EFFECT OF LEGUMINOUS TREE LEAVES AS NITROGEN ADDITIVE TO PENNISETUM PURPUREUM SILAGE

BY

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ABSTRACT

A study comprising of two experiments was conducted to investigate the effectiveness of three leguminous tree leaves as nitrogen additive (NA) in presence or absence of molasses (as WSC additive) on fermentation and nutritive value of napier (*Pennisetum purpureum*) silage. *Gliricidia sepium*, *Leucaena leucocephala* and *Sesbania sesban* leaves were used in experiment I while *L. leucocephala* leaves was used in experiment II. Polyvinyl chloride (PVC) cylinders ($1.9x10^{-2}m^3$) were used as laboratory silos for experiment I and earth pit silos ($1m^3$) for experiment II.

A 2 x 3 x 3 factorial experiment which comprised of fourteen treatments was adopted in experiment I. Napier grass was ensiled in combination with leaves from *G.sepium, L. leucocephala* and *S.sesban* as NA. Each combination received three levels of NA (0, 15 and 30% w/w) and two levels of molasses (0 and 3% w/w).

A 2 x 3 factorial experiment that comprised of six treatments was adopted in experiment II. One combination in experiment I was ensiled in pilot scale silos, where *L.leucocephala* was chosen as (NA). Same levels of inclusion of Leucaena and molasses in experiment I were used in this experiment and the grass alone without and with molasses served as control.

The effect of molasses, NA type, NA inclusion level and interactions were observed in experiment I while the effect of molasses and NA inclusion level as well as the interaction between the two were observed in experiment II. Parameters observed in both experiments were sensoric tests, dry matter, chemical composition, *in vitro* dry matter and organic matter digestibility. Dry matter losses and acceptability test were observed in the second experiment only.

In experiment I, addition of molasses and combination of molasses and NA inclusion level improved aroma of the silages. Nitrogen additive types and inclusion levels had no significant ($P > 0.05$) effect on sensoric qualities of the napier silages.

Molasses addition improved dry matter, chemical composition and digestibility of the napier silage. Addition of Leucaena increased DM, CP, NDF, ADF and lowered IVDMD and IVOMD as compared to addition of Gliricidia and Sesbania. Inclusion of 15 and 30 percent NA improved silage DM, chemical composition and digestibility. Combination of molasses and NA type had significant ($P < 0.05$) improvement on WSC, ADF, IVDMD and IVOMD while combination of molasses and NA inclusion level had significant ($P < 0.05$) improvement on WSC, NDF, IVDMD and IVOMD. The NA types at 15 and 30% inclusion had more improvement on DM, chemical composition, IVDMD and IVOMD than 0% inclusion level. All NA types at 15 and 30% inclusion levels had higher CP, WSC, IVDMD, IVOMD and lower NDF than the control, 30% Gliricidia and 30% Sesbania being the highest among the combinations.

Fermentation products were significantly ($P < 0.05$) improved by addition of molasses and nitrogen additives. Combinations of NA with molasses gave better fermentation quality than those without molasses.

In experiment II, molasses addition improved aroma of the silage however, it didn't affect appearance and texture. Sensoric qualities were neither affected by NA inclusion level nor the combination of molasses and NA level.

Water soluble carbohydrates, ADF, IVDMD and IVOMD were improved by molasses addition. However, in this experiment, DM, CP, ash and NDF were not improved by addition of molasses. The CP, IVDMD and IVOMD increased with increasing NA inclusion level while NDF and ADF decreased with increasing NA inclusion level. The combination of molasses and NA inclusion level had significant ($P < 0.05$) improvement on WSC, ADL and IVDMD.

Fermentation products were improved by both molasses and NA inclusion. However, they were not affected by the combination of molasses and NA inclusion levels. Dry matter losses were significantly lowered by molasses addition (18.5% vs 20.7%). The DM losses decreased with increasing NA inclusion (16.7, 18.9 and 23.3 percent for 30, 15, and 0 percent NA inclusion, respectively). The combinations of molasses and NA inclusion have the DM losses ranged from 16.6% to 27.0%. The intake rate was higher in molassesed than unmolassesed silage. It was also higher in 30 and 15 percent NA levels than 0% NA. The combination of molasses and NA levels had significant effect on intake rate, $15NA^+$, $30NA^+$ and $30NA^-$ having higher intake rate than other combinations.

It was concluded that 30% NA inclusion could be used to conserve well silage of napier grass. Further findings are required to establish the NA inclusion level in other common grasses such as Guatemala grass (*Tripsucum laxum*) and Guinea grass (*Panicum maximum*).

DECLARATION

I, MARTHA WILLIAM TESHA, do hereby declare to the SENATE of Sokoine University of Agriculture that the work presented here is my own original work and has not been submitted for a higher degree in any other university.

Signature ....

Date 3-11-1999......................

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DEDICATION

This work is dedicated to my husband Emmanuel L.K. Laisser and to my sons Loitarasakaki Baraka and William Emmanuel.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF APPENDICES

ABBREVIATIONS AND SYMBOLS

%	-	Percent
$0NA^+$	-	0% Nitrogen additive plus molasses
$0NA^-$	-	0% Nitrogen additive without molasses
$15G^+$	-	15% Gliricidia plus molasses
$15G^-$	-	15% Gliricidia without molasses
$15L^+$	-	15% Leucaena plus molasses
$15L^-$	-	15% Leucaena without molasses
$15NA^+$	-	15% Nitrogen additive plus molasses
$15NA^-$	-	15% Nitrogen additive without molasses
$15S^+$	-	15% Sesbania plus molasses
$15S^-$	-	15% Sesbania without molasses
$30G^+$	-	30% Gliricidia plus molasses
$30G^-$	-	30% Gliricidia without molasses
$30L^+$	-	30% Leucaena plus molasses
$30L^-$	-	30% Leucaena without molasses
$30NA^+$	-	30% Nitrogen additive plus molasses
$30NA^-$	-	30% Nitrogen additive without molasses
$30S^+$	-	30% Sesbania plus molasses
$30S^-$	-	30% Sesbania without molasses
ADF	-	Acid detergent fibre

ADL	-	Acid detergent lignin
ANOVA	-	Analysis of Variance
AOAC	-	Association of official analytical chemists
CF	-	Crude fibre
CP	-	Crude protein
DHP	-	3-hydroxy-4 (1H)-pyridone
DM	-	Dry matter
DMD	-	Dry matter digestibility
EE	-	Ether extract
g	-	gram
g/min.	-	gram per minute
GLM	-	General Linear Model
IVDMD	-	*In vitro* dry matter digestibility
IVOMD	-	*In vitro* organic matter digestibility
kg	-	kilogram
m	-	metre
MOL	-	Molasses
N	-	Nitrogen
NA	-	Nitrogen additive
NAIL	-	Nitrogen additive inclusion level
NATP	-	Nitrogen additive type

CHAPTER ONE

INTRODUCTION

A major problem which hinders expansion of ruminant production in tropical countries is lack of good quality pasture particularly during the dry season of the year (Mannetje, 1982). Seasonal rainfall in most tropical countries results in a variable supply of pasture (Crowder and Chedda, 1982). This emphasizes the need for conservation of forage in form of hay or silage for periods of feed shortage.

Successful conservation of forage as hay or silage depend greatly on the environmental conditions and type of forage (Wilkins, 1988). In most areas of the tropics, it is difficult to conserve grasses as hay (Crowder and Chedda, 1982) because the appropriate stage of growth for haymaking coincides with rain season that makes the drying process rather difficult (Webster and Wilson, 1980). These grasses can therefore be conserved as silage.

Silage is a moist succulent feed produced as a result of controlled fermentation of fresh forage when stored in a silo under anaerobic conditions (Wilkins, 1988; McDonald *et al.*, 1995). The major aim of silage making is to conserve the forage at an optimum stage of growth for utilization during seasons when the forage is unavailable (Jarrige *et al.*, 1982). However, silage production is not a common practice among livestock farmers in the tropics (Panditharatne *et al.*, 1986). This could be attributed to the lack of technology for simple methods of making silage that can be easily adopted by farmers (Sarwatt, 1995; Maeda, 1996).

Most of the tropical grasses are inherently low in nutritive value (Ulyatt, 1973; Kidunda and Lwoga, 1988) especially water soluble carbohydrates and protein (Humphreys, 1978) and changes during ensiling reduce their nutritive value even further (Thomas *et al.*, 1980; Crowder and Chedda, 1982; Rooke, *et al.*, 1985; McDonald *et al.*, 1991). *Pennisetum purpureum* for instance, when harvested in mature stage is nutritionally low in nitrogen (Webster and Wilson, 1980; Muinga *et al.*, 1992) and when fed to ruminants as silage do not meet their nitrogen requirement (Catchpoole, 1970; Aguilera, 1975).

There is an extensive degradation of forage protein to non-protein nitrogen which normally occurs during ensiling (Aguilera, 1975; Wilkinson *et al.*, 1976), initially as a result of proteolysis by plant enzymes and subsequently by clostridial fermentations (McDonald *et al.*, 1991). The problem is compounded further by degradation of residual plant protein in the rumen (Thomas *et al.*, 1980) which reduces the efficiency of dietary energy capture and the rate of microbial protein synthesis (Rooke *et al.*, 1985). The high degradability of silage nitrogen often necessitates the use of expensive protein/nitrogen supplements or additives to maximize animal production whenever silage is used (Beever, 1980).

Few studies have investigated the possibility of ensiling grass/legume mixtures to overcome the need for addition of expensive protein/nitrogen supplements or additives (Tjandraatmaadja *et al.*, 1991; 1993; Fleischer and Tackie, 1993). The authors reported silages of high nitrogen content made from tropical grasses in combination with legumes. Grasses and legumes used were sorghum, Leucaena and Gliricidia (Tjandraatmaadja *et al.*, 1991), Digitaria, Setaria, Leucaena and Gliricidia (Tjandraatmaadja *et al.*, 1993), sorghum and Leucaena (Fleischer and Tackie, 1993).

There is a need to extend investigations using the popular tropical grass species for instance, *Pennisetum purpureum*. This grass is among the most abundant forage species utilized by the dairy farmers in Eastern and Central Africa (Walters, 1985; Kayongo, 1991) and is probably the commonest grass which could be used for silage making.

Therefore the objectives of the present study were:

1. To study the effects of including *Gliricidia sepium, Leucaena leucocephala* and *Sesbania sesban* leaves as nitrogen additive on the fermentation and nutritive value of *Pennisetum purpureum* silage.

2. To estimate the optimum inclusion level of *Gliricidia sepium, Leucaena leucocephala* and *Sesbania sesban* leaves required in ensiling *Pennisetum purpureum* silage.

CHAPTER TWO

LITERATURE REVIEW

2.1 Tree Legumes as Livestock Feed

Leaves and pods from tree legumes form a natural part of the diet of many ruminant species (Trung, 1989) and have been used traditionally as sources of forage for domesticated livestock in several parts of the world particularly Africa (Le Houerou, 1980), Asia and the Pacific (Skerman, 1977; NAS, 1979; Brewbaker, 1986). Tree legumes have been mostly used as feed for ruminants although there are some reports of their inclusion in the diets of non-ruminants (pigs and poultry) (Trung, 1989). The leaves, stems and fruits may be used either as a complete feed or as a supplement to other feeds (Norton, 1994). In some legume species however, a major limitation to the use of one or more of these components is the presence of toxic and/or anti-nutritive factors (Norton, 1994).

2.1.1 Chemical composition of forage tree legumes

Much of information available on the chemical composition of tree foliage is from proximate analysis i.e. EE, CP, CF, (Göhl, 1981, Le Houerou, 1980a). This information is of limited value as a predictor of nutritive value (Van Soest, 1994). Analyses based on detergent extraction are more useful as they separate plant dry matter into a completely digestible fraction (neutral detergent solubles) (NDS) representing cell contents, and a partially digestible fraction (neutral detergent fibre) (NDF) representing plant cell walls (Van Soest, 1994).

Table 1 shows the chemical composition of selected tree legume species foliage as summarized by Norton (1994). The author reported that, the chemical composition of most tree legumes vary with soil type (location), plant part (leaf, stem, pods), age of leaf and season. Further variability in composition might be introduced when subjective selection of the edible fractions of tree foliage is made (Bamualim *et al.*, 1980). According to the author the edible fractions tend to contain higher stem contents and to be of lower nutritive value than leaves alone.

The protein content of forage tree legume leaves (12-30%) is usually high (Norton, 1994) compared with that of mature grasses (3-10%) (Crowder and Chedda, 1982). Feeds containing less than 1.3% N (8% CP) are considered deficient as they cannot provide the minimum rumen ammonia levels required for microbial protein synthesis by a ruminant (Milford and Minson, 1966). All forage tree legumes have N contents higher than this value (Norton; 1994) and may be judged adequate in protein.

2.1.2 Digestibility and voluntary intake of tree legume foliage

The digestibility of plant material in the rumen is related to the proportion and lignification of plant cell walls (NDF) (Van Soest, 1994). Forage species with a low NDF content (20-35%) are usually of high digestibility and species with high lignin contents are often of low digestibility (Norton, 1994). Bamulim *et al.*, (1980) showed that the lignin content of tree foliage was negatively correlated (r = - 0.92) with feed digestibility in nylon bags. Stems have higher lignin contents than leaves, and are less digestible.

Table 2 shows values for *in vitro*, *in sacco* and *in vivo* digestibility estimates and voluntary intake of tree legume species foliage by different ruminant species. It is worth noting that these data were collected under a range of conditions, and only general conclusions can be drawn about the comparative value of the different species.

Low intakes associated with high feed digestibilities may be related to the presence of compounds which are appetite depressants for instance, tannin or alkaloids (Norton, 1994). High feed intakes and low feed digestibilities may be related to rapid rates of passage of feed through the rumen, such as when small leaflets of pinnate leaves are being consumed.

2.1.3 Limitations to nutritive value of forage tree legumes

Some tree legumes contain anti-nutritive factors that adversely affect feeding value. The significance of these factors however, becomes more evident when tree foliage is the only feed consumed or when supplemented in higher levels as the case of Leucaena with mimosine (Norton, 1994).

It is known that Gliricidia is a high quality forage but of low palatability when first introduced to animals (Chadhokar, 1982). The odour of the leaves of Gliricidia has been attributed to its low palatability (Brewbaker 1986). Once adopted it however, appeared that there were no long-term detrimental effects on sheep and cattle (Chadhokar, 1982). Tannin content of Gliricidia leaves does not appear to interfere with plant protein availability but may be one of the factors affecting palatability

Table 1: The chemical composition (g/kg DM) of foliage from selected tree legume species

Species	Crude protein (NX6.25)	Fat	Ash	NDF	Crude fibre	ADF	Lignin	Tannin
G. sepium	275		60	255		216	94	34
	150		55	272		212	55	
	276	24						
			104					
	234	14		231				
			107					
	261		22		186	232	88	
	256					279	91	
	183		59	656		357		20
L.leucocephala	267		57	312		226	99	37
	203	55	93	183				
	269			383		266	68	
	258		69	309		234	87	55
S.sesban	213		80	219		153	36	ND
	263	9			122			
			100					
	194		74		329			
	152	16	86		353			

Source: Norton, 1994.

Table 2: Some values for *in vitro*, *in sacco* and *in vivo* digestibility and voluntary intakes of ruminants given forage tree legume species[*].

Species	Digestibility (%)					Voluntary intake (g/kg LW)	Animal species
	In vitro DM	*In sacco*		*In vivo*			
		DM	N	DM	N		
G. sepium	68.2						
		79.1	84.1[a]				goat
		68.2					cattle
	66.0					34.6	goat
						33.0	sheep
						16.7	sheep
				54.8		25.8	sheep
				56.3	84.6	32.6	goat
L.leucocephala	68.8						
		52.7					
		82.1	83.3[a]				goat
				68.0		35.6	goat
				63.2		31.9	sheep
						27.4	sheep
				54.8	65.0		cattle
S. sesban	67.8						
		90.6	96.0				goat

[*] All values are for fresh foliage unless stated otherwise [a] contains tannin

Source: Norton, 1994.

(Norton, 1994). Coumarins have been found in Gliricidia leaves (Griffiths, 1962), these compounds are precursors of phyto-oestrogens which have been reported to cause infertility and abortion in sheep at Australia (Cox and Braden, 1974). However, Chadhokar (1982) fed diets containing 75% Gliricidia to pregnant sheep and did not find the problems of infertility or abortion.

Leucaena leucocephala has been found to contain the non-protein amino acid mimosine (ß-[N-(3-hydroxy – 4-oxopyridyl)]-α-aminopropionic acid) (Norton, 1994). Mimosine acts by interfering in cellular mitosis, and the symptoms of toxicity are alopecia, reduced appetite, reduced weight gain and often death. The severity of toxicity is related to the level of Leucaena consumed and diets containing less than 30% are generally considered safe for ruminants (Norton, 1994).

It is now known that in areas where Leucaena is indigenous (Central America), and in parts of Asia, ruminants consuming Leucaena appear able to degrade the ruminal metabolite of mimosine, 3-hydroxy- 4(1H)-pyridone (DHP), to harmless end-products (Jones and Lowry, 1984). The authors associated this capacity with a specific bacterial population in the rumen of these adapted animals.

Sesbania sesban is potentially useful forage source and it has no tannin (Norton, 1994). However, it contains compounds potentially toxic to non-ruminants (Reed and Soller, 1987). Getteridge and Shelton (1991) in a yearlong field grazing cattle in *S. sesban* found no indication of toxicity.

2.1.4 Potential of forage tree legumes as a nitrogen additive in tropical grass silages

In anticipation of low level of protein in tropical grass silages due to loss of protein, which is degraded to ammonia during an anaerobic fermentation, nitrogen additives are often added. The inclusions of both Gliricidia and Leucaena have been found to produce highly palatable silages, which promote animal weight gain (Tjandraatmadja *et al.*, 1991; 1993).

Table 3 shows results from studies where tropical grasses and leguminous tree leaves were used to make silage fed to sheep. When compared with Sorghum silage, which is usually recommended for tropical areas, the leguminous tree leaves - Pangola (*Digitaria decumbens*) silages were superior. However, when lower quality grasses were used Setaria (*Setaria sphacelata*) molasses was necessary to produce silages, which promoted animal weight gain.

In other studies with these silages, feed protein degradabilities were determined as 78% for pangola silage, 65% for Leucaena + pangola and 75% for Gliricidia + pangola silage (Tjandraatmadja *et al.*, 1993). These values suggest that, unlike pangola silage, a significant component of the original bypass protein in the Leucaena and Gliricidia added silages was retained after fermentation. These studies therefore suggest that tree legume leaves may contribute to protein improvement to tropical silages.

2.2 The ensilage process

The major aim of ensiling forage is to preserve the material with a minimum loss of nutrients. This can only be achieved if anaerobic conditions are maintained. However, ensilage process is influenced by microbiological and biochemical activities which are carried out from the harvesting of the forage to the ultimate use of the silage. The majority of microorganisms on the surface of a forage require oxygen to survive and die off rapidly in the early stages of ensilage (Henderson, 1991). Of the microorganisms, which remain, the lactic acid bacteria, enterobacteria, yeasts, clostridia and moulds are the most important. Biochemical activities occurring after a plant is cut and during conservation results: from the continuing metabolism of plant cells; from the plant enzymes of the dead tissue; and from the microorganisms present on the plant. This can be easily summarised as shown in Table 4.

2.3 Silage quality

The term silage quality is usually used to denote the extent to which silage fermentation has been successful. Several techniques are available for evaluating the quality of silage. These involve physical evaluation, chemical analyses and determination of nutritive value of the fermentation products.

Table 3: The use of Leucaena and Gliricidia leaves in silage making

Grass	Forage tree	Molasses	Intake (g/kg/day)	DMD (%)	Weight change	Nitrogen balance (% intake)	Rumen ammonia (mg/N/l)
Pangola hay	-	-	23.9	64.7	-	16.6	134
Pangola silage	-	+	16.1	60.6	-	6.8	100
	L. leucocephala	-	22.9	54.9	+	28.8	88
	L. leucocephala	+	22.7	56.4	+	33.6	77
	G. sepium	-	23.1	53.3	+	36.4	77
	G. sepium	+	25.4	55.8	+	40.3	88
Setaria hay	-	-	16.7	40.6	-	< 0	31
Setaria silage	-	+	19.0	56.5	-	6.7	54
	L. leucocephala	-	22.1	53.6	0	31.0	77
	L. leucocephala	+	21.1	57.4	+	35.5	105
	G. sepium	-	17.1	54.4	-	33.3	80
	G. sepium	+	21.8	60.4	+	36.2	114
Sorghum silage	-	-	15.9	57.1	-	< 0	46

Source: Tjandraatmadja *et al.*, 1991.

Table 4: Factors influencing the ensilage of forages

Enzymes	Micro-organisms
Respiratory	Lactic acid bacteria
Proteolytic	Enterobacteria
Polysaccharide-degrading	Clostridia
	Fungi
	Yeasts
	Moulds
	Bacillus
	Listeria
	Acetic acid bacteria
	Propionic acid bacteria

Source: Henderson, 1991.

2.3.1 Physical evaluation of silage quality

Physical evaluation of silage quality entails several simple sensoric tests to evaluate physical properties of silage mainly in terms of appearance, smell and texture (McCullough, 1976). Three distinct types of silage: good, poor and overheated silages have been identified by McCullough (1976) on the basis of silage appearance, temperature, smell, taste and texture. Zimmer (1959) categorized a variety of silages on the basis of smell, texture and colour. He graded them as very good; good to satisfactory; medium to hard and spoiled. Similar classification of silages was made by Otieno *et al.*, (1990) basing on appearance and smell.

2.3.2 Evaluation of silage quality by indices and chemical analyses

Several indices in conjunction with chemical analyses have been employed to evaluate and express the quality of silage. These include concentration of fermentation acids (Flieg, 1938), pH values and DM content (Virtanen, 1952; Van Soest, 1994) and ammonia Nitrogen content (Nilson *et al.*, 1956) or a combination of these and other criteria (Ekern *et al.*, 1975).

2.3.2.1 Concentration of fermentation acids

The concentration of fermentation acids has been used synonymous with 'Flieg Index'. Flieg developed this index in 1938 basing on the proportion of lactic acid, acetic acid and butyric acid (expressed in milliequivalents). Higher scores which normally denote better quality was given to higher proportions of lactic and acetic acids to butyric acid. Zimmer (1966) modified the Flieg index basing on the proportion of the acids and classified silage as very good (81-100%); good (61-80%); medium (41-60%); bad (21-40%); and very bad (0-20%).

2.3.2.2 pH value and dry matter content

The pH and DM content of silage has been widely used to evaluate the quality of silage and they are considered as the simplest indices of silage quality to measure (Virtanen, 1952; Dijkstra, 1957; Weissbach *et al.*, 1974; Van Soest, 1994). The best quality silage of low dry matter should have the pH values less than 4.4 whereas for high-dry matter silage should have the pH values less than 5.0 for achieving satisfactory fermentation (Virtanen, 1952; Dijkstra, 1957; Van soest, 1994). However, Weissbach *et al.*, (1974) found that the critical pH value required for silage stability fell from 5.0 for forage with 50% DM down to 4.1 for forage with 15% DM.

2.3.2.3 Concentration of ammonia Nitrogen

The concentration of ammonia nitrogen expressed as percent of total Nitrogen in silage has been used as a criterion for assessing silage quality. A threshold level of 8% ammonia nitrogen (as percent of total N) has been reported by Nilson *et al.*, (1956) as a measure of well-preserved silage. Carpintero *et al.*, (1969) reported ammonia nitrogen content of less than 11% as the measure of well preserved silage. Likewise, Wilkins (1988) reported the silage containing between 8 and 10% of the total N as ammonia being considered as well preserved. He further noted that a low figure of NH_3N content as a proportion of total N of silages reflects limited breakdown of the N fraction.

2.3.2.4 Combination of different indices to evaluate the silage quality

For accurate evaluation of silage, a combination of the above indices should be employed. Catchpoole and Henzel (1971) summarised observations of products from tropical silages as shown in Table 5 indicating good quality for direct cut grass silage.

Table 5: Silage quality based on combination of indices

Parameter	Value
pH	< 4.2
Lactic acid	3 - 13%
Acetic acid	0.5 - 0.8%
Butyric acid	< 0.2%
NH_3N (as % of total N)	< 11%

Source: Catchpoole and Henzel, 1971.

Breirem and Saue (1973) went further in evaluating silage quality and proposed an arbitrary classification as shown in Table 6.

Table 6: Silage quality based on some important fermentation products

Silage quality	Butyric acid concentration (%)	Ammonia nitrogen (as % of total N)
Not acceptable	above 0.6	above 18
Poor	0.3 - 0.6	12.1 - 18
Acceptable	0.1 - 0.3	8.1 - 12.1
Good	max 0.1	max 8

Source: Breirem and Saue, 1973.

2.4 Nutritive value of silages

The nutritive value of silage refers to its chemical composition and feeding value (McDonald *et al.*, 1991; Woolford, 1984). The nutritive value of particular silage is influenced by the nature of the crop at the time of harvesting and the changes during the storage period. The main factors influencing the nutritive value of growing crops and hence the nutritive value of silage include: buffering capacity of the plants, physical structure, level of fermentable substrate in the form of WSC and DM content (McDonald *et al.*, 1991). Changes during the storage period occur as a result of the activities of the plant and microbial enzymes.

2.4.1 Dry matter content of the silage

The importance of dry matter content in silage fermentation has well been documented (McDonald *et al.*, 1991; McCullough, 1983; Woolford, 1984). The critical pH value below which clostridial growth is inhibited varies directly with the moisture content of the plant material and if soluble carbohydrate levels are not high, the ensiling crops having low DM (< 22%) will encourage a clostridial fermentation resulting in high DM losses and a silage of low nutritive value (McCullough, 1983; Wooldford, 1984).

Ensiling of crops having low DM results in production of large volumes of effluent which is difficult to dispose off but also carries with it in solution, valuable and highly digestible nutrients. Optimum DM levels between 25 and 35% have been indicated by Dixon (1982) and McCullough (1983).

2.4.2 Nitrogen content of the silage

The amount of nitrogen contained in silage vary depending on the nitrogen or protein content of the ensiled forage material (Webster and Wilson, 1980; Randhawa *et al.*, 1992; Sarwatt *et al.*, 1989).

When forage is ensiled the plant proteases hydrolyse the forage protein, particularly the chloroplast proteins (Bousset *et al.*, 1972; McDonald and Edwards, 1976). As a consequence, silages contain a large but variable proportion of non-protein nitrogen and soluble nitrogen including ammonia. Hydrolysis of protein ceases when the pH has fallen to 4 (McDonald *et al.*, 1991). However, additives such as formic acid, formaldehyde or both restrict the degradation of amino acids by proteolytic clostridia in the silo by 55-70 percent (Jarrige *et al.*, 1982; Siddons *et al.*, 1979).

Tjandraatmadja *et al.*, (1993) found the addition of leguminous tree leaves at ensiling to increase the total nitrogen content of the silage by 6.60 – 7.90% TN and reduce the content of ammonia nitrogen by 5 - 12 g/kg TN. Dulphy *et al.* (1985) working with Red clover (*Trifolium pratense*) based silages found the increase of total nitrogen by 6 g/kg DM resulted from the addition of Red-clover in grass during ensiling. Daniel (1985) found the increase in CP content of silage by 300g/kg DM as a result of adding 25% White clover (*Trifolium repens*) or Field bean (*Vicia sativa*) in maize silage. Likewise, Carneiro *et al.* (1985) reported a substantial increase of the CP content of elephant grass silage resulting from the addition of legume, Lablab (*Lablab purpureus*) at 40% during ensiling.

Nitrogen content of the silage also varies depending on the fermentation pattern of the silage. For instance, the nitrogenous components of lactate silages are mainly in a non-protein soluble form, which are mostly present in the form of amino acids. On the other hand, the clostridial silages have high content of ammonia nitrogen (McDonald *et al.*, 1991).

2.4.3 Carbohydrate content of silage

Carbohydrates form the major component of plant tissues and they constitute up to 50% or more of the dry matter of the forages (Chuch and Pond, 1988). These are mainly classified as structural and non-structural carbohydrates. The former include hemicellulose, pectin and cellulose (the cell wall constituents) whereas the later is composed mainly of water-soluble carbohydrates (Bailey, 1973). Due to fermentation process taking place during ensiling, the structural carbohydrate fraction may slightly be altered especially the hemicellulose (McDonald *et al.*, 1991).

Water-soluble carbohydrates and hemicellulose are the main substrate for lactic acid bacteria during the silage fermentation (McDonald *et al.*, 1991). Increased concentration of NDF, ADF and ADL (the cell wall constituents) in silages compared to the corresponding ensiled forages has been documented (Singh and Pandit, 1978; Sarwatt *et al.*, 1992). Sarwatt *et al.* (1992) found higher concentration of NDF, ADF and ADL averaging 633.2, 382.4 and 84.7 gkg^{-1} DM in Rhodes grass (*Chloris gayana*) silage respectively compared with 647.9, 355.5 and 76.6 gkg^{-1} DM respectively found in its corresponding ensiled forage. Singh and Pandit (1978) observed similar trend while working on sorghum ensiled with urea and molasses. The increases are likely to be created by losses of cell contents in gaseous form or effluent leaving the largest portion of dry matter occupied by cell wall contents.

2.4.4 Voluntary dry matter intake of silage

The potential of the forages for animal production is greatly determined by their dry matter intake. The intake is influenced by the characteristics of the animal and also of the forage (Dulphy, 1980). The intake of forages is limited by the rate of removal of forage particles from the reticulo – rumen (Campling, 1970). The rate of removal is related to the chemical composition of the forage; particle size; digestion of constituents; and reduction rate of indigestible components of the forage (Dulphy, 1980).

The intake of DM of silage by ruminants is thought to be controlled by the same basic mechanisms regulating forage intake (Farnhan and Thomas, 1978) but several workers have noted that intakes of silage DM are generally lower than those of fresh or dried forage made from similar material Wilkins, 1974; McDonald and Edwards, 1976; Wilkinson *et al.*, 1976; Rogers *et al.*, 1979; Dulphy, 1980; Forbes, 1986). Primary factors that have been suggested as being responsible for the reductions of intake of ensiled forages include moisture content of the ensiled material (Gordon *et al.*, 1961; 1965; Thomas *et al.*, 1961; Wilkins *et al.*, 1971), silage pH (McLeod et al., 1970; Thomas and Wilkinson, 1975; Shaver *et al.*, 1985), organic acid contents (Thomas and Chamberlain, 1983) and products of protein breakdown (Vetter and Von Glan, 1978; Thomas and Chamberlain, 1983).

A positive linear relationship between silage DM and DM intake has been reported (Wilkins *et al.*, 1971). This was supported by Olubanjo *et al.*, (1989) working on small ruminants in which an average daily DM intake of 62.7 and 54.9 g/kg $W^{0.75}$ were observed when animals were fed napier silage with about 19.2% DM, and a ration constituting of 50% napier plus 50% cocoa pod silage with 16.9% DM, respectively. The low intake of silage is likely due to differences in the end products of silage fermentation resulting from the more extensive fermentation common to silages with low DM as compared with silages with high DM. Thomas and Chamberlain (1983) have reported a negative linear relationship between intake and total organic acids and NH3N concentrations in silage. However, from their findings, the silages with higher proportion of lactic acid in the total acidity was recorded to have higher voluntary intake than the one with higher proportion of acetic acid in total acidity. Similar trend was observed by Sarwatt *et al.*, (1992).

Most of tropical grass silages are believed to have lower contents of protein due to low protein values of their respective forages. Incorporation of urea or legumes with high protein value has been found to improve the nitrogen content of the silage, thereby improving silage dry matter intake (Chandler *et al.*, 1975; Shaver *et al.*, 1985; Tjandraatmadja *et al.*, 1993; Sarwatt, 1995; Araujo-Febres *et al.*, 1996).

As it was mentioned earlier on, silage pH is among the primary factors that have been found to be responsible for reduction of intake of ensiled material. Ingestion of low pH lactate silages has been found to alter the normal rumen pH, which ultimately lower the voluntary dry matter intake (McLeod *et al.*, 1970; Thomas and Wilkinson, 1975). Alleviation by partial neutralisation of the free acid content of silage has been attempted and resulted in large increases of DM intake (McLeod and Wilkins, 1970; McLeod *et al.*, 1970; Shaver *et al.*, 1985; Thomas and Wilkinson, 1975; Wilkins, 1974).

2.4.5 Acceptability of Forages

Acceptability of forage usually describes aspects of quality that reflect palatability and voluntary intake and the degree to which the particular forage is eaten (Van Soest, 1994). Palatability designates those characteristics of a feed which involve a sensory response in the animal, and is considered to be the corollary of the animal's appetite for the feed (Baumont, 1996). Grovum (1984) and Baumont (1996) reported that when only one feed is given to animals fed indoors, acceptability can be evaluated from feeding rate at the beginning of the meal and not by voluntary intake which integrates acceptability and post ingestive effects. Most of the acceptability studies were therefore suggested to be short-term with time scales of minutes or hours (Baumont, 1996).

Several studies have shown that the acceptability of a given feed by an animal would be influenced by physical characteristics such as its texture (Grovum and Chapman, 1988), particle size, resistance to fracture and DM content (Baumont, 1996). The authors suggested that these factors contribute to the sensory response invoked by the animal. They further pointed out that these factors influence ease of prenhension and mastication, as the animals prefer a physical form which they can eat faster.

Acceptability of the feed can also be affected by its taste or odour (Arnold *et al.*, 1980; Grovum 1984). Grovum (1984) found that the intake rate of coarse chopped straw by sheep was only 0.91 g/min, but this was increased to 5.17 g/min simply by spraying the straw immediately before feeding to add monosodium glutamate at the rate of 4% of the air dry weight of the untreated straw. Arnold *et al.*, (1980) found that the voluntary intake of some forages was increased by adding the odour of butyric acid and amyl acetate. They also pointed out that the smell, taste and touch of the feed might be more important in determining the amount eaten when food is plentiful.

Acceptability of the forage is also affected by physiological state of an animal. Suzuki *et al.*, (1969) found a significant difference between cows in the rate of eating. They found that dry pregnant cows had a lower rate of eating than lactating cows with both silage and hay. The dry pregnant cows started each meal eating slowly and continued to eat for a longer time than lactating cows, on both silage and hay, the rate of eating was highest during the first or second five minutes and then declined continuously until the end of eating. This can be expected from differences in their body needs, abundance of the feed and ruminating efficiencies (Van Soest, 1994).

2.4.6 Digestibility of silage

Lowered digestibility of silage due to bad preservation has been reported by Givens *et al.* (1983) working with Perennial ryegrass (*Lolium perene*) silage. According to the authors *in vivo* digestibility of the badly preserved silage (with butyric acid, and ammonia N averaging 3 gkg^{-1} and 36.4% of total N, respectively) was found to be lower, averaging 64.5% of DM compared with the expected temperate standards of 70-75% of DM. Wilkinson *et al.*, (1980) showed that the digestibility of lucerne silages which had undergone clostridial fermentation was 4% lower than those which had undergone lactic fermentation.

Losses during ensilage lead to the decrease in nutritive value of the conserved forage and as a result most of the digestible components of the forage especially proteins and water-soluble carbohydrates decrease while fibre content increases. This lead to reduction in organic matter and digestibility. This has been shown by Sarwatt *et al.*,

(1992) working on tropical fodders. The authors reported slightly higher *in vitro* DM digestibility in fresh forages ranging between 59.7 to 70.4% compared with 56.4 to 64.1% in their corresponding silages.

Wilting prior to ensiling also has small effect on digestibility. In 18 paired comparisons, digestibility of wilted silages was lower than that of unwilted silages by 2% (Zimmer and Wilkins, 1984).

It is obvious that growing environment and climatic conditions existing at the time of harvesting, herbage maturity during harvesting hence fibre fractions of the forage and the fermentation patterns in the silo are the main factors which influence the digestibility of the silage.

2.5 Silage additives

Silage additives have been developed over the years to take some of the risk out of the ensilage process and to improve the nutritive value of silages (Henderson, 1993). Ideally, a silage additive should be safe to handle, reduce DM losses, improve the hygienic quality of the silage, limit secondary fermentation and improve aerobic stability, increase the nutritive value by increasing the efficiency of utilization of the silage and give a farmer returns greater than the costs of the additive (Done and Appleton, 1990; Otieno *et al.*, 1990; Tobioka *et al.*, 1993; Van Soest, 1994; Harrison *et al.*, 1995; Sarwatt, 1995; Maeda *et al.*, 1997).

McDonald *et al.* (1991) classified silage additives into five main categories. The first category is fermentation stimulants while the second category is fermentation inhibitors. Both are concerned with fermentation control by stimulating lactic acid fermentation and partial or completely inhibition of microbial growth (inhibitors). The third category is aerobic deterioration inhibitors. This combine additives which inhibit aerobic deterioration of silage. The fourth category is nutrients. This include nutrients which when added to the crops at the time of ensiling improve the nutritive value of resulting silage. The fifth category is absorbents. This include absorbents which when added to low DM forages to reduce effluent and thus minimises nutrient losses and pollution of water courses.

Van Soest (1994) however, classified the silage additives into three main categories as: stimulators of fermentation which encourage a lactic acid fermentation; inhibitors of fermentation which inhibit partially or completely microbial growth; and substances or materials whose principal purpose is to alter composition of silage.

2.5.1 Effect of molasses on quality and nutritive value of silage

Molasses is the carbohydrate source used most frequently, and is of particular benefit when applied to crops low in soluble carbohydrates (McDonald *et al.*, 1991). In order to obtain maximum benefit, molasses must be used in relatively higher concentrations about 40-50 gkg^{-1} of forage (Henderson, 1993). The author also reported that, if the treated crops has a very low DM content, a considerable proportion of the added carbohydrate may be lost in the effluent during the first few days of ensilage.

Sunarso *et al.*, (1995) found that addition of 4 and 6% molasses at the time of ensiling *Setaria sphacelata* grass with DM between 25 to 30% produced good quality silage with pH values ranging from 3.9 to 4.2. Likewise, Sarwatt *et al.* (1992) ensiled Rhodes grass having 32% DM mixed with 3% molasses and obtained silage with pH 4.6, NH_3N 4.8 (% of TN) and lactic, acetic and butyric acids concentrations of 47.1, 23.8 7 and 1.5 gkg^{-1} DM respectively. The author also reported increase in voluntary intake by 124.9 gDM/day) and *in vitro* DMD by 3.10%. Improvement in silage palatability and acceptance has been observed by Otieno *et al.* (1990) by ensiling elephant grass with 5% molasses. They also reported improved DM content from 16 to 19.8% and lowered pH from 5.2 to 4.2.

Addition of too little molasses has been observed to give worse results than none at all, and may even increase the concentration of butyric acid (Catchpoole and Henzel, 1971). Singh and Pandit (1978) did not reveal any significant changes in the pattern of fermentation between the sorghum ensiled with 1% molasses and the control which had no molasses.

Conclusions made by several workers have however, revealed that stable silages of higher pH, with low to medium concentration of volatile fatty acids and moderate to high amounts of ammonia nitrogen were recovered from some tropical grasses without use of molasses (Catchpoole and Henzell, 1971; Wylie, 1975; Skerman and Riveros, 1988; Tjandraatmadja *et al.*, 1993). The main reason which have been given to be responsible for maintenance of such stability is decrease in water activity which is coupled with increase in osmotic pressure of the plant cell sap there by limiting growth of clostridia organisms (Wylie, 1975). Specific reasons are however not yet established, though in some literature acetic acid has been attributed to the maintenance of such stability of tropical forage silages (Aguilera, 1975).

Tjandraatmadja *et al.* (1993) reported that with a high quality grass such as Pangola (*Digitaria decumbens*) there were no advantage gained by adding molasses. The authors suggested that molasses may be better used directly as a supplement for animals because in their study they found molasses to increase ethanol and decrease water soluble carbohydrates content particularly where legumes were not included in the forage mixture.

2.5.2 Effect of legumes on quality and nutritive value of silages

Several studies on grass silages have revealed improved quality of silage especially increase in protein or total nitrogen content and voluntary dry matter intake by including the legumes (Obeid *et al.*, 1992; Davies and Onwuka, 1993; Evangelista and Lima, 1993; Fleischer and Tackie, 1993; Tjandraatmadja *et al.*, 1993; Imura *et al.*, 1997).

Tjandraatmadja *et al.* (1993) have found that silages made from mature tropical grasses (Pangola and Setaria) were improved by addition of leaves from the tree legumes (*Leucaena leucocephala* and/or *Gliricidia sepium*), producing silages of low pH (3.8 –

4.4) and ammonia content and high total N content (> 20 gN/kg DM).

Fleischer and Tackie (1993) working with sorghum ensiled with 30% Leucaena reported total N, ammonia N and IVDMD averaging 3.62, 7.56% and 42.3% respectively compared with 0.92, 12.5% and 38.9%, respectively found in control, that is, sorghum ensiled without Leucaena. The authors also reported the decrease in cell wall proportion as the consequence of including Leucaena where NDF, ADF and cellulose were 60.9, 43.2 and 25.0 %DM, respectively compared with 73.5, 48.1 and 35.9 %DM, respectively found in the control.

Legume (*Vicia Sativa*) included in maize at 25% inclusion level has been found to increase the CP content of the resulting silage by 30 g/kgDM (Daniel, 1985). Similar observation has been reported by Carneiro *et al.*, (1983) working with elephant grass ensiled with *Lablab purpureus* at 40% by weight. The authors found a significant increase in CP, however they didn't observe improvement in digestibility and intake of DM. Imura *et al.* (1997) working with bahi grass (*Palpalum notatum*) and barnyard millet (*Echinochloa crus-galli*) ensiled with Phasey bean (*Macroptilium lathyroides*) at 25%, 50% and 75% inclusion level found improved silage quality in terms of composition of organic acids.

Evangalista and Lima (1993) found the inclusion of soy bean at 20%, 40%, and 60% in napier grass during ensiling to increase dry matter, crude protein content, dry matter intake and digestibility regardless of the level of inclusion. Szentpetery *et al.* (1989)

using the same legume mixed with maize and sorghum found that the crude protein content of the mixed silages were 4.6 – 6.3% better than that of the pure maize and sorghum silages.

Increased protein or nitrogen content of grass silage due to inclusion of legumes has been attributed to the fact that legumes contain more protein/nitrogen as compared to grasses (Gutteridge and Shelton, 1994). Increased protein/nitrogen content in grass-legume silages is consequently associated with improved voluntary intake and digestibility due to the fact that the CP/nitrogen content of the silage will reach or be raised to the threshold level of 7% CP (1.1. – 1.3 %N) suggested by Milford and Minson (1966). Below that threshold, microbes will not function well hence digestibility and intake will be reduced.

Despite the benefits of grass-legume silages, ensilage of legumes has been claimed to be characterized by extensive protein hydrolysis during fermentation (McCarrick, 1962; McDonald, 1981; Kass and Rodriguez, 1987; McDonald *et al.*, 1991). Presence of tannin and maintenance of anaerobic conditions have however, been found to play a major role in limiting proteolysis in some legumes during ensiling (Albrecht and Muck, 1991; Tjandraatmadja *et al.*, 1993).

2.6 Losses of dry matter and nutrients during ensilage

Losses of dry matter occurring during ensilage have been documented by several workers (Watson and Nash, 1960; Pizzaro and Vera, 1980; Webster and Wilson, 1980;

Zimmer and Wilkins, 1984; Woolford, 1984; Lampila *et al.*, 1988). Watson and Nash (1960) found from a series of experiments that the dry matter losses for silage made from fresh forage ensiled without additives to be higher (averaged 16.1%) than those from wilted forage (averaged 13.4%). Lampila *et al.*, (1988) reported similar findings of DM losses averaging 19.0 and 13.4% from unwilted and wilted silage respectively.

The extent of DM loss in silage is largely determined by: plant enzymes; moisture content and nutrients composition of ensiled crop; temperature; pH; air/oxygen ratio maintained inside the silo; and ensiling technique applied (Zimmer, 1980). The losses during ensilage normally represent the digestible fraction of the herbage (Wilkins, 1988), therefore it is obvious that whenever losses are substantial, the reduction in nutritive value of the silage is considerable.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Materials

3.1.1 Forages used

Napier grass (*Pennisetum purpureum*) was used in this study. This forage was already established at Magadu dairy farm of the department of Animal science and production at Sokoine University of Agriculture - Morogoro.

3.1.2 Management of the forages used

Weeding of the Napier grass plot was done in late September 1997. The grass was cut at a height of about 15 cm above the ground using bush knives. This was done to ensure uniform regrowth of the grass. Farmyard manure was applied at a rate of 12 tonsDM/ha. Irrigation using hosepipe was applied occasionally till the beginning of the rains in late October. The napier grass for ensilage was harvested after 9 weeks of new regrowth using bush knives, when most of the plants had attained a height of 1.2 - 1.5 metres.

3.1.3 Additives used

Leguminous tree leaves (*Gliricidia sepium, Leucaena leucocephala* and *Sesbania sesban*) and cane molasses were the additives used in this study. Fresh Gliricidia (*Gliricidia sepium*), Leucaena (*Leucaena leucocephala*) and Sesbania (*Sesbania sesban*) leaves (leaflets plus petioles) were hand harvested and incorporated into some of the silage treatments at a rate of 15% or 30% of the total weight of the forage material ensiled.

Molasses was applied at a rate of 3% of the total weight of the forage material ensiled. Since molasses is thick and viscous, dilution before application was made to ensure uniform spread. Molasses was diluted with water at a ratio of 1:1.

3.2 Types of silos used in this study

3.2.1 Laboratory silos

Polyvinyl chloride (PVC) cylinders of average $1.9x10^{-2}m^3$ were used as laboratory silos in this study. The open end of each cylinder was sealed by polyethylene sheet held securely by strong rubber bands immediately after sealing.

3.2.2 Pilot scale silos

Earth pit silos of $1m^3$ were made using hand hoe and spade. Each silo was furnished with polyethylene sheet in all sides so as to protect the ensiled material from soil contamination.

3.3 Experiments

The study was carried in two experiments, experiment II and I. In experiment I forages were ensiled in laboratory silos whereas in experiment II forages were ensiled in pilot scale silos. The second experiment was conducted so as to mimic the field condition as well as to produce enough silage for acceptability test by dairy heifers.

3.4 Experiment I: Laboratory silos

3.4.1 Treatments

Napier grass (*P. purpureum*) was ensiled in combination with three leguminous tree leaves (*G.sepium, L. leucocephala* and *S.sesban*) as nitrogen additive (NA). Each main treatment received three levels of nitrogen additive (0, 15 and 30 % w/w) in combination with two levels of molasses (0 and 3 % w/w) as water soluble carbohydrate (WSC) additive. For the purpose of saving costs of ensiling six combinations of 0% Gliricidia, Leucaena and Sesbania plus 0 and 3% molasses, napier grass without and with 3% molasses served as control silage. Therefore the treatments were as shown below.

$0NA^{+}$ - 97% grass, 3% molasses - control

$15G^{+}$ - 82% grass, 15% Gliricidia, 3% molasses

$30G^{+}$ - 67% grass, 30% Gliricidia, 3% molasses

$15L^{+}$ - 82% grass, 15% Leucaena, 3% molasses

$30L^{+}$ - 67% grass, 30% Leucaena, 3% molasses

$15S^{+}$ - 82% grass, 15% Sesbania, 3% molasses

$30S^{+}$ - 67% grass, 30% Sesbania, 3% molasses

$0NA^{-}$ - 100% grass - control

$15G^{-}$ - 85% grass, 15% Gliricidia, 0% molasses

$30G^{-}$ - 70% grass, 30% Gliricidia, 0% molasses

$15L^{-}$ - 85% grass, 15% Leucaena, 0% molasses

$30L^{-}$ - 70% grass, 30% Leucaena, 0% molasses

$15S^{-}$ - 85% grass, 15% Sesbania, 0% molasses

30S$^-$ - 70% grass, 30% Sesbania, 0% molasses

Treatments were randomly allocated to silos and replicated twice. The total number of silos was 28.

3.4.2 The ensiling process

The grass was chopped at 2.5cm length with a tractor driven chaff cutter and materials in each treatment were mixed thoroughly prior to ensiling. For each treatment 4kg of the materials were compacted in laboratory silos followed by application of pressure exerted by 4kg sand contained in polyethylene bags throughout the fermentation period which took 45 days.

3.4.3 Sampling, quality test and laboratory analyses

Samples of pre-ensiling materials from each treatment were taken for chemical composition analysis and *in vitro* dry matter and organic matter digestibility (IVDMD and IVOMD). The compositional analyses included determination of dry matter (DM), crude protein (CP), ash, neutral detergent fibre (NDF), acid detergent fibre (ADF), acid detergent lignin (ADL) and water soluble carbohydrates (WSC).

Immediately after opening each silo, spoiled silage was separated from well preserved silage and then a sample of about 400g of well preserved silage was taken. The sample was divided into four sub-samples and put in polyethylene bags and stored in a deep freezer at -10^{0}C until when they were used for laboratory work which include chemical analyses (DM, CP, ash, NDF, ADF, ADL, WSC) and determination of pH, volatile fatty acids (VFA), lactic acid, ammonia nitrogen (NH_3N), IVDMD and IVOMD. Qualities of the silages were also evaluated by sensoric tests soon after opening the silos. All portions of silage that were considered spoiled were weighed and dried. One sub-sample of well preserved silages was air dried in a well ventilated room. The dried sub-samples were grounded to pass through 1mm screen in a hammer mill, ready for chemical composition analyses and IVDMD and IVOMD. The spoiled silage from each treatment was mixed thoroughly and a composite sub-sample of about 200g placed in a polyethylene bag for determination of DM used in calculation of DM losses.

The pH reading was made using a pH meter (model 219 - Mk2; Pye Unicam). Samples of 40g from each silo were soaked in 200ml of cool distilled water for 12 hours. The mixture was then filtered and the supernatant divided into 4 aliquots for determination of pH.

Due to perturbation of freeze-drying equipment, the dry matter of the samples was determined by drying them in a well-ventilated room. The ammonia nitrogen (expressed as a percent of total N) of silages from frozen samples was determined by the routine Kjeldahl method (AOAC, 1990). Frozen samples were taken to the University of Dar es Salaam in a cool ice box for determination of volatile fatty acids (VFA) and lactic acid. Both VFA and lactic acid were determined according to the procedure of Playne (1985).

Crude protein and ash were analysed according to the standard procedures (AOAC, 1990). Fibre fractions that are, NDF, ADF and ADL were determined by the methods described by Goering and Van Soest (1970). Water soluble carbohydrates (WSC) contents were determined spectro-photometrically according to the methods described by Thomas (1977). IVDMD was determined by Tilley and Terry (1963) method.

3.4.4 Sensoric test

Immediately after opening each silo, the silage was assessed in terms of appearance, smell and texture. Assessment was done by a panel of six assessors, each marked a score grade card for each treatment (appendix 1).

3.4.5 Experimental design and statistical analysis

3.4.5.1 Experimental design

A 3 x 3 x 2 factorial experiment was conducted in a completely randomised design. The factors were 3 types of nitrogen additives i.e. tree legume species, 3 levels of inclusion of tree legume leaves and 2 level of inclusion of molasses.

3.4.5.2 Statistical analysis

General linear model (GLM) procedures of statistical analysis system (SAS, 1988) with SS1 option for analysis of variance was used in analysis of data on dry matter, chemical composition, IVDMD and IVOMD of silage treatments. The mathematical model used was as follows:

$Y_{ijkl} = \mu + A_i + B_j + C_k + AB_{ij} + AC_{ik} + BC_{jk} + ABC_{ijk} + e_{ijkl}$

Where,

Y_{ijkl} = An l^{th} record obtained from i^{th} nitrogen additive NA) included at j^{th} level and treated with k^{th} level of water soluble carbohydrate (WSC).

μ = General mean.

A_i = Effect due to i^{th} nitrogen additive (NA).

B_j = Effect due j^{th} level of nitrogen additive (NA).

C_k = Effect due k^{th} level of WSC.

AB_{ij} = Interaction between i^{th} NA and j^{th} level of NA.

AC_{ik} = Interaction between i^{th} NA and k^{th} level of WSC.

BC_{jk} = Interaction between j^{th} level of NA and k^{th} level of WSC.

ABC_{ijk} = Interaction between i^{th} NA, j^{th} level of NA and k^{th} level of WSC.

e_{ijkl} = Random error.

3.5 Experiment II: Pilot scale silos

3.5.1 Treatments

One combination in experiment I was ensiled in pilot scale silos. In this case, *L.leucocephala* was chosen as NA. Same levels of inclusion of Leucaena and molasses in experiment I were used in this experiment. The grass alone without and with molasses served as control. Therefore there were six treatments as shown below.

$0NA^+$ 97% grass, 3% molasses - control

$15NA^+$ - 82% grass, 15% NA (Leucaena), 3% molasses

$30NA^{+}$ -	67% grass, 30% NA (Leucaena), 3% molasses
$0NA^{-}$	100% grass - control
$15NA^{-}$ -	85% grass, 15% NA (Leucaena), 0% molasses
$30NA^{-}$ -	70% grass, 30% NA (Leucaena), 0% molasses

3.5.2 The ensiling process

The napier grass for ensilage was harvested after 9 weeks of new regrowth using bush knives, when most of the plants had attained a height of 1.2 - 1.5 metres and chopped as in experiment I. Materials in each treatment were mixed thoroughly prior to ensiling. Allocation of treatments to each silo was at random with two replications per treatment resulting in a total number of 12 silos. Filling of silos was done in layers of 15cm in depth, each layer being compacted after filling. Each silo was filled with 100kg of ensiling material. After complete filling, sealing of silos was done by covering them with polyethylene sheet, then about 500kg of soil was added on top to exert pressure throughout the fermentation period of 112 days. Continuing rains (*El Nino*) made impossible to open the earth pit silos after 45 days.

3.5.3 Sampling, quality test and laboratory analyses

On the opening of the silos, spoiled and well preserved silages were separated. Spoiled silage was mixed, weighed and sub-sampled then air dried as for experiment I. The well preserved silages were sampled then sub-sampled for sensoric test and laboratory analyses following the same procedures as in experiment I. The remaining portion of the well preserved silage from each treatment was used for acceptability (intake rate) test with cattle.

3.5.4 Determination of dry matter losses

The amount of dry matter lost from the silos was calculated by considering the difference between the DM contained in the original ensiled material and the DM of silage recovered. The DM losses were calculated as follows:

kgDM loss = kgDM of ensiled forage - kgDM of recovered silage

Where,

kgDM of recovered silage = kgDM of good silage + kgDM of spoiled silage.

The DM loss was finally expressed as percentage of DM present in the original forage material.

3.5.5 Silage acceptability test

The acceptability of the silages by the animals was evaluated in terms of intake rate.

3.5.5.1 Experimental animals and their management

Six heifers (Friesian * Ayrshire crosses) were selected from Magadu dairy farm at Sokoine university of Agriculture. The body weights of heifers ranged between 189 to 258kg. The experiment was conducted in May 1998 when the heifers were 13 to 20 months of age. Prior to the experiment, the animals were dewormed using 7.5% Levamisole hydrochloride.

Since animals were fed on green pastures before the beginning of the experiment they were accustomed to the experimental diet for 7 days prior to the experiment. Each animal was confined in individual stalls during the morning and supplied with the silage similar to the test silage. Water was provided *ad libitum* throughout the experiment. The heifers were not provided with any feed from 1600 to 0730 hours next mornings so as to activate intake of the experimental silage meals.

3.5.5.2 Feeding of the experimental silage meals

The silos were opened at 0630 hours on each day of experiment. Spoiled and well preserved silage were separated and sub-sampled for analysis as described in section 3.4.3. The remaining portion of well preserved silage from silos was weighed into polyethylene bags each containing 1500g meal for each of the six animals. Weighing was repeated until 3 meals for each animal from each treatment were prepared. Two treatments were fed in each morning therefore each animal received six meals of the test silage.

The experiment begun at 0730 hours with one person holding a stop watch, 6 men each took a single polyethylene bag with 1500g of experimental silage meal and spread the meal on the polyethylene sheet covering the feeding trough present in each individual animal stall. The heifers were allowed to eat every meal for 5 minutes only. At the end of 5 minutes, the animals were prevented to take any more bite, and refusals together with spilled silage on the platform and on the floor collected.

The collected uneaten silage was weighed and the weight subtracted from 1500g to get the intake in 5 minutes. The intake rate of the silage treatment was then calculated as:

Intake rate (g/minute) = grams eaten (for 5 minutes)/ 5 minutes

The gram of fresh silage eaten was multiplied by the corresponding DM content of each silage treatment to get dry matter intake rate per minute (gDM/minute).

3.5.6 Experimental design and statistical analysis

3.5.6.1 Experimental design

2 x 3 factorial experiment were conducted in a completely randomised design. The factors were 2 levels of inclusion of molasses and 3 levels of inclusion of nitrogen additive (Leucaena leaves).

3.5.6.2 Statistical analysis

General linear model (GLM) procedures of statistical analyses system (SAS, 1988) with SS1 option for analysis of variance was used in analysis of data on dry matter, chemical composition, IVDMD and IVOMD of silage treatments. Mathematical model used was as follows:

$$Y_{ijk} = \mu + A_i + B_j + AB_{ij} + e_{ijk}$$

Where,

Y_{ijk} = An k^{th} record obtained from i^{th} level of nitrogen additive (NA) treated with j^{th} level of molasses (WSC additive).

μ = General mean.

A_i = Effect due to i^{th} level of nitrogen additive (NA).

B_j = Effect due j^{th} level of molasses (WSC additive).

AB_{ij} = Interaction between i^{th} level of NA and j^{th} level of molasses (WSC additive).

e_{ijk} = Random error.

CHAPTER FOUR

RESULTS

4.1 Experiment I: Laboratory silos

4.1.1 Chemical composition, dry matter, *in vitro* dry matter and organic matter digestibility of the forages and additives used in silage making

Chemical composition, dry matter, *in vitro* dry matter and organic matter digestibility of the forages and additives used in making silage of different treatments are shown in Tables 7, 8 and 9. There were variations between treatments, the controls tended to have lower DM, CP and WSC relative to other treatments. The ash, NDF, and ADF tended to decrease whereas ADL tended to increase with increasing leguminous tree leaves inclusion level.

4.1.2 Sensoric evaluation of napier silage

Results of the effect of molasses inclusion and combination of the molasses and nitrogen additives on sensoric qualities of the napier silage are shown in Tables 10 and 11. Molasses added silage had higher scores in smell than unmolassesed silage. There were no significant ($P > 0.05$) difference between the molasses added and unmolassesed silage in terms of appearance and texture. Sensoric parameters of the napier silage were neither affected ($P > 0.05$) by nitrogen additive types nor nitrogen additive inclusion levels.

Table 7: Chemical composition of the forages in treatment combinations at the time of ensiling

Treatment	CP	WSC	Ash	NDF	ADF	ADL
	gkg^{-1} DM					
0NA^{+}	109.8	73.1	156.6	646.5	457.3	79.8
15G^{+}	123.5	99.8	153.2	563.2	368.6	76.8
30G^{+}	165.4	140.4	143.6	537.4	328.4	83.3
15L^{+}	147.6	74.0	151.5	579.2	438.1	118.3
30L^{+}	192.2	103.2	136.9	559.0	425.7	181.6
15S^{+}	151.6	93.9	151.1	591.2	392.1	67.6
30S^{+}	183.6	108.7	143.6	466.9	319.1	76.1
0NA^{-}	110.5	58.3	162.2	697.3	499.8	86.6
15G^{-}	121.8	71.7	150.2	609.7	440.8	96.0
30G^{-}	163.6	80.4	141.4	477.4	318.9	98.7
15L^{-}	137.5	75.8	148.6	667.9	419.9	122.1
30L^{-}	192.6	96.6	139.6	569.8	413.4	171.4
15S^{-}	144.8	76.3	150.1	565.4	420.4	70.4
30S^{-}	181.8	93.2	125.5	448.9	338.2	72.6
SEM	2.65	1.22	3.86	20.52	18.70	6.25

NA, G, L, S = nitrogen additive, Gliricidia, Leucaena and Sesbania, respectively; 0,15,30 preceding NA, G, L and S represent proportion (%) inclusion of nitrogen additive, Gliricidia, Leucaena and Sesbania, respectively; $^{+,-}$ indicate with and without molasses, respectively.

Table 8: **Dry matter, *in vitro* dry matter and organic matter digestibility of the forages in treatment combinations at the time of ensiling**

Treatment	DM	IVDMD	IVOMD
		%	
$0NA^+$	17.0	55.2	56.5
$15G^+$	20.7	67.2	65.2
$30G^+$	21.8	71.4	71.9
$15L^+$	20.1	56.5	56.3
$30L^+$	24.9	58.3	58.9
$15S^+$	20.0	63.2	63.9
$30S^+$	19.5	65.0	64.2
$0NA^-$	17.1	45.3	44.9
$15G^-$	19.3	48.6	48.6
$30G^-$	23.9	59.9	61.1
$15L^-$	18.9	44.0	45.2
$30L^-$	25.8	54.3	54.4
$15S^-$	19.2	59.8	57.1
$30S^-$	19.6	64.8	63.0
SEM	0.48	0.96	0.92

NA, G, L, S = nitrogen additive, Gliricidia, Leucaena and Sesbania, respectively; 0,15,30 preceding NA, G, L and S represent proportion (%) inclusion of nitrogen additive, Gliricidia, Leucaena and Sesbania, respectively; $^{+,-}$ indicate with and without molasses, respectively.

Table 9: Mean dry matter, chemical composition, in vitro dry matter and organic matter digestibility of additives used in silage making

Parameter	Gliricidia	Leucaena	Sesbania	Molasses
DM (%)	30.7	39.0	28.9	71.2
$gkg^{-1}DM$				
CP	189.2	212.4	181.9	16.8
WSC	201.9	152.4	179.9	304.2
Ash	126.3	111.0	79.6	124.3
NDF	457.7	468.3	298.9	
ADF	283.2	294.8	190.8	
ADL	104.2	123.9	56.1	
IVDMD (%)	78.8	56.7	74.2	
IVOMD (%)	76.3	54.1	73.0	

Table 10: Mean effect of molasses inclusion level on smell of the napier silage

Molasses inclusion	With	Without	SEM
Scores[1]	3.1^{a}	2.7^{b}	0.06

[1] The scores used were defined as 1 = poor; 2 = moderate; 3 = good; 4 = very good; [a-b] Means within the same row followed by different superscripts are significantly ($P < 0.05$) different; NA = nitrogen additive; SEM = standard error of the means.

Results of the effect of molasses and NA inclusion levels on sensoric parameters of the napier silage showed slight differences in sensoric parameters between treatments however, silage with $0NA^{-}$ tended to have lower scores relative to other treatments. There were no significant ($P > 0.05$) difference in sensoric parameters of the napier silage between the combinations of molasses and NA type, NA type and inclusion level and molasses, NA type and inclusion level.

Table 11: Mean effect of molasses and nitrogen additive inclusion levels on sensoric parameters of the napier silage

Treatment	Appearance	Smell	Texture
		Scores[1]	
$0NA^+$	3.3^a	3.3^a	2.8^a
$15NA^+$	3.1^{ab}	3.0^{ab}	2.6^{ab}
$30NA^+$	2.8^b	2.8^{bc}	2.6^{ab}
$0NA^-$	2.8^b	2.5^d	2.5^b
$15NA^-$	3.1^{ab}	2.9^{bc}	2.7^{ab}
$30NA^-$	2.9^b	2.7^{cd}	2.7^{ab}
SEM	0.11	0.10	0.07

[1] The scores used were defined as 1 = poor; 2 = moderate; 3 = good; 4 = very good; NA = nitrogen additive; 0,15,30 preceding NA represent proportion (%) inclusion of nitrogen additive; $^{+,-}$ indicate with and without molasses, respectively; $^{a-d}$ means within the same column followed by different superscript letters are significantly ($P < 0.05$) different; SEM = standard error of the means.

4.1.2 Dry matter, chemical composition, *in vitro* dry matter and organic matter digestibility of the napier silage

4.1.3.1 Effect of molasses

Results of the effect of molasses inclusion level on dry matter, chemical composition, *in vitro* dry matter and organic matter digestibility of the napier silage are shown in Table 12. The molasses added silage had significantly ($P < 0.01$) higher DM than unmolassesed silage. The CP and WSC content were significantly ($P < 0.01$) higher in molasses added than unmolassesed silage. The case was opposite for NDF, ADF and

ADL. Molassesed silage had significantly ($P < 0.01$) lower NDF, ADF and ADL than unmolassesed silage. Molasses added silage was significantly ($P < 0.01$) more digestible than unmolassesed silage. There were no significant ($P > 0.05$) difference between molasses added and unmolassesed silage in terms of ash content.

Table 12: Mean effect of molasses inclusion level on dry matter, chemical composition, *in vitro* dry matter and organic matter digestibility of the napier silage

Parameter	Molasses inclusion		SEM
	With	Without	
DM(%)	18.0^{a}	15.9^{b}	0.14
In gkg^{-1} DM			
CP	112.0^{a}	105.7^{b}	1.54
WSC	55.3^{a}	42.7^{b}	0.58
Ash	132.9^{a}	133.9^{a}	1.07
NDF	513.8^{b}	581.8^{a}	4.19
ADF	324.9^{b}	378.0^{a}	3.14
ADL	42.0^{b}	48.6^{a}	0.96
IVDMD(%)	57.9^{a}	47.5^{b}	0.36
IVOMD(%)	57.3^{a}	49.4^{b}	0.33

$^{a\text{-}b}$ means within the same row followed by different superscripts are significantly ($P < 0.01$) different; SEM = standard error of the means.

4.1.3.2 Effect of nitrogen additives

Results of the effect of NA type and NA inclusion level on dry matter, chemical composition, *in vitro* dry matter and organic matter digestibility are shown in Tables 13 and 14. Silage with Leucaena had significantly ($P < 0.05$) higher DM than silage with

Gliricidia and Sesbania. Leucaena and Sesbania added silage had significantly ($P < 0.05$) higher CP content than Gliricidia added silage. Sesbania added silage had higher WSC than Gliricidia and Leucaena added silage. Gliricidia added silage had the highest ash content, followed by Leucaena whereas Sesbania added silage had the lowest ash content. Leucaena added silage had higher NDF and lower ADF than Gliricidia and Sesbania added silage. Gliricidia and Leucaena added silage had higher ADL content than Sesbania added silage. Gliricidia and Sesbania added silage were more digestible than Leucaena added silage.

Silage with 30% NA had the highest DM, followed by 15% NA silage and 0% NA silage had the lowest DM. The trend was similar for CP and WSC. Silage with 0 and 15 percent NA had higher ash content than 30% NA silage. Silage with 0% NA had the highest NDF and ADF, followed by 15% NA silage whereas 30% NA silage had the lowest NDF and ADF content. Silage with 30% NA was the most digestible, followed by 15% NA silage whereas 0% NA silage was the least digestible.

Table 13: Mean effect of nitrogen additive type on dry matter, chemical composition, *in vitro* dry matter and organic matter digestibility of the napier silage

Parameter	Nitrogen additive type			SEM
	Gliricidia	Leucaena	Sesbania	
DM(%)	16.6[b]	17.7[a]	16.5[b]	0.17
In gkg^{-1} DM				
CP	99.4[b]	114.4[a]	112.8[a]	1.89
WSC	47.3[b]	45.6[b]	54.0[a]	0.71
Ash	139.9[a]	132.2[b]	128.1[c]	1.31
NDF	537.5[b]	575.1[a]	530.9[b]	5.13
ADF	357.8[a]	344.9[b]	351.5[a]	3.85
ADL	50.5[a]	50.9[a]	34.5[b]	1.17
IVDMD(%)	54.7[a]	48.9[b]	54.4[a]	0.45
IVOMD(%)	55.1[a]	49.9[b]	55.1[a]	0.41

[a-c] means within the same row followed by different superscripts are significantly ($P < 0.05$) different; SEM = standard error of the means.

4.1.3.3 Effect of the combination of molasses and nitrogen additives

Results of the effect of the combination of molasses and nitrogen additives on chemical composition and *in vitro* digestibilities are shown in Tables 15, 16, 17, 18 and 19. Mean effect of the combination of molasses and NA type (Table 15) indicated that unmolassesed silage with Gliricidia had significantly ($P < 0.05$) lower WSC content than other treatments. Unmolassesed silage with Gliricidia and Sesbania had the highest ADF content, followed by unmolassesed silage with Leucaena whereas molassesed silage

Table 14: Mean effect of nitrogen additive inclusion level on dry matter, chemical composition, *in vitro* dry matter and organic matter digestibility of the napier silage

Parameter	Nitrogen additive inclusion (%)			SEM
	0	15	30	
DM(%)	14.7^{c}	17.0^{b}	19.2^{a}	0.17
In gkg^{-1} DM				
CP	78.6^{c}	117.4^{b}	136.3^{a}	1.89
WSC	33.5^{c}	50.2^{b}	63.3^{a}	0.71
Ash	136.6^{a}	136.5^{a}	127.1^{b}	1.31
NDF	633.6^{a}	530.8^{b}	479.6^{c}	5.13
ADF	405.5^{a}	343.0^{b}	305.8^{c}	3.85
ADL	34.7^{c}	48.3^{b}	53.0^{a}	1.17
IVDMD(%)	46.7^{c}	53.3^{b}	58.2^{a}	0.45
IVOMD(%)	49.7^{c}	53.2^{b}	57.1^{a}	0.41

$^{a-c}$ means within the same row followed by different superscripts are significantly ($P < 0.05$) different; SEM = standard error of the means.

with Gliricidia, Leucaena and Sesbania had the lowest ADF content. Molassesed Gliricidia, Leucaena and Sesbania silage were consistently and significantly ($P < 0.05$) more digestible than unmolassesed napier silage with the same leguminous tree leaves. Napier silage with Leucaena were least digestible whether with or without molasses. There were no significant ($P > 0.05$) difference between treatments in terms of DM, CP, ash, NDF and ADL contents of the napier silage.

Table 15: Mean effect of molasses inclusion level and nitrogen additive type on chemical composition, *in vitro* dry matter and organic matter digestibility of the napier silage

Treatment	WSC	ADF	IVDMD	IVOMD
	gkg^{-1}DM		%	
G^+	58.7^a	323.5^c	61.7^a	60.8^a
L^+	50.9^b	327.6^c	53.3^c	52.8^c
S^+	56.2^a	323.5^c	58.7^b	58.2^b
G^-	35.9^d	392.2^a	47.9^e	49.3^d
L^-	40.3^c	362.3^b	44.6^f	47.0^e
S^-	51.8^b	379.6^a	50.1^d	52.0^c
SEM	1.01	5.44	0.63	0.57

G, L, S = Gliricidia, Leucaena and Sesbania, respectively; $^{+,-}$ indicate with and without molasses, respectively; $^{a-f}$ means within the same column followed by different superscript letters are significantly ($P < 0.05$) different; SEM = standard error of the means.

Results of effect of combination of molasses and NA inclusion levels (Table 16) showed that water soluble carbohydrates were consistently and significantly higher in molassesed treatments with 30 and 15% NA than unmolassesed treatments with 30 and 15% NA. Silages with $0NA^-$ and $0NA^+$ had the lowest WSC content, $0NA^-$ being lower than $0NA^+$. Silage with $0NA^-$ and $0NA^+$ had consistently and significantly higher NDF content than other treatments, $30NA^+$ silage having the lowest content. Silage with $30NA^+$ was the most digestible, followed by $15NA^+$ silage then $0NA^+$ and $30NA^-$, whereas $15NA^-$ and $0NA^-$ silage were the least digestible.

The mean effect of the combination of NA type and inclusion

Table 16: Mean effect of molasses and nitrogen additive inclusion levels on chemical composition, *in vitro* dry matter and organic matter digestibility of the napier silage

Treatment	WSC	NDF	IVDMD	IVOMD
	gkg^{-1}DM		%	
0NA^{+}	36.1^{e}	595.1^{b}	54.3^{c}	55.2^{c}
15NA^{+}	58.8^{b}	489.2^{d}	58.0^{b}	57.2^{b}
30NA^{+}	71.0^{a}	457.2^{e}	61.4^{a}	59.5^{a}
0NA^{-}	30.9^{f}	671.2^{a}	39.1^{e}	44.3^{e}
15NA^{-}	41.5^{d}	572.3^{c}	48.5^{d}	49.3^{d}
30NA^{-}	55.6^{c}	502.1^{d}	54.9^{c}	54.7^{c}
SEM	1.01	7.25	0.63	0.57

NA = nitrogen additive; 0,15,30 preceding NA represent proportion (%) inclusion of nitrogen additive; $^{+,-}$ indicate with and without molasses, respectively; $^{a-f}$ means within the same column followed by different superscript letters are significantly ($P < 0.05$) different; SEM = standard error of the means.

level (Table 17 and 18) showed that silage with 30% Sesbania and Leucaena had the highest CP content followed by 15% Leucaena and Sesbania and 30% Gliricidia silage then 15% Gliricidia whereas 0% NA silage had the lowest CP content. There were significant differences in WSC between the treatments. Under each NA type silage with 30% NA inclusion level tended to have significantly higher WSC than those with 15% NA inclusion level. Among the treatments silage with 30% Sesbania had the highest WSC whereas 0NA silage had the lowest WSC content. The 0NA silage had higher ash and NDF content than other treatments. The silage with 15 and

30% Leucaena and Gliricidia had higher ADL content than other treatments. The 30L silage had the highest DM content followed by 30G, 15L, and 30S then 15G and 15S whereas 0NA silage had the lowest DM content. The 30G silage were the most digestible followed by 15G and 15S then 30L whereas 15L and 0NA silage were the least digestible. There was no significant (P > 0.05) difference between treatments in terms of ADF content.

Table 17: Mean effect of nitrogen additive type and inclusion on chemical composition of the napier silage

Treatment	CP	WSC	Ash	NDF	ADL
			gkg^{1} DM		
0NA	78.6^{d}	33.5^{f}	136.6^{b}	633.1^{a}	34.7^{c}
15G	101.6^{c}	51.2^{d}	144.7^{a}	514.8^{c}	56.5^{b}
30G	118.0^{b}	57.3^{c}	138.5^{b}	464.6^{d}	60.4^{ab}
15L	120.2^{b}	39.0^{e}	135.7^{bc}	556.9^{b}	54.9^{b}
30L	144.4^{a}	64.5^{b}	124.2^{cd}	535.3^{bc}	63.2^{a}
15S	113.3^{b}	60.3^{c}	129.2^{c}	520.7^{bc}	33.4^{c}
30S	146.5^{a}	68.3^{a}	118.6^{d}	439.0^{d}	35.5^{c}
SEM	3.27	1.23	2.28	8.88	2.03

NA, G, L, S = nitrogen additive, Gliricidia, Leucaena and Sesbania, respectively; 0,15,30 preceding NA,G,L and S represent proportion (%) inclusion of nitrogen additive, Gliricidia, Leucaena and Sesbania, respectively; $^{a-e}$ means within the same column followed by different superscript letters are significantly (P < 0.05) different; SEM = standard error of the means.

Results of the effect of molasses inclusion level, types and inclusion levels of nitrogen additive on water soluble carbohydrates, *in vitro* dry matter and organic matter digestibility of the napier silage indicated slight differences in WSC content between treatments however, unmolassesed treatments except 30S⁻ had consistently and significantly lower contents than molassesed treatments except 0NA⁺. There were significant differences in digestibility between treatments. The 30G⁺, 15G⁺, 30S⁺, 15S⁺ and 30S⁻ silage were more digestible than other treatments.

Table 18: Mean effect of nitrogen additive type and inclusion on dry matter, *in vitro* dry matter and organic matter digestibility of the napier silage

Treatment	DM	IVDMD	IVOMD
		%	
0NA	14.7^{e}	46.7^{d}	48.1^{d}
15G	16.6^{d}	55.7^{b}	54.7^{c}
30G	18.6^{b}	61.9^{a}	60.8^{a}
15L	17.7^{c}	48.2^{d}	48.4^{d}
30L	20.8^{a}	51.9^{c}	51.7^{c}
15S	16.7^{d}	55.9^{b}	56.2^{b}
30S	18.2^{bc}	60.7^{a}	59.5^{c}
SEM	0.29	0.77	0.72

NA, G, L, S = nitrogen additive, Gliricidia, Leucaena and Sesbania, respectively; 0,15,30 preceding NA, G, L and S represent proportion (%) inclusion of nitrogen additive, Gliricidia, Leucaena and Sesbania, respectively; $^{a-e}$ means within the same column followed by different superscript letters are significantly ($P < 0.05$) different; SEM = standard error of the means.

Table 19: Mean effect of molasses inclusion level, type and inclusion level of nitrogen additive on water soluble carbohydrates, *in vitro* dry matter and organic matter digestibility of the napier silage

Treatment	WSC	IVDMD	IVOMD
	gkg^{-1}DM	%	
0NA^{+}	36.1^{fg}	54.3^{e}	55.2^{de}
15G^{+}	66.7^{bc}	63.9^{b}	63.3^{ab}
30G^{+}	73.4^{a}	66.9^{a}	64.1^{a}
15L^{+}	45.8^{e}	50.7^{f}	49.4^{g}
30L^{+}	71.0^{ab}	54.9^{e}	53.8^{ef}
15S^{+}	63.9^{c}	59.4^{cd}	59.0^{bc}
30S^{+}	68.7^{bc}	62.4^{bc}	60.6^{b}
0NA^{-}	30.9^{h}	39.1^{h}	44.3^{i}
15G^{-}	35.7^{gh}	47.5^{fg}	46.1^{hi}
30G^{-}	41.1^{ef}	57.0^{de}	57.6^{cd}
15L^{-}	32.2^{gh}	45.8^{g}	46.3^{hi}
30L^{-}	58.0^{d}	48.8^{fg}	48.3^{gh}
15S^{-}	56.8^{d}	52.4^{ef}	53.4^{d}
30S^{-}	67.9^{bc}	59.0^{d}	58.3^{bc}
SEM	1.75	1.09	1.00

NA, G, L, S = nitrogen additive, Gliricidia, Leucaena and Sesbania, respectively; 0,15,30 preceding NA, G, L and S represent proportion (%) inclusion of nitrogen additive, Gliricidia, Leucaena and Sesbania, respectively; $^{+,-}$ indicate with and without molasses, respectively; $^{a-i}$ means within the same column followed by different superscript letters are significantly ($P < 0.05$) different; SEM = standard error of the means.

4.1.4 Fermentation products of the napier silage

4.1.4.1 Effect of molasses

Results of the effect of molasses inclusion level on fermentation products of the napier silage are shown in Table 20. The molasses added silage had significantly lower pH, NH_3N, acetate and higher lactate than unmolassesed silage. There were no significant ($P > 0.05$) difference in terms of butyrate content between molasses added and unmolassesed silage.

Table 20: Mean effect of molasses inclusion level on fermentation products of the napier silage

Parameter	Molasses inclusion		SEM
	With	Without	
Ph	4.04^b	4.91^a	0.041
NH_3 N (as %TN)	1.8^b	3.4^a	0.15
In gkg^{-1} DM			
Lactate	10.4^a	9.1^b	0.24
Acetate	0.7^b	1.0^a	0.09
Butyrate	0.1^a	0.2^a	0.07

$^{a-b}$ means within the same row followed by different superscript are significantly ($P < 0.05$) different; SEM = standard error of the means.

4.1.4.2 Effect of nitrogen additives

Results of the effect of nitrogen additive type as well as NA inclusion level on fermentation products of the napier silage are shown in Tables 21 and 22. Ammonia nitrogen was significantly ($P < 0.05$) higher in Sesbania added silage than in Gliricidia and Leucaena added silage. There were no significant ($P > 0.05$) differences in terms of pH, lactate, acetate and butyrate content between nitrogen additive types.

Table 21: Mean effect of nitrogen additive type on fermentation products of the napier silage

Parameter	NA type			SEM
	Gliricidia	Leucaena	Sesbania	
pH	4.40^{a}	4.54^{a}	4.50^{a}	0.049
NH_3 N (% TN)	2.3^{b}	2.3^{b}	3.3^{a}	0.19
In gkg^{-1} DM				
Lactate	10.3^{a}	9.2^{a}	9.8^{a}	0.29
Acetate	0.8^{a}	1.0^{a}	0.8^{a}	0.12
Butyrate	0.1^{a}	0.1^{a}	0.1^{a}	0.09

$^{a-b}$ means within the same row followed by different superscripts are significantly ($P < 0.05$) different; SEM = standard error of the means.

Results of the effect of nitrogen additive inclusion level on fermentation products of the napier silage showed that silage with 0% NA had higher NH_3N than 15 and 30 percent NA silage. The 15% NA silage had significantly ($P < 0.05$) higher lactate than 0 and 30 percent NA silage. There were no differences in terms of pH, acetate and butyrate content between nitrogen additive inclusion levels.

Table 22: Mean effect of nitrogen additive inclusion level on fermentation products of the napier silage

Parameter	Nitrogen additive inclusion (%)			SEM
	0	15	30	
pH	4.55[a]	4.41[a]	4.48[a]	0.049
NH_3N,(% TN)	3.3[a]	2.4[b]	2.3[b]	0.19
In gkg^{-1} DM				
Lactate	9.2[b]	10.7[a]	9.4[b]	0.29
Acetate	0.8[a]	0.8[a]	1.0[a]	0.12
Butyrate	0[a]	0.1[a]	0.2[a]	0.08

[a-b] means within the same row followed by different superscript are significantly ($P < 0.05$) different; SEM = standard error of the means.

4.1.4.3 Effect of the combination of molasses and nitrogen additives

Results of the effect of the combination of molasses and nitrogen additives on fermentation products of the napier silage are shown in Tables 23 and 24. Silage with $0NA^-$ had the highest pH, followed by $15NA^-$ and $30NA^-$ silage whereas $0NA^+$, $15NA^+$ and $30NA^+$ silage had the lowest pH. The $0NA^-$ silage had the highest NH_3N content. The silage with $15NA^+$ had higher lactate and lower acetate content than other treatments. Butyrate content of the napier silage was not affected by neither molasses inclusion nor nitrogen additive inclusion level.

Silage with $30L^+$, $30S^-$, $15L^-$, $15G^-$ and $30G^-$ had higher acetate content relative to other treatments. The pH, NH_3N, lactate and butyrate content were not significantly ($P > 0.05$) affected by molasses inclusion, nitrogen additive type and nitrogen additive inclusion levels. There were no significant ($P > 0.05$) differences in sensoric parameters between the combinations of molasses and NA types and those of NA types and inclusion levels.

Table 23: Mean effect of molasses and nitrogen additive inclusion levels on fermentation products of the napier silage

Treatment	pH	NH3N (% TN)	Lactate	Acetate	Butyrate
				gkg^{-1}DM	
0NA^{+}	4.00^{c}	2.1^{cd}	10.2^{b}	0.7^{bc}	0
15NA^{+}	3.97^{c}	1.8^{d}	12.1^{a}	0.4^{c}	0
30NA^{+}	4.18^{c}	1.8^{d}	9.0^{bc}	1.0^{ab}	0.2^{a}
0NA^{-}	5.11^{a}	4.5^{a}	8.2^{c}	0.8^{abc}	0
15NA^{-}	4.84^{b}	3.0^{b}	9.4^{bc}	1.3^{a}	0.2^{a}
30NA^{-}	4.78^{b}	2.9^{bc}	9.8^{b}	1.0^{ab}	0.3^{a}
SEM	0.071	0.27	0.41	0.16	0.12

NA = nitrogen additive; 0,15,30 preceding NA represent proportion (%) inclusion of nitrogen additive; $^{+,-}$ indicate with and without molasses, respectively; $^{a-d}$ means within the same column followed by different superscript letters are significantly ($P < 0.05$) different; SEM = standard error of the means.

Table 24: Mean effect of molasses inclusion, nitrogen additive type and inclusion level on fermentation products of the napier silage

Treatment	Acetate
	$gkg^{-1}DM$
$0NA^{+}$	0.7^{bcde}
$15G^{+}$	0.3^{e}
$30G^{+}$	0.6^{cde}
$15L^{+}$	0.4^{de}
$30L^{+}$	1.9^{a}
$15S^{+}$	0.4^{de}
$30S^{+}$	0.5^{de}
$0NA^{-}$	0.8^{bcde}
$15G^{-}$	1.4^{abc}
$30G^{-}$	1.2^{abcd}
$15L^{-}$	1.5^{ab}
$30L^{-}$	0.6^{cde}
$15S^{-}$	0.9^{bcde}
$30S^{-}$	1.3^{abc}
SEM	0.28

NA, G, L, S = nitrogen additive, Gliricidia, Leucaena and Sesbania, respectively; 0,15,30 preceding NA,G,L and S represent proportion (%) inclusion of nitrogen additive, Gliricidia, Leucaena and Sesbania, respectively; $^{a\text{-}e}$ means within the same column followed by different superscript letters are significantly ($P < 0.05$) different; SEM = standard error of the means.

4.2 Experiment II: Earth pit silos

4.2.1 Chemical composition, dry matter, *in vitro* dry matter and organic matter digestibility of the forages and additives used in silage making

Chemical composition, dry matter, *in vitro* dry matter and organic matter digestibility of the forages and additives used in making silage of different treatments are shown in Tables 25, 26 and 27. The CP and WSC content tended to increase with increasing NA and molasses inclusion levels. Ash content in the forages tended to decrease with increase in NA inclusion. Grass with $0NA^+$ and $0NA^-$ had higher NDF content relative to other treatments. Variation in ADF content between treatments were however, narrow. Grass with $0NA^+$ and $0NA^-$ tended to have higher values of ADF relative to other treatments. There were slight variations in ADL content between treatments however, grass with $30NA^+$ had higher ADL relative to $0NA^+$ grass. Grass with $30NA^+$ had higher DM content relative to other treatments. Grass with $30NA^+$, $15NA^+$, $30NA^-$ and $0NA^+$ were more digestible relative to grass with $0NA^-$ and $15NA^-$.

4.2.2 Sensoric evaluation of the napier silage

Results of the effect of molasses inclusion level on smell of the napier silage are shown in Table 28. The molasses added silage had higher scores than unmolassesed silage. Molasses inclusion level did not affect appearance and texture. Sensoric parameters of the napier silage were neither affected ($P > 0.05$) by nitrogen additive inclusion level nor the combination of molasses and nitrogen additive.

Table 25: Chemical composition of the forages in treatment combinations at the time of ensiling

Treatment	CP	WSC	Ash	NDF	ADF	ADL
	gkg^{-1}DM					
0NA$^+$	77.1	83.4	133.3	625.1	386.6	41.6
15NA$^+$	153.0	90.0	125.2	502.0	309.0	48.0
30NA$^+$	167.9	95.5	112.4	456.8	278.7	54.4
0NA$^-$	74.2	62.9	125.0	695.2	435.9	53.3
15NA$^-$	144.1	72.1	111.3	511.8	320.8	47.3
30NA$^-$	150.0	75.1	115.9	543.5	337.4	51.7
SEM	2.40	1.77	3.41	22.67	16.69	3.38

NA = nitrogen additive; 0,15,30 preceding NA represent proportion (%) inclusion of nitrogen additive; $^{+,-}$ indicate with and without molasses, respectively.

Table 26: Dry matter, *in vitro* dry matter and organic matter digestibility of the forages in treatment combinations at the time of ensiling

Treatment	DM	IVDMD	IVOMD
	%		
0NA$^+$	15.9	50.7	51.6
15NA$^+$	17.1	60.5	59.5
30NA$^+$	17.9	64.2	63.2
0NA$^-$	15.1	50.7	49.7
15NA$^-$	16.5	52.7	52.2
30NA$^-$	16.9	54.4	54.3
SEM	0.75	0.87	0.76

NA = nitrogen additive; 0,15,30 preceding NA represent proportion (%) inclusion of nitrogen additive; $^{+,-}$ indicate with and without molasses, respectively.

Table 27: Mean dry matter, chemical composition, in vitro dry matter and organic matter digestibility of additives used in silage making

Parameter	Leucaena	Molasses
DM (%)	25.1	73.8
$gkg^{-1}DM$		
CP	250.3	23.0
WSC	85.8	306.4
Ash	82.2	138.6
NDF	299.1	
ADF	434.9	
ADL	168.6	
IVDMD (%)	61.7	
IVOMD (%)	66.7	

Table 28: Mean effect of molasses inclusion level on smell of the napier silage

Molasses inclusion	With	Without	SEM
Scores[1]	3.4^{a}	3.1^{b}	0.09

[1] The scores used were defined as 1 = poor; 2 = moderate; 3 = good; 4 = very good; [a-b] Means within the same row are significantly ($P < 0.05$) different; SEM = standard error of the means.

4.2.3 Dry matter, chemical composition, *in vitro* dry matter and organic matter digestibility of the napier silage

4.2.3.1 Effect of molasses

Results of the effect of molasses inclusion level on dry matter, chemical composition, *in vitro* dry matter and organic matter digestibility of the napier silage are shown in

Table 29. The molasses added silage had significantly (P < 0.05) higher WSC content than unmolassesed silage. Unmolassesed silage had higher ADF and ADL content and were less digestible than molasses added silage. Molasses inclusion level did not affect (P > 0.05) DM, CP, ash and NDF of napier silage between the treatments.

4.2.3.2 Effect of nitrogen additive

Results of the effect of nitrogen additive inclusion level on chemical composition, in vitro dry matter and organic matter digestibility of the napier silage are shown in Table 30. The CP content was highest in the silage with 30% NA, followed by silage with 15% NA whereas silage with 0% NA had the least CP content. Silage with 15% NA had the highest WSC content, followed by silage with 0% NA while silage with 30% NA had the lowest WSC content. Silages with 0 and 15 percent NA had higher NDF content than that with 30% NA. Silage with 0% had higher ADF and lower ADL than those with 15 and 30 percent NA. Silage with 30% NA was the 0% NA had higher ADF and lower ADL contents than those with most digestible followed by 15% NA silage while 0% NA silage was the least digestible. Ash and DM content of the napier silage were not affected (P > 0.05) by nitrogen additive inclusion level.

Table 29: Mean effect of molasses inclusion level on dry matter, chemical composition, *in vitro* dry matter and organic matter digestibility of the napier silage

Parameter	Molasses inclusion		SEM
	With	Without	
DM (%)	15.4^{a}	15.5^{a}	0.45
$gkg^{-1}DM$			
CP	95.4^{a}	91.0^{a}	3.80
WSC	55.5^{a}	50.3^{b}	0.85
Ash	115.8^{a}	119.4^{a}	4.75
NDF	621.9^{a}	633.8^{a}	7.84
ADF	429.5^{b}	461.3^{a}	8.29
ADL	127.1^{b}	135.5^{a}	1.28
IVDMD (%)	56.0^{a}	49.6^{b}	0.64
IVOMD (%)	55.8^{a}	48.3^{b}	0.71

$^{a-b}$ Means within the same row are significantly ($P < 0.05$) different; SEM = standard error of the means.

4.2.3.3 Effect of the combination of molasses and nitrogen additive

Results of the effect of molasses inclusion and nitrogen additive inclusion levels on chemical composition and *in vitro* dry matter digestibility of the napier silage are shown in Table 31. Silage with $15NA^{+}$ had the highest WSC content, followed by $0NA^{+}$ silage then $15NA^{-}$ and $0NA^{-}$ silages followed by $30NA^{-}$ whereas $0NA^{+}$, $0NA^{-}$ and $15NA^{-}$ were the least digestible. There were no significant ($P > 0.05$) difference between treatments in terms of DM, CP, ash, NDF and ADF contents of the napier silage.

Table 30: Mean effect of nitrogen additive inclusion level on chemical composition and *in vitro* dry matter and organic matter digestibility of the napier silage

Parameter	Nitrogen inclusion (%)			SEM
	0	15	30	
DM (%)	14.5^{a}	15.2^{a}	16.7^{a}	0.55
In gkg^{-1}DM				
CP	60.1^{c}	99.4^{b}	120.1^{a}	4.83
WSC	56.6^{b}	60.8^{a}	40.5^{c}	1.04
Ash	125.3^{a}	117.4^{a}	110.3^{a}	5.81
NDF	659.5^{a}	629.7^{a}	594.3^{b}	9.61
ADF	510.1^{a}	428.5^{b}	397.6^{b}	10.15
ADL	48.0^{b}	172.8^{a}	173.2^{a}	1.56
IVDMD (%)	46.4^{c}	53.7^{b}	58.3^{a}	0.79
IVOMD (%)	47.1^{c}	51.8^{b}	57.2^{a}	0.87

$^{a-c}$ Means within the same row are significantly ($P < 0.05$) different; SEM = standard error of the means.

4.2.4 Fermentation products of the napier silage

4.2.4.1 Effect of molasses

Results of the effect of molasses inclusion level on fermentation products of the napier silage are shown in Table 32. Unmolassesed silage had significantly ($P < 0.05$) higher pH, NH_3N and lower lactate than molasses added silage. Molasses inclusion did not affect the acetate and butyrate content of the napier silage.

Table 31: Mean effect of molasses and nitrogen additive inclusion levels on chemical composition and *in vitro* dry matter digestibility of the napier silage

Treatment	WSC	ADL	IVDMD
	$gkg^{-1}DM$		%
$0NA^{+}$	62.1^{b}	48.5^{d}	47.3^{c}
$15NA^{+}$	67.5^{a}	161.9^{c}	58.9^{a}
$30NA^{+}$	35.4^{e}	171.1^{b}	61.8^{a}
$0NA^{-}$	51.2^{c}	47.5^{d}	45.2^{c}
$15NA^{-}$	54.2^{c}	175.3^{b}	48.5^{c}
$30NA^{-}$	45.7^{d}	183.7^{a}	54.7^{b}
SEM	1.48	2.21	1.12

NA = nitrogen additive; 0,15,30 preceding NA represent proportion (%) inclusion of nitrogen additive; $^{+,-}$ indicate with and without molasses, respectively; $^{a-e}$ Means within the same column are significantly ($P < 0.05$) different; SEM = standard error of the means.

Table 32: Mean effect of molasses inclusion level on fermentation products of the napier silage

Parameter	Molasses inclusion		SEM
	With	Without	
PH	4.23^{b}	4.78^{a}	0.125
NH3N (% TN)	2.4^{b}	4.3^{a}	0.36
In $gkg^{-1}DM$			
Lactate	14.8^{a}	11.6^{b}	0.39
Acetate	0.6^{a}	0.5^{a}	0.04
Butyrate	0.7^{a}	0.5^{a}	0.23

$^{a-b}$ Means within the same row are significantly ($P < 0.05$) different; SEM = standard error of the means.

4.2.4.2 Effect of nitrogen additive

Results of the effect of nitrogen additive inclusion level on fermentation products of the napier silage are shown in Table 33. Silage with 30% NA had significantly ($P < 0.05$) higher pH and lactate than silage with 0 and 15 percent NA. Silage with 15 and 30 percent had significantly ($P < 0.05$) higher acetate than 0% NA silage. Butyrate and NH_3N of the napier silage were not affected by NA inclusion level.

Table 33: Mean effect of nitrogen additive inclusion level on fermentation products of the napier silage

Parameter	Nitrogen additive inclusion (%)			SEM
	0	15	30	
PH	4.09^{b}	4.56^{b}	4.88^{a}	0.154
NH_3N (% TN)	2.9^{a}	3.6^{a}	3.6^{a}	0.45
In $gkg^{-1}DM$				
Lactate	12.7^{b}	12.4^{b}	14.5^{a}	0.48
Acetate	0.3^{b}	0.8^{a}	0.7^{a}	0.05
Butyrate	0.08^{a}	0.8^{a}	0.1^{a}	0.28

$^{a-b}$ Means within the same row are significantly ($P < 0.05$) different; SEM = standard error of the means.

4.2.4.3 Effect of the combination of molasses and nitrogen additive

There were no significant ($P > 0.05$) differences between treatments in terms of fermentation products.

4.2.5 Dry matter losses of the napier silage

4.2.5.1 Effect of molasses

Results of the effect of molasses inclusion on dry matter losses of the napier silage are shown in Table 34. The %DM loss was significantly ($P < 0.05$) higher in molasses added silage than unmolassesed silage.

Table 34: Mean effect of molasses inclusion level on dry matter loss of the napier silage

Parameter	Molasses inclusion		SEM
	With	Without	
kgDM ensiled	16.5[a]	16.3[a]	0.11
kgDM of silage recovered	13.1[a]	13.3[a]	0.13
% DM loss	20.7[a]	18.5[b]	0.37

[a-b] Means within the same row are significantly ($P < 0.05$) different; SEM = standard error of the means.

4.2.5.2 Effect of nitrogen additive

Results of the effect of nitrogen additive inclusion level on dry matter losses are shown in Table 35. The %DM loss was highest in silage with 0% NA, followed by 15% NA silage while 30% NA silage were the least.

Table 35: Mean effect of nitrogen additive inclusion level on dry matter loss of the napier silage

Parameter	Nitrogen additive inclusion (%)			SEM
	0	15	30	
kgDM ensiled	15.4^{c}	16.5^{b}	17.2^{a}	0.14
kgDM of silage recovered	11.8^{b}	13.4^{b}	14.3^{a}	0.15
% DM loss	23.3^{a}	18.9^{b}	16.7^{c}	0.46

$^{a-c}$ Means within the same row are significantly ($P < 0.05$) different; SEM = standard error of the means.

4.2.5.3 Effect of the combination of molasses and nitrogen additive

Results of the effect of molasses inclusion level and nitrogen additive inclusion level on dry matter losses of the napier silage are shown in Table 36. The %DM loss was highest in $0NA^{+}$ silage followed by $0NA^{-}$ and $15NA^{-}$ silages whereas $30NA^{+}$ and $30NA^{-}$ silages were the lowest.

Table 36: Mean effect of molasses and nitrogen additive inclusion levels on dry matter loss of the napier silage

Parameter	Treatment						SEM
	0NA+	15NA+	30NA+	0NA-	15NA-	30NA-	
kgDM ensiled	15.8^a	16.6^a	17.2^a	15.1^a	16.5^a	17.3^a	0.19
kgDM of silage recovered	11.5^a	13.5^a	14.4^a	12.2^a	13.4^a	14.4^a	0.22
% DM loss	27.0^a	18.7^{bc}	16.6^c	19.5^b	19.1^b	16.8^c	0.65

NA = nitrogen additive; 0,15,30 preceding NA represent proportion (%) inclusion of nitrogen additive; $^{+,-}$ indicate with and without molasses, respectively; $^{a-c}$ means within the same row followed by different superscript letters are significantly ($P < 0.05$) different; SEM = standard error of the means.

4.2.6 Intake rate of the napier silage

4.2.6.1 Effect of molasses

Results of the effect of molasses inclusion on intake rate of the napier silage are shown in Table 37. The intake rate of fresh silage and silage dry matter were significantly higher in molasses added silage than in unmolassesed silage.

Table 37: Mean effect of molasses inclusion level on intake rate of the napier silage

Silage eaten	Molasses inclusion		SEM
	With	Without	
Fresh silage ($gmin.^{-1}$)	180.4[a]	164.6[b]	4.67
gDM intake/min.	28.5[a]	25.8[b]	0.75

[a-b] Means within the same row are significantly (P < 0.05) different; SEM = standard error of the means.

4.2.6.2 Effect of nitrogen additive

Results of the effect of nitrogen additive inclusion level on intake rate of the napier silage are shown in Table 38. The silage dry matter intake was higher in silage with 30 and 15 percent NA than 0% NA silage. The intake rate of fresh silage was not affected by NA inclusion level.

Table 38: Mean effect of nitrogen additive inclusion level on intake rate of the napier silage

Silage eaten	NA inclusion (%)			SEM
	0	15	30	
Fresh silage ($gmin.^{-1}$)	169.4[a]	172.7[a]	175.4[a]	5.72
gDM intake/min.	24.9[b]	27.3[a]	29.2[a]	0.92

[a-b] Means within the same row are significantly (P < 0.05) different; SEM = standard error of the means.

4.2.6.3 Effect of the combination of molasses and nitrogen additive

Results of the effect of molasses inclusion and nitrogen additive inclusion level on intake rate of napier silage are shown in Table 39. Silage with $15NA^+$, $30NA^+$, $30NA^-$ and $0NA^+$ had higher intake rate relative to silage with $0NA^-$ and $15NA^-$.

Table 39: Mean effect of molasses and nitrogen additive inclusion levels on intake rate of the napier silage

Treatment	$0NA^+$	$15NA^+$	$30NA^+$	$0NA^-$	$15NA^-$	$30NA^-$	SEM
gDM intake/min.	26.2^{bc}	30.4^{a}	28.8^{ab}	23.6^{c}	24.1^{c}	29.6^{ab}	1.29

NA = nitrogen additive; 0,15,30 preceding NA represent proportion (%) inclusion of nitrogen additive; $^{+,-}$ indicate with and without molasses, respectively; $^{a-c}$ means within the same column followed by different superscript letters are significantly ($P < 0.05$) different; SEM = standard error of the means.

CHAPTER FIVE

DISCUSSION

5.1 Experiment I: Laboratory silos

5.1.1 Chemical composition, dry matter, *in vitro* dry matter and organic matter digestibility of the forages used in silage making

The higher CP, WSC and DM contents in NA added treatments relative to control treatments could be attributed to addition of CP, WSC and DM from NA additives. This was revealed from the higher CP, WSC and DM contents in nitrogen additives relative to the grass alone. Lower ash, NDF, ADF and higher ADL contents in NA added treatments relative to controls could be attributed to lower contents of ash, NDF, ADF and higher ADL found in nitrogen additives relative to grass.

5.1.2 Sensoric evaluation of the napier silage

The observed higher scores of smell in molasses added than unmolassesed napier silage could be attributed to the good aroma added by molasses. These results were in consistence with findings reported by Sunarso *et al.* (1995) who ensiled napier grass without and with 5% molasses. Insignificant differences in appearance and texture between molasses added and unmolassesed silages in this study indicated little effect of molasses on appearance and texture.

The results indicated insignificant differences in sensoric qualities between nitrogen additive type as well as nitrogen additive inclusion level. Probably fermentation was satisfactory in all NA types and inclusion levels.

The sensoric qualities in unmolassesed silage at all NA inclusion levels were rather poor as compared to other combinations. Possibly lack of molasses in these combinations lead to unfavourable fermentation which lowered the sensoric qualities of the silages.

5.1.2 Dry matter, chemical composition, *in vitro* dry matter and organic matter digestibility of the napier silage

5.1.3.1 Effect of molasses

The DM content of molasses added silage was slightly higher than that of unmolassed silage (18.0 vs 15.9 %). The trend was similar for CP and WSC (112.0 and 55.3 vs. 105.7 and 42.7 gkg^{-1}DM, respectively). These findings revealed improvement in DM, CP and WSC contents by 2.1%, 6.3 and 12.6 gkg^{-1}DM, respectively from addition of molasses. Similar observations were made by Maeda *et al.* (1997) who ensiled napier grass without and with 5% molasses.

Addition of molasses also produced silages with lower contents of NDF, ADF and ADL. The present findings are also supported by Otieno *et al.* (1990) and Maeda *et al.* (1997) who reported lower NDF, ADF and ADL contents for molasses treated napier silage than untreated silage. Improved digestibility of napier silage due to addition of molasses was consistence with observations made by Maeda *et al.* (1997).

5.1.3.2 Effect of nitrogen additives

Results indicated higher DM content in Leucaena added silage than Gliricidia and Sesbania added silages. The similar trend was observed in these additives before ensiling. The CP was higher in Leucaena and Sesbania added silages than in Gliricidia silage. This trend was inconsistent with the additives before ensiling. Possibly proteolysis was higher in Gliricidia added silage than in Leucaena and Sesbania added silages. The WSC was higher in Sesbania added silage than in Gliricidia and Leucaena added silages. Gliricidia and Leucaena added silage could have suffered more WSC loss in effluent than Sesbania added silage.

Lower NDF and higher ADF contents were observed in Gliricidia and Sesbania added silages compared to Leucaena added silage. These could have influenced digestibility since Gliricidia and Sesbania added silages had higher IVDMD and IVOMD than Leucaena silage. Skerman and Riveros (1988) have documented the high fibre content of Leucaena to reduce its digestibility. Reduced digestibilities of Leucaena added silage could also be attributed to the presence of digestibility depressants for instance, tannins, which were not measured in this study. Barry and Forss (1983) have documented reduction of organic matter digestibility by tannin content in browse species. In particular, presence of tannins up to 10% in *L.leucocephala* has been documented by Ahn *et al.* (1989) and Norton (1994). The ADL content was higher in Gliricidia and Leucaena added silages than in Sesbania added silage. The same trend was shown in these additives before ensiling.

The DM, CP and WSC contents were highest in 30% NA followed by 15% NA and were lowest in 0% NA. These results indicated improvement in DM, CP and WSC by increasing NA inclusion level. The DM was improved by 2.3 and 4.5 percent, CP by 38.8 and 57.7 $gkg^{-1}DM$, WSC by 16.7 and 29.8 $gkg^{-1}DM$ by including 15 and 30 percent NA, respectively. The 0 and 15 %NA silages have higher in ash content than 30% NA silage. This could be attributed to the lower ash content in nitrogen additives observed before ensiling.

The decreased NDF and ADF contents with increasing NA inclusion level were observed in this study. The opposite trend was observed in ADL content. The lowered content of NDF by 102.8 and 154.0 $gkg^{-1}DM$, ADF by 62.5 and 99.7 $gkg^{-1}DM$, and increased ADL content by 13.6 and 18.3 $gkg^{-1}DM$ due to the addition of 15 and 30 percent NA inclusion, respectively, were also observed in this study. Increased IVDMD by 6.6 and 11.5 %, IVOMD by 3.5 and 7.4 % caused by addition of 15 and 30 percent NA, respectively, have also been observed. Improvement in digestibility might be associated with the lowered NDF and ADF contents observed.

5.1.3.3 Effect of the combination of molasses and nitrogen additives

The combination of molasses and nitrogen additives gave silages with higher WSC, IVDMD, IVOMD and lower NDF and ADF contents as compared to other combinations. The observed improved digestibility might be attributed to easier breakdown of these silages by rumen microbes as they contain lower NDF and ADF. Higher WSC could also be attributed to improved digestibility by supplying enough fermentable sugars for rumen microbes to work effectively.

Improved silage from the combination of different NA types and inclusion levels compared to the silage without NA had also observed in this study. Inclusion level of 30% had higher CP and WSC than 15% inclusion at all NA types. This could be attributed to added contents of CP and WSC from large proportions (30% vs 15%) of NA types included. It could also be attributed to fermentation pattern as these combinations may have suffered little degradation hence little losses than in other combinations.

Higher NDF content was observed in 15L, 30L and 0NA than in other combinations. Possibly this lowered the digestibility as it was observed in these combinations compared to others. Both Gliricidia and Leucaena at 15 and 30 percent have higher ADL than 0NA, 15S and 30S. The differences observed could be attributed to the different ADL content of the NA types. Sesbania had lower ADL than Gliricidia and Leucaena before ensiling. Combinations of 30L, 30G and 30S have better DM than others. This improvement could be attributed to the DM added by the high proportion of NA included.

Results showed improved WSC in all molassesed treatments at all NA types and inclusion levels. The values observed in this study were a bit higher compared to those reported by Tjandraatmadja *et al.* (1993) who ensiled Pangola and Setaria grass combined with Leucaena, Gliricidia and molasses. The differences might be caused by difference in fermentation pattern of different grasses (Pangola and Setaria) used.

Molassesed Gliricidia and Sesbania at both 15 and 30 percent inclusion level have better digestibility than other combinations. Low digestibilities observed in molassesed Leucaena combinations could be attributed to presence of digestibility depressants such as tannin, which was not measured in this study. Low digestibilities observed in unmolassesed combinations could be attributed to lack of enough fermentable sugars for rumen microbes to work effectively.

5.1.4 Fermentation products of the napier silage

5.1.4.1 Effect of molasses

Molassesed silage had better fermentation quality (lower pH, NH_3N, acetate, higher lactate and low butyrate) than unmolassesed silage. These findings were consistent with the findings reported by various researchers working on ensilage of tropical forages (Otieno *et al.*, 1990; Tjandraatmadja *et al.*, 1993; Sarwatt, 1995; Sunarso *et al.*, 1995; Maeda *et al.*, 1997). However, as seen from Table 20, silages without molasses were also well preserved despite of high pH.

5.1.4.2 Effect of nitrogen additives

Higher NH_3N in Sesbania added silage than Gliricidia and Leucaena added silages had been observed although all NA types have NH_3N content which was within the range (< 11%) of good quality silage (Catchpoole and Henzel, 1971). There were insignificant differences in other fermentation products between NA types though all were within the accepted ranges of good quality silage.

Addition of NA reduced NH_3N. These findings were in agreement with those reported by Tjandraatmadja *et al.* (1993). The results from this study showed very little increase in lactate content by addition of NA (1.5 and 0.2 $gkg^{-1}DM$ for 15 and 30 percent NA, respectively). This could probably be attributed to low ability of NA to provide substrate for lactate formation.

5.1.4.3 Effect of the combination of molasses and nitrogen additives

Results showed improved silage fermentation in NA plus molasses combinations as indicated by lower pH, NH_3N, butyrate and higher lactate relative to silages with NA but without molasses. Similar trend was reported by Tjandraatmadja *et al.* (1993).

There were insignificant differences between combinations of molasses, NA type and inclusion level in terms of pH, NH_3N, lactate and butyrate. Variation in acetate content between combinations was very narrow however, $30L^+$, $15G^-$, $30G^-$ and $15L^-$ revealed higher acetate content relative to other combinations. Probably fermentation pattern in these combinations favoured more acetate production.

5.2 Experiment II: Earth pit silos

5.2.1 Chemical composition, dry matter, *in vitro* dry matter and organic matter digestibility of forages and additives used in silage making

The higher CP and WSC observed to increase with increasing NA and molasses inclusion could be attributed to the added CP and WSC from NA and molasses. Decreased ash content with increasing NA inclusion might be attributed to low ash content found in NA. Higher NDF and ADF contents observed in $0NA^+$ and $0NA^-$

relative to other treatments might be attributed to higher NDF and ADF contents of the grass relative to NA. Higher DM observed in NA treatments relative to controls might be attributed to added DM from NA.

5.2.2 Sensoric evaluation of the napier silage

Molassesed napier silage showed slightly higher scores for smell than unmolassesed silage. This could be attributed to the pleasant estry aroma added by molasses. Insignificant differences in appearance and texture indicated little influence of molasses in these parameters. Nitrogen additive inclusion level had very little effect on sensoric parameters of the napier silage as indicated by insignificant ($P>0.05$) differences in these parameters between NA inclusion levels.

5.2.2 Dry matter, chemical composition, *in vitro* dry matter and organic matter digestibility of the napier silage

5.2.3.1 Effect of molasses

Results showed insignificant difference in DM between molasses added silage and unmolassesed silage. Otieno et al. (1990) and Maeda et al. (1997) reported the opposite trend. This could be attributed to high DM losses in effluent caused by the diluted molasses added silage. The WSC reserved in molasses treated silage obtained in this work was higher by 5.2 $gkg^{-1}DM$ above that remained in unmolassesed silage. This might be due to additional soluble sugars from molasses. Insignificant differences in CP content between molassesed and unmolassesed silage were observed however, molassesed silage had 4.4 $gkg^{-1}DM$ CP above that of unmolassesed silage.

Slightly lower NDF and ADF in molassesed compared to unmolassesed silage was observed in this study. This could be attributed to increased acidity which stimulated further hydrolysis of linked sugar molecules in the cell wall causing further breakdown of hemicellulose. Breakdown of up to 50% of hemicellulose during silage fermentation has been documented by McDonald *et al.* (1991).

The IVDMD and IVOMD of the napier silage was improved by addition of molasses. This could be attributed to the provision of useful energy substrate for ruminal microbes by the added molasses. The importance of nutrient additives such molasses as useful energy substrate for ruminal microbes have been documented by McDonald *et al.* (1973).

5.2.3.2 Effect of nitrogen additive

Nitrogen additive inclusion levels had insignificant effect on silage DM although 30%NA silage had 2.2% and 15%NA silage had 0.7% DM above that of 0%NA silage. Inclusion of 15 and 30 percent NA increased the CP content of the silage by 39.3 and 60.0 gkg^{-1}DM, respectively. Higher WSC was observed in 0 and 15 percent NA silages compared to 30%NA silage. Possibly WSC was more used by microbes in 30%NA silage compared to others. The findings indicated insignificant differences in ash content between NA inclusion levels however, it tended to decrease with increasing NA inclusion level. Similar trend was observed before ensiling.

Inclusion of 15 and 30 percent NA reduced NDF by 29.8 and 65.2 gkg^{-1}DM, ADF by

81.6 and 112.5 gkg^{-1}DM, respectively. Inclusion of 15 and 30 percent NA was observed to improve IVDMD by 7.3 and 11.9 percent and IVOMD by 4.7 and 10.1 percent, respectively. Improved digestibilities in NA added silages could be attributed to increased CP and reduced NDF and ADF by NA inclusion. The 15 and 30 percent NA silages were however, observed to have higher ADL than 0% NA silages.

5.2.3.3 Effect of the combination of molasses and nitrogen additive

The 30NA^{+} and 30NA^{-} combinations showed lower WSC than others. Possibly WSC was much more used in these treatments or they suffered more effluent loss which would have taken away most of WSC.

The 0NA^{+} and 0NA^{-} were observed to have lower ADL as compared to other treatments. The same trend was observed for these treatments before ensiling. The IVDMD was observed as the highest in 15NA^{+} and 30NA^{+} followed by 30NA^{-} whereas rest of the treatments was the least. These findings indicated better IVDMD in NA combined with molasses than NA without molasses.

5.2.4 Fermentation products of the napier silage

5.2.4.1 Effect of molasses

Addition of molasses was observed to reduce pH, NH_3N and increase lactate content. This was possibly caused by additional WSC content in the herbage from molasses which in turn favoured lactate fermentation and stabilized the acidity (pH 4.23 vs 4.78) in molassesed silage. Despite of high pH, unmolassesed silages were also well preserved as indicated by insignificant differences in butyrate and NH_3N observed.

5.2.4.2 Effect of nitrogen additive

Results revealed higher pH in 30% NA silage as compared to 0 and 15 percent (4.88 vs 4.09 and 4.56, respectively) silages. This could be caused by the differences in buffering capacity, which limit the easiness with which a pH could be lowered during fermentation. The NH_3N was not significantly different between NA inclusion levels however, all NA inclusion levels have NH_3N which were within the standards of well preserved silage (< 8%) as described by Catchpoole and Henzel (1971).

5.2.5 Dry matter losses of the napier silage

5.2.5.1 Effect of molasses

Results of the effect of molasses on dry matter losses revealed higher %DM loss in molassesed silage than unmolassesed silage. Possibly effluent was higher in molassesed than in unmolassesed silage which could have caused greater DM loss. Percent DM losses from both silages were however, within the normal range of 10 to 20 percent reported by Crowder and Chheda (1982) and Lampila *et al.* (1988) from the well preserved unwilted tropical grasses.

5.2.5.2 Effect of nitrogen additive

Results of the effect of NA inclusion level on dry matter losses showed decreasing percent DM loss with increasing NA inclusion level. This might be caused by increased amount of DM ensiled and DM of silage recovered which was observed to increase with increased NA inclusion level.

5.2.5.3 Effect of the combination of molasses and nitrogen additive

The percent DM loss was highest in $0NA^+$ followed by $0NA^-$ and $15NA^-$ silages and lowest in $30NA^-$ and $30NA^+$ silages. Combinations with higher DM losses could possibly be due to high effluent production.

5.2.6 Intake rate of the napier silage

5.2.6.1 Effect of molasses

Intake rate of both fresh silage and silage dry matter was higher in molassesed than unmolassesed silage. This could be attributed to the nice aroma of molasses treated silage which might have arouse appetite of the heifers thereby increase it's acceptance to the animals. These findings were in agreement with those reported by Sarwatt (1995) and Maeda (1996).

5.2.6.2 Effect of nitrogen additive

The silage DM intake rate was higher in 30 and 15 percent NA than 0% NA silage. Since the intake rate of fresh silage was insignificantly different between NA inclusion levels, the differences observed in silage DM intake rate could be attributed to the differences in DM content.

5.2.6.3 Effect of the combination of molasses and nitrogen additive

Results revealed improved DM intake rate in $15NA^+$, $30NA^+$ and $30NA^-$ compared to other combinations. This could be attributed to the improved aroma and DM content from both molasses and NA added.

CHAPTER SIX

CONCLUSIONS AND RECOMMENDATIONS

This study aimed at investigating the possibility of using *G.sepium*, *L.leucocephala* and *S.sesban* leaves as nitrogen additive (NA) in presence or absence of molasses (as WSC additive) to boost fermentation and nutritive value of napier silage, hence estimating the optimum inclusion level of these leguminous tree leaves required in ensiling napier grass.

Results obtained in this study indicated that napier grass can be conserved well when leguminous tree leaves are included at 15 and 30% (fresh weight basis) as NA. The results showed that leguminous tree leaves could successfully be used to improve quality of napier silage through increasing DM, CP, IVDMD, IVOMD, DM intake rate and reducing NDF, ADF, NH_3N and DM losses. Quality improvement in NA included silages increased with increasing NA inclusion level.

Results of the combination of nitrogen additives and molasses indicated improvement in WSC, NDF, IVDMD, IVOMD, pH, DM losses and DM intake rate. The DM, CP, ash, ADF, acetate and butyrate were however, insignificantly affected. The more improved quality in terms of WSC, NDF, IVDMD, DM losses and DM intake rate were observed in $30NA^+$ and $15NA^+$ followed by $30NA^-$ than in rest of the combinations. This suggests that inclusion of 3% molasses and reduction of NA from 30% to 15% results into added quality in WSC, IVDMD, DM intake rate and reduced NDF and DM losses above that could be obtained from 30% NA inclusion without molasses.

From this study, the recommended optimum inclusion level of nitrogen additives in napier silage should be 30% because this level produced silage of good quality and showed the lowest DM losses and highest intake rate. Fifteen percent NA inclusion might be used in combination with molasses if and only if the costs of including additional 15% NA outweigh the costs of including 3% molasses however, added advantage of improved silage DM and CP will be missed.

Further investigations are recommended to establish the optimum level of inclusion of these nitrogen additives in other grass species like Guatemala grass (*Tripsacum laxum*) and Guinea grass (*Panicum maximum*). These grasses are among the most abundant forage species utilized by the dairy farmers in Tanzania.

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APPENDICES

Appendix 1: The chart for sensoric evaluation of napier silage recovered from the silos in experiment I

Date................. Assessor No........... Silo type............

Treatment/Replication		Condition scores										
		Appearance				Smell				Texture		
		1	2	3	4	1	2	3	4	1	2	3
ONA^{+}	R1											
	R2											
15G^{+}	R1											
	R2											
30G^{+}	R1											
	R2											
15L^{+}	R1											
	R2											
30L^{+}	R1											
	R2											
15S^{+}	R1											
	R2											
30S^{+}	R1											
	R2											
ONA^{-}	R1											
	R2											
15G^{-}	R1											
	R2											
30G	R1											
	R2											
15L	R1											
	R2											
30L	R1											
	R2											
15S	R1											
	R2											
30S^{-}	R1											
	R2											

Score grades:
Appearance:
1=Poor - spoiled silage, dark brown in colour with mould growth
2=Moderate - greenish with some mould growth
3=Good - Yellowish green to brown colour
4= Very good - well pickled silage, yellowish green to light brown colour

Smell:
1=Poor - foul smell associated with putrefaction
2=Moderate - moderate pungent smell of ammonia
3=Good - moderate pleasant aroma
4=Very good - pleasant estery aroma (typical silage smell)

Texture:
1=Poor - slimy and watery
2=satisfactory - less slimy and wet
3=Good - non-slippery and slightly wet

NB: Tick the appropriate score.

Appendix 2: The chart for sensoric evaluation of napier silage recovered from the silos in experiment II

Date................. Assessor No........... Silo type...........

Treatment/Replication		Condition scores										
		Appearance				Smell				Texture		
		1	2	3	4	1	2	3	4	1	2	3
0NA^{+}	R1											
	R2											
15NA^{+}	R1											
	R2											
30NA^{+}	R1											
	R2											
0NA^{-}	R1											
	R2											
15NA^{-}	R1											
	R2											
30NA^{-}	R1											
	R2											

Score grades:
Appearance:
1= Poor - dark brown in colour with mould growth
2= Moderate - greenish with some mould growth
3= Good - Yellowish green to brown colour
4= Very good - well pickled silage, yellowish green to light brown colour

Smell:
1=Poor - foul smell associated with putrefaction
2=Moderate - moderate pungent smell of ammonia
3=Good - moderate pleasant aroma
4=Very good - pleasant estery aroma.

Texture:
1=Poor - slimy and watery
2=Satisfactory - less slimy and wet
3=Good - non-slippery and slightly wet

NB: Tick the appropriate score.

Appendix 3: **ANOVA for effect of molasses, nitrogen additive type, nitrogen additive inclusion level and combination of molasses and nitrogen additives on sensoric qualities of napier silage in experiment I**

Dependent Variable: Appearance

Source	DF	Type I SS	Mean Square	F Value	Pr > F
MOL	1	1.67129630	1.67129630	3.67	0.0570
NATP	2	0.11111111	0.05555556	0.12	0.8853
NAIL	2	1.69444444	0.84722222	1.86	0.1587
ASSR	5	5.76388889	1.15277778	2.53	0.0304
MOL*NATP	2	0.48148148	0.24074074	0.53	0.5906
MOL*NAIL	2	4.50925926	2.25462963	4.95	0.0080
NATP*NAIL	4	1.77777778	0.44444444	0.97	0.4224
MOL*NATP*NAIL	4	2.96296296	0.74074074	1.62	0.1696

Dependent Variable: Smell

Source	DF	Type I SS	Mean Square	F Value	Pr > F
MOL	1	6.68518519	6.68518519	17.17	0.0001
NATP	2	0.19444444	0.09722222	0.25	0.7793
NAIL	2	1.19444444	0.59722222	1.53	0.2184
ASSR	5	4.33333333	0.86666667	2.23	0.0534
MOL*NATP	2	0.67592593	0.33796296	0.87	0.4215
MOL*NAIL	2	4.28703704	2.14351852	5.50	0.0047
NATP*NAIL	4	2.61111111	0.65277778	1.68	0.1571
MOL*NATP*INCR	4	2.68518519	0.67129630	1.72	0.1463

Dependent Variable: Texture

Source	DF	Type I SS	Mean Square	F Value	Pr > F
MOL	1	0.29629630	0.29629630	1.49	0.2241
NATP	2	0.11111111	0.05555556	0.28	0.7569
NAIL	2	0.00000000	0.00000000	0.00	1.0000
ASSR	5	4.22222222	0.84444444	4.24	0.0011
MOL*NATP	2	0.48148148	0.24074074	1.21	0.3009
MOL*NAIL	2	1.81481481	0.90740741	4.56	0.0117
NATP*NAIL	4	1.22222222	0.30555556	1.53	0.1939
MOL*NATP*NAIL	4	1.40740741	0.35185185	1.77	0.1372

Appendix 4: ANOVA for the effect of molasses, nitrogen additive type, nitrogen additive inclusion level and combination of molasses and nitrogen additives on DM, chemical composition, *in vitro* dry matter and organic matter digestibility of napier silage in experiment I

Dependent Variable: DM

Source	DF	Type I SS	Mean Square	F Value	Pr > F
MOL	1	36.6025000	36.6025000	111.01	0.0001
NATP	2	10.4872222	5.2436111	15.90	0.0001
NAIL	2	119.7205556	59.8602778	181.55	0.0001
MOL*NATP	2	0.0316667	0.0158333	0.05	0.9532
MOL*NAIL	2	1.3816667	0.6908333	2.10	0.1520
NATP*NAIL	4	7.8344444	1.9586111	5.94	0.0031
MOL*NATP*NAIL	4	1.3566667	0.3391667	1.03	0.4194

Dependent Variable: CP

Source	DF	Type I SS	Mean Square	F Value	Pr > F
MOL	1	349.06694	349.06694	8.14	0.0106
NATP	2	1631.48167	815.74083	19.02	0.0001
NAIL	2	20108.70167	10054.35083	234.38	0.0001
MOL*NATP	2	228.78389	114.39194	2.67	0.0968
MOL*NAIL	2	107.67722	53.83861	1.26	0.3089
NATP*NAIL	4	1097.60167	274.40042	6.40	0.0022
MOL*NATP*NAIL	4	176.44944	44.11236	1.03	0.4195

Dependent Variable: WSC

Source	DF	Type I SS	Mean Square	F Value	Pr > F
MOL	1	1428.840000	1428.840000	234.60	0.0001
NATP	2	471.348889	235.674444	38.70	0.0001
NAIL	2	5377.075556	2688.537778	441.43	0.0001
MOL*NATP	2	529.526667	264.763333	43.47	0.0001
MOL*NAIL	2	251.646667	125.823333	20.66	0.0001
NATP*NAIL	4	691.737778	172.934444	28.39	0.0001
MOL*NATP*NAIL	4	280.406667	70.101667	11.51	0.0001

Dependent Variable: ASH

Source	DF	Type I SS	Mean Square	F Value	Pr > F
MOL	1	10.2400000	10.2400000	0.49	0.4909
NATP	2	860.4616667	430.2308333	20.78	0.0001
NAIL	2	720.1216667	360.0608333	17.39	0.0001
MOL*NATP	2	67.8750000	33.9375000	1.64	0.2219
MOL*NAIL	2	110.3750000	55.1875000	2.66	0.0969
NATP*NAIL	4	462.9116667	115.7279167	5.59	0.0042
MOL*NATP*NAIL	4	44.8150000	11.2037500	0.54	0.7076

Dependent Variable: NDF

Source	DF	Type I SS	Mean Square	F Value	Pr > F
MOL	1	41650.0069	41650.0069	132.00	0.0001
NATP	2	13638.3872	6819.1936	21.61	0.0001
NAIL	2	146578.8572	73289.4286	232.27	0.0001
MOL*NATP	2	1702.7906	851.3953	2.70	0.0944
MOL*NAIL	2	2480.1739	1240.0869	3.93	0.0383
NATP*NAIL	4	10431.2494	2607.8124	8.26	0.0006
MOL*NATP*NAIL	4	1260.3061	315.0765	1.00	0.4339

Dependent Variable: ADF

Source	DF	Type I SS	Mean Square	F Value	Pr > F
MOL	1	25429.61778	25429.61778	143.05	0.0001
NATP	2	1002.50389	501.25194	2.82	0.0860
NAIL	2	60954.19056	30477.09528	171.44	0.0001
MOL*NATP	2	1768.36056	884.18028	4.97	0.0191
MOL*NAIL	2	865.59389	432.79694	2.43	0.1159
NATP*NAIL	4	814.38778	203.59694	1.15	0.3672
MOL*NATP*NAIL	4	1137.12778	284.28194	1.60	0.2177

Dependent Variable: ADL

Source	DF	Type I SS	Mean Square	F Value	Pr > F
MOL	1	385.467778	385.467778	23.39	0.0001
NATP	2	2106.965000	1053.482500	63.91	0.0001
NAIL	2	2184.326667	1092.163333	66.26	0.0001
MOL*NATP	2	78.590556	39.295278	2.38	0.1206
MOL*NAIL	2	68.408889	34.204444	2.08	0.1545
NATP*NAIL	4	1094.983333	273.745833	16.61	0.0001
MOL*NATP*NAIL	4	114.237778	28.559444	1.73	0.1867

Dependent Variable: IVDMD

Source	DF	Type I SS	Mean Square	F Value	Pr > F
MOL	1	966.1736111	966.1736111	405.34	0.0001
NATP	2	258.4516667	129.2258333	54.21	0.0001
NAIL	2	795.6650000	397.8325000	166.90	0.0001
MOL*NATP	2	54.3038889	27.1519444	11.39	0.0006
MOL*NAIL	2	116.1072222	58.0536111	24.36	0.0001
NATP*NAIL	4	135.8733333	33.9683333	14.25	0.0001
MOL*NATP*NAIL	4	42.0677778	10.5169444	4.41	0.0116

Dependent Variable: IVOMD

Source	DF	Type I SS	Mean Square	F Value	Pr > F
MOL	1	555.3877778	555.3877778	281.76	0.0001
NATP	2	219.4616667	109.7308333	55.67	0.0001
NAIL	2	326.6250000	163.3125000	82.85	0.0001
MOL*NATP	2	60.5772222	30.2886111	15.37	0.0001
MOL*NAIL	2	55.5505556	27.7752778	14.09	0.0002
NATP*NAIL	4	126.4133333	31.6033333	16.03	0.0001
MOL*NATP*NAIL	4	85.7944444	21.4486111	10.88	0.0001

Appendix 5: **ANOVA for the effect of molasses, nitrogen additive type, nitrogen additive inclusion level and combination of molasses and nitrogen additives on fermentation products of napier silage in experiment I**

Dependent Variable: Ph

Source	DF	Type I SS	Mean Square	F Value	Pr > F
MOL	1	6.71673611	6.71673611	224.58	0.0001
NATP	2	0.13211667	0.06605833	2.21	0.1387
NAIL	2	0.11765000	0.05882500	1.97	0.1688
MOL*NATP	2	0.18953889	0.09476944	3.17	0.0662
MOL*NAIL	2	0.40317222	0.20158611	6.74	0.0065
NATP*NAIL	4	0.12788333	0.03197083	1.07	0.4006
MOL*NATP*NAIL	4	0.10702778	0.02675694	0.89	0.4874

Dependent Variable: NH_3N

Source	DF	Type I SS	Mean Square	F Value	Pr > F
MOL	1	21.62250000	21.62250000	50.25	0.0001
NATP	2	7.86888889	3.93444444	9.14	0.0018
NAIL	2	7.30388889	3.65194444	8.49	0.0025
MOL*NATP	2	1.58000000	0.79000000	1.84	0.1881
MOL*NAIL	2	3.27166667	1.63583333	3.80	0.0420
NATP*NAIL	4	4.09777778	1.02444444	2.38	0.0900
MOL*NATP*NAIL	4	1.87333333	0.46833333	1.09	0.3918

Dependent Variable: Lactate

Source	DF	Type I SS	Mean Square	F Value	Pr > F
MOL	1	15.21000000	15.21000000	14.86	0.0012
NATP	2	7.26055556	3.63027778	3.55	0.0503
NAIL	2	16.52388889	8.26194444	8.07	0.0031
MOL*NATP	2	3.65166667	1.82583333	1.78	0.1964
MOL*NAIL	2	21.21500000	10.60750000	10.37	0.0010
NATP*NAIL	4	7.17444444	1.79361111	1.75	0.1825
MOL*NATP*NAIL	4	5.07333333	1.26833333	1.24	0.3296

Dependent Variable: Acetate

Source	DF	Type I SS	Mean Square	F Value	Pr > F
MOL	1	1.00000000	1.00000000	6.16	0.0231
NATP	2	0.24888889	0.12444444	0.77	0.4789
NAIL	2	0.44055556	0.22027778	1.36	0.2823
MOL*NATP	2	0.62000000	0.31000000	1.91	0.1768
MOL*NAIL	2	1.37166667	0.68583333	4.23	0.0312
NATP*NAIL	4	0.15444444	0.03861111	0.24	0.9132
MOL*NATP*NAIL	4	2.23333333	0.55833333	3.44	0.0295

Dependent Variable: Butyrate

Source	DF	Type I SS	Mean Square	F Value	Pr > F
MOL	1	0.10027778	0.10027778	1.11	0.3059
NATP	2	0.00500000	0.00250000	0.03	0.9727
NAIL	2	0.26166667	0.13083333	1.45	0.2609
MOL*NATP	2	0.09388889	0.04694444	0.52	0.6032
MOL*NAIL	2	0.08388889	0.04194444	0.46	0.6357
NATP*NAIL	4	0.24333333	0.06083333	0.67	0.6187
MOL*NATP*NAIL	4	0.65444444	0.16361111	1.81	0.1704

Appendix 6: ANOVA for effect of molasses, nitrogen additive inclusion level and combination of molasses and nitrogen additive on sensoric qualities of napier silage in experiment II

Dependent Variable: Appearance

Source	DF	Type I SS	Mean Square	F Value	Pr > F
MOL	1	0.12500000	0.12500000	0.42	0.5190
NAIL	2	0.08333333	0.04166667	0.14	0.8694
ASSR	5	1.95833333	0.39166667	1.32	0.2684
MOL*NAIL	2	0.58333333	0.29166667	0.98	0.3805

Dependent Variable: Smell

Source	DF	Type I SS	Mean Square	F Value	Pr > F
MOL	1	1.12500000	1.12500000	4.16	0.0458
NAIL	2	1.36111111	0.68055556	2.51	0.0893
ASSR	5	1.23611111	0.24722222	0.91	0.4786
MOL*NAIL	2	0.75000000	0.37500000	1.39	0.2580

Dependent Variable: Texture

Source	DF	Type I SS	Mean Square	F Value	Pr > F
MOL	1	0.68055556	0.68055556	3.05	0.0859
NAIL	2	0.00000000	0.00000000	0.00	1.0000
ASSR	5	2.45833333	0.49166667	2.20	0.0656
MOL*NAIL	2	0.11111111	0.05555556	0.25	0.7806

Appendix 7: **ANOVA for the effect of molasses, nitrogen additive inclusion level and combination of molasses and nitrogen additive on DM, chemical composition, *in vitro* dry matter and organic matter digestibility of napier silage in experiment II**

Dependent Variable: DM

Source	DF	Type I SS	Mean Square	F Value	Pr > F
MOL	1	0.00270000	0.00270000	0.00	0.9638
NAIL	2	9.75606667	4.87803333	4.04	0.0772
MOL*NAIL	2	1.42940000	0.71470000	0.59	0.5823

Dependent Variable: CP

Source	DF	Type I SS	Mean Square	F Value	Pr > F
MOL	1	56.333333	56.333333	0.65	0.4508
NAIL	2	7443.886667	3721.943333	42.95	0.0003
MOL*NAIL	2	28.186667	14.093333	0.16	0.8535

Dependent Variable: WSC

Source	DF	Type I SS	Mean Square	F Value	Pr > F
MOL	1	63.9408333	63.9408333	14.67	0.0087
NAIL	2	919.3816667	459.6908333	105.49	0.0001
MOL*NAIL	2	336.7616667	168.3808333	38.64	0.0004

Dependent Variable: ASH

Source	DF	Type I SS	Mean Square	F Value	Pr > F
MOL	1	37.8075000	37.8075000	0.28	0.6159
NAIL	2	448.9016667	224.4508333	1.66	0.2667
MOL*NAIL	2	986.8950000	493.4475000	3.65	0.0918

Dependent Variable: NDF

Source	DF	Type I SS	Mean Square	F Value	Pr > F
MOL	1	439.230000	439.230000	1.19	0.3171
NAIL	2	8543.121667	4271.560833	11.58	0.0087
MOL*NAIL	2	2032.145000	1016.072500	2.75	0.1418

Dependent Variable: ADF

Source	DF	Type I SS	Mean Square	F Value	Pr > F
MOL	1	3049.64083	3049.64083	7.40	0.0347
NAIL	2	26990.73167	13495.36583	32.73	0.0006
MOL*NAIL	2	461.13167	230.56583	0.56	0.5988

Dependent Variable: ADL

Source	DF	Type I SS	Mean Square	F Value	Pr > F
MOL	1	210.84083	210.84083	21.55	0.0035
NAIL	2	41666.98667	20833.49333	2129.67	0.0001
MOL*NAIL	2	285.54667	142.77333	14.59	0.0050

Dependent Variable: IVDMD

Source	DF	Type I SS	Mean Square	F Value	Pr > F
MOL	1	122.8800000	122.8800000	49.32	0.0004
NAIL	2	286.9816667	143.4908333	57.59	0.0001
MOL*NAIL	2	37.7150000	18.8575000	7.57	0.0229

Dependent Variable: IVOMD

Source	DF	Type I SS	Mean Square	F Value	Pr > F
MOL	1	168.7500000	168.7500000	56.03	0.0003
NAIL	2	202.4316667	101.2158333	33.61	0.0006
MOL*NAIL	2	19.0050000	9.5025000	3.16	0.1158

Appendix 8: **ANOVA for the effect of molasses, nitrogen additive inclusion level and combination of molasses and nitrogen additive on fermentation products of napier silage in experiment II**

Dependent Variable: pH

Source	DF	Type I SS	Mean Square	F Value	Pr > F
MOL	1	0.91300833	0.91300833	9.66	0.0209
NAIL	2	1.24745000	0.62372500	6.60	0.0306
MOL*NAIL	2	0.20081667	0.10040833	1.06	0.4029

Dependent Variable: NH_3N

Source	DF	Type I SS	Mean Square	F Value	Pr > F
MOL	1	11.60333333	11.60333333	14.56	0.0088
NAIL	2	1.22000000	0.61000000	0.77	0.5056
MOL*NAIL	2	1.88666667	0.94333333	1.18	0.3686

Dependent Variable: Lactate

Source	DF	Type I SS	Mean Square	F Value	Pr > F
MOL	1	31.68750000	31.68750000	34.66	0.0011
NAIL	2	9.65166667	4.82583333	5.28	0.0476
MOL*NAIL	2	7.66500000	3.83250000	4.19	0.0726

Dependent Variable: Acetate

Source	DF	Type I SS	Mean Square	F Value	Pr > F
MOL	1	0.05333333	0.05333333	5.33	0.0603
NAIL	2	0.44666667	0.22333333	22.33	0.0017
MOL*NAIL	2	0.04666667	0.02333333	2.33	0.1780

Dependent Variable: Butyrate

Source	DF	Type I SS	Mean Square	F Value	Pr > F
MOL	1	0.27000000	0.27000000	0.84	0.3961
NAIL	2	1.75500000	0.87750000	2.71	0.1447
MOL*NAIL	2	0.03500000	0.01750000	0.05	0.9478

Appendix 9: **ANOVA for the effect of molasses, nitrogen additive inclusion level and combination of molasses and nitrogen additive on dry matter losses of napier silage in experiment II**

Dependent Variable: kgDM ensiled

Source	DF	Type I SS	Mean Square	F Value	Pr > F
MOL	1	0.14083333	0.14083333	1.86	0.2219
NAIL	2	6.45166667	3.22583333	42.54	0.0003
MOL*NAIL	2	0.30166667	0.15083333	1.99	0.2174

Dependent Variable: kgDM silage recovered

Source	DF	Type I SS	Mean Square	F Value	Pr > F
MOL	1	0.10083333	0.10083333	1.05	0.3446
NAIL	2	12.82666667	6.41333333	66.92	0.0001
MOL*NAIL	2	0.34666667	0.17333333	1.81	0.2428

Dependent Variable: %DM loss

Source	DF	Type I SS	Mean Square	F Value	Pr > F
MOL	1	15.18750000	15.18750000	18.03	0.0054
NAIL	2	89.03166667	44.51583333	52.84	0.0002
MOL*NAIL	2	39.81500000	19.90750000	23.63	0.0014

Appendix 10: ANOVA for the effect of molasses, nitrogen additive inclusion level and combination of molasses and nitrogen additive on intake rate of napier silage in experiment II

Dependent Variable: Intake rate of fresh silage

Source	DF	Type I SS	Mean Square	F Value	Pr > F
MOL	1	4512.50000	4512.50000	5.74	0.0195
NAIL	2	439.58333	219.79167	0.28	0.7570
BWT	1	42889.72853	42889.72853	54.55	0.0001
MOL*NAIL	2	1152.08333	576.04167	0.73	0.4846

Dependent Variable: Intake rate of silage DM

Source	DF	Type I SS	Mean Square	F Value	Pr > F
MOL	1	127.733472	127.733472	6.35	0.0142
NAIL	2	222.860278	111.430139	5.54	0.0060
BWT	1	1074.671053	1074.671053	53.46	0.0001
MOL*NAIL	2	154.350278	77.175139	3.84	0.0266