

Effect of ensiling *Acacia nilotica* and *Vachellia karroo* pods and leaves with molasses and urea on the chemical composition, secondary compounds and *in vitro* degradability of silage

By

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DECLARATION

I Nosipho Morin Twalo, hereby declare that this thesis is submitted in partial fulfilment of the requirements of the degree of Masters of Science in Agriculture (Animal Science) at the University of Fort Hare. This dissertation is my own work and has not been submitted previously for any degree at this or any other university. It is original in design and in execution, and all reference material contained in this dissertation has been duly acknowledged.

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ABSTRACT

Effect of ensiling using molasses and urea inclusion level on chemical composition, secondary compounds and in vitro degradability of *Acacia nilotica* and *Vachellia karroo* (Pods and Leaves)

By

Twalo Nosipho

Summary

The study aims to evaluate chemical composition, in vitro degradability, secondary compounds, and pH of *Acacia nilotica* and *Vachellia karroo* (pods and leaves). Two trees per browse species were selected, for harvesting leaves and pods. A completely randomized design was used where each treatment was replicated three times. Then 50% of grass and 50% of pods and leaves were mixed with 3% molasses and urea. The *Acacia nilotica* and *Vachellia karroo* (pods and leaves) were harvested just before winter (March-April 2018) at Honey Dale Farm. Kikuyu (*Pennisetum clandestinum*) was also harvested at the Research Farm of the University of Fort Hare. The fresh Kikuyu grass was cut into small pieces together with *Acacia nilotica* and *Vachellia karroo* (pods and leaves). The samples were packed and compacted by hand, pressing into one (1) litre consol jars with airtight fitting lids. The jars were kept at room temperature for 42 days. The silage was analyzed for chemical composition (CP, NDF, ADF, DM, ADL, ash, OM and pH), secondary compounds (condensed tannins and phenolics) and in vitro degradability.

Phenolics showed significant differences ($P < 0.05$) in urea and molasses treatments. On browse trees *Acacia nilotica* leaves with urea (0.34), *Acacia karroo* pods leaves with molasses (0.42) and *Acacia Karroo* pods with molasses (0.45) had low levels of phenolic compounds compared to *Acacia nilotica* pods with molasses (0.55).

Treatments showed significant differences ($P < 0.05$) in tannin content *Acacia karroo* leaves with urea (0.22), *Acacia nilotica* pods with urea (0.23) and leaves with molasses (0.23) and *Acacia karroo* leaves with molasses (0.28) had low levels compared to *Acacia nilotica* leaves with urea (0.39). In addition, pH showed significant differences ($P < 0.05$) between all the treatments. *Acacia nilotica* pods with urea (4.91), *Acacia nilotica* leaves with molasses (5.23), *Acacia nilotica* leaves with urea (5.27), and *Acacia karroo* leaves with molasses (5.40) had low levels compared to *Acacia nilotica* pods with molasses (6.66).

The browse silage results showed significant differences ($P < 0.05$) among all the treatments in their dry matter content. *Acacia karroo* leaves mixed with molasses, and *Acacia nilotica* leaves mixed with urea had higher ($P < 0.05$) dry matter content than others. There were highly significant differences ($P < 0.05$) between neutral detergent fibre (NDF), ash acid detergent lignin (ADL), crude protein (CP), organic matter (OM), and acid detergent fibre (ADF) was also significantly different ($P < 0.05$). *Acacia karroo* pods ensiled with urea (11.90%), *Acacia karroo* leaves with urea (9.89%), and *Acacia nilotica* pods with urea (9.21%) had higher CP content than other treatments. The digestibility of treatments compared to time were significantly different ($P < 0.05$) and within the columns, although there was no significant difference ($P > 0.05$) in the 12th and 36th hours. *Acacia karroo* leaves mixed with molasses had the highest (488.23) on the 0 hour and *Acacia Nilotica* pods with urea on the 12th hour (144.10). In conclusion, ensiling using urea and molasses had a positive effect on secondary compounds, chemical composition and digestibility.

Keywords: *Acacia nilotica*, *Acacia karroo*, chemical composition, condensed tannins, phenolics, degradability, silage, urea, molasses

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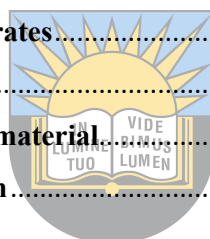
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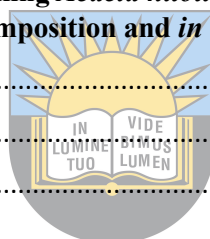
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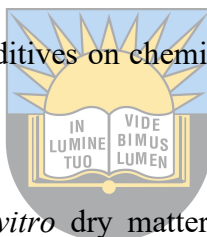


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Abbreviations list

ADF- acid detergent fibre

ANF- anti-nutritional factors

AU- absorbance units

AOAC- Association of Official Analytical Chemists

CG- cyanogenic glycosides

CO₂- carbon dioxide

CH₄- methane

Cm- centimetres

CP- crude protein

CT- condensed tannins

DM- dry matter

DMD- dry matter digestibility

etc. - etcetera

g- Grams

HCl- hydrochloric acid

H₂O- water

ICT- Insoluble/unextractable-condensed tannin

IVDMD- *In vitro* dry matter digestibility

Kg- kilograms



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ml- millilitres

mg- milligram

nm- nanometre

N- Nitrogen

NDF- neutral detergent fibre

NH₃ - ammonia

NRC- national research council

PEG- Polyethylene glycol

PVP- Polyvinylpyrrolidone

SCT- soluble/extractable-condensed tannins

SE – standard error

SPh- Soluble phenolic

TP- total phenolic

UV- ultra violet

V/V- volume per volume

WSC-water-soluble carbohydrates

W/W- weight for weight



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1 CHAPTER ONE

1.1 Background

Silage is the fermentation of fresh forage material that contains relatively high moisture content (Titterton and Bareeba, 2000). Multipurpose trees such as *Vachellia karroo* have become an alternative ruminant feed in semi-arid conditions (Ben Salem *et al.*, 2010). Crude protein from browse trees ranges from 10-25% dry matter and can be a reliable feed source to sustain livestock production (Aynalem and Taye, 2008). However, most browse trees contain secondary compounds (Salem, 2005). These secondary compounds reduce the nutritional value, making them unavailable to ruminal microorganisms. The foliage of trees such as different *Acacia* species and the pods and fruits can substitute for concentrate supplement (Adugna, 2007). When ensiling, if silage is stable after opening, it contains no spoilage microorganisms (Wilhelm and Wurm, 1999). Yeast and moulds cause dry matter loss in silage. Their activity shows a rise of temperature above ambient temperature; the sample starts to deteriorate, and silage becomes aerobically unstable. *Acacia* species are dominant in Sub-Saharan Africa (Mahesh and Satish, 2008).

When pastures are deficient in quantity, trees provide forage. The protein content is relatively high than in grasses (Otte and Chilonda, 2002). Browse from these trees offers vitamins and minerals (Singh and Yadav, 2009). *Acacia* pods could serve as a protein source for poor-quality roughages. Previous research (Banso, 2009) revealed that freezing and drying are some of the methods that conserve browse. These methods require labour. Silage is the most used technique because of affordability. Before ensiling, crops need to be wilted to reduce water content to levels that result in desirable silage. A problem that affects silage is fungal growth. Fungi affect silage by deterioration forming mycotoxins and resulting in dry matter loss (Buxton *et al.*, 2003). The silage needs to be free from oxygen to inhibit the growth of moulds. Compaction

and sealing of silos minimize the entry of air into the silo. Clostridia has a significant impact on the loss of nutrients during fermentation.

Silage additives such as salts rapidly lower pH where other bacteria struggle to survive except for lactic acid bacteria (McDonald *et al.*, 1991). Wet crops have a desirable pH of less than four, but the final pH depends on the buffering capacity and dry matter content of crops. Good silage can be achieved by good ensiling practices such as airtight sealing and wilting (Buxton *et al.*, 2003). Ensiling reduces secondary compounds (Huisden *et al.*, 2014). Ngwa *et al.*, (2004) found a reduction in the concentration of cyanogenic glycosides (CG) in *Acacia sieberiana* pods. This research focuses on the chemical composition of the leaves and pods of *Vachellia karroo* and *Acacia nilotica*, availability, and possibilities of conserving tree species with additives.



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1.2 Problem statement

The use of browse is not focusing on livestock due to a lack of understanding of its utilization, especially in smallholder farmers, more research needs to be conducted on ensiling tree species and using browse as source of protein to livestock. Browse here refers to fruits, leaves, and pods. The amount of protein during the dry season is too low to maintain animals. Although there is published information on the supplementation of livestock when using local trees such as *Vachellia karroo* leaves, most studies focus on supplementation at research stations, and additives are not used (Robles *et al.*, 2008). Browse in the rural area is not primarily used to feed animals due to secondary compounds toxic to animals.

1.3 Justification

There is insufficient documented scientific information on ensiling browse tree pods and leaves than grass and other materials. In ruminants, it provides nutrients when supplemented. Pods form part of a diet for different herbivores in Africa. This is a compelling reason to research detoxification to improve feeding value. Silage can detoxify anti-nutritional factors and reduce the risk of cyanide poisoning.



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1.4 Objectives

- To determine the effect of ensiling on secondary compounds and pH of *Acacia nilotica* and *Vachellia karroo* pods and leaves
- To determine the effect of ensiling on chemical composition and *in vitro* degradability of *Acacia nilotica* and *Vachellia karroo* pods and leaves

1.5 Hypotheses

- Ensiling does not reduce secondary compounds and pH of *Acacia nilotica* and *Vachellia karroo* pods and leaves

- Ensiling does not alter the chemical composition and no significant differences *in vitro* degradability of pods and leaves of *Acacia nilotica* and *Vachellia karroo*



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2 CHAPTER TWO: literature review

2.1 Introduction

When crushed with seed, Acacia pods provide protein that ranges from 10-20% (Timberlake *et al.*, 1999). In general, pods contain high levels of protein and minerals than grasses (Seymour and Suzanne 2003). Farmers harvest pods and mill them with sulphur to supplement cattle feeds in times of drought. Secondary metabolites reduce the digestibility and acceptability of herbivores when they are present in high amounts (Rubanza *et al.*, 2007). Cutting and ensiling with additives can improve microbial protein synthesis and maintain plant protein in the rumen (Kumar, 1992). Ensiling is a process that cannot be controlled when performed if ensiled under incorrect conditions, poor quality can occur so use of additives helps with silage quality in ensiling (Kung *et al.*, 2003). Chemical additives and microbial inoculants help with stimulating and inhibiting undesirable microorganisms (Kung *et al.*, 2003). Saeed (2015) demonstrated that more palatable feed could be produced by ensiling. For instance, nitrogen increased when urea was added to sorghum silage (Filya, 2001). The current study will determine the effects of adding of adding urea and molasses to browse tree pods and leaves silage on; *in vitro* degradability, chemical composition, and secondary compounds.

2.2 Description of browse trees

2.2.1 *Acacia nilotica*

Acacia nilotica originates from Africa within North and South and subtropical areas (Orwa *et al.*, 2009). The crown is flat, and branches are facing downward. The bark is dark brown, and when the tree matures, it turns to blackish grey. Its root system is affected by environmental conditions and the genus of the species. During drought, they develop a taproot, and in wet conditions, they develop lateral roots (Van Wyk *et al.*, 2000). The flowers grow from spring to summer, depending on the rainy season. The pods form a beaded necklace form. As shown in Plate 1, the pods have curved and are fleshy when they are still young and later become blackish. They break up and fall onto the ground from March to September. When crushing the green pods, a sticky fluid shows (Thomas and Grant, 2004).



Plate 1: *Acacia nilotica* pods and leaves (Van Wyk *et al.*, 2000)

2.2.2 *Vachellia karroo*

Vachellia karroo is indigenous to South Africa and is a sweet thorn, is found along rivers and drainage lines (International Legume Database and Information Service, 2010). *Vachellia karroo* varies in height from a shrub to a tree, generally between 1 and 15m tall, but can grow up to 25m tall (Archibald and Bond, 2003). Young trees have red bark, but as the tree grows, the bark turns dark (Joffe, 2001). The leaves are finely textured and dark green. During the rainy season, the tree develops yellow flowers (Barnes *et al.*, 1996). Its pods are crescent-shaped, as shown in Plate 2, flattened 5-10cm long and 5-8mm wide. Its leaves remain in the tree throughout the year sometimes. They are drought tolerant because of taproot that takes water and nutrients from the soil (Barnes *et al.*, 1996). The tree adapts to the different soil types (sand or heavy clays) and annual rainfall of 200mm-1500mm. *Vachellia karroo* contains long thorns (2 - 5 cm); however, its thorns make it challenging to handle.

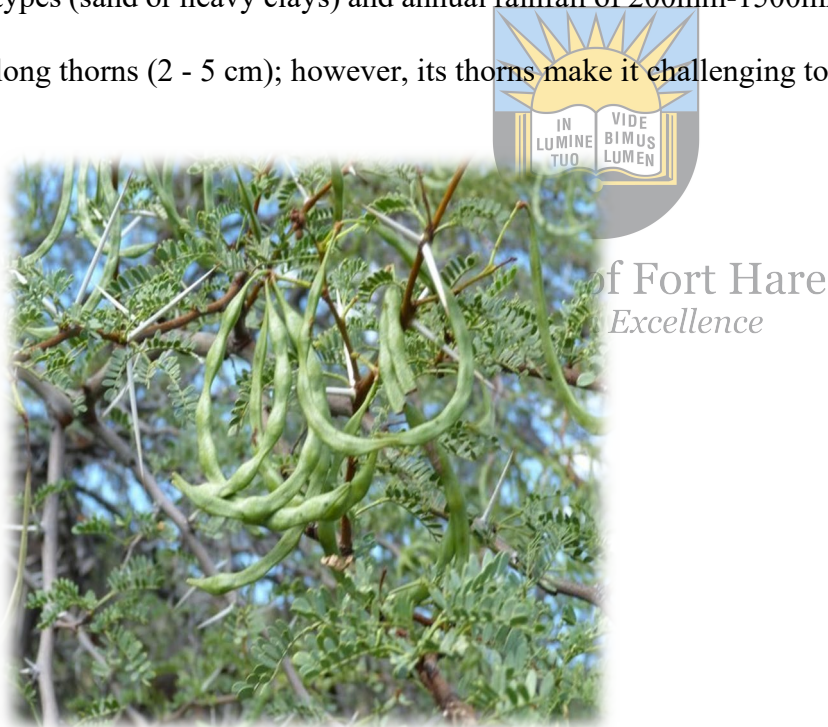
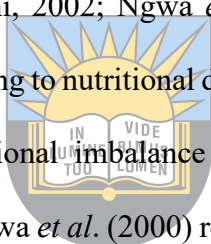


Plate 2: *Vachellia karroo* pods and leaves (Joffe, 2001)

2.3 Importance of browse trees

The balance between the nutritional contents of the plant, such as digestibility of nutrients, and the quantity ingested by the animal determines the feeding value of fodder (Adesogan *et al.*, 2006). High nutritional value promotes high livestock production. The amount of nutrients consumed influences the performance of ruminants. Nitrogen is the critical element during the dry season, and tropical forages such as hay and straw are low in nitrogen (Lazzarini *et al.*, 2013). The crude protein of hay and straw ranges from 20-50g crude protein/kg DM, and that does not meet the minimum crude protein required, which is 80g CP/kg DM for ruminal microbes to function (Annison and Bryden, 1998). *Acacia Karroo* is three times rich in CP than cereal grains (Table 1) (Chepape *et al.*, 2011). The leaves contain high levels of fatty acids, minerals, and crude protein (Halimani, 2002; Ngwa *et al.*, 2002). Pastures that have poor management result in low intake, leading to nutritional deficiencies and reduction of production (Cudjoe and Mlambo, 2014). Nutritional imbalance affects production by increasing the occurrence of metabolic disorders. Ngwa *et al.* (2000) reported factors that reduce productivity as low quality of pastures, nature of forage, and inadequate intake. Mlambo *et al.* (2008) reported forage quality as decreasing animal production. Nutritious trees are important for supporting all-year-round production of animals in arid and semi-arid regions (Tefera *et al.*, 2008). Still, the value of the chemical composition is unknown and needs to be investigated if browse is to be used as a protein supplement (Mlambo *et al.*, 2008).



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Table 1: Chemical composition of *Vachellia karroo* leaves

	Composition (%)
DM	97.0
CP	12.6
ADF	32.4
NDF	38.0
CT	2.0
TP	1.9

Source; Brown *et al.*, 2016

**Table 2: Chemical composition of selected common legumes (g/kg DM)**

Species	DM	CP	CF	NDF	ADF	Ash	Source
<i>Vachellia karroo</i>	922	154	119	450	300	110	Halimani <i>et al.</i> (2005); Ravindran and Blair (1992)
<i>Acacia nilotica</i>	947	198	-	399	227	56.5	Kambashi <i>et al.</i> (2016); Piva (1992);
<i>Vachellia tortilis</i>	944	218	-	494	298	65.0	Piva (1992); Mukumbo <i>et al.</i> (2014)

DM: Dry matter, CP: Crude protein, CF: crude fibre, NDF: Neutral detergent fibre, ADF: Acid detergent fiber

2.4 Making Silage

Ensiling keeps forage for later use (Stewart, 2011). Silage has different phases, namely, aerobic phase, anaerobic and stable phase. During the phases, there is a change in pH, carbohydrate, and organic acid concentrations and temperature and O₂ levels (Wilkinson and Davies, 2012). The environment has to be favourable with a temperature that ranges from 30-48°C. Good silage contains high lactic acid levels and low levels of butyric acid and ammonia (Wilkinson *et al.*, 2003; Downing *et al.*, 2008).

2.5 Factors affecting silage making

2.5.1 Water-soluble carbohydrates

Silages prepared from high water-soluble carbohydrates (WSC) grasses have better quality than silages made from low WSC grasses (Downing *et al.*, 2008). Annual grasses and corn produce stable silage as they contain sugar that is above 5% dry matter (Rustas *et al.*, 2010). Legumes do not ensile well because they have a small amount of sugar in their tissues. Therefore, limited WSC content restricts fermentation, while undesirable microorganisms use excess WSC during storage (Cherney and Cherney 2003). The concentration of the WSC is influenced by forage type, the stage of maturity, time of harvesting during the day, and environmental conditions (Buxton and O'kiely 2003). For instance, time of harvesting affects the WSC content, with perennial ryegrass harvested in the daytime having more WSC content than that harvested in the morning (199 vs. 144 g kg⁻¹) (Downing *et al.*, 2008). The primary effect of WSC during ensiling is the rapid decline in pH just after ensiling (McDonald *et al.*, 1991). WSC impacts lactic acid and acetic acid concentrations during ensiling (Merry *et al.*, 2006).

2.5.2 Buffering Capacity

Forage material can withstand variation in pH. Each forage has its buffering capacity (Kung and Shaver, 2004). More acid is required in fresh forages to reduce pH than forages with low buffering capacity. Forage has been reported to have protein and buffering capacity

concentration of 5.6 and 4.1 higher than in grains (Moharrery, 2007). Buffering capacity is one factor that influences the status of ruminal acid-base (Le Ruyet *et al.*, 1992). Buffer value index provides an evaluation of diet changes in the quality of acid-base status. The required percentage of water-soluble carbohydrates in grass and legumes is 6-8 and 10-12.

2.5.3 The moisture content of material

Moisture is the difference between weight before and after drying the material. Moisture makes it hard to compact, exclude air, and provide enough moisture to promote lactic acid fermentation. The optimum moisture content of chopped material is about 65% (Gay *et al.*, 2003); the percentage that falls within this range restricts butyric acid from forming. The fermentation process helps eliminate oxygen trapped in the mass of forage when packing (Chambliss, 2002). Ensiling forage with a moisture content greater than 70% is not good (Chambliss, 2002). High moisture level favours undesirable fermentation. Silage that results from a moisture percent less than 70% contains less acid and concentration of butyric acid with high ammonia nitrogen. High moisture content leads to silage that has a strong rancid odour and fermentation being prolonged with the breakdown of protein and energy loss. It is important to consider moisture, especially when a crop has high crude protein content, buffering capacity, and low sugar content. In contrast, when ensiling forage with low moisture content (less than 50%) can result in restricted fermentation and production of unstable silage that contains low lactic acid concentration and is less acidic (O'Brien *et al.*, 2007a). Mould and spontaneous heating are common in these silages.

2.5.4 Dry matter concentration

It is best to harvest during the day when small amounts of moisture have exposure to sunlight. Forage with moisture content that is greater than 70% result in poor silage. Those with less than that reduce the fermentation of clostridia (Muck *et al.*, 2003). Before forage ensiling, wilting is essential. Forages with less percentage of moisture have difficulties when it comes

to packing. It is difficult to get rid of the air within the forage mass. Yeast and mould growth lead to poorer fermentation when visible in the silage. Therefore, harvesting is better when moisture concentrations are low and considering the maturity of forage. When forage has dry matter great than 55%, water must be added to achieve the required moisture for bacteria and improvement of packing (Wilkinson *et al.*, 2003).

2.5.5 Packing density

Packing density determines the porosity at which air moves from the container and the spoilage that occur during storage. Porosity measures space between particles of the material and is influenced by new weight density (Holmes and Muck, 2008 Poor packing density reduces the effectiveness of silage inoculants. Dry matter of forage when harvesting needs to be 30-40% with adequate packing (Holmes, 2006). Compaction has to be good to remove the air inside (Mushi *et al.*, 2000). High density restricts the air, and it decreases when dry matter increase.

2.5.6 Temperature

When there is a temperature rise, microorganisms occur in the silage. The higher the temperature, the greater the growth rate of microorganisms. When temperatures are high from the beginning of fermentation, the acidification is greater (Bjorge, 1996). High temperatures cause the growth of clostridia, increasing butyric acid and ammonia, affecting silage quality. Ensiling in temperatures below 37.8°C is the best. The silage produced at high temperatures can be palatable though the quality is poor (Kunkle and Chambliss, 2002). Kunkle and Chambliss (2002) reported heating as damaging protein and reducing the availability of carbohydrates and this can result to poor silage.

2.6 Effect of additives on silage

Additives control the fermentation process and retain nutrients from the forage before ensiling. They make it easier for lactic bacteria growth to dominate, producing lactic acid that ensures

good silage (Oliveira, 1995). There is an improvement in nutrient composition, quick fermentation, and bunk life when used. Use of additives can also increase the intake and performance of animals through the positive effects on silage quality (Merry *et al.*, 1993).

2.6.1 Molasses

It improves silage by promoting lactic fermentation, reduction of pH, inhibits the growth of clostridia and proteolysis (Thomas *et al.*, 2003). Forage crops with harmful WSC benefit from the use of molasses. Keady (1996) published a review where the conclusion on the use of molasses was positive and dry matter intake was improved.

2.6.2 Urea

Ammonia additions result in addition of crude protein (Filya *et al.*, 2002), extension in bunk life with less moulding and heating during ensiling (Whiter *et al.*, 1999); and decreased protein degradation (Meeske *et al.*, 2002). The use of urea improved the bunk life of corn silage.

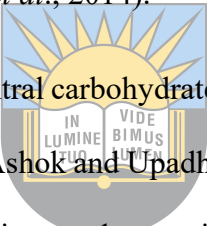
2.7 Effect of anti-nutritive factors on livestock

Legumes have high protein levels and can be used as supplements to meet nutrient deficiencies when secondary compounds are controlled. Tannins bind with proteins in plants (Makkar, 2003; Min *et al.*, 2003). In some plants, the presence of these compounds limits their utilization (Forbey *et al.*, 2009). Feed anti-nutritional factors (ANFs) are generated by normal metabolism that affects optimum nutrition (Becerra, 2015). Ruminants are less affected by toxicity than poultry and pigs. Tannins, saponins, and cynides make up ANFs (Osman, 2007). Secondary compounds amount greater than 45 g/kg DM have adverse effects in ruminants (Getachew *et al.*, 2002).

2.7.1 Tannins (Condensed and Hydrolysed)

Condensed tannins are plant secondary compounds found in all plant parts (Hassanpour *et al.*, 2011). Waghorn (2008), reported a research regarding condensed tannins synthesis, including

protection against herbivores. Structurally, they are oligomers and polymers of flavonoid units linked with a covalent bond (two carbon atoms) (Tharayil *et al.*, 2011; Monagas *et al.*, 2010). Condensed tannins have a high molecular weight that ranges from 500 to 20,000 Dalton (Okuda and Ito, 2011). They are responsible for the anti-nutritional effects in ruminants (Makkar, 2003). Anti-nutritive effects associated with the ability to combine with polymers, thus delaying digestion (Naumann *et al.*, 2013). When the nutritional value is affected, feed intake and digestibility decrease (Gxasheka *et al.*, 2015). They also affect palatability by being apparent to ruminants as bitterness (Lamy *et al.*, 2011). CTs can bind to digestive enzymes, thus reducing their activities (Lu, 2011). Literature also states that a high intake of tannins in the diet can cause liver and kidney damage, lesions associated with haemorrhagic gastroenteritis, and mortality (Karimi *et al.*, 2014).



Hydrolyzable tannins are made of a central carbohydrate core that contains phenolic carboxylic acids and hexahydroxydiphenic acid (Ashok and Upadhyaya, 2012; Plate 3). HTs break down easily by enzymes that are not present in cereals meaning they do not bind in the rumen. As a result, they are associated with a positive impact on animal nutrition (Bhatta, 2002). When they are hydrolyzed, gallic acid and sugars are formed. However, condensed tannins are dimers of catechin and epicatechin (Clinton, 2009). Tannins are essential in animal nutrition. They become available in the small intestine for digestion when not degraded in the rumen (Alipour and Rouzbehan, 2010). The pH found in rumen ranges from 5.5 to 7.0, making tannins bind with proteins, slowing microbial degradation of proteins (Makkar, 2003). The pH of 2.5 to 3.5 in the abomasum dissociates tannin protein complexes. There are advantages and disadvantages of consuming tannins depending on the amount and state of the animal (Amlan and Jyotisna, 2010a; Waghorn, 2008; Makkar, 2003).

Additionally, the response of ruminants consuming diets that contain condensed tannins is inconclusive. Hervas *et al.* (2003) reported that high consumption of tannins produces toxicity,

while others found no evidence (Krueger *et al.*, 2010). Therefore, attention needs to be paid to amounts to prevent reduction in animal performance (Theodoridou *et al.*, 2010).

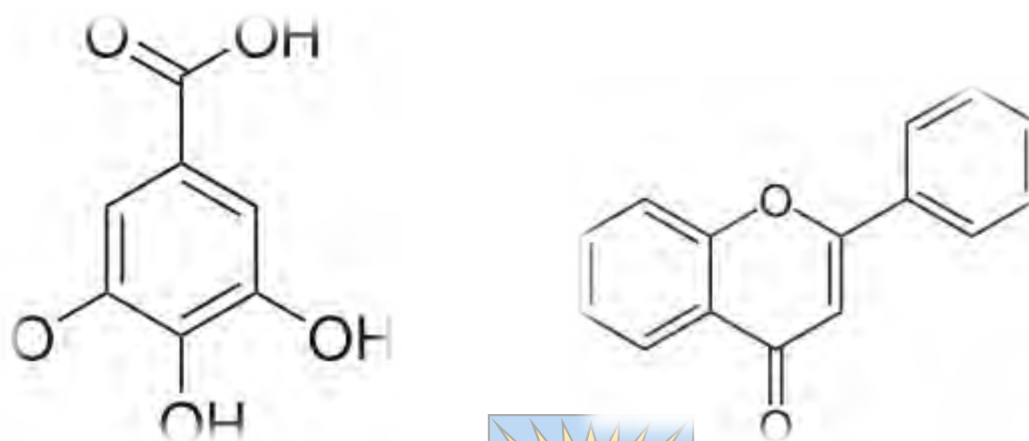


Plate 3: Hydrolysable

Source: Ashok and Upadhayaya (2012)



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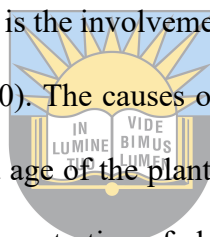
2.7.2 Saponins

Saponins are detergents in plants, they reduce palatability, thus affecting feed intake (Makkar and Becker, 1999). They have been reported to suppress protozoal activity and hence reduce methane production (Moss *et al.*, 2000). The presence of saponins improves absorption of nitrogen because there is less ammonium excretion in urine (Santoso *et al.*, 2004). Saponins do not affect protozoa numbers (Hess *et al.*, 2003a) because of the number of bacteria in the rumen

that break down saponins into free glycosyl and saponins fractions (Newbold *et al.*, 1997). The complexation of essential minerals, steroids, and saponins is limited by bacterial growth inhibition (Simon *et al.*, 2002). Goel and Makkar (2012) summarized six studies that reported a decrease in methane from about 6-27% of total production in three of the studies, OM digestibility was decreased (Goel and Makkar, 2012).

2.7.3 Phenolics

The arrangement is from low molecular weight, and classification is done based on the number and structure of carbons. There are two groups, namely, flavonoids and non-flavonoids. Flavonoids have fifteen carbons with aromatic rings that are connected by a three-carbon bridge. Leaves and fruits contain a high concentration of flavonoids and these compounds play a role as secondary metabolites. There is the involvement of diverse processes of pigmentation and disease resistance (Pierpoint, 2000). The causes of variation of phenolic compounds can be differences in the environment and age of the plant (Mueller-Harvey, 2006). Old plants in soils with low fertility have a high concentration of phenolic during the dry season (Scogings and Mopipi, 2008).



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2.8 Ways of inactivating tannins-protein complexes

Several ways have been tested to inactivate toxic tannins to improve the nutritional value of feedstuffs. These methods include the use of binding agents (polyethylene glycol, polyvinylpyrrolidone, polyvinyl polypyrrolidone, acetic acid, sodium hydroxide), heating, soaking in water and drying, wood ash, chopping and storage, urea, solid-state fermentation, and use of enzymes (Makkar, 2003). The methods are commonly grouped into physical and chemical methods.

2.8.1 Physical method

Condensed tannins in acacia foliage can be affected by drying (Salem *et al.*, 2000; Makkar, 2003). At 90 °C for 24 hours, tannin content was reduced from leaves of cassava and *Leucaena*. Grinding and chopping decrease the effects of tannins in leaf meals (Wina *et al.*, 2005). Soaking is a recommended method for reducing the detrimental impact of tannins (Medugu *et al.*, 2012). Chopping fresh leaves, water soaking, and storage can be of practical use because they require minor changes in everyday farm practices (Bhat *et al.*, 2013). Moreover, these methods can affect tannin content in Acacia foliage (Salem *et al.*, 2005).

2.8.2 Chemical method

Several chemical methods have been tested (Alipour and Rouzbehan, 2007; Salem, 2005; Kyarisiima *et al.*, 2004). Kyraissima *et al.* (2004) reported that wood ash treated sorghum reduces tannin level in feed resources rich in tannin. Urea to neutralize tannin-rich leaf meals is recommended and makes extra nitrogen available to the animals (Sahnoune *et al.*, 1991). Moreover, urea is effective due to the higher pH caused by the evolution of urea-ammoniation. Tannin-binding agents and enzymes can be used to neutralize anti-nutritive factors (Schons *et al.*, 2012; Tshabalala *et al.*, 2013). Polyethylene glycol, polyvinylpyrrolidone (PVP), charcoal, and sodium hydroxide are examples of tannin-binding agents. Challenges of chemical methods include the loss of soluble nutrients, analyses, and laboratory work which is unaffordable to resource-poor farmers.

2.9 *In vitro* degradability

In vitro degradability determines digestibility by simulating the rumen and digestive juices (Oba and Allen, (1999). *In vitro* methods evaluate the quality of feed regarding nutritive value (Kamalak *et al.*, 2005). They are less expensive and require less time, giving accurate experimental conditions (Getachew *et al.*, 1998). Nitrogen solubility is determined by buffer nitrogen giving protein fraction of non-conventional protein feed (Licitra *et al.*, 1996). Buffer

solubility imitates the environment found in the rumen, and bicarbonate buffer breaks down protein at 37°C (controlled temperature).

2.10 Conclusion

Indigenous tree species are well adapted to the environment and add substantially to livestock diets throughout the year. Browse is a protein supplement that can be harvested locally in summer and stored for use in winter when the quality and quantity of grazing and or browse are limited. Feed with high nutritional value is essential and fodder conservation can help avoid the loss of nutrients from time of availability. Additives help decrease mould during storage and increase nutritional content, but they are not replacements for good silage. Small-scale farmers experience a limited quantity of feed in winter.



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3 CHAPTER THREE: Effect of ensiling *Acacia nilotica* and *Vachellia karroo* pods and leaves with urea and molasses on secondary compounds of silage

3.1 Abstract

The objective of the study was to investigate the effect of ensiling *Acacia nilotica* and *Vachellia karroo* pods and leaves with molasses and urea on tannins, phenolics and pH values of silage. The pods and leaves were harvested in April-March from both browse trees. These parts of browse trees were mixed with 3% of molasses and urea. The samples were then packed and compacted by hand, pressing into 1 litre consol jars with airtight fitting lids. After closing, the jars were kept at room temperature for 42 days. The results of the effect of ensiling browse trees pods and leaves with additives (urea and molasses) on phenolic compounds are presented in Table 3.1. There were significant differences ($P < 0.05$) between treatments (silage mixtures). For pods, *Acacia nilotica* pods with molasses had the highest value (0.55) while for leaves *Vachellia karroo* leaves with urea recorded highest phenolic compounds content (0.52), while *Acacia nilotica* pods with urea (0.14) had low levels than other treatments. In tannins, there were significant differences ($P < 0.05$) between treatments. *Acacia nilotica* leaves with urea recorded the highest tannin levels (0.39) while *Acacia nilotica* leaves with molasses had the lowest tannin levels (0.15). For the pods, *Acacia nilotica* pods with urea recorded the highest tannin levels (0.23), and *Vachellia karroo* pods ensiled with molasses (0.13) had the lowest levels of tannins ($P > 0.05$) compared to other treatments. In addition, pH had significant differences ($P < 0.05$) between all the treatments. *Vachellia karroo* leaves with urea (6.06) had the higher pH value ($P < 0.05$) than *Vachellia karroo* leaves with molasses (5.40). In contrast, *Acacia nilotica* leaves with urea (5.27), and *Acacia nilotica* leaves with molasses (5.23) had low pH levels. For pods, *Acacia nilotica* pods with molasses recorded the highest pH (6.66) than *Acacia karroo* pods with urea (5.92) while *Acacia nilotica* pods with urea had the lowest pH (4.91). In conclusion, urea performed better than molasses in tannins for both *Acacia karroo*

and *Acacia nilotica*. For phenolics, urea (*Acacia karroo*) performed better in leaves and molasses (*Acacia nilotica*) in pods. Urea (*Acacia karroo*) performed better in leaves and molasses (*Acacia nilotica*) in pods for pH.

Keywords: Silage, Condensed tannins, Phenolics, pH, Urea, Molasses, *Acacia nilotica*, *Vachellia karoo*



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3.2 Introduction

In South Africa, browse trees have different types of secondary compounds. Secondary compounds are natural substances that act as Anti-nutritional factors (Gobbo-Neto and Lopes, 2007). These substances affect animal nutrition, feed intake, and utilization (Allen and Segarra, 2001). Phenols contain a group of defensive compounds that host plant resistance against herbivores (Ballhorn *et al.*, 2009). Browse species provide nutrients, including fibre and protein (Clauss *et al.*, 2008, Mbatha *et al.*, 2012). Ensiling can be affected by buffering capacity and dry matter content for rich protein plants (McDonald *et al.*, 2002). Natural and chemical additives are used to improve silage quality (Titterton 1999). The inclusion of additives reduces pH, inhibits the growth of microorganisms, and preserves carbohydrates and proteins (Zhang *et al.*, 2000a; Saarisalo, 2007). Good quality silage results in edible silage with an ideal dry matter and well-preserved nutrients (Latorre, 2015). Fajemisin *et al.* (2013) used urea, and it improved the feeding value during the dry season when feeding low-quality crops to ruminants. Molasses contains water-soluble carbohydrates and reduces pH in treated silages (Fajemisin *et al.*, 2013). It also increases the palatability due to its high sugar content and acts as a binder for the densification of straw (Garg *et al.*, 2005). Low pH preserves protein by inhibiting plant proteases. Whereas focus has been given to grass silage (van Niekerk *et al.*, 2007, Cardens *et al.*, 2003 and McDonald *et al.*, 1991), there is a limited information regarding the use of urea and molasses on secondary compounds in *Acacia nilotica* and *Vachellia karroo*. Hence the objective of the current study was to determine the effect of ensiling *Acacia nilotica* and *Vachellia karroo* pods and leaves with urea and molasses on secondary compounds in silage.

3.3 Materials and Method

3.3.1 Ethical considerations

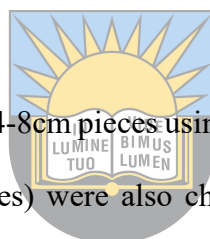
Ethical clearance was applied for and obtained from the University of Fort Hare (Certificate reference number: GAJ011STWA01).

3.3.2 Description of the study

The study was conducted in the Animal Nutrition Laboratory (Department of Livestock and Pasture) at the University of Fort Hare Alice, Eastern Cape Province. Kikuyu grass (*Pennisetum clandestinum*), *Acacia nilotica* and *Vachellia karroo* (pods and leaves) were harvested from the University of Fort Hare Research Farm (Honey Dale farm) just before winter (March-April).

3.3.3 Silage preparation

Fresh Kikuyu grass was chopped into 4-8cm pieces using a paper guillotine and *Acacia nilotica* and *Vachellia karroo* (pods and leaves) were also chopped. The samples were packed and compacted by hand, pressing into 1 litre consol jars with airtight lids. The jars were kept at room temperature for 42 days.



3.3.4 Experimental design

A completely randomized design was used where each treatment was replicated three times. Then 50% of kikuyu grass, 50% of pods and leaves were mixed with 3% of molasses and urea. Silage mixture 1: Silage mixture 2: Silage mixture 3: Silage mixture 4: Silage mixture 5: Silage mixture 6: Silage mixture 7:

3.3.5 Fermentation and secondary compounds

The pH of the silage mixtures were determined by adding 100ml of distilled water to 25g of each sample in a beaker, and a pH meter glass electrode was inserted to determine the pH. The soluble phenolic compounds were determined by extracting samples three times with 10ml of aqueous acetone using the Folin-Ciocalteu method (Singleton and Rossi, 1965). Absorbance was read at 675 nm using a spectrophotometer. The aqueous acetone (5ml) was used to extract 40mg of samples. At 550 nm per 40 mg of sample absorbance unit for condensed tannin concentration was reported.

3.4 Statistical analysis

Effect of ensiling on secondary compounds was analysed using SAS/JMP Version 14.0 software 2018; the following statistical model was used:

$$Y_{ij} = \mu + T_i + e_{ij}$$

Where:

Y_{ij} = Measured parameter (pH, tannins and phenolics)

μ = mean

T_i = treatments (1& 2)

e_{ij} = residual error

$$Y_{ij} = \mu + T_i + e_{ij}$$

3.5 Results

3.5.1 Phenolics

The results of the effect of ensiling browse trees pods and leaves with additives (urea and molasses) on phenolic compounds are presented in Table 3.1. There were significant differences ($P < 0.05$) between treatments (silage mixtures). *Acacia Karroo* leaves ensiled with

urea recorded highest phenolic compounds content (0.52), followed by *Acacia karroo* leaves with molasses (0.42), while *Acacia Nilotica* leaves with urea was high (0.34), compared to *Acacia nilotica* with molasses (0.15). *Acacia nilotica pods* with molasses had the highest value (0.55) than *Acacia karroo pods* with urea (0.47) and *Acacia karroo pods* with molasses (0.45). In contrast, *Acacia nilotica pods* with urea (0.14) had low levels than other treatments.



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Table 3.1: Effect of ensiling *Acacia nilotica* and *Vachellia karroo* pods and leaves with urea and molasses on phenolics content

Silage mixture	Phenolics (g/kg DM)
<i>A.N</i> PM	0.55 ^a
<i>A.K</i> LU	0.52 ^{ab}
<i>A.K</i> PU	0.47 ^{ab}
<i>A.K</i> PM	0.45 ^b
<i>A.K</i> LM	0.42 ^{bc}
<i>A.N</i> LU	0.34 ^c
<i>A.N</i> LM	0.15 ^d
<i>A.N</i> PU	0.14 ^d
S.E	0.029
Level of significance	**



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^{abcd} Means with different superscripts within columns are significantly different according ($P < 0.05$). S.E= standard error. Silage mixture 1: ANPM: *Acacia nilotica* pods plus molasses, Silage mixture 2: AKLU: *Acacia karroo* leaves plus urea, Silage mixture 3: AKPU: *Acacia karroo* pods plus urea, Silage mixture 4: AKPM: *Acacia karroo* pods plus molasses, Silage mixture 5: AKLM: *Acacia karroo* leaves plus molasses, Silage mixture 6: ANLU: *Acacia nilotica* leaves plus urea, Silage mixture 7: ANLM: *Acacia nilotica* leaves plus molasses, Silage mixture 8: ANPU: *Acacia nilotica* pods plus urea

3.5.2 Tannins

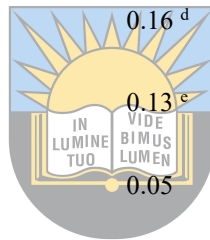
The results of the effect of ensiling browse trees pods and leaves with additives (urea and molasses) on condensed tannins are presented in Table 3.2. There were significant differences ($P < 0.05$) between treatments. *Acacia nilotica* leaves ensiled with urea recorded the highest tannin levels (0.39) compared to *Vachellia karroo* leaves with molasses (0.28) and *Acacia karroo* leaves with urea (0.22), while *Acacia nilotica* leaves with molasses had the lowest tannin levels (0.15). For the pods, *Acacia nilotica* pods with urea recorded the highest tannin levels (0.23), followed by *Acacia nilotica* pods with molasses (0.16), *Vachellia karroo* pods with urea (0.16), and *Vachellia karroo* pods ensiled with molasses (0.13) had the lowest levels of tannins ($P > 0.05$) compared to other treatments.



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Table 3.2: Effect of ensiling *Acacia nilotica* and *Vachellia karroo* pods and leaves with urea and molasses on tannin content

Silage mixture	Tannins (g/kg DM)
<i>A. K LM</i>	0.28 ^a
<i>A. N PU</i>	0.23 ^b
<i>A. N LM</i>	0.23 ^{bc}
<i>A. K LU</i>	0.22 ^c
<i>A. N PM</i>	0.16 ^d
<i>A. K PU</i>	0.16 ^d
<i>A. K PM</i>	0.13 ^e
S.E	0.05



Level of significance

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abcedef Means with different superscripts within columns are significantly different according (P<0.05). S.E= standard error. Silage mixture 1: ANPM: *Acacia nilotica* pods plus molasses, Silage mixture 2: AKLU: *Vachellia karroo* leaves plus urea, Silage mixture 3: AKPU: *Vachellia karroo* pods plus urea, Silage mixture 4: AKPM: *Vachellia karroo* pods plus molasses, Silage mixture 5: AKLM: *Vachellia karroo* leaves plus molasses, Silage mixture 6: ANLU: *Acacia nilotica* leaves plus urea, Silage mixture 7: ANLM: *Acacia nilotica* leaves plus molasses, Silage mixture 8: ANPU: *Acacia nilotica* pods plus urea

3.5.3 pH levels

The results of the effect of ensiling browse trees pods and leaves with additives (urea and molasses) on pH are presented in Table 3.3. There were significant differences ($P < 0.05$) between all the treatments. *Vachellia karroo* leaves with urea (6.06) had the higher pH value ($P < 0.05$) than *Acacia karroo* leaves with molasses (5.40). In contrast, *Acacia nilotica* leaves with urea (5.27), and *Acacia nilotica* leaves with molasses (5.23) had low pH levels. *Acacia nilotica* pods with molasses recorded the highest pH (6.66) than *Acacia karroo* pods with urea (5.92) and *Acacia karroo* pods with molasses (5.67), while *Acacia nilotica* pods with urea had the lowest pH (4.91).



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Table 3.3: Effect of ensiling *Acacia nilotica* and *Vachellia karroo* pods and leaves with urea and molasses on pH levels

Silage mixture	pH levels
<i>A.N</i> PM	6.66 ^a
<i>A.K</i> LU	6.06 ^b
<i>A.K</i> PU	5.92 ^c
<i>A.K</i> PM	5.67 ^d
<i>A.K</i> LM	5.40 ^d
<i>A.N</i> LU	5.27 ^{de}
<i>A.N</i> LM	5.23 ^e
<i>A.N</i> PU	4.91 ^e
S.E	0.56
Level of significant	***



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^{abcdef} Means with different superscripts within columns are significantly different according (P<0.05). S.E= standard error. Silage mixture 1: ANPM: *Acacia nilotica* pods plus molasses, Silage mixture 2: AKLU: *Vachellia karroo* leaves plus urea, Silage mixture 3: AKPU: *Vachellia karroo* pods plus urea, Silage mixture 4: AKPM: *Vachellia karroo* pods molasses, Silage mixture 5: AKLM: *Vachellia karroo* leaves plus molasses, Silage mixture 6: ANLU: *Acacia nilotica* leaves plus urea, Silage mixture 7: ANLM: *Acacia nilotica* leaves plus molasses, Silage mixture 8: ANPU: *Acacia nilotica* pods plus urea

3.6 Discussion

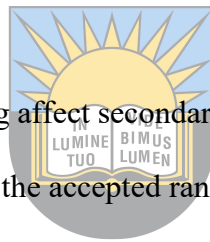
Condensed tannins affect the ingestion of feeds (Dube *et al.*, 2001) when the levels are high in the browse, but they are beneficial to animals at low levels of inclusion in diets (Foo *et al.*, 1996). In this study, the recorded levels of condensed tannins were low in *Vachellia karroo* pods, which were ensiled with molasses. Based on the literature, when levels of tannins are low, they are considered not to depress feed intake (Mbomi *et al.*, 2011). Therefore, both browse contained CTs at levels beneficial to ruminants. CTs at low levels produce a low protein binding effect (Olafadehan, 2013). However, these results disagree with those reported by (Abdulrazak *et al.*, 2000a, b), where phenolics and tannins in some browse were high after ensiling with molasses and urea. The low levels of tannins are beneficial to ruminants as they produce a low protein binding effect (Olafadehan, 2013). The intake of condensed tannins at a moderate level (2-4% DM) in the feed has been observed to have a beneficial effect on the metabolism of proteins, reducing bloat, and affect gastrointestinal parasites (Muir, 2011; Robins and Brook, 2005; Rakesh *et al.*, 2000). Ruminant animal diets ranges from 20 to 40g/kg DM for tannins (Yoshida, 2000). Therefore, this silage is nutritionally safe based on the levels of secondary compounds recorded. Degradation of protein in the rumen gets reduced when there are low tannins (Aron and Kennedy, 2008). The amount of protein degraded in the rumen depends on microbial proteolytic activity and accessibility of feed to microbes (NRC, 1985). The effect of tannins depends on the amount consumed by animals. However, the rate at which the amount is consumed results in toxicity. For example, the amount eaten in a rush could have a negative impact, whereas the same amount, when eaten slowly, would cause no harm

Ideally, silage has a normal pH that ranges from 3.8 – 5.0. (Eisner *et al.*, 2006). In the current study, the results fell within the range, *Acacia nilotica* pods with urea (4.91) and *Acacia nilotica* leaves with molasses (5.2). Silage with a pH higher than 4.8 involves ensiling crop with dry

matter greater than 50%, thus restricting fermentation. However, a high pH due to restricted fermentation is not indicating that silage is poor. Silage cannot be subject to development of mould, which can weaken quality of feed since the low levels in pH restrict microorganisms from developing, such as clostridia (Amer *et al.*, 2012). According to Petterson (1988), silage with a pH below 4.5 is considered high quality. When silage pH is below 4.5, it is preferred because it limits proteolytic bacterial activities (van Man and Wiktorsson, 2002). Measurement of pH provides a fast and most straightforward way to determine the quality of silage. There are many ways to determine pH on farms; bromophel blue papers and bromocresol green ranging from 2.8-4.4 and 4.2-5.6. Poor fermentation can be caused by low dry matter in silage.

3.7 Conclusion

The use of urea, molasses, and ensiling affect secondary compounds and pH. The study shows low levels of tannins while pH was in the accepted range. With the values obtained, the silage would still be ingested by animals. This study needs to further research secondary compounds before and after ensiling and other fermentation parameters such as lactic acid to evaluate silage quality fully.



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4 CHAPTER THREE: Effect of ensiling *Acacia nilotica* and *Vachellia karoo* pods and leaves with urea and molasses on chemical composition and *in vitro* degradability of silage

4.1 Abstract

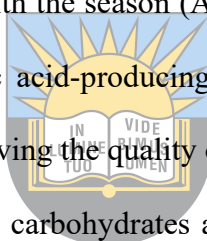
The objective of the study was to investigate the effect of ensiling *Acacia nilotica* and *Vachellia karoo* pods and leaves with molasses and urea on chemical composition and *in vitro* degradability of silage. The pods and leaves were harvested in April from both browse trees. The browse materials were mixed with 3% of molasses or urea. The samples were then packed and compacted by hand pressing into 1 litre consol jars with airtight fitting lids. After closing, the jars were kept at room temperature for 42 days. The silage was analysed for chemical composition and *in vitro* degradability. The chemical compositions of browse pods and leaves silages are presented in Table 4.1. There were significant differences ($P < 0.05$) between all the treatments. For dry matter, *Vachellia karoo* with molasses (95.29), and *Acacia nilotica* leaves with urea (95.15) had high dry matter content ($P < 0.05$) than other treatments. While *Acacia nilotica* pods with molasses had high DM% (94.08) compared to *Vachellia karoo* pods with molasses (93.64), *Acacia nilotica* pods with urea (92.90) and *Vachellia karoo* pods with urea (92.87) were the lowest. There were significant differences ($P < 0.05$) between NDF, *Vachellia karoo* leaves with urea had high percentage (65.99) compared to *Vachellia karoo* leaves with molasses (54.18) and *Acacia nilotica* leaves with urea (48.93). In comparison, *Acacia nilotica* pods with urea (62.00) and *Acacia nilotica* pods with molasses (55.92), while for *Vachellia karoo* pods with molasses (45.19) and *Vachellia karoo* pods with urea (48.18) had the low percentages ($P > 0.05$). For crude protein, *Vachellia karoo* leaves with molasses (11.41), *Vachellia karoo* leaves with urea (9.89), *Acacia nilotica* leaves with molasses (8.60) had a high CP content than *Acacia nilotica* leaves with urea (7.84). At the same time, *Vachellia karoo* pods with urea (11.90), *Acacia nilotica* pods with urea (9.21), *Vachellia karoo* pods

with molasses (9.18), had high CP content than *Acacia nilotica* pods with molasses (7.31). For ADL, *Acacia nilotica* pods with urea (20.77) had high ADL levels than *Acacia nilotica* pods with molasses (17.13), *Vachellia karroo* pods with molasses (15.46) and *Vachellia karroo* pods with urea (13.36). For leaves, *Vachellia karroo* leaves with molasses (16.15), *Acacia nilotica* leaves with urea (15.56), *Acacia nilotica* leaves with molasses (10.73) had high ADL levels than *Vachellia karroo* leaves with urea (8.49). Ash also had significant differences ($P < 0.05$) between the treatments (silage mixtures). The *in vitro* digestibility silage is shown in Table 4.2. There was an interaction between treatments and time (hours). The *in vitro* digestibility of treatments against time was significantly different ($P < 0.05$) within columns. At 0-hour, *Acacia nilotica* leaves with urea (201.80) had high level than *Acacia nilotica* leaves with molasses (184.67), and *Vachellia karroo* leaves with urea (148.67). In contrast, *Vachellia karroo* pods with molasses recorded (488.23) while *Acacia nilotica* pods with urea (134.63) had low level. *Acacia nilotica* leaves with urea recorded (248.13) at 6 hours high ($P < 0.05$) value compared to *Acacia nilotica* leaves with molasses (225.18). In contrast, *Acacia nilotica* pods with molasses (416.95) recorded the higher value compared to *Vachellia karroo* pods with molasses. There were no significant differences ($P > 0.05$) in 12 hours. *Acacia nilotica* leaves with urea (313.95) recorded higher value than other silage mixtures. In conclusion, *Acacia nilotica* and *Vachellia karroo* (pods and leaves) can be considered of greater nutritional value than grasses with low digestibility and highly fibrous.

Keywords: Silage, Urea, Molasses, Chemical composition, Degradability, *Acacia nilotica*, *Vachellia karroo*

4.2 Introduction

Browse trees could be used as feed supplements, especially in high-producing animals, if their digestibility is improved (Wanapat *et al.*, 2009). Many studies have investigated the improvement of the feeding value of forage (Shreck, 2013; Wanapat *et al.*, 2009). One of the methods used was treating feed with urea through ammoniation (Diaz *et al.*, 2013; Mapato *et al.*, 2010; Moreira Filho *et al.*, 2013). Urea improves digestibility by breaking bonds of lignin, cellulose, and hemicellulose (Vadiveloo, 2003). It also plays a role in synthesizing microbial protein by converting ammonia into rumen microbial protein (Mapato *et al.*, 2010; Schroeder and Titgemeyer, 2008; Shreck, 2013). Ruminants digest silage from grass and tree legumes, improving production (Helander *et al.*, 2014). The chemical composition of forage plants can be affected by species and changes with the season (Arzani *et al.*, 2006). Ensiling minimizes nutrient wastage by promoting lactic acid-producing bacteria (Baytok *et al.*, 2005). Urea increases nitrogen content, thus improving the quality of fermentation of forage (Filya, 2001). Molasses is a source of water-soluble carbohydrates and is used with urea to prevent silage from instability (Jaurena and Pichard, 2001). Molasses has been used in silages to increase dry matter content and fermentation rate (Soderholm *et al.*, 1998). Analysis of crude protein in feed samples or silage is essential to determine needs of animals. Knowledge of protein degradation is important to make balanced diets. The *in vivo* method is said to be costly and time-consuming, but it is more accurate. Evaluation of in Sacco method by Orskov and McDonald (1979) has been performed (Norman *et al.*, 2010. Hedqvist and Uden, 2006) and is much cheaper than *in vivo*. The in Sacco involves incubating porous bags that contain feed in the rumen of fistulated animals. But because of theoretical drawbacks, it has been questioned (Dewhurst *et al.*, 1995). Other methods to substitute in Sacco have been investigated, such as N buffer solubility of feed components (Broderick, 1983). Hence the aim is to determine the



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effect of ensiling on chemical composition and in vitro degradability of *Acacia nilotica* and *Vachellia karroo* pods and leaves

4.3 Materials and Method

See on chapter three

4.3.1 Chemical Composition

Crude protein, organic matter, dry matter, and ash content of the silages were carried out in triplicates as described by AOAC (1999). The fibre components, including NDF and ADF, were determined according to Van Soest (1991). North West University Laboratory carried out the analysis.

4.3.2 Dry matter degradability

After F57 Ankom bags were inserted in the jars, carbon dioxide was purged from the jars which were then placed into the incubation chamber. A Daisy II incubator consisting of a thermostatic chamber (39°C) with four rotating jars was used. Filter bags were withdrawn at 0, 6, 12, 24, 36, and 48 hours after inoculation. Withdrawn bags were washed with demineralized water and put in the oven at 45°C overnight, and N was determined on the residues using the macro-Kjeldahl method (AOAC, 1999 method no. 984.13).

4.3.3 Statistical analysis

The data was analysed using SAS/JMP Version 14.0 software 2018; the chemical composition was analysed using the following statistical model:

$$Y_{ij} = \mu + T_i + e_{ij}$$

Where:

Y_{ij} = Measured parameter (Pods and Leaves)

μ = mean

T_i = treatments $j = (1,2)$

e_{ij} = residual error

The data was analysed for *in vitro* degradability using SAS/JMP Version 14.0 software 2018;
the *in vitro* degradability was analysed using the following statistical model:

$$Y_{ij} = \mu + T_i + H_j + (T*H)_{ij} + E_{ij}$$

Where:

Y_{ij} = Measured parameter (pods and leaves)

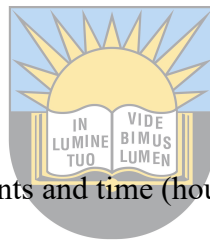
μ = mean

T_i = treatments $i = (1, 2)$

H_j = time $j = (1,2,3,4,5,6)$

$(T*H)_{ij}$ = interaction between treatments and time (hours)

e_{ij} = residual error



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4.4 Results

4.4.1 Chemical composition of silage (browse trees)

The chemical compositions of browse pods and leaves silages are presented in Table 4.1. There were significant differences ($P < 0.05$) between all the treatments. For dry matter, *Vachellia karroo* with molasses (95.29), and *Acacia nilotica* leaves with urea (95.15) had high dry matter content ($P < 0.05$) than other treatments. While *Acacia nilotica* pods with molasses had high DM% (94.08) compared to *Vachellia karroo* pods with molasses (93.64), *Acacia nilotica* pods with urea (92.90) and *Vachellia karroo* pods with urea (92.87) were the lowest. There were significant differences ($P < 0.05$) between NDF, *Vachellia karroo* leaves with urea had high percentage (65.99) compared to *Vachellia karroo* leaves with molasses (54.18) and *Acacia nilotica* leaves with urea (48.93). In comparison, *Acacia nilotica* pods with urea (62.00) and *Acacia nilotica* pods with molasses (55.92), while for *Vachellia karroo* pods with molasses (45.19) and *Vachellia karroo* pods with urea (48.18) had the low percentages ($P > 0.05$). For crude protein, *Vachellia karroo* leaves with molasses (11.41), *Vachellia karroo* leaves with urea (9.89), *Acacia nilotica* leaves with molasses (8.60) had a high CP content than *Acacia nilotica* leaves with urea (7.84). At the same time, *Vachellia karroo* pods with urea (11.90), *Acacia nilotica* pods with urea (9.21), *Vachellia karroo* pods with molasses (9.18), had high CP content than *Acacia nilotica* pods with molasses (7.31). For ADL, *Acacia nilotica* pods with urea (20.77) had high ADL levels than *Acacia nilotica* pods with molasses (17.13), *Vachellia karroo* pods with molasses (15.46) and *Vachellia karroo* pods with urea (13.36). For leaves, *Vachellia karroo* leaves with molasses (16.15), *Acacia nilotica* leaves with urea (15.56), *Acacia nilotica* leaves with molasses (10.73) had high ADL levels than *Vachellia karroo* leaves with urea (8.49). Ash also had significant differences ($P < 0.05$) between the treatments (silage mixtures).

Table 4.1: Effect of ensiling using additives on chemical composition of *Acacia nilotica* and *Vachellia karoo* (pods and leaves)

Browse species	%DM	%NDF	%ADF	%ASH	%ADL	%CP	OM g/kg
<i>A. N</i> PM	94.08 ^{ab}	55.92 ^b	39.93 ^{ab}	9.60 ^c	17.13 ^b	7.31 ^f	844.84 ^a
<i>A. K</i> LU	94.25 ^{ab}	65.99 ^a	37.25 ^{abc}	14.96 ^a	8.49 ^d	9.89 ^{bc}	792.88 ^b
<i>A. K</i> PU	92.87 ^b	45.18 ^d	33.94 ^c	9.02 ^c	13.46 ^{bcd}	11.90 ^a	838.54 ^a
<i>A. K</i> PM	93.64 ^{ab}	48.19 ^d	34.47 ^{bc}	8.99 ^c	15.46 ^{bc}	9.18 ^{cd}	846.48 ^a
<i>A. K</i> LM	95.29 ^a	54.18 ^{bc}	38.11 ^{abc}	11.93 ^b	16.15 ^b	11.41 ^a	833.58 ^a
<i>A. N</i> LU	95.15 ^a	48.93 ^{cd}	33.02 ^c	11.62 ^b	15.56 ^{bc}	7.84 ^{ef}	835.33 ^a
<i>A. N</i> LM	94.75 ^a	57.23 ^b	63.56 ^{bc}	14.88 ^a	10.73 ^{cd}	8.60 ^{de}	798.66 ^b
<i>A. N</i> PU	92.90 ^b	62.00 ^a	42.71 ^a	9.26 ^c	20.77 ^a	9.21 ^{cd}	836.43 ^a
S.E	0.52	1.72	1.85	0.28	1.60	0.29	5.15
Level of significant	**	***	*	***	**	***	***

ab c d e f Means with different superscripts within columns are significantly different according (P<0.05). S.E= standard error. DM: dry matter percent; ADF: acid detergent fibre; NDF: neutral detergent fibre; CP: crude protein; ADL: acid detergent lignin; OM: organic matter. ANPM: *Acacia nilotica* Pods Molasses, AKLU: *Acacia karroo* Leaves Urea, AKPU: *Acacia karroo* pods Urea, AKPM: *Acacia karroo* Pods Molasses, AKLM: *Acacia karroo* Leaves Molasses, ANLU: *Acacia nilotica* Leaves Urea, ANLM: *Acacia nilotica* Leaves Molasses, ANPU: *Acacia nilotica* Pods Urea

4.4.2 In vitro digestibility

The *in vitro* digestibility silage is shown in Table 4.2. There was an interaction between treatments and time (hours). The *in vitro* digestibility of treatments against time was significantly different ($P < 0.05$) within columns. At 0-hour, *Acacia nilotica* leaves with urea (201.80) had high level than *Acacia nilotica* leaves with molasses (184.67), and *Vachellia karroo* leaves with urea (148.67). In contrast, *Vachellia karroo* pods with molasses recorded (488.23) while *Acacia nilotica* pods with urea (134.63) had low level. *Acacia nilotica* leaves with urea recorded (248.13) at 6 hours high ($P < 0.05$) value compared to *Acacia nilotica* leaves with molasses (225.18). In contrast, *Acacia nilotica* pods with molasses (416.95) recorded the higher value compared to *Vachellia karroo* pods with molasses. There were no significant differences ($P > 0.05$) in 12 hours. *Acacia nilotica* leaves with urea (313.95) recorded higher value than other silage mixtures.



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Table 4.2: Effect of ensiling using additives on in vitro dry matter degradability of *Acacia nilotica* and *Vachellia karroo* (pods and leaves)

Treatment	0	6	12	24	36	48
A.N LM	184.67 ^{B b}	225.18 ^{B b}	235.95 ^{A b}	378.34 ^{AB ab}	338.88 ^{A ab}	496.52 ^{A a}
A.K PM	488.23 ^{A a}	167.71 ^{B c}	258.62 ^{A bc}	316.60 ^{ABCabc}	351.21 ^{A ab}	393.18 ^{AB ab}
A.KPU	219.15 ^{B b}	233.12 ^{B b}	234.80 ^{A b}	444.08 ^{A a}	311.59 ^{A sb}	411.08 ^{AB a}
A.NPM	126.22 ^{B b}	416.95 ^{A a}	145.34 ^{A b}	194.89 ^{c b}	246.22 ^{A ab}	306.32 ^{B ab}
A.NLU	201.80 ^{B b}	248.13 ^{A a}	313.95 ^{A ab}	326.28 ^{ABC ab}	327.16 ^{A ab}	403.42 ^{AB a}
A.KLU	148.67 ^{B b}	121.81 ^{B b}	176.37 ^{A b}	219.45 ^{BC b}	296.74 ^{A ab}	402.10 ^{AB a}
A.NLC	123.52 ^{B b}	280.16 ^{B ab}	192.11 ^{A ab}	249.14 ^{BC ab}	277.79 ^{A ab}	345.59 ^{AB a}
A.NPU	134.63 ^{B a}	161.04 ^{B a}	144.10 ^{A a}	200.39 ^{BC a}	207.84 ^{A a}	278.96 ^{B a}
S.E	64.45	64.45	64.45	64.45	64.45	64.45
Level of significant	*	*	Ns	*	Ns	**

^{abc} means with different superscript within row are significantly different (P<0.05).

^{ABC} means with different superscript within column are significantly different (P<0.05).

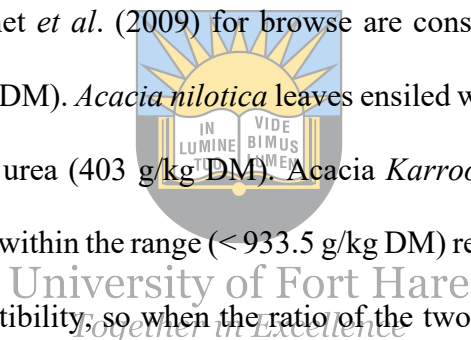
S.E standard error. ANPM: *Acacia Nilotica* Pods Molasses, AKLU: *Acacia Karroo* Leaves Urea, AKPU: *Acacia karroo* pods Urea, AKPM: *Acacia Karroo* Pods Molasses, AKLM: *Acacia Karroo* Leaves Molasses, ANLU: *Acacia Nilotica* Leaves Urea, ANLM: *Acacia Nilotica* Leaves Molasses, APU: *Acacia Nilotica* Pods Urea

4.5 Discussion

Chemical composition determines the quality of browse silage feed. In this study, dry matter content ranged from 95.29 to 94.25. *Acacia Karroo* leaves ensiled with molasses (95.29), and *Acacia nilotica* leaves with urea (95.15) had higher dry matter content than *Vachellia karroo* leaves with urea (94.25) and *Acacia nilotica* leaves with molasses (94.75). High dry matter means more intake of silage (Aderinboye *et al.*, 2016). Legume trees have been used as feed for livestock because they contain high protein contents even in the dry season (Tolera *et al.*, 1997). The values of CP in table 4.1 have shown to be above the minimum level required (7%) for microbial activities in the rumen (Njidda, 2011), suggesting that all the treatments can maintain ruminants. The high protein content acts as an advantage to microorganisms in the rumen when the concentration of tannins is average (Aderinboye *et al.*, 2016). The study reported that the protein was high (>14%) in the leaves; thus, the plants can be used as supplements to roughages. Values obtained for OM ranged from 792 to 844 g/g, which were similar to the findings of (Njida *et al.*, 2012), where the results ranged from 742 to 868 g/kg. NDF is a dominant factor that determines the quality of any forage. When forage contains 40% of NDF is considered high in quality, unlike 60% (Shaver *et al.*, 2002). The study's values were not more than 65, % and they fall between the acceptable levels (65%). Legume silages containing 60% of NDF and less are considered good (Hoffman *et al.*, 2001). NDF in the current study was higher than the range (15.4-61.9) percent reported by Mtui *et al.* (2009). Results for ADF (33-63) percent were higher than those reported by Rubanza *et al.* (2003) and Mokoboki *et al.* (2011). Meaning, environmental conditions such as moisture and the stage at which the browse was harvested could be the reason why the percentage of ADF was high (Norton and Waterfall 2000). Low fibre (NDF and ADF) contents suggest high nutritional value since fibre plays a significant role in digestibility and intake. Acid detergent lignin reported from the current study was below 20% and was higher than 11% reported by Mtui *et al.* (2009); therefore, they would not harm the availability of crude protein. Ash was high,

which means minerals are also high in the silage so that this silage can be used as a supplement to ruminants fed on low-quality roughages.

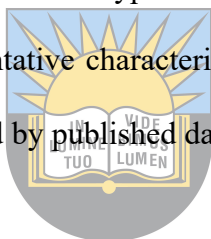
Digestibility is an essential factor when measuring the nutrition value of animal feed. In this study, dry matter disappearance increased as incubation was increasing. *Acacia nilotica* leaves ensiled with urea (248.13 g/kg DM) at 6 hours recorded the highest ($P<0.05$) compared to *Acacia nilotica* leaves with molasses (225.18 g/kg DM). In comparison, *Acacia nilotica* pods ensiled with molasses (416.95 g/kg DM) recorded the highest than *Acacia Karroo* pods with molasses. This could be a result of ruminal microbial composition speeding the degradation of samples. All the treatments (0-48 hours) had high levels of DMD. The high DMD was comparable to results (300-847) g/kg DM reported by (Madibela *et al.*, 2006). The digestibility values reported by Yayneshet *et al.* (2009) for browse are consistent with the results in the present study (123-488 g/kg DM). *Acacia nilotica* leaves ensiled with molasses (496 g/kg DM), *Acacia nilotica* leaves with urea (403 g/kg DM), *Acacia Karroo* leaves with urea (402 g/kg DM) (IVDMD₄₈ values) fall within the range (<933.5 g/kg DM) reported by Aster *et al.* (2012). Sugar and fibre affect digestibility, so when the ratio of the two is low, then DMD becomes higher (Rodrigues *et al.*, 2001). The addition of urea to the silage increased digestibility due to increasing cell wall hydrolysis. Contrary to Keskin *et al.* (2005), these results reported that urea or urea plus molasses to sorghum silages decreased the IVDMD compared with the control.



4.6 Conclusion

Ensiling can be used as an option to enhance nutrient utilization of *Acacia nilotica* and *Acacia Karroo* (pods and leaves) for ruminants. The application of urea and molasses improved the digestibility of silage. So the addition of additives affects the in vitro degradability of silage. Crude protein was well preserved in both browse trees. *Vachellia karroo* leaves with urea and molasses had a higher CP content than *Acacia nilotica* leaves with urea and molasses. Good management of silage and the use of additives can improve the quality of silage produced. So silage can be used to improve the feeding value of browse with the use of additives. The making of silage from urea and molasses could contribute to browse use as feed for ruminants better.

Further work to test other additives on silage for ruminant growth performance should be pursued. Determining an appropriate additive type and fermentation period to silage crops is essential in improving silage's fermentative characteristics and nutritional values. Producers should use additives that are supported by published data.



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5 Chapter 5

5.1 General discussion

During the dry season, crude protein in the grass and other plants becomes insufficient for animals. Browse trees are green all year with a high crude protein even in the winter season when grasses have peaked. Browse trees contain tannins, which restrict the use of leaves and pods. Hence, the study's objective was to evaluate the effect of ensiling pods and leaves of *Acacia nilotica* and *Vachellia karroo* mixed with grass, using molasses and urea as additives. In chapter 2, the study reviewed the importance of browse trees in feeding animals and factors that affect the ensiling process. Shortage of crude protein was a major challenge, which affects livestock, especially in dry seasons. Although some of these browse trees contain tannins that can be toxic to animals when ingested in large amounts, browse trees play a vital role in maintaining livestock in small and large-scale farming.

In Chapter 3, the effect of ensiling using the following additives (urea and molasses) on secondary compounds and pH was determined. There were significant differences between treatments in phenolics and tannins. In pH all the treatments were significantly different. This was attributed to the use of additives. According to (Santos *et al.*, 2005), using molasses increases WSC content, resulting in the reduction of pH increasing DM content. The levels of condensed tannins below the range of 100g/kg DM are considered to depress the intake of browse trees (Mbomi *et al.*, 2011). Therefore, condensed tannins found in the current study are primarily preferred in animals.

Further research (Chapter 4) was done to determine nutritional composition and digestibility. There were significant differences among all the treatments in their dry matter content. *Acacia Karroo* leaves mixed with molasses, and *Acacia nilotica* leaves mixed with urea had higher dry matter content. While *Acacia Karroo* leaves with urea, and *Acacia nilotica* pods with urea had higher CP content than other treatments. The digestibility of treatments against time was

significantly different within the columns. These results were in line with the results reported by (Madibela *et al.*, 2006), indicating that ensiling and additives affect nutritive value and secondary compounds in plants.

5.2 Conclusion

According to this current study, urea and molasses can be used when ensiling to improve the feeding value of browse (leaves and pods) by reducing secondary compounds. Crude protein was within the accepted range. The current study also established that digestibility is affected by ensiling and the use of molasses and urea. It is crucial to produce feed with high nutritional value and microbiological quality to ensure good animal health and growth. Additives can be valuable tools in improving silage quality, such as increasing nutritional content or decreasing molding during storage and feed-out; however, the use of additives does not replace good management practices. Producers should use additives that are supported with published data.

5.3 Recommendations

According to the present study, communal farmers can harvest *Acacia nilotica* and *Vachellia karoo* (pods and leaves) in rangelands and feed livestock during the dry season when grasses fail to retain their quality. When harvesting pods and leaves, care should be taken as they contain thorns that can cause harm. It is advisable to mix the pods or leaves with grass to avoid the toxicity of tannins. Further research should include the use of levels for each additive when ensiling browse.



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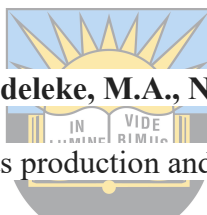
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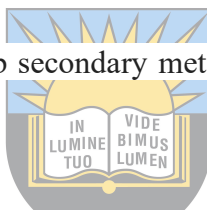
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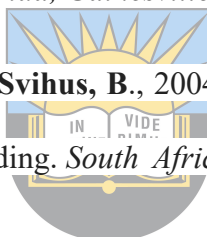
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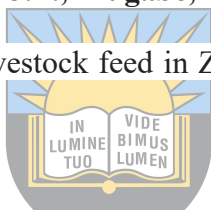
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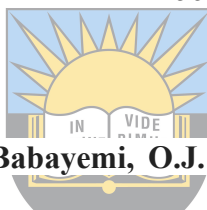
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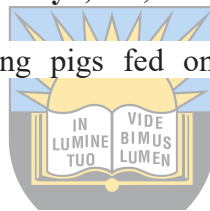
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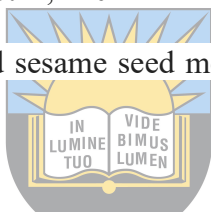
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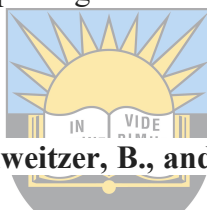
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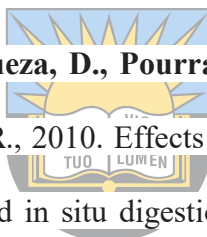
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APPENDICES

Appendix 1: Ethical clearance certificate AREC-150311-008

Certificate Reference number: GAJ011STWA01

Appendix 2:

Table 3.1: Effect of ensiling using additives on phenolics in pods and leaves of *Acacia nilotica* and *Vachellia karroo*

Source	Nparm	DF	Sum of Squares	F Ratio	Prob > F
Treatm	7	7	0,35443994	29,8628	<,0001*

ent



Table 3.2: Effect of ensiling using additives on tannins in *Acacia nilotica* and *Vachellia karroo* (pods and leaves)

Source	Nparm	DF	Sum of Squares	F Ratio	Prob > F
Treatment	7	7	0,09489275	231,2342	<,0001*

Table 3.3: Effect ensiling using additives on pH levels of *Acacia nilotica* and *Vachellia karroo* (pods and leaves)

Source	Nparm	DF	Sum of Squares	F Ratio	Prob > F
Treatment	7	7	4,6031437	103,8645	<,0001*

Table 4.1: Effect of ensiling using additives on chemical composition of *Acacia nilotica* and *Vachellia karroo* (pods and leaves)

DM% content

Source	Nparm	DF	Sum of Squares	F Ratio	Prob > F
Treatment	7	7	12,236435	3,2647	0,0596

NDF% content

Source	Nparm	DF	Sum of Squares	F Ratio	Prob > F
Treatment	7	7	740,97031	17,8046	0,0003*

ADF% content

Source	Nparm	DF	Sum of Squares	F Ratio	Prob > F
Treatment	7	7	155,90544	3,2422	0,0607

ASH% content

Source	Nparm	DF	Sum of Squares	F Ratio	Prob > F
Treatm	7	7	88,689674	82,6654	<,0001*

ent

ADL% content

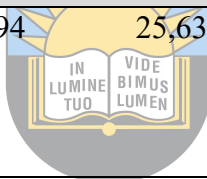
Source	Nparm	DF	Sum of Squares	F Ratio	Prob > F
Treatme	7	7	204,17699	5,6687	0,0130*

nt

CP % content

Source	Nparm	DF	Sum of Squares	F Ratio	Prob > F
Treatm	7	7	29,870994	25,6375	<,0001*

ent



OM (g/kg content

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Source	Nparm	DF	Sum of Squares	F Ratio	Prob > F
Treatme	7	7	5970,8466	16,0689	0,0004*
nt					

Table 4.2: Effect of ensiling using additives on *In vitro* dry matter degradability of *Acacia nilotica* and *Vachellia karroo* (pods and leaves)

Source	Nparm	DF	Sum of Squares	F Ratio	Prob > F
Treatment*	35	35	367677,22	1,2644	0,2230
Time(Hours)					
Treatment	7	7	214455,07	3,6876	0,0029*
Time(Hours)	5	5	356548,96	8,5832	<,0001*



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