Effects of nutrient-tannin interactions on intake and germination of woody plant species by ruminants

by

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Declaration: Plagiarism

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I proclaim that the dissertation is my own particular work. This dissertation has not been submitted for any other degree or examination at any other institution.

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Declaration 2: Publications

Khanyisile Mbatha, Julius Tjelele and Ntuthuko Mkhize (Supervisors) contributed helpful comments and suggestions to the manuscripts. The two papers chapters presented in this dissertation were formatted according to the requirements of the particular journal to which they were submitted or published.

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Publication 2: Formatted for submission to **Animal Production Science**

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Dedication

I would like to devote this dissertation to my mother, Lentheng Monegi and my siblings, Michelle Monegi, Emmanuel Monegi and Ramokone Monegi. Appreciation to my friends Kabelo Molopo, Thapelo Masuku, Tiisetso Phala, Lebogang Skhosana and Abia Mabotha for their unwavering support throughout my studies.

Abstract

Woody plant encroachment is one of the major problems worldwide because it affects negatively the herbaceous layer, which provide forage for livestock production. However, the role of ruminants particularly browsers in the dispersal of woody plant seeds still remains a concern for farmers interested in grass production. Seedpods of various woody plant species constitute a crucial part of the diet of herbivores during the dry season because of their high nutritional quality compared to herbaceous material. The interaction of associated diet quality, seed characteristics and animal species among other factors play a pivotal part in the success of livestock faecal seeds dispersion. Furthermore, dispersed seeds that successfully grow into mature woody plants become an important source of protein for herbivores. The use of woody plants as a source forage is known to be limited by plant secondary metabolites (PSMs) such as condensed tannins.

The objectives of this study were to determine 1) the effects of condensed tannins and crude protein of *Vachellia tortilis* and *Dichrostachys cinerea* pods in seed recovery and germination fed to goats, and 2) the effects of diet mixing on the feed intake of plant species by goats. In the first experiment, a total of 12 female indigenous goats and 12 female Pedi sheep were utilised in this study, with the average body weights of 29.50 kg ± 1.60 (S.E) and 28.70 kg ± 1.60, respectively. Twelve goats were grouped into two groups of six goats per group, one group was fed *D. cinerea* pods and the other group was fed *V. tortilis* pods. The group of 12 sheep were divided similarly, the one group was fed *D. cinerea* pods and the other group was fed *V. tortilis* pods. Each animal was given *V. tortilis* and *D. cinerea* pods at 2.50% of their body weight. All animals were allowed to consume *D. cinerea* or *V. tortilis* pods within 24 h, after which the remaining pods were collected and weighed. Faecal collection

commenced immediately after the 24 h pods feeding and was carried on until no seeds were discovered in faeces. All faeces extracted from sheep and goats were collected daily in the morning from the faecal bags.

In the second experiment, a total of 24 indigenous goats with average body weight of 26.6 kg ± 0.51 were utilised. Goats were arbitrarily selected and grouped into four groups of six goats per group (goats were placed individually in 2 m^2 pens). Each group was fed one of the following diets: diet one - *Searsia lancea*, diet two - *S. pyroides*, diet three - *Euclea crispa* and diet four - was a combination of the three plant species (*Searsia lancea, S. pyroides* and *Euclea crispa*). *Searsia lancea*, *S. pyroides* and *E. crispa* branches were collected every morning prior to feeding, and were weighed before offering the animals. Refusals were gathered and weighed, and intake was calculated as distinction between weight in and refusals. Plant species foliage were analysed for crude protein, condensed tannin, acid detergent lignin, acid detergent fibre and neutral detergent fibre.

During the first experiment, the cumulative percentage seed recovery of *V. tortilis* from goats (46.00 % ± 1.90) and sheep (52.00 % ± 2.93) was significantly higher than *D. cinerea* from goats (13 % ± 1.47) and sheep (24.00 % ± 1.16). Germination percentage of *D. cinerea* seeds that passed through the gastro-intestinal tract of goats (33.12 % ± 2.94) and sheep (36.00 % ± 2.68) was significantly higher than *V. tortilis* seeds that passed through the gastro-intestinal tract of goats (28.98 % ± 2.68) and sheep (23.04 % ± 2.81). Average *D. cinerea* (34.56 % ± 1.99) and *V. tortilis* (26.02 % ± 2.10) seeds that went through the gastro-intestinal of goats and sheep had a significantly higher germination rate than the control (i.e. no passage through the gut; *D. cinerea* = 2.31 % ± 1.55, *V. tortilis* = 5.07 % ± 2.68). The high mean cumulative percentage seed recovery of *V. tortilis* (18.80 %) may be attributed to the relatively higher crude protein than *D. cinerea* (12.20 %). This may encourage animal seed dispersal and germination of woody plant species with relatively high crude protein content.

In the second experiment, *Searsia lancea* contained 8.50 % CP, 21.46 % acid detergent fibre (ADF), 12.50 % ADL and 39.37 % NDF. *Searsia pyroides* had 9.03 % CP, 27.07 % ADF, 10.89 % ADL and 40.30 % NDF. *Euclea crispa* had 6.19 % CP, 26.20 % ADF, 16.63 % ADL and 30.02 % NDF. Mixed diet (combination of the three plant species) had 8.96 % CP, 23.72 % ADF, 11.13 % ADL and 38.28 % NDF. *Searsia lancea* had 2.70 % of CTs while *S. pyroides* had 5.20 % CT, *E. crispa* had 6.44 % CT and mixed diet had 7.20 % CT. The mean dry matter intake varied significantly among dietary groups ($P < 0.001$). Similarly, goats offered a mixed diet consumed more CTs ($P < 0.01$) than those offered individual forage species.

The high mean cumulative percentage seed recovery of *V. tortilis* may be attributed to the higher crude protein of *V. tortilis* (18.80 %) than *D. cinerea* (12.20 %). Higher passage rate may encourage animal seed dispersal and germination of plant species. The results from experiment two support the postulation that animals foraging in mixed diet systems consume more PSMs and achieve higher dry matter intake than animals confined to monocultures or single species feeding systems. Given that woody plant encroachment is already reducing farm-grazing capacities in African savannas and this problem is predicted to double by 2050, strategies that improve herbivore ability to consume woody plants will increase forage availability and inform bush control programmes and policies. Moreover, the concomitant increase in CTs by goats exposed to diets with diverse species also has positive implications for animal.

Key words: African savannas; complementarity; condensed tannins; crude protein; germination percentage; herbivory; plant defence; seed dispersal; seedpods, seed viability, woody plant encroachment.

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Chapter 1

1.1. Introduction and literature review

Savannas are ecosystems or landscapes with a continuous herbaceous layer and woody plants, usually dominated by herbs, grasses and forbs (Scholes and Archer 1997). Savannas cover 54% of southern Africa and 60% of sub-Saharan Africa (Scholes 1997; Scholes and Archer 1997). Being so widespread in Africa, savannas support large, rapidly growing human populations and their associated livelihoods (Scholes and Archer 1997). However, human exercises have prompted to alteration of savannas, either through vegetation expulsion, wood gathering, through plant invasions, reduction of wild ungulates, overgrazing and global climate change (Scholes and Archer 1997; Smit et al. 1999; Ward 2010).

1.2. Woody plant encroachment

Woody plant encroachment is one of the common problems related with reduced grazing areas, and is reported throughout southern African savannas (Smit 2004). Woody plant encroachment is a state where trees and shrubs occupy the grassland and/or increase in density, resulting in reduced production of herbaceous plants (Ward 2005). This poses a challenge to land users because it negatively affects the herbaceous layer, which provides a proportion of forage for livestock production (Wigley et al. 2010). Thus, woody plant encroachment has negative effects on rangeland quality by reducing carrying capacity for livestock production. Furthermore, woody plant encroachment also restricts production of herbaceous understory and access to quality forage (Hagos and Smit 2005; Ward 2005). This may prompt to the suppression of palatable herbaceous material by encroaching woody species (Ward 2005).

It is most certainly not only the extent of woody plant encroachment that is a challenge but also the rate at which it occurs (Kraaij and Ward 2006). The question of what causes woody plant encroachment remains unanswered and this hinders the development of effective management (Wiegand et al. 2006; Ward et al. 2013). The problem of encroaching woody plant species is too complicated to be associated with only one factor (Ward et al. 2013).

1.2.1. Seed dispersal and germination

There have been multiple studies on woody plant encroachment, particularly on animal seed dispersal (Roques et al. 2001; Smit 2004; Tjelele et al. 2012; Tjelele et al. 2014). Mammalian herbivores such as goats, sheep and cattle are among the most important herbivores leading to the increase of woody plant encroachment through dispersal of viable seeds (Tjelele et al. 2012; Tjelele et al. 2014). However, the process of animal seed dispersal is not as straight forward because there are a number of components such as diet quality, seed traits and animal species that play a significant role (Tjelele et al. 2014).

Seed dispersal, seed germination and seedling recruitment are essential in plant population dynamics because of their impact on the dispersion and abundance of woody plant species (Salazar 2010). Pods of various plant species constitute an essential part of the diet of herbivores especially throughout the dry season because of their high protein quality compared to herbaceous material (Janzen 1984; Tjelele et al. 2014). Some pods have seeds that are hard-coated which are not easily destroyed after been ingested and recovered from the faeces (Or and Ward 2003).

Hard-coated seeds within the pods are scarified during mastication and passage through the digestive system of mammalian herbivores (Bodmer and Ward 2006). However, some of seeds might be damaged while they pass through the digestive tract of mammalian herbivores while others are defecated unharmed in the faeces (Or and Ward 2003). These may frequently be related to a number of interacting components, for example, seed traits (seed hardness, shape and density), diet quality, animal species and body size (Castro et al. 2008; Murray et al. 1994; Bodmer and Ward 2006). Mammalian herbivores feeding on hard-coated seeds may encourage seed germination by scarifying the seed coat, and as a result increasing water uptake during passage through the digestive system (Tjelele et al. 2012).

1.2.2. Woody plants as forage for livestock

Woody plants are an essential component of the diets of mixed-feeders and browsers, particularly throughout the dry season when the nutritive value and amount of grass material declines (Pawelek et al. 2008; Abdulrazak et al. 2000). Leaves, tender shoots, and pods are parts of woody plants that are normally used as feed (Aganga and Tshwenyane 2003). Thus, savanna woody pants serve as major source of feed for livestock since their crude protein is relatively high throughout the most part the year (Ramirez et al. 1993). However, it is also known that their limitation as a source of feed is due to anti-quality factors such as physical and chemical defences (Cooper and Owen-smith 1986; Boege and Maquis 2005).

1.2.2.1. Physical defences

Physical defence may be characterized as any morphological or anatomical quality that presents a wellness advantage to the plant by straightforwardly discouraging browsers and other mixed-feeders from feeding on it (Boege and Maquis 2005). Various plant parts that may play a major role as feeding deterrents includes spines and thorns (spinescene), layer of hairs (trichomes), and hard leaves (sclerophylly) (Hanley et al. 2007). Physical structures of plants (such as spinescence) are generally considered to be more effective against herbivore feeding, and have been demostrated to influence the feeding behaviour of browsers and mixed-feeders (Cooper and Owen-smith 1986).

Mammalian herbivores can forage more on plants that are not physically protected compared to plants with physical defences (Cooper and Owen-Smith 1986). Spinescene removal experiments demonstrated the protective value of these structures (Cooper and Ginnett 1998; Wilson and Kerley 2003). A study by Cooper and Ginnett (1998) showed that removal of thorns allowed southern plain woodrats access to raisins pinned on the branches of the *Vachellia rigidula* species. Furthermore, the removal of thorns from a range of spinescent shrub species increased the rate of herbivory by indigenous bushbucks and Boer goats (Wilson and Kerley 2003).

Leaf trichomes may also play a part in herbivore defence (Haberlandt 1914). However, trichomes like most different sorts of physical defence play double roles. For instance, a study in North America found that leaf hairs of *Verbascum thapsus* go about as a structural defence against grasshoppers, while likewise securing immature foliage against dehydration (Woodman and Fernandes 1991). Thus, it is broadly acknowledged that trichomes may act as an essential plant defence against herbivory (Handley et al. 2005).

In addition, sclerophylly can also be a major feeding deterrent (Turner 1994). Hard leaves and shoots decrease both the palatability and digestibility of forage

(Robbins 1993), which may negatively affect herbivore production (Perez-Barberia and Gordon 1998). The digestibility of cellulose particularly is a crucial problem for herbivores (Hochuli 1996; van Soest 1982). The presence of cellulose in plant tissues is associated with lignin, hemicellulose and silica (van Soest 1982). High fibre concentration is a trait of plants that show greater mechanical anti-quality (Laca et al. 2001). Therefore, browse plants with high fibres are usually hard for browsers and mixed-feeders to forage as a result of the physical strength associated with fibres. However, indigestibility of plants alone may not deter herbivores from foraging on plants (Forsyth et al. 2005).

1.2.2.2. Chemical defences

The evolutionary connections between browsers/mixed-feeders and plants have brought about a variety of adjustments and associations (Aganga and Tshwenyane, 2003; Kaitho, 1997). For example, herbivores grazing pressure prompted to the evolution of chemical defences in plants (Bryant and Kuropat 1980). Thus, plants possess several chemicals that can reduce plant quality and palatability. At high dosage they may cause toxicities and discourage herbivory. These chemicals are named anti-quality factors because they limit the quality of forage (Lauchbaugh et al. 2001). They are not included in the essential metabolic processes supporting plant growth, development or reproduction (Lauchbaugh et al. 2001). Thereby, they are called plant secondary metabolites. Plant secondary metabolites have negative impacts on the key biochemical processes, survival, growth or selective behaviour of herbivores (Launchbaugh 1996).

Terpenes, glycosides, oxalates, alkaloids, and tannins are the most important categories of plant secondary metabolites (Pfister et al. 2001; Majak et al. 2001;

Cheeke 1998). Although most of these chemicals are found in almost all categories of plants, tannins are the fundamental plant secondary metabolites that exist in woody plant species in savannas and probably influence the nutritive value of browse as animal feed enormously. According to Makkar 2003, tannins are the main reason woody plants are less utilised as livestock forage in savanna ecosystems.

Tannins are naturally existing plant polyphenols that can have a major effect on the nutritional quality of forage (Reed 1995). Tannins are grouped into two classes: condensed tannins and hydrolysable tannins. They may have both unfavourable and advantageous influences depending on their quantities and nature besides different variables, for example, composition of the diet, physiological state of the animal and animal species (Makkar 2003).

Condensed tannins are not exceptionally toxic, but negatively affect intake, digestion and tend to be poorly absorbed, which therefore reduces the protein status of animals (Mueller-Harvey 2006). According to Silanikove et al. 2001; Kaitho et al. 1998, tannins have the affinity with proteins and they are not available for physiological needs of animals.

Animals increase their desire for feedstuffs related with advantageous influences; for example, feed that meet their nutrient requirements. However, animals encountering hunger are expected to endure the effects of condensed tannins, as long as their nutrients and water requirements are met (Baraza et al. 2009). These dietary components (i.e. proteins, energy and water) are required for physiological functions of herbivores such as assisting with the detoxification process (Freeland and Janzen 1974).

However, tannin-rich plants can be beneficial to the health and welfare of herbivores by reducing parasitism in ruminants (Mbatha et al. 2002). Nematodes, found in the gastrointestinal tract and lungs of herbivores cause major losses in production because of the threat they pose to the economic viability of livestock. Therefore, herbivores foraging on tanniferous plants may mitigate the detrimental effects of gastrointestinal parasitism (Hoste et al. 2006; Hoste et al. 2012; Mbatha et al. 2002; Novobilsky et al. 2011 Niezen et al. 2002). Lisonbee et al. 2009, reported that sheep that were suffering from internal parasites showed an increase in preference for high tannin plant species than sheep uninfected by parasites. However, when the infection was no longer present preference for high tannin plant species was lost.

Furthermore, condensed tannins mitigate bloating in ruminants (Waghorn 1991). The extent of bloating in ruminants is controlled by reducing gas production in the rumen, polysaccharide slime and microbial activities (Min et al. 2005). Thus, condensed tannins may serve as rich source of bioactive products that can affect herbivores either positively or negatively.

Searsia lancea, _Searsia pyroides_, _Euclea crispa_, _Dichrostachys cinerea_ and _Vachellia tortilis_: common encroaching plant species in South Africa

Searsia lancea is a small to medium-sized tree, normally up to 8 m in height but sometimes it may be taller (Coates-Palgrave 2002). *Searsia lancea* normally occurs over a number of altitudes and habitats (i.e. in open woodland, along river and stream banks and usually on termite mounds). *Searsia pyroides* is frequently a shrub, or a small bushy to spreading tree up to 6 m in height (Coates Palgrave 2002). It occurs in a variety of habitats ranging from coastal dunes through montane grassland, even open woodland, to fringing forest and bushveld and dry thornveld. *Searsia pyroides* is frequently associated with *Vachellia karroo*, often on termite mounds or along watercourses and to semi-arid desert areas (Coates Palgrave 2002). *Euclea crispa* is dense upright 1-2 m in height, usually forming almost pure base over a small area, or a slender tree 8-20 m high (Coates-Palgrave 2002). It occurs in bush clumps in grassland, open woodland and bushveld, often among rocks and at forest margins.

Dichrostachys cinerea is a shrub or small *Vachellia*-like tree of up to 5-6 m height (Coates Palgrave 2002). It is more predominant at lower altitudes, existing on a number of soils in wooded grassland. *Dichrostachys cinerea* often forms secondary bush in deteriorating areas, becoming problematic when it grows into inaccessible bushes (Coates Palgrave 2002). *Vachellia tortilis* (previously known as *Acacia tortilis*) species are more or less evergreen, medium to large trees, height differs from 5-20 m (Coates Palgrave, 2002). Over their extensive range, the smaller trees are often round-crowned, the larger specimens are usually flat-topped and widespread in low-altitude, dry areas, in variety of types of woodland (Coates Palgrave, 2002).

1.3. Motivation

This study determined the role of pod's nutrients and condensed tannins on seed recovery and germination. A tool that can be utilised to increase the use of woody plants species was also determined.

This study expected to contribute to a greater understanding of some of the aspects that underlie woody plant encroachment, and the objectives of this study were:

1) To determine the effects of crude protein and tannins from the pods on seed recovery and germination of *Vachellia tortilis* and *Dichrostachys cinerea*,

2) To determine the effects of diet mixing on the intake of browse species by goats,

1.4. Literature cited

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Chapter 2

Effects of Condensed Tannins and Crude Protein on Seed Recovery and Germination of *Dichrostachys cinerea* and *Vachellia tortilis* Fed Ruminants

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2.1. Abstract

Herbivores feeding on seedpods of woody plants may spread unharmed seeds that can possibly germinate and recruit into mature trees. The quality of the diet such as pods chemical composition may be the most crucial determinant of success of livestock faecal seed dispersal. The objective of this study was to determine the effects of condensed tannins and crude protein of *Vachellia tortilis* and *Dichrostachys cinerea* pods in seed recovery and germination fed to goats and sheep. These animals were each fed either *D. cinerea* or *V. tortilis* seedpods at 2.50 % of their body mass. Pods were mixed fed with *Eragrostis curvula* hay to facilitate intake and mimic grazing under natural conditions. The cumulative percentage seed recovery of *V. tortilis* from goats (46.00 % ± 1.90) and sheep (52.00 % ± 2.93) was significantly higher than *D. cinerea* from goats (13 % ± 1.47) and sheep (24.00 % ± 1.16). Germination percentage of *D. cinerea* seeds that passed through the gastro-intestinal tract of goats (33.12 % ± 2.94) and sheep (36.00 % ± 2.68) was significantly higher than *V. tortilis* seeds that passed through the gastro-intestinal tract of goats (28.98 % ± 2.68) and sheep (23.04 % ± 2.81). *Dichrostachys cinerea* (34.56 % ± 1.99) and *V. tortilis* (26.02 % ± 2.10) seeds that passed through the gastro-intestinal tract of goats and sheep had a significantly higher germination percentage than the control (i.e. no passage through the gut; *D. cinerea* = 2.31 % ± 1.55, *V. tortilis* = 5.07 % ± 2.68). The high mean cumulative percentage seed recovery of *V. tortilis* may be attributed to the higher crude protein of *V. tortilis* (18.80 %) that was higher than *D. cinerea* (12.20 %). This may encourage animal seed dispersal and germination of woody plant species.

Keywords: tannins, protein, germination percentage, seed dispersal, seedpods, seed viability, woody plant encroachment

2.2. Introduction

Seedpods of a variety of plant species form a crucial part of the diet of sheep and goats during the dry season because of their high quality (i.e. high protein) value compared to grasses (Janzen 1984; Rohner and Ward 1999). However, herbivores feeding on seedpods of woody plants may spread undamaged seeds that can possibly germinate and recruit into mature trees (Tews et al. 2004). Endozoochory and seed germination are major processes in plant population dynamics because they normally have an effect on the dispersion and abundance of plant species (James et al. 2011; Tjelele et al. 2015).

Diet of high digestibility passes more rapidly through the digestive system of animals and may play a meaningful role in seed recovery and germination (Robbins 1993; Tjelele et al. 2014). Faster passage rate may result in low scarification and low seed germination percentages (Bodmer and Ward 2006). However, this is dependent on a combination of different factors. For instance, Cipollini and Levey 1997; Murray et al. 1994, stated that plant secondary metabolites such as glycoalkoloids may influence retention time in the gut of an animal and, thereby dispersal of seeds in the rangelands. Consequently, high tannin-rich plants may induce mammalian herbivores to leave plants that bear pods early in a feeding bout, thereby, improving dissemination of seeds away from the parent plant (Cipollini and Levey 1997). Understanding the tannin and crude protein interaction and their influence on retention time, seed viability, seed recovery, and germination following ingestion by herbivores may contribute to the understanding of encroaching tree species.

The objective of this study was to determine the effects of crude protein and condensed tannins in seed recovery and germination of *Vachellia tortilis* and *Dichrostachys cinerea* pods fed to ruminants. We hypothesised that the 1) relatively high protein and low tannin concentration of either *V. tortilis* or *D. cinerea* pods may result in higher seed recovery, 2) faster passage rate of seeds may result in low scarification and low germination percentage.

2.3. Materials and methods

2.3.1. Pods collection and viability tests

Dry mature pods of *D. cinerea* were collected from trees and under trees at Kwa-Mhlanga, Mpumalanga Province (28°30´E, 25° 15´S), South Africa, where these species occur abundantly. *Vachellia tortilis* pods were acquired from Silver Hill Seeds Company in Kenilworth, Cape Town, South Africa. *Dichrostachys cinerea* seeds have a length and width of 4.26 mm ± 0.10 and 1.76 mm ± 0.10 and *V. tortilis* seeds have 3.4 mm ± 0.16 and 1.6 mm ± 0.20, respectively. *Vachellia tortilis* and *Dichrostachys cinerea* seeds were examined for viability prior to the commencement of the experiment to determine their germination potential. Seeds of both species were scarified using sand paper and soaked in distilled water for 18 hours at room temperature (Tjelele et al. 2014). Seeds were soaked in 1% tetrazolium solution (2, 3, 5-triphynyl chloride) for 18 hours in an incubator at 30°C. Seeds that were subjected to viability tests were cut longitudinally through the endosperm to expose the embryo through a microscope to assess viability (International Seed Testing Association 1985).

2.3.2. Experimental animals and feeding

The seed recovery trial was carried out at the Agricultural Research Council, Irene Experimental Farm, Gauteng Province, South Africa. A total of 12 female indigenous goats and 12 female Pedi sheep were utilised in this study, with the average body weights of 29.50 kg ± 1.60 (S.E) and 28.70 kg ± 1.60, respectively. Twelve goats were grouped into two groups of six goats per group, one group was fed *D. cinerea* pods and the other group was fed *V. tortilis* pods. The group of 12 sheep were divided similarly, the one group was fed *D. cinerea* pods and the other group was fed *V. tortilis* pods. Each animal was fed *V. tortilis* and/or *D. cinerea* pods at 2.50% of their body mass. To estimate the number of seeds from the pods offered to the animals, 50 *D. cinerea* and 50 *V. tortilis* pods were collected and weighed. Seeds were removed from these pods, counted and the average number of seeds per pod was used to estimate the number of seeds fed to each animal (Miller 1995).

Each animal was fed experimental diet (*Eragrostis curvula* hay) mixed with either *D. cinerea* or *V. tortilis* pods, individually in 2 m^2 pens. Sheep and goats were fed *Eragrostis curvula* hay for seven days prior to the commencement of the experiment to allow them to acclimatize to experimental conditions and remove any possible seeds from the digestive tract. Sheep and goats were fed *Eragrostis curvula* hay and water *ad libitum* throughout the experiment (i.e. during acclimatization and seed recovery trial). The Animals Ethics Committee of the University of South Africa under permit number 2015/CAES/015 and the Agricultural Research Council under permit number APIEC15/003 approved the handling of animals and experimental procedures.

On each day during the seed recovery trial, a random grab sample of *E. curvula* hay, *D. cinerea* pods and *V. tortilis* pods were collected and stored pending the analysis. For each feed and pod species, the bulked samples were analysed for crude protein using Kjeldahl block digestion method (Association of Official Analytical Chemists 2000). Neutral detergent fibre was analysed using tector fibertec system (Van Soest et al. 1991). *Dichrostachys cinerea* and *V. tortilis* pods were analysed for condensed tannins (CT) as determined by acid-butanol proanthocyanidin assay with purified sorghum as a standard for CT estimation (Hagerman1995).

2.3.3. Seed recovery from the faeces

All animals were allowed to consume *D. cinerea* or *V. tortilis* pods within 24 h, after which the remaining pods were collected and weighed. Faecal collection commenced immediately after the 24 h pods feeding and was carried on until no seeds were found in faeces. All faeces extracted from sheep and goats were collected daily in the morning from the faecal bags. Faeces were immersed in cold water until soft and then washed with tap water through a wire strainer until the water was clear (Tjelele et al. 2012). A cabinet with light source below a glass surface was used to separate seeds from faecal remains. Undamaged and partially damaged seeds retrieved from animals per day were calculated and kept in brown paper bags in a cool dry place awaiting the germination trial.

2.3.4. Germination trial

Germination tests were handled at the Agricultural Research Council, Roodeplaat Farm Forage Genebank, Gauteng Province, South Africa according to the International Seed Testing Association standards (1985). The germination tests used round plastic dishes (12 mm) containing one disc of germination paper and 5 ml of distilled water. Each plastic dishes contained three sets of 25 seeds from each animal species.

Germination tests were carried out in germination chamber kept at a temperature of 20-30°C with 16 hours of dark period and eight hours of light period. The germination trial was monitored daily for 30 days and all germinated seeds were recorded and removed (International Seed Testing Association 1985). The percentage germination was calculated at the end of germination tests as the number of seeds germinated divided by the total number of seeds placed in a square plastic dish multiplied by 100 (Armke and Scott 1999). All seeds that did not germinate at the end of 30 days were subjected to viability tests.

Seed germination was determined on 1) control (seeds that did not pass through the gut of goats or sheep), 2) *D. cinerea* seeds recovered from goats, 3) *V. tortilis* seeds recovered from goats, 4) *D. cinerea* seeds recovered from sheep, 5) *V. tortilis* seeds recovered from sheep.

2.3.5. Statistical analysis

A 2×2 factorial design analysis of variance with the following factors: seed species (*V. tortilis* and *D. cinerea*) and animal species (goats and sheep). The experiment was separated into two parts i.e. seed recovery and germination trial. The total amount of seeds recovered from goats and sheep were considered independent variables. Seed germination was determined on 1) control, 2) *D. cinerea* and *V. tortilis* seeds recovered from goats and 3) *D. cinerea* and *V. tortilis* seeds recovered from sheep. Each animal (sheep and goats) (*n*=12) was considered an experimental unit. Distinctions between means were considered significant at 5% level. All the data were analysed by analysis of variance (ANOVA) using SPSS 2013 statistical software.

2.4. Results

2.4.1. Seed viability and chemical composition

Dichrostachys cinerea and *V. tortilis* seeds prior to feeding of animals had a viability of 91.50 % and 88.50 %, respectively. The two seed pods species (*D. cinerea* and *V. tortilis*) had relatively high crude protein content; with *V. tortilis* (18.80 %) having a higher crude protein than *D. cinerea* (12.20 %) and *Eragrostis curvula* hay had the lowest crude protein level of 6.60 %. *Dichrostachys cinerea* pods had higher condensed tannins concentration (10. 00%) than *V. tortilis* pods (1.00 %). *Eragrostis curvula* hay had higher neutral detergent fibre (74.40 %) than *D. cinerea* (33.70 %) and *V. tortilis* (30.30 %).

2.4.2. Seed pod consumption and recovery

Sheep consumed slightly more *D. cinerea* and *V. tortilis* pods (668.00 g ± 10.70 (SE), 658.30 g ± 10.50) than goats (654.00 g ± 10.44; 650g ± 10.40), respectively. The cumulative percentage seed recovery of *V. tortilis* from goats (46.00 % ± 1.90) and sheep (52.00 % ± 2.93) was significantly higher than cumulative percentage seed recovery of *D. cinerea* from goats (13.00 % ± 1.47) and sheep (22.00 % ± 1.16) (Fig. 2.1).

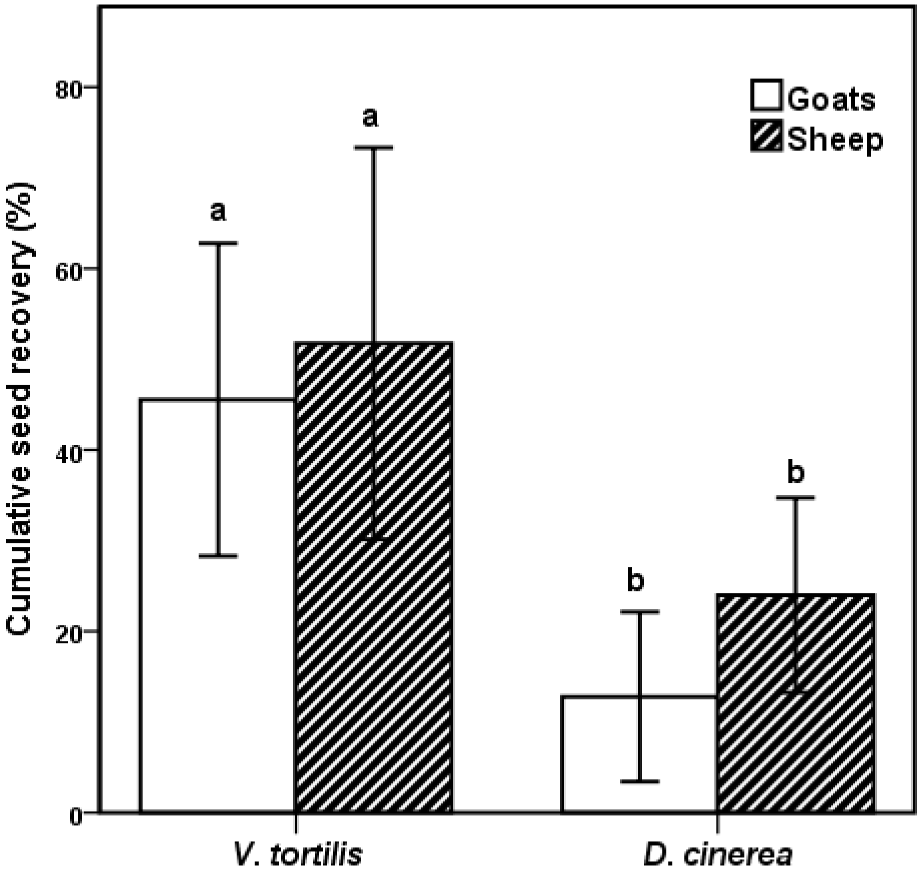

Fig. 2.1. Interaction effects of animal species and pod seed species on mean cumulative percentage seed recovery, and errors bars represent standard errors (S.E).

2.4.3. Germination trial

There were more viable *V. tortilis* and *D. cinerea* seeds (96.00 % and 85.00 %, respectively) than dead seeds after 30 days of germination trial. Germination percentage of *D. cinerea* seeds that passed through the gastro-intestinal tract of goats (33.12 % ± 2.94) and sheep (36.00 % ± 2.68) was significantly higher than *V. tortilis* from the gut of goats (28.98 % ± 2.68) and the gut of sheep (23.04 % ± 2.81). Overall, *V. tortilis* (26.02 % ± 2.10) and *D. cinerea* (34.56 % ± 1.99) seeds that passed through the gastro-intestinal of goats and sheep germinated significantly higher ($P < 0.01$, Fig. 2.2.) than the untreated seeds/control (*V. tortilis* 5.07± 2.68; *D. cinerea*

2.311 ± 1.55). Significantly, more *V. tortilis* seeds (46.00 %) were retrieved from goats than *D. cinerea* seeds (13.00 %). The same was true for *V. tortilis* seeds (52.00 %) from sheep than *D. cinerea* seeds (22.00 %; Fig. 1). No significant effects of animal species were found on percentage seed recovery ($P > 0.05$).

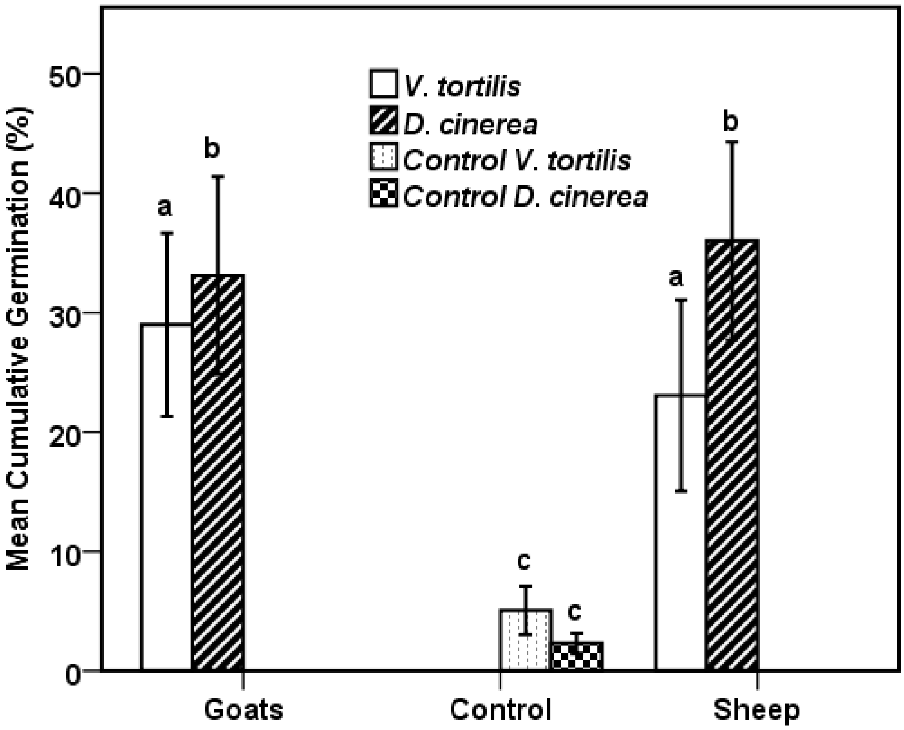

Fig. 2.2. Mean cumulative percentage germination of *D. cinerea* and *V. tortilis* seeds that passed through the gastro-intestinal of goats and sheep and, untreated seeds (i.e. no passage of *D. cinerea and V. tortilis* through the gut). Errors bars represent standard errors (S.E).

2.5. Discussion

The relatively high seed viability of *V. tortilis* (96 %) and *D. cinerea* (85 %) post the germination trial shows that the passage through the gut of these livestock did not compromise germination potential. However, it must be noted that the dispersal of viable seeds does not ascertain seed germination (James et al. 2011). Seed germination under natural conditions depends on appropriate environmental and seed survival conditions (James et al. 2011; Grellier et al. 2012).

Pods of *D. cinerea* and *V. tortilis* both contained relatively high crude protein with the lowest observed in *D. cinerea* pods. However, the differences observed in the amount of crude protein between *D. cinerea* and *V. tortilis* did not affect pods consumption by sheep and goats. The quality of associated diet is one of the paramount determinants of successful livestock faecal seed dispersal (Whitacre and Call 2006). Seeds ingested with associated high forage quality, in this study *D. cinerea* and *V. tortilis* pods tend to pass faster through the digestive (i.e. high seed recovery) with less damage to the seed coat (Tjelele et al. 2014). Feed of high digestibility, high crude protein and low fibre passes more rapidly through the gastro-intestinal tract of animals, presumably carrying more seeds (van Soest 1994; Simao Neto and Jones 1987; Tjelele et al. 2014). Depending on the hardness of the seed coat, seeds that reside in the digestive tract for longer tend to germinate better (Whitacre and Call 2006). *Dichrostachys cinerea* seeds has hard-seeded coat, which may have resulted in resistance to damage in the gut (Gardener et al. 1993; Tjelele et al. 2014). The hypothesis that the relatively high crude protein of either *V. tortilis* or *D. cinerea* pods may result in higher seed recovery was supported by the results acquired in this study. These results were consistent with the results acquired by Tjelele et al. 2014, which demonstrated the role that crude protein plays in seed passage rate.

The retention time of feeds in the digestive tract of goats is usually shorter than that of sheep (McGregor and Whitting 2013). However, the seed recovery and germination percentage of *V. tortilis* and *D. cinerea* did not differ between the animal species. This is possibly because animal physiology is not the only determinant but the interaction of different determinants such as associated diet quality (i.e. pods), and characteristics of seeds may increase seed recovery (Whitacre and Call 2006). For example, smaller seeds usually have higher recovery during the first few days after consumption than later in the period because they more readily separate from fibrous digesta in the rumen (Gardener et al.1993; Whitacre and Call 2006).

2.6. Management implications

Pod quality (i.e. low condensed tannins and high crude protein) and passage through the gastro-intestinal tract of sheep and goats played a major role in seed recovery and germination, which may increase the dispersal of viable *V. tortilis* and *D. cinerea* seeds. This suggest that goats and sheep feeding on pods high in crude protein and low condensed tannins passes more quickly through the gut, resulting in low scarified seeds and therefore low germination potential. Moreover, this may be one of the plant's evolutionary strategies to disperse their seeds away from the parent plants.

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Chapter 3

Effects of diet mixing on intake of tannin-rich plants by goats

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3.1. Abstract

The use of woody plants as a source forage is known to be limited by plant secondary metabolites (PSMs) such as condensed tannins. To overcome the negative effects of PSMs, herbivores have developed a number of behavioral and metabolic coping means. One of these means is increasing the variety of dietary plants in ways that possibly increase their ability to consume chemically defended plants. This study tested the prediction that goats offered diverse woody plant species in the diet consume more forage and condensed tannins than those exposed to single plant species diets. A total of 24 indigenous goats with an average body weight of 26.6 kg ± 0.51 (SEM) were individually kept in 2 m^2 pens and randomly categorized into four groups of six goats each. The four groups were offered either *Searsia lancea*, *Searsia pyroides*, *Euclea crispa*, and a mixture of all three diets. *Searsia lancea* contained 2.70 % (CT), *S. pyroides* 5.20 % (CT), *E. crispa* 6.44 % (CT) while the mixed diet contained 7.20 % (CT). The mean dry matter intake varied significantly among dietary groups (P < 0.001). Goats offered mixed diet i.e. *S. lancea*, *S pyroides* and *E. crispa* had the highest dry matter intake than goats separately offered *S. lancea*, *S pyroides* and *E. crispa*. Similarly, goats offered a mixed diet consumed more CT ($P < 0.001$) than those offered single browse species. These results support the postulation that animals foraging in mixed diet systems consume more PSMs and achieve higher feed intake than animals confined to monocultures or single species feeding systems. Management strategies that improve herbivore ability to consume woody plants will increase forage availability and inform bush control programmes.

Additional keywords: African savannas; complementarity; herbivory; plant defence; ruminants

3.2. Introduction

Woody plants and shrubs serve as a crucial source of protein for goats in arid and semi-arid environments, their intake by herbivores is limited by a widespread of plant secondary metabolites (PSMs) (Rogosic *et al*. 2008) and mainly condensed tannins in African savannas. To overcome negative effects of these PSMs, mammalian herbivores have developed various behavioral and metabolic coping strategies (Coley and Barone 1996; Rogosic *et al*. 2008). One of these strategies is increasing the variety of dietary plants in ways that possibly increase their ability to consume chemically defended plants. The explanation for this is based on the limitation of detoxification theory (Freeland and Janzen 1974). This theory projects that the quantity of feed that herbivores can consume depends on the detoxification rate of PSM's contained in the feed. One of the hypotheses developed from this theory is that herbivores consume more PSM-rich feeds of wide range of chemical diversity because detoxification would be diffused over many metabolic pathways (Marsh *et al*. 2006). Thus, reduce constraints on enzymes and substrates. Furthermore, different PSMs are to a lesser degree toxic when consumed as a dilute mixture than when one PSM is consumed in great amounts (Freeland and Janzen 1974).

Even though the effects of diet mixing on the intake of PSM-rich plants and shrubs have been shown previously, nothing is known as yet, about the effects of diet mixing on herbivores browsing in African savannas. Most of the work so far on diet mixing was done in North America and the Mediterranean ecosystems that are endowed by completely different groups of PSMs than those in the African savannas (Rogosic *et al*. 2006, 2007; Villalba *et al*. 2004; Papachristou *et al*. 2007; Saric *et al*.

2014). African savanna woody plants are predominantly defended by carbon-based PSMs (Scogings *et al*. 2014; Bryant *et al*. 1983; Bryant *et al*. 1992), opposed to nitrogen-based PSMs (Villalba *et al*. 2004; Windels *et al*. 2003). Given these differences, the detoxification limitation theory may not apply in savanna ecosystems because condensed tannins, which predominantly defend woody plants in African savannas, are generally not toxins (Mueller-Harvey 2006). Instead, CTs are digestibility-reducers that, at high dosage can affect intake and become toxic. They are structurally large and tend to be poorly absorbed which makes it hard to believe that they can be detoxified (Mueller-Harvey 2006).

Goats (*Capra hircus*) are important and common domestic mixed-feeders in African savannas (Kerley *et al*. 1995). They have the character of surviving on harsh and degraded rangelands (Devendra 1978). Woody plants are an important constituent of the diet of goats though they are mixed-feeders. Goats usually graze during wet season and switch to browsing during dry season. Thus, exposure to a number of plant species may help goats choose combinations that may result in more balanced diets with less detrimental effects of PSMs (Rogosic *et al*. 2007).

The objective of this study was to determine the effects of diet mixing on the intake of browse species (i.e. *Searsia lancea*, *Searsia pyroides* and *Euclea crispa)* by goats. It was hypothesized that, (1) goats offered single plant species will have less intake compared to goats offered mixed plant species, and (2) goats offered mixed plant species may consume more condensed tannins.

3.3. Materials and Methods

3.3.1*. Study area*

The study was conducted at the Agricultural Research Council (ARC) Roodeplaat Experimental Farm, Gauteng Province, South Africa (28°19´E, 25°35´S). The natural vegetation component of the farm used for livestock production and game encompasses an area of approximately 2100 ha. The vegetation type of Roodeplaat is Marikana Thornveld (Mucina and Rutherford 2006), and is characterized by open *Vachellia karroo* and *Vachellia caffra* woody plant species (Mucina and Rutherford 2006). Shrubs are dense along drainage lines and rocky outcrops. The general soil type is Hutton. The mean annual rainfall is 646 mm (November and March). The minimum and maximum summer and winter temperatures are 20-29 °C and 2-16 °C, respectively.

3.3.2. *Experimental design*

A total of 24 indigenous goats aged between 1.5 and 2 years with average body mass of 26.6 kg ± 0.51 were utilised in the study. All goats were dewormed prior to the experiment. Goats were randomly categorized into four groups of six goats per group. Each group was fed one of the diets (diet one; *Searsia lancea*; diet two 2; *Searsia pyroides*, diet three 3; *Euclea crispa* and diet four; was a combination of the three diets (*S. lancea, S. pyroides* and *E. crispa*).

Each animal was individually placed in a 2 m^2 pens and offered fresh clean water *ad libitum* throughout the experiment. Goats were acclimatized to the experimental conditions for seven days prior to data collection, and each animal was offered the experimental diet from 9:00 to 12:00. Fresh *S. lancea*, *S. pyroides* and *E. crispa* branches (< 50 cm long and 12mm diameter) were collected every morning prior to feeding. The experimental diets were weighed before feeding to the animals. Refusals were collected and weighed. Feed intake was calculated as distinction between weight in and refusals. Goats were provided with a maintenance diet of *Medicago sativa ad libitum* from 14:00 -17:00. Refusals were gathered and no other nourishment was offered until the following day. This was done to prevent goats from feeding during the night so that voluntary feed intake on the experimental diet was not affected in the following morning. The feeding experiment lasted for seven days. The Animals Ethics Committee of the University of South Africa (2015/CAES/015) and the Agricultural Research Council (APIEC15/003) approved the experimental procedures.

Feed intake were reported on dry matter basis. To calculate the dry matter feed intake of the diets, fresh leaves of the three diets and the combination of the three diets were weighed and oven dried. Plant samples were oven-dried at 30°C for one week. After the diets were removed from the oven, they were weighed again to calculate the dry matter of the diets.

3.3.3. *Chemical composition*

Each day during the experiment, a random grab sample of each diet was taken and bulked in a sealed bag pending the analysis. *Searsia lancea*, *S. pyroides*, *E. crispa* and a combination of were analysed for crude protein (CP) using Kjeldahl block digestion method (AOAC 2000) neutral detergent fibre (NDF) using tector fibertec system (Van Soest *et al*. 1991). Acid detergent fibre (ADF) and acid detergent lignin (ADL) using heat treatment of the samples with sulphuric acid containing cetyltrimethyl ammonium bromide (Goering and Van Soest, 1970).

Condensed tannins were determined by acid-butanol proanthocyanidin assay with purified sorghum as a standard for CT estimation (Hagerman 1995). Condensed tannins intake was also calculated. The intake of CT was calculated as: browse intake*(CT content/100) (Mkhize *et al*. 2016). The results were then used to compare the intake of CT between the experimental diets.

3.3.5. *Statistical analysis*

Univariate analysis of variance (ANOVA) was used to evaluate significant differences between dietary groups on the DM and CT intake. Intake was considered the dependent variable and the diets considered the independent variable. Each goat (*n* = 24) was considered as an experimental unit. Distinctions between means were considered significant at 5 % level. The SPSS (2013) was used for data analysis.

3.4. Results

3.4.1. Chemical composition

Table 1: chemical composition of *S. lancea*, *S. pyroides*, *E. crispa* and the mixed diet.

Parameters	***S. lancea***	***S. pyroides***	***E. crispa***	**Mixture of *S. lancea*, *S. pyroides* and *E. crispa***
CP (%)	8.50	9.03	6.19	8.96
NDF (%)	39.37	40.30	30.02	38.28
ADF (%)	21.46	27.07	26.20	23.72
ADL (%)	12.50	10.89	16.63	11.13
CT (%)	2.70	5.20	6.44	7.20

3.4.2. Browse *intake*

The overall intake varied among experimental diets (*P* < 0.01) with the highest being the mixed treatments (Figure 3.1). Goats offered mixed diet i.e. *S. lancea*, *S pyroides* and *E. crispa* had the highest dry matter intake than goats individually offered *S. lancea*, *S pyroides* and *E. crispa*. There were also significant differences in the intake of condensed tannins between the experimental diets (*P* < 0.01). Goats offered a mixed diet consumed more condensed tannin than goats offered single forage species. *Serasia pyroides* and *E. crispa* had higher condensed tannin intake than *S. lancea*. However, no significance difference was found between *S. pyroides* and *E. crispa* on condensed tanning intake (Figure 3.2). Goats had an average body weight of 26.6 kg (SE ± 0.51) at the beginning of the experiment and an average body weight of 28.2 kg (SE ± 0.43) at the end of the experiment.

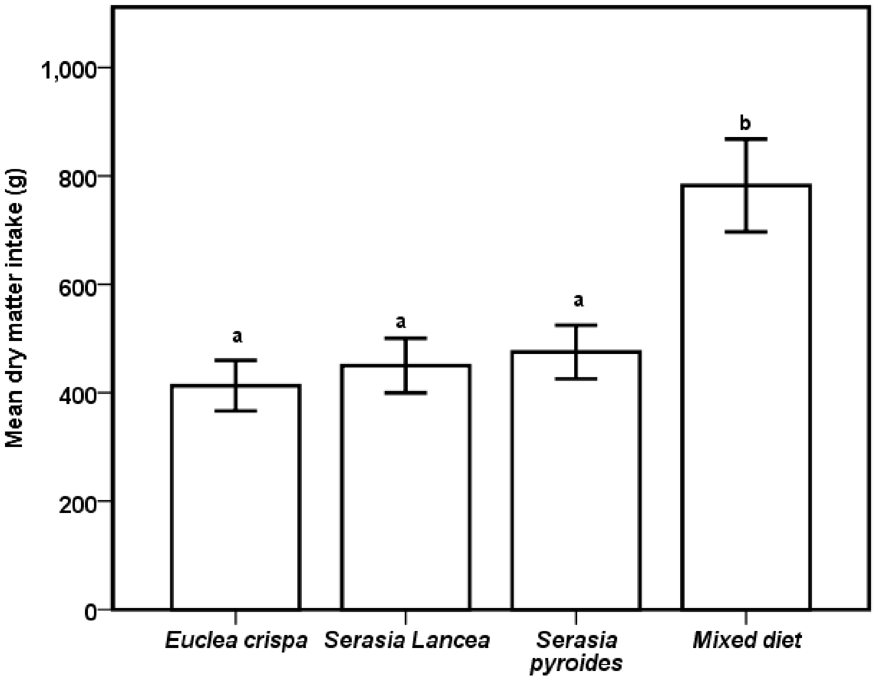

Figure 3.1. Mean of browse intake of *E. crispa*, *S. lancea* and *S. pyroides* and a mixture of all browse species by goats. The letters represent significant differences among different treatments, and errors bars represent standard error.

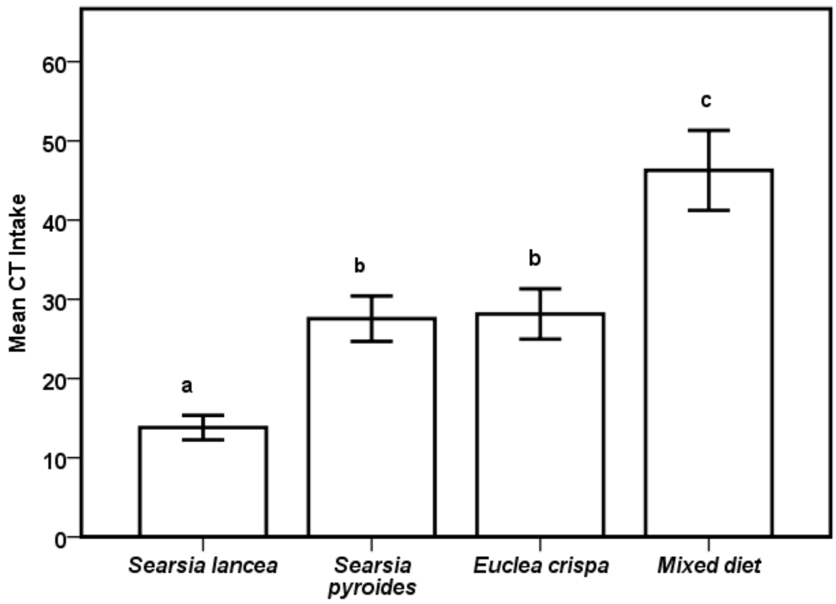

Figure 3.2. Mean of condensed tannins intake of *Euclea crispa, Searsia lancea* and *Seasia pyroides* and a mixture of all browse species. The letters represent significant differences among different treatments, and errors bars represent standard error.

3.5. Discussion

The chemical composition of forage play a significant role in feed intake and dry matter digestibility among others. High fibre content that result in low feed digestion may reduce feed intake, and thereby leads to reduced nutrients intake (Warghorn 2008). High fibre content may increase chewing duration and rumination required to thoroughly process the feed (Laca *et al*. 2001; Warghorn 2008). However, fibres contained in the study diets alone may not justify the significant feed intake observed in this study, as this may depend on different components (Forsyth *et al*. 2005). For instance, plant species offered to the animals in this study had relatively high crude protein, which has the minimum requirements of 7% crude protein except for *E. crispa*. Condensed tannin levels exceeding 5% in forages may reduce protein and digestibility; however, it must be noted that these factors alone may not deter herbivores from foraging on browse material (Forsyth *et al*. 2005) especially for mixed-feeder such as goats. Intake of tannin-rich plants may depend on various associating elements such as crude protein and alternative forage.

The significant intake of mixed forages (i.e. *S. lancea*, *S. pyroides*, *E. crispa*) and condensed tannins than single browse species by goats in this study is in accordance with the concept that herbivores select different feed items to complement their nutrient requirements; however, keeping away from over-ingestion of plant secondary metabolites (Mkhize *et al*. 2015; Bailey and Provenza 2008). These results were consistent with results from other studies demonstrating that a mixed diet may allow herbivores to increase their intake of different PSMs (Rogosic *et al*. 2006; Villalba *et al*. 2004). The concomitant increase in dry matter feed intake and CTs intake by goats exposed to diets with diverse species has positive implications for animal production (i.e., bypass protein and reducing internal parasite burden) (Chalupa 1975; Lisonbee *et al.* 2009).

The results acquired in the current study suggest that diet mixing can also be used as a tool to increase the intake of condensed tannins rich woody plants in African savannas (Figure 3.1). Most PSMs are classified as toxins because they can be absorbed, detoxified and removed from the body through the liver (Marsh *et al*. 2003, 2005). However, CTs are not absorbable (Marsh *et al*. 2003). Thus, the detoxification hypothesis may not justify the intake of tannin-rich feeds in response to diet mixing in this study. To explain the increased consumption of browse by goats offered a mixed diet, a few alternative explanations may be proposed. Firstly, goats offered a mixed diet might have consumed more browse material as means of replacing lost protein during the secretion of tannin binding salivary protein (Yisehak *et al*. 2011). Therefore, this might be a trade-off between gaining more crude protein for physiological needs and losing salivary protein. The flavour-nutrient-tannin interaction may also justify the results acquired in this study (Rogosic et al. 2006). A mixed diet presumably contains different flavours which may encourage herbivores to increase their dry matter feed intake than single diets. Alternatively, hunger may motivate further feeding and habituation (Szentesi and Bernays 1984).

Mixed diet provides herbivores with chemical diversity that helps them cope better with chemical defences. Monoculture/single species systems may limit intake and compromise nutrition. Providing goats with a mixed diet increased overall total intake of browse material. Thus, animal practitioners in African savannas should provide animals with mixed browse materials and not feed them individual plants species. The results obtained in this study provided crucial information for the management of woody plant encroachment and herbivore production in African savannas. Woody plant encroachment is already reducing farm-grazing capacities in African savannas and this problem is predicted to double by 2050.Therefore, strategies that improve herbivore ability to consume woody plants will increase forage availability and inform bush control programmes and policies.

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Chapter 4

4.1. Synthesis

The primary objective of this study was to comprehend the interactive effect of crude protein and condensed tannins on intake, seed recovery and germination of woody plant species. Several studies have been conducted to understand mechanisms such as seed characteristics, diet quality and animal species in isolation on seedling recovery and seedling germination. There are a number of factors separate or combination, which may result dispersal of viable woody plant seeds in the rangelands. For instance, plant secondary metabolites such as glycoalkoloids alone or in combination with other factors may influence retention time in the gut of an animal and the dispersal of seeds far from the parent plant. There is an increasing rate and degree of woody plant encroachment in grasslands and savannas at the expense of herbaceous layer. This pattern is anticipated to increase due to various components including among others, animal seed dispersal.

To better understand mechanisms underlying seed recovery and seed germination, I studied 1) the effects of crude protein and condensed tannins in seed recovery and germination of *Vachellia tortilis* and *Dichrostachys cinerea* pods fed to ruminants and 2) effects of diet mixing on the intake of browse species by goats.

Despite the extensive occurrence of woody plant encroachment (Hoffman and Ashwell 2001), its dynamics are not entirely understood, especially processes that prompt to woody plant encroachment by animal seed dispersal (Higgins et al. 1996; Ward 2005; Scholes and Archer 1997; Sharam et al. 2006). It must be noted that seed recovery and seedling establishment are essential process in plant population dynamics since they affect the distribution and abundance of plant species (James, et al. 2011). In addition, foliage and pods of many woody plant species serves as a source of protein for browsers, and thus animals could exacerbate woody plant encroachment through the dispersal of viable seeds by animals. In chapter two, I indicated that seed recovery of *V. tortilis* from goats and sheep was higher than *D. cinerea* from the same animal species. Interestingly, *D. cinerea* seeds retrieved from goats and sheep had more germination rate than *V. tortilis* seeds. The results from this study supported the hypothesis that the relatively high protein of the seedpods species enhances seed passage (Whitacre and Call 2006; Tjelele et al. 2014). Thus, high quality pods may escape the rumen faster and germinate away from the parent plant, depending on other factors such as environmental factors (Bodmer and Ward 2006; Grellier et al. 2012; James et al. 2011). In addition, the relatively high-condensed tannins in *D. cinerea* pods explained the lower seed recovery than *V. tortilis* and thereby resulting in high germination rate (James et al. 2011).

I indicated in chapter three that mixed diet (i.e. *S. lancea*, *S. pyroides* and *E. crispa*) allow animals to increase intake of high-tannin rich browse plants while avoiding over-ingestion of plant secondary metabolites (Villalba et al. 2004; Bailey and Provenza 2008; Rogosic et al. 2007; Mkhize 2015).

4.2. Management implications

It is evident from this study (Chapter two) that sheep, goats and pod quality played an important role on the recovery of viable woody plant seeds. Together these factors may facilitate woody plant encroachment. Thus, this suggests that mammalian herbivores foraging on certain woody plant pods are likely to disperse intact seeds that can remain viable and potentially germinate. This mechanism may have both positive and negative effects on the abundance of plant species. It may increase the abundance of woody plant species in ways that may worsen woody plant encroachment in ecosystems and/or increase the biodiversity of plant species in these ecosystems.

The results obtained in chapter three showed that diet mixing could increase the use of tannin-rich plants that are usually avoided (Villalba et al. 2004, Regosic et al. 2006, 2007). This in combination with other treatments (e.g. fire) may be used as a management tool for woody plant encroachment. This would not only contribute to the management of increasing woody plants but also as feed for browsers and mixed feeders. Mixed diet provides herbivores with chemical diversity that helps them cope better with chemical defences. Monoculture/single species systems may limit intake and compromise nutrition. This may allow them to forage more and mitigate the possibility of any deterring factors.

4.3. Future research

In rangelands, goats have been thought to be helpful agents of woody plant control (Trollope et al. 1989; O'Connor 1996). However, their ability has been shown to be mostly limited by plant secondary metabolites, for instance, condensed tannins that are reported to reduce the woody plant intake (Scogings et al. 2014; Owen-Smith 1993; Cooper and Owen-Smith 1985). Therefore, to achieve increased livestock production further research is needed.

4.3.1. Can nutrients and polyethylene glycol supplementation increase the intake of mixed diets by ruminants?

There is scientific proof that nutrient supplementation could permit animals to ingest more PSMs and enhance nutrient balance (Foley et al. 1999; Illius and Jessop1996). Freeland and Janzen (1974), who projected herbivores to increase the intake of plants containing PSMs as long as their ability to neutralize and eliminate these toxins is not surpassed. This hypothesis offers an exciting possibility to enhance the utilization of woody plants that are routinely avoided by herbivores and improve livestock productivity.

Furthermore, polyethylene glycol has been proven to greatly enhance feed intake and effectiveness of browse by goats. For instance, goats supplemented with PEG increased forage intake (Silanikove at al. 1996). Polyethylene glycol also enhances the organic matter digestibility of woody plant species. Polyethylene glycol binds tannins irreversibly over an extensive variety of conditions (Gilboa et al. 2000). Polyethylene glycol has a high affinity for binding condensed tannins and preventing the emergence of tannin-protein association. Therefore, its presence lessens the formation of protein-tannin complexes. According to Rogosic et al. (2008), supplementing herbivores fed mixed forage (Mediterranean shrubs) with PEG only has small beneficial effects on feed intake. However, the influence of PEG supplementation on the intake of mixed diets in African savannas are not yet known. Thus, more research is needed to determine the effect of nutrient and PEG supplementation on the intake of mixed diet offered browsers. Applying these practices together may allow herbivores to forage even better.

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