with *Leucaena diversifolia* and fed to sheep (Hindrichsen *et al.*, 2002) and native pasture hay supplemented with *Acacia angustissima*, *Calliandra calothyrsus* and *Leucaena leucocephala* fed to goats (Hove *et al.*, 2001). Similar results were reported by Kaitho *et al.* (1998) after supplementing teff straw with *Leucaena leucocephala*, *Leucaena pallida*, *Chamaecytisus palmensis* and *Sesbania sesban* in a feeding trial using Ethiopian highland sheep. The dry matter intake as a percentage of the live weight for the three diets (3.0, 3.3 and 3.4% for NG, NGL and NGM respectively) agreed with expected voluntary intakes for adult sheep (Church and Pond, 1988).

The dry matter and organic matter digestibility coefficients (0.52-0.57 and 0.61-0.66) obtained in this study were similar to those obtained by Adesogan *et al.* (2002) for pea-wheat bi-crop and perennial Ryegrass silage. In the current study, supplementation with *Lablab* did not affect ($P \leq 0.05$) dry matter digestibility of the silage. Dry Matter digestibility of *Mucuna*-supplemented silage tended to be lower than for non-supplemented Napier silage and this was attributed to the higher fibre content of this silage compared to *Lablab*-supplemented silage. Organic matter digestibility was significantly higher ($P<0.05$) in *Lablab*-supplemented compared to *Mucuna*-supplemented silage, which was not different from the non-supplemented silage. Adesogan *et al* (2002) also recorded marginal increases in digestibility of legume supplemented wheat silage (wheat-pea bi-crop). Tolera and Sundstol (2000) also reported linear increase in the digestibility of maize stover diets supplemented with graded levels of Desmodium hay. The lower digestibility of Mucuna-supplemented silage in the current study may probably be a result of higher fibre content of the *Mucuna* forage (ADF

389 compared to 303 and 253 gkg^{-1} DM for Napier grass and Lablab respectively), attributable to more advanced stage of maturity at harvest.

Though not significantly different, the daily digestible dry matter and organic matter intakes in the current study tended to be higher for legume supplemented (DDMI: 960 and 850, DOMI: 893 and 801 $gday^{-1}$ for Lablab- and Mucuna-supplemented silage respectively) than the non-supplemented silage (802 and 765 $gday^{-1}$). This means that, despite the lower digestibility of nutrients and DM for Mucuna-supplemented silage, the actual DM and OM digested (g/day) tended to be higher than for the control silage (Table 17).

The digestibilities of the ash-free fibre fractions (ADF, NDF and CF) were all above 60% indicating a high content of potentially fermentable carbohydrates (Cellulose and hemicellulose) and low lignin content in the fibre (Van Soest, 1994). Mucuna-supplemented silage had significantly lower ($P \leq 0.05$) digestibility of crude fibre and ADF than the non-supplemented silage whereas that of *Lablab*-supplemented silage was not significantly different. Generally, there was a tendency for lower fibre digestibility in legume-supplemented silage, again, indicating a higher lignin activity in legumes. This agrees with the general observation that legumes have lower cell wall components and higher lignin than grasses (Van Soest, 1994).

6.3.3 Nitrogen balance

The advantage of legume inclusion in grass-based diets (fresh forage or silage) is the increased DMI, which results in higher digestible nutrient uptake. Nitrogen balance measurements (Table 5.24) showed higher intake of nitrogen by the NGL- and NGM-fed sheep, which also resulted in higher nitrogen balances. Apart from increasing the nitrogen intake, supplementation with legumes increased faecal N losses ($P \leq 0.05$) with Mucuna supplementation causing the highest losses followed by lablab and the control. However, when expressed as a percentage of the N intake, *Mucuna*-supplemented and control silage had similar (49.3 and 49.5% respectively) whereas *Lablab* had slightly lower (42%) though non-significant proportionate N losses. Without analysis for NDF-bound nitrogen in the faeces, it may not be possible to comment on the source of the faecal nitrogen excreted. Faecal nitrogen may be of microbial (rumen, caecal and ileal micro-organisms), endogenous or feed origin (Ørskov and Miller, 1988). Metabolic fecal nitrogen increases with feed intake and this may partly account for the higher losses in supplemented silages, which also tended to have higher DM intake.

There was no dietary influence on the loss of nitrogen in urine but the supplemented silages tended to lead to higher losses. Though the supplemented silages led to higher total nitrogen losses, this parameter tended to be lower than that of the control when expressed as a percentage of the N intake. The amount of N retained expressed as a percentage of N intake was highest for NGL (26%) and lowest for NG (19%); Mucuna-supplemented silage had a retention of 22%. It can be concluded that inclusion of legumes in Napier silage leads to increase in nitrogen retention. The values of protein retained (27.9, 48.4 and 39.4 g /animal/day for NG, NGL and NGM respectively) were unexpectedly high for adult sheep and could not be explained by wool growth. The undocumented losses of nitrogen may be attributed to evaporative losses from urine during sample collection. It should also be noted that nitrogen analyses in faecal material was done on air-dried samples from which some ammonia nitrogen may have been lost. However, the samples for the three dietary treatments were processed using identical methods and therefore the results can still be used to compare the three treatments.

6.3.4 Implications of use of Napier grass silage in dairy cattle feeding

6.3.4.1. Supply of metabolisable energy

The energy requirement of dairy cattle is the composite of requirement for maintenance, milk production, weight gain, and conceptus (foetus and foetal membranes). However, this may be reduced to requirements for maintenance and lactation in case of empty adult lactating cows. The requirement may be given in metabolisable energy units or in net energy for lactation (NEL) (GfE, 2001). Below is an example of how metabolisable energy requirements for an adult cow of small breed weighing 400kg and producing 10 kg of 3.5% butter fat milk may be calculated.

Maintenance requirements = 0.488MJ ME * $Kg^{0.75}$ per day

= $0.488*400^{0.75}$

= 43.6 MJ ME

Energy content of milk = 0.4*3.5+1.5 (0.5*butter fat*1.5)

=2.9 MJ $NELkg^{-1}$

ME required =2.9/0.6

=4.83 MJ $MEkg^{-1}$ milk

The estimated daily dry matter intake for cows having the above-mentioned attributes (body weight and production) is about 12.5 kg dry matter (NRC, 2001). Therefore, the NG silages (average 7.5 MJ $MEkg^{-1}$ DM) would supply the animal with 12.5*7.5=94 MJ. If the maintenance requirement of 43.6 MJ is subtracted, then the balance available for milk production is 50 MJ ME. The cow is therefore able to meet its maintenance requirements and the supply of approximately 50/4.83 = 10 kg milk.

Estimation of ME from digestible nutrients (GfE, 2001) is both time consuming, laborious, expensive and requires large quantities of test feed (Osuji *et al.*, 1993). In view of the foregoing, in vitro methods have been developed which have the advantage of being faster, inexpensive and allow the standardization of experimental conditions more precisely than in *in vivo* trials. The *in vitro* gas production technique (Menke *et al.*, 1979) has been adapted for direct estimation of ME from the gas production from a standard sample weight and incubation time (Hohenheimer Futterwert Test (Steingass and Menke, 1986). It has been employed routinely for the evaluation of temperate feedstuffs in a number of research laboratories. In the present study, the gas produced after fermentation for 24 hours was 33, 34 and 31 ml for Napier-alone, NGL and NGM silages (Table 5.18). The ranking was, therefore, the same as for apparent digestibilities of DM and organic matter obtained from the *in vivo* trial (Table 5.17). The ME content calculated from gas production and digestible nutrients were significantly higher for *Lablab*-supplemented silage followed by the control and the *Mucuna*-supplemented forage in that order. ME values derived from gas production were slightly lower (7.4, 7.7 and 7.2) but very close to values derived from the *in vivo* study (7.8, 8.0 and 7.5) and the ranking of the three silages was maintained. The foregoing confirms that the gas production method may confidently be used not only to study fermentation kinetics but also to estimate the ME of tropical feeds including tropical grass silage.

Supplementation of Napier grass with *Mucuna pruriens* forage at 20% level during ensiling led to a reduction in silage metabolisable energy but supplementation with Lablab forage increased silage ME in this study. This was mainly attributed to the lower digestibility of the Mucuna-supplemented silage since metabolisable energy is calculated from the digestible crude nutrients (GfE, 2001).

6.3.4.2. Protein supply

The net protein required by the animal is estimated as the sum of net protein for maintenance and for production (growth, milk production and conception products). The net protein requirement for maintenance is estimated as the sum of endogenous and surface losses of nitrogen from the animal multiplied by the factor 6.25 (GfE, 2001). The endogenous losses include metabolic faecal nitrogen (MFN) and endogenous urinary nitrogen (UNe). Surface losses (VN) include nitrogen lost in hair and scurfing of outer skin layers. The following equations are used in estimating the different nitrogen fractions:

UNe (g/day) = 5.9206 log LWt-6.76 (1)

MFN (g/day) = 2.19 x kg DMI (2)

VN (g/day) = $0.018LWt^{0.75}$ (3)

For a cow weighing 400 kg and consuming 12.5 kg dry matter per day, the net protein requirement for maintenance calculated from the above equations is: UNe+MFN+VN = 235g CP/day.

The protein requirement for milk production is calculated using the total milk produced per day and the protein content of milk. The protein content of milk varies between cattle breeds with low levels (3.3%) in the Holstein and high values in the Jersey breed (4%). The other cattle breeds have values that lie between these two extremes. If an average of 3.4% crude protein in milk is assumed, then the daily requirement for protein is 34g/kg milk. If such a cow produces 10 kg of milk per day, then the net crude protein requirement for milk production would be 340g/day. The total net crude protein requirement is, therefore, 235+340 ≈ 575 g /day.

It should be noted that the protein ingested by a ruminant is different both in total amount and in the amino acid composition of the protein (metabolisable protein) that reaches the duodenum. This is because rumen microbes break down intake crude protein and synthesize microbial protein from the resultant ammonia and amino acids. They also make use of ammonia recycled into the rumen from the blood stream. Some of the nitrogen from the feed may be lost as ammonia, which is converted to urea in the liver and excreted in urine. The metabolisable protein reaching the duodenum comprises of undegraded feed protein (UDP) and microbial crude protein (MCP) as well as endogenous crude protein. It may be higher, equal or lower than the intake crude protein. This means that the rumen nitrogen balance

(RNB) may be positive, zero or negative. Since metabolisable protein is the one available to the host animal, it is only logical that ruminant protein requirements be expressed in terms of metabolisable protein reaching the absorption site in duodenum.

For ruminants reared on pasture forage, rumen degradability of the protein determines its usefulness to the animal. Degradation of protein to ammonia in the rumen has to be coupled with fermentable carbohydrate supply. The latter provides the energy required and the carbon skeletons for microbial protein synthesis and growth. Deficiency in fermentable carbohydrates in the rumen leads to accumulation of NH_3, which is absorbed into circulatory system and converted (at an energy and amino acid cost) to urea in the liver. Some urea is recycled into the rumen via saliva and rumen wall but some is inevitably lost in urine and contributes to environmental pollution. It is, therefore, important to optimize the ratio of nitrogen to energy in the rumen. Poppi and McLennan (1995) reported the critical maximum ratio to be 210g CP/kg DOM above which losses of protein or incomplete transfer of protein from the rumen to the intestines occurs. Such feed leads to highly positive rumen nitrogen balance values (Lebzien *et al*, 1996; GfE, 1997). In the present study, all the silages had ratios lower than the 194g CP/kg DOM (Table 5.21) recommended for moderate milk production (Tamminga, 1989). However, all the silages had CP:DOM ratios within range recommended for optimum fermentation and rumen nitrogen balance (152, 173 and 188 g/kg DOM for the control, *Lablab*- and *Mucuna*-supplemented silages). It can be concluded that legume supplementation of Napier grass silage at the time of ensiling improved the protein:energy ratio to values close to adequate for moderate milk production. Moderate milk production under the Kenyan smallholder conditions translates to an average of 10 kg milk per cow and day. The data on protein/energy ratio, therefore, agrees with the metabolisable energy calculations above where the Napier grass silages were shown to supply at least 50 MJ ME daily above maintenance, which is enough for the production of 10 kg of milk. However, this is only achievable at an assumed daily dry matter intake of 12.5 kg, which may not be achievable on sole diets of Napier grass silage. Muia et al. (2001) reported a total dry matter intake of 11.6 and 10.1 kg/day in Friesian steers (445 kg average live weight) fed on medium age and old Napier grass (>15 weeks old). The intake increased to 15.3 kg/day when the diet was supplemented with poultry litter-based concentrate. Observations on smallholder dairy farms in Kenya have shown that animals are fed on older, stemmy NG leading to failure of animals to achieve enough DM intake to meet their maintenance needs and have extra energy for milk production.

The legumes in this study were included at a rate of 20% on green weight basis. Data shows that these legumes may be included at higher levels without pushing the CP:DOM ratio above 210 g/kg beyond which significant losses of protein from the rumen occur (Poppi and McLennan, 1995). This would of course depend on the degradability of the legume protein and whether other fermentable energy sources are included in the diet. Different legumes have different rates of degradability in the rumen and the presence of condensed tannins plays a major role in nitrogen degradation in the rumen as has been shown elsewhere in this study. Suffice it to mention that higher losses are likely to occur with Napier grass silage supplemented with *Lablab* than that supplemented with *Leucaena* or *Calliandra*, which contain tannins. The added advantage of using the legumes in mixed silages is that there is minimal substitution of the basal feedstuff by the legumes as would occur if the components are fed separately (Kaitho *et al.*, 1998).

6.3.5. Conclusions

1. Legume supplementation improved the CP:digestible organic matter ratio.
2. The dry and organic matter intakes tended to be higher in legume-supplemented than in Napier grass-alone silages.
3. Digestible DM and OM intakes (g/day) tended to be higher in legume-supplemented Napier grass silage.
4. Lablab supplementation significantly increased the ME content of silage compared to Mucuna-supplemented and non-supplemented Napier grass silage.
5. Legume supplementation significantly increased nitrogen intake and retention

6.4 Experiment 4: Effect of tannins in protein-rich forage on *in vitro* gas production kinetics, estimated metabolisable energy and fermentation of other substrates

6.4.1 Chemical composition of browse

The protein-rich forage (PRF) used in this trial were either leguminous multipurpose trees and shrubs (MPTS; *Acacia tortilis, Acacia angustissima, Leucaena leucocephala*), non-leguminous MPTS (*Morus alba*) or herbaceous legumes (*Lablab purpureus, Crotalaria ochroleuca* and *Mucuna pruriens*). The multipurpose trees and shrub forage used in this trial (*Leucaena, Morus alba and A. angustissima*) had higher crude protein than the herbaceous species (*Lablab, Crotalaria* and *Mucuna*) with the exemption of *Acacia tortilis* (Table 5.26). The CP values of *A. angustissima* and *Calliandra* (260 and 258 gkg^{-1} DM respectively) were higher than 219 and 119 gkg^{-1} DM reported by Hove *et al.* (2001) and *Morus alba* had higher CP than that reported by Yao *et al.* (2000) and Roothaert (1999); 249 versus 219 and 151g/kg DM respectively. The CP value for *Lablab* (174g/kg DM) was higher than that reported by Njarui *et al.* (2000) but lower than Muinga *et al.* (2000); 16 and 22% respectively.

Among the PRF studied, *Mucuna pruriens* had the highest NDF and ADF content and *Morus alba* the least (497 and 218 versus 389 and 140 respectively). The fibre levels observed were consistent with the general observation of lower NDF and ADF contents in legumes and non-grass fodders compared to tropical grasses (Van Soest, 1994; Kariuki *et al.*, 1998). From the chemical composition, it would be expected that the PRFs would have a higher nutritional value compared to conventional tropical forage such as Napier grass (CP <10, NDF >600 and ADF >400 g/kg DM). However, this did not apply when the PRFs were incubated in buffered rumen fluid (Tables 5.27 and 5.30).

6.4.2 *In vitro* gas and volatile fatty acid production from browse plants

There was a great variation in gas production between the different plant species, which ranged from 3.6-50.3 ml/200mg DM after 24 hours incubation (Table 5.27). Whereas all the PRFs exhibited an exponential gas production pattern, the net gas production (corrected for gas production of blank incubations) from *A. angustissima* increased slightly and then showed a reducing trend as the incubation time increased (Figure 5.1). This indicates that the gas production from blank incubations (buffered rumen fluid without sample) surpassed that from

incubations of *A. angustissima*. This can be attributed to inhibition of microbial activity by chemical factors in *A. angustissima*.

The main factor that may cause inhibition of rumen microbial activity by tropical browse is condensed tannin (Gupta and Haslam, 1979; Kumar and Vaithiyanathan, 1990; Khazaal and Ørskov, 1994; Getachew *et al*, 2000). However, the tannin content in forage has been found to be poorly correlated with the digestibility of dry matter and nitrogen (McSweeney *et al.*, 1999; Jones *et al.*, 2000). Khazaal and Ørskov (1994) assessed the effect of antinutritive factors in several browse species using the in vitro gas production technique and polyvinylpolypyrrolidone as a tannin binder. They reported weak correlation between gas production and total extractable tannins (TET), phenols (TEPH), condensed tannins (TECT), proanthocyanidins (TEPA) and total proanthocyanidins (TOPA). They concluded that the lack of correlation was due to the fact that the 'nature' of tannins differs from one species to another. This, therefore, supports the argument that the determination of tannin activity in a potential feedstuff is more important than the quantification of tannin content *per se*. For the evaluation of ruminant feedstuffs, the *in vitro* gas production technique offers a rapid and inexpensive means of ranking feedstuffs according to the effect of tannins on rumen fermentation.

In the current study, PEG-6000 was used to rank the plant samples in order of tannin activity and to demonstrate the effect of tannins on various parameters of *in vitro* fermentation. When the PRFs were incubated with an equal amount (200mg) of PEG-6000, the gas production increased most significantly in 4 leguminous browse species (Table 5.27); namely *Leucaena*, *Acacia tortilis*, *Calliandra* and *Acacia angustissima* (23, 54, 112 and 475% respectively). The percentage increase of gas production from *Leucaena* and *Calliandra* are close to values reported by Baba *et al.* (2002; 51 and 149% respectively). In their study, they used higher sample weights (500 mg) and incubation medium volume (40 ml) but the ratio of legume to PEG was 1:1 similar to the current study. On the other hand, there was minimal increase in gas production from the herbaceous legumes and *Morus alba*. In terms of condensed tannin activity, the 4 leguminous browse species may be ranked in terms of percentage increase in gas volume on incubation with PEG. Tannin analyses were not done in the present study.

The effect of tannin activity on the *in vitro* volatile fatty acid (VFA) production (Table 5.28) followed similar pattern to that observed for gas production. Negative values of butyric,

isobutyric and isovaleric acids were observed for *Acacia angustissima* and *Calliandra calothyrsus* incubated without PEG. The negative values were obtained after correction of the observed VFA concentrations by subtraction of the VFA from blank incubations and can be interpreted to mean that there was inhibition of both carbohydrate and protein degradation by tannin in the two legumes. Getachew *et al.* (2000) reported similar findings on *Acacia cyanophylla* and *Acioa barteri* forage incubated without tannin binder. Inclusion of PEG increased total VFA production by 25, 167 and 437% for *Leucaena leucocephala*, *Calliandra calothyrsus* and *Acacia angustissima* respectively, which is quite close to the increases in gas production (23, 112 and 475%) for the same species. This corroborates the work of Blümmel *et al.* (1999) who reported a very close association between *in vitro* VFA production and gas production for a wide range of feeds. In their study, gas volumes could be accurately predicted from the amounts and proportions of acetic, propionic, butyric and isobutyric acids in the *in vitro* system. These findings support the *in vitro* gas production technique as the best *in vitro* method for assessing the degradability of tannin containing feeds. Getachew *et al.* (2000) reported that, despite large increases in gas production in the presence of PEG, the apparent *in vitro* digestibility did not change, a fact attributed to the formation of insoluble tannin-PEG complexes which caused an under-estimation of the degraded sample.

6.4.3. Rate of gas production

The rate of gas production was significantly affected by the legume type and treatment with PEG. The pattern of gas production from *A. angustissima* without PEG (Fig. 5.1) could not be fitted to an exponential regression function and hence the rate of gas production could not be calculated by use of an exponential model. It should be noted that the gas production was very low and not different from zero after 24 hours of incubation. In general, the herbaceous legumes and *Morus alba* had higher rates of gas production than leguminous MPTs incubated without PEG (Table 5.27). However, on incubation with PEG, *A. angustissima* had the highest rate (12.8%/hour) followed by *Leucaena* (10.8%/hour), *A. tortilis* (6.7%/hour) and *Calliandra* (3.5%/hour).

The time required to release 70% of the asymptotic gas volume (T70) is a parameter that can be related to the potential degradation of plant material in the rumen and has a bearing on the retention time of the particular feed in the rumen. The T70 was calculated from the rate of gas production and averaged 12 hours for herbaceous PRFs and *M. alba*. The shrub legumes when

incubated without PEG had an average T70 of 16 hours apart from *Calliandra* (97 hours) and *A. angustissima* whose data could not fit an exponential model. When incubated with PEG, the T70 reduced to 11, 15 and 35 hours for *Leucaena*, *Acacia tortilis* and *Calliandra* respectively. This parameter is comparable to the time required for 70% of the substrate to be degraded in the rumen or the time taken for 70% of the substrate dry matter to disappear from nylon bag in the *in sacco* technique (Ørskov and McDonald, 1979). It was chosen in this study as it corresponds to the rumen degradability of good quality forage measured by the nylon bag technique. Kariuki *et al.* (1993; 1998) reported potential degradabilities of 67% for Bana grass and French Cameroon varieties of Napier grass and 69.8 and 77% for Lucerne and sweet potato vines respectively. In general, feed passage from the rumen of cattle depends on the intake level and the structural composition of the feed (Van Soest, 1994). Rumen solid outflow rates may be classified into three general categories; 2, 5, and 8% representing situations of low, medium and high dietary intake (ARC, 1984). This means that the mean retention time of solid particles in the rumen is expected to be 50, 20 and 12.5 hours for the three categories respectively. All the herbaceous PRFs investigated in this study as well as *Leucaena*, *Acacia tortilis* and *A. angustissima* evolved 70% of the potential gas production in less than 20 hours. This is within the mean retention time of particles for animals having the medium level of intake and therefore solid particle passage rate which represent animals in the small holder dairy production systems of the Kenyan highlands.

6.4.4 Metabolisable energy

Metabolisable energy refers to the feed energy available for intermediary metabolism, a considerable fraction of which is retained in the animal's tissues for maintenance and growth or leaves the animal in form of products e.g. milk. Knowledge of ME content of feedstuffs allows more accurate formulation of rations for animals. As described elsewhere in the current study (experiment 2), the Hohenheimer Futterwertstest (HFT) uses *in vitro* gas production to estimate metabolisable energy of animal feeds using regression equations, which have been validated using in vivo data (Menke and Steingass, 1987, Getachew *et al* 2002). It has mainly been used for conventional feedstuffs and rarely for tropical PRFs, which may contain antinutritive factors. The alternate incubation of the test material without and with added PEG or any other tannin binder gives information of how much ME is unavailable due to the effect of tannin activity. In the present study, the metabolisable energy calculated from gas production from PRFs without and with added PEG (Table 5.27) showed significant

increases in this parameter when leguminous MPTS forage were incubated with PEG. The ranking of the MPTS based on the effect of PEG was similar to that obtained for gas production above. However, the percentage increases in ME were comparatively smaller than that realised for gas production viz: 57, 37, 25 and 8% in *A. angustissima*, *Calliandra*, *A. tortilis* and *L. leucocephala*. This may be explained by the fact that all the MPTS had high crude protein content, which is taken into account when calculating ME and therefore partly compensates for the reduced gas production.

Apart from *Leucaena*, the MPTS had ME values below 6.5 MJ/kg DM when incubated without PEG. This is well below the ME value estimated for Napier grass and Napier grass based silage in Experiments 2 and 3. Addition of PEG in the incubation mixture increased the ME to an average of 8 MJ/kg DM which is within the range calculated for Napier grass at the optimal stage of growth (8 weeks) and higher than the average for Napier grass silage (7.5 MJ/kg DM) (Table 5.19). The herbaceous legumes, *Morus alba* and *Leucaena* had higher ME values than Napier grass. The use of these forages to supplement Napier grass based diets, therefore, would not only increase the crude protein concentration but also the metabolisable energy of the diet with concomitant better utilisation.

6.4.5 Effect of tannin activity on the *in vitro* fermentation of Napier grass

The effect of the activity of tannins in *Acacia angustissima* foliage on the *in vitro* fermentation of Napier grass was evaluated (Table 5.30). Inclusion of *A. angustissima* in Napier grass at a rate of 1:1 decreased gas production from 23 to 9.4 ml at 12 hours and from 34.8 to 11.8 ml at 24 hours of incubation, indicating that the legume affected negatively the fermentation of Napier grass. The inclusion of PEG in the fermentation medium increased the gas production of the mixed samples (Napier grass and *Acacia angustissima*) to 40.4 ml at 12 hours and 56.9 ml at 24 hours of incubation. The latter values were very close to those expected from summing the gas production of the individual plant species in the presence of PEG viz: 35.2+ 20.7 at 12 hours and 35.2+ 20.7 ml at 24 hours, which confirms that in the absence of tannin activity, the gas production of mixed feedstuffs is additive (Schöner, 1981). This was expected since tannins bind dietary protein, are reactive with bacterial cell wall as well as secreted microbial enzymes (McSweeney *et al.*, 2001). According to Barry and McNabb (2000), condensed tannins occur in two forms in plant material; insoluble CT bound to protein and fibre and free CT. The free CT is the one that inactivates microbial enzymes

therefore reducing carbohydrate digestion in the rumen. Insoluble CT merely makes the protein in tanniniferous legume forage unavailable for bacterial break down. In the current study, the negative effect on gas production was different for the two substrates. The reduction of gas production of starch by the inclusion of *A. angustissima* foliage in the absence of PEG (19% at 12 hours and 18% at 24 hours of incubation) was less than for Napier grass (59 and 66% at 12 and 24 hours respectively). These observations led to the following postulates: i). that tannin activity affects amylolytic bacteria and enzymes more than cellulolytic bacteria and cellulases, and ii). that the differential effect on starch and Napier grass derives from the fact that soluble tannins take some time to dissolve into solution to react with bacterial cell walls and since starch breakdown happens at a faster rate (21%/hour compared to 5.3 and 5.9 %/hour for NG8 and NG12 respectively, Table 5.14), the effect is less than on fibrous feeds that require bacteria to attach on the feed particles; this allows time for the tannins to complex with the bacteria and their enzymes thereby hampering the fermentation process.

In practical ruminant nutrition, the inclusion level of PRFs in Napier grass rations for optimal rumen function is an important consideration. The present study attempted to answer this by constituting miniature rations (approximately 200mg) comprising of increasing fractions of the PRF (0 to 100%) and Napier grass (8 weeks maturity). Incubation of graded levels of *A. angustissima, Calliandra and Leucaena* combined with Napier grass in standard gas production tests showed that the gas production decreased as the proportion of the legume in the diet increased (Fig. 5.2-5.5). This decrease was most pronounced for *A. angustissima* and *Calliandra* and was attributed to the negative effects of tannin activity on rumen fermentation. Though expected, the decrease in gas production and ME when the level of Acacia was increased was not linear; it was more rapid at lower levels of acacia than at higher levels. In the absence of PEG, it was observed that the decrease could not be accounted for solely by the proportional contribution of the two components to gas production and the ME. Therefore, the additivity of gas production of feedstuffs in diet mixtures (Schöner, 1981) may not occur when tanniniferous feedstuffs are used which again points to the fact that tannins also affect negatively the fermentation of other ingredients. The pattern of changes in gas production and ME with increasing levels of *Calliandra* and *Leucaena* without PEG was more linear indicating that the reductions were mainly attributable to non-availability of fermentable nutrients due to bound tannins. In the presence of PEG (Fig. 5.2, 5.3 and 5.4), gas production decreased with increasing legume inclusion but at a slower and more uniform rate attributable

to progressive reduction in fermentable carbohydrates. Inclusion of the relatively tannin-free *Morus alba* (Fig. 5.5) increased the gas production and ME in the absence of PEG.

The effect of tannin activity on the fermentation of Napier grass diets supplemented with tanniniferous legume forage may be otherwise presented as the reduction of gas production or metabolisable energy in the absence of a tannin binder (Fig. 5.6 and 5.7). As earlier reported, the tannin effect on gas production and ME was greatest for *Acacia angustissima* followed by *Calliandra* and *Leucaena*. The curves show that the tannin effect increased slowly at first and then rapidly when *Acacia angustissima* and *Calliandra* were included above 25%. There was not much change when *Leucaena* was used and the amount of reduction did not go above 10%. This implies that, in practical animal feeding using *Acacia angustissima* and *Calliandra* as protein supplements in forage-based diets, the legumes should not be included at levels beyond 12.5% of the diet due to the reduction in ME. The effect of graded levels of legume forage on the ratio of N:ME in Napier grass-based rations (Fig. 5.8) show that, apart from *C. calothyrsus* and *A. angustissima*, the legume forage could be included up to 25% without going beyond the N:ME ratio recommended for optimum supply of energy and nitrogen to rumen microbes (1.97 g/MJ, GfE, 1997). *Morus alba* can be included at levels as high as 50% without adversely affecting the N:ME ratio.

6.4.6 *In vitro* degradability of Nitrogen

The ME estimation using the gas production technique uses crude protein as one of the variables in the regression equation. However, the degradability of the crude protein is not considered (Menke and Steingass, 1987). This may not be crucial in normal feedstuffs where a considerable proportion of the UDP is, rightfully, assumed to be utilisable together with microbial protein in the lower gut. There is, however, still controversy in regard to the high UDP levels in leguminous browse (especially high-tannin-activity plants) and its fate after escaping the rumen fermentation. Several authors have reported on the importance of tannins in ensuring the 'bypass' or escape of intake crude protein from the rumen to the lower gut (Barry and Manley, 1984; Gupta and Haslam, 1979). In small amounts (2-4% on DM basis), tannins may have beneficial effects by protecting feed protein from rumen degradation and therefore increase the by pass protein (Barry *et al.*, 1986). Animals feeding on tannin rich diets have been reported to have higher supply of non-ammonia nitrogen at the post rumen stage,

which is due to protection of protein in the rumen (Barry *et al.*, 1986) and the increase in microbial biomass.

The above phenomenon is explained by the defaunating effect of saponins and some other secondary plant factors. Protozoa account for 90% of bacterial protein turnover in the rumen (Wallace and McPherson, 1987). The source of nitrogen for rumen protozoa usually derives from bacteria engulfed and digested by them (Ørskov, 1982). Wallace and McPherson (1987) reported that the rate of breakdown of bacterial protein in rumen fluid was positively related to the number of small entodiniomorphid protozoa. Therefore, defaunation leads to increased microbial protein yield due to reduced microbial protein degradation in the rumen. This in turn improves the protein nutrition of animals feeding on tanniniferous forage. Barry *et al.* (1986) concluded that the choice of an optimum concentration of condensed tannin in browse plants should depend on a balance between its positive effect in increasing duodenal NAN flow and the negative effect of depressing energy and organic matter digestibility.

Other researchers have reported that the increased NAN at the duodenum is actually due to an increase in UDP as opposed to increased microbial biomass. Most of this NAN, which is actually NDF-associated N (Hove *et al.*, 2001), is refractory to host enzymes in the lower GIT and therefore, the net effect is an increase in faecal nitrogen. Barry and McNabb, (1999) reported that whilst tannin activity increased abomasal NAN flow in sheep fed on *Lotus pedunculatus*, this was counteracted by reduced apparent digestibility in the small intestines. This was attributed to the effects of condensed tannins in not releasing some of the protein in the small intestines or causing an increase in endogenous protein secretion or inactivating digestive enzymes.

The *in vitro* degradability of nitrogen (IVDN, Figure 6.1) values were generally low for the plants investigated (-13, 5, 6 and 34% for *A. angustissima*, *Calliandra*, *A. tortilis* and *Leucaena* respectively) incubated without PEG (Table 5.31). The negative value for *A. angustissima* is explained by the fact that the blank incubations had higher ammonia values than the y-intercept of the regression of ammonia nitrogen against gas production. This actually means that not only was the crude protein in *A. angustissima* refractory to rumen microbial degradation, the tannin activity inhibited the microorganisms in such a way that they could not degrade the little protein available in the incubation medium hence the higher NH_3-N in blank incubations. The percentage increases in IVDN values on incubation of the forages with PEG were >1000, 600,

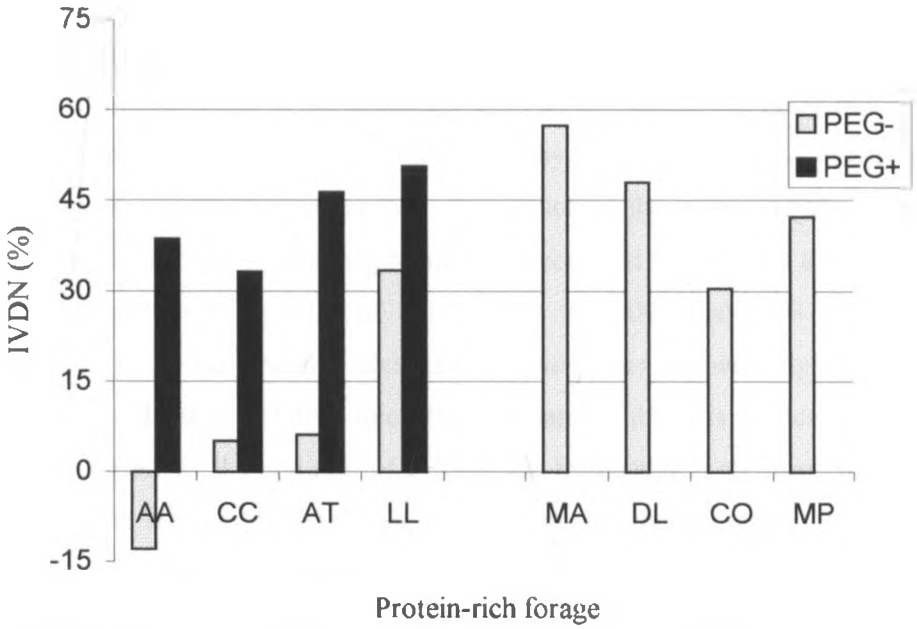

Figure 6.1 In vitro nitrogen degradability of tropical PRF

AA, Acacia angustissima; CC, Calliandra callothyrsus; AT, Acacia tortilis; MA, Morus alba; DL, Dolichos lablab; CO, Crotalaria ochroleuca and MP, Mucuna pruriens

550 and 52% for *A. angustissima*, *A. tortilis*, *calliandra* and *Leucaena* respectively. The new values were comparable to those obtained for low-tannin-activity PRF in this study viz: 31, 42, 48 and 57% for *Crotalaria*, *Mucuna*, *Lablab* and *Morus alba* respectively.

Getachew *et al.* (2000) measured the in vitro degradability of nitrogen (IVDN) of tropical legume forages after 24-hour incubation in buffered rumen fluid using the same method. They found low values of below 40% for some *Acacia* and *Desmodium* species incubated without PEG. On inclusion of PEG in the incubations, the IVDN values increased more than two-fold for most of the plant species investigated thereby demonstrating the effects of tannins in protecting plant proteins from microbial degradation in the rumen. In the same experiment, the efficiency of nitrogen uptake (mg N /ml gas) decreased for some of the plant species when incubated with PEG from 0.09 to 0.05, 0.054 to 0.046 and 0.08 to 0.06 for *Acacia cyanophylla*, *Acioa barteri* and *Desmodium uncinatum* respectively. This indicates that microbial N uptake and therefore microbial biomass synthesis is reduced when the effect of tannins is removed by the addition of PEG in the fermentation mixture. Similar results have been reported in studies by Baba *et al.* (2002) who observed a reduction of the partitioning factor (mg DM degraded/ml gas produced) of tropical leguminous browse plants when incubated with PEG. The partitioning factor (PF) (Blümmel *et al.*, 1997) is a recent concept which expresses the conversion of energy from truly degraded substrate to yield gas and consequently volatile fatty acids. The counter side to this is that the substrate truly degraded and not converted to short chain fatty acids is available for microbial growth. In the current study however, there were no significant changes in the efficiency of nitrogen uptake, which were between 0.03 and 0.06 mg/ml and therefore similar to values obtained by Getachew *et al.* (2000). However, the inclusion of PEG in the incubations increased the calculated microbial N uptake ($P<0.001$) for all the tanniniferous legumes with the biggest increase recorded in *A. angustissima*, *A. tortilis*, *Calliandra*, and *Leucaena* in that order. The herbaceous legumes and *Morus alba* generally had higher estimates of microbial N uptake than the browses.

6.4.7 Conclusions

1. In terms of tannin activity, the MPTS investigated in this study may be ranked in ascending order as follows: *Leucaena leucocephala*, *Acacia tortilis*, *Calliandra calothyrsus and Acacia angustissima*

2. Tannin activity in *A. angustissima* significantly reduced the *in vitro* fermentation of Napier grass and wheat starch
3. Based on *in vitro* fermentation observations, *Acacia angustissima* and *Calliandra calothyrsus* may not be included in Napier-based diets above the level of 12.5%
4. The non-leguminous multipurpose tree, *Morus alba*, has a high potential as a protein supplement in ruminant diets
5. Among the herbaceous legumes, *Lablab* was the best in terms of *in vitro* gas production, metabolisable energy content and *in vitro* degradability of nitrogen

7. GENERAL DISCUSSION

7.1 Introduction

The smallholder dairy sector, which accounts for 80% of the milk produced in Kenya is practiced in the high agricultural potential areas where the human population pressure is high. The average land holdings are 2-3 ha per family unit. Due to the need to maximize use of available land, animals are usually housed in zero grazing units in a 'cut and carry' system where the forage is brought to the animals. Availability of feed and its seasonal distribution are the major constraints to productivity. This seasonal pattern of feed availability and quality leads to similar fluctuations in milk production and household income. The main forage type on smallholder dairy farms is Napier grass. There is usually excess growth of Napier grass in the main wet season (April to June) which if not harvested overgrows and deteriorates in quality. The excess Napier grass, if well preserved, would serve as a dry season feed, thereby reducing feed supply fluctuations.

7.2 Nutritional attributes of Napier grass

A lot of work has been undertaken on the nutrient composition and feeding value of Napier grass (Anindo and Potter 1986; Kariuki, 1998; Muia, 2000). The factors affecting Napier grass quality include soil fertility and fertilizer/manure application (Snijders *et al.*, 1992), age at harvesting and the soil moisture (Muia *et al.*, 1999). Using the CP:organic matter method, Muia *et al.* (1999) concluded that the optimum age for the harvesting of Napier grass is 7 to 8 weeks in medium rainfall areas and 9 to 10 weeks in high rainfall areas. In the current study, standard agronomic procedures were used and irrigation was done weekly for 4 hours in the absence of rainfall.

The main effect of advancing stage of growth of forages is an increase in the cell wall components (cellulose, hemicellulose and lignin). This is accompanied by a reduction in minerals, crude protein content, and digestible soluble cell components (starch, water soluble carbohydrates) and an increase in the biomass. The decrease in forage quality with maturity is associated with a decline in quality of both leaves and stems and an increase in the proportion of stems. Whole plant forage quality is influenced to a greater extent by stem than by leaf

composition (Cowan and Lowe, 1998) since leaf quality declines at a slower rate with maturity than stem quality. The leaves are consistently higher in those parameters associated with higher nutritive value e.g. crude protein and digestibility, undergo more rapid and extensive degradation in the rumen, require less energy for chewing and are eaten in greater quantities than stem.

Tropical grass leaves contain a relatively higher proportion of bundle sheath and vascular tissue and a lower proportion of thin-walled mesophyll cells compared with temperate grass leaves (Wilson *et al.*, 1989: Van Soest, 1994). This makes them more fibrous i.e. they have higher proportions of cell wall components (NDF, ADF and lignin) than temperate grass species at similar stages of maturity. The high proportion of cell wall components in tropical grasses is associated with low soluble nutrients especially water soluble carbohydrates and protein. In the present study, increasing age from 4 to 12 weeks increased the fiber fractions (NDF: 547-608 and ADF: 283-323 gkg^{-1} DM for NG4 and NG12 respectively) of Napier grass and caused a reduction in crude protein from 133 to 89 gkg^{-1} DM. The water-soluble carbohydrate (WSC) content increased from 32 to 40 gkg^{-1} DM for NG4 and NG 12 respectively in agreement with reports by McDonald *et al.* (1991) and Mühlbach (2000).

It would be of interest to the farmer to feed the Napier grass at the optimum stage of growth in regard to dry matter yield, nutrient composition and digestibility. However, due to rapid growth in the wet season and few animals per farm holding, Napier grass tends to overgrow and is therefore fed to animals at maturities much higher than the optimum. There is little re-growth of Napier grass in the dry season and the animals are fed on Napier grass at maturities much younger than the optimum. Ensiling of Napier grass is an option available to the farmer to preserve the forage at the optimum stage of growth and to take advantage of the high biomass productivity and high nutrient content. Silage making is more appealing than hay making due to problems associated with drying of the thick-stemmed Napier grass and the field losses that would occur in prolonged field drying in the rainy season.

7.3 Napier grass silage and its improvement with molasses and protein-rich forage

In silage making, the level of water-soluble carbohydrates (WSC) in the herbage is critical. They include simple sugars (glucose, fructose and sucrose) and organic acid intermediates of sugar metabolism (malate, citrate, oxaloacetate) as well as some complex carbohydrates such as fructans that the plant synthesizes as an energy store (Thomas and Morrison, 1981). Water-soluble carbohydrates are highest in grasses grown at high light intensities and low temperatures (McDonald et al., 1991). Tropical grasses generally have low WSC content in the range 30 to 100 gkg^{-1} DM (Cowan and Lowe, 1998). In the current study, molasses was included as a source of easily fermentable carbohydrates and the treated silage had significantly lower pH and ammonia nitrogen content. Based on the results, it was concluded that Napier grass should not be ensiled in the absence of a source of water-soluble carbohydrates. Apart from cane molasses, other alternatives reported include various starch-rich additives such as cassava tuber meal (Panditharatne *et al.*, 1986) and caged broiler waste (Mthiyane *et al.*, 2001). Where available, cane molasses is the best additive since lactic acid bacteria do not ferment starch readily (Elferink *et al.*, 2000).

The problem of high fiber and low CP in tropical grasses was covered in the literature review and by Kariuki (1998) and Muia (2000). Napier grass is usually low in CP and the use of mineral fertilizers is one of the agronomical practices employed to increase the CP content of Napier grass (Snijders *et al.*, 1992). Fertilization of pastures is, however, expensive and most small-holder farmers do not practise it. To meet the animals' nitrogen requirements, the Napier grass-based rations should be supplemented with protein-rich sources such as soya bean meal, cotton seed cake, fish meal, etc. These protein sources are expensive and usually out of reach of the smallholder farmers. Alternative high protein supplements are in the form of protein-rich forages (PRF), which may be in the form of multipurpose trees and shrubs (MPTS) or herbaceous forage legumes. Research on new MPTS and nitrogen-fixing legumes in agroforestry and soil conservation research programs is ongoing (Roothaert *et al.*, 1997; Maobe *et al.*, 1998). Most of these have high N content and have potential as protein supplements in ruminant diets. Kariuki *et al.* (1998) reported increased N, DM and organic matter intake in heifers fed Napier grass supplemented with Desmodium and Lucerne while Muia (2000) reported increased DM intake in Friesian cows supplemented with poultry litter-

based concentrate and sunflower seed meal. Data have also been reported on the improvement of Napier grass and maize stover diets by the inclusion of *Leucaena* (Muinga *et al.*, 1995; Abdulrazak *et al.*, 1996; Nherera *et al.*, 1998; Kaitho *et al.*, 1998; Hove *et al.*, 2001; Hindrichsen *et al.*, 2002), *Gliricidia sepium* (Abdulrazak *et al.*, 1996), *Calliandra calothyrsus* (Nherera *et al.*, 1998; Hove *et al.*, 2001), *Acacia angustissima* (Hove *et al.*, 2001), *Crotalaria ochroleuca* (Sarwatt, 1990, 1992), *Mucuna pruriens* (Muinga, 2000; Njarui *et al.*, 2000), *Lablab purpureus* (Muinga *et al.*, 2000; Umunna *et al.*, 1995) and *Sesbania sesban* (Umunna *et al.*, 1995; Kaitho *et al.*, 1998).

In this study, the effect of inclusion of four different legume forages (*Lablab purpureus, Crotalaria ochroleuca, Mucuna pruriens and Leucaena leucocephala)* in Napier grass at the time of ensilage was investigated. In the first experiment, silage was made in miniature polythene silos. Inclusion of legume forage increased the protein content of the Napier grass silage and did not affect the fermentation quality of the silage (pH and ammonia nitrogen content). However, in the absence of molasses, the legume-supplemented silage was of low fermentation quality similar to non-supplemented silage. Legume inclusion reduced the fiber content of the silage and increased the *in vitro* fermentation rate. These observations led to the conclusion that legume supplemented silages are of better nutritive value than non-supplemented ones. In experiment 3, three types of silages (Napier, Napier+Lablab and Napier+Mucuna) were made in pit silos and fed to sheep. The voluntary dry matter intake was high for all the silages (74-83 $g/kg^{0.75}$) and the inclusion of legume tended to increase the DM, OM, DDM and DOM intake and significantly increased the intake of crude protein from 145 for non-supplemented silage to 183 g/animal/day for legume-supplemented silage. The digestibility of DM, OM and the fibre components were increased by the inclusion of Lablab whereas inclusion of Mucuna had no effect. The results are similar to those reported by Adesogan *et al.* (2002) using pea-supplemented wheat silage fed to sheep. Though more labour-intensive, the preparation of legume-grass mixed silages offers a means of preserving the grass and the legume at the optimum stage of growth as well as increasing the intake of dry matter and protein and the nutrient digestibility. The alternative is to ensile Napier grass alone and to supplement it with legume forage at the time of feeding. This is more appealing since multipurpose trees and shrubs (*Leucaena, Calliandra, Morus alba*) still produce some biomass in the dry season. However, for annual herbaceous legumes such as *Mucuna,*

Crotalaria and *Lablab*, preservation in form of silage or hay is necessary. Legume hay is usually problematic due to leaf shattering losses during field drying and transportation (Church and Pond, 1988; Wilkins, 1988) hence ensilage in combination with the grass is the better option.

7.4 *In vitro* fermentation kinetics of fresh forages and silages and the effect of tannin activity

The difficulties of ME determination by use of *in vivo* techniques were outlined in the literature review. In this study, the ME values of silage estimated by the gas production method (Menke and Steingass, 1987) were close to, but lower than, those obtained from the *in vivo* method (7.4, 7.7 and 7.2 versus 7.8, 8 and 7.6 $MJkg^{-1}$ for NG, NL and NM silage respectively). The ranking was, however, maintained and the *in vitro* values approximated 96% of the *in vivo* values. It was, therefore, concluded that this method may be used for the estimation of ME in mixed silages. The ME content of all the other silages and silage raw materials were therefore estimated using the *in vitro* gas production technique. The legumes (*Crotalaria, Lablab, Leucaena* and *Mucuna*) were observed to have higher ME than Napier grass 8.8, 9.7, 9.0 and 8.6 versus 8.1 $MJkg^{-1}$ respectively, which indicated their potential as diet supplements. They also had significantly higher rates of fermentation than the Napier grass, meaning that legume inclusion would improve the nutrient utilization in higher producing cows whose intake and dietary outflow rates are higher (Shannak *et al.*, 2000). Legume inclusion also tended to increase the ME content of Napier grass silage. The differences in the nutrient content and *in vitro* gas production of *Mucuna* forage and *Mucuna*-supplemented silage in experiments 2 (maturity: 110 days, CP: 193 g/kg DM and GP: 36 ml) and 3 (maturity: 121 days, CP: 166g/kg DM and GP: 31 ml) indicate that the stage of growth of the herbaceous PRF is an important consideration in determining its use as a supplement. The findings from experiments 1, 2 and 3 led to the *in vitro* investigation of other PRF (*Acacia angustissima*, *Acacia tortilis*, *Morus alba* and *Calliandra calothyrsus*) as potential supplements to Napier grass in ruminant diets.

Reports in the literature (McSweeney *et al.*, 1999; Getachew *et al.*, 2000; Odenyo *et al.*, 1997; Tolera *et al.*, 1997; Khazaal et al., 1994) indicate high tannin content in most tropical browse

species. However, tannin content *per se* determined by the conventional methods (Butanol HCl and Vanillin) was found not to be directly correlated to the effect of the tannins on digestibility (Ahn *et al.*, 1989; Abdulrazak *et al.*, 2000). This is attributed to differences in biological activity of tannins from different plant species and accessions. It is, therefore, important to test the potential protein supplements in terms of their biological activity on rumen microbial activity. *In sacco* methods and *in vitro* methods that require filtering for digestibility determinations may not be appropriate for the measurement of DM digestibility and degradability of nitrogen of tanniniferous material since free tannins solubilize into the medium and pass through the filter though they are not digested (Getachew *et al.*, 2000). The use of the gas production technique in combination with a tannin binder is simpler and gives more information about tannin activity than the conventional *in vitro* techniques or direct analyses for tannins (Getachew *et al.*, 1998a). In the current study, polyethylene glycol (PEG), a tannin binder was used to study the effect of tannin activity on the *in vitro* fermentation kinetics of the PRF. Inclusion of PEG in the incubation medium caused significant increases in gas production for *Acacia angustissima* (475%), *Calliandra* (112%), *Acacia tortilis* (54%) and *Leucaena* (23%). Increases in gas production for *Morus alba* and the herbaceous legumes on inclusion of PEG were not significant. These increases were reflected in the total and individual volatile fatty acid production of the legumes which increased by 437, 167 and 25% for *Acacia angustissima*, *Calliandra* and *Leucaena* respectively. These findings further confirm the close association between gas production and substrate fermentation as outlined by Blümmel *et al.* (1999b).

The effect of tannin activity in the leguminous PRF on the *in vitro* fermentation of Napier grass, which is the basal feed supplemented, was investigated. It was observed that inclusion of *A. angustissima* at a rate of 1:1 to Napier grass reduced gas production by 59% after 12 hours of incubation and by 66% after 24 hours indicating that chemical factors in *A. angustissima* inhibited rumen microbial activity. The effect was reversed by the inclusion of PEG, confirming that free tannin activity was the cause of the inhibition. Using the same technique, miniature rations combining graded levels of tanniferous legumes (*A. angustissima*, *Calliandra calothyrsus* and *Leucaena leucocephala*) were prepared and investigated. Increasing the proportion of *A. angustissima* and *Calliandra calothyrsus* reduced gas production of the rations and this was reversed by the inclusion of PEG. On the other

hand, inclusion of *Leucaena* and *Morus alba* which had minimal tannin activity increased the ME of the rations.

It was observed that the ratio of N:ME in the mixed rations increased rapidly with increasing PRF level especially for the tanniniferous PRF. To keep this ratio below 2 (Poppi and McLennan, 1995), the inclusion of tanniniferous PRF in the diet should not be above 25%. *Morus alba*, however, may be included at levels up to 50% of the ration without the risk of nitrogen loss from the rumen. It should however be noted that the tanniniferous PRF also have very low nitrogen degradability in the rumen (<10% IVDN for *A. angustissima, Calliandra calothyrsus* and *A. tortilis* in the present study). This is likely to modify the nitrogen energy relationships in the rumen, favouring better utilization of the available N. However, this is only possible if the activity of free tannins is inhibited by the use of a tannin binder so that microbial fermentation is not suppressed.

7.5 General conclusions and practical implications

The current study has shown that it is possible to make good quality PRF-enriched Napier grass silage when both Napier grass and PRF are available at the optimum stage of growth. Though a performance trial was not performed, the higher DM and nitrogen intake of PRF supplemented silage as well as the higher nitrogen retention and improved ratio of CP to Digestible organic matter in the PRF-silage indicate that animals fed such diets would perform better than animals fed non-supplemented silage or poor quality Napier grass. This was supported by *in vitro* data on a wider array of PRF, which showed significantly higher rates of fermentation of PRF and PRF-supplemented silages in comparison to Napier grass and non-supplemented silages. The benefits of use of PRF as feed supplements depend on the tannin activity especially for leguminous MPTs as was demonstrated in experiment 4. The use of mulberry (*Morus alba*) as a supplement in Napier grass diets is recommended based on the *in vitro* findings.

8 RECOMMENDATIONS AND SCOPE FOR FURTHER RESEARCH

1. Since it was shown that relatively good silage could be made from wilted Napier grass treated with 3% molasses, it would be worthwhile to experiment with other molasses levels and wilting durations. Lower levels of molasses, without compromising silage quality, would reduce the cost of ensilage.

2. Inclusion of legume silage at the time of ensiling improved the level of crude protein in silage without negatively affecting silage fermentation quality. The use of other types of protein-rich forages e.g. *Morus alba* at graded rates of inclusion should be investigated. This would give a clearer picture on the effect of supplementation on the voluntary intake, digestibility and nitrogen balance in animals fed on Napier grass silage.

3. An economic evaluation on the preservation of Napier grass and legumes in mixed silage at the smallholder farm level should be done. This should be accompanied by technology adoption studies

4. The measurement of nitrogen degradability of tropical PRF using the modified gas production technique should be validated using data from *in vivo* and *in sacco* studies. This would give more information on whether the higher UDP levels lead to higher metabolisable crude protein levels at the duodenum and whether this translates into higher animal performance.

5. Further investigations should be done on the high-tannin activity PRF to determine the effect of season, leaf maturity and effect of processing on rumen fermentation.

6. The effect of PEG and other tannin binders on *in vivo* nutrient digestibility and voluntary intake of high-tannin activity PRF should be investigated

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APPENDICES

App. 1: ANOVA for the effects of age of Napier grass, molasses and legume inclusion on silage pH

a. Main effects of Napier age and molasses

Effect	Condition	Sign.	P-value
Napier age	-Molasses	NS	0.094
	+Molasses	**	0.006
	NG4	***	<0.001
Molasses	NG8	***	<0.001
	NG12	***	<0.001

b. Main effect of legume forage inclusion

condition	Sign.	P-value
-Molasses	NS	0.328
+Molasses	***	<0.001

c. interactions

Interaction	Sign.	P-value
Napier age*Molasses inclusion	***	<0.001
Napier age* Legume inclusion	***	<0.001
Napier age * storage time	NS	0.215
Molasses * Legume	***	<0.001
Storage time * molasses inclusion	***	<0.001

App. 2: Analysis of Variance for the effect of age of Napier grass and inclusion of molasses on crude protein of silage

Effect	condition	Significance	P-value
Napier grass age	-molasses	*	0.039
	+ molasses	**	0.005
Molasses	NG8	NS	0.463
	NG12	NS	0.806
Napier age*Molasses inclusion		NS	0.196
Napier age* Legume inclusion		***	<0.001
Molasses * Legume		NS	<0.057

App. 3: Analysis of Variance for the effect of age of Napier grass and inclusion of molasses on the ammonia nitrogen content of silage

Effect	condition	Significance	P-value
Napier grass age	-molasses	NS	0.406
	+ molasses	NS	0.388
Molasses	NG8	**	0.008
	NG12	*	0.014
Napier age*Molasses inclusion		*	0.021
Napier age* Legume inclusion		***	<0.001
Molasses * Legume		NS	<0.065

App. 4: ANOVA for the effects of age of Napier grass and the inclusion of molasses on detergent fibres

a. NDF

Effect	Condition	Sign.	P-value
NG age	-M	*	0.025
	+ M	***	0.003
Molasses	NG8	*	0.011
	NG12	NS	0.092

b. ADF

Effect	Condition	Sign.	P-value
NG age	-M	***	0.004
	+ M	***	0.005
Molasses	NG8	*	0.013
	NG12	***	0.004

App. 5: ANOVA for effect of age of Napier grass and molasses inclusion on 24-hour gas production, rate of gas production and proportion of gas volume produced after 24 hours of incubation

a. 24-hour gas production

Effect	Condition	Sign.	P-value
NG age	-M	NS	0.354
	+ M	*	0.047
Molasses	NG8	**	0.007
	NG12	***	0.000

b. Rate of gas production (%/hour)

Effect	Condition	Sign.	P-value
NG age	-M	NS	0.128
	+ M	NS	0.175
Molasses	NG8	***	0.000
	NG12	***	0.000

c. % total gas achieved in 24hours

Effect	Condition	Sign.	P-value
NG age	-M	NS	0.150
	+ M	NS	0.113
Molasses	NG8	***	0.000
	NG12	***	0.001

App. 6: Gas production[1] from silages (experiment 3) expressed as a percentage of the extent of gas production (A+B)

		Incubation time (hours)						
Silage	A+B[2] (ml)	4	8	12	24	28	32	36
NG	45.7 ±1.5	17.7 ±0.4	28.8 ±0.6	45.3 ±0.7	71.5 ±0.5	76.0 ±0.6	79.7 ±1.0	81.6 ±1.4
NGL	42.9 ±2.2	22.6 ±1.9	38.8 ±3.8	55.9 ±3.8	80.2 ±3.4	84.8 ±2.9	88.9 ±2.9	90.5 ±2.6
NGM	43.3 ±1.5	19.6 ±2.2	33.6 ±2.8	50.3 ±3.8	76.2 ±4.2	81.0 ±4.0	85.0 ±3.9	87.1 ±3.6

[1] n = 3 (samples from 3 digestibility trial periods)

[2] extent of gas production

App. 7: Gas production (% of potential gas production) and rate of gas production from silages in experiments 1 and 2, (without molasses)

Napier age	Silage	Rate (%/hr)	A+B[1] (ml)	Incubation time (hours) 4	8	12	24	28	32	36
8										
	NG	3.6 ±0.4	54.3 ±6.6	9.3	15.9	28.6	57.5	61.7	65.5	68.5
	NGC	4.8 ±0.0	44.6 ±1.0	14.7	24.3	39.3	68.7	73.2	76.7	79.0
	NGL	4.4 ±0.1	45.6 ±0.7	13.6	22.5	36.6	66.1	70.1	73.8	76.5
	NGLL	4.1 ±0.5	49.4 ±0.9	12.6	21.9	34.4	62.8	67.6	71.3	74.1
	NGM	3.8 ±0.4	48.5 ±2.6	10.4	18.1	30.0	59.7	64.2	67.5	70.5
12										
	NG	2.6 ±1.4	53.5 ±0.8	9.3	14.7	27.1	55.7	60.0	63.9	67.7
	NGC	3.5 ±0.9	49.4 ±4.0	10.9	17.0	28.8	56.6	60.4	64.5	67.2
	NGL	4.3 ±0.2	48.9 ±1.5	12.5	22.0	35.7	64.5	69.3	73.0	75.8
	NGLL	3.8 ±0.3	52.8 ±1.2	12.6	20.3	33.0	60.4	64.5	68.9	71.7
	NGM	4.0 ±0.2	45.6 ±1.6	8.0	15.7	31.5	59.2	64.8	69.1	71.9

[1] extent of gas production

App. 8: Gas production (% of potential gas production) and rate of gas production from silages in experiments 1 and 2, (with molasses)

				Incubation time (hours)						
Napier age	Silage	Rate (%/hr)	A+B[1] (ml)	4	8	12	24	28	32	36
8										
	NG	5.2 ±0.3	46.2 ±1.6	15.1	26.8	42.6	71.2	61.7	76.2	79.5
	NGC	6.4 ±0.0	39.7 ±0.3	17.9	32.3	51.0	78.2	73.2	82.7	86.2
	NGL	5.2 ±0.1	44.5 ±0.9	16.6	27.5	43.3	71.8	70.1	75.6	79.7
	NGLL	6.1 ±0.9	41.9 ±2.6	18.8	32.9	48.6	74.2	67.6	78.9	82.5
	NGM	5.2 ±0.1	44.5 ±0.9	20.1	31.8	47.4	75.6	64.2	80.3	83.4
12										
	NG	4.6 ±0.2	49.5 ±0.8	11.0	20.2	36.9	66.7	60.0	70.8	75.2
	NGC	5.0 ±0.3	47.7 ±0.1	13.6	24.5	41.2	69.7	60.4	73.7	77.8
	NGL	5.3 ±1.2	46.4 ±0.6	14.8	26.8	43.7	70.6	69.3	76.0	79.9
	NGLL	6.2 ±0.7	45.9 ±1.9	18.1	32.3	49.7	77.1	64.5	81.5	84.9
	NGM	6.0 ±0.4	43.8 ±1.1	19.5	32.8	50.8	75.8	64.8	81.3	84.6

[1]extent of gas production; Means ± standard deviation

App. 9: Gas production (% of potential gas production) and rate of gas production from protein-rich forages (experiment 4)

PRF	Rate (%/hr)	A+B[1] (ml)	Incubation time (hours) 4	8	12	24	28	32	36
*Acacia angustissima**									
Acacia angustissima+PEG	12.8	21.7	49.3	70.9	80.6	96.3			
Calliandra calothyrsus	1.2	50.3	10.5	13.4	15.3	22.3	25.7	27.2	29.0
Calliandra calothyrsus+PEG	3.5	52.5	17.6	28.6	37.5	59.0			
Leucaena leucocephala	6.7	39.6	26.7	42.4	57.3	81.0	85.2	88.4	91.2
Leucaena leucocephala+PEG	10.8	44.8	30.7	54.6	71.1	91.8			
Acacia tortilis (leaves)	3.3	34.5	24.2	35.4	42.1	60.2	65.7	70.0	73.8
Acacia tortilis+PEG	8.1	37.4	27.3	47.4	61.9	85.3			
Acacia tortilis (pods)	6.5	50.1	29.4	44.6	58.9	80.8	85.0	88.6	91.6
Morus alba	13.5	53.7	33.3	61.4	78.1	93.7	96.8	98.7	100.5
Lablab purpureus	11.7	48.8	31.2	58.2	73.1	91.8	95.0	97.4	99.9
Crotalaria ochroleuca	9.6	44.9	30.7	53.2	69.4	88.9	93.0	96.5	98.3
Mucuna pruriens	10.1	35.9	31.2	54.7	70.8	88.7	93.2	96.6	98.8

* Gas production pattern from *A. angustissima* incubated without PEG did not fit the exponential model