Unlocking resources in savannas:

How goats and other mixed feeders overcome the negative effects of tannins

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This research was conducted under the auspices of the C.T. de Wit Graduate School for Production Ecology and Resource Conservation (PE&RC)

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Thesis

submitted in fulfilment of the requirements for the degree of doctor

at Wageningen University

by the authority of the Rector Magnificus

Prof. Dr M.J. Kropff,

in the presence of the

Thesis Committee appointed by the Academic Board

to be defended in public

on Thursday 7 May 2015

at 11 a.m. in the Aula.

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Unlocking resources in savannas: How goats and other mixed feeders overcome the negative effects of tannins
110 pages.

PhD thesis, Wageningen University, Wageningen, NL (2015)
With references, with summaries in Dutch and English

ISBN: 978-94-6257-427-2

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Chapter One

General Introduction

Ntuthuko R. Mkhize

Evolutionary theory predicts any organism to have been selected through natural selection for maximizing its inclusive fitness and optimizing food intake and diet selection (Gordon 2003, Gordon and Prins 2008). Foraging is therefore a central process in resource ecology since it leads to growth, survival and reproduction of the animals (Prins and Van Langevelde 2008). However, while foraging to meet their nutritional requirements, herbivores face the cost of (among others) plant physical and chemical defences (Rooke et al. 2004). Physical/mechanical defences such as thorns and spines reduce the amount of food that herbivores can harvest per unit of time, thereby reducing the acceptability of forages to herbivores (Cooper and Owen-Smith 1986, Papachristou et al. 2003). While mechanical defences restrict the harvesting rate, chemical defences are especially significant because plants produce a variety of secondary metabolites that can potentially cause sickness and even kill the animal. Plant secondary metabolites are thought to be a plant's evolutionary response to herbivore and fungal attacks (Bryant et al. 1991b, Hattas 2014). Given that woody plants produce relatively higher concentrations of plant secondary metabolites in comparison to graminoids (Bryant et al. 1991a, Bryant et al. 1992), chemical defences are even more important in savanna rangelands that are increasingly getting dominated by woody plants (via bush encroachment). While foraging in savanna ecosystems, herbivores (whether livestock or game) encounter plant species that differ in their concentrations of nutrients and plant secondary compounds and hence have to adopt foraging and physiological strategies to deal with changes in forage quality and defences.

Until 1959, plant secondary metabolites were simply referred to by many scientists as plant waste products of primary plant metabolism (Fraenkel 1959). Scientists initially thought that these chemicals were deposition excess carbon fixed by photosynthesis (Iason 2005). In those days, plant secondary metabolites were conveniently grouped as glucosides, saponins, tannins, alkaloids, essential oils organic acids and thousands many others. It was almost 60 years ago when Fraenkel (1959) recognised the leading ecological role of secondary metabolites in the interactions between plants and herbivores. Since then countless papers have reported plant secondary metabolites as plant defences against a broad range of herbivores and funguses, and science has achieved broad insights into the diversity of plant secondary metabolites and herbivore adaptations to these chemicals (Bryant et al. 1983, Bryant et al. 1989, Foley 1999). The initial acceptance of a co-evolutionary arms race between plants and herbivores, with antagonism as the pivotal interaction, led to a general view that plant secondary metabolites were toxins that needed be avoided, tolerated or overcome by consumers (Forbey and Hunter 2012). However, later work on plant-herbivore interactions identified these chemicals either as feeding deterrents (DeGabriel et al. 2014), toxins (Marsh et al. 2014), digestibility reducers (Foley 1999), feeding or oviposition cues, and signals for communicating to neighbouring plants and natural enemies of herbivores (Dicke 2009, Moore and DeGabriel 2012). There was later a general trend towards acceptance that plant secondary metabolites could have beneficial effects. It is ironical that the chemicals that were once thought to be the waste products of inconsequential value are now pivotal to the ways in which plants interact with their environment (Iason et al. 2012).

In savanna ecosystems, foraging ecology of especially browsers and mixed feeders is strongly shaped by digestibility reducers (e.g., condensed tannins) and other carbon-based secondary metabolites (Bryant et al. 1983, Cooper et al. 1988, Bryant et al. 1992, Scogings et al. 2014). Just how plant characteristics such as condensed tannins affect mammalian herbivores has been explored extensively, mostly through experimentation with captive animals which simplifies the complexity of natural systems. These experiments have shown that condensed tannins act within the digestive system (gut) of herbivores (especially ruminants) by either forming complexes with forage substrates (such as proteins) and preventing degradation (Robbins et al. 1987a, Robbins et al. 1987b) or inhibiting digestive degradation by the herbivores' endogenous or ruminally-derived digestive enzymes such as proteinase inhibitors (Makkar 2003). The complex interactions between condensed tannins and nutrients (especially protein) have been shown to ultimately reduce the apparent protein digestibility in many foregut and hindgut-fermenting species of placental mammals when offered tannin-rich forages (Iason 2005, Foley 1999). Tannins can also shift the site of protein digestion via increasing the ruminal escape proteins and nitrogen excretion from urine to faeces of ruminants (Woodward and Reed 1997, Kaitho et al. 1998).Tannins further bind carbohydrates to some extent (Mueller-Harvey 2006) which may have negative implications for fermentation and thus energy value of tannin-rich forages. Pen experiments have not only shown tannins to act as digestibility reducers, but also as feeding deterrents (McArthur et al. 1991) that affect intake, feeding behaviour and diet choice of herbivores (Bedoya-Perez et al. 2014).

Data obtained from captive animals have demonstrated that particular nutrients can help herbivores counteract the negative effects of specific toxins and tannins (Villalba et al. 2002a, Baraza et al. 2005, Villalba and Provenza 2005). These interactions among nutrient and plant secondary metabolite are believed to change the ways in which herbivores perceive the costs and benefits of eating certain plants and thus ultimately altering forage intake and diet choices (Wang and Provenza 1997, Mote et al. 2007). The theoretical framework for how herbivores evolved these behavioural and physiological strategies to counteract the negative effects of plant secondary metabolite in general, was first developed more than 40 years ago when Freeland and Janzen (1974) drew together some disparate toxicological observations. They predicted mammalian herbivores to continue consuming the toxin-containing plants as long as their physiological capacity to neutralize and excrete the toxins is not exceeded (Freeland and Janzen 1974). It is now a well-established notion that processes such as synthesis of detoxification enzymes, supply of carbohydrates and amino acid precursors for conjugation and excretion, and maintenance of the acid balance to compensate for acidic end-product formation can all deplete nutrients (Freeland 1975, Freeland et al. 1985a, Freeland et al. 1985b, Illius and Jessop 1995, 1996). Thus processes associated with detoxification of toxins and deactivation of digestibility reducers such as tannins reduce the energy and protein that otherwise would be available to the herbivores for their growth, maintenance or reproduction.

Supplementing animals with nutrients such as proteins and energy is believed to replace nutrients depleted by the detoxification or deactivation processes, and to increase herbivores' capacity to detoxify toxins and deactivate tannins, in ways that increase the consumption of chemically defended forages (Dziba et al. 2007, Rogosic et al. 2011). While this is suggesting a potential for rangeland and herbivore managers to manipulate the utilization of chemically defended vegetation, no rigorous test of this nutrient-plant secondary metabolite interaction hypothesis has been conducted in the African savannas. Contrary to other ecosystems, savannas are dominated by woody plants that are endowed predominantly with condensed tannins. Although condensed tannins and toxins are all plant secondary metabolites (from plant ecologists' perspective), according to animal ecologists tannins and toxins differ immensely in how they induce their effects on the herbivores. These chemicals also differ markedly in how they are physiologically dealt with by herbivores. Although there is evidence that protein supplementation improves intake of diets rich in condensed tannins (Banner et al. 2000, Villalba et al. 2002b, Rogosic et al. 2009), it is not yet clear whether these supplements replace proteins precipitated by condensed tannins (Villalba et al. 2002c) or the nutrients depleted via detoxification of the toxins coexisting with tannins in many forages. This information is crucial in developing management strategies aimed towards increasing defoliation of the encroaching woody plants in savannas. Moreover, this information will guide future attempts to model the roles played by tannins on plant-herbivore interactions, both from the plant and herbivore perspectives.

Supplementing herbivores that forage on tannin-rich systems with polyethylene glycol (PEG) has been proposed to alleviate the adverse effects of tannins in studies of common brushtail possums (Foley and Hume 1987, Marsh et al. 2003) and many other herbivore species. PEG is a polymer that binds condensed tannins irreversibly over a wide range of pH levels ,and it presence reduces the formation of protein-tannin complexes (Makkar et al. 1995b). Studies with domestic ruminants suggest that feeding PEG to wild ruminants could have adverse effects, depending on the tannin content and activity (Foley et al. 2007). Some believe that addition of PEG could decrease productivity of wild ruminants especially when heavily infested by internal parasites that could be killed by consuming tannin-rich forages (Silanikove et al. 2001, Perevolotsky et al. 2006). However, supplying ruminants with PEG in captivity, has increased the intake and digestibility of tannin-rich forages (Kumar and Vaithiyanathan 1990) and performance (wool production and live weight) (Barry 1985, Min et al. 1999). Under controlled conditions animals have been shown to self-regulate their intake of PEG depending on the amounts of food and tannin ingested (Villalba and Provenza 2001). This implies that animals may be able to recognise the benefits of ingesting the amounts of PEG related to the amount of tannin ingested. The main gap in the current understanding of how PEG influences foraging behaviour and productivity of herbivores is reconciling results obtained from pens with those from the field with free-ranging herbivores.

It is clear (from the literature reviewed here) that condensed tannins are important mediators of browse-browser interactions in the African savanna ecosystems. However, knowledge in this field is still based on the interactions between single (or a few) plant species and captive herbivores that are mostly offered diets containing tannins under highly simplified conditions. There is no doubt that a considerable amount of work done with animals in controlled conditions has contributed a better understanding of how animals might forage under certain conditions. However, although controlled experiments are a necessary first step in isolating and characterising the actions of tannins, they greatly oversimplify the complex interactions that occur between wild herbivores and plants in the field (Moore and DeGabriel 2012). The next challenge for ecologists is to translate the roles of tannins observed in controlled experiments at small temporal scales into the field. Until then, it will remain a daunting (or even an impossible) task to confidently link condensed tannins, nutrients and herbivore productivity (DeGabriel et al. 2014).

While experiments with captive animals usually describe plant-herbivore interactions over very short time intervals, in nature these interactions are continuous and the effects of tannins can be long-lasting (Cheeke 1996). Animal foraging preferences are dynamic and they often change with seasons or reproductive states of the animals. There is compelling evidence in literature that herbivores can better meet their needs when offered a variety of forages that differ in nutrients and plant secondary metabolites than when constrained to a single food (Provenza et al. 2007). Given that variety of plant species is a rule rather than an exception in nature, we should not expect such simple relationships between forage quality and foraging by free-ranging herbivores as demonstrated in captivity (Foley et al. 2007, DeGabriel et al. 2014).

Research Objectives

The main objectives of this research were;

(1) To investigate how condensed tannins influence foraging behaviour and growth performance of free-ranging ruminant herbivores, and

(2) To determine the effects of supplements on use of woody plants and intake rates of condensed tannins by free-ranging herbivores.

A clearer understanding of how chemical characteristics (e.g., condensed tannins) of woody plants interact with browsers and mixed feeders will present an opportunity for improving herbivore productivity and guide future modelling of browse-browser interactions in savanna rangelands dominated or encroached by chemically defended tree species.

Putting the study animal in context

Although a domestic goat (*Carpa hircus*) was used as a model mixed feeding ruminant, the results presented in this thesis are also applicable to a wide range of wild herbivores with similar morphological, behavioural, and physiological traits. The choice of a study animals was influenced by a number of factors; Firstly, docility of this animal species and the potential ease with which I could manipulate the initial conditions for the experimentation rendered goats ideal for this work (Bailey and Provenza 2008). Goats are found under a wide range of climatic conditions and are a species of domestic animals with the largest distribution in the world (Alexandre and Mandonnet 2005). About 88% of world's goat population is located in Africa and Asia, and the majority in the tropics and sub-tropics. Goats are probably the most important domestic browsers in African savannas (Nyamangara and Ndlovu 1995) and most of them are located in the arid and semi-arid zones of Sub-Saharan Africa (Lebbie and Ramsay 1999). Particularly in South Africa there are over 6 million goats that are owned by two distinct sectors; the commercial and the small-scale, non-commercialised farmers (Roets and Kirsten 2005). These animals are generally kept for provision of milk, meat, hair (mohair, cashmere) and skins (Ngambi et al. 2013). With the majority of goats owned by small-scale subsistence farmers, goat production is a major source of income and food for families in South Africa. Therefore, this thesis is not only of academic value to ecologists interested in how tannins mediate plant-herbivore interactions. In addition, to its ecological value, this research provides solutions that may improve goat productivity in degraded rangelands, with concomitant improvement in the livelihoods of the goat farming populations.

Goats have been considered as useful agents of woody plant control, especially following fire or mechanical clearing (Du Toit 1972, Trollope 1980). While this may generally be due to their reputation to survive environmentally harsh conditions, it is specifically due to their ability to compile varied diets that are relatively high in browse (Ramírez-Orduña et al. 2008). During the dry season, browse becomes the most important source of forage because crude protein in browse is relatively constant during the year and is usually higher than that of grasses in the dry season (Ramírez et al. 1993). Numerous studies have cited condensed tannins and other phenolic compounds as potential constraints to the success of bush control programs that use goats and other browsing herbivores. In this thesis I show the potential effects of using herbivores to control chemically defended woody plants on herbivore productivity.

Thesis Outline

This introductory chapter has set the scene for the subject of the succeeding chapters. In this chapter I presented what is known about the effects of condensed tannins on foraging behaviour and productivity of large herbivores. I highlighted how knowledge in this area is strongly based on laboratory and pen experiments mostly with captive herbivore. I presented how data from these experiments may oversimplify the complexity associated with natural systems and I proposed that this thesis addresses the current lack of field data to an extent. In chapters two, three and four I report data on different components of the same field experiment. In chapter two I analysed the effects of tannins on the amount of time spent by free-ranging animals foraging on either grasses or woody plants. In this chapter I address the hypothesis that mixed feeders exposed to high levels of condensed tannins spend most of their foraging time grazing, less time browsing and reduce their foraging time. In chapter three, I show how mixed feeders alter their food intake rates, bite rates, bout length and numbers, number of dietary species, and diet composition as a strategy to regulate their intake of condensed tannins. In chapter four, I studied the growth performance consequences of condensed tannins. This chapter gives an opportunity to indirectly assess if the foraging behaviour responses presented in chapters two and three have any serious consequences for herbivore fitness. In chapter five, I investigated the effects of supplementing animals with high-protein and high-energy sources on foraging behaviour, diet composition and tannin intake rates under natural conditions. I concluded this thesis with chapter six, in which I summarized and integrated all results obtained in this study. I also attempt to discuss new perspectives obtained through this thesis to the understanding of chemical mediation of plant-herbivore interactions.

All data were collected between January 2012 and July 2013 at the Agricultural Research Council experimental farm in Roodeplaat near Pretoria, South Africa (Figure 1).

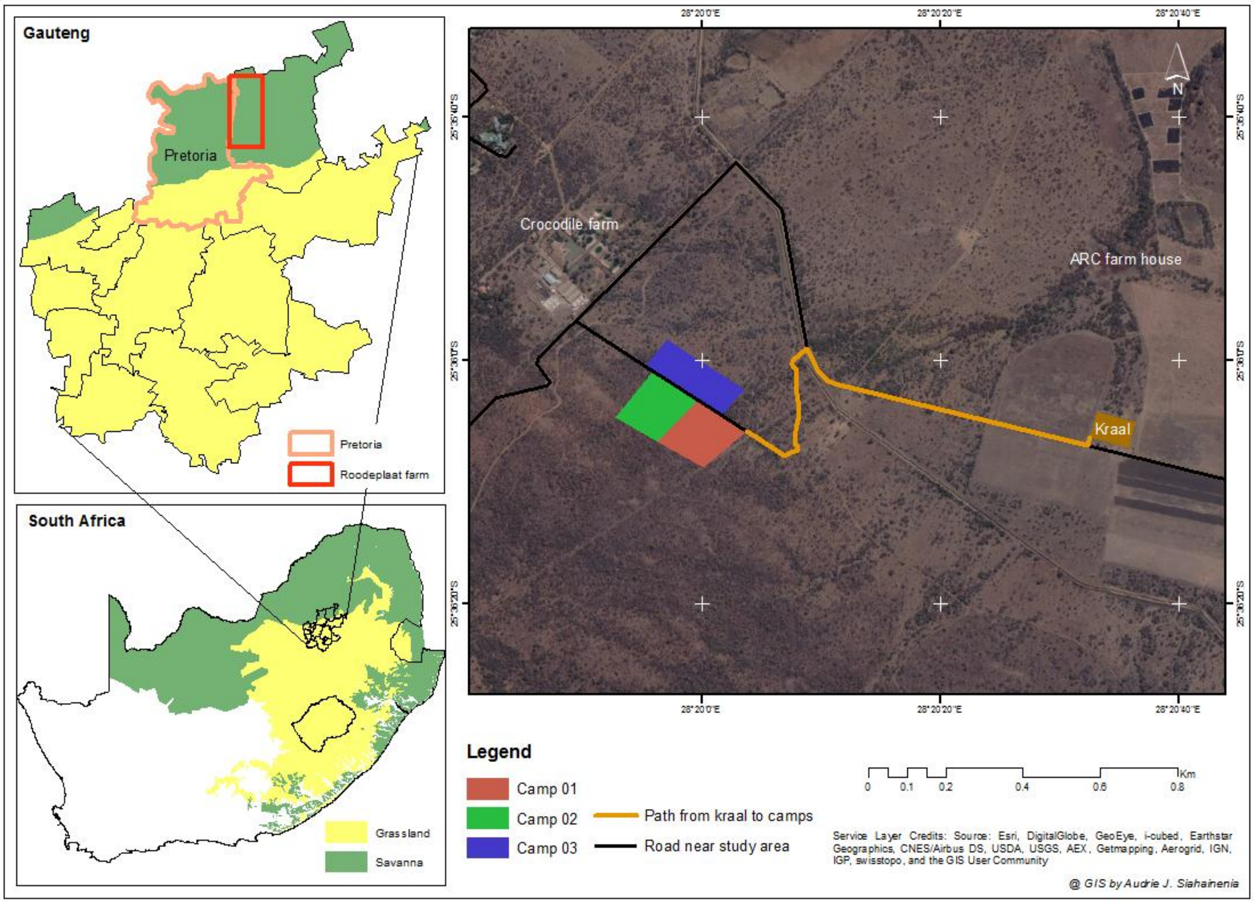

Figure 1: A map showing the country, province, city, biome and layout of the experimental site where the study area is located.

Chapter Two

Condensed tannins reduce browsing and increase grazing time of free-ranging goats in semi-arid savannas

Ntuthuko R. Mkhize, Ignas M.A. Heitkönig, Peter F. Scogings, Luthando E. Dziba, Herbert H.T. Prins, Willem F. de Boer

Abstract

Tannin concentrations fluctuate considerably in space and time within and among plant species, with consequences for forage quality of herbivores. The extent to which these fluctuations influence foraging activities of mixed feeders is not fully understood. While accounting for the effects of the time of the day and season, we tested the hypothesis that mixed feeders exposed to high levels of condensed tannins (i) spend less of their foraging time browsing, (ii) spend more time grazing, and (iii) reduce their total foraging time, especially during the dry season when grasses dry out and deciduous trees lose leaves. We orally dosed 15 goats with (i) 20g of condensed tannins extract (high tannin exposure), another 15 goats (ii) with 20g of polyethylene glycol, which known for neutralizing the effects of tannins, and the last group of 15 goats (iii) with 50 ml of water (control). We recorded the time spent on grazing, browsing and these two activities together (i.e., foraging) for 30 days in the dry and wet season. As expected, dosing goats with condensed tannins reduced their browsing time (%) and increased their grazing time. Goats dosed with polyethylene glycol increased their browsing time and lowered the time they spent grazing. Animals dosed with polyethylene glycol foraged for longer than animals from other treatment groups in the dry season. Goats dosed with condensed tannins increased their foraging time in the wet season. Overall, all treatment groups spent a similar amount of time foraging, indicating an instinctive drive by mixed feeders to maintain high total foraging time while avoiding over ingestion of tannin-rich forages. We concluded that tannins do not supress total foraging time for free-ranging mixed feeders. Instead, they influence the amount of time animals spend foraging on either herbaceous or woody forages.

Keywords: Feeding behaviour, Bush encroachment, Herbivore, Polyethylene glycol, Pre-dosing

Introduction

Browsing herbivores have been in a co-evolutionary arms race with their woody food resources for a long time (van Soest 1994, du Toit and Olff 2014), and consequently woody plants evolved an array of physical and chemical defences against herbivore attack (Gordon and Prins 2008, du Toit and Olff 2014). In Southern Africa, condensed tannins (CTs) are known to be the main chemical components affecting the nutritional value and acceptability of browse (Cooper and Owen-Smith 1985, Owen-Smith 1993, Basha et al. 2012). A number of field studies in the region have reported negative relationships between dietary condensed tannin concentrations [CT] and selection of browse by African antelopes (Cooper and Owen-Smith 1985), and giraffe (Furstenburg and van Hoven 1994). Numerous feeding experiments with domestic goats (*Capra hircus*) have also reported similar patterns (Dziba et al. 2003, Scogings et al. 2004, Shrader et al. 2008). Plant [CT] is a variable trait, varying with plant species (Hattas and Julkunen-Tiitto 2012), plant part (Furstenburg and van Hoven 1994), season (Cooper et al. 1988, Scogings et al. 2013), tissue developmental stages (Hattas et al. 2011), environmental conditions, and exposure to defoliation (Scogings 2005, Wessels et al. 2007, Kohi et al. 2011). While these variations in the plant [CT] may induce alterations in the activity patterns and foraging behaviour of browsing herbivores, we know of no studies that have elucidated the presumed alterations under natural conditions.

Although the effects of biotic factors such as forage quantity, nutrient and plant secondary metabolites (PSMs) on foraging behaviour are generally understood (especially in grazing mammals), there is limited insight on the effects of CTs on foraging behaviour under field conditions. Moreover, few studies have considered the variety of intertwined mechanisms used by mixed feeders to circumvent the negative effects of tannins (Estell 2010). These mechanisms range from the physiological secretion of proline-rich salivary proteins (Hanovice-Ziony et al. 2010b, Canon et al. 2013) to the behavioural strategies (discussed in chapter three) such as avoidance, regulation of intake below a certain thresholds, cautious sampling, altering sizes and patterns of feeding bouts, diet switching, or consuming diverse / complementary diets (Provenza et al. 2003, Marsh et al. 2006a, Jansen et al. 2007, Estell 2010).

Free-ranging herbivores are known to show daily and seasonal activity patterns (Shi et al. 2003) that optimize their nutrient intake, especially in a dynamic environment with varying levels of constraints (Shrestha et al. 2014). Considering that CTs are among the constraints, it is of importance to know the extent to which herbivores mitigate their negative effects. CTs are regarded as digestibility reducers (Dearing et al. 2005a) and thus reduce the nutritive value of browse forages. The resultant low nutrient concentrations in forage may require herbivores foraging on tannin-rich forages to either harvest and process larger volumes of food to meet their nutrient requirements (Dearing et al. 2005a) or they may need to feed selectively to avoid over-ingesting CTs (Iason 2005). Either way, this likely imposes longer or shorter foraging times as a way to counter the negative effect of CTs on the nutritional value of the food.

The main objective of this study was to determine the effects of CTs on foraging activity time budgets by mixed feeders in a semi-arid savanna. While accounting for the effects of time of day and season, we used yearling female goats to test the hypothesis that mixed feeders exposed to high levels of CTs (i) reduce the time they spend on browsing, (ii) increase time spent on grazing, and thereby (iii) achieve reduced total foraging time. We hypothesized that this is a strategy used by mixed feeders to cope with tannin-rich foraging environments in a natural foraging context. This is based on the idea that herbivores must minimize CT intake while maximizing nutrient intake (Freeland and Janzen 1974, Jansen et al. 2007). We thus, expected goats exposed to low tannin levels to spend more time browsing (Landau et al. 2002) and less time grazing. Forage availability for both herbaceous and woody vegetation declines in the dry season due to drying out of grasses and leaf loss by deciduous woody species in semi-arid savannas. Given that evergreen species, which retain their forage throughout the year, are highly chemically defended, we expected goats exposed to high CT levels to forage for even less time during the dry season. Even though we used goats as model organisms, this work is of importance to understanding other mixed feeders, such as impala or steenbok or even eland (McNaughton and Georgiadis 1986, Hofmann 1989).

Methods and materials

Study area

This study was conducted at Roodeplaat Experimental Farm of the Agricultural Research Council located in northern Gauteng, South Africa (25º20´-25º40´E; 28º17´-28º25´S). The climate in the study area is semi-arid with a mean annual rainfall of 646 mm. Daily maximum temperatures in the study area range between 20-29ºC in wet season and between 2-16ºC in dry season. The main growing season starts from November to April, and the dormant season starts in May and reaches its peak in July. The natural vegetation of the farm used for livestock production and game covers 2100 ha. The vegetation of Roodeplaat falls within the savanna biome classified as Marikana Thornveld (Mucina and Rutherford 2006). The rangeland is dominated by *Vachellia karroo, Vachellia tortilis, Ziziphus mucronata,* and some *Euclea* species. The herbs found in the study sites include *Lippia rehmannii* and *Tarconanthus camphoratus*. The dwarf shrub *Aloe greatheadii* var. *davyana* is also abundant in the farm. For nomenclature of plants we followed Coates Palgrave (2002) and Kyalangalilwa et al., (2013).

Study design

We used 45 yearling female goats ranging from 8 to 12 months old with an initial body weight of 14.9 (SD ± 3.7) kg. All animals were weighed one day before the experiment and were allocated to 3 treatment groups of equal number (N=15). This allocation was done such that the mean body weight for all groups was similar. These treatment groups were maintained throughout the experiment. All study animals were treated for internal and external parasites before the experiment and had *ad libitum* access to water throughout the experiment. Every morning between 07:00 and 08:00, 15 goats were orally dosed with 20 g of polyethylene glycol (PEG 6000) dissolved in 50 ml of water, while another 15 were dosed with 50 ml of water plus 20 g of CTs (extracted from mimosa bark) and the last 15 received 50 ml of water (control). The mimosa extract was obtained from the bark of Black Wattle (*Vechellia mearnsii*) tree and contained a minimum of 66% CT (http://www.mimosa-sa.com). Three grazing camps/paddocks of similar size (±1.7 ha) were fenced and each stocked with fifteen goats (i.e., 5 from each treatment group) daily from 08:00 until 16:00. From 08:00 all goats were allowed to forage freely in the field until 16:00 when they were kraaled to avoid predation.

Data collection

Behaviour activities of goats in three paddocks were monitored for 30 days during the dry (between June-August 2012) and for 30 days during wet (between January-March 2013) seasons. All study goats were marked with paint on their flanks to facilitate identification during observations. The goats were conditioned for a period of two weeks before the actual observations, to habituate them to the presence of observers and to allow close monitoring of behaviour.

After the conditioning period, fifteen goats (5 per treatment) were allowed to forage together in each of the three paddocks for the duration of the experiment. On each day, nine goats (3 from each treatment group) were randomly selected and observed. The three goats observed per treatment group would be foraging in different paddocks. Of the nine goats observed each day, three (one from each treatment group) were observed in the early morning (08:00 to 10:30), three (one from each treatment group) observed in the late morning (10:30 to 12:00) and the other three (one from each treatment group) observed in the afternoon (12:00 to 15:30). Each goat was observed for fifteen minutes sequentially by one observer and one recorder throughout the experiment. The same people observed and recorded the behavioural activities throughout the experiment. The observations were conducted for thirty days and thus a total of 270 observations (90 per treatment) were made in each season. The Observer XT 10.5 (Noldus 1991b) in combination with a Psion Work-about handheld computer (Noldus 1991b, Zimmerman et al. 2009) was used to record the time (seconds) spent on different behavioural activities. The foraging activities recorded were grazing (consumption of only grasses), browsing (consumption of forage from woody plants). Non-foraging activities that were recorded include standing (without eating and sometimes while ruminating), walking (moving from one place to another), resting/laying down (sometimes while ruminating), searching (moving the head sideways in between different forage plants), drinking, rasping (scratching the head or body against plants), and socialising (touching other animals in a playful or aggressive manner). Non-feeding activities were overridden by foraging in cases where they occurred at the same time. For current purposes, data for all non-feeding activities were pooled. In addition to behavioural activities, the observation date, starting and ending times, treatment, paddock and the goat identification number were recorded.

Data analysis

The percentage (%) of time spent on grazing, browsing or foraging (grazing + browsing) was analysed using the general linear model option of SPSS, v20 (IBM SPSS Statistics; Chicago, IL, USA), with a manual backward selection of significant variables. In each model, season (dry and wet), treatment (PEG, CT and control) and time of day (early morning, late morning and afternoon) were used as fixed factors, while the paddock (1, 2 and 3) served as a random factor. The unstandardized residuals for all the models followed a normal distribution and the results of Levene's test showed equal variances. The Sidak comparison test was used for differences among the treatments and seasons.

Results

The percentage of time spent on total foraging depended significantly on the interaction between season and treatment (Table 1), with goats dosed with CT spending less time on foraging during the dry season compared to the wet season (Figure 1). The opposite was true for goats supplemented with PEG, while time spent by the control group on total foraging remained unchanged throughout the seasons. Although there were clear seasonal effects within treatments, no significant differences were observed between the control group and the two extremes in CT exposure level (i.e., CT and PEG). Time allocated to foraging was highest in early mornings and lowest in the afternoons irrespective of the season or treatment (Table 1).

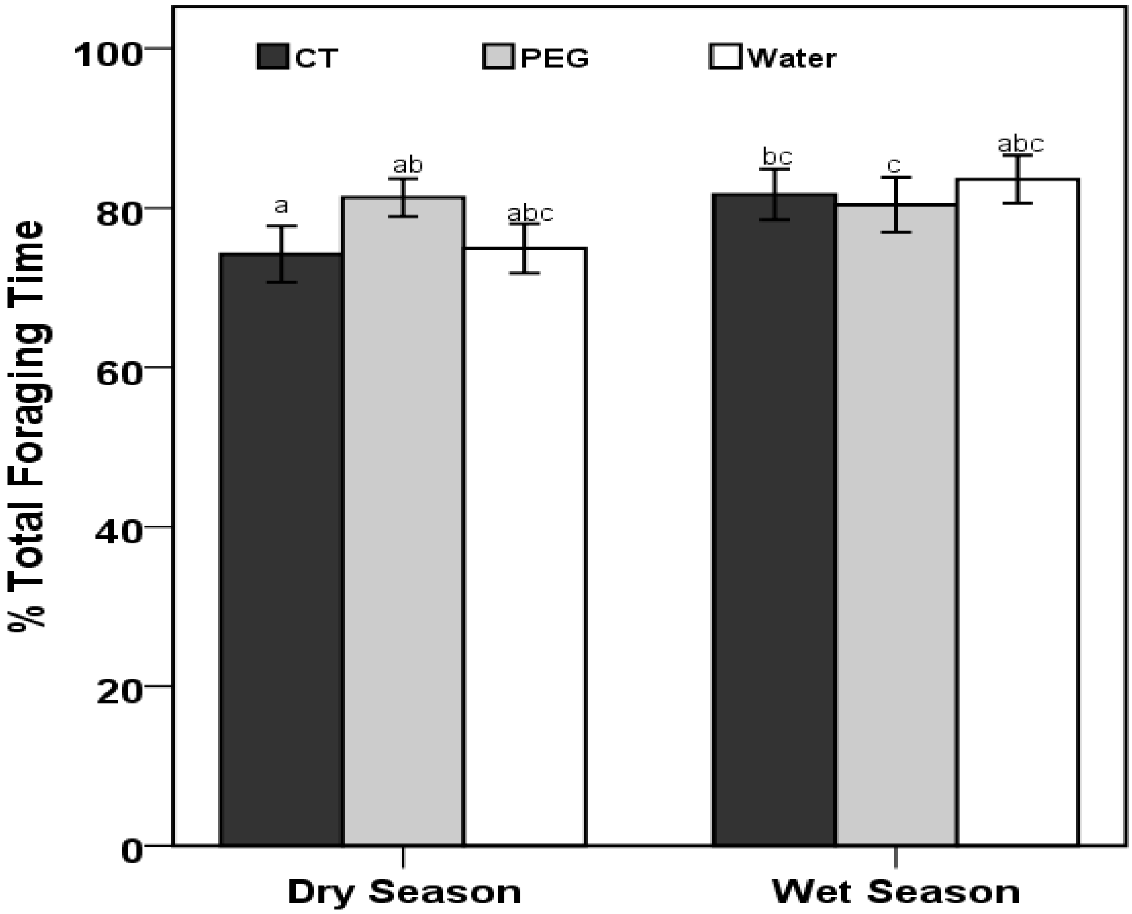

Figure 1: Mean time (± 95% CL) spent on foraging activities by goats dosed with condensed tannins (CT), polyethylene glycol (PEG) and water (as a neutral control) in the dry and wet season. The different superscripts represent significant differences between treatments and seasons.

Of all the time goats spent foraging, 57% was devoted to browsing. The percentage of time spent browsing was influenced by a three-way interaction between season, treatment and time of day (Table 1). Supplementing animals with PEG resulted in them spending more time on browsing equally throughout the seasons (Figure 2), with small variations among different times of day. Although time spent by CT and control goats on browsing decreased as the day progressed, browsing time by PEG goats remained relatively constant throughout the day. The percentage of the time that goats allocated to browsing was always shorter than that allocated by the PEG goats.

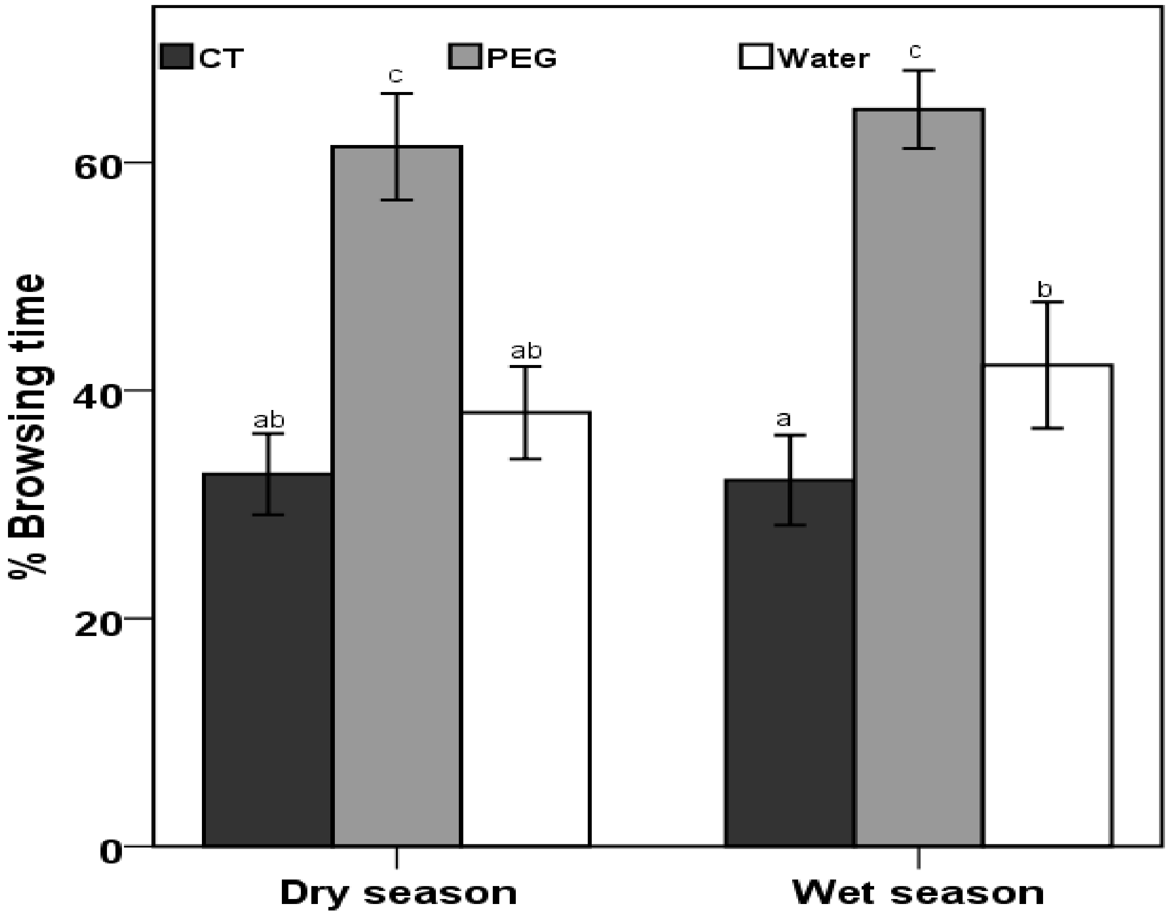

Figure 2: Time (± 95% CL) spent on browsing by goats dosed with condensed tannins (CT), polyethylene glycol (PEG) and water during the dry and wet season. The different superscripts represent significant differences between seasons and treatments.

Table 1. Results from the GLM, testing for the effect of daily administration of free-ranging goats with 20g of condensed tannins (CT), 20g of polyethylene glycol (PEG) and 50ml of water on foraging, browsing and grazing time (%) during early morning, late morning and afternoon in the dry and wet seasons.

Variable	**Source of Variation**	$F_{(df)}$	***P-value***
Foraging	Season	$_{1,533}$ = 16.27	0.000
	Treatment	$_{2,533}$ = 1.71	0.181
	Session	$_{2,533}$ = 20.76	0.000
	Season*Treatment	$_{2,533}$ = 5.70	0.003
Browsing	Season	$_{1,523}$ = 1.96	0.162
	Treatment	$_{2,523}$ = 118.95	0.000
	Session	$_{2,523}$ = 12.38	0.000
	Season*Treatment*Session	$_{12,523}$ = 1.85	0.038
Grazing	Season	$_{1,523}$ = 2.73	0.099
	Treatment	$_{2,523}$ = 99.32	0.000
	Session	$_{2,523}$ = 0.51	0.601
	Season*Treatment*Session	$_{12,523}$ = 1.86	0.037

The percentage of time spent by goats on grazing significantly varied with the interaction between season, treatment and time of the day (Table 1). Goats supplementing with CT grazed for more than double (2.6 times) the time gazed by goats supplemented with PEG (Figure 3). Goats dosed with CT grazed relatively more in the wet than in the dry season, while the opposite was true for goats dosed with PEG. While the percentage of time allocated to grazing by the control and PEG animals decreased gradually from early morning to afternoon in both seasons, it remained relatively similar throughout the day for animals supplemented with CT.

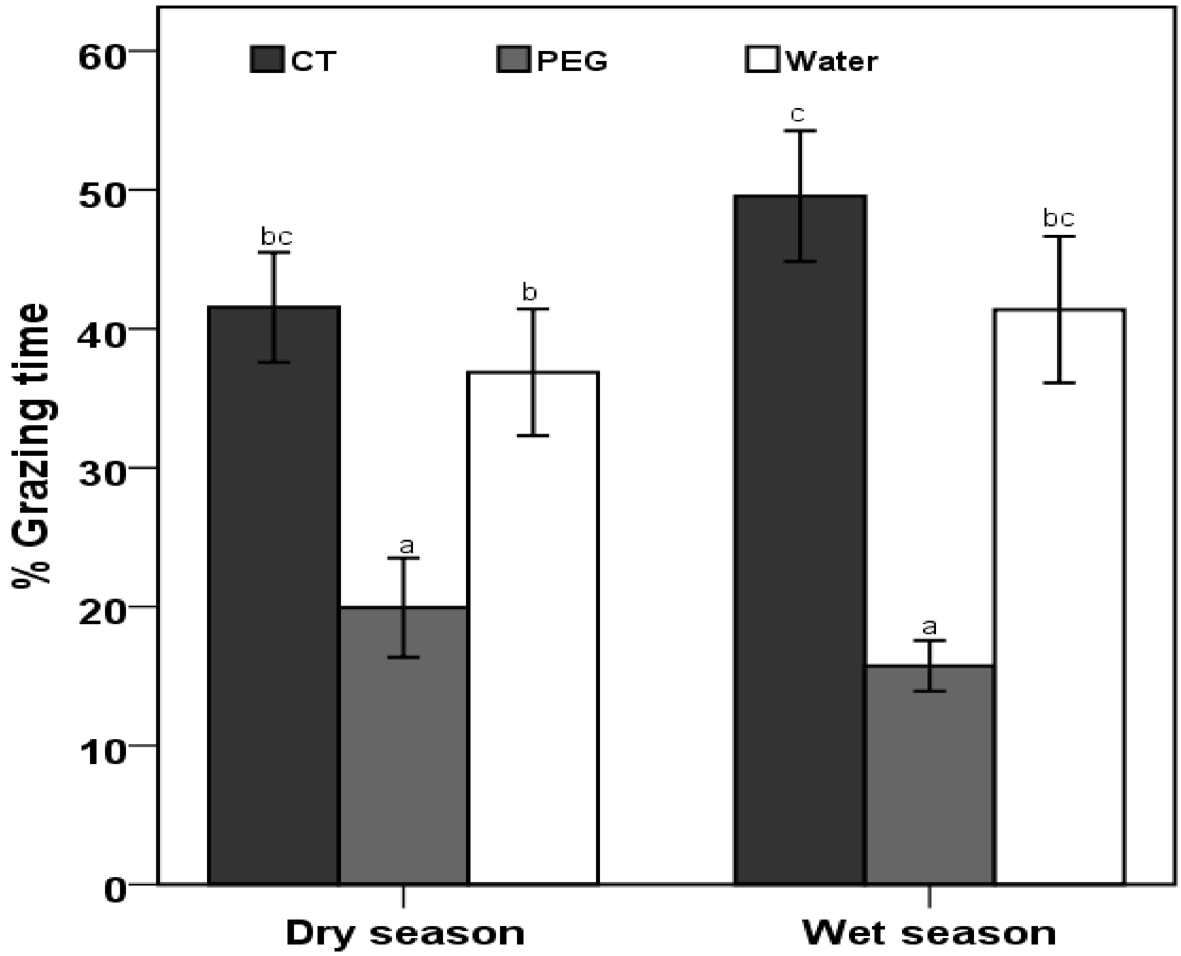

Figure 3: Time (± 95% CL) spent on grazing by goats dosed with condensed tannins (CT), polyethylene glycol (PEG) and water during the dry and wet. The different superscripts represent significant differences between treatments and seasons.

Discussion

In this study, we used domestic goats to investigate the effects of CTs on the browsing, grazing and total foraging time budgets of mixed feeders, as an important step towards a better understanding of the behavioural responses of mixed feeding herbivores to plant chemical defences. Through dosing the goats with PEG, water and tannins, we created groups of animals with low, medium, and high exposure levels to CTs respectively.

Our results supported the hypothesis that CTs reduce browsing time and increase time spent on grazing. Goats are generally small-sized mixed feeders that are relatively sensitive to forage quality fluctuations (Hofmann 1989). They flexibly switch from grazing to browsing and *vice versa* in ways that meet their high nutrient requirements (Hofmann 1989, Kos et al. 2012). Experimentally exposing goats to high CT levels likely imposed an increased foraging constraint and made it difficult for them to meet their nutrient requirements from browse (Chapter four). Browsing likely increased the consumption of tannin-rich browse over and above the high content of tannins in their rumen and intestinal tract following the CT dosage. Goats, as expected, shifted towards spending more time on grass which is nearly always tannin-free (Ellis 1990). Grasses generally have a higher digestibility than browse although grasses contain more cell wall and lignified fibres than browse especially during the dry season (van Soest 1994, Duncan and Poppi 2008). The observed time spent grazing by the CT dosed goat shows that CTs can influence whether a mixed feeder will browse or graze. Goats used time switching strategies that likely influenced their foraging behaviour to reduce tannin consumption and minimize their tannin intake (Dearing et al. 2005a, Iason 2005). Moreover, the consistently high time spent grazing by CT dosed goats even during the dry season may indicate that the importance of CTs as foraging constraint surpasses that of the cell wall material and lignified fibres.

Studies have shown animals to increase forage intake in chemically defended environment by mixing plant species that containing a variety of nutrients and PSMs in their diets (Freeland and Janzen 1974, Provenza et al. 2003). For example, increasing dietary botanical diversity has resulted to high tannin-rich shrub intake by the Mediterranean sheep and goats (Rogosic et al. 2006a, Rogosic et al. 2006c, b). Since the current study was conducted in a multi-species savanna with free-ranging goats whose nutrition was entirely dependent on their ability to utilise forage resources from the field, we consider diet mixing as a possible explanation for the relatively high browsing time invested by the control goats throughout the seasons.

We predicted that the time spent by the CT-dosed goats on total foraging would be lower than that spent by their PEG and control counterparts especially during the dry season. This prediction was premised on the fact that small-sized mixed feeders (such as goats) are poorly able to process high-fibre diets (Silanikove 2000, Kos et al. 2012), and that browse availability reduces in the dry season when deciduous species lose leaves. We therefore expected animals exposed to high levels of CTs to spend more time searching for high quality forages that contain low or no tannins and fibres, as they seek to meet their nutrient requirements while circumventing the CT constraint. The observed treatment x season interaction corroborated our prediction. For example, the time spent by CT-dosed goats on foraging was significantly lower in the dry than the wet season. The opposite was observed for the PEG goats. The observed similar total foraging time in this study for all treatment groups indicates a clear compromise that mixed feeders make on daily basis to maximize their foraging time while dealing with biotic constraints (Shi et al. 2003).

Acknowledging the inextricable effects of season and time of day on our variables of interest (percentage of time browsing, grazing and total foraging), we included these factors in our analysis. Animals across all treatment groups invested most of their browsing and grazing in the early morning and least time was spent foraging in the afternoon, which agreed with results from other studies (Shi et al.

2003, Bakare and Chimonyo 2011). The animals had been fasted overnight, which partly explains why they prioritised foraging in the morning and most of their afternoons on non-feeding activities.

In this study we used goats to show the importance of CTs on the resource use by mixed feeders. The overall conclusion in this study was that tannins do not supress total foraging time for free-ranging mixed feeders. Instead, they influence the amount of time animals spend foraging on either herbaceous or woody forages. The ability of large herbivores to utilise browse resources is important for African savanna rangelands degraded by bush encroachment. Woody plant encroachment is a major threat to livestock production and biodiversity of both farmed and conserved savannas rangelands (Trollope 1980, Ward 2005, Gray and Bond 2013). The encroaching woody species invade into open grasslands or thicken up in wooded areas (Trollope 1980), suppressing the palatable grasses and herbs for grazing herbivores. Because browse is generally a poor substitute for grasses as a forage resource, especially for sheep and cattle, bush encroachment significantly reduces stocking rates of grazers (Gray and Bond 2013). These consistent with an idea that mixed feeders such as goats can be useful agents of woody plant control, especially following fire or mechanical scrub clearing (Du Toit 1972, Teague 1989, Magadlela et al. 1995, Luginbuhl et al. 1998). However, the current results suggest that bush control programs may successfully increase browse utilization through supplementing mixed feeding herbivores with PEG.

Acknowledgements

Experimental procedures followed in this study were approved by the Animal Ethics Committee of the ARC under permit number APIE11/039. Lucas Letsoalo and Piet Monegi are thanked for their assistance during data collection. Although this study was supported by the National Research Foundation (NRF) of South Africa, any opinions, findings and conclusions or recommendations expressed in this material are of the authors and the NRF does not accept any liability in regard thereto. The Wageningen University through its PhD sandwich fellowship provided to the first author.

Chapter Three

Seasonal regulation of condensed tannin consumption by free-ranging goats in a semi-arid savanna

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Abstract

Although condensed tannins (CTs) are known to reduce forage intake by mammalian herbivores in controlled experiments, few studies have tested these effects in the field. Thus the role of CTs on foraging ecology of free-ranging herbivores is inadequately understood. To investigate the effects of CTs under natural savanna conditions, we pre-dosed groups of goats with polyethylene glycol (PEG, a CT-neutralising chemical), CT powder or water before observing their foraging behaviour. While accounting for the effects of season and time of the day, we tested the hypothesis that herbivores forage in ways that reduce the intake rate (g DM per minute) of CTs. We expected pre-dosing goats with CTs to reduce CT intake rates by (1) consuming diets low in CTs, (2) reducing bite rates, (3) increasing the number of foraging bouts, or (4) reducing the length of foraging bouts. Lastly, (5) expected CT to have no influence the number of dietary forage species. In both wet and dry seasons, pre-dosing goats with CTs resulted in lower CT consumption rates compared to PEG goats which seemed relieved from the stress associated with CT consumption. During dry season, the number of dietary forage species was similar across treatments, although goats that were dosed with PEG significantly increased this number in the wet season. Dosing goats with PEG increased the number and length of browsing bouts compared to goats from the other treatments. Pre-loading goats with PEG also tended to increase bite rates on browse forages, which contributed to increased consumption rates of CTs. Based on the behavioural adjustments made by goats in this study, we concluded that herbivores under field conditions foraged in ways that reduced CTs consumption.

Keywords: Bite rate, Intake rate, Feeding bout, Polyethylene glycol, Herbivore

Introduction

Condensed tannins (CTs) are widely distributed among the nutritionally important forages in the African savanna rangelands (Mueller-Harvey 2006, Barbehenn and Constabel 2011), and their consumption by mixed feeding and browsing herbivores is therefore unavoidable (Provenza 1996, Sorensen et al. 2005a, Sorensen et al. 2005b). To reduce the deleterious effects of dietary CTs, herbivores are purported to regulate their daily intake of browse species such that CT intake is minimized without compromising the overall dry matter intake (Wiggins et al. 2003, Marsh et al. 2006b). Although intake regulation of CTs is expected to involve altering of meal patterns, these short-term alterations are not clearly understood. Feeding experiments show captive herbivores to reduce the intake rate (g DM per minute) of forages containing compounds with known anti-nutritional, toxic or digestibility-reducing effects (Marsh et al. 2007, Foley 1999).

The length of feeding bouts has been reported to be shorter for animals exposed to toxin-containing forage sources than those exposed to toxin-free forages (Dearing et al. 2005b, Foley et al. 2007, Foley 1999). Marsh et al. (2007) reported high concentrations of formylated phloroglucinol compounds to cause koalas to eat more slowly, eat shorter meals and eat less per meal. Moreover, Wiggins et al (2003) indicated that plant secondary metabolites not only constrain overall intake, but also alter feeding behaviour of the animals. Altered feeding patterns are believed to reduce the negative influence of PSMs on intake (Wiggins et al. 2006a, Wiggins et al. 2006b). However, we know of no rigorous tests of these short-term behavioural alterations over the timescale of feeding bouts and inter-bout intervals with regard to CTs (Foley 1999). Given that rumen microbes are not capable of degrading CTs (Makkar et al. 1995a, Makkar et al. 1995c), these compounds are unlikely to be absorbed and transported to liver cells, and therefore may not induce foraging alterations similarly to the plant secondary metabolites (i.e., toxins) that are detoxified through activation of liver enzymes (Makkar 2003). Therefore, CTs are different from toxins and are expected to affect foraging behaviour differently.

Forages that are rich in CTs likely require longer chewing and digestive processing time than similar forages that contain little or no CTs (Terrill et al. 1994). Thorough chewing by herbivores with CT-binding salivary proteins (Juntheikki et al. 1996, Shimada 2006, Hanovice-Ziony et al. 2010b) is required to facilitate effective insalivation of food during mastication, which may lower intake rates (g DM per minute) through reducing the bite rates (Foley 1999). Moreover, in the presence of CTs in the rumen (pH 5.5-7.0), most of the dietary proteins and carbohydrates remain bound and protected from microbial degradation (Hagerman et al. 1992). However, some of the bound protein are released later in the abomasum (pH 2.5 to 5.1) enabling protein digestion and amino acid absorption in the small intestines (Barry and Manley 1984). We therefore, hypothesized that herbivores foraging in African savanna rangelands that are dominated by CT-rich woody plants would forage in ways that reduce their intake rates (g DM per minute) of CTs.

Mixed feeders are known to consume varied diets (i.e., mixed diets) as a means to maintain high forage intake from plants that are rich in different secondary metabolites while avoiding excessive ingestion of individual plant secondary metabolites (Bailey and Provenza 2008). This has been explained in terms of the detoxification limitation hypothesis (Freeland and Janzen 1974) which predicts varied diets to spread detoxification of toxins over many metabolic pathways , thereby reducing constraints on liver enzymes and substrates (Marsh et al. 2006a). While this hypothesis has been tested mostly on toxins (Dearing et al. 2000, Marsh et al. 2005, 2007), the extent to which CTs influence the number of dietary species and diet composition in the field is poorly understood. Given that CTs are not toxic and thus are not detoxified via the liver, we would not expect goats that are foraging in CT-rich environments to increase dietary species diversity. Instead we predicted goats in the African savannas to deal with CT constraint by switching their diets from CT-rich to CT-poor forages.

To determine the effects of CTs on short-term foraging behaviour and diet composition of free-ranging goats in a semi-arid African savanna, we pre-dosed goats with (1) polyethylene glycol (PEG, an anti-tannin agent dissolved in water), (2) CT powder dissolved in water or (3) only water. While accounting for the effects of season and time of the day, we tested the hypothesis that free-ranging goats in the African savannas forage in ways that reduce the intake rate of CTs. We predicted that pre-dosing goats with CTs will lead animals to (a) maintain the number of dietary forage species, (b) consume diets lower in CTs (c) reduce bite rates, (d) increase the number of foraging bouts, or (e) reduce their foraging bout length. We defined a bout as a period of continuous foraging on a particular forage species separated by either a non-foraging activity (De Castro 1975) or by foraging on a different plant species. We used goats as an important model organism for understanding feeding behaviour of mixed feeders, such as impala, lamas, steenbok, deer or eland (McNaughton and Georgiadis 1986, Hofmann 1989).

Materials and Methods

Study area

We carried out a field experiment at the Roodeplaat Experimental Farm located in Pretoria, South Africa (25º20´-25º40´E; 28º17´-28º25´S). The climate is semi-arid with a mean annual rainfall of 646 mm and mean daily maximum temperatures between 20-29ºC in January and 2-16ºC in July (Panagos et al. 1998). The main wet season occurs from November to April, and the dry season starts in May and reaches its peak in July. The vegetation of the farm falls within the savanna biome and is classified as Marikana

Thornveld (Mucina and Rutherford 2006). The rangeland is dominated by *Vachellia karroo*, *Vachellia tortilis, Ziziphus mucronata* and some *Euclea* species. Nomenclature of plants followed Coates Palgrave (2002) and Kyalangalilwa et al. (2013).

Study design

Forty five (45) yearling female goats ranging from 8 to 12 months old with an initial body weight of 14.9 (standard deviation ± 3.7) kg were used in this experiment. Goats were allocated to three treatment groups such that all groups had an equal number of goats (N=15) and a similar mean body weight. Fifteen goats received a daily oral dose with 20g of polyethylene glycol (PEG 6000) dissolved in 50 ml of water whereas another 15 were dosed with 50 ml of water plus 20 g of CT extract (from mimosa bark) (MIMOSA Extract Company (Pty) Ltd., Pietermaritzburg, South Africa) and the last 15 received 50 ml of water (control) before they were released to the field. The mimosa extract was obtained from the bark of the Black Wattle (*Vachellia mearnsii*) tree and contained a minimum of 66% CTs on dry matter basis. Three grazing camps/paddocks of similar size (1.8 ha) were fenced and stocked with 15 goats (i.e., 5 from each treatment group) daily from 08:00 until 16:00. All study goats were treated for internal and external parasites before the experiment and had *ad libitum* access to water throughout the experiment. From 08:00 onwards, all goats were allowed to forage freely in the field until 16:00 when they were corralled to avoid predation. The goats were corralled 1.2 km away from the camps and they received free-access to water and no feed while in the corral. The experiment was approved by the Animal Ethics Committee of the ARC under permit number: APIEC11/039.

Data collection

The foraging behaviour of goats in the field was recorded during the dry (June-August 2012) and wet (January-March 2013) seasons. To aid easy identification during observations, we marked all study goats with paint on the flanks. To habituate the goats to the presence of observers and to allow close monitoring of behaviour, we subjected them to a two week conditioning period before the actual observations. On each day of the actual observation, nine goats (i.e., 3 from each treatment group) were randomly selected and observed. The three goats observed per treatment group would be foraging in different paddocks. Of the nine goats observed each day, three (one from each treatment group) were observed in the early morning (08:00 to 10:30), three observed in the late morning (10:30 to 12:00) and the other three observed in the afternoon (12:00 to 15:30).

One observer followed each goat for fifteen minutes, while assisted by one recorder throughout the experiment. The same team observed and recorded the foraging behaviour throughout the experiment. Per observation, we identified and counted the number of dietary forage species, and recorded the date, starting time, treatment, paddock and the goat number for each observation. The observations were conducted for thirty days and thus a total of 270 observations lasting 15 min each (i.e., 9 observations per day x 30 days) were made per season. We used the Observer XT 10.5 (Noldus 1991a) in combination with a Psion Work-about handheld computer (Noldus 1991a, Zimmerman et al. 2009) to record the forage species being eaten, bite rate (number of bites per minute during foraging), number of times a goat foraged from each species (number of bouts) and the amount of time (seconds) the goat spent on each foraging bout (bout length). Grazing was recorded as one species (i.e., "grass") without distinguishing the different species.

To estimate the tannin concentration [CT] of the diet consumed by the study goats, we sampled leaves of all plant species included by goats in the diet, and analysed them for CTs. In each season, a minimum of 8 leaf samples (each with fresh weight of 20g) were collected from unbrowsed branches at about 1.2 m height or lower. Collected leaves were oven-dried at 60 ^{o}C till completely dry. Dried samples were finely ground to pass through a 1mm screen and stored in plastic honey jars pending chemical analysis. Condensed tannins were determined using the acid-butanol assay method (Porter et al. 1986). Since it was not possible to purify all forage species consumed by goats, a purified sorghum was used as a standard for CT estimation (Hattas and Julkunen-Tiitto 2012). This analysis allowed us to estimate the [CTs] (mg/g equivalent on dry matter basis) for each plant species, which we further report as [CTs] (mg/g). Sampling was carried out during dry and wet season separately and the lab analyses were conducted at the Botany laboratory of the Department of Biological Sciences, University of Cape Town.

To estimate the amount of CTs consumed (g DM) per minute, we multiplied the total number of bites taken from each plant species by the mean [CT] (mg/g DM) and by the mean bite size (g DM) for each plant species in each season. Since it was not possible to estimate bite sizes from the field, we, estimated the bite size in a pen experiment. Bite size was not only important in estimating the amount of food (g DM) per bite (which was used in estimating CT intake), but it was also important in estimating intake rates achievable from each forage species. During each of the two seasons (one day after the field observations), we selected 10 of the study goats and penned them individually under a shelter. At least 10 un-browsed branches of each of the plant species that were included by goats in the diet during field observations were collected from the sites in which the field observations were done. We then estimated the bite sizes (g DM per bite) according to Mkhize et al. (2011).

For each plant species we estimated the average percentage inclusion in the diet of each goat. This percentage was calculated as the quotient of the consumption (g DM) of each plant species and total consumption of all species during an observation multiplied by 100.

Data analysis

Differences in (1) CT intake rate, (2) bite rates, (3) length of feeding bouts and (4) intake (g DM) by goats from different treatment groups were analysed with linear models using a manual backward selection of variables. In each model, season (dry and wet), treatment (PEG, CT and control) and time of day (early morning, late morning and afternoon) were fixed factors, while the paddock served as a random factor. The unstandardized residuals of CT intake rates model were normally distributed only after a natural log transformation. All other response variables met the normality and variance homogeneity assumptions without any transformation. A generalized linear model, with a Poisson distribution was used to analyse the effect of season, treatment and time of the day on (1) the number of forage species in the diet and (2) the number of foraging bouts. We applied a Sidak test for pairwise comparisons between different treatment groups, seasons and times of the day. We also conducted a simple regression analysis of CT content and consumption. All analyses were performed using SPSS, v20 (IBM SPSS Statistics; Chicago, IL, USA).

Results

There was a significant interaction between treatment and season on CT intake rates of free-ranging goats ($F_{2,533} = 11.69$; $P < 0.001$; Figure 1). All goats, except for those that were dosed with PEG, achieved lower CT intake rates in the wet than in the dry season (Figure 1). In both seasons, control and CT-dosed goats tended to consume CTs at lower rates than the PEG-dosed goats. Although further analysis showed a gradual decline in CT intake rates from early-morning to the afternoon across all treatments, the time of the day did not significantly influence CT intake rates.

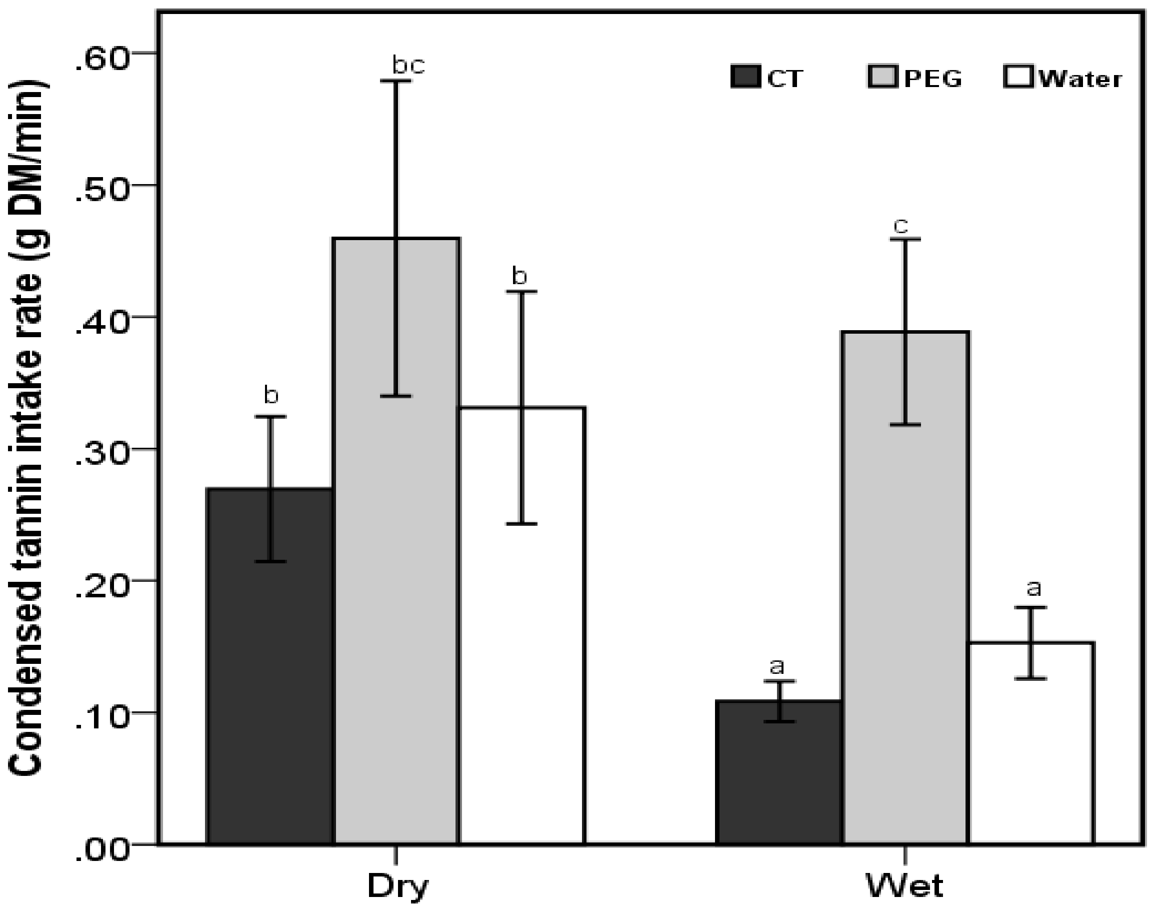

Figure 2: Mean (±95% CI) condensed tannin intake rate (g DM per minute foraging) of free-ranging goats that were orally dosed with 20g of condensed tannins (CT), 20g of polyethylene glycol (PEG) and 50ml of water daily during the dry and wet season. Letters represent significant differences among seasons and treatments.

The number of forage species included by goats from all treatment groups was the same during the dry season and slightly, but not significantly increased for CTs and control goats in wet season. Dosing goats with PEG significantly increased the number of dietary plant species included in the diet during the wet season (Wald X^2 = 15.53; P < 0.001, Figure 2). Goats dosed with PEG consumed more browse and relatively less grass than the other treatment groups. All browse species were eaten less by the control goats and those pre-dosed with CTs than goats dosed with PEG, independently of the [CT] in the dietary plants ($F_{2,141}$ = 4.83; P = 0.009). The [CT] of browse species did not have any relationship with the percentage contribution of the species in the diet (R^2 = 0.035; P = 0.380, Table 1).

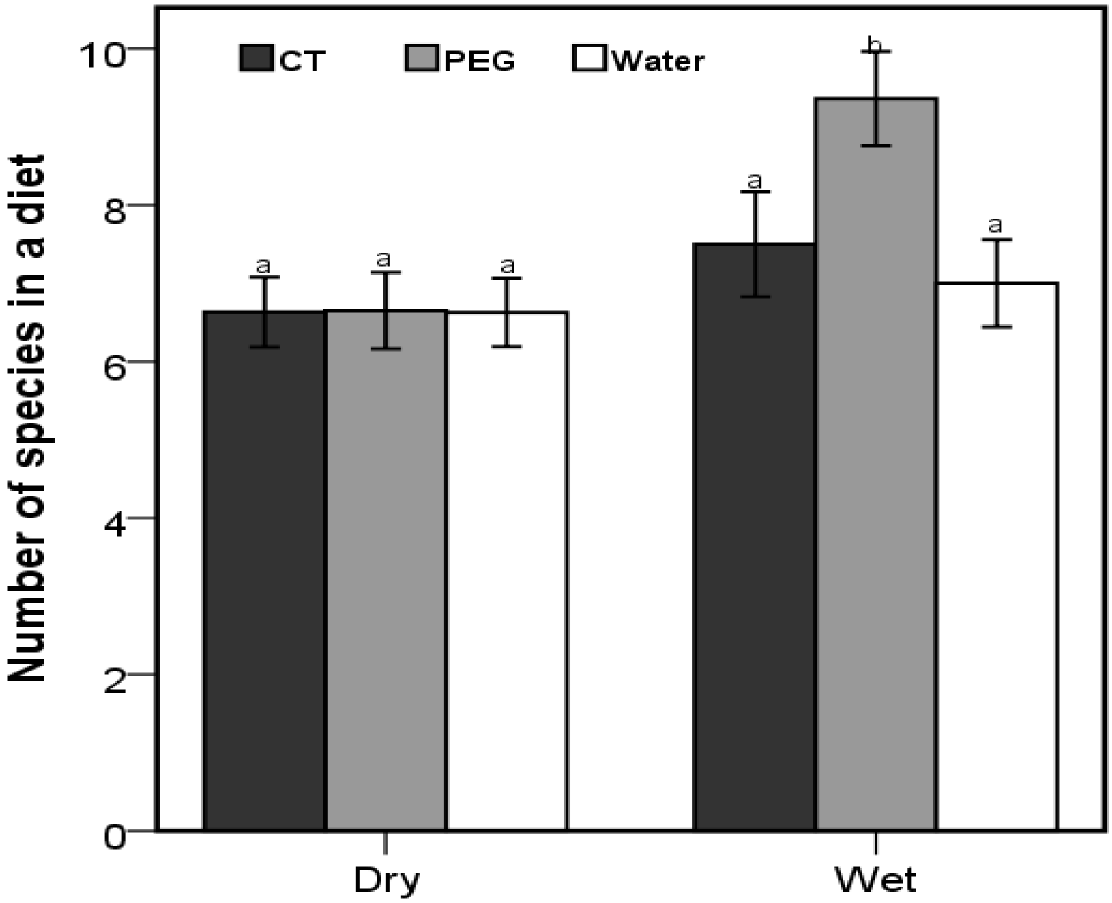

Figure 2: The mean (±95% CI) number of forage species included by free-ranging goats in their diets during a 15 minutes observation. The goats were orally dosed with 20g of condensed tannins (CT), 20g of polyethylene glycol (PEG) and 50ml of water daily during the dry and wet seasons. Letters represent significant differences among seasons and treatments.

The distinctive nature of biting by goats when grazing and browsing necessitated separate bite rate analysis for browsing and grazing. Bite rate on browse forages was significantly influenced by the interaction between season and treatment ($F_{2,517}$ = 3.15; P = 0.044; Figure 3). The bite rates achieved by goats across the treatment groups were consistently lower in the dry season and almost doubled in the wet season (Figure 3). Interestingly, the bite rates while grazing were only influenced by season ($F_{1,530}$ = 8.75; P = 0.003), with significantly higher rates in the dry than in the wet season.

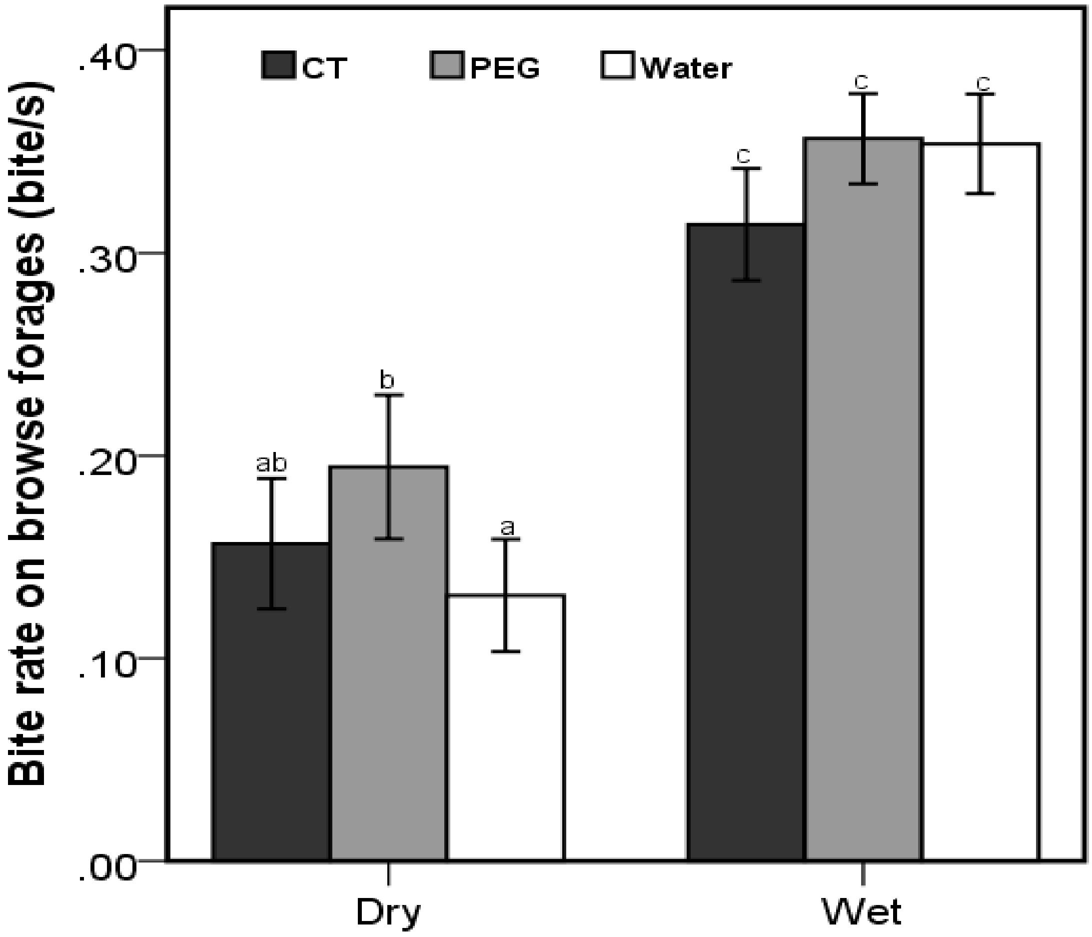

Figure 3: Mean (±95% CI) bite rates while browsing on woody plants by free-ranging goats that were orally dosed with 20g of condensed tannins (CT), 20g of polyethylene glycol (PEG) and 50ml of water daily during the dry and wet season. Letters indicate significant differences among the seasons and treatments.

Although the number of browsing bouts was affected by the interaction between season and treatment (Wald X^2 = 171.47; P < 0.001), only the goats that were dosed with PEG during dry season had a significantly higher number of browsing bouts (Figure 4). The number of browsing bouts was also influenced by the interaction between the season and time of the day (Wald X^2 = 19.42; P < 0.001), with significant differences during the late morning and afternoon foraging periods (Figure 5). The season x treatment interaction also influenced the number of grazing bouts (Wald X^2 = 10.17; P = 0.006), with the CT-goats recording the highest and the PEG goats recording the lowest number of grazing bouts in both seasons (Figure 6). Browsing bout length differed significantly among seasons and treatments ($F_{2,3414}$ = 7.81; P < 0.001), with the goats treated with PEG achieving the longest and CT-dosed goats achieving the shortest bouts in both seasons (Figure 7). The opposite was found for the grazing bout length (Figure 8) which was also affected by the interaction between season and treatment ($F_{2,530}$ = 8.09; P < 0.001).

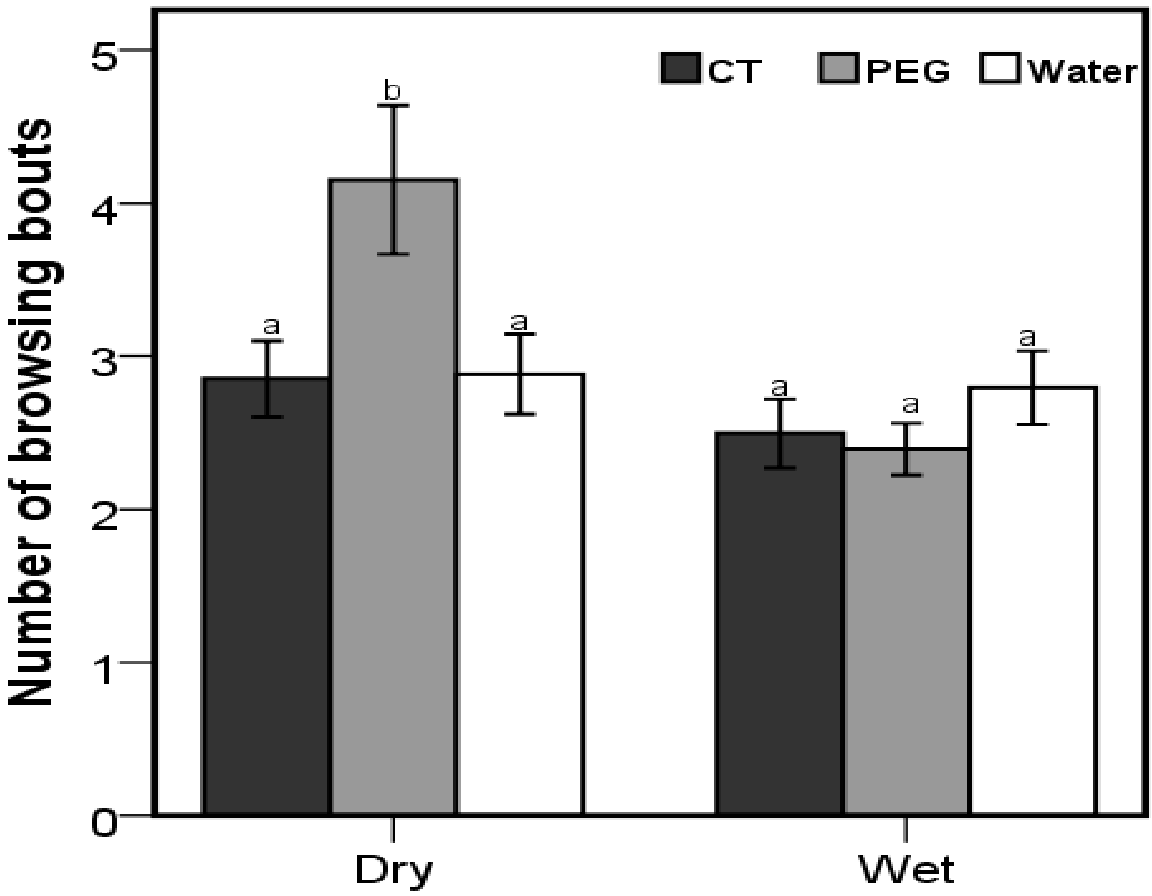

Figure 4: Mean (±95% CI) number of browsing bouts during a 15 minute observation of free-ranging goats that were orally dosed with 20g of condensed tannins (CT), 20g of polyethylene glycol (PEG) and 50ml of water daily in dry and wet seasons. Letters indicate significantly different means among the seasons and treatments.

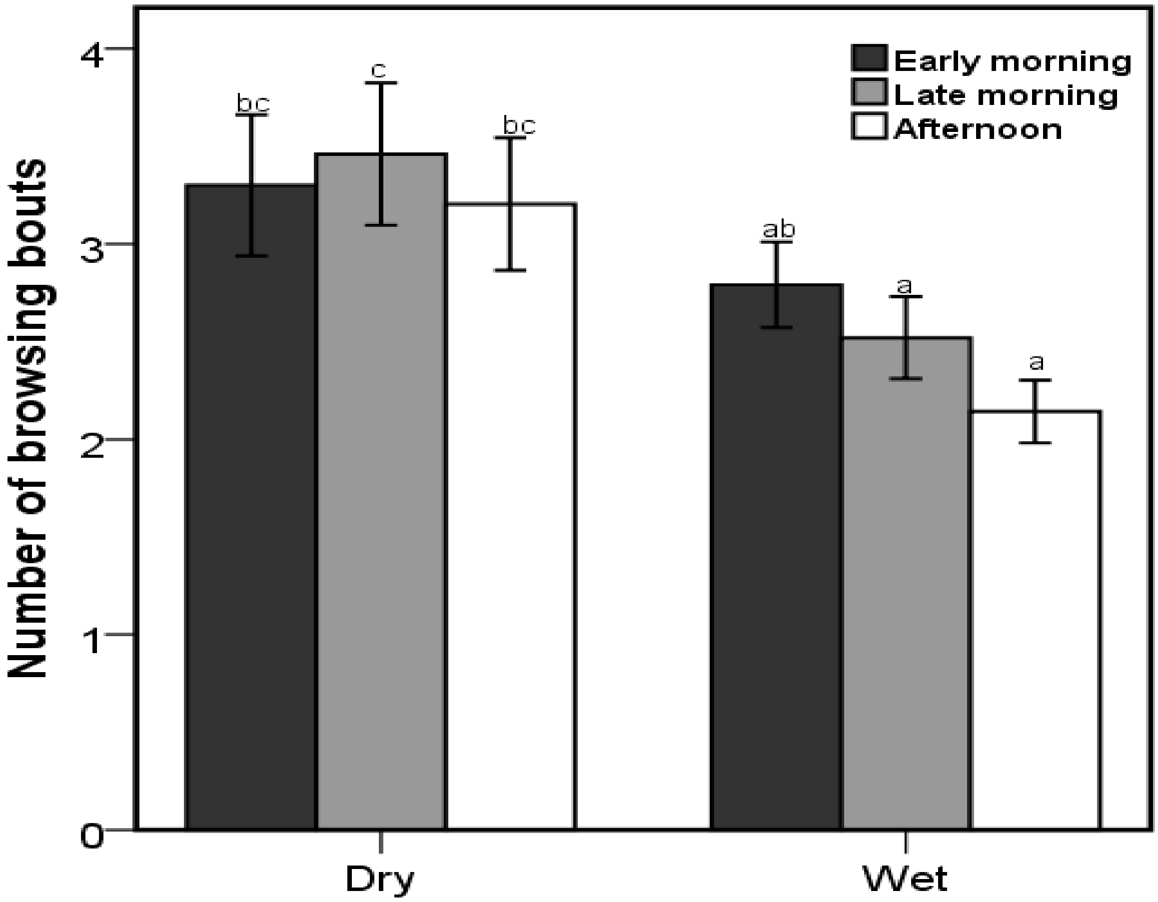

Figure 5: Mean (±95% CI) number of browsing bouts during a 15 minute observation of free-ranging goats in the wet and dry season and in early morning, late morning and afternoon. Letters indicate significantly different means among the seasons and treatments.

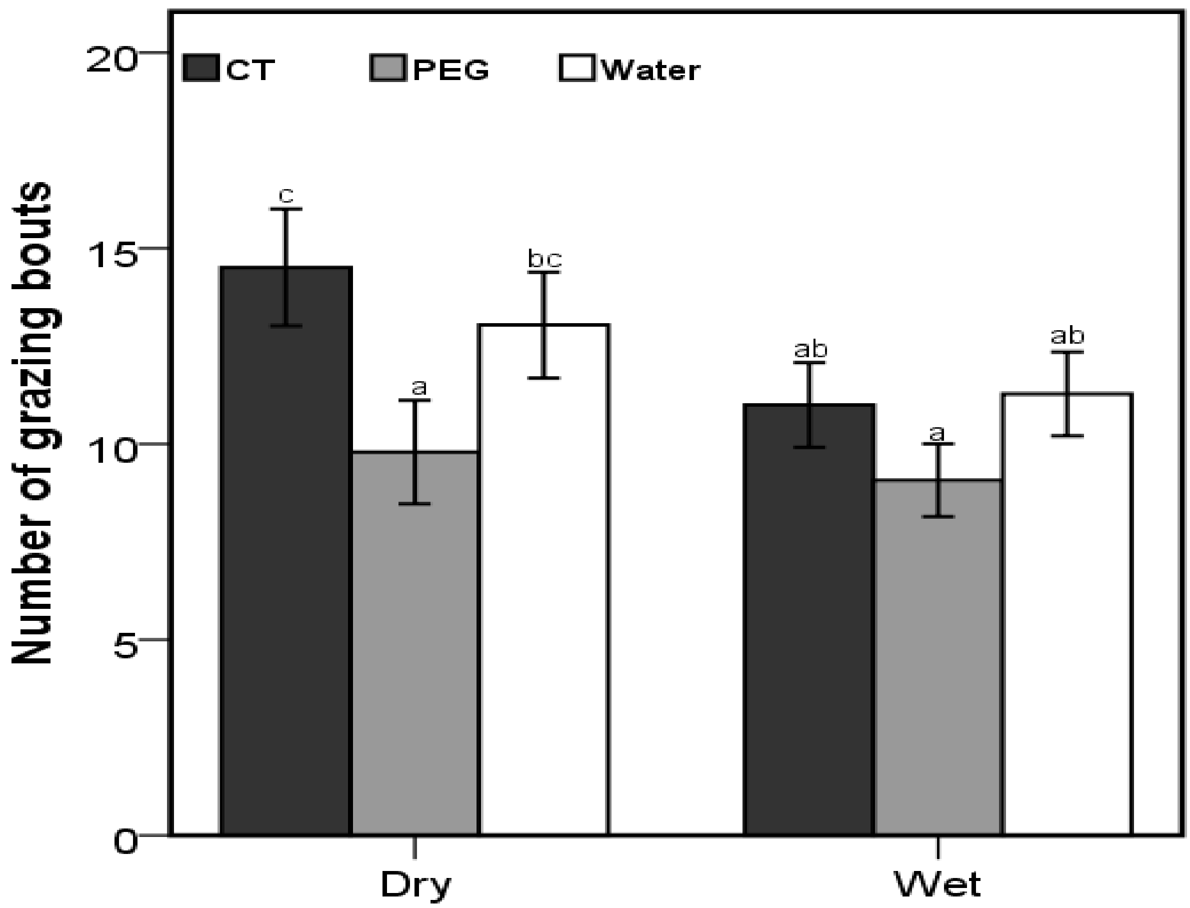

Figure 6: Mean (±95% CI) number of grazing bouts per 15 minute observation period for the free-ranging goats that were orally dosed with 20g of condensed tannins (CT), 20g of polyethylene glycol (PEG) and 50ml of water daily during the dry and wet seasons. Letters represent significant differences among seasons and treatments.

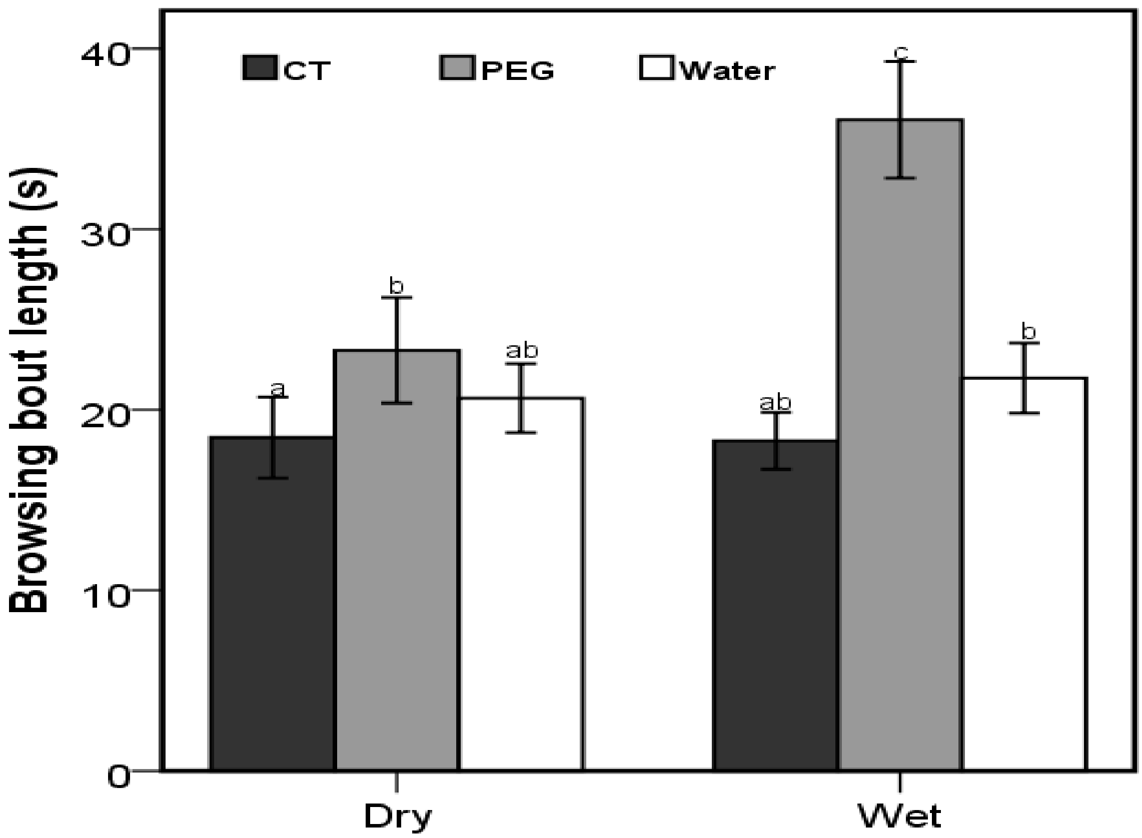

Figure 7: Mean (±95% CI) browsing bout length (s) for the free-ranging goats orally dosed with 20g of condensed tannins (CT) 20g of polyethylene glycol (PEG) and 50ml of water daily during the dry and wet seasons. Letters represent significant differences among seasons and treatments.

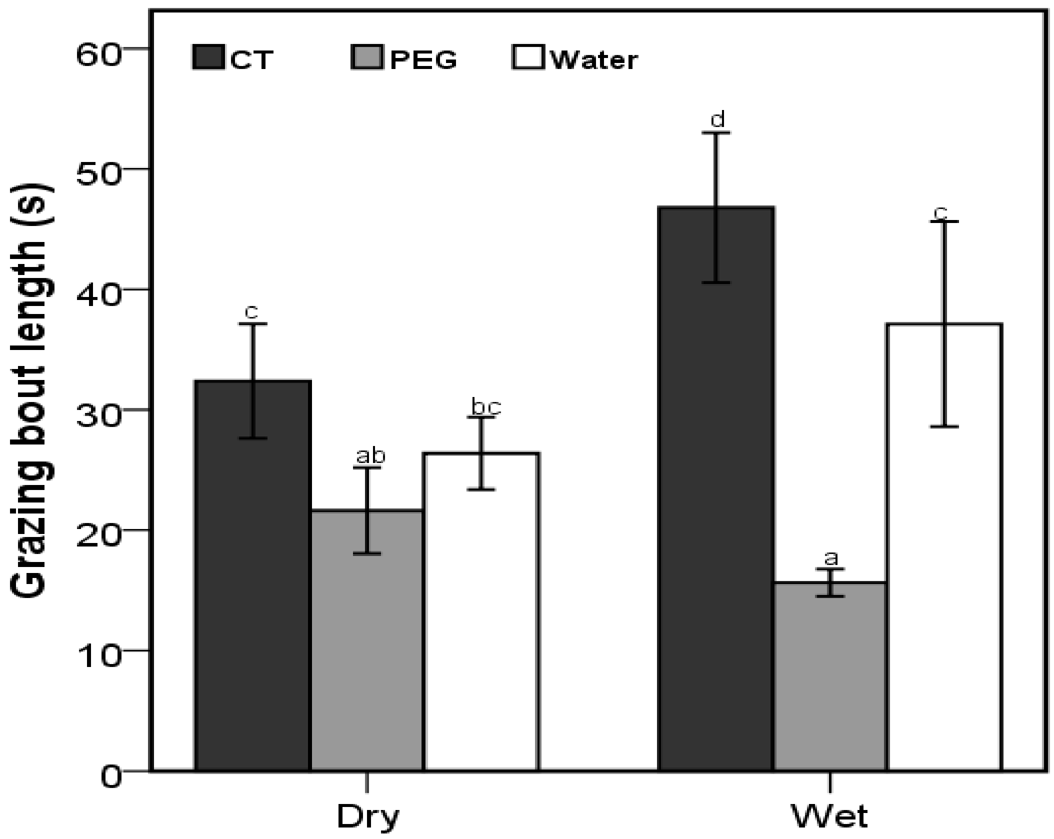

Figure 8: Mean (±95% CI) grazing bout length (s) for the free-ranging goats orally dosed with 20g of condensed tannins (CT) 20g of polyethylene glycol (PEG) and 50ml of water daily during the dry and wet seasons. Letters represent significant differences among seasons and treatments.

Table 1: Condensed tannin (CT) composition (mg/g) of forage plants and average % consumption (g DM) for each plant species consumed by goats dosed with CT, PEG and water per observation during dry and wet seasons.

	CT composition	**Dry season (% g DM intake in the diet)**			**CT composition**	**Wet season (% g DM intake in the diet)**		
Forage Species	**mg/g (N)**	**CT-group**	**PEG-group**	**Water-group**	**mg/g (N)**	**CT-group**	**PEG-group**	**Water-group**
Senegallia/Acacia caffra	149.93 (6)	1.59	11.16	2.50	141.71 (6)	4.86	6.00	7.19
Vachellia/Acacia karroo	103.06 (9)	0.85	5.70	2.07	133.95 (6)	2.35	6.09	7.65
Vachellia/Acacia nilotica	4.94 (8)	1.59	5.92	1.18	18.82 (5)	0.95	2.58	0.57
Vachellia/Acacia robusta	16.27 (10)	1.92	4.77	1.80	13.33 (6)	2.31	5.40	6.02
Vachellia/Acacia tortilis	21.90 (4)	0.65	2.07	0.06	51.25 (6)	0.76	2.64	1.92
Aloe greatheadii	0.70 (1)	5.56	3.63	3.48	2.60 (3)	3.95	2.50	3.48
Berchemia zeyheri	28.19 (1)	10.66	12.34	9.16	54.26 (3)	1.21	10.69	5.29
Carissa bispinosa	95.57 (10)	1.22	2.86	2.08	111.73 (5)	0.41	2.41	0.43
Combretum apiculatum	50.42 (8)	30.52	43.50	31.60	56.10 (4)	32.62	59.08	0.00
Combretum zeyheri	17.54 (6)	3.24	13.03	0.35	26.43 (6)	4.21	23.11	1.68
Dichrostachys cinerea	38.61 (5)	0.42	18.08	2.48	75.39 (6)	2.93	7.50	11.36
Dombeya rotindifolia	52.81 (9)	12.11	16.36	9.00	62.25 (6)	10.10	26.87	51.66
Ehretia rigida	1.40 (9)	7.61	8.45	5.50	2.25 (6)	4.20	8.91	6.78
Euclea crispa	69.02 (12)	6.11	7.78	10.38	70.49 (6)	2.46	10.40	8.39
Grass		57.99	33.91	54.37		66.37	22.09	55.90
Grewia flava	48.56 (5)	0.53	0.80	0.56	67.03 (6)	3.80	7.29	5.09
Gymnosporia buxifolia	68.92 (9)	3.04	12.36	2.57	68.57 (6)	3.10	9.90	4.89
Pappea capensis	48.46 (11)	7.13	9.81	4.22	78.46 (6)	2.59	4.05	3.85
Searsia/Rhus lancea	129.35 (9)	18.27	19.35	27.64	28.73 (6)	9.08	20.69	17.65

Searsia/Rhus Leptodictya	175.69 (14)	5.21	1.32	1.80	76.91 (6)	0.84	4.87	2.55
Searsia/Rhus pyroides	63.49 (2)	0.35	0.75	0.23	93.57 (4)	6.98	10.09	8.40
Scolopia zeyheri	72.79 (7)	0.68	1.45	1.19	83.64 (4)	1.17	2.06	0.90
Herbs	7.07 (7)	4.58	5.35	5.18	6.30 (5)	4.28	6.97	5.22
Ziziphus mucronata	40.51 (10)	12.20	25.08	17.03	41.87 (6)	5.81	11.40	9.33

Discussion

We tested the hypothesis that the foraging behaviour of free-ranging goats enables them to regulate intake rates of CTs in the African savannas. Condensed tannins are well known to influence foraging behaviour in controlled feeding experiments (Min et al. 2003, Waghorn 2008). As predicted, goats that were pre-dosed with CTs achieved lower intake rates of CTs than the PEG group. Reduced consumption rates of CTs by CT-dosed and control goats were likely a physiological strategy to contend with the adverse effects of CTs on nutrient metabolism (Villalba et al. 2002b).

PEG is potentially a powerful anti-CT agent in the context of animal nutrition (Foley et al. 2007), and was used it in this study to experimentally create different levels of CT stress among the study goats. While dosing goats with CTs constrained their CT intake rate, PEG goats seemed to be relieved from this CT constrain. PEG is known to preferentially bind CTs, thereby reducing the CTs' detrimental protein-binding effects (Villalba and Provenza 2002, Decandia et al. 2008, Rogosic et al. 2008b). Our results suggest a limited ability of goats to reduce CT intake rate in the dry season, and we found higher amounts and rates of CT intake during dry season. This was in line with findings of other studies (Nyamangara and Ndlovu 1995, Jansen et al. 2007). One explanation for this is the loss of leaves among deciduous trees during the dry season. Goats have fewer options and have to optimally utilize those available options to meet their nutritional needs. One coping strategy, which is discussed in detail later, is to reduce the length, while increasing the number, of feeding bouts during the dry season.

Cautious sampling and diet mixing by herbivores have been assumed to generally increase intake of forages that are rich in secondary metabolites (Marsh et al. 2006b, McLean and Duncan 2006). As expected, the current results did not support this notion owing to the plant secondary metabolites in question being only digestibility reducers and not toxins. Pre-dosing goats with CTs or PEG did not influence the botanical diversity of their diets. However, the results show the number of dietary species to be consistently higher for all treatments in the wet than in the dry season. Season may have influenced the number of dietary forages through reducing browse forage availability in dry season. This is a common phenomenon for the seasonal semi-arid African savannas (Owen-Smith and Cooper 1985, Bryant et al. 1989). Deciduous species lose leaves during dry season, thus limiting the forage options for mixed feeders and browsing herbivores to a few evergreen woody species and grass. Dosing goats with CTs in the wet season forced them to focus their foraging on grass (Chapter two). It also increased the grazing bout length and the number of grazing bouts at the expense of browsing bouts. This may indicate the existence of a threshold for CT intake above which, higher dietary species diversity does not benefit herbivores. Hence the number of dietary species was similar between the CT-dosed goats and the control goats. The high species diversity among PEG dosed goats may suggest that goats were more tolerant for

CT rich forages which enabled them to include more species in their diet. On the other hand, pre-dosing goats with CTs forced goats to select and consume a diet that is lower in CTs in the field.

Herbivores can modify their intake patterns as an important strategy to minimize consumption of plant secondary metabolites (Estell 2010). Specific behavioural modifications that goats use to regulate intake of plant secondary metabolites include adjusting their total intake, intake rate, length and/or number of bouts, length between feeding bouts, or to switch their diet composition (Wiggins et al. 2003, Marsh et al. 2006b, Wiggins et al. 2006b, Estell 2010). We predicted pre-dosing goats with CTs to increase the number of foraging bouts while at the same time reducing the length of foraging bouts, as a means to regulate intake rates of CTs. Bite rates appeared to be influenced more by the season than by treatment. We also predicted that pre-dosing goats with CTs would reduce bite rates, thereby reducing their intake rates, especially intake rates of CT rich species. A clear difference between the goats dosed with PEG and those from the other two groups indicated some potential effects of CTs on browse bout and browse bout length. For example, PEG goats achieved the highest number of bouts during the dry season, the season in which they achieved the highest CT intake rates. The control group and the CT-dosed goats achieved less frequent bouts in the dry season compared to PEG dosed goats possibly due to them achieving CT satiation levels sooner than their PEG counterparts. This supports previous studies that have shown CTs to limit diet intake although this study is further demonstrating these effects under field conditions. There were no differences in number of feeding bouts during the wet season. The higher number of grazing bouts by CT-dosed and control goats shows that these goats preferred to graze than browsing, possibly due to higher CT content in browse compared to grass (see Table 1). The consistent similarities in terms of bite rates, bout number and bout length between CT and control groups may suggest a similar CT stress level between these two groups. This may therefore indicate that our CT treatment was not as effective in stressing foraging behaviour as anticipated, or that the control goats were already heavily CT-stressed. Our results were obtained from free-ranging goats, which allowed the goats to make a broader choice from the available forage species than those in previous studies in pens (Dearing et al. 2000). Previous studies on the effects of plant secondary metabolites on meal patterns were done with captive animals, which may have had fewer behavioural options than free-ranging animals. Although our study did not show CTs to significantly reduce bite rates as we expected, the results indicated that CTs do not only constrain overall intake of CT-rich plants but they also alter their short-term foraging behaviour.

These results support our hypothesis that the foraging behaviour of free-ranging herbivores in the African savannas enables them to control intake rates of CTs. This study demonstrates that pre-dosing herbivores with CTs reduced their consumption rate of woody plants (CT-containing forages) in favour of grass. We interpret this reduction in CT intake rate as the need for herbivores to regulate CT intake (Marsh et al. 2007) in an effort to decrease the digestibility-reducing effects. We demonstrated that pre-dosing herbivores with CTs leads to significant alterations by animals in numbers of foraging bouts and length of foraging bouts. We explained the observed foraging alterations as means to regulate intake rates of CTs. Although pre-dosing goats with CTs did not reduce bite rates, reduced bout length or increased the number of foraging bouts, pre-dosing with PEG evidently decreased CT-stress in the field. Thus we concluded that herbivores under natural conditions alter their bite rate, bout number and bout length in ways that regulate CT consumption.

Acknowledgements: We are grateful to the National Research Foundation (NRF), South Africa, for financial support. We thank Carolien Kooiman, Dorian van Dalen, Lucas Letsoalo, Piet Monegi, Marvin Mahvungu, Michael Mokwala, Veronica Rakoena, Tebogo Matlou and Sikelela Simelane for their assistance with data collection. Any conclusions or recommendations expressed here are those of the authors and the NRF does not accept any liability in regard thereto.

Chapter Four

Effects of condensed tannins on body weight, faecal nitrogen and nutritionally related blood metabolites of free ranging yearling goats in a semi-arid African savanna

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Abstract

Current understanding of the effects of condensed tannins (CTs) on productivity of mixed-feeding ruminants is largely based on simple laboratory and short-term feeding experiments. These experiments do not adequately capture the complex behavioural and physiological responses of mixed-feeders to plant secondary chemistry. In a field experiment with goats, we tested whether CTs suppress the growth performance of mixed-feeding large herbivores. We also tested the prediction that CTs reduce blood circulatory nutrient and increase nitrogen (N) excretion via faeces. We divided forty five yearling female goats into 3 groups that were orally dosed daily with either CTs, polyethylene glycol 6000 (PEG, a polymer that neutralizes dietary tannins), or water (control). We measured the average daily gains, body weights, faecal nitrogen and four blood metabolites from each goat during the dry and wet seasons. In each season body weight significantly increased over time. The average daily gain was consistently highest for animals dosed with PEG and lowest for those dosed with CT. Goats dosed with CT excreted the most faecal nitrogen and had the least blood protein concentrations, while the opposite was true for PEG goats in both seasons. Blood urea and non-esterified fatty acids indicated a negative influence of CT on energy and protein metabolism. We concluded that CTs limit growth and PEG mitigates the negative effects of CTs on growth performance of free-ranging mixed feeding ruminants.

Keywords: intermediate feeder**,** ruminant**,** body weight, blood metabolite, digestion, PEG

Introduction

Trees and shrubs are an important food base for large wild and domestic herbivores in African savanna ecosystems (Bergstrom 1992). Although they provide a valuable source of protein especially in the dry season when the availability and nutritive value of the herbaceous forages are limiting (Le Houérou 1980a, b, Basha et al. 2013), these forages are nearly always endowed with tannins. Tannins are the most abundant secondary metabolites produced by plants and are found in approximately 80% of woody and 15% of herbaceous dicotyledonous species (Bryant et al. 1991b). Being widespread in nutritionally important forages, tannins probably play a large ecological role in mediating woody plant-browsing herbivore interactions (Barbehenn and Constabel 2011), in African savannas characterised by bush encroachment (Ward 2005).

Until recently, tannins were assumed to have only adverse effects on herbivores despite their very low toxicities (Provenza and Villalba 2010). Thanks to relentless research efforts, it is now known that tannins can either be detrimental and beneficial to the herbivores and environment, depending on the tannin type and concentration in the forages (Min et al. 2003, Piluzza and Bullitta 2010). For instance, condensed tannins (CTs) are more effective than the hydrolysable tannins in reducing mammalian herbivore attack (Barroso et al. 2001) but not particularly toxic to the animals (Hagerman et al. 1992, Silanikove et al. 1996a, Barbehenn et al. 2006).

At low to moderate quantities (20-45 g CT/kg DM), CTs bind with and provide protection to the dietary proteins from degradation by rumen microbes thereby increasing the efficiency of protein digestion and absorption later in the small intestines (Waghorn 2008, Piluzza et al. 2013). Forages containing low levels of CTs may lower the internal parasite burden with positive consequences for animal growth performance (Lisonbee et al. 2009b, Piluzza et al. 2013). On the other hand, at high concentrations (>55 g CT/kg DM), tannins are known to reduce feed intake, and reduce live-weight gain with detrimental consequences for productivity (Waghorn and McNabb 2003). Increased faecal nitrogen excretions (Kumar and Vaithiyanathan 1990, Owens et al. 2012a), reduced growth hormone titre (Barry 1984) and reduced blood nitrogen (Silanikove et al. 1997) have also been observed from animals exposed to tannin-rich forages. In addition to forming irreversible complexes with dietary proteins, excessive CTs reduce lipid digestion (Barry and Manley 1986) and bind carbohydrates to form indigestible complexes with the cell wall material (Reed et al. 1990). These properties of CTs explain the low nutritional value of tannin-rich forages and why CTs reduce the condition and productivity of the large herbivores.

Polyethylene glycol (PEG) binds tannins irreversibly over a wide range of conditions and reduces the formation of protein-tannin complexes (Silanikove et al. 1996b, Silanikove et al. 1997). It has widely been used to counteract the negative effects of tannins especially on ruminants. Some studies have shown PEG to vastly improve browse utilization (Silanikove et al. 1996b, Gilboa et al. 2000) while others reported no improvements at all (Barahona et al. 1997, Bhatta et al. 2002, Bhatta et al. 2004).

Our understanding of the effects of tannins and PEG on mixed feeding herbivores is largely based on results from simple laboratory and short-term feeding experiments. The applicability of these experiments to the complex and diverse natural environments (typical to the African savannas) is however limited by the number of plant species or diets that can experimentally be used. Herbivores foraging in a single-species vegetation are less able to manifest their physiological and behavioural adaptations to secondary chemistry than those foraging in heterogeneous ecosystems (Moore and Foley 2005, Moore et al. 2005). Mixed feeders such as goats are known to generally cope better with plant secondary metabolites when exposed to forages of higher chemical diversity than just a single plant species (Papachristou et al. 2007). Numerous studies have shown that forage mixtures produce less deleterious effects than tannin-rich plants fed as sole feeds (Dube et al. 2001, Villalba et al. 2006, Melaku and Betsha 2008). Therefore, animals foraging in diverse natural environments may benefit from diet mixing and respond differently to tannins than usually shown by the feeding experiments.

Our main objective in this study was to determine the effects of CTs on growth performance of free-ranging mixed feeding herbivores in a semi-arid savanna. Body weight changes and condition determine key life history processes such as reproduction and mortality (Prins 1989b). Body weight has been shown to correlate significantly with gestation time, age at first reproduction, life expectancy at birth and lifespan for many large herbivores (Prins 1989c, b). Moreover, the rate of growth for growing animals reflects total intake and availability of nutrients in the diet (Reed 1995). We combined body weight measurements with those of blood metabolites to increase the accuracy of assessing the nutritional state and welfare of free-ranging herbivores (Chester-Jones et al. 1990, Ndlovu et al. 2007). We thus compared average daily gains, body weight, faecal nitrogen, blood glucose, blood urea, blood total protein, and blood non-esterified fatty acids (NEFA) of 45 free-ranging yearling goats.

To achieve our objective, we tested the hypothesis that CTs impose nutritional limits to the growth performance of free-ranging herbivores by either reducing nutrient absorption and/or increasing faecal nitrogen excretion. Based on this hypothesis we predicted CTs to reduce the average daily gains, or levels of blood protein and blood glucose. We further predicted tannins to increase faecal nitrogen excretion rates, and levels of blood urea and blood NEFA of free-ranging goats.

Material and Methods

Study area

Fieldwork was conducted during the dry (June to August 2012) and wet (January to April 2013) seasons at the Roodeplaat Experimental Farm of the Agricultural Research Council in South Africa (25˚20'-25˚40'S; 28˚17'-28˚25'E). The climate in the study area is semi-arid with a mean annual precipitation of 646 mm and an average daily temperature of 29 ºC (Panagos et al. 1998). The natural vegetation of the farm (2067 ha) is classified as a Marikana Thornveld by Mucina and Rutherford (2006). The dominant tree species whose foliage or fruits were consumed by goats in this study included 5 species of *Vachellia* (formely nemed: *Acacia),* 3 species of *Searsia (*formerly named *:Rhus)*, two species of *Combretum*, *Dichrostachys cinerea, Ziziphus mucronata, Gymnosporia buxifolia, Scolopia zeyheri, Ehretia rigida, Carissa bispinosa, Dombeya rotundifolia, Pappea capensis, Grewia flava, Berchemia zeyheri,* and some *Euclea* species. A number of shrubs/herbs were consumed, the main of which were *Lippia rehmannii,* and *Tarconanthus camphoratus*. A dwarf shrub *Aloe greatheadii* var. *davyana* was also present in the study area. Plant nomenclature and further details on the phenology and morphology of these plant species can be found in Coates Palgrave (1977, 1985) and Kyalangalilwa et al. (2013).

Experimental design

We used 45 yearling, female goats ranging from 10 to 18 months old with an initial body weight of 14.9 kg (SD ± 3.7). During the dry season, all animals were weighed one day before the experiment and were allocated to 3 treatment groups so that all groups had an equal number of animals (N=15) and a similar mean within 95% CL body weight per group. Every morning between 07:00 and 08:00, 15 animals were orally dosed with 20g of polyethylene glycol (PEG 6000) dissolved in 50 ml of water while another 15 were dosed with 50 ml of water plus 20g of CT (extracted from mimosa bark) and the last 15 received only 50 ml of water (control). The mimosa extract, which contained a minimum of 66% condensed tannin (http://www.mimosa-sa.com) was obtained from the bark of the Black Wattle (*Vachellia*/*Acacia mearnsii*) trees grown in the local plantations. The three treatment groups were maintained throughout the experiment. Three grazing paddocks of similar size (±1.7 ha) were fenced off and stocked with fifteen animals (i.e., 5 from each treatment group) from 08:00 until 15:30 daily. All animals were treated for internal and external parasites before the experiment and had *ad libitum* access to water throughout the experiment. From 08:00 all animals were allowed to forage freely in their respective paddocks until 15:30 when they were kraaled to avoid predation and theft.

Data collection

During the dry season, all animals were weighed on days: 0, 12, 22, 38 and 45 of the experiment, and they were all weighed on days: 0, 7, 21, 27, 34, 40, 45 and 65 .in the wet season. Average daily gains per animal were calculated as the difference between the initial and final body weights divided by the duration of the experiment (in days) in each season.

Faecal samples were collected on days: 0, 25, 45 and 65 from all animals in wet season. However, the experiment was terminated earlier (i.e., on day 45) in the dry season due to logistical reasons. All collected samples were oven dried at 40 ^{o}C until completely dry, and then were milled and analysed for nitrogen by the micro Kjeldahl method (AOAC 1997).

Blood samples were collected from all animals by jugular venepuncture with an evacuated tube system three times during the dry season and four times in wet season. This sampling was done at 07:00 in the morning after the animals had fasted overnight. The blood variables studied were chosen for various nutritionally related reasons: Firstly, total protein reflects availability of protein and the decline in its concentration indicate protein deficiency (Ndlovu et al. 2007). Secondly, high blood urea levels indicate a high protein intake or excessive mobilization of muscle (Chimonyo et al. 2002). In ruminants a decrease in the blood urea concentration is related to low dietary protein intake due to recycling of urea from blood back to the rumen when dietary protein is low (Kohn et al. 2005). Thirdly, insufficient nutrient intake can reduce circulatory glucose and cholesterol levels. Lastly, NEFAs are released into the circulation as a direct result of lipid catabolism (Ndlovu et al. 2007). Ethylene diamine tetra acetic acid (EDTA) tubes were used to collect blood for glucose determination while clot activator tubes were used to collect blood for determining the concentrations of serum urea, total protein and non-esterified fatty acids (NEFA). All blood metabolites were assayed by the Cobas Integra 400/700/800 analyser using only the standard methods. Blood sampling was conducted by a trained veterinary assistant under permit number: APIEC11/039 provided by the Animal Ethics Committee of the ARC.

Data analysis

A General Linear Model with treatment and season as fixed factors, paddock in which the animals foraged as random factor, and the average daily gain as the dependant variable was used. GLMs with repeated measures were used to test for differences in body weight, faecal nitrogen excretion, blood glucose, blood urea, blood total protein and blood NEFA. In each model, time (in days) was used as the within subject variable and treatment as the between-subject factor. Separate models were used for different seasons. In all models, unstandardized residuals showed normal distribution, while the assumptions of variance homogeneity and sphericity were also met. A Sidak *post hoc* test was used for pairwise comparisons between the treatments means. All statistical analysis were performed using the SPSS, v20 (IBM SPSS Statistics; Chicago, IL, USA).

Results

Average daily gain and body weight

The average daily weight gain significantly varied between treatments ($F_{2,84}$= 7.07; P = 0.001), with the highest gain being observed for goats dosed with PEG and the lowest gain for animals dosed with condensed tannin (Figure 1). No seasonal effect was detected on the average daily weight gain. Repeated measures of body weight for all treatment groups showed a significant increased with time both in dry ($F_{1,42}$= 23.04); P < 0.001) and the wet ($F_{1,42}$= 25.47; P < 0.001) seasons. Although insignificant (P > 0.05) some animals CT-dosed group tended to lose weight and grow slower rate than their counterparts. The increase in body weight tended to level off for CT animals in the dry season after 22 days (Figure 2). We also observed a significant interaction effect of time and treatment on body weight only in the wet season ($F_{2,42}$ = 5.76; P = 0.006).

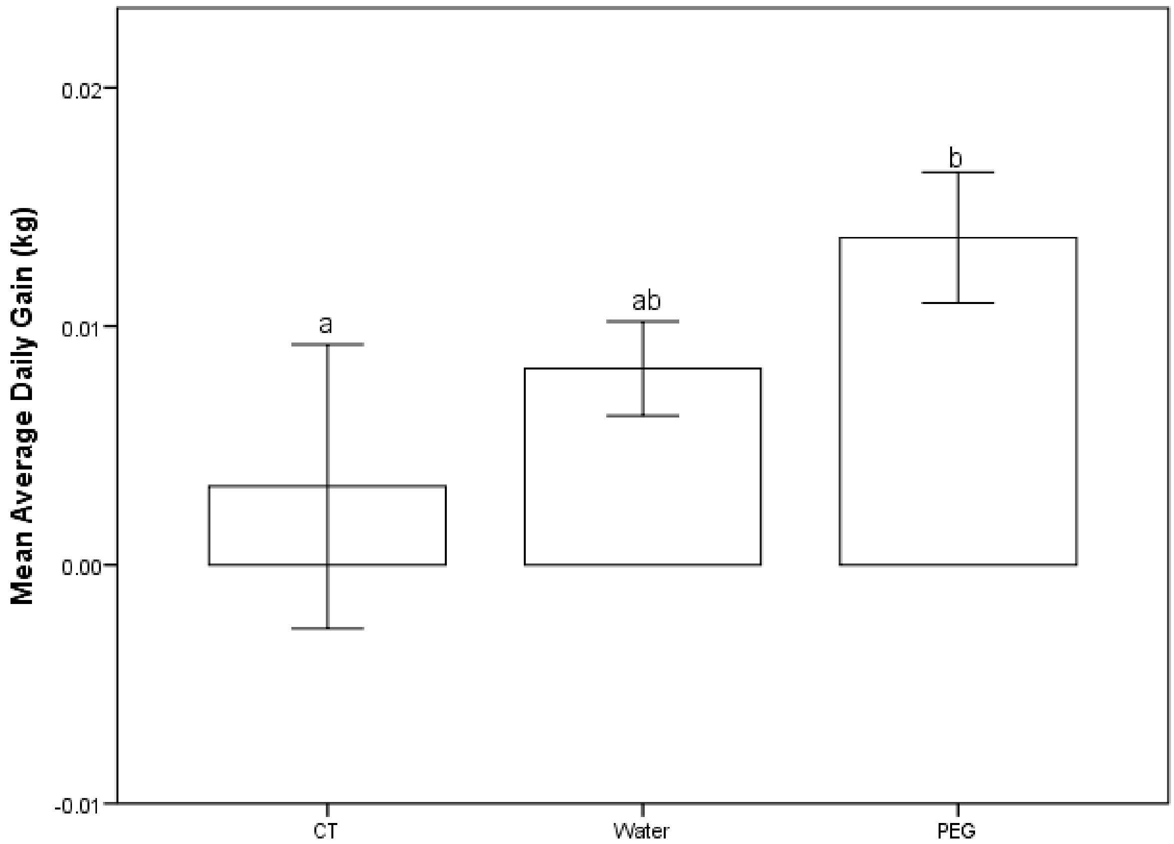

Figure 3: Mean average daily gain (kg) of free-ranging goats orally dosed with 20g of condensed tannins (CT), 20g of polyethylene glycol (PEG) and 50ml of water daily during the experiment. Error bars represent 95% a confidence interval.

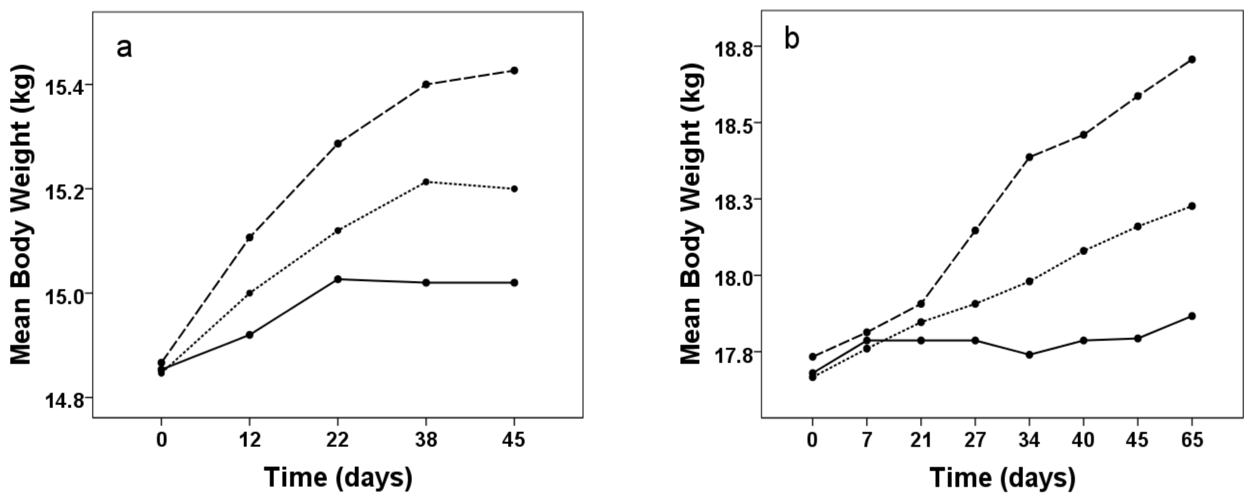

Figure 2: Changes in mean body weight of free-ranging goats, which were orally dosed with either 20g of condensed tannins (solid line), 20g of polyethylene glycol (long-dashed line) or 50ml of water (short-dashed line) over time (days) during the dry (a) and wet (b) season.

Faecal nitrogen

During the dry season, we observed a significant influence of time x treatment interaction on faecal nitrogen content ($F_{2,42}$= 12.14; P < 0.001) excreted by goats. An increase in nitrogen excretion by the CT dosed goats was observed over time while a decrease was true for goats treated with PEG. Similar patterns were observed in the wet season ($F_{2,42}$= 29.41; P < 0.001). The differences in faecal nitrogen content between the three treatment groups seem to have increased after 24 days (Figure 3).

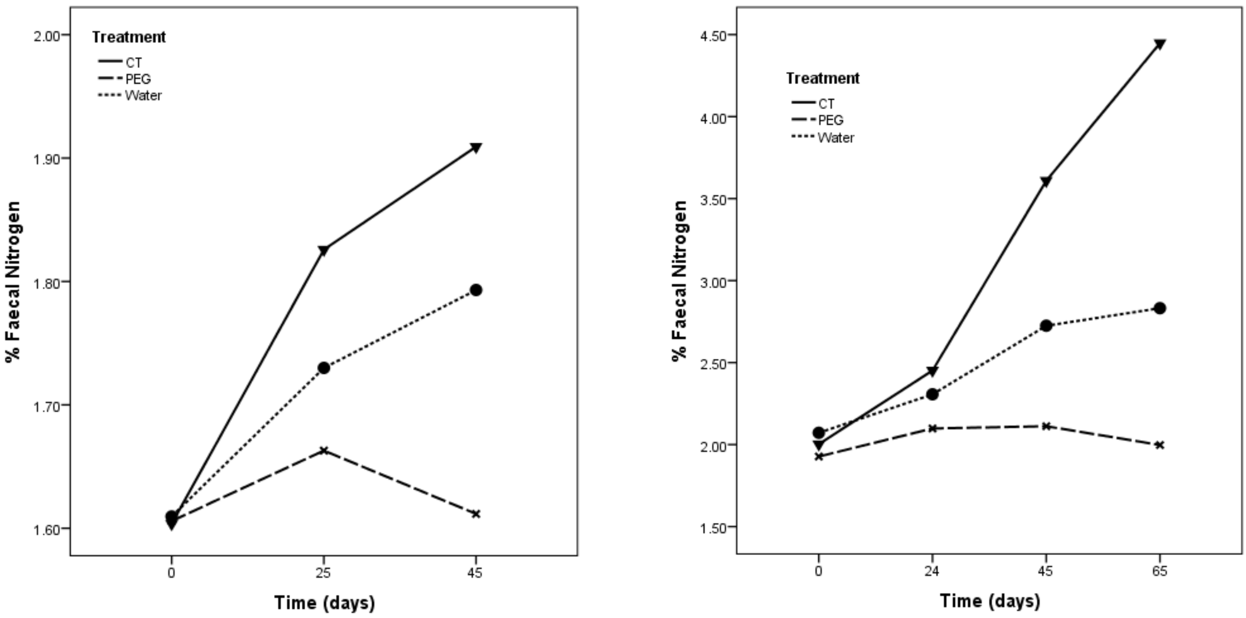

Figure 3: Changes in faecal nitrogen content excreted by free-ranging goats over time during the dry (left) and wet season (right) of the three groups of goats: orally dosed with 20g of condensed tannins (CT), 20g of polyethylene glycol (PEG) and 50ml of water.

Blood metabolites

In the dry season there was an effect of treatment x time interaction ($F_{2,42}$= 3.66; P= 0.034) on total protein. CTs drastically reduced the blood total protein as time increased. On the other hand, the blood total protein of the goats dosed with PEG increased over time. In the same season blood total protein was lowest for animals dosed with CTs and highest for the PEG dosed animals ($F_{2,42}$= 3.57; P= 0.037). In wet season, blood total protein increased over time ($F_{1,42}$= 41.69; P<0.001), without any differences between treatments. Blood urea and NEFA contents were affected by the time x treatment interaction in dry season (Figure 4), with these metabolites occurring in higher concentrations for CT treated goats (Table

1). Blood glucose content equally increased over time for all treatments. None of the blood metabolites was influenced by the treatment in the wet season (Table 1 and Figures 4).

Table 1: Results from the GLM, testing for the effect of time (day) and daily administration of free-ranging goats with 20g of condensed tannins (CT), 20g of polyethylene glycol (PEG) and 50ml of water on blood metabolites during the dry and wet seasons

Variable	**Source of Variation**	**Dry Season**		**Wet Season**	
		$F_{(df)}$	*P-value*	$F_{(df)}$	*P-value*
Total Protein	Time	$0.23_{(1,42)}$	0.635	$41.69_{(1,42)}$	0.000
	Treatment	$3.57_{(2,42)}$	0.037	$0.51_{(2,42)}$	0.605
	Time x Treatment	$3.66_{(2,42)}$	0.034	$0.60\ (_{2,42})$	0.554
Urea	Time	$109.76_{(1,42)}$	0.000	$196.82_{(1,42)}$	0.000
	Treatment	$4.92_{(2,42)}$	0.012	$0.84_{(2,42)}$	0.439
	Time x Treatment	$3.72_{(2,42)}$	0.033	$2.00_{(2,42)}$	0.148
NEFA	Time	$61.55_{(1,42)}$	0.000	$29.14_{(1,42)}$	0.000
	Treatment	$10.36_{(2,42)}$	0.000	$1.08_{(2,42)}$	0.348
	Time x Treatment	$5.44_{(2,42)}$	0.008	$0.65_{(2,42)}$	0.530
Glucose	Time	$17.58_{(1,42)}$	0.000	$25.34_{(1,42)}$	0.000
	Treatment	$0.81_{(2,42)}$	0.922	$1.36_{(2,42)}$	0.268
	Time x Treatment	$0.06_{(2,42)}$	0.944	$0.17_{(2,42)}$	0.846

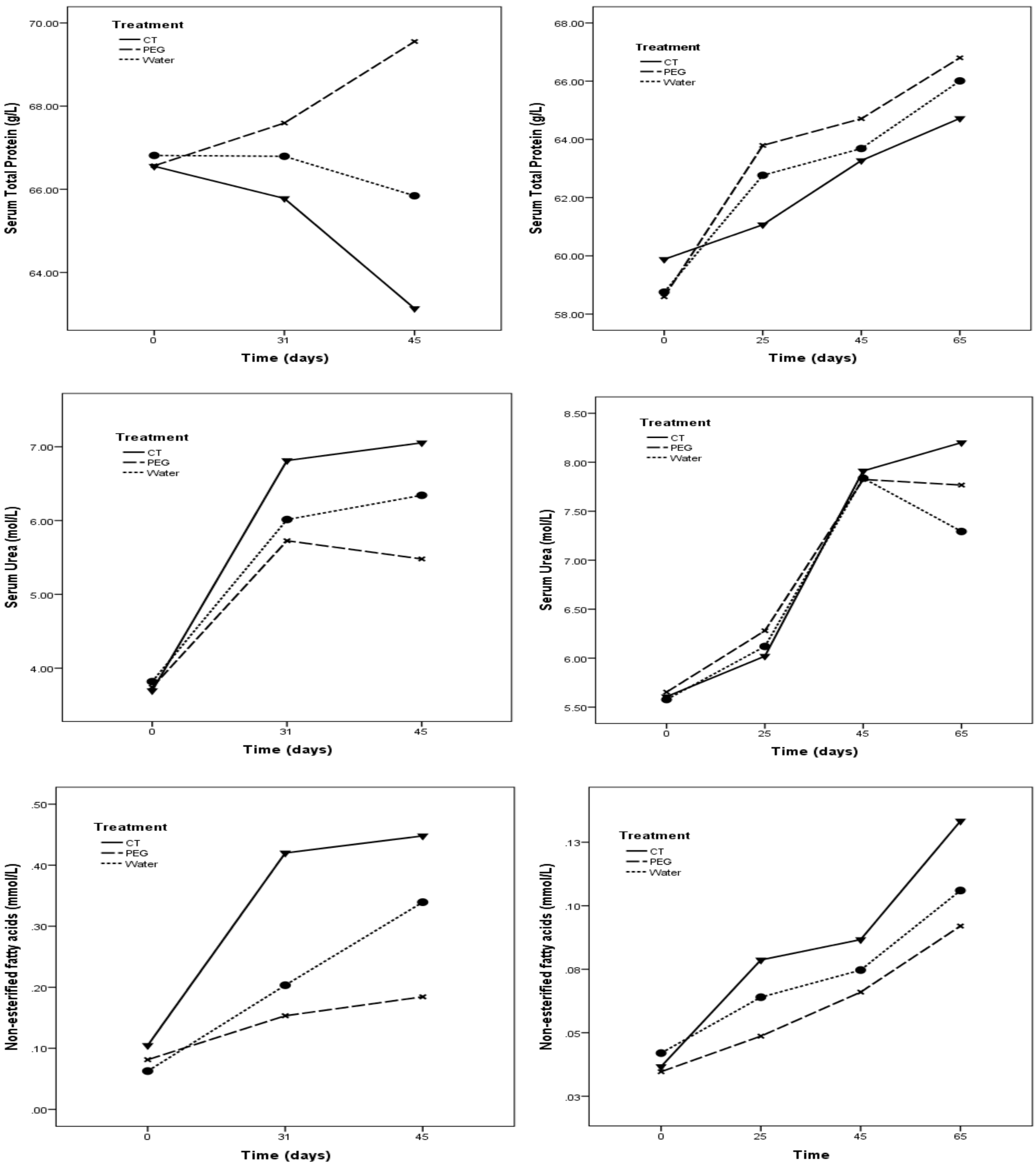

Figure 4: Changes in blood total protein, urea and NEFA of free-ranging goats over time during the dry (left) and wet season (right) of the three groups of goats: orally dosed with 20g of condensed tannins (CT), 20g of polyethylene glycol (PEG) and 50ml of water.

Discussion

Although plant secondary chemistry undoubtedly plays a pivotal role in herbivory, the effects of CTs on herbivore productivity and the mechanisms by which free ranging mixed feeders cope with tannins are not well understood. The overall hypothesis that CTs supress herbivore growth performance by reducing nutrient absorption and increasing faecal nitrogen excretion was supported in our experiments.

Effects of CTs on average daily gain and body weight

Although PEG, which makes the tannins inert by chemically binding to them (Priolo et al. 2000, Provenza et al. 2000), caused an increase in the average daily gain in body weight as expected, the CT treatment did not significantly reduce the average daily weight gain of free-ranging goats. However, given that we studied yearling animals that were still growing, we assess the effect of CTs on body weights through comparing the animals treated with PEG with those treated with CT. The animals dosed with PEG achieved significantly higher body gains than their counterparts, indicating a huge growth potential achievable by herbivores in the absence of tannins. Previous studies found PEG supplementation to improve average daily gain in cattle, sheep and goats (Motubatse et al. 2008, Waghorn 2008, Yisehak et al. *In press*). Our results implicate CTs to significantly limit the average daily gain and repeated measurements of body weight (Figure 2). These findings are supported by other studies that reported significant reductions in live weight gains from low CT (20-45 g CT/kg DM) (Min et al. 2003, Waghorn and McNabb 2003) and others that reported improvements in live weight gain from high CT concentrations (>55 g CT/kg DM) concentrations (Olivares-Perez et al. 2013, Piluzza et al. 2013).

We explain the slow growth rather than the body weight loss of CT dosed animals as a result of the physiological status (growing) of the study animals. Moreover, the impact of CT on growth performance was likely mitigated by the opportunity that these free ranging animals had to choose other diets, such as herbs and grasses that generally contain low or zero tannins. This likely enabled goats dosed with tannins to consume a varied diet and thus still acquire the nutrients that are needed to support moderate growth rates, mitigating the CT effects (Villalba et al. 2011). There is evidence in literature that herbivores grazing in monocultures are constrained to consume high concentrations of secondary chemicals, with negative impact on their body condition (Provenza et al. 2003, Papachristou et al. 2007).

Effects of CTs on faecal N excretion

These results are consistent with our prediction that CTs increase faecal nitrogen excretions. At high concentrations CTs are known to irreversibly bind to dietary proteins in ways that reduce protein availability for digestion and absorption (Min et al. 2003). PEG appeared to consistently reduce severity of nitrogen loss by goats in both seasons. These patterns support earlier observations from pen studies where shrubs containing high tannin concentrations reduced protein digestibility and nitrogen retention (Hagerman et al. 1992, Kaitho et al. 1998). Our results also show that PEG significantly reduced nitrogen excretion in ways that subsequently improved the average daily gain. The faecal nitrogen excretion results are therefore in line with our predictions and may explain the observed body weight differences between the treatment groups.

Effects of CTs on blood metabolites

Our hypothesis that CTs depress protein and energy absorption was partly supported by the current results. The results indicate higher NEFA levels for goats dosed with condensed tannin than those animals dosed with PEG in the dry season. This shows that tannins may trigger nutrient body reserve depletion and thus negatively influence growth performance in the long term (Ndlovu et al. 2007). NEFAs are released into the circulation as a direct result of lipid catabolism (Chimonyo et al. 2000). Wang and Provenza (1996) found that CTs had no effect on NEFA concentrations, which agrees with our observations in the wet season. Plasma urea nitrogen concentration is a useful indicator for the protein status of animals. Although our study animals did not seem to exceed the normal ranges for this metabolite (Kohn et al. 2005), goats dosed with CTs consistently recorded higher urea values than goats dosed with PEG throughout both seasons. High blood urea levels could indicate a high protein intake or excessive mobilization of muscle (Chimonyo et al. 2002, Kohn et al. 2005). Wang (2013) observed reduced plasma urea and glucose concentrations in relation to intake of CTs. Since the study goats foraged in a diverse savanna rangeland, CT dosed animals might have focused their diet on nutritious alternatives such grasses and other forages with low tannins. In this study we observed a potential for CT to reduce nutrient absorption and alter nutrient blood balance, with negative implications for growth performance of mixed feeding ruminants.

Conclusions

CT increased faecal nitrogen excretion and slightly reduced blood protein and nutrient concentrations, in ways that were enough to suppress the average daily body gain and body weights of goats. However, the fact that the study animals were still growing might have mitigated the negative effects of CTs, given that mixed feeders have the ability to opportunistically graze or browse, benefitting from the large natural variation in forage quantity and quality. Thus, the availability of alternative diets might have mitigated the effects of tannins, indicating that the effect of diet composition on productivity of mixed feeding herbivores in the field is important. We concluded that CTs limit growth while PEG mitigates the negative effects of CTs on growth performance of free-ranging mixed feeding ruminants.

Acknowledgements

This study was partly funded by the Wageningen University (the Netherlands) and National Research Foundation (NRF), South Africa. The opinions, findings and conclusions or recommendations expressed are those of the authors and therefore the NRF does not accept any liability in regard thereto. Authors are grateful to Lucas Letsoalo and Piet Monegi for helping with data collection.

Chapter Five

Nutrient supplementation enhances shrub use by goats: Implications for bush control in semi-arid Savannas

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Abstract

Large herbivores are purported to continue consuming toxin-containing forages as long as their capacity to neutralize, detoxify and excrete dietary toxins is not exceeded. This capacity depends on the availability of liver enzymes, energy and amino acid precursors. While this may explain increased intake of toxin-rich forages by herbivores supplemented with nutrients, a different effect may emerge in rangelands dominated by forages that are rich in condensed tannins which are not as degradable and readily absorbable as toxins. In a field experiment, we investigated the effects of supplementing animals with a high-energy source (yellow maize grain) and a high-protein source (soybean meal) on browse intake, foraging behaviour and diet composition of goats in a semi-arid savanna. Results confirmed our prediction that nutrient supplementation increased the percentage of time animals spent browsing and subsequently enhanced browse intake. Supplemented animals consumed more condensed tannins than animals that were not supplemented. Animals from supplemented groups tended to compose different diets from animals that received no supplement. We contend that supplements replaced the nutrients that are routinely bound and rendered indigestible by condensed tannins. Therefore, supplemental nutrients likely increased the intake of tannin-rich forages through delaying a negative post-ingestive feedback (aversion) from dietary tannins. We concluded that nutrient supplementation increased browse consumption by goats. Given that chemically defended woody plants are predicted to continue encroaching in the semi-arid savanna rangelands, these results suggest a potential for browsers and mixed feeders to serve as biological bush control agents.

Keywords: Ruminant, Ungulates, Nutrient-toxin Interactions, Livestock Production

Introduction

With woody expansion predicted to increase globally (Hughes 2003), it will soon not be possible to sustain animal production from the shrinking grass layer alone (Gordon and Prins 2008, Estell et al. 2012). The increasing demand for animal protein to feed the growing human population (FAO 2009) and the increasing ruminant populations (Rischkowsky et al. 2006) will further complicate the situation. The challenge facing farmers, managers, and ecologists alike will be to mitigate the predicted forage shortfall through enhancing the utilization of the shrubs that start to dominate global rangelands (Gordon and Prins 2008, Estell et al. 2012). This need is increasingly being acknowledged, particularly in the shrub-dominated rangelands of southern Africa. Browse has been reported to provide nutrients such as protein, vitamins and minerals that are sometimes in short supply in grasslands especially during the dry seasons (Le Houérou 1980b, Prins 1996b, Mapiye et al. 2011). Although shrubs are known to enable browsers and mixed feeders to survive critical periods of prolonged drought (Franzel et al. 2005), the extent to which domestic and wild herbivores consume these shrubs varies widely depend on the shrub and animal species (Papachristou et al. 2005). Cattle have been reported to spend 4-7% of their time browsing, while goats browse for 43-52%, and sheep are intermediate at 4-28% (Prins et al. 2000, Sanon et al. 2007). Goats are mixed feeders with a narrow mouth, mobile lips and tongue (Solaiman 2010), which help them to deal with plant physical defences such as thorns (Gowda 1997, Gowda et al. 2003). Additionally, goats are known to possess proline-rich proteins in their saliva (Juntheikki et al. 1996, Shimada 2006), which help them utilise chemically defended forages better than other domestic herbivores and many wild grazers (Hanovice-Ziony et al. 2010a, Solaiman 2010).

Although goats are anatomically and physiologically suited to thrive in shrub-dominated ecosystems, the extent to which they consume savanna shrubs (Bakare and Chimonyo 2011, Perez et al. 2013, Hacker and Alemseged 2014) is still largely limited by the presence of plant secondary metabolites (PSMs) (Kaitho et al. 1997a, Scogings et al. 2014). Plant secondary metabolites have been shown to affect both domestic and wild animals' intake, food preference, behaviour, and nitrogen retention rumen function (Mueller-Harvey 2006, Estell 2010, Foley 1999). Consequences for consuming plant secondary metabolites range from beneficial, to negligible or acutely toxic, depending on the particular PSM and the amount consumed (Villalba and Provenza 1999). In southern African savannas, browse species are well endowed with carbon-based secondary metabolites (Scogings et al. 2014), a group that includes many different phenolic compounds such as furanocoumarins, low molecular weight phenolics (Hattas et al. 2011, Hattas 2014), condensed tannins and hydrolysable tannins (Cooper and Owen-Smith 1985, Owen-Smith 1993, Scogings et al. 2004), and condensed and hydrolysable tannins (Cooper and Owen-Smith 1985, Owen-Smith 1993, Scogings et al. 2004). Alkaloids have also been observed among African woody species (Scogings 2005).

Therefore in order to better utilise savanna browse, African browsers and mixed feeders should counteract a complex system of plant chemical defence.

There is evidence that supplementing herbivores with nutrients does not only improve their nutrient balance (Illius and Jessop 1996, Foley 1999), but it also increases the amount of PSMs that animals can consume (Provenza et al. 2003). Intake of PSM-rich forages is assumed to be controlled by nutrient-PSM interactions that increase or reduce animal's preference for food and habitat (Provenza et al. 2003, Iason 2005). The rate at which PSM-rich foods can be eaten depends on how quickly the animal can detoxify, deactivate or eliminate ingested PSMs from the body (Foley et al. 1995). The process of transforming the more toxic compounds into less toxic water soluble compounds requires nutrients, especially energy, protein and water (Illius and Jessop 1995). This implies that nutrient deficiencies will negatively affect the efficiency of animals to detoxify and eliminate PSMs from the body. Logically, supplementing animals with food sources that are high in these nutrients has been reported to increase the animals' capacity to detoxify dietary PSMs (Provenza et al. 2003, Marsh et al. 2006a). While nutrient supplementation has been shown to increase utilization of terpene rich sagebrush by sheep in North America (Dziba et al. 2007) and chemically defended and unpalatable shrubs in Mediterranean systems (Banner et al. 2000, Rogosic et al. 2008a, Rogosic et al. 2011, Saric et al. 2013), no study has tested this prediction in the context of African savannas.

The influence of nutrient supplementation on PSM-rich forage intake depends on the kind and amount of nutrients and PSMs the animal is exposed to (Villalba et al. 2002a). Although diverse PSMs are prevalent among the African savanna woody plants, condensed tannins are thought to be the main chemical component affecting leaf defoliation by browsing ruminants (Owen-Smith et al. 1993, Chapman et al. 2010, Scogings et al. 2011). Unlike most PSMs that are categorised as toxins owing to their property of being easily absorbed, detoxified and eliminated from the body via the liver (Marsh et al. 2003, 2005), condensed tannins are not absorbable (Makkar 2003). Since rumen microbes are not capable of degrading condensed tannins (Lopez-Andres et al. 2013), tannins are unlikely to be absorbed and transported to liver cells (Makkar et al. 1995a, Makkar et al. 1995c). Therefore, detoxification through activation of liver enzymes cannot explain changes in intake of tannin-rich forages in response to nutrient supplementation. Ruminants have been purported to learn about the consequences of food ingestion (Provenza 1995) and discriminate between the post-ingestive effects of energy and protein (Villalba and Provenza 1999). Feeding experiments with sheep reported supplementation with high-protein and high-energy concentrates to improve utilization of tannin-rich shrubs (Villalba et al. 2002b, Villalba et al. 2002c). This supplementation-induced improvement in tannin-rich shrub intake can also be explained in terms of the supplemental nutrients, especially protein replacing either the tannin-bound proteins (Makkar et al. 1988, Min et al. 2003) or the tannin-binding salivary proteins in ways that delay a negative aversion for tannin-rich forages (Shimada 2006, Yisehak et al. 2012). Therefore, a high-protein supplement is expected to enhance consumption of tannin-rich shrub intake than a high-energy supplement (Figure 5).

In this study, we investigated the effects of high-protein and high-energy concentrate supplementation on woody plant use by free-ranging goats in a South African semi-arid savanna. Tropical savannas are highly seasonal and forage availability and quality is generally reduced during the dry season, when grass dries out and deciduous shrubs lose leaves (Prins and Beekman 1989). The remaining evergreen species are highly defended, either mechanically and or by PSMs, resulting in reduced defoliation (Prins 1996a, Ganqa et al. 2005). As a result, decreases in herbivore productivity are reported mainly during the dry season (Prins 1989a, Mapiye et al. 2011, Sebata et al. 2011). Here we conducted a field experiment under circumstances where both browse and grass were available. We predicted that supplementing animals with high-protein and high-energy concentrates would (1) increase the percentage of foraging time of supplemented animals and consequently, (2) increase browse intake. We also predicted (3) increase the amount of condensed tannins consumed and therefore result in (4) supplemented animals to include a larger percentage of tannin-rich forages in their diet than animals not supplemented resulting in (4) an increase in the amount of condensed tannins consumed (g DM). Lastly, given high prevalence of condensed tannins in the African savanna browse and the higher affinity that tannins have for dietary proteins, we expected (5) a high-protein supplement would increase browse use more than high-energy supplement.

Materials and Methods

We conducted a field experiment that lasted for 15 days in July 2013 at the Roodeplaat Experimental Farm of the Agricultural Research Council in Pretoria, South Africa (25º20´-25º40´E; 28º17´-28º25´S). The climate is semi-arid with an average annual rainfall of 646 mm and daily mean temperatures ranging between 2-16 ºC in July. The main dry season in the study area starts in May and reaches its peak in July. The vegetation is classified as savanna (Mucina and Rutherford 2006) and the rangeland in the study area is dominated by *Vachellia karroo*, *Vachellia tortilis, Ziziphus mucronata,* and some *Euclea* species (Coates Palgrave 2002, Kyalangalilwa et al. 2013).

Experimental design and habituation period

A camp of 1.8 ha was fenced with mesh wire and stocked with 15 indigenous female goats (South African veld goats) that were 12 months old and with an initial body weight of 20.6 (SD ± 2.5) kg. Animals were subjected to a two week habituation that was aimed at (1) establishing the correct amount of supplement to offer, (2) familiarizing the goats with presence of observers and (3) familiarizing goats with the terrain. Every morning during this period, five goats individually received yellow maize grain (i.e., a high-energy supplement), non-dehulled soybean meal (i.e., a high-protein supplement) or no supplement (the control group). Soybean meal typically contains 51.8 % DM crude protein (CP) and 18.2 MJ/kg DM digestible energy (Heuzé et al. 2012), whereas maize grain typically contains 10.5 % DM CP and 16.2 MJ/kg DM digestible energy (Heuzé and Tran 2013). Thus, both supplements are high in energy, but maize grain is much lower in CP. The CP composition of the grass and browse species in the study area range between 15 to 6.5% (Mkhize, unpublished data). Based on observations during the habituation period, each goat was offered 100 g of supplement which was rapidly consumed (in less than 15 minutes) by all animals. This amount was small enough to avoid gut-fill or substitution of forage consumption in the field. From 08:00 in the morning, all animals were allowed to forage together freely in the camp until 16:00 when they were corralled. All animals were treated for internal and external parasites prior to starting the experiment. They all received *ad libitum* access to water throughout the experiment. The handling and treatment procedures were approved by the Animal Ethics Committee of the ARC under permit number APIEC11/039.

Data collection

Following a two week habituation period, direct observation of foraging behaviour and diet composition was conducted. The treatment groups established during the habituation period were maintained throughout the experiment. Each day, nine goats (three per treatment) were observed for 15 days. To control for possible effect of time of the day (i.e., time since supplementation was offered) on feeding behaviour, we observed three goats (one from each treatment group) in the early morning (08:00 to 10:30), late morning (10:30 to 12:00) and afternoon (12:00 to 15:30). An observer followed each goat for fifteen minutes per observation, while assisted by a recorder. The same people observed and recorded the foraging behaviour throughout the experiment.

During each observation, we used the Observer XT 10.5 (Noldus 1991a) in combination with a Psion Work-about handheld computer (Noldus 1991a, Zimmerman et al. 2009) to record the activity (grazing, browsing or non-feeding activities). For browsing activity we identified the forage species, and the number of bites taken. We recorded grazing activity as one species (namely, “grass”) without identifying the grass species. The number of bites and the time animals spent on grass were recorded. For non-foraging activities we recorded standing (standing without eating and sometimes while ruminating), walking (moving from one place to another), resting/laying down (sometimes while ruminating), searching (moving the head sideways in between different forage plants), drinking, rasping (scratching the head or body against plants), and socialising (touching other animals in a playful or aggressive manner). However, for the purpose of this paper, we pooled all non-feeding activities. In cases where feeding and non-feeding activities did not occur mutually exclusively, feeding always overrode non-feeding activities. In addition to behavioural activities, the Observer XT 10.5 recorded the date, starting/stopping time, treatment, and the goat identification number for each observation.

To calculate the tannin consumption, we needed to know condensed tannin concentration for each plant species. Therefore, we sampled leaves of all dietary woody species and analysed them for condensed tannin concentration according to Porter et al. (1986). To estimate the amount of tannins consumed during a 15 minute observation, we multiplied the total number of bites taken from each plant species by the mean condensed tannin concentration and the mean bite size of that particular plant species. Since it was not possible to estimate bite sizes in the field, we conducted a feeding experiment and obtained bite size estimates under controlled conditions. One day after the field observations, we individually penned nine of the 15 goats used in the field for behaviour observation (3 from each treatment). We offered these goats at least 10 un-browsed branches of each of the forage species that were included in diets during field observations, and estimated bite sizes (g DM per bite) according to Mkhize et al. (2011). All branches were collected from the camp in which the field observations took place.

To estimate the average percentage inclusion of each plant species in the diet of each goat per observation, we divided the dry matter intake from each plant species in each observation with the total intake from all plant species during that particular observation.

Data analysis

A general linear model procedure of SPSS, v20 (IBM SPSS Statistics; Chicago, IL, USA) was used to analyse the (1) percentage of time spent on (a) browsing and (b) foraging (foraging = grazing + browsing), and (2) browse intake as response variables. Treatment (high-energy supplement, high-protein supplement or no supplement) and time of the day (early morning, late morning and afternoon) were used as fixed factors, while goat ID was used as random factor in the models. To compare the three treatments in terms of percentage inclusion of each plant species in the diet, general linear models were used, followed by Bonferoni multiple comparison test (adjusted alpha = 0.003). In all models the Shapiro-Wilk test was used to test if unstandardized residuals followed a normal distribution, and the Levene's test was used to test for equality of variances. The natural log transformation for percentage inclusion data for several plant species worked successfully as they generated residuals that followed a normal distribution. A Sidak test was applied for pairwise comparisons between different treatments and between different times of the day. We performed a Kruskal-Wallis test to investigate the effect of treatment on condensed tannin intake.

Results

Supplemental energy and proteins did not influence the amount of time spent by the animals on total foraging ($F_{2,78}$ = 1.48; P = 0.233). Animals in this study spent 70% of their time in the field foraging and consequently 30% on non-foraging activities. No significant interactions were found between treatment and time of day on all dependant variables. Therefore, the effects of these two independent variables are reported separately.

The percentage of time spent by animals on browsing significantly increased as a result of supplementing with high-protein and high-energy sources ($F_{2,76}$ =7.96; P = 0.001) by reducing their grazing activity, but supplementation did not influence the time animals spent on non-foraging activities (Figure 1). Independent of the treatment, animals browsed for longer times ($F_{2,76}$= 6.03; P = 0.004) during the early morning than in the late morning. Although browsing time decreased later in the morning, it increased again in the afternoon (Figure 2).

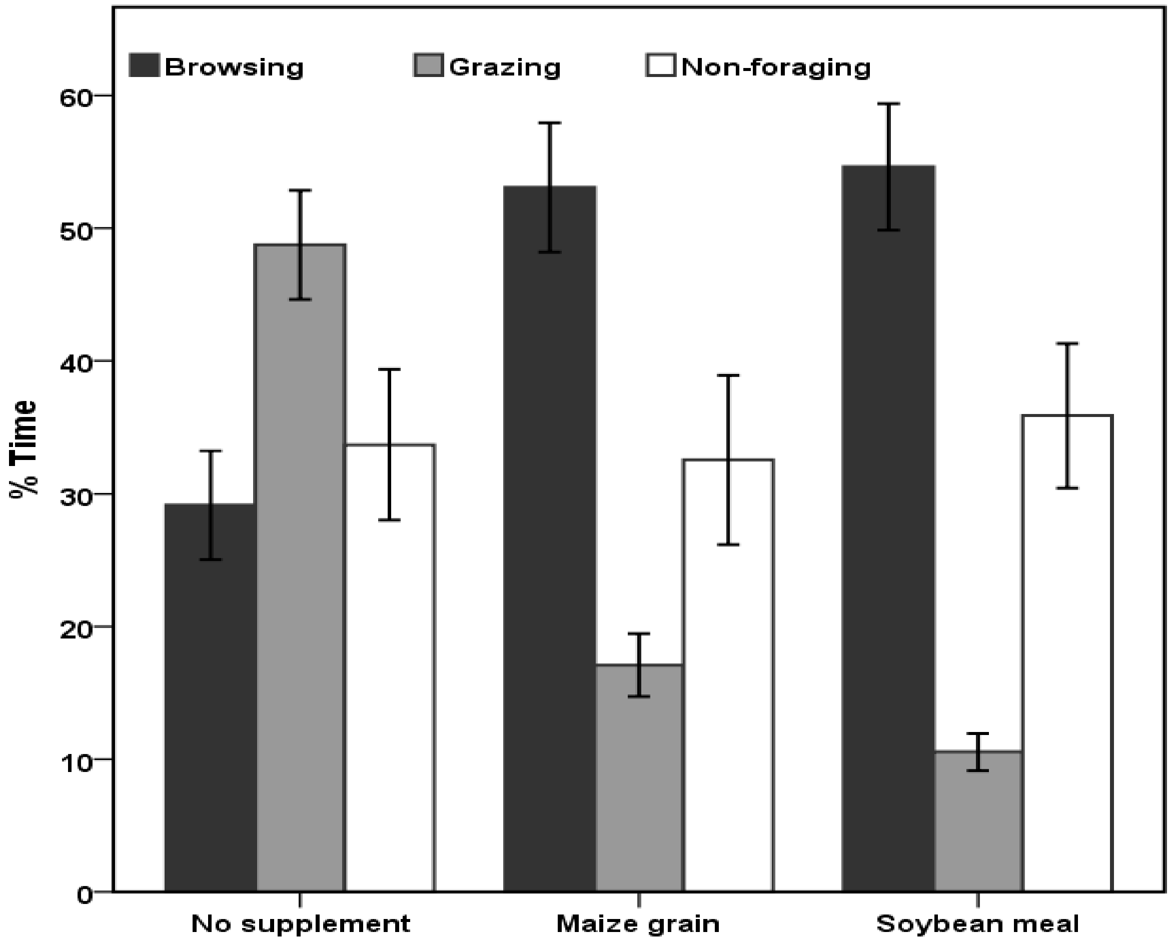

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Figure 4: Mean (±95% CI) percentage of time spent on browsing, grazing and non-foraging activities by the free-ranging goats that were either given a high-energy supplement (i.e., yellow maize grain), high-protein supplement (i.e., non-dehulled soybean meal) or no supplement (i.e., control).

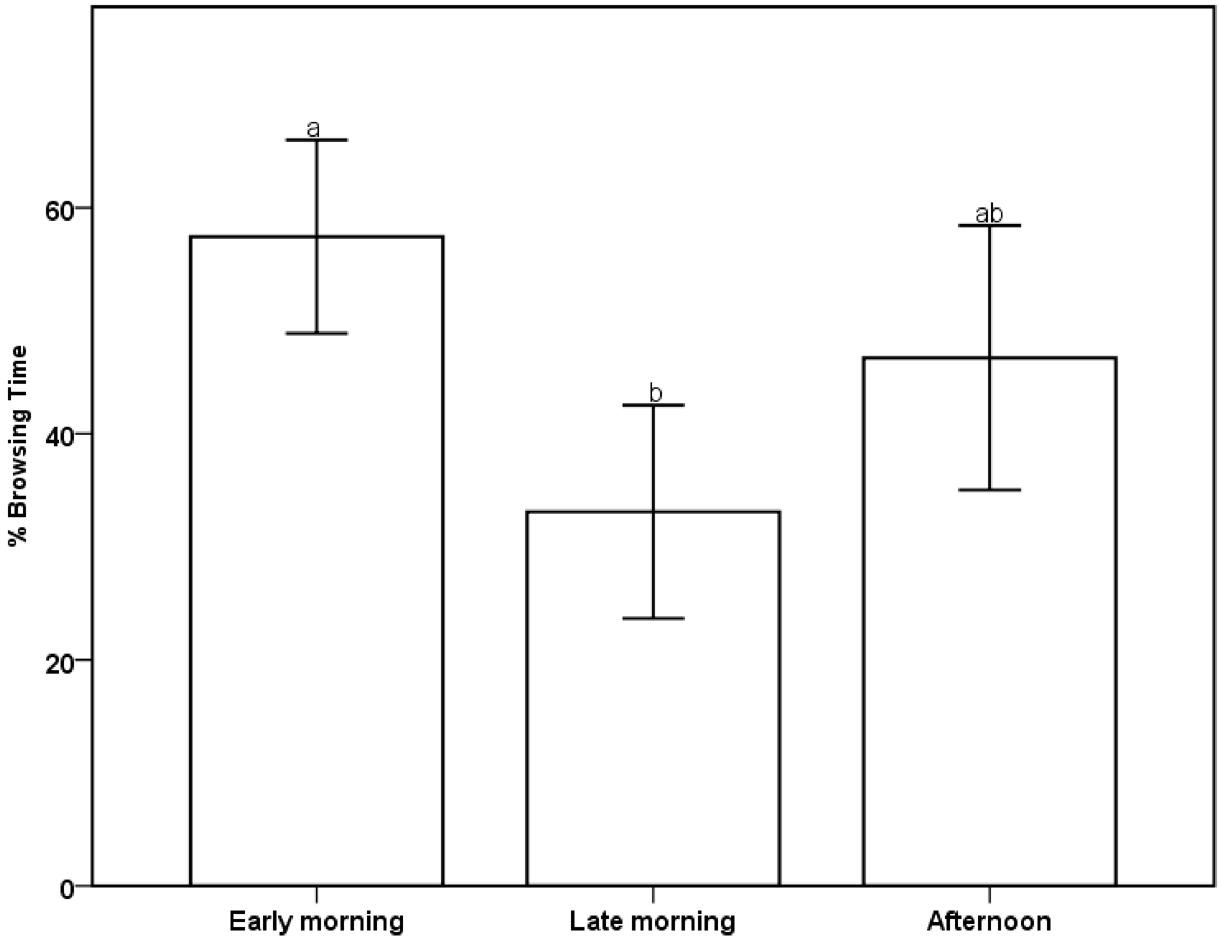

Figure 2: Mean (±95% CI) percentage of time spent on browsing activity by free-ranging goats during early morning (i.e., 08:30 to 10:30), late morning (i.e., 10:30 to 12:00) and afternoon (from 12:00 to 15:30) of the field experiment at Roodeplaat Farm. Letters represent significant differences among different times of the day (P<0.05).

Browse intake was increased with high-energy and high-protein supplementation ($F_{2,78}$ = 3.52; *P* = 0.034; Figure 3). Similarly, supplementing animals with high-protein or high-energy sources influenced browse intake, i.e., intake was highest in the early morning and lowest in the late morning ($F_{2,78}$ = 7.17; *P* = 0.001).

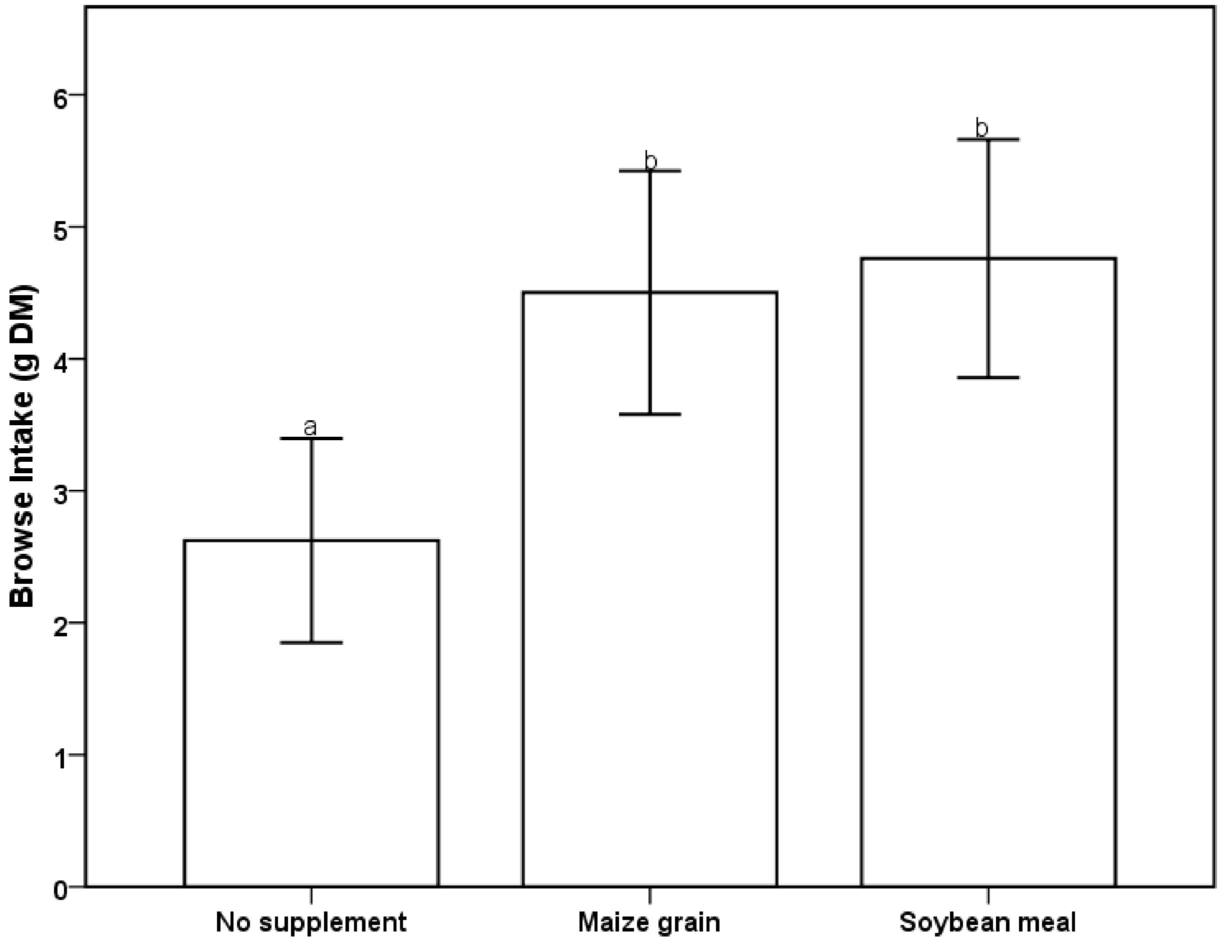

Figure 3: Mean (±95% CI) intake rate of browse materials during foraging by free-ranging goats that were either given a high-energy supplement (i.e., yellow maize grain), high-protein supplement (i.e., non-dehulled soybean meal) or no supplement (i.e., control). Letters represent significant differences among different treatments (P<0.05).

Dietary condensed tannin intake significantly increased (Chi-square = 5.75; *df* = 2; *P* = 0.041) in response to high-energy and high-protein treatments (Figure 4). Condensed tannin intake significantly varied among different times of the day ($F_{2,82}$ = 10.37; P < 0.001), with most tannin intake being recorded in the early-morning and least intake in the late morning.

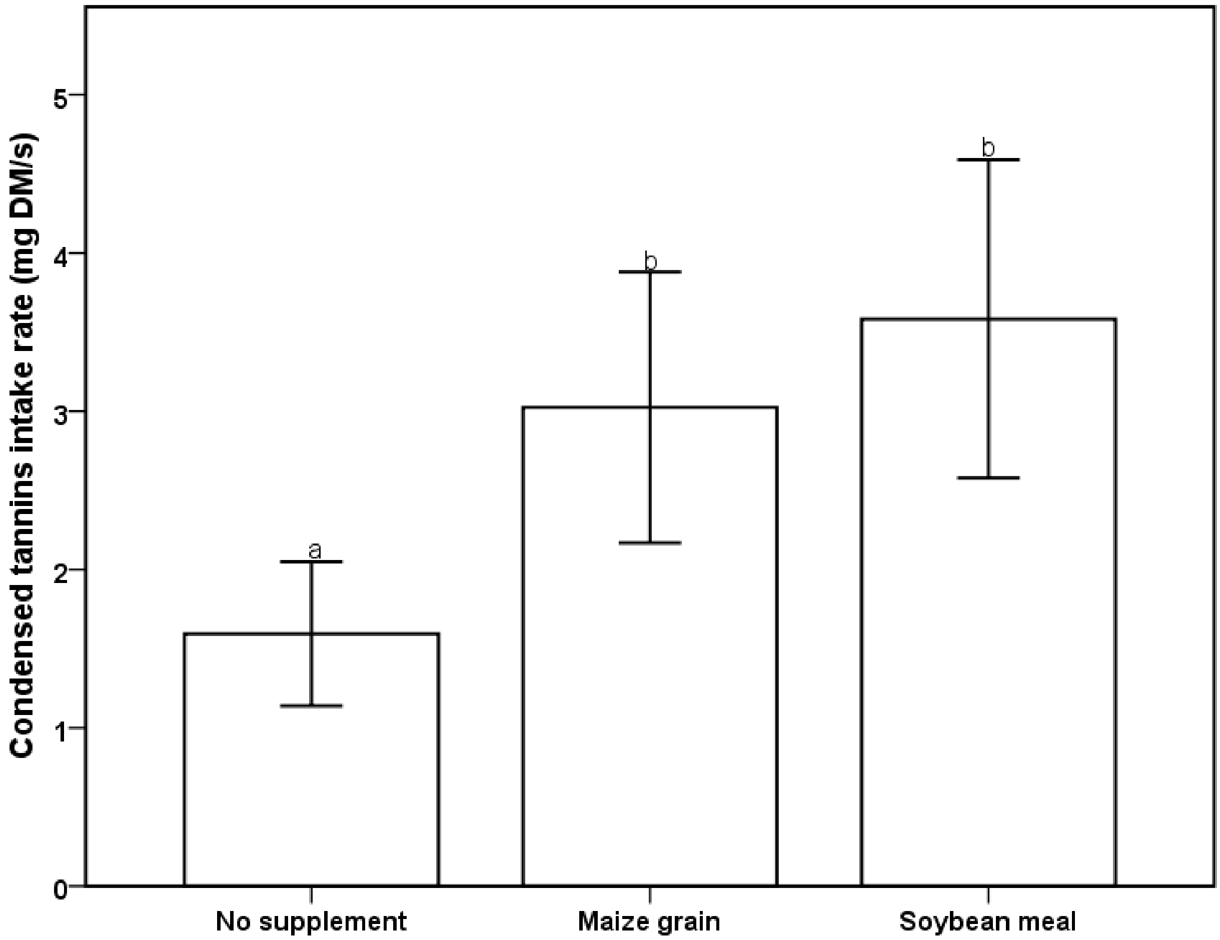

Figure 4: Mean (±95% CI) condensed tannin intake rates of browse materials during foraging by free-ranging goats that were either given a high-energy supplement (i.e., yellow maize grain), high-protein supplement (i.e., non-dehulled soybean meal) or no supplement (i.e., control). Letters represent significant differences among different treatments (P<0.05).

Although descriptive analysis indicated that animals from different treatment groups consumed the same forages in their diet, results from a general linear model analysis showed control animals to include some of these forages at significantly different percentage of their diets than the supplemented animals (Table 1). Significant differences were found mostly for plant species with either highest or lowest condensed tannins. For example, control goats included significantly lower percentage of forages with high CT concentrations such as *Euclea crispa* ($F_{2,43} = 12.14$; $P < 0.001$), *Gymnosporia buxifolia* ($F_{2,49} = 3.61$; $P = 0.034$) and *Ziziphus mucronata* ($F_{2,67} = 7.69$; $P = 0.001$) than supplemented animals. Tannin-poor forages tended to feature in higher percentages in diets of control than supplemented animals. For example, grass ($F_{2,79} = 21.84$; $P < 0.001$) was significantly higher in the diet of control than supplemented animals.

Table 1: Condensed tannin concentrations [CT] in forage plants in order of descending [CT] and dry matter percentage inclusion of each forage in the diet of goats supplemented with nothing, high protein and high energy concentrates. Different superscripts within the same row represent significantly different % inclusion of the forage plant in the diet by animals between different treatment groups (P<0.05).

		Treatment (% inclusion in diet)		
Forage Species	**CT composition (mg/g)**	**Control**	**High Protein**	**High Energy**
Carissa bispinosa	95.6	0.1[a]	3.1[b]	1.2[b]
Scolopia zeyheri	72.8	0.4[a]	0.0[a]	0.2[a]
Euclea crispa	69.0	2.4[a]	29.9[b]	16.3[b]
Gymnosporia buxifolia	68.9	1.1[a]	4.7[b]	3.9[b]
Grewia flava	48.6	0.5[a]	0.8[a]	0.8[a]
Pappea capensis	48.5	1.4[a]	1.5[a]	0.8[a]
Ziziphus mucronata	40.5	8.8[a]	18.6[b]	28.6[b]
Barchemia zeyheri	28.2	0.4[a]	1.8[a]	0.9[a]
Vachellia robusta	16.3	1.3[a]	4.1[a]	2.4[a]
Herbs	7.1	6.1[a]	9.4[a]	8.2[a]
Vachellia nilotica	4.9	0.4[a]	0.6[a]	0.5[a]
Grass	2.2	73.7[a]	20.3[b]	29.5[b]
Ehretia rigida	1.4	1.0[a]	1.0[a]	1.7[a]
Aloe greatheadii	0.7	2.4[a]	4.3[a]	5.0[a]
	Total	100	100	100

Discussion

Our prediction that supplemental nutrients would increase the amount of time animals spend on browsing was supported by the results. These results are consistent with findings from a similar field study that showed ewes supplemented with both high-protein and high-energy sources to spend more time on browsing sagebrush, a shrub that contains terpenes, than non-supplemented animals (Dziba et al. 2007). It is, however, important to note that total foraging time did not change even though supplemented animals spent more time on browsing than non-supplemented animals. Since resultant foraging time was equal across the treatment groups, these results suggest that the main effect of tannins or nutrient supplementation is on the relative amount of time spent browsing or grazing. High-energy supplements increased the incidence of browsing by goats similarly to a high-protein supplementation throughout the experiment, indicating that either of the two supplements supplied adequate nutrients required for the mechanism that led to enhanced tolerance to increased time spent on browse (Rogosic et al. 2011). Our study showed that supplemented animals browse more in the morning (shortly after the supplementation), slow down on browsing in the middle of the day only to increase browsing behaviour again in the afternoon. This may be associated with animals regulating intake of PSMs and keeping the levels below certain thresholds (Marsh et al. 2005). The differences between different times of the day agree with our previous results in the same area that showed goats spend more time browsing during the early morning than other times of the day irrespective of the treatment (see chapter two).

Our results supported the prediction that supplementation would increase dry matter intake of savanna shrubs. Similarly, sheep and goats supplemented with energy and protein ate double the amount of sagebrush than non-supplemented animals (Banner et al. 2000, Villalba et al. 2002b). Furthermore, supplementing sheep with barley (an energy source) in the Mediterranean rangelands increased average daily intake of chemically defended shrubs (Rogosic et al. 2006b, Rogosic et al. 2011, Saric et al. 2013). Against our prediction, the results showed no differences between high-energy and high-protein supplements in terms of improving shrub intake. The basis for our prediction was that protein supplement would replace the nitrogen lost through protein-tannin complexing. In a long term supplemental nutrients could probably replace protein lost by secreting tannin-binding salivary protein (Shimada 2006, Hanovice-Ziony et al. 2010b, Yisehak et al. 2011) and thus allow goats to bind more tannins and eat more shrubs than animals supplemented with a high-energy source (Figure 5). Although shrub intake still improved, protein replacement cannot explain it. This observation was also inconsistent with the findings from Villalba et al.(2002a) who reported protein supplementation to increase intake of a diet containing condensed tannins more than a high-energy supplement. Although Villalba et al.(2002b) also reported improved increased intake of sagebrush by goats and sheep when fed a protein supplement, they generally found energy to have no effect on sagebrush intake. Explaining this discrepancy can be a complex exercise given that these two supplements also differed in other respects such as different minerals, fibres and amino acids in different quantities. The clearest differences between the two supplements used in our study were in CP content (10.5% for maize grain vs.51% for soybean meal) and minerals that were often lower for maize than for soybean. (Heuzé et al. 2012, Heuzé and Tran 2013). Nevertheless, both supplements likely contained a combination of nutrients that were required by goats to contend with dietary PSMs and increase shrub intake.

The amount of PSMs that animals can ingest depends on the nutritional composition of the PSM-containing food relative to the nutritional composition of the alternative forage (Villalba and Provenza

2005). Supplementation significantly increased tannin intake in our study, supporting our prediction. A previous study showed that supplementing goats with a high-carbohydrate or a high-protein food, neutralized the effect of tannins in ways that increased intake of tannin-rich plants (Silanikove et al. 1997). In another study, the increased intake of one-seed juniper by sheep and goats that received supplemental protein was explained as the apparent role of added protein in offsetting loss of plasma amino-acids due to PSM detoxification (Utsumi et al. 2010, Utsumi et al. 2013). Similar results have been reported on quebracho tannin consumption by sheep and goats (Villalba et al. 2002a), and intake of forages rich in alkaloids, saponins and tannins by sheep (Owens et al. 2012b). Our results show a tendency for control animals to include species that are rich in condensed tannins to a lesser extent than supplemented animals. The main differences among the treatment groups were observed on percentage inclusion of tannin-rich (i.e., higher for supplemented goats) and tannin-poor (i.e., higher for control goats) forages in the diets.

Overall, results in this study showed that protein and energy supplementation improves the use of high condensed tannin containing savanna shrubs by mixed feeding herbivores. The fact that shrub and condensed tannin intake increased as a result of supplementation may indicate that post-ingestive feedback is at least partially involved in how animals deal with chemically defended forages (Provenza 1995). Our results also indicate that without nutrient supplementation the limits set by tannins have negative effects on intake of chemically defended plant species.

From an evolutionary point of view, these results show that it is likely that at least some mixed feeders (including goats) evolved from grazers and not from browsers. It is the grass that allows them to tackly the tannins in the browse and based on these results we postulate that protein rich-grass allow these mixed feeders to have easier access to the browse. We thus postulate that these mixed feeders could expand into browse-rich environments (niches) because of the successful combination of the grass proteins and grass energy with the nutrients that had to be unlocked for digestion through overcoming the "tannin-lock" in the browse. Our results thus make an evolutionary pathway from browser to mixed feeder less likely.

Responses of goats to nutrient supplementation highlight opportunities for managing the savannas. There is opportunity for using mixed feeders such as goats, as well as browsers, to manage the chemically defended savanna rangelands in ways that create a more diverse mix of plant species in the vegetation and enhancing herbivore production. Woody plant encroachment is predicted to increase in savannas (Ward 2005, O'Connor et al. 2014) and condensed tannins are among the factors limiting the ability of large herbivores to consume the encroaching plants. Results from the current study have serious implications for management of woody vegetation in southern African savannas (Milton and Dean 1995, Dziba et al. 2003). Supplementing animals with either maize grain or soybean meal seems to alleviate the aversive effects of dietary PSMs and may, therefore, increase efficiency of mixed feeding herbivores as biological bush control agents.

Acknowledgements

Authors are grateful to Lucas Letsoalo and Piet Monegi for helping in the field. The study was funded by the Wageningen University (the Netherlands), as well as the Agricultural Research Council, and National Research Foundation, South Africa.

Chapter Six

Synthesis: How mixed feeders utilise chemically defended shrubs

Ntuthuko R. Mkhize

Introduction

Understanding the factors that influence foraging behaviour of herbivores is a central goal in nutritional ecology (DeGabriel et al. 2013). Several studies with captive animals have revealed a plethora of effects of nutrients and plant secondary metabolites on forage intake, diet composition, digestive physiology and reproduction. This gives a good reason to expect variations in forage characteristics, such as forage quality, to limit productivity of free-ranging livestock (Foley et al. 2007) and wild herbivore populations (Degabriel et al. 2009). However, since experiments with captive animals tend to oversimplify the complexity of natural ecosystems (Calisi and Bentley 2009), it has so far proven very difficult to link concentrations of dietary plant secondary metabolites and nutrients with herbivore population regulation in the wild (DeGabriel et al. 2013, Bedoya-Perez et al. 2014). Despite this difficulty, research has shown plant secondary metabolites to act as feeding deterrents (DeGabriel et al. 2014), toxins (Marsh et al. 2014), digestibility reducers (Makkar 2003, Foley 1999), feeding or oviposition cues (Moore and DeGabriel 2012) and as signals for communicating to neighbouring plants and natural enemies of herbivores (Dicke 2009, Iason et al. 2012, Moore and DeGabriel 2012). In this thesis I contributed insights on the effects of digestibility reducers (specifically condensed tannins) on foraging behaviour and growth performance of mixed feeding ruminant herbivores under field conditions. Furthermore, I explored ways in which current knowledge of herbivore adaptations to digestibility reducers (in particular) and toxins (in general) could be used to increase intake of chemically defended woody plants by large herbivores.

The first three chapters were based on a single field experiment in which goats (as model mixed feeders) were orally dosed (daily) with either condensed tannins or with polyethylene glycol (a tannin-neutralizing chemical) or water (control). The fact that (in the first place) I expected to observe differences in foraging behaviour and subsequently growth performance among the animals from different treatment groups, should clearly indicate that I premised this thesis on post-ingestive feedback as an important basis for palatability (Provenza 1995, 1996). Given that palatability is a nebulous term, I will dedicate the remaining part of this paragraph to discussing what palatability is in light of the research being reported in this thesis. Animal scientists often explain palatability as a hedonic response of an animal to its food depending on the flavour, texture and the relish with which an animal is consuming the food (Kaitho et al. 1996, Kaitho et al. 1997b, Strengbom et al. 2003). From this, one can deduce that according to animal scientists, herbivores simply eat food that taste good and avoid foods that taste bad (Owen-Smith and Cooper 1987b, Strengbom et al. 2003, Gordijn et al. 2012). On the other hand, plant ecologists describe palatability as plant attributes that alter acceptability or attractiveness to the animals including chemical composition, growth stage and associated plants (Owen-Smith and Cooper 1987a, Cooper et al. 1988, Bernays and Raubenheimer 1991, Taha 1992). While forage characteristics are important to a great extant in determining plant palatability, there is increasing evidence that neutrally mediated interactions between the senses (i.e., taste and smell) and the viscera enable herbivores (especially ruminants) to sense the consequences of food ingestion (Cheeke 1980). These interactions are thought to operate in ways that affect food selection and intake (Rigos et al. 1999). It was probably out of this evidence that Provenza (1995) redefined palatability as the interrelationship between a food's flavour (odour, taste, and texture) and the post-ingestive effects of nutrients and plant secondary metabolites. Both the food's flavour and post-ingestive feedback are influenced by the chemical characteristics of forages and an animal's nutritional state and past experiences with the food. The research I reported in this thesis is generally in line with the notion that animals dynamically select various food items to match their nutrient needs while avoiding over-ingesting plant secondary metabolites (Bailey and Provenza 2008).

My main aim in this chapter is to summarize and integrate the results obtained in the preceding chapters, and to discuss new perspectives I contributed via this thesis to current understanding of the effects of condensed tannins and toxins on how herbivores use shrubs in savannas. Given the importance of savannas as a major terrestrial biome, I will (throughout this chapter) draw inference from my findings in this thesis to discuss opportunities for managing savanna rangelands dominated by chemically defended woody plants that are also considered unpalatable to large herbivores. The ultimate goal of my discussion is to clearly show that knowledge of how plant secondary metabolites affect foraging can be used to increase productivity of large herbivores in general, and specifically ruminants in degraded savanna rangelands. The idea I wish to get across is that herbivore production can be increased while simultaneously improving savanna rangeland productivity. While many believe that these two objectives are mutually exclusive, I propose that they can be complementary if the biochemical linkages between plants and herbivores are well understood, and managed appropriately.

Overview of foraging and nutritional studies on tannins

Condensed tannins occur widely in woody plants that are eaten by domestic and wild herbivores in African savannas, and have been the focus of much research by both animal scientists and ecologists. While animal scientists have looked at tannins with the focus of improving livestock production, ecologists have always studied tannins from the perspective of evolution and the functioning of ecosystems. Although ecological and animal science studies often have different goals, their mutual goal has always been to know how dietary and foraging parameters are affected by tannins and how these effects translate into productive processes for herbivores. In the case of ecology, productive processes are survival, growth (chapter four) and population dynamics of herbivores. The foraging parameters of interest have often differed between these two disciplines. For example, ecological studies have focused on diet selection and diet composition (chapters three & five), while the goal of animal sciences has been to understand food intake (chapters five) or digestible food intake which is of greater nutritional relevance. As a result, ecological studies have been less served by intensive nutritional research, although they have additionally investigated how tannins affect whether or not a plant will be eaten or damaged by herbivores (chapter two), and which plant parts are likely to be eaten more. Numerous ecological field studies in African savannas have reported negative relationships between dietary tannin concentrations and preference by browsers and mixed feeders (Cooper and Owen-Smith 1985, Owen-Smith 1993, Furstenburg and van Hoven 1994). Consequently, ecologists have tended to regard tannins as entirely negative merely based on correlative field studies which proved neither causes nor effects. On the other hand, the challenge with animal science studies has been translating result from pens and laboratory studies into free-ranging situations (DeGabriel et al. 2014). Yet, surprisingly there has been little cross-fertilization between these two research areas (Foley et al. 2007). In this thesis I borrowed examples, theoretical frameworks and methodologies from both these disciplines and addressed a broad question: *how to (1) minimize the negative effects of tannins and toxins, (2) enhance utilization of locally available plant resources that may be chemically defended, in ways that subsequently (3) enhance herbivore productivity?*

Tannins effects on foraging behaviour and growth performance

Capitalising on the above mentioned lack of a trans-disciplinary approach, I tested the effects of tannins on free-ranging herbivores and attempted to explain results in ways that make sense to both ecologists and animal scientists. In chapters two, three and five, I investigated how foraging behaviour parameters such as bite rates, intake rates, foraging bouts and foraging time are influenced by condensed tannins, polyethylene glycol, energy and protein supplementation. This is an important question since the process of finding and harvesting food is at the basis of animal growth and reproduction of livestock and wild herbivores. The rate at which herbivores consume their food determines the amount of time they will spend on foraging to meet their metabolic needs for energy and nutrients (Shipley et al. 1994). Thus intake rates determine the amount of time that is available for non-foraging activities such as resting, mating and avoiding predation. Contrary to numerous results from pen studies that showed tannins to reduce intake by animals that are usually confined to a single or a few forages, I showed (in chapter two) that mixed feeders (such as goats) do not necessarily reduce their foraging time in response to high concentrations of dietary condensed tannins. Instead they switch from spending their foraging time on browse to grass. I explained this change in behaviour switching from browse to grass, as a strategy to minimize condensed tannin intake. There is ample evidence that animals do not completely avoid tannins, but instead they keep tannin intake below certain thresholds (Jansen et al. 2007). A long standing hypothesis for diet selection is that herbivores forage in ways that maximize nutrient intake while minimising the intake of plant secondary metabolites (Westoby 1974, 1978). Although, I did not test any nutrient maximization prediction in this thesis, the postulation about switching being a strategy to minimise tannin intake was supported by the findings of chapter three.

In chapter three, I indicated that goats exposed to high concentrations of condensed tannins significantly decrease their intake rate of tannins especially during the wet season. Regulation of forage intake rates and dietary choices combines short-term control of feeding behaviour related to the body's homeostatic regulation, and long-term control that depends on general requirements and body reserves (Baumont et al. 2000). In chapter three I showed that goats increase their bite rates during the wet season likely due to increased forage availability and better forage quality during this season. The key finding from chapter three was that mixed feeders alter their bite rates, bout numbers and bout lengths as a strategy to regulate tannin intake. In order to understanding these foraging behaviour results (chapters two and three) in terms of the effects that tannins subsequently have on the animals' fitness, I measured growth performance parameters such as body weights, blood metabolites and dietary nitrogen retention. In chapter four, I did not only show that high condensed tannin exposure reduces the average daily body gains by free-ranging mixed-feeding herbivores, but I also explained the mechanism for this body weight reduction using faecal nitrogen and blood metabolites data. Firstly, using nitrogen faecal excretion as a rough indication for protein-tannin complexes, I showed that mixed feeding herbivores loose a significant amount of protein as a result of their exposure to high levels of dietary tannins. Blood urea and non-esterified fatty acids indicated a negative influence of high levels of condensed tannins on energy and protein metabolism (chapter four). As mentioned in chapter four, condensed tannins can either be detrimental or beneficial to herbivores depending on the concentrations in the dietary forages (Min et al. 2003). Tannins form insoluble complexes with dietary proteins, resistant to ruminal fermentation, which decreases ammonia evolution in the rumen and subsequently urinary urea excretion (Dawra et al. 1989, Duckstein et al. 2012). Excessive protein-tannin binding can however reduce subsequent enzymatic protein degradation in the small intestine which will reduce amino acid uptake (Mueller-Harvey 2006). Chapter four also confirmed that tannins increase faecal protein excretion.

Foraging behaviour of animals that were dosed with tannin powder (the high tannin exposure group) was characterised by less browsing and more grazing and these animals exhibited some signs of stress in terms of body weight gain and levels of nutritionally related blood metabolites. Given that protein utilization is a particularly important factor in livestock production (for example), tannins seem to have an important role in increasing the nutrient use efficiency achievable by optimizing diet composition, thereby reducing nutrient wastage from ruminants into the environment.

Mechanical and chemical woody plant defences

Shrubs and trees dominate the vegetation in savanna ecosystems. Goats and other mixed feeding herbivores and pure browsers utilise browse from numerous woody species to satisfy their needs for nutrients. These forages play an important role especially in areas with long dry periods because they provide green and nutritious forage for livestock and wild herbivores throughout the year (evergreen species) or at specific critical periods of the year (deciduous species). Despite a strong focus by this thesis on tannins, savanna woody plants possess a wide variety of other chemical and physical properties that are known to simultaneously (with tannins) affect foraging behaviour.

Mechanical defences such as spines and thorns are very common among savanna woody plants and influence browsing rates by reducing bite mass and decreasing biting and chewing rates (Cooper and Owen-Smith 1986). Spines also slow chewing rates by requiring herbivores to carefully manoeuvre their mouths among thorns to avoid injury (Cooper and Owen-Smith 1986, Wilson and Kerley 2003). Many studies have also shown plant morphology to influence browsing rates and daily intake in different ways (Papachristou et al. 2005). For example, animals tend to achieve higher bite rates and reduced intake rates from consuming forages whose leaves grow on old short shoots than when foraging on plants with leaves that grow on young edible shoots that allow bigger bite rates and relatively high intake rates (Dziba et al. 2003, Mkhize et al. 2011, Sebata and Ndlovu 2012). This trend is also in line with the idea that animals select forages from which they can harvest bigger bite sizes. Although this sometimes decreases the bite rate, it increases instantaneous intake rates and daily food intake (Perevolotsky et al. 1998). Forage availability declines over time reducing the rate of food intake because bite sizes decline while bite rates increase (Shipley et al. 1999, Mkhize et al. 2011). In my study, availability may have confounded the intake rate because bite rates do not fully compensate for smaller bite sizes when forage availability has declined (Papachristou et al. 2005). Factors such as plant phenology may also influence availability of leaf material for mixed feeders and browsing herbivores during dry seasons (Papachristou et al. 2003). For example, deciduous species provide less forage material during the dry season compared to evergreen species whose material is available throughout the year (Scogings et al. 2004). Forage digestibility and intake of browse species are also affected by lignin and other fibre contents (van Soest 1994), as high levels of indigestible compounds are known to decrease the digestive benefits of browse by tying up nutrients, and thereby reduce forage preference and intake (Ndindana et al. 2002). While diet selection of herbivores is determined by multiply factors (Pretorius et al. 2012), this thesis focused on the effects of condensed tannins.

Potential to increase shrub use in savanna rangelands by herbivores

Woody plant encroachment has increased in both farmed and conserved savannas all over the world in the last century (Ward 2005, Gray and Bond 2013), the highest increases of woody expansion are particularly evident in the arid and semi-arid savanna ecosystems (Joubert et al. 2012, Smit and Asner 2012). Based on past trends and future predictions, woody expansion is likely to continue with concomitant declines of the grasslands (Hughes 2003, Johns et al. 2003). Given the economic importance of savannas in Africa as rangelands, agricultural lands and conservation areas, the current and predicted expansion of woody dominance over grasses in these ecosystems exposes farmers and managers to potential economic and ecological losses. Besides environmental repercussions such as loss of biodiversity (O'Connor and Crow 1999, Bond and Parr 2010, Parr et al. 2012), and altered ecosystem services such as water supply and carbon sequestration (Coetsee et al. 2013, Gray and Bond 2013), bush encroachment exerts negative economic consequences on rangelands via stocking rate restrictions (Roques et al. 2001, Gray and Bond 2013). In evolutionary terms, these changes might benefit herbivores that are better adapted to woody forages (such as browsers and mixed feeders) at the expense of grazers.

Invasion of woody vegetation into grasslands and savannas is generally thought to lead to increases in the amounts of carbon stored in these ecosystems (Jackson et al. 2002). If predictions of doubling of the present atmospheric CO_2 concentrations within the next 50 years or so are accurate (Bond and Midgley 2000, Bond et al. 2003, Bond and Midgley 2012), then the elevated atmospheric CO_2 may increase the relative carbon availability (Lawler et al. 1997). At the same time, there is evidence that plants growing under conditions of high relative carbon availability will contain more carbon-based allelochemicals (terpenes, phenolic compounds) and have lower concentrations of nutrients than plants growing under low relative carbon availability. These changes in the balance between the concentrations of carbon-based secondary metabolites and nutrients create variations in the relative carbon availability, which might lead to severe consequences such as reduced digestibility, growth rates and final body size for herbivores (including browsers and mixed feeders).

Concurrent with the woody expansion in savannas, is the increasing societal demand for animal protein and livestock products (Estell et al. 2012), which is expected to continue through to 2030 (FAO 2009). Growth of rural and peri-urban small scale agriculture in sub-Saharan Africa over the years has been paralleled with increases in small ruminant numbers (Rischkowsky et al. 2006). Specifically in Africa, goat numbers have increased over the past 30 years (McDermott et al. 2010, Estell et al. 2012). By 2030, numbers of cattle, buffaloes, sheep and goats in the developing world alone are projected to exceed those of the entire world at the beginning of the 21th century (Herrero et al. 2010). About 3.2 billion tons of additional animal forage per year will be needed to feed these additional numbers (Estell et al. 2012). The main question is how to reconcile a world with declining grasslands, expansion of woody vegetation that is increasingly arming itself with carbon-based secondary metabolites, and the concurrent increase in demand for forage to feed the increasing numbers of livestock and wild herbivores? Generalizing from the findings of this thesis, I provide support for already documented strategies and mechanisms that can be used to enhance shrub use by especially ruminant herbivores. In my thesis I propose that part of the forage requirements can be met via manipulating foraging behaviour from compiling grass-based to browse based diets.

Although shrubs and trees generally contain ample nutrients, they are well defended from herbivory via chemical (i.e., plant secondary metabolites) and/or physical (e.g., spinescence, morphology) traits (Papachristou et al. 2003, Provenza et al. 2007). Most savanna shrub species are consumed by livestock and wild herbivores to some extent, but consumption is often low and usually varies with the shrub and animal species and availability of alternative forages (Papachristou et al. 2007). In this thesis I found support for the idea that supplementing animals with either nutrients or additives may minimize the negative effects of condensed tannins and increase shrub intake (Villalba and Provenza 2002, Villalba et al. 2002a, Dziba et al. 2007). Charcoal, alkaline treatments and polyethylene glycol (this thesis) have been shown to decrease plant secondary metabolite absorption via different mechanisms (Rogosic et al. 2008b, Rogosic et al. 2009). In chapters two to four, I did not only study the negative effects of condensed tannins on browsing time, browsing rate, foraging bouts, diet composition, and growth performance of goats, I further presented a potential to use polyethylene glycol to increase browsing time, increase tannin intake rates and increase the amount of dietary browse in ways that improve body weight gains by mixed feeders. Polyethylene glycol is arguably the most studied additive both in pen and field studies for enhancing shrub use by sheep (Titus et al. 2000, Villalba and Provenza 2002), goats (Titus et al. 2001, Salem et al. 2006), and cattle (Henkin et al. 2009). The main challenge will be to introduce this technology into the game production sector. Development of PEG-containing supplement block (Ben Salem et al. 2005) can be used to influence livestock and wild herbivore distribution in ways that influence the amount of grazing pressure.

We are only beginning to appreciate how the interactions among plant secondary metabolites and nutrients influence forage intake and selection and thus mediate plant-herbivore interactions. The rate at which forages that contain plant secondary metabolites are consumed has been purported to depend on how quickly an animals can detoxify and eliminate the toxins from the body (Marsh et al. 2006a, Marsh et al. 2014). This theoretical framework has been used before to explain increased consumption of compounds as diverse as lithium chloride, menthol, terpenes, tannins and saponins in response to supplementation with especially energy and protein sources (Provenza et al. 2003). I started chapter five by challenging the use of this detoxification limiting hypothesis to explain consumption of all secondary metabolites (including tannins), and disregarding their mode of action. I argued that condensed tannins are not absorbed and therefore cannot be detoxified in the liver as has been proposed for toxins. The results of chapter five however showed that indeed supplemental energy and proteins increase the ability of animals to consume forages that are high in tannins. Given that tannins are well known to inhibit utilization of nutrients (such as proteins and carbohydrates) which may serve as cost of ingesting tannin-rich forages, I explained the results of chapter five partly as a way by herbivores to compensate for the nutrients for which the utilisation has been inhibited by tannin-nutrient complexes (see chapter five). These results offer an exciting potential to increase intake of chemically defended savanna shrubs (or encroaching species) that are habitually avoided. Since bush encroachment can directly and indirectly affect the biodiversity of millions of hectares in African savannas (Ward 2005), these results indicate an opportunity to increase utilization of encroaching species that may reduce biodiversity and come to dominate the savanna landscapes.

Implications for herbivore health, nutrition and environment

The benefits of ameliorating the negative effects of condensed tannins with supplemental nutrients and PEG are not only limited to those measured in this thesis namely; improved growth performance (i.e., body weight gain, reduced faecal excretion, and improve circulatory nutrients) as presented in chapter four. The main message that is being communicated by supplemental nutrient and PEG results from chapter two to five is that increased shrub consumption will increases tannin intake (see chapters three to five). Although, tannins were historically thought by agriculturalists and ecologists alike only to adversely affect the nutrition and health of herbivores, they are increasingly being recognised as important compounds in health and nutrition. Countless studies have shown herbivores to eat plant that are high in tannins as a way to reduce internal parasite burden (Min et al. 2003, Provenza and Villalba 2010, Villalba et al. 2013). Sheep with parasite burden have been reported to manifest greater pretences for tannin-rich forages than non-parasitized sheep, yet lost preference when the infection was terminated (Lisonbee et al. 2009a). Furthermore, condensed tannins alleviate bloating by binding to proteins in the rumen (Waghorn 1991). Specifically, condensed tannins reduce microbial activities, polysaccharide slime and gas production in the rumen in ways that potentially reduce the incidence of bloating (Min et al. 2005b). A study in which steers were grazing on bloat-inducing rangelands were given a daily tannin dose, found that tannins improved animal performance and minimized frequency of bloating (Min et al. 2005b). Of ecological importance, another study with sheep showed that animals learn to avoid foods that are associated with rumen distension (i.e., bloat) and develop preferences for forages eaten during relief from distension (Villalba et al. 2009). This suggests that herbivores can and will self-medicate for bloat and internal parasite burden when given the opportunity. More research is needed to investigate the medicinal use of tannins and other plant secondary metabolites in forages of herbivores. Implications of this knowledge for managing livestock and game and rangelands in savanna systems also need further investigation.

Nutritionally, condensed tannins render proteins unavailable for digestion and absorption until they reach a more acidic abomasum, which enhances nutrition by providing high-quality protein to the small intestines (Min et al. 2003). This high-quality bypass protein is known to enhance immune responses and increase resistance to gastrointestinal nematodes (Min et al. 2005a).

Lastly, the world population of (at least) domesticated ruminants is thought to be responsible for more than 15 % of all methane emissions due to anaerobic enteric fermentation of feed (Bodas et al. 2012). This constitutes a major criticism for the modern ruminant production. This is because the impacts of greenhouse gases on climate change and on natural resources are huge and dire. Dietary tannins may be a natural way to reduce methane emissions in ruminants (Pinares-Patino et al. 2003). Although research is still needed to identify the mode of action of tannins in decreasing methanogenesis, as well as to characterize the effects of different levels of condensed tannins to develop practical means of exploitation, there have been reports of lower methane emissions by ruminants consuming tannin-rich forages (Puchala et al. 2005, Animut et al. 2008). So, given all these benefits associated with increased consumption of tannins, the research reported in this thesis should be viewed as a contribution not only from a nutritional ecology perspective, but also from animal health, and environmental points of view.

In conclusion, I have shown that condensed tannins limit growth performance of free-ranging mixed feeders, possibly via reducing the amount of time animals spend browsing and through inducing alterations of bite rates, bout numbers and lengths, number of dietary species and diet composition. I also showed that when dosed with PEG or supplemented with either an energy or protein source, animals are able to adjust their feeding behaviour in ways that are likely to improve their growth performance. This study indicates a potential to improve herbivore productivity in rangelands that are degraded by bush encroachment. It also presents ways in which defoliation of encroaching woody species could be increased. I propose that future research direction be to determine the extent to which the shift from a grass-based to browse-based diet could contribute towards using goats and other mixed feeders as agents for bush control.

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Summary (English)

Savanna ecosystems are important for a wide range of activities such as livestock grazing, tourism, game ranching and nature conservation. Although savannas are usually characterised by a harmonious coexistence of grasses and trees, tree densities are increasing at the expense of grasses, and this trend is predicted to continue due to global warming and other factors. Woody plants are an important source of nutrition for many browsers and mixed feeders in savannas. However, woody plants possess a wide variety of chemical, and other properties that reduce forage value and serve as foraging deterrents to herbivores. While foraging to meet their nutrient requirements, herbivores have to contend with the negative effects of plant secondary metabolites. In African savannas, condensed tannins are among the primary determinants of leaf palatability for browsing herbivores; hence tannin-rich leaves are usually unattractive for most herbivores.

Current understanding of how these chemicals influence the interactions between woody plants and large herbivores is still based on data from either short-term laboratory experiments, mostly with confined animals or few correlative field studies that only explore relationships between tannin concentrations of plants with their intake. Controlled experiments have shown condensed tannins to act both as digestibility reducers inhibiting the foraging value of browse, and as feeding deterrents, affecting intake, feeding behaviour and diet choice. Data from captive animals indicate that particular nutrients can help herbivores cope with the adverse effects of specific toxins and tannins. Moreover, supplementing herbivores foraging on tannin-rich systems with polyethylene glycol (PEG) can alleviate the adverse effects of tannins and enhance woody plant use. Although these experiments are a necessary first step in isolating and characterising the effects of condensed tannins, they oversimplify the complex interactions that occur between wild herbivores or livestock and plants. The challenge for research is to translate the roles of tannins in plant-herbivore interactions from controlled experiments to field conditions.

The overall aims of this research were:

1. To investigate how condensed tannins influence foraging behaviour and growth performance of free-ranging ruminant herbivores, and
2. To determine the effects of supplements on use of woody plants and intake rates of condensed tannins by free-ranging herbivores in a semi-arid savanna.

The specific questions that I addressed in this thesis were:

1. Do free-ranging mixed feeders seasonally change their foraging time expenditure in response to dietary condensed tannins?

2. How do herbivores seasonally alter their feeding behaviour (i.e., bite rates, feeding bout length, feeding bout number, number of dietary forages and diet composition) in response to differences in condensed tannin concentration?
3. Do mixed feeders seasonally regulate intake rates of condensed tannins in the field?
4. Do condensed tannins suppress growth performance, increase faecal nitrogen excretion and/or influence blood circulatory nutrients of mixed feeders under natural conditions?
5. Does nutrient supplementation increase the ability of herbivores to consume chemically defended plants?

To address these questions, I conducted field experiments and collected data on goats as models for all mixed feeders that share similar characteristics with goats. I experimentally increased the level of condensed tannins to which study animals were exposed in the field by orally dosing 15 goats with 20g condensed tannin powder extracted from a bark of tannin-rich species. I dosed another 15 goats with 20 g of PEG in an attempt to neutralize tannin effects, and another group of 15 goats was dosed only with water and served as a control group. I also compared feeding behaviour of goats supplemented with a protein-rich source, an energy-rich source, and goats that were not supplemented to address the last of my research questions.

The results obtained supported my predictions that mixed feeders exposed to high levels of condensed tannins spent more time grazing and less time browsing compared to animals with low tannin exposure. However, the findings did not support my expectation for tannins to reduce overall foraging time. Therefore, I concluded that condensed tannins do not necessarily suppress foraging, but only influences the amount of time animals spend foraging on either herbaceous or woody forage. These findings also supported my hypothesis that herbivores forage in ways that minimize their intake rate of condensed tannins. Furthermore, animals altered their foraging behaviour depending on the treatment groups they were allocated to, and compiled diets that indicated tannin minimization as a goal. Moreover, I also found support for the notion that condensed tannins are digestibility reducers. It was clear that free-ranging animals are able to employ their behavioural adaptations to chemical defences in ways that mitigate the negative physiological effects on their presumed ultimate fitness. I also presented possible effects of nutrient-tannin/toxin interactions on herbivores in African savannas. In the supplementation experiment, proteins and energy equally increased browse consumption by herbivores, with a concomitant increase in tannin intake rates.

I explain these results in light of the ongoing bush encroachment in the African savannas. The expected increase in the availability of browse will probably impose a selection pressure for herbivores that can better utilise the encroaching woody plants known to be endowed with tannins and other carbon-based secondary metabolites. In my synthesis, I draw inference from these results and generalise about the health, nutritional, and environmental benefits that are possible from managing our rangelands and herbivores in ways that increase utilization of chemically defended plants. For example, increased consumption of tannin-rich forage will not only improve nutrition, but it will also reduce internal parasite burden, and reduce bloating by ruminant herbivores while simultaneously reducing the methane emissions that lead to global warming.

Samenvatting (Dutch)

Savanne ecosystemen zijn belangrijk voor een groot aantal activiteiten zoals, begrazing met vee, toerisme, het fokken van wild en natuurbeheer. Savannes worden meestal gekarakteriseerd door een harmonieuze samenleving van grassen en bomen, maar de dichtheid aan bomen neemt momenteel toe ten koste van de grassen, waarschijnlijk als gevolg van klimaatverandering en andere factoren. Houtige planten zijn een belangrijke bron van nutriënten voor herbivoren op savannes die ook bladeren eten van bomen en struiken, waardoor ze onder andere een grote variatie aan chemische afweermechanismes hebben ontwikkeld om hun voedingswaarde te verlagen en om zichzelf minder aantrekkelijk te maken voor deze herbivoren. Hierdoor moeten herbivoren tijdens het foerageren rekening houden met de negatieve gevolgen van plantaardige secundaire metabolieten. Op de Afrikaanse savannes wordt de verteerbaarheid van bladeren voor een groot deel bepaald door gecondenseerde tannines, met als gevolg dat tannine-rijke bladeren vaak worden vermeden door herbivoren.

Onze huidige kennis over de rol van chemicaliën bij de interactie tussen houtige planten en grote herbivoren is gebaseerd op korte laboratoriumexperimenten met dieren met een beperkte bewegingsvrijheid en een paar correlatieve veldstudies die alleen hebben gekeken naar de relatie tussen de tannine-concentraties van planten en hoeveel ze gegeten werden. Uit gecontroleerde experimenten is gebleken dat gecondenseerde tannines de verteerbaarheid van voedsel reduceren waardoor ze de voedingswaarde voor herbivoren verlagen, ook maken ze voedsel minder aantrekkelijk, waardoor ze de inname, het foerageergedrag en de dieetkeuze van herbivoren beïnvloeden. Experimenten met dieren in gevangenschap laten zien dat bepaalde nutriënten ervoor kunnen zorgen dat herbivoren minder last hebben van de effecten van gifstoffen en tannines in hun voedsel. Daarnaast is gebleken dat het voeren van polyethyleenglycol (PEG) aan herbivoren die foerageren in een tannine-rijke omgeving, de negatieve effecten van tannines vermindert en ervoor zorgt dat deze dieren meer houtige planten gaan eten. Hoewel deze experimenten nodig zijn als eerste stap in het begrijpen van het effect van gecondenseerde tannines, simplificeren ze de complexe interacties tussen wilde herbivoren of vee en planten. De uitdaging voor onderzoek is dan ook om de rol van tannines in plant-herbivoor interacties te vertalen van gecontroleerde experimenten naar veldomstandigheden.

Het doel van mijn onderzoek was:

1. Om een beter beeld te krijgen van de invloed van gecondenseerde tannines op het foerageergedrag en de groei van loslopende herkauwers, en
2. Om beter te begrijpen wat het effect is van het voeren van voedingssupplementen op het eten van houtige planten en de inname van gecondenseerde tannines van loslopende herkauwers in een halfdroge savanne.

De specifieke vragen die ik in deze thesis behandel zijn:

1. Veranderen loslopende geiten hun foerageertijdsbesteding als reactie op gecondenseerde tannines in hun dieet afhankelijk van het seizoen?
2. Hoe veranderen geiten hun foerageergedrag (bijv. bijtsnelheid, lengte van foerageerrondes, aantal foerageerrondes, aantal voedselcomponenten en dieetcompositie) als reactie op verschillen in gecondenseerde tannine-concentraties in hun voedsel afhankelijk van het seizoen?
3. Reguleren geiten hun inname van gecondenseerde tannines in het veld afhankelijk van het seizoen?
4. Onderdrukken gecondenseerde tannines de groei, stimuleren ze de stikstof uitscheiding via feces en/of beïnvloeden ze de nutriënten in het bloed van geiten onder natuurlijke omstandigheden?
5. Kunnen herbivoren makkelijker chemisch beschermde planten eten na supplementatie met nutriënten?

Om deze vragen te beantwoorden heb ik veldexperimenten uitgevoerd en metingen gedaan aan geiten, als model voor herbivoren die bladeren eten van bomen en struiken. Ik heb de hoeveelheid gecondenseerde tannines waaraan dieren in het veld werden blootgesteld experimenteel verhoogd door 15 geiten een orale dosis van 20g gecondenseerde tanninepoeder, onttrokken uit de schors van een tanninerijke plantensoort, te geven. Ik heb 15 andere geiten een dosis van 20g PEG gegeven in een poging om tannine-effecten te neutraliseren en daarnaast heb ik 15 geiten een dosis met alleen water gegeven, zodat ze konden fungeren als controle groep. Voor mijn laatste onderzoeksvraag heb ik geiten voedingssupplementen gegeven, een eiwitrijk supplement, een energierijk supplement of water als controle, en daarna hun foerageergedrag vergeleken.

De resultaten van hoofdstuk 2 ondersteunen mijn voorspelling dat geiten die worden blootgesteld aan hoge gecondenseerde tannine-concentraties meer tijd spenderen met gras eten, en minder met het eten van bladeren, takken en schors, dan dieren die worden blootgesteld aan lage tannine-concentraties. In tegenstelling tot mijn verwachting verlaagden hoge tannine-concentraties de foerageertijd echter niet. Daarom concludeer ik dat gecondenseerde tannines niet perse de foerageertijd veranderen, maar wel de tijd die dieren spenderen aan het foerageren op gras of houtige planten. Deze bevindingen ondersteunen ook mijn hypothese dat herbivoren tijdens het foerageren proberen om hun inname van gecondenseerde tannines te minimaliseren. Daarnaast veranderden dieren hun foerageergedrag afhankelijk van de groep waarin ze waren ingedeeld, waarbij hun dieet suggereerde dat ze het minimaliseren van gecondenseerde tannines nastreefden. Ook vond ik aanwijzingen dat gecondenseerde tannines de verteerbaarheid van voedsel reduceren. Het was duidelijk dat loslopende dieren negatieve fysiologische effecten van chemische verdediging van planten verzachten door aanpassingen in hun gedrag. Ik presenteer ook mogelijke effecten van nutriënt-tannine/gifstof interacties op herbivoren op de Afrikaanse savannes. Zowel de toevoeging van eiwitten als energierijke supplementen zorgden voor een toename in de opname van houtige planten door herbivoren, met een toename van tannine inname als gevolg.

Ik probeer deze resultaten te duiden in het kader van de aanhoudende toename aan bomen op de Afrikaanse savannes. De verwachte toename aan beschikbaar voedsel bestaande uit houtige planten zal waarschijnlijk een selectie druk uitoefenen op herbivoren die dit voedsel, rijk aan tannines en andere koolstof houdende secundaire metabolieten, beter kunnen benutten. In mijn synthese trek ik conclusies uit mijn resultaten en ga ik in op de mogelijke voordelen voor gezondheid, voeding, en milieu, die voort kunnen komen uit een ander beheer van (semi-)natuurlijke weidegebieden en herbivoren, op een manier waarbij het gebruik van zich chemisch verdedigende planten wordt verhoogd. Zo zal het verhogen van inname van tannine-rijk voedsel niet alleen zorgen voor een betere voeding van herbivoren, maar zal het bijvoorbeeld ook zorgen voor een verlaging van endoparasieten en een verlaging van dyspepsie en methaanuitstoot van herkauwers, gekoppeld aan klimaatverandering.

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Acknowledgements

Embarking on this PhD was not only a journey to becoming an independent scientist, but it also marked the beginning of a continuing journey towards finding myself. At this stage, I want to pause and acknowledge the debt of gratitude I owe to the many selfless and talented individuals who contributed enormously towards this accomplishment. Someone once said "look at a man the way he is, he only becomes worse. But look at him as if he was what he could be, and then he becomes what he should be". That's exactly what my supervisors: Prof. Herbert Prins, Dr Fred de Boer, Dr Ignas Heitkönig, Prof. Peter Scogings and Dr Luthando Dziba did to me. They looked at me beyond my faults and saw my needs.

Thanks Peter for the ideas you shared and for always showing interest in my development. Peter has always expressed belief and trust in me even when I (myself) did not believe in my own ability to succeed. It was Peter who introduced me to Fred, a person who epitomises everything that has ever been sought for by any student and everything that has ever been written in any book about good student supervision. Thanks Fred for the speed with which you gave feedback, the thoroughness in your comments, the honesty in your constructive criticisms and the openness in allowing me to scrutinise your advice and independently decide where I wanted to take the project. Fred introduced me to Ignas who was always willing to offer guidance, wisdom and a bit of humour throughout the journey. Many times when I felt weak or scared, Ignas would somehow see me through and calm me down. When I slowed, Ignas energized me. His critical thinking made me feel very confident about my work every time he offered a green light on a chapter. When I occasionally veered from the path or made a wrong turn, both Ignas and Fred would patiently sit me down and pull me back on track. I will forever be grateful to Luthando for being such a good mentor throughout my career. His advice during this PhD has always been intelligent and surely made me a better scientist. Thanks Luthando also for the coaching you offered as I was applying for the NRF grant.

I consider myself among the most fortunate students to have gotten a chance to interact with and learn from a professor as resourceful and influential as Herbert Prins. Herbert taught me the value of keeping in mind the bigger picture while not compromising the technical integrity of the finer details. His lessons did not only shape my thinking about science, but also made me more confident (although I know he still wishes I was more assertive). Thanks Herbert for accepting and embracing me as your pupil.

Another important reason I consider myself to have been extremely privileged as a PhD student is the fact that I got a rare opportunity to mingle with the Resource Ecology Group (REG) staff and students. I am deeply indebted to Frank, Milena, David, Ron, Sip, Pim, Patrick, Gerda, Patricia, Herman and Anne-Marie for always keeping their doors open every time I needed help. I thank Joke Jansen and Marion Rodenburg for making sure that I didn't have to wait for Patricia or Gerda (in their absence) in order to get answers on any administrative issue.

Claudius and his friendly colleagues at PE&RC did a fantastic job in boosting my confidence via those PhD weekends, parties and workshops they organised magnificently. It was empowering to discuss the potential PhD challenges with other PhD candidates (Claudius did not like us using the word “students”). The courses you organised surely improved both my hard and soft skills immensely. But, you guys still need to explain to me what you really meant by “T-shaped” skills☺.

Fellow PhD students and post-doc fellows made my stay in the Netherlands feel like a home away from home. During my first visit in August 2010, I benefited from the talents of Henjo, Ralf, Edson, Benson, Rudy, Vincent, Daniel, Robert, Dorit and Abel Ramoelo. I thank Yong, Tessema, and Zheng for helping with my registration, securing of a bike, knowing of my way around Wageningen and for being good office mates. When proposal writing gave me sleepless nights, it was comforting to know that Anil, Audrie, Edwardo, Jasper, Kyle, Xavier and Lennart understood my frustrations and were always willing to extend their help. My second visit to the Netherlands introduced me to new wonderful REG family members who offered all the technical and emotional support I needed. My sincere gratitude goes to Helen, Martijn, Mikhail, Sintayehu, Tsewang, Yussuf, Yorick, Tibor, Bernardo, Rob and Milene. Thanks Iris for always taking the trouble of getting us together to discuss pertinent PhD career and development issues. Thanks Jente and Tim for your interest in my work and for your friendship. I salute Joost for organising all those bike trips, bush walks, movies and dinners which saved me from a potential home sickness. Although not a REG members, Lu Huicui and colleagues from other research groups are thanked for their friendship. Yin was the last person I met before leaving Holland, but the relationship we forged will hopefully last for a very long time.

The families of my friends and colleagues generously opened their homes and hearts to me while in the Netherlands. This gave me a closer look at the Dutch culture and the cultures of all those non-Dutch families I got exposed to. I thank Fred’s wife and their two daughters, Ignas’ wife and son, Lennart’s wife (Marijke) and son (Kai), Audrie and his wife, Bram van der Braak and Anne, Dick van der Wal and his wife Maaike, Maiga Fernades and her husband, Carolien and Dorian. Those dinners, bible classes, swimming lessons, and piano lessons added value to my PhD experience.

I got a chance to work with Dr Dawood Hattas and his colleagues at the University of Cape Town on condensed tannin analysis using the facilities of the Department of Biological Sciences. Here I record my sincere appreciation to Dawood for the patience, accuracy and enthusiasm with which he taught the laboratory procedures to me and my colleagues. I am grateful to his family for the hospitality they showed us every time we visited Cape Town. I also collaborated with Dr Dibungi Luseba, Mr Scelo Dludla and Prof. Khanyisile Mbatha through student supervision. I learnt some invaluable lessons from you about the value and ethic of student supervision and mentorship.

I shared this PhD journey with very kind friends; Kim Hamunyela (my mentor), Melvin, Precious, Collins, Catherine, Fine, Valerie, Koketso, Lihle, Kuna, Lina, Judy, Rita, Promise, Gilbert, Marble, Nelson, Bongani, Vusi and many others. Thanks guys for the debates, trips and dinner parties we enjoyed together.

I enjoyed immeasurable technical and logistical assistance from my colleagues at ARC-Irene. Thanks a lot to Julius (my partner in crime), Sikhalazo (my boss), Glibert, Mike, Flip, Lilian, Malenyalo, Dan (my other boss), Florence, Claude, Roger (Rest in Peace), Leone Kruger, Lucky Mohale, Douglas Nkosi, Douglas Tester, Motsamai, Anza, Arnold, Mr Sewnath, Sonyboy, Matume Maonatlala, Eric Mathisa, James Joubert and his team from Farm Section. I respectfully extend my thanks to the ARC-API management, colleagues from Nutrition and Rangelands building, staff members of finance, Human Resources and Analytical Services for being very supportive to me in the past five years. I know that saying "THANK YOU" will not be enough given the amount of help I received from Jan Manganye and his hard working team. I also thank Dannie, Dinah, Marike, Marcia and Gerrie for always helping out when I ran into trouble with the goats. Ms Johanna Mnisi generously shared her work space with my field assistants.

This PhD gave me an opportunity to interact with a group of very talented Diploma, B-Tech, BSc and MSc students to whom I owe a considerable debt of gratitude. I thank Dorian and Carolien (*tough cookie*) for setting the ball rolling in the field. Many thanks go to Sikelela, Veronica, Thobekile, Xolani, Jacobeth, Kate, Marvin, Tebogo, Thabo, Alfred, Asanda, Bob, Malebo and Boipelo for the hard times they endured while running my experiments in Roodeplaat. It will be difficult to express how I appreciate the assistance received from Peit Monegi and Lucas Letsoalo throughout this PhD project. They took complete ownership of the project and gave everything they had to making sure that we succeed. I hope that they have learnt from me as much as I did from them. This thesis is as much theirs as it is mine!!!

Lastly but definitely not the least, I thank my family for the moral support. When I was 10, my mom (Xolisile Mkhize) would jokingly say I was too weak for any physically challenging job. She would then advise that I work hard at school and become a doctor (which according to her was not physically taxing). However, later when she realised that I was too scared of blood, she would ask: "what kind of a doctor WILL you be if you are so scared of blood?" Well mom, I hope that I have responded to your question. Hopefully, I have made you proud. Thank you for always being the wind beneath my wings!! I appreciate Khetha, Asanda, Aphelele, Sania and Leeto for the understanding and patience without which I would not have been able to finish this work. I hope you will find this book appropriate as a token of my sincere apology for all the time of separation, the frustrations and sacrifices that you went through during the process of completing this study.

Curriculum Vitae

Ntuthuko Mkhize was born and raised in a township called Ntuzuma in Durban, South Africa on 04 October 1981. He started schooling in 1989 and received primary education from Phikiswayo Primary School until 1993 and Thobile Senior Primary School until 1996. In 2001, he received a Senior Certificate (known as Matric in South Africa) with distinctions in mathematics and biology from Mandlenkosi High School. Starting from the age of 15, Ntuthuko would do some gardening jobs during school holidays and weekends to help his unemployed parents pay for his school fees. Given that his parents (who had been unemployed since he was a kid) relied on short-term gardening and washing jobs to feed a family of five, it was impossible for them to afford Ntuthuko any university education. However, his matric results afforded him the only break he ever needed. In 2002 he earned a bursary which saw him register with the University of Zululand and completing a four-year BSc degree in Agriculture (Animal Science) in 2005. In 2006 Ntuthuko received a National Research Foundation's Scarce Skills Scholarship which helped him complete a BSc Honours in Agriculture (Animal Production) in the same year, from the same university. While finishing his Honours degree, Ntuthuko got permanently employment as a researcher in Animal Science by the KwaZulu Natal Department of Agriculture and Environmental Affairs (KZN-DAEA) in July 2006. In 2007 he registered a master's degree in animal production with the University of Zululand and had to juggle his studies and a full-time job until February 2008 when he resigned from the KZN-DAEA. In March 2008, Ntuthuko joined the Professional Development Programme of Agricultural Research Council (ARC) as a candidate researcher. This position allowed him to focus solely on his MSc studies which he completed in the same year with a first class mark and an award for best presentation of an MSc thesis in the Faculty of Science and Agriculture. In May 2010, Ntuthuko got employed by the ARC as a Researcher in Rangelands Management, a position he still holds. Just three months later, he won a PhD sandwich fellowship from Wageningen University, the Netherland and has since been juggling his studies and a job until 2015 when he successfully completed this PhD thesis. Ntuthuko's motto in life is: "the ONLY limit to the height of our achievement is the reach of our dreams and our willingness to work hard for them".

Publications

Mkhize N.R., Heitkönig I.M.A., Scogings P.F., Dziba L.E., Prins H.H.T. and de Boer W.F. (accepted). Condensed tannins reduce browsing and increase grazing time of free-ranging goats in semi-arid savannas. *Applied Animal Behaviour Science*.

Mkhize N.R., Scogings P.F., Nsahlai I.V. and Dziba L.E. (2014) Diet selection of goats depends on season: roles of plant physical and chemical traits, *African Journal of Range & Forage Science*, 31:3, 209-214, DOI: 10.2989/10220119.2014.901417.

Mkhize N.R., Scogings P.F., Dziba L.E. and Nsahlai I.V. (2011) Season and plant species influence foraging efficiency of Nguni goats in pens. *African Journal of Range & Forage Science*: 28(1): 29–34.

PE&RC Training and Education Statement

With the training and education activities listed below the PhD candidate has complied with the requirements set by the C.T. de Wit Graduate School for Production Ecology and Resource Conservation (PE&RC) which comprises of a minimum total of 32 ECTS (= 22 weeks of activities)

Writing of project proposal (4.5 ECTS)

- Plant secondary metabolites and goat productivity: implications for bush control in African Savannas

Post-graduate courses (5 ECTS)

- Introduction to R for statistical analysis; PE&RC (2013)
- Linear models; PE&RC (2014)
- Generalized linear models; PE&RC (2014)
- Mixed models; PE&RC (2014)
- Consumer-resource interactions; PE&RC (2014)

Laboratory training and working visits (2 ECTS)

- Condensed tannin analysis using acid-butanol assay protocol; University of Cape Town-Department of Biological Sciences, South Africa (2013)

Invited review of (unpublished) journal manuscript (2 ECTS)

- African Journal of Range and Forage Science (AJRFS): bush encroachment (2012)
- AJRFS: rangeland productivity (2013)

Deficiency, refresh, brush-up courses (3 ECTS)

- Ecological methods (2010)
- A 9-month certificate programme in introduction geographic information systems; University of Pretoria, South Africa (2010)

Competence strengthening / skills courses (4.5 ECTS)

- Information literacy including endnote introduction; Wageningen Library (2010)
- PhD Competence assessment; Wageningen Library (2011)
- Writing grant proposal; Wageningen in’to Languages (2013)
- PhD Scientific writing; Wageningen in’to Languages (2014)

PE&RC Annual meetings, seminars and the PE&RC weekend (2.2 ECTS)

- PE&RC Weekend; first year (2010)
- PE&RC Weekend; last year (2013)
- PE&RC Mini-symposium: how to write a world class paper (2013)
- WGS Workshop; last stretch of the PhD (2014)

Discussion groups / local seminars / other scientific meetings (4.5 ECTS)

- Ecological Theory and Application Discussion Group (2010-2014)
- Agricultural Research Council-Journal Club (2011-2013)

International symposia, workshops and conferences (9 ECTS)

- Grassland Society of Southern Africa (GSSA) annual congress (2011)
- Grassland Society of Southern Africa (GSSA) annual congress (2012)
- Grassland Society of Southern Africa (GSSA) annual congress (2013)
- Netherlands Annual Ecology Meeting (NAEM) (2014)

Supervision of MSc students (6 ECTS)

- T.J.P. van Dalen: bite sizes of goats in relation to condensed tannins and other plant characteristics (2012)
- C.H. Kooiman: effects of chemical and structural defence mechanisms on feeding behaviour of free-ranging goats in African Savannas (2012)

The research described in this thesis was financially supported by the **National Research Foundation** via a Thuthuka grant numbered 80538 and by the **C.T. de Wit Graduate School for Production Ecology and Resource Conservation** through a PhD sandwich grant numbered PE&RC 10054.

Cover design: **Elsa van Niekerk**
Photos used in the cover were taken by Peter Scogings, Luthando Dziba and Flip Breytenbach
Printed by GVO Drukkers & Vormgevers B.V.