Alternative use of various plant parts of *Vachellia nilotica* as fodder for small ruminants: Implications for woody plant encroachment

by

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DECLARATION 1: PLAGIARISM

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I declare that the above dissertation is my own work and that all the sources that I used or quoted have been indicated and acknowledged by means of complete reference/s.

I further declare that I submitted the dissertation to originality checking software and that it falls within the accepted requirements for originality.

I further declare that I have not previously submitted this work, or part of it, for examination at UNISA for another qualification or at any other higher education institution.

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PUBLICATIONS

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ABSTRACT

Woody plant encroachment is regard as a common problem in South African rangelands, however edible twigs and seed pods from these plants are generally regarded as an important source of protein to livestock during the dry season. Livestock, especially small ruminants feed more on the seed pods of these woody plant, which, in turn, results in the dispersal of scarified seeds back in the rangelands through their feces. This, in turn, results in further woody plant encroachment and bush thickening. The current study aimed to explore the use of *Vachellia nilotica* plant parts (seed pods and edible twigs) as fodder for small ruminants to fill the dry season feed shortage. To achieve this aim, the study examined the possibility of reducing the dispersal of viable seeds in rangelands and controlling bush encroachment by including *Vachellia nilotica* seeds into livestock feeds. The seed pods and edible shoots of *Vachellia nilotica* were harvested separately, and analyzed for mineral, protein and fibre contents at different seed pod inclusion rates, with or without the addition of feed additive. Based on the chemical analysis, six diets (1) 100% *V. nilotica* seed pods (un-chipped), 2) 100% *V. nilotica* seed pods (chipped), 3) 100% *V. nilotica* seed pods (un-chipped) + feed additive, 4) 100% *V. nilotica* seed pods (chipped) + feed additive, 5) 4:4 (*V. nilotica* shoots: *V. nilotica* chipped seed pods) and 6) 4:4 (*V. nilotica* shoots: *V. nilotica* chipped seed pods) + feed additive) were selected to feed 24 goats and measure the number of seeds that was recovered and germinated after ingestion. Results from the study showed significant differences ($p < 0.05$) with higher concentrations of CP, TDN, K, Mg, P and Zn in the 100% *V. nilotica* seed pods compared to the 100% *V. nilotica* shoots. Furthermore, a relatively high ($p < 0.05$) digestible dry matter (DDM), digestible organic matter (DOM), metabolizable energy (ME), net energy for lactation (NE_L), and net energy for growth (NE_G) were observed in the 100% *V. nilotica* seed pods, shoots + seed pods and 100% *V. nilotica* seed pods. The concentrations of N, P, K, and CP from the 100% shoots fodder significantly increased ($p < 0.05$) upon the addition of feed additives (i.e.

Voermol LS33). The highest seed pod inclusion of 100% shoot fodder, the content level of DDM, TDN, DOM, ME, NE_L, and NE_G significantly increased ($p < 0.05$). No significant differences ($p \geq 0.05$) were observed in mineral content between the 100% shoot and the shoots + seed pod fodders, irrespective of the seed pod inclusion levels. In the feeding trial, no differences ($p \geq 0.05$) were observed in feeds consumed (58- 83%) and remained (17-35%) among the six experimental diets. However, seeds ingested were significantly higher ($p < 0.05$) when the seed pods were chipped, and this also influenced significantly ($p < 0.05$) the number of seed recovered in the diets compared to the control. Less than 2% of the chipped seed pods diet were recovered after ingestion, while 3% and 6% of seeds in the diets containing whole seed pods with or without Voermol LS33. The study found that chipping seed pods before incorporating them into livestock diets led to a significant decrease in the number of seeds recovered. Therefore, chipping the seed pods prior to adding to the shoot material in diets significantly reduces the number of seeds that could potentially be dispersed through endozoochory, thereby reducing bush encroachment. However, it should be taken into consideration that the majority of the 2% of seeds recovered were dormant but still viable. Another aim of the study was to explore the possibility of whether *V. nilotica* edible shoots (chipped) can be preserved in the form of silage. The ensiling lasted for 60 days, and fermentation characteristics, dry matter, mineral nutrients and aerobic stability were analysed within 4 treatments: 1) control (shoots only without molasses), 2) shoots + seed pods (without molasses), 3) shoots + molasses (without seed pods), and 4) shoots + molasses + seed pods. The study found that dry matter content of *V. nilotica* materials in all treatments significantly differ ($p < 0.05$) at ensiling, but they all fall within the recommended DM range of 45-55% for high DM legume silages. The same was true for CP (7-8%), ADF, NDF and ash content. Although significant differences were also observed in pH level between days and treatments, it was within the

recommended level of 4.3-5.0. Therefore, results from this work show that all parameters measured fall within the recommended range of well-fermented and preserved legume silage. These findings demonstrate that *V. nilotica* can be effectively preserved as silage in the wet season in order to feed during dry season or to counter the feed shortage during dry season. However, more research is necessary to determine whether the addition of inoculants will likely affect the nutritional quality of the silage produced.

Key words: encroachment, germination, livestock, seed pods, silage

DEDICATIONS

I dedicate this thesis to my parents Mr. Makasela Jameson and Mrs. Tintswalo Noria Manganyi.

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Chapter 1: General introduction and literature review

1.1 Bush encroachment in South Africa

Savannas are ecosystems characterized by trees, shrubs, grasses, herbs, and forbs; which serve as a major source of feed for both human and wild animals (Acocks, 1988; Stevens et al. 2017; Sebata, 2017). Savannas occupy about 60% of Sub-Saharan Africa, 54% of southern Africa and 34% of South Africa, and they support the majority of the world's livestock production (Scholes and Archer, 1997). Previous research showed that savannas have experienced rapid conversion from continuous grass layer and a discontinuous layer of woody plants due to bush encroachment (Smit, 2012; Belayneh and Tessema, 2017). Thus, bush encroachment is one of the most prevalent form of land degradation in African rangelands. (Ward, 2005; Scholes, 2009).

Encroachment of woody plants is one of the frequent issues linked to deteriorating of rangelands conditions and has been reported throughout southern African savannas (Smit, 2004). It has a negative impact on rangelands by reducing carrying capacity for livestock and subsequently increasing the costs associated with livestock management (Ward, 2005; Hagos and Smit, 2005; Kgosikoma et al. 2012). The interaction and/or individual factors, such as decline in soil moisture, continuous overgrazing, fire suppression, nutrients and global warming, have been associated with woody plant encroachment and bush thickening (Scholes and Archer, 1997; van Auken, 2009). In addition, Gxasheka et al. (2017) reported that factors such as heavy grazing and drought encourage the establishment of woody seedling by reducing grass cover.

A well-known factor leading to woody plant encroachment and thickening is the distribution of viable seeds by livestock (Whitacre and Call, 2006; Tjelele et al. 2015). Studies has shown that ruminants particularly goats, sheep and to a certain extent cattle are distributors of viable seed in the rangeland because they consume a significant volume of woody plant seeds during the dry season (Frost et al. 2013; Tjelele, 2014; Tjelele et al. 2015; Marchetto et al. 2020). On the other hand, the twigs and seed pods of woody plants and shrubs are important sources of feed for domestic animals due to their high protein content (Du et al. 2023). Consequently, the use of trees and shrubs as animal feed is becoming more widely acknowledged, and they are good source of protein, especially under harsh environmental conditions, and periods of drought (Heuzé et al. 2016). This is particularly true when there is insufficient grazing to meet the maintenance needs for producing livestock (Abdalla et al. 2014).

1.2 Common encroaching species and their use as an alternative feed to livestock

Encroaching woody species such as *Vachellia nilotica*, *V. tortilis*, *V. erioloba*, *V. karroo* and *Dichrostachys cinerea*, amongst others, have been used as potential supplements to livestock during the dry season in the form of cut-and-carry resource, leaf meal and seed pods (Mapiye et al. 2010; Uguru et al. 2014; Mnisi and Mlambo, 2016). Seed pods from species such as *V. nilotica*, *V. tortilis*, and *V. karroo* are browsed directly from the tree/shrubs by goats, and/or can be eaten from the ground by other livestock species (Chepape et al. 2011; Koireng et al. 2015). Furthermore, more recent fodder flow activities include the use of these seeds and seed pods as supplements for livestock during the dry season (Mlambo et al. 2011; Ravhuhali et al. 2020). In each of these scenarios, some of the seeds consumed by these animals escape digestion and are dispersed intact with solid excreta and may germinate and establish in the rangelands leading to woody plant encroachment (Gill and Al-shankiti, 2015). The spread and speed at which woody plant encroachment is occurring calls for an urgent need to develop management strategies such as new feed resources to mitigate fluctuating quality and quantity of natural feed resources (Vandermeulen et al. 2017; Du et al. 2023).

Edible twigs of woody plants can be harvested in summer and preserved using various methods including among others silage for use during feed scarcity (SANA, 2015; Du et al. 2023). Silage is one of the common preservation techniques, and its goal is to preserve the forage crop (i.e. grass or woody material) as similar to its original quality as possible (Wilkinson and Davies, 2012; Nkosi et al. 2016). Ensiling is a growing practice for preserving forages worldwide in the smallholder sector (Wilkinson and Toivonen, 2003; Kung et al. 2018; Jones, 2021; Jade, 2022) but has been long practiced by the larger agricultural sector. Additionally, silage is produced by controlled fermentation of a crop of high moisture content, and its production involves the achievement of anaerobic conditions and discouraging the activity of undesirable microorganisms. The characteristics rapid drop in pH of the ensiled material is considered a better way to conserve forage crops (McDonald et al. 1991).

1.3 *Vachellia nilotica* as one of the common encroaching plant species in South African rangelands

Vachellia nilotica is one of the encroaching browse tree species, and it is typically found in most parts of the bush veld of South Africa (Venter and Venter, 2000). This species can grow and reach up to 10 m height with an average of 4-7 m in height (Van Wyk et al. 2000; Fagg and Mugedo, 2005). Flowers are scented, bright yellow in colour and their pods are shaped like a necklace with constrictions between the seeds. *Vachellia nilotica* edible plant parts including leaves, twigs and pods are an important source of protein supplements, especially in dry areas (Randford et al. 2001; Orwa et al. 2009; SANA, 2015). Le Houerou (1980) reported that *V. nilotica* pods and leaves contain about 12.4% crude protein and 7.2 MJ/kg of energy. Several studies explored factors governing woody plant encroachment, specifically seed dispersal by livestock (Cain et al. 1998; Tjelele, 2014; Tjelele et al. 2015). Despite the various research interventions, the rate and extent of woody plant encroachment is worsening, and on the side feed shortage, especially during drought and dry periods is a concern for livestock farmers.

1.4 Rationale and motivation

The poor quality and insufficient feed that is available from natural pastures during the dry season is one of the major limiting factors in extensive livestock production systems in South Africa (Mudzengi et al. 2020). This challenge legitimizes efforts towards the search for alternative and locally accessible supplements for sustaining and enhancing the productivity of ruminants in the face of inadequate feed resources. During the dry season, conventional feeds are often used as a reliable feed resource. Browse tree species are a potential source of feed during the dry season for ruminant survival (Mnisi and Mlambo, 2016; Stevens et al. 2017). Edible plants part of encroaching *Vachellia s*pecies (twigs, leaves and seed pods) are good sources of protein to livestock, and therefore are proposed as a feed supplement to improve livestock production in dry season (Uguru et al. 2014; Ravhuhali et al. 2020; Marchetto et al. 2020).

Under rangeland conditions the seed pods of these encroaching tree species are consumed in large quantities by livestock. However, this can also result in the spread of the encroaching species throughout the rangelands as the seeds pass through the livestock gut, they are scarified and those that are not completely digested can still be viable in the livestock faeces and in this way, can be spread throughout the rangeland (Tjelele, 2014; Gill and Al-shankiti, 2015; Tjelele et al. 2015; Sebata, 2017; Marchetto et al. 2020). Although smallholder and resource poor livestock producers occasionally use protein-rich supplementary feeds and concentrates during these dry periods to supplement the poor quality of forage, these sources are often expensive and inaccessible to financially constrained farmers. Therefore, the nutritional evaluation of nonconventional feed resources is essential to minimize feed costs for farmers, while optimizing livestock production.

1.5 Aim and objectives

The study's main objective was to determine the utilization of encroaching *V. nilotica* components i.e. seed pods and edible twigs as fodder and at the same time reduce the spread of viable seeds in rangelands which can cause further bush encroachment. To achieve this, the following objectives were pursued:

1. To determine the nutritional quality and the potential use of *V. nilotica* as fodder for ruminants.
2. To investigate the effect of seed ingestion by goats on seed recovery
3. To assess the potential of feed additives and ensiling as complementary methods for preservation of *V. nilotica* fodder.

1.6 Hypotheses

The study hypothesized that:

1. Feed additives will improve the nutritional qualities of the fodder and digestibility of seed pods consumed by goats and reduces seed viability and dispersal.
2. Chipping, ensiling and feed additives will improve the nutritional quality of *V. nilotica* for livestock production.

This study includes one published, and one submitted (Under review) paper chapters for various journals. As a result, they have different formatting. Therefore, some information may be repeated, particularly when describing the study area and references.

Chapter 2

The potential for endozoochorous dispersal of *Vachellia nilotica* seeds by goats: Implications for bush encroachment

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2.1 Abstract

Seed dispersal has become an important component in understanding plant–animal interaction. Recently, there have been concerns about the role of ruminants, particularly browsers, in the dispersal of woody plant seeds. This study aimed to determine whether including *Vachellia nilotica* seeds in livestock diets could reduce the spread of viable seeds in the rangelands and control bush encroachment. The shoots and seed pods of *V. nilotica* were harvested and analyzed for fibre, protein, and mineral nutrients at different seed pods inclusion rates, with or without a feed additive. Six diets were selected for feeding 24 goats and quantifying seed recovery and germination after ingestion. Results indicated that including seed pods and feed additives to *V. nilotica* shoots significantly improved the quality of the fodder. Chipping the seed pods prior to including them in the diet resulted in 13% intact seed recovery, and approximately 2% of these seeds were recovered after ingestion. These recovered seeds were mostly still viable but still dormant as seed coats were not sufficiently damaged after ingestion. Therefore, chipped pod inclusion to ruminant diets can effectively reduce seed dispersal in rangelands.

Keywords: chipping, feed additives, seed germination, seed pods, woody thickening

2.2 Introduction

Many researchers consider bush encroachment as a significant rangeland problem that negatively affects livestock production and the livelihoods of farmers (Mlambo et al. 2004; O'connor et al. 2014; Smits and Prins, 2015). This is because bush encroachment suppresses the growth of herbaceous vegetation and therefore reduce productivity and the quality of rangeland forage resources (O'connor et al. 2014; Stevens et al. 2016). Furthermore, increasing tree densities in rangelands reduce forage accessibility to livestock, further negatively affecting the utilization of rangelands by livestock (Lesoli et al. 2013; Smits and Prins, 2015; Abbas et al. 2019). Although there has been significant financial investment into the eradication of the encroaching woody plant species, the interventions have not yielded conclusive management strategies due to the persistent and adaptive nature of the encroaching species (O'connor et al. 2014). In addition, extensively reared livestock often make use of the encroaching tree species, making them useful resources to farmers, which makes it difficult to motivate the eradication of the encroaching woody plant species (Abbas et al. 2019).

Seed dispersal by herbivores has become an important issue in plant ecology, where both wild game animals and livestock play an important role in endozoochorous seed dispersal (Abbas et al. 2019). Many reasons have been given for the increased rate and extent of encroaching species (O'connor et al. 2014). One among many drivers of this is the influence of endozoochory, i.e. seeds that are ingested and subsequently dispersed by livestock (Or and Ward, 2007; Tjelele, 2014; Tjelele et al. 2015). This is because the pods of many woody plants form an important part of the diet of livestock and wildlife during the dry season due to their high nutritive value (Tjelele, 2014) compared to grasses and herbaceous vegetation in this season. However, some of the seeds that remain intact after ingestion pass through the gastro intestinal tract, where they are scarified, which in turn allows for greater efficiency in the dispersal and effective seedling establishment of the encroaching plant species (Tjelele, 2014; Tjelele et al. 2015).

Endozoochorous seed dispersal is influenced by several factors, such as seed size, hardiness, animal species and amount of seeds consumed, survival from chewing and rumination (Tjelele, 2014; Tjelele et al. 2015; Jaganathan et al. 2016; Abbas et al. 2019). Other studies show that small seeds (<2.5 mm in width) are most likely to escape chewing and rumination compared to large seeds, whereas seeds with a hard coat are more likely to pass through the gut without physical damage (Frost et al. 2013; Jaganathan et al. 2016; Marchetto et al. 2020). To limit further spreading of encroaching tree species in rangelands and, at the same time, improve rangeland conditions and reduce dry season feed shortage, the active harvesting of the encroaching tree species as a feed source has been proposed (Mnisi and Mlambo, 2016; Mnisi and Mlambo, 2017; Mokoboki et al. 2019). There are positive effects that thinning and/or eradication of the encroaching trees have on rangeland condition (O'connor et al. 2014; Mnisi and Mlambo, 2017; Mapiye et al. 2019). However, there is limited information on how these trees could potentially be used as alternative feed sources by livestock, other than their use as leaf meal (Abbas et al. 2019; Mapiye et al. 2019).

It has been observed that encroaching tree species are highly nutritious and have a high potential of being used as supplementary feed (Gebeyew et al. 2015; Ravhuhali et al. 2020). However, some of the woody plant species have very low dry matter digestibility and high levels of secondary compounds and, thus, have low potential for use as feed sources without additional processing (Mlambo et al. 2011). The digestibility of these fodder trees can, however, be improved through different processing techniques. Furthermore, most studies focus on using the trees as a cut-and-carry resource without the addition of feed additives and mostly neglect the impacts of seed ingestion on the potential for contributing to further bush encroachment (Mapiye et al. 2010; Mapiye et al. 2019;). This, in turn, leaves a knowledge gap of whether or not feed additives can enhance the utilization of woody plant seeds as ruminant feed.

It is yet to be validated whether or not feeding woody plant seeds to ruminants can be used as an alternative strategy to minimize bush encroachment in light of the possible spread thereof through endozoochory. This study evaluated the nutritional quality of *V. nilotica* fodders (edible shoots and twigs) with and without seed pods and the inclusion of feed additives. After nutrient composition analysis, the best quality treatments were selected for feeding experiment based on their protein, fibre and energy content. The study intended to answer the following questions: (1) will the inclusion of seed pods as well as feed additives improve the nutritional quality of the *V. nilotica* fodders and (2) whether the inclusion of the chipped seed pods and feed additive could reduce seed recovery and viable seeds.

2.3 Materials and methods

2.3.1. *Seed Collection, Preparation, and Initial Viability Screening*

Vachellia nilotica seed pods were hand-picked at the Agricultural Research Council (ARC) Roodeplaat experimental farm (28°19´E, 25°35´S) in Pretoria, South Africa. Seed pods were separated from the shoots and stored in a cool, dry area pending feed formulation. Five replicates of whole seed pods were weighed to a mass of 250 g, and the number of seed pods within each replicate was counted (Lacey et al. 1992; Tjelele et al. 2015). Thereafter, the seeds were then extracted from the seed pods, and the number of seeds counted. An additional ten replicates of 250 g seed pods were chipped using a Tandem 6.5 hp chipper, and the number of whole seeds (i.e., undamaged seeds) recovered was quantified after chipping. Thereafter, a representative number of seeds from the chipped and un-chipped treatments were used to determine the initial viability of the seeds. A sample of un-chipped seeds were scarified by clipping the seed coat with a clipper to expose the embryo. The seeds were immersed in a 1% Tetrazolium chloride solution (3,5-triphynyl chloride) for 18 h in a dark germination chamber and stored at room temperature. In order to reveal the embryo, each seed was longitudinally dissected through the endosperm, and evaluated for staining through a light microscope (ISTA, 1985; 2012). Seeds that stained red were regarded as viable, while unstained seeds were regarded as dead.

2.3.2 *Feed formulation and Nutritional Quality Determination*

Edible *Vachellia nilotica* shoots consisting of edible branches and leaves (30 cm long and approximately 1.0 cm diameter) were harvested using the tree pruner at the end of the wet season (March–April 2021) at the fruiting stage (i.e. plants with seed pods). During this time, all plant material still containing green leaves were harvested, but the seed pods were harvested towards the end of this period at maturity. The shoots and pods were collected from 70 different trees; seed pods were collected when matured and dry and were kept separate from shoots and merged differently to form a composite sample. The samples of shoots were chipped using a woodchipper (Tandem 6.5 hp chipper/shredder) and mixed thoroughly to obtain a uniform mixture. A uniform sample of 250 g chipped shoots was used as the base, and chipped seed pods were included in a 4:1, 4:2, 4:3, and 4:4 ratio. For each feed treatment formulated, the seed pods were chipped separately and included into the chipped shoots and mixed. Three replicates of each treatment were developed, as well as two control treatments (250 g chipped shoots and 250 g chipped seed pods), and a feed additive (Voermol LS33) at a recommended rate of 800 mL/10 kg DM for the small stock was added in all six treatments, resulting in a total of 12 feed treatments (i.e., six with the feed additives and six without the feed additives). A sub-sample of 150 g of each feed was collected, oven-dried at 60° C until a constant mass was achieved and milled to pass through a 3 mm mesh and stored for chemical analyses. From the dried and milled feed samples, a 0.5 g sub-sample was digested using a technique described by Zasoski et al. (1977). After digestion, an aliquot of the digested solution was used for the determination of calcium (Ca), magnesium (Mg), phosphorus (P), potassium (K), sodium (Na), manganese (Mn), iron (Fe), zinc (Zn), and copper (Cu) using an ICP-OES (Inductively Coupled Plasma Optical Emission Spectrometer—Agilent 725 (700 Series), Agilent Technologies, Santa Clara, CA, USA). The ICP-OES can determine the quantity of each element in each sample simultaneously. Prior to analyses, the instrument was calibrated against a series of standard solutions containing all the elements of interest in alignment with the operating procedures of the manufacturer. Furthermore, 8–12 g of the plant samples were used to determine the total nitrogen (N) concentrations using the dry oxidation method (Jimenez and Ladha, 1993; Majejovic, 1995) in a Flash 2000 CHNS-O Analyzer (Thero Scientific, Waltham, MA, USA). For each analysis, the instrument was calibrated against a known standard (Phenylalanine) which contained 8.48% N. Total N was converted to crude protein (CP) by multiplying %N with 6.25 (Meissner, 2000). Neutral detergent fibre (NDF) and acid detergent fibre (ADF) were determined using a Dosi fibre analyzer system (Labex (Pty) Ltd., Edenvale, South Africa) according to the methods of van Soest et al. (1991). The NDF and ADF values obtained were used to calculate the digestible dry matter (DDM) (Rasby et al. 2008), metabolizable energy (ME) (Meissner et al. 2000), total digestible nutrients (TDN) (Schoeder, 2009), digestible forage energy (DFE) (Meissner et al. 2000), digestible organic matter (DOM) (Meissner et al. 2000), net energy for lactation (NEL) (Rasby et al. 2008), net energy for maintenance (NEM) (Rasby et al. 2008), and net energy for gain/growth (NEG) (Rasby et al. 2008) using Equations (1-8) below.

1. DDM (%) = 88.9 – (ADF × 0.779)

2. ME (Mcal/kg DM) = (1.01× DFE) – 0.45

3. TDN (%) = 87.84 – (0.7 × ADF)

4. DFE (Mcal/kg DM) = 0.04409 × TDN

5. DOM (%) = TDN ÷1.05

6. NE_L (Mcal/kg DM) = 1.044 – (0.0119 × % ADF)

7. NE_M (Mcal/kg DM) = ((1.37 ×ME) – (0.3042 × ME) + (0.051 × ME)) – 0.508

8. NE_G (Mcal/kg DM) = ((1.42 × ME) – (0.3836 × ME) + (0.0593 × ME)) – 0.7484

2.3.3 Feeding Trial and Seed Recovery

Based on the nutritional analyses, six experimental diets, each representing an experimental treatment, were used based on their protein level for the feeding trial (Table 2.1). The feeding and seed recovery trial was conducted at the ARC, Irene experimental farm, Gauteng province, South Africa. A total of 24 female indigenous veld goats (South African veld goats) of approximately two years of age with an average body weight of 29.6 ± 1.33 kg were used in the study. The 24 goats were divided into four groups of six per group. Each animal in a group was regarded as a replicate. The animals were acclimatized to the experimental conditions for 14 days prior to the start of data collection, during which time they were fed chipped *V. nilotica* shoots and grass hay. The goats were housed in individual metabolic pens (2 m ×1 m) with slatted floors, each fitted with feed and water troughs in a well-ventilated covered area. After acclimatization, each animal was fed the experimental diet at 3% of their body weight and grass hay as a basal diet. All experimental animals were allowed to consume their assigned diets within 24 h, after which the remaining materials were collected (Tjelele, 2014). Left-over feed was weighed, and feed intake was determined by calculating the difference between the feed offered to the animals and the remaining feed. The number of seeds in the feeds was also quantified. The fecal collection commenced immediately after the experimental feeding period started and continued until no seeds were recovered in the feces for three consecutive days. The number of seeds recovered per day per animal was recorded in order to calculate the seed recovery percentage. The collected feces were immersed in cold water until soft and then washed with tap water through a wire mesh until the water was clear. A cabinet with a light source below a glass surface was used to separate seeds from the fecal remains. Seeds recovered from each animal per day were counted and stored in brown paper bags prior to the germination trial. Using these counts, the number of days when the first seeds were recovered from the fecal matter after ingestion was recorded. At the end of the trial, the number of days to 10%, 50%, and 90% of the total number of seeds recovered was calculated. From these calculations, the time taken from 10% to 90% seed recovery was determined and used as an indication of seed recovery uniformity".

Table 2.1. Experimental diets used for feeding goats.

Experimental Diets	
1	100% *V. nilotica* seed pods (un-chipped)
2	100% *V. nilotica* seed pods (chipped)
3	100% *V. nilotica* seed pods (un-chipped) + Feed Additive
4	100% *V. nilotica* seed pods (chipped) + Feed Additive
5	4:4 (*V. nilotica* Shoots: *V. nilotica* chipped seed pods)
6	4:4 *(V. nilotica* Shoots: *V. nilotica* chipped seed pods) + Feed Additive

2.3.4 Germination of Recovered Seeds

Germination tests were performed at the ARC National Forage Genebank Seed Laboratory according to the International Seed Testing Association standards (ISTA, 2012). The recovered seeds per animal per day were counted and stored in a brown paper bag resulting in six replicates per experimental feed treatment pending germination trial. The three control treatments, i.e., unscarified seeds, seeds that passed through the chipper, and mechanically scarified seeds by scarifying the seed coat with a clipper, were created. All seeds were germinated in 12 cm petri dishes on a single disk of Whatman No. 1 filter paper. The petri dishes were maintained in seed germination chambers at a temperature of 25° C (Tjelele, 2014) for the duration of the trial. The seeds were watered with 5 mL of distilled water (dH_2O), and watering was done as needed throughout the duration of the germination period. Every day for 28 days, the germination of the seeds was monitored, once a radicle protrudes, the seed was removed from the petri-dish, on a daily basis to minimize excessive water uptake by germinated seeds. The total germination percentage was calculated at the end of the germination period following the technique by Armke and Scott (1999). After 28 days, all the seeds that did not germinate were counted and put through a viability test using tetrazolium chloride solution (Tjelele, 2014). Seeds were scarified and soaked in 1% tetrazolium solution (3,5-triphynyl chloride) for 18 h in an incubator at 25° C. Thereafter, each seed was cut longitudinally through the endosperm to expose the embryo, and staining was recorded by viewing the seeds under a stereo microscope (ISTA, 1985; 2012).

2.3.5 *Statistical Analysis*

Nutritional quality, seed recovery, and seed viability data were subjected to a one-way analysis of variance (ANOVA) followed by a Fishers' LSD post hoc test to separate means and identify statistically significant differences ($p < 0.05$) between the different treatments.

2.4 Results

2.4.1. *Seed Pods and Seed Characteristics*

The collected seed pods contained, on average, 10 ± 0.2 seeds per pod and had an average weight of 2.7 ± 0.1 g. After chipping ten replicates of 250 g intact seed pods, with each replicate containing approximately 1108 ± 24 seeds, approximately 141 ± 22 intact seeds (13 ± 0.3%) were recovered. The viability of the intact seeds recovered after chipping was 97%, and the unscarified seeds tested (after removal from the seeds pods) had a viability of 96%.

2.4.2. *Nutritional Quality of V. nilotica Fodders*

Significantly ($p < 0.05$) higher concentrations of N, K, Mg, P, Zn (Table 2.2), CP, and TDN (Table 2.3) were found in the 100% *V. nilotica* seed pods compared to the 100% *V. nilotica* shoots. Furthermore, the *V. nilotica* fodders formulated from 100% *V. nilotica* seed pods also had significantly ($p < 0.05$) higher digestibility, lower ADF and NDF, higher DDM, DOM, and higher energy content (ME, NEL, and NEG) compared to the fodders containing 100% *V. nilotica* shoots (Table 2.3). When the seed pods were added to the shoots, N, P, K and CP content significantly increased ($p < 0.05$) from the 100% pure shoot fodder (Table 2.2) and (Table 2.3). The treatment with 100% shoot fodders at the highest seed pod inclusion levels, i.e., 4:4 shoots/pods significantly increased ($p < 0.05$) the content level of the DDM, TDN, DOM, ME, NE_L, and NE_G (Table 2.3). No significant differences ($p \geq 0.05$) were found in any of the other mineral nutrients between the 100% shoot fodders and the shoots + seed pod fodders, irrespective of the seed pod inclusion levels (Table 2.2). Neutral detergent fibre content significantly decreased ($p < 0.05$) when seed pods were added to the shoots, irrespective of the seed pod inclusion levels, while ADF content only decreased at the highest seed pod inclusion levels (Table 2.3).

The addition of the feed additive (Voermol LS33- molasses-based protein, vitamin, and mineral supplement) to the 100% *V. nilotica* seed pods and 100% *V. nilotica* shoot fodders significantly increased ($p < 0.05$) the concentrations of N, K, Mg, Na, Mn, and Zn. Phosphorus concentrations were only significantly increased ($p < 0.05$) when the additive was added to the shoots (Table 2.2). Similarly, the addition of the feed additives to the 100% seed pods and 100% shoot fodders significantly ($p < 0.05$) increased the CP content (Table 2.3). Feed additive added to 100% seed pods significantly decreased ($p \geq 0.05$) ADF and NDF content; however, no significant differences ($p \geq 0.05$) were observed on DDM, TDN, DOM, NE_L, and NE_G, whereas there was a significant increase ($p < 0.05$) when feed additives were added to the 100% *V. nilotica* shoot fodders (Table 2.3). The addition of feed additive to the fodders created from the pure *V. nilotica* shoot + the different seed pod inclusion levels, irrespective of the inclusion levels, resulted in significantly higher ($p < 0.05$) concentrations of K, Mg, Na, Mn, Zn, and Cu (Table 2.2) and CP (Table 2.3), while N only increased at the 4:1 and 4:2 seed pod inclusion levels (Table 2.2) and DDM, TDN, DOM, NE_L, and NE_G only increased at the 4:2 and 4:3 seed pod inclusion levels (Table 2.3).

Table 2.2. Mean (±SEM) mineral nutrient content in experimental diets created from *V. nilotica* shoots and seed pods with or without the addition of a feed additive (Voermol LS33). Different letters for each variable measured indicate statistically significant differences ($p<0.05$) between different experimental diets within a column. P = probability, F = ratio of statistics.

Experimental Diet	N%	K (g/kg DM)	Ca (g/kg DM)	Mg (g/kg DM)	P (g/kg DM)	Na (g/kg DM)	Fe (g/kg DM)	Mn (g/kg DM)	Zn (g/kg DM)	Cu (g/kg DM)
100% Seed pods	2.2 ± 0.1^{b}	16.1 ± 0.3^{f}	5.8 ± 0.3^{a}	16.7 ± 0.2^{cd}	1.7 ± 0.1^{f}	0.1 ± 0.003^{a}	0.06 ± 0.003^{a}	0.02 ± 0.001^{a}	0.03 ± 0.001^{b}	0.01 ± 0.0004^{a}
100% Shoots	1.1 ± 0.1^{a}	6.7 ± 0.1^{a}	6.4 ± 0.4^{a}	11.6 ± 1.6^{ab}	0.7 ± 0.1^{a}	0.2 ± 0.003^{a}	0.16 ± 0.02^{bc}	0.03 ± 0.005^{bc}	0.03 ± 0.001^{a}	0.01 ± 0.0004^{b}
4:1 (Shoots:Seed pods)	1.5 ± 0.01^{a}	8.6 ± 0.2^{b}	6.6 ± 0.2^{a}	14.3 ± 1.3^{bc}	0.8 ± 0.03^{b}	0.1 ± 0.004^{a}	0.18 ± 0.02^{bcd}	0.03 ± 0.004^{c}	0.02 ± 0.001^{a}	0.01 ± 0.0011^{b}
4:2 (Shoots:Seed pods)	1.4 ± 0.02^{a}	10.0 ± 0.2^{c}	6.2 ± 0.03^{a}	12.6 ± 0.1^{ab}	0.9 ± 0.01^{bc}	0.2 ± 0.01^{a}	0.19 ± 0.02^{cd}	0.03 ± 0.001^{bc}	0.03 ± 0.001^{ab}	0.01 ± 0.0003^{b}
4:3 (Shoots:Seed pods)	1.5 ± 0.03^{b}	10.7 ± 0.03^{cd}	5.8 ± 0.1^{a}	11.8 ± 0.3^{a}	1.0 ± 0.02^{de}	0.2 ± 0.01^{a}	0.16 ± 0.03^{bc}	0.03 ± 0.002^{ab}	0.03 ± 0.0004^{a}	0.01 ± 0.0005^{b}
4:4 (Shoots:Seed pods)	1.5 ± 0.04^{b}	10.7 ± 0.03^{cd}	5.1 ± 0.2^{a}	12.2 ± 0.5^{ab}	1.1 ± 0.01^{e}	0.2 ± 0.01^{a}	0.15 ± 0.004^{bc}	0.03 ± 0.001^{ab}	0.02 ± 0.001^{a}	0.01 ± 0.0005^{b}
100% Seed pods + LS33	2.5 ± 0.04^{c}	17.4 ± 0.3^{g}	5.7 ± 0.3^{a}	20.3 ± 0.2^{f}	1.6 ± 0.1^{f}	1.5 ± 0.2^{b}	0.08 ± 0.002^{a}	0.04 ± 0.001^{c}	0.04 ± 0.002^{c}	0.01 ± 0.0005^{b}
100% Shoots + LS33	2.3 ± 0.01^{b}	11.1 ± 0.1^{d}	8.3 ± 0.1^{a}	22.9 ± 1.0^{g}	0.9 ± 0.03^{cde}	2.1 ± 0.1^{c}	0.23 ± 0.01^{cd}	0.07 ± 0.004^{f}	0.05 ± 0.002^{c}	0.02 ± 0.0013^{d}
4:1 (Shoots:Seed pods) + LS33	2.1 ± 0.18^{b}	13.0 ± 0.6^{e}	6.5 ± 0.1^{a}	19.7 ± 1.2^{ef}	0.9 ± 0.02^{bc}	2.6 ± 0.4^{d}	0.19 ± 0.01^{cd}	0.06 ± 0.003^{e}	0.06 ± 0.004^{d}	0.02 ± 0.0003^{cd}
4:2 (Shoots:Seed pods) + LS33	1.9 ± 0.03^{b}	13.2 ± 0.3^{e}	5.7 ± 0.2^{a}	18.2 ± 0.3^{def}	1.0 ± 0.1^{e}	1.7 ± 0.1^{bc}	0.16 ± 0.01^{bc}	0.05 ± 0.001^{d}	0.05 ± 0.001^{c}	0.01 ± 0.0006^{c}
4:3 (Shoots:Seed pods) + LS33	2.0 ± 0.03^{b}	13.9 ± 0.4^{e}	5.7 ± 0.1^{a}	17.3 ± 0.3^{de}	1.1 ± 0.02^{e}	1.9 ± 0.1^{bc}	0.15 ± 0.01^{bc}	0.05 ± 0.001^{d}	0.05 ± 0.003^{c}	0.01 ± 0.0004^{c}
4:4 (Shoots:Seed pods) + LS33	2.0 ± 0.11^{b}	13.8 ± 0.2^{e}	5.5 ± 0.2^{a}	17.7 ± 0.8^{de}	1.0 ± 0.1^{e}	1.8 ± 0.4^{bc}	0.14 ± 0.01^{b}	0.05 ± 0.003^{d}	0.05 ± 0.004^{c}	0.01 ± 0.0005^{c}
Significance	$F_{(11,36)} = 9.5$	$F_{(11,36)} = 118.2$	$F_{(11,36)} = 1.0$	$F_{(11,36)} = 21.1$	$F_{(11,36)} = 54.0$	$F_{(11,36)} = 38.1$	$F_{(11,36)} = 9.5$	$F_{(11,36)} = 32.9$	$F_{(11,36)} = 32.3$	$F_{(11,36)} = 16.5$
	$p \leq 0.001$	$p \leq 0.001$	$p = 0.474$	$p \leq 0.001$	$p \leq 0.001$	$p \leq 0.001$	$p \leq 0.001$	$p \leq 0.001$	$p \leq 0.001$	$p \leq 0.001$

N = Nitrogen, K = Potassium, Ca = Calcium, Mg = Magnesium, P = Phosphorus, Na = Sodium, Fe = Iron, Mn = Manganese, Zn = Zinc, Cu = Copper.

Table 2.3. Mean (±SEM) crude protein, fibre, digestibility, and energy content in experimental diets created from *V. nilotica* shoots and seed pods with or without the addition of a feed additive (Voermol LS33). Statistically significant differences ($p<0.05$) between different experimental diets are indicated by different letters for each variable measured. P = probability, F = ratio of statistics.

Experimental Diet	CP%	ADF%	NDF%	DDM%	TDN%	DOM%	DFE (Mcal/kg DM)	ME (Mcal/kg DM)	NE_L (Mcal/kg DM)	NE_M (Mcal/kg DM)	NE_G (Mcal/kg DM)
100% Seed pods	13 ± 0.4^{de}	28.3 ± 0.9^{a}	29.3 ± 1.1^{ab}	66.1 ± 2.1^{de}	67.4 ± 0.6^{cd}	64.2 ± 0.6^{cd}	3.0 ± 0.03^{a}	2.6 ± 0.03^{b}	0.7 ± 0.01^{d}	2.3 ± 0.03^{a}	2.1 ± 0.03^{d}
100% Shoots	7 ± 0.8^{a}	39.6 ± 2.9^{b}	48.6 ± 3.1^{e}	58.0 ± 2.3^{a}	60.1 ± 2.0^{a}	57.2 ± 1.9^{a}	2.7 ± 0.09^{a}	2.2 ± 0.09^{a}	0.6 ± 0.03^{a}	2.0 ± 0.10^{a}	1.7 ± 0.10^{a}
4:1 (Shoots:Seed pods)	9 ± 0.1^{b}	35.9 ± 0.8^{b}	39.7 ± 2.7^{d}	60.9 ± 0.6^{ab}	62.7 ± 0.6^{ab}	59.7 ± 0.5^{ab}	2.8 ± 0.02^{a}	2.3 ± 0.03^{a}	0.6 ± 0.01^{ab}	2.1 ± 0.03^{a}	1.8 ± 0.03^{ab}
4:2 (Shoots:Seed pods)	9 ± 0.1^{b}	36.0 ± 1.1^{b}	39.5 ± 0.4^{d}	60.9 ± 0.9^{ab}	62.7 ± 0.8^{ab}	59.7 ± 0.7^{ab}	2.8 ± 0.03^{a}	2.3 ± 0.03^{a}	0.6 ± 0.01^{ab}	2.1 ± 0.04^{a}	1.8 ± 0.04^{ab}
4:3 (Shoots:Seed pods)	9 ± 0.2^{b}	31.9 ± 0.4^{b}	35.8 ± 1.6^{abc}	61.0 ± 0.3^{ab}	62.7 ± 0.3^{ab}	59.8 ± 0.3^{ab}	2.8 ± 0.01^{a}	2.3 ± 0.01^{a}	0.6 ± 0.01^{ab}	2.1 ± 0.01^{a}	1.8 ± 0.01^{ab}
4:4 (Shoots:Seed pods)	10 ± 0.2^{b}	29.3 ± 1.9^{a}	30.0 ± 1.1^{ab}	66.1 ± 1.5^{de}	67.3 ± 1.3^{cd}	64.1 ± 1.3^{cd}	3.0 ± 0.06^{a}	2.6 ± 0.06^{b}	0.7 ± 0.02^{d}	2.3 ± 0.07^{a}	2.0 ± 0.06^{d}
100% Seed pods + LS33	16 ± 0.3^{f}	25.6 ± 1.2^{a}	30.0 ± 1.6^{ab}	65.8 ± 0.9^{cde}	67.1 ± 0.8^{cd}	63.9 ± 0.8^{cd}	3.0 ± 0.04^{a}	2.5 ± 0.04^{b}	0.7 ± 0.01^{cd}	2.3 ± 0.04^{a}	2.0 ± 0.04^{cd}
100% Shoots + LS33	14 ± 0.1^{e}	30.1 ± 0.7^{a}	32.9 ± 0.9^{bc}	65.5 ± 0.5^{cd}	66.8 ± 0.5^{cd}	63.6 ± 0.5^{cd}	2.9 ± 0.02^{a}	2.5 ± 0.02^{ab}	0.7 ± 0.01^{cd}	2.3 ± 0.02^{a}	2.0 ± 0.02^{cd}
4:1 (Shoots:Seed pods) + LS33	12 ± 1.1^{c}	34.5 ± 1.4^{b}	48.1 ± 1.4^{bcd}	62.0 ± 1.1^{bc}	63.7 ± 1.0^{bc}	60.7 ± 1.0^{bc}	2.8 ± 0.04^{a}	2.4 ± 0.05^{a}	0.6 ± 0.02^{bc}	2.2 ± 0.05^{a}	1.9 ± 0.05^{bc}
4:2 (Shoots:Seed pods) + LS33	12 ± 0.2^{c}	29.7 ± 2.7^{a}	30.5 ± 1.0^{ab}	65.8 ± 2.1^{cde}	67.0 ± 1.9^{cd}	63.9 ± 1.8^{cd}	3.0 ± 0.08^{a}	2.5 ± 0.08^{b}	0.7 ± 0.03^{cd}	2.3 ± 0.09^{a}	2.0 ± 0.09^{cd}
4:3 (Shoots:Seed pods) + LS33	12 ± 0.2^{c}	27.2 ± 1.2^{a}	31.1 ± 1.0^{ab}	67.7 ± 1.0^{de}	68.8 ± 0.9^{d}	65.5 ± 0.8^{d}	3.0 ± 0.04^{a}	2.6 ± 0.04^{b}	0.7 ± 0.01^{d}	2.4 ± 0.04^{a}	2.1 ± 0.04^{d}
4:4 (Shoots:Seed pods) + LS33	12 ± 0.7^{c}	24.9 ± 1.1^{a}	37.9 ± 4.2^{cd}	69.5 ± 0.9^{e}	70.4 ± 0.8^{d}	67.1 ± 0.8^{d}	3.1 ± 0.03^{a}	2.7 ± 0.04^{b}	0.8 ± 0.01^{d}	2.5 ± 0.04^{a}	2.2 ± 0.04^{d}
Significance	**$F_{(11,36)}= 29.0$** **$p \leq 0.001$**	**$F_{(11,36)}= 8.0$** **$p \leq 0.001$**	**$F_{(11,36)}= 10.1$** **$p \leq 0.001$**	**$F_{(11,36)}= 8.1$** **$p \leq 0.001$**	**$F_{(11,36)}= 7.9$** **$p \leq 0.001$**	**$F_{(11,36)}= 7.4$** **$p \leq 0.001$**	**$F_{(11,36)}= 1.0$** **$p = 0.474$**	**$F_{(11,36)}= 3.6$** **$p = 0.004$**	**$F_{(11,36)}= 8.3$** **$p \leq 0.001$**	**$F_{(11,36)}= 1.0$** **$p = 0.474$**	**$F_{(11,36)}= 8.0$** **$p \leq 0.001$**

CP = Crude protein, ADF = Acid detergent fibre, NDF = Neutral detergent fibre, DDM = Digestible dry matter, TDN = Total digestible nutrients, DOM = Digestible organic matter, DFE = Digestible forage energy, ME = Metabolizable energy, NE_L = Net energy for lactation, NE_M = Net energy for maintenance, NE_G = net energy for gain/growth.

2.4.3. *Selection of Diets, Feeding Trial, and Seed Recovery after Ingestion*

Vachellia nilotica feeds were selected based on relatively high of protein, energy and low fibre level for the feeding trial as experimental diets (Table 2.3). Generally, feed intake did not differ statistically among the six experimental diets ($p > 0.05$; Table 2.4). However, the number of seeds ingested was significantly higher ($p < 0.05$) when the seed pods were chipped, irrespective of the addition of the feed additives (Table 2.4). The number of seeds recovered was significantly lower ($p < 0.05$) in the diets containing chipped seed pods compared to unchipped seeds, i.e. whole seed pods (Table 2.4). Less than 2% of the chipped seed pods were recovered after ingestion, while 3% and 6% of seeds in the diets containing whole seed pods with or without Voermol LS33 were recovered (Table 2.4). No differences ($p \geq 0.05$) were found between the experimental diets in regard to when the first seeds were recovered from the feces (Table 2.4), while significant differences ($p < 0.05$) were observed in the number of seeds recovered between experimental diets. However, it was evident that the seed retention rate generally remained longer ($p < 0.05$) in the digestive tract of goats when they were fed diets containing whole seed pods, irrespective of the addition of the feed additive (Table 2.4). Furthermore, uniformity in seed recovery, calculated as the time taken between 10% and 90% of seeds recovered, indicated that the diets containing chipped seed pods with the addition of the feed additives resulted in a significantly ($p < 0.05$) shorter retention period in the gut, i.e., two days, while the % recovery of the seed per day was spread over 5–6 days long (Table 2.4).

Table 2.4. Mean (±SEM) feed, seed ingested, and seed recovery from the goats. Statistically significant differences ($p \geq 0.05$) between different experimental diets are indicated by different letters for each variable measured. P = probability, F = ratio of statistics.

Treatments	Feed ingested	Feed Remains	% seed ingested	% Seed remains	% Recovery	1st recovery	50% recovery	90% recovery	Uniformity
Whole Seed pods	64.7 ± 9.8[a]	35.3 ± 9.8[a]	58.8 ± 9.7[a]	41.2 ± 9.7[b]	3.5 ± 1.3[b]	1.7 ± 0.2[a]	3.8 ± 0.3[c]	5.7 ± 0.3[c]	3.0 ± 0.4[cd]
Whole Seed pods + LS33	65.7 ± 9.7[a]	34.3 ± 9.7[a]	57.4 ± 9.9[a]	42.6 ± 9.9[b]	6.1 ± 2.2[c]	1.0 ± 0.0[a]	3.5 ± 0.2[bc]	6.2 ± 0.6[c]	4.0 ± 0.6[d]
Chipped seed pods	68.9 ± 10.9[a]	31.1 ± 10.9[a]	90.1 ± 6.7[b]	9.9 ± 6.7[a]	0.6 ± 0.2[a]	1.5 ± 0.2[a]	2.3 ± 0.5[a]	3.7 ± 1.0[a]	2.2 ± 0.9[bc]
Chipped seed pods + LS33	83.0 ± 8.6[a]	17.0 ± 8.6[a]	85.2 ± 6.7[b]	14.8 ± 6.7[a]	0.8 ± 0.3[a]	1.5 ± 0.2[a]	2.8 ± 0.4[ab]	3.8 ± 0.3[b]	1.8 ± 0.2[a]
Chipped (shoots, seed pods)	57.9 ± 6.4[a]	42.1 ± 6.4[a]	90.8 ± 6.8[b]	9.2 ± 6.8[a]	0.8 ± 0.2[a]	1.7 ± 0.3[a]	3.0 ± 0.4[bc]	4.3 ± 0.5[b]	2.3 ± 0.6[bc]
Chipped (shoots, seed pods) + LS33	78.1 ± 7.6[a]	21.9 ± 7.6[a]	71.4 ± 8.3[b]	28.6 ± 8.3[a]	1.9 ± 1.2[a]	1.8 ± 0.3[a]	3.2 ± 0.5[bc]	4.0 ± 0.5[b]	1.7 ± 0.4[a]
Significance	**$F_{(5,36)}$= 1.10 p = 0.382**	**$F_{(5,36)}$= 1.08 p = 0.391**	**$F_{(5,36)}$= 3.58 p = 0.012**	**$F_{(5,36)}$= 3.58 p = 0.012**	**$F_{(5,36)}$= 3.55 p = 0.012**	**$F_{(5,36)}$= 0.61 p = 0.693**	**$F_{(5,36)}$= 3.60 p = 0.014**	**$F_{(5,36)}$= 10.31 p ≤ 0.001**	**$F_{(5,36)}$= 6.13 p ≤ 0.001**

The time to different germination percentages (i.e. 10 % – 90 %) were calculated using equation 1 (Farooq *et al.* 2004), where final germination percentage was greater than 50 %. Germination uniformity was then calculated as the time (days) taken to go from 10 % to 90 % of the final germination percentage.

2.4.4 Germination Potential of Recovered Seeds

The unscarified seeds that were not fed to the goats were mostly dormant (88%), with only 3.2% of the seeds germinated (Table 2.5). Mechanically scarified seeds had a germination percentage of 79% (Table 2.5). Although chipping of the seed pods significantly increased ($p < 0.05$) the germination potential compared to control (unscarified seeds), more than 65% of the recovered seeds remained dormant (Table 2.5). Approximately 80% of the recovered seeds from whole seed pods diets were dormant, which was similar to the control (unscarified seeds) treatment (Table 2.5).

Table 2.5. Mean (±SEM) germination of seed recovered from the feces. Statistically significant differences ($p \geq 0.05$) between different experimental diets are indicated by different letters for each variable measured. P = probability, F = ratio of statistics.

Treatments	Germination (%)	Dormant seed (%)	Dead seed (%)
Seed pods	14.1 ± 2.8^{c}	83.0 ± 3.6^{d}	3.0 ± 1.3^{a}
Seed pods + LS33	8.7 ± 1.1^{b}	87.6 ± 1.7^{d}	3.7 ± 0.7^{a}
Chipped seed pods	16.8 ± 1.1^{c}	79.5 ± 3.0^{c}	3.7 ± 2.6^{a}
Chipped seed pods + LS33	17.1 ± 2.1^{c}	77.1 ± 2.4^{c}	5.7 ± 2.7^{a}
Chipped (shoots+ seed pods)	14.4 ± 2.2^{c}	77.8 ± 3.0^{c}	7.8 ± 1.0^{a}
Chipped (shoots+ seed pods) + LS33	13.1 ± 2.1^{c}	79.4 ± 3.2^{c}	7.4 ± 2.3^{a}
Unscarified	3.2 ± 1.0^{a}	88.4 ± 3.5^{d}	8.4 ± 2.9^{a}
Scarified	78.8 ± 2.2^{e}	0.0 ± 0.0^{a}	21.2 ± 2.2^{b}
Chipped	29.6 ± 2.9^{d}	65.2 ± 3.4^{b}	5.2 ± 1.0^{a}
Significance	**$F_{(8,45)}$= 121.3 $p \leq 0.001$**	**$F_{(8,45)}$= 89.6 $p \leq 0.001$**	**$F_{(8,45)}$= 7.8 $p \leq 0,001$**

2.5 Discussion

2.5.1 *Nutritional Quality of Vachellia nilotica Fodders*

Browse plants such as *V. nilotica* are major sources of livestock feed during the dry season, partially due to their ability to retain their nutritional value during the dry season contrary to grasses (Aruwayo and Adeleke, 2020). This, along with their rate and extent of encroachment, have a potential to be used as source of feed for livestock. At the end of the growing season, the nutritional value of browse plants may not be sufficient to sustain livestock. An example of this is reported by Britz et al. (2022), who indicated that maturing of browse plants resulted in a decline in the nutritional quality in terms of their mineral nutrients, digestibility, protein, and energy content. Thus, the best time to harvest the material for fodder is during the vegetative or early reproductive stages (Ravhuhali et al. 2020; Mudzengi et al. 2020; Britz et al. 2022). However, other studies have shown that some of the browse seed pods during the end of the wet season have higher nutritional value and could be used to improve the quality of the fodders from encroaching tree species (Mlambo et al. 2004; Uguru et al. 2014; Mokoboki et al. 2019; Ravhuhali et al. 2020; Mudzengi et al. 2020;). These findings are in accordance with the findings of the current study, where results indicated that seed pods generally contained relatively higher quality mineral nutrients, crude protein, and lower amounts of fibre and increased digestibility and energy content compared to the shoots. Both seed pods and shoots in the current study contained sufficient concentrations of K, Ca, Mg, Fe, Mn, Zn, Cu, and crude protein to meet the minimum requirements of 5–15 g/kg, 1.8–10 g/kg, 1 g/kg, 0.03–0.1 g/kg, 0.02–0.04 g/kg, 0.02–0.05 g/kg, 0.005–0.1 g/kg, and 7–8%, respectively, to maintain livestock condition (Meissner, 2000; Meissner et al. 2000). However, only the 100% seed pod diet contained sufficient P concentrations (1.6–6 g/kg) to meet the maintenance requirements of ruminants. In addition, neither the seed pods nor the shoots contained sufficient Na concentrations to meet the minimum requirements of 0.4–1.8 g/kg to maintain livestock conditions (Meissner, 2000). The fibre content in the shoots of *V. nilotica* had 28% (ADF) and 29% (NDF), and the seed pods was 39% (ADF) and 48% (NDF), which both fall within the adequate range of 19–40% ADF and 25–40% NDF, respectively, for normal rumen functions (Van Soest et al. 2008; McDonald et al. 2010; Uguru et al. 2014; Spencer, 2018). Small ruminants such as goats and sheep require high concentrations of degradable fibre in their daily diets for rumen function (Spencer, 2018). However, a high level of fibre is often associated with decreased forage intake (Rinehart, 2008; McDonald et al. 2010; Spencer, 2018). Furthermore, forages with a digestible dry matter (DDM) content of greater than 60% are regarded as high-quality forages as intake will not be impacted (Van Soest et al. 1991; Spencer, 2018). According to the study results, the *V. nilotica* shoots alone contained 58% DDM while the seed pods had a DDM content of 66%, indicating the importance of the inclusion of the seed pods in livestock diets. The energy content of the *V. nilotica* shoots and seed pods individually was sufficient to sustain the energy requirements for small ruminants (goats/sheep) during the dry season (Meissner, 2000). In addition, both shoots and seed pods had sufficient metabolizable energy (ME) content to meet the energy requirements of lambs up to 20 kg (3.9–10.5 MJ kg–1 DM) as well as those of 40–60 kg dry ewes (7.6–10.2 MJ kg–1 DM). However, neither shoots nor pods were found to have sufficient ME content (14.5–17.7 MJ kg–1 DM) to sustain pregnant and lactating (15.5–19.4 MJ kg–1 DM) ewes. The nutritional value of shoots with the addition of seed pods was found to be higher compared to shoots alone, and it contained sufficient levels of mineral nutrients, CP, digestibility, and energy content to maintain livestock conditions during the dry season (Meissner, 2000; Meissner et al. 2000; Rinehart, 2008). Furthermore, the addition of seed pods to the shoots improved the mineral nutrient content of the forages and was found to meet the minimum requirements to maintain small stock conditions. This was true for all mineral nutrients, except for P and Na, which was below the minimum requirements levels of small stock (Meissner, 2000; Meissner et al. 2000). Moreover, the addition of the feed additives to the pure seed pods and pure shoots further increased the nutritional quality of the *V. nilotica* shoots, resulting in a CP content that was suitable for maintaining highly productive livestock herds, which have a minimum requirement of 13–14% CP.

2.5.2 *Feed Intake, Seed Recovery, and Seed Germination Potential of Recovered Seeds*

Hard-coated seeds tend to be protected against damage during ingestion and rumination, which in turn results in the recovery of more undamaged seeds in the feces (Abdel, 1997; Whitacre and Call, 2006; Tjelele, 2014). Therefore, diets containing the chipped seed pods were found to be consumed in higher amounts than unchipped pods, irrespective of the addition of the feed additive in this study. Furthermore, the seed recovery was relatively high for whole seed pod diets and low for chipped seed pod diets. This might be because chipping the seed pods before feeding results in easy ingestion, making it easier to digest and thus reducing the recovery of intact seeds. The first seed recovery for all treatments ranged between 1–2 days; however, the time to 90% recovery was longer for diets that contained whole seed pods compared to chipped seed pods. Seed recovery and survival after passage through the gut depends on factors such as the hardness of the seed coat, the size of the seeds, the associated diet fed with the seed pods as well as the number of seeds ingested (Whitacre and Call, 2006; Bodmer and Ward, 2006; Tjelele, 2014). Furthermore, seed recovery and germination after ingestion may be influenced by factors such as chewing and rumination (Mlambo et al. 2005; Tjelele et al. 2015). Results from the current study showed that there was a low percentage of intact seeds that passed through the digestive tract of goats, especially from chipped seed pods diets. However, those seeds remained viable and had substantial germination potential. Although the relative viability of seeds that passed through the rumen was lower than those that were mechanically scarified and chipped seeds, it was significantly higher than untreated seeds. The relatively low viability of ingested seeds is a good indication for the likelihood of these seeds being dispersed by animals away from the parent tree. Therefore, the study partially supported the hypothesis that feed additives will improve the digestibility of seed pods consumed by goats, thus reducing seed dispersals and viability.

2.6 Conclusions

Edible *V. nilotica* shoots in this study were found to contain low crude protein content for maintaining livestock conditions during the dry season. However, adding seed pods to the shoots significantly increased the nutritional quality. Chipping of the seed pods before inclusion in livestock diets resulted in significantly lower numbers of seeds recovered, with more than 85% of seeds being broken and damaged to the point where they did not germinate. Feeding the remaining seeds led to a further reduction in seeds recovered, with only 2% of the whole seeds fed to the livestock being recovered. Therefore, processing the seed pods prior to adding these to the shoot material in diets already significantly reduces the number of seeds that could potentially be dispersed throughout the rangeland. However, it is important to remember that the majority of the 2% of seeds recovered were dormant but still viable. These seeds could potentially still lead to further bush encroachment. Therefore, further research is required to determine whether these seeds passing through the gut of the livestock will survive the dry season to germinate and establish in the next wet season.

2.7 Acknowledgments

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Chapter 3

Ensiling as a technique for preservation of the nutritional quality of *Vachellia nilotica*

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3.1 Abstract

Fodder tree species are considered to have high nutritional value and contribute significantly in feeding livestock to overcome nutrient deficiencies, particularly during the dry seasons. The study was conducted to explore the possibility of preserving chipped *Vachellia nilotica* shoots as silage. Edible plant material of *V. nilotica* (shoots and seed pods) were harvested separately, chipped and ensiled in 1.5 L anaerobic glass jars with four treatment applications, (1) control (shoots with no additive), (2) shoots + seed pods (3) shoots + molasses, and (4) shoots + molasses + seed pods. The jars were stored at room temperature (25˚C), and the ensilage lasted for 60 days. Each treatment had three jars opened on days 3, 7, 21, and 60 to determine fermentation characteristics. *V. nilotica* silages were subjected to a 5 days aerobic stability test at day 60. The samples were analyzed for their chemical composition, mineral nutrients, fermentation characteristics and aerobic stability. The study found that dry matter content of *V. nilotica* significantly differ ($p < 0.05$) at ensiling but they all fall within the recommended DM range of 45-55% for high DM silages. The same was true for CP (7-8%), ADF (35-42%) and NDF (46-50%). Although significant differences were also observed in pH level between days and treatments, it has dropped as expected to the recommended level (4.3-5.0). Butyric acid was < 0.01%, acetic acid ranged between 0.4- 0.6% and propionic acid was not detected. These findings demonstrated that *V. nilotica* edible parts can be effectively preserved as legume silage to bridge the dry season gap.

Key words: aerobic stability, fermentation, molasses, nutritive value, silage

3.2 Introduction

Leguminous fodder shrubs and trees are potential supplements to overcome nutrient deficiencies in extensive livestock production systems because they are considered to have high protein concentrations (Du et al. 2023). Furthermore, leguminous woody plants may reduce feeding costs for emerging livestock farmers, especially during the dry seasons, when the herbaceous plants in rangelands are dormant (Mlambo et al. 2004; Mokoboki et al. 2019; Ravhuhali et al. 2020). Although the production period of trees extends for longer periods into the dry season compared to grasses, higher quantities of edible biomass are produced during the wet season, with biomass and the quality of the edible portions gradually decreasing as the growing season advances to dry season (Mbatha and Bakare, 2018). In the dry season, protein-rich seed pods are available in large quantities, and these are known sources of fodder for ruminants (Tjelele, 2014; Tjelele et al. 2015).

These seed pods and edible twigs alone, do not provide adequate quantity and quality fodder needed to fill the dry season feed shortage gap (Tainton, 2000; Wilkinson and Toivonen, 2003). Therefore, due to the relatively high nutritional value of these fodder plants during the wet season, there is a need to harvest and preserve these fodders during the wet season to ensure the availability of high-quality feed for ruminants during the dry season. One possible means of doing this is through ensiling, which is a method, used to preserve the nutritional quality of fodders for feeding of livestock during the dry season (Kung et al. 2018).

Ensiling has become a popular and growing practice for preserving forages across the world (Wilkinson and Toivonen, 2003). Ensilage is an alternative method to conserve forage nutrients for use during time of feed scarcity (Kung and Shaver, 2001; Wilkinson and Davies, 2012; Jones et al. 2021). Additionally, silage is the term used for material produced by controlled fermentation of a crop of high moisture content (Kung et al. 2001; Kung et al. 2018). The production of silage involves the achievement of anaerobic conditions and discouraging the activity of undesirable microorganisms, by rapid dropping in pH of the ensiled material (McDonald et al. 1991).

The most commonly ensiled forage species are mainly grasses, cereals (oats and barley), grains (e.g. maize) (Jones et al. 2021). Similarly, woody plants contain forage nutrients required by livestock but are often utilized as fresh twigs and leaves (Zhang et al. 2019; Du et al. 2023). However, there is currently limited information on silage from indigenous browse species in southern Africa (Trytsman et al. 2023). This is probably because most browse species are classified as poor ensilage material, due to low water-soluble-carbohydrate (WSC) content, which is essential for successful ensilage (McDonald, 1991; Tjandraatmadja et al. 1993; Nussio, 2005). This study aimed to explore the possibility of ensiling chipped *V. nilotica* shoots material. We hypothesized that the material will preserved well, however, due to the low WSC content nutritional quality of ensiled material may not be as good as the raw material.

3.3 Materials and methods

3.3.1 Collection of Forage Material and Ensiling Procedure

Fresh plant materials (shoots and seed pods) of *Vachellia nilotica* were harvested separately and chipped at the Agricultural Research Council – Roodeplaat Experimental Farm, which is located in Pretoria, South Africa. Edible branches of 30 cm long and a maximum stem diameter of 1.0 cm were harvested from several *V. nilotica* plants in the wet season using a tree pruner. The harvested plant materials were chipped with a woodchipper (Tandem 6.5hp chipper / shredder) to pass through a 1 cm sieve size. The chipped *V. nilotica* plant materials were mixed thoroughly to obtain a uniform mixture. Molasses syrup was added to *V. nilotica* material before ensiling. The additives were added to the chipped materials to make four treatments: (1) control (shoots only without molasses), (2) shoots + seed pods (without molasses), (3) shoots + molasses (without seed pods), and (4) shoots + molasses + seed pods. Molasses was added at a recommended inclusion level of 4% (w/w) of the plant materials in order to increase the amount of WSC, which serve as substrates for production of lactic acid during the ensiling process. Molasses has a high viscosity, and to enable ease application over the chipped plant materials, it was mixed with warm water at a ratio of 1:2 (w/w). Therefore, 360 g of molasses was mixed with 720 g of distilled water to obtain a water of 1080 g and applied to 9 kg of fresh plant materials per treatment before ensiling. To ensure uniform application of moisture for all treatments, the control was treated by applying 1080 g of distilled water on 9 kg of fresh *V. nilotica* chipped materials.

The chipped and uniformly mixed materials were ensiled in 1.5 L anaerobic glass jars (J. Weck, GmBHu. Co., Wehr-Oflingen, Germany) equipped with lids to inhibit gas release with an average of 903.63 ± 10.27 g/L density of material per jar. A total of 48 jars were filled (i.e. 12 jars/treatment) with the chipped materials, and stored at room temperature of 25˚C. The chipped plant materials were ensiled in a completely randomized design with 3 replicates per treatment. Three jars per treatment were opened at day 3, 7, 21, and 60 to determine fermentation characteristics. After 60 days of ensilage, aerobic stability of the *V. nilotica* plant silage was determined by aerobic exposure of the silage for five days based on the protocol described by Ashbell et al. (1991).

3.3.2 Chemical analyses of pre-ensiled and ensiled plant materials

Triplicate samples were collected from pre-ensiled plant materials of each treatment for the determination of chemical composition. Furthermore, triplicate samples were collected from each treatment for the determination of fermentation characteristics, aerobic stability and chemical composition of the *V. nilotica* silage. The protocol described by Suzuki and Lund (1980) was used to determine pH, WSC, volatile fatty acids (VFAs), lactic acid (LA) and ammonia-N (NH_3-N) and Water-soluble carbohydrate content was determined by phenol-sulphuric acid method according to Dubois et al. (1956) and LA was determined by the colorimetric method of Barker and Summerson (1941) as modified by Pryce (1969). Volatile fatty acids were determined with a Varian 3300 FID detector gas chromatograph (Varian Associates, Inc., Palo Alto, CA, USA) by the procedure of Suzuki and Lund (1980). Ammonia-N was determined by distillation using a Buchi 342 apparatus and a Metrohm 65 Dosimat with an E526 titrator according to AOAC (ID 941. 04, 2016). The technique was based on the method of Pearson and Muslemuddin (1968) for determining volatile nitrogen. Carbon dioxide production, yeast and mold counts were determined from the aerobically exposed silage at day 60, based on the protocols described by Ashbell et al. (1991). Counts of yeast and mold were conducted according to ISO 21527-1 (2008). Lactic acid bacteria (LAB) count was conducted according to ISO 15214 (1998).

The DM content of fresh *V. nilotica* plant materials and silage was determined by drying the samples at 60 °C until a constant weight, and only the DM of silage samples was corrected for the loss of volatiles by using the equation of Porter and Murray (2001). After drying, samples of fresh *V. nilotica* plant materials and silage were ground to pass through a 1mm sieve (Wiley mill, Standard Model 3, Arthur H. Thomas Co., Philadelphia, PA, USA) for chemical analyses. Acid detergent fibre (ADF) content of fresh *V. nilotica* plant materials and silage samples were determined using a Fibretec System 1010 (FOSS Analytical AB, Sweden) by boiling samples in an acidic solution followed by filtration (ID 973.18, AOAC, 2016; 2019). Neutral detergent fibre (NDF) content of fresh *V. nilotica* plant materials and silage samples were determined by using amylase and sodium sulphate (Van Soest et al. 1991).

For mineral analysis, a 0.5 g sub-sample was digested using a technique described by Zasoski et al. (1977). After digestion, an aliquot of the digested solution was used for the determination of calcium (Ca), magnesium (Mg), phosphorus (P), potassium (K), sodium (Na), manganese (Mn), iron (Fe), zinc (Zn), and copper (Cu) using an ICP-OES (Inductively Coupled Plasma Optical Emission Spectrometer-Agilent 725 (700 Series), Agilent Technologies, Santa Clara, CA, USA). The ICP-OES can determine the quantity of each element in each sample simultaneously. Prior to analyses, the instrument was calibrated against a series of standard solutions containing all the elements of interest in alignment with the operating procedures of the manufacturer. Furthermore, 8–12 g of the plant samples were used to determine the total nitrogen (N) concentrations using the dry oxidation method (Jimenez and Ladha, 1993; Majejovic, 1995) in a Flash 2000 CHNS-O Analyzer (Thero Scientific, Waltham, MA, USA). For each analysis, the instrument was calibrated against a known standard (Phenylalanine) which contained 8.48% N. Total N was converted to crude protein (CP) by multiplying %N with 6.25 (Meissner, 2000).

3.3.3 Statistical analysis

was used for all statistical analyses. All data were subjected to a one-way analysis of variance (ANOVA) using SPSS Version 22 (SPSS Inc., Chicago, IL, USA, 2013). A Fishers' LSD post hoc test was used to separate treatment means at ($p < 0.05$)

3.4 Results and Discussion

Generally, the recommended dry matter (DM) for a good legume silage range from 30-40 and 45-55 % for low- and high - DM silage, respectively at ensiling (Kung et al. 2018). The DM content of *V. nilotica* plant material in this study at day 0 of ensiling (Table 3.1) and after 60 days of ensiling (Table 3.2) were significantly higher ($p < 0.05$) for the control treatment (shoots only with no molasses) compared to the other treatments (i.e. shoots + seed pods; shoots + molasses; shoots + molasses + seed pods). The DM content of the freshly chopped *V. nilotica* at day 0 of ensiling was 43% (Table 3.1), which falls within the recommended range for high DM legume silages. Losses in the nutritional quality of silages happen when silage DM is less than 25%, which induces clostridia activities (Wilkinson, 2005).

Table 3.1: Mean (±SEM) chemical composition of silage at day 0. Different letters for each variable measured indicate statistically significant differences ($p < 0.05$) within a column.

Treatments	**DM%**	**CP%**	**Ash%**	**ADF%**	**NDF%**	**WSC (g/kg DM)**	**LA (g/kg DM)**
Shoots (Control)	44.04 ± 0.30[b]	7.2 ± 0.10[a]	3.8 ± 0.03[a]	41.6 ± 0.68[b]	49.8 ± 2.17[a]	10.4 ± 3.30[a]	1.22 ± 0.06[a]
Shoots + seeds	43.05 ± 0.26[a]	7.6 ± 0.15[a]	3.9 ± 0.02[a]	38.9 ± 0.97[ab]	48.5 ± 2.22[a]	10.1 ± 0.74[a]	1.36 ± 0.12[ab]
Shoots + Molasses	42.82 ± 0.11[a]	7.2 ± 0.15[a]	4.2 ± 0.03[b]	38.6 ± 0.49[a]	49.5 ± 2.02[a]	19.9 ± 1.37[b]	1.96±0.35[bc]
Shoots + Molasses + seeds	43.06 ± 0.17[a]	6.9 ± 0.54[a]	4.5 ± 0.01[c]	36.4 ± 1.09[a]	45.9 ± 0.27[a]	30.5 ± 4.30[c]	2.09±0.13[c]
Significance	**$F_{(3.12)} = 16.00$**	**$F_{(13,12)} = 0.96$**	**$F_{(13,12)} = 165.6$**	**$F_{(13,12)} = 6.37$**	**$F_{(13,12)} = 0.95$**	**$F_{(13,12)} = 11.72$**	**$F_{(13,12)} = 4.84$**
	$p < 0.001$	**$p = 0.459$**	**$p < 0.001$**	**$p = 0.016$**	**$p=0.462$**	**$p = 0.003$**	**$p = 0.033$**

DM= dry matter, CP= crude protein, ADF= Acid detergent fibre, NDF= neutral detergent fibre, WSC= water-soluble carbohydrates and LA= Lactic acid.

One of the key criteria in evaluating the quality of silage fermentation is the pH level. Legume forages are known to be rich in proteins. However, 10-20% of the buffering effect of plant constituents on pH can be attributed to proteins (McDonald et al. 2010). A terminal pH level of 4.5 is associated with reduced amount of water-soluble carbohydrates and increased buffering ability (Nkosi et al. 2016; Kung et al. 2018). A rapid decline of pH to below 5 during the early stages from day 0 to 21 days of ensiling is required to ensure good silage quality and inhibit the activity of undesirable microorganisms (McDonald et al. 1991). In the current study, there was a gradual decrease in pH from day 0 to 65 and significant differences ($p < 0.05$) were observed from day 3 to 65 between the different days within each treatment (Figure 3.1). Significant differences ($p < 0.05$) of pH were observed in shoots (without molasses); shoots + seed pods (without molasses) treatments except for the shoots with molasses treatment (Figure 3.1) between the different treatments within each day. The pH of *V. nilotica*, ensiled for 60 days was 4.3 for shoots, 4.3 for shoots + seed pods, 4.4 for shoots + molasses and 4.3 for shoots + molasses + seed pods treatments respectively (Figure 3.1). These results fall within the normal range of 4.3 – 5.0 for the legume silage (Kung and Shaver, 2001; Kung et al. 2018).

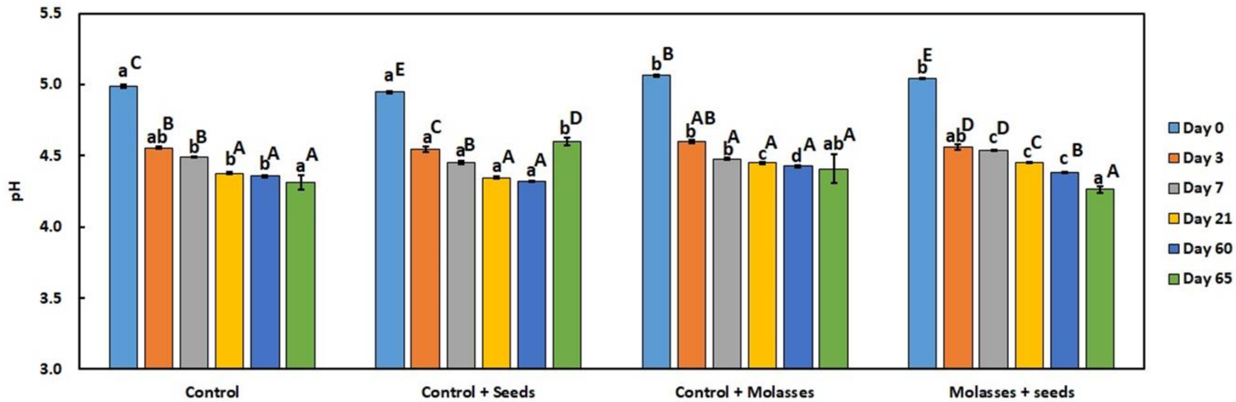

Figure 3.1: pH level for determining silage fermentation from day 0-65. Different lower case letters indicate significant differences ($p < 0.05$) between the different treatments within each day. Significant differences ($p < 0.05$) between the different days within each treatment is indicated by different upper case letters.

In addition, silages that are undergoing a clostridia fermentation have a relatively high pH as the lactic acid is converted to butyric acid (Kung and Shaver, 2001). The butyric acid content of *V. nilotica* was <0.1% (Table 3.2), which was within the recommended level of 0% for legume silages with a DM content of >45%. The acetic acid content of *V. nilotica* ranged between 0.4 - 0.6%, which falls within the recommended range of 0.5 - 2% for high DM legume silages (Kung et al. 2018). Furthermore, propionic acid was not detected in this study, and this is supported by the finding of Kung et al. (2018) for a good legume silage. Volatile fatty acids (VFA's) should be less than 0.1 or not detected at all because they are not required in high quantity for legume silage. The Ammonia-N (NH_3-N) content as a % of total nitrogen in this study ranged from 0.1- 0.3% and this value falls with the recommended levels of <12% for high DM legume silages (McDonald et al. 2002). This is also reflected in the high proportion of crude protein retained in the silage at day 60 (Table 3.2) in comparison to the fresh forage at day 0 (Table 3.1). The pH of *V. nilotica* silage did not change in the aerobic stability test, and this explains the limited carbon dioxide production during the 5 days of exposure. This resistance to a change in the pH of *V. nilotica* silage can be attributed to the high buffering capacity of *V. nilotica* as a forage legume. The pH value, butyric acid, acetic acid, and ammonia-N nitrogen contents are indicative of a well-fermented *V. nilotica* silage.

Water-soluble carbohydrates are regarded as essential substrates for growth of lactic acid bacteria (LAB) for proper fermentation (McDonald et al. 1991), and low levels may restrict LAB growth. The level of WSC and LA in this study differed significantly ($p < 0.05$) between the different treatments, where the shoots + molasses + seed pods treatment had a higher WSC and LA content compared to the other treatments (i.e. shoots (control), shoots + seed pods and shoots + molasses) at ensiling (Table 3.1). However, no significant differences ($p > 0.05$) in WSC content were observed between the different treatments after 60 days of ensiling (Table 3.2). A WSC content of 60 - 70 g WSC/kg DM is recommended for achieving well-preserved silage (Lunden-Pettersson and Lindgren, 1990). A high level of WSC at day 60 may indicate residual WSC, which makes the silage prone to aerobic deterioration (McDonald et al. 2010). In the current study, at the start of ensiling, the WSC content of the plant material ranged between 10 and 30 g/kg DM which is below the recommended level of WSC; and at the end of the ensiling period the WSC content ranged between 9.5 and 16.6 g/kg DM further decreasing from the recommended level. The same pattern was true for lactic acid, although there were no significant differences between shoots + molasses + seed pods; shoots + molasses treatments (Table 3.1).

Hargreaves et al. (2009) reported that one of the most important compositional factors that affect the nutritional quality of legume silage is crude protein (CP). The recommended level of crude protein is 7-8%, required for maintenance of ruminant livestock (Meissner, 2000). The study showed no significant differences ($p \geq 0.05$) on the CP content at day 0 of ensiling (i.e. 6.9-7.2%;

Table 3.1). However, significant differences were observed after 60 days ensiling (7.6-8.1%; Table 3.2). Manganyi et al. (2023) showed that the nutritional quality of the shoots of *V. nilotica* was approximately 7%, which corresponds to the results from the current study (Table 3.2). Furthermore, Manganyi et al. (2023) also found that when adding seed pods to the shoots, the quality could be increased to 9%, which is slightly higher than what was observed in this study (Table 3.2). However, after ensiling for 60 days, results from this study showed that CP was at 8%, which was slightly above the recommended level for maintenance of ruminant livestock condition (Meissner, 2000), especially during the dry season.

Although *Vachellia* species are known as a good crude protein source for livestock (Tefera et al. 2008; Uguru et al. 2014), its nutritive value is limited by among others its high levels of lignin and fibres and secondary compound such as tannins, which decrease digestibility and intake (Rinehart, 2008). In the current study, no significant differences ($p \geq 0.05$) were observed in ADF, NDF and ash content at ensiling (Table 3.1). However, significant differences ($p < 0.05$) were observed in NDF and ash content after 60 days (Table 3.2). Jade (2022) reported that *Vachellia* leaf silage is least desirable in regard to NDF and ADF levels. However, in this study, it was found that although ADF levels of the shoots (control), shoots + seed pods, shoots + molasses + seed pods treatments fell within the recommended ranges of 19 - 40% except for shoots + molasses treatment, NDF levels exceeded 40% which is the top range (25 – 40 %) of the recommended levels for normal ruminant function (Rinehart, 2008). In this study *V. nilotica* shoots had 38% ADF and 52% NDF, and the shoots + seed pods treatment had 40% ADF and 46% NDF, the shoots + molasses treatment had 43% ADF and 51% NDF and the shoots + molasses + seed pods treatment had 36% ADF and 47% NDF. These higher NDF values indicate that intake of these silages may be reduced but with adequate ADF level, the digestibility and energy content of the silages produced is sufficient (McDonald et al. 2010).

Table 3.2: Mean (±SEM) chemical composition, fermentation characteristics and aerobic stability of silage at day 60. Different letters for each variable measured indicate statistically significant differences ($p < 0.05$) within a column.

Treatments	DM %	CP%	Ash %	ADF %	NDF %	WSC (g/kg DM)	Y&M (g/kg DM)	NH3-N (%total N)	AA (g/kg DM)	PA	BA
Shoots (control)	46.3 ± 0.85^{c}	8.1 ± 0.45^{b}	3.9 ± 0.05^{a}	37.6 ± 6.86^{a}	52.4 ± 0.49^{b}	9.5 ± 0.57^{a}	6.84 ± 0.0^{a}	0.13 ± 0.007^{a}	0.5 ± 0.01^{a}	ND	<0.1
Shoots + Seeds	44.1 ± 0.25^{b}	8.1 ± 0.15^{b}	4.0 ± 0.02^{a}	39.5 ± 0.39^{a}	46.8 ± 0.59^{a}	16.6 ± 2.16^{a}	7.98 ± 0.01^{b}	0.33 ± 0.005^{d}	0.6 ± 0.01^{b}	ND	<0.1
Shoots + Molasses	42.6 ± 0.43^{a}	6.6 ± 0.06^{a}	4.1 ± 0.01^{b}	42.6 ± 0.47^{a}	50.5 ± 0.82^{b}	14.8 ± 1.96^{a}	8.04 ± 0.01^{d}	0.27 ± 0.003^{c}	0.4 ± 0.02^{a}	ND	<0.1
Shoots +Molasses+Seeds	43.0 ± 0.49^{a}	7.6 ± 0.17^{ab}	4.4 ± 0.05^{c}	36.0 ± 0.11^{a}	47.2 ± 0.49^{a}	16.6 ± 4.39^{a}	8.02 ± 0.01^{c}	0.19 ± 0.013^{b}	0.4 ± 0.04^{a}	ND	<0.1
Significance	$F_{(3,12)} = 17.0$	$F_{(3,12)} = 8.50$	$F_{(3,12)} = 34.8$	$F_{(3,12)} = 0.68$	$F_{(3,12)} = 19.3$	$F_{(3,12)} = 1.61$	$F_{(3,12)} = 7304.6$	$F_{(3,12)} = 114.08$	$F_{(3,12)} = 17.63$		
	$p < 0.001$	$p = 0.007$	$p < 0.001$	$p = 0.588$	$p < 0.001$	$p = 0.261$	$p < 0.001$	$p < 0.001$	$p = 0.01$		

DM= dry matter, CP= Crude protein, ADF= acid detergent fibre, NDF= Neutral detergent fibre, WSC= water-soluble carbohydrates, Y&M= Yeast and molds, NH3-N= Ammonia nitrogen, AA= Acetic acid, PA= Propionic acid and BA= Butyric acid.

With regard to mineral nutrient content of the silage produced, it was found that there were significant differences ($p < 0.05$) in the mineral nutrient content of the different treatments (Table 3.3). Mineral nutrients such as K, Ca, Mg, Fe, Mn, Zn, and Cu, were found to be sufficiently high to meet the minimum requirements of K = 5–15 g/kg, Ca = 1.8–10 g/kg, Mg = 1 g/kg, Fe = 0.03–0.1 g/kg, Mn = 0.02–0.04 g/kg, Zn = 0.02–0.05 g/kg, and Cu = 0.005–0.1 g/kg to maintain livestock condition (Meissner, 2000). However, other minerals such as Na and P content were insufficient to meet the minimum requirements of 0.4-1.8 g/kg and 1.6-6 g/kg, respectively, for maintaining livestock condition (Meissner, 2000). Prior work done by Manganyi et al. (2023) on *V. nilotica* reported similar results, with Na and P content in the fodders were insufficient. Mineral nutrient content of forages is often influenced by soil conditions and the availability of nutrients in the soil (Khan et al. 2006; Ravhuhali et al. 2020). Certain plant species take up mineral nutrients effectively in poor soils while others are unable to do so, which, in turn, could lead to nutritional deficiencies in livestock making use of the forages.

Table 3.3: Mean (±SEM) mineral nutrients of silage at day 60. Different letters for each variable measured indicate statistically significant differences ($p < 0.05$) within a column.

Treatments	K (g/kg DM)	Ca (g/kg DM)	Mg (g/kg DM)	P (g/kg DM)	Na (g/kg DM)	Fe (g/kg DM)	Mn (g/kg DM)	Zn (g/kg DM)	Cu (g/kg DM)
Shoots (control)	8.4 ± 0.09^{a}	8.9 ± 0.03^{c}	1.4 ± 0.01^{a}	1.1 ± 0.01^{b}	0.2 ± 0.01^{a}	0.2 ± 0.01^{b}	0.02±0.0001	0.02±0.0004	0.006 ± 0.0003^{a}
Shoots + Seeds	8.4 ± 0.10^{a}	8.5 ± 0.05^{b}	1.3 ± 0.01^{a}	1.2 ± 0.08^{b}	0.2 ± 0.00^{a}	0.1 ± 0.01^{ab}	0.02±0.0002	0.02±0.0008	0.006 ± 0.0000^{ab}
Shoots + Molasses	9.3 ± 0.53^{b}	8.2 ± 0.03^{a}	1.5 ± 0.01^{b}	0.9 ± 0.03^{a}	0.2 ± 0.03^{b}	0.1 ± 0.010^{a}	0.02±0.0003	0.02±0.0003	0.006 ± 0.0003^{b}
Shoots+Molasses+Seeds	10.8 ± 0.12^{c}	8.4 ± 0.12^{ab}	1.6 ± 0.02^{c}	1.1 ± 0.02^{b}	0.3 ± 0.01^{c}	0.1 ± 0.000^{ab}	0.02±0.0003	0.02±0.0007	0.007 ± 0.0001^{c}
Significance	**$F_{(3,12)}$= 16.454**	**$F_{(3,12)}$= 19.143**	**$F_{(3,12)}$= 75.494**	**$F_{(3,12)}$= 4.437**	**$F_{(3,12)}$= 15.072**	**$F_{(3,12)}$= 5.511**	**$F_{(3,12)}$= 0.000**	**$F_{(4,15)}$= 0.000**	**$F_{(3,12)}$= 8.529**
	p<0.001	**p<0.001**	**p<0.000**	**p<0.041**	**p<0.001**	**p<0.024**	**p<0.000**	**P<0.000**	**p<0.007**

K= Potassium, Ca= Calcium, Mg= Magnesium, P= Phosphorus, Na= Sodium, Fe= Iron, Mn= Manganese, Zn= Zinc and Cu= copper.

3.5 Conclusion

This study aimed to determine whether ensiling could be used as a means of preserving *V. nilotica* plant material for use during the dry season. The results from this work shows that the majority of parameters such as pH, dry matter, voluntarily fatty acids and crude protein were within the recommended range of well-fermented and preserved legume silage. The results in this study show that *V. nilotica* can be successfully preserved as silage during the wet seasons for feeding livestock during the dry seasons. It is concluded that *V. nilotica* can be preserved as silage with or without the inclusion of seed pods and molasses. Nevertheless, additional research is required to determine whether adding inoculants will possibly have an effect on the nutritional quality of the silage produced. Additionally, feed intake study and the impact of silage from woody plants on animal performance should also be included for future work.

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Chapter 4

General discussions, implications and recommendations.

4.1 Synthesis

One of the main factors affecting livestock production is the fluctuating supply and quality of forage, which is exacerbated by the changing climate. Additionally, an increase in global population further increases the demands for meat products (Du et al. 2023), which in the process worsen the degraded rangelands. *Vachellia nilotica* is an encroaching tree species widely distributed in semi-arid rangelands (Mlambo et al. 2005; Mlambo et al. 2011; Smit and Prins, 2015), including the Agricultural Research Council – Roodeplaat Farm where the current study was conducted. Although *V. nilotica* is regarded as an encroacher species it is well known that it also contributes significantly to livestock diets especially small ruminants that feed mostly on the pods and leaves of this species during the dry season (Lesoli et al. 2013; Mnisi and Mlambo, 2016; Stevens et al. 2017). This means that *V. nilotica*, could be used as a natural fodder resource by farmers to fill the dry season feed gap, thereby minimizing further encroachment and thickening. In extensive livestock production systems have played a significant role in seed dispersal of viable encroaching tree species through endozoochory as they feed on the seed pods during the dry season and subsequently disperse the seed back in the rangeland (Frost et al. 2013; Tjelele at al. 2015).

This work was done to determine whether *V. nilotica* plant materials (seed pods and edible twigs) nutritional quality and utilization can be used as an alternative source of fodder during dry season and at the same time to try and minimize further bush encroachment through endozoochory by processing the seed pods first before feeding to the livestock. Specifically, the following questions were asked:

1. What are the nutritional composition of *V. nilotica* shoots and seed pods and will the addition of feed additives improves its quality?

2. Will adding chipped seed pods to the diets prior to feeding have an effect on the number of seeds recovered and its germination potential: (Chapter 2: The potential for endozoochorous dispersal of *V. nilotica* seeds by goats: Implications for bush encroachment)?
3. Rather than feeding the material as is, could silage be an alternative option to preserve the nutritional quality of the fodders (Chapter 3: Ensiling as a technique for preservation of the nutritional quality of *V. nilotica*)?

The findings in chapter 3 of this study showed that, *V. nilotica* shoots contained inadequate amount of crude protein content for livestock maintenance requirements during the dry season. Addition of seed pods to the shoots resulted in an increased crude protein level to meets the maintenance requirements for small ruminants. Furthermore, addition of feed additive resulted in further increases in the nutritional quality but did not have an impact on feed intake. Chipping of the seed pods prior to feeding the livestock also increased seed ingestion, reduced seed recovery significantly, and reduced the time that seeds were retained in the gut, regardless of the inclusion of a feed additives. Chipping the seed pods, resulted in only 13% intact seed recovery and ingestion of these only resulted in approximately 2% of seeds offered to the livestock passing through the digestive system. This advocates that chipping of the seed pods prior to inclusion in livestock diets will further reduce the number of seeds that could be spread through the rangelands. However, the recovered seeds from the livestock after ingestion were dormant but still viable and could germinate and establish.

Chapter 3 of this study explored the possibility of ensiling the *V. nilotica* edible twigs (shoots) with or without the addition of seed pods and molasses. Ensiling has become one of the best popular and growing techniques in preserving the forage plants in the world and it is best practiced

(McDonald et al 1991; Wilkinson and Toivonen, 2003). Findings in the study showed that the overall results fall within the recommended range of well-fermented and preserved legume silage, with protein ranged from 7-8 % which is sufficient to meet the baseline requirements for livestock production during the dry season. It is concluded that *V. nilotica* can be preserved as silage with or without addition of seed pods and molasses.

4.2 Implications and recommendations

This study evidenced that *V. nilotica'* shoots only, contained low nutrients for minimum requirements for livestock production. however, the addition of seed pods and additives improved the level of nutrients. This implies that, when cutting and chipping *V. nilotica* shoots as fodder for supplements during the dry season, one should bear in mind the addition of seed pods to improve the protein content. Furthermore, chipping the seed pods prior to adding them to the diet, significantly increased the feed intake and reduced the number of viable seeds recovered. These results indicate reduction of seed dispersal in the rangeland, when chipping the seed pods.

From the results obtained in this study, it can be recommended that the *V. nilotica* shoots (chipped) should be added with chipped seed pods and additives to increase the level of protein and other nutritional parameters required for livestock production. Silage production can be another alternative method to preserve the *V. nilotica* edible material in order to fill the dry season feeds shortage.

4.3 Future research

Although this study answered several questions regarding the quality and potential use of *V. nilotica*, several further questions arose from this work. Nutritional qualities may differ with phenological stages (Britz et al. 2022). Therefore, further research is needed to answer the question of whether harvesting the shoots at different phenological stages could potentially result in higher quality fodders without having to incorporate the seeds pods. This in turn, would completely reduce the risk of spreading the seeds through endozoochory. An important observation was that although very few seeds were recovered after ingestion, the majority of these seeds were still viable and therefore might results in further bush encroachment. More investigation is needed to find out if the retrieved seeds can withstand the dry season and grow and establish themselves during the wet season. Seed pods are fed to livestock in the dry season, although some of the seeds passing through the digestive system already has their dormancy broken down, it is not likely that they would survive the dry season. Another interesting future study will be to evaluate whether the inclusion of inoculants will possibly have an influence on the silage's nutritional quality, and how will the silage have an impact on animal performance, meat quality, methane production and emission. In addition, it is suggested to explore different conservation methods of these materials in the form of pelleting and to evaluate the nutritional quality of the products is crucial.

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