

The Effect of Condensed Tannins on Goats' Body Weight

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IN

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The Effect of Condensed Tannins on Goats' Body Weight

A thesis

BY

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DECLARATION

I (**Sicelo Phumlani Dladla**) hereby declare that this thesis is entirely my original work with the exception of such references and quotations that have been attributed to their authors or sources. This thesis has never been submitted for any degree of examination in any university.

Candidate's signature:

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Co-supervisor:

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DEDICATION

I dedicate this thesis to the African child who, with all his/her hard work, dedication and brains but could not afford the university education because of poverty and lack of funds. This is how we define life, as being unfair, however, with patience and dedication the future is everyone's for the taking.

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SUMMARY

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Tannins are one of the most common plant secondary compounds. Amongst the tannins are condensed tannins (CT), which occur nearly in every plant species browsed by goats. Condensed tannins have a potential to bind proteins, reduce the feed intake and consequently reduce the body weight gain of goats. The study was conducted in order to determine the effects of different condensed tannin concentrations on the body weight gain of goats.

The first experiment was conducted for three weeks in order to determine the effect of different condensed tannin concentrations in browse species. To eliminate the large chemical and physical variation in browse species and to have more control in feeding, the second experiment with artificial diets was conducted for four weeks. In each experiment, twenty-four goats were fed four different levels of condensed tannin-containing diets with six goats in each diet group.

In the first experiment, a marginal negative relationship between condensed tannin concentrations and the body weight gain of goats was observed. There was no significant relationship between condensed tannin concentration and the browse intake, pellet intake and lucerne intake, protein intake, protein retention and protein loss by goats.

In the second experiment, a significant negative effect of different CT concentrations on the body weight gain, pellet intake of goats as well as on the protein intake, faecal protein loss and protein retention of goats was observed. Protein retention and faecal protein loss were all affected by CT concentrations and yet did not affect growth. However, feed intake was affected by CT concentrations and affected growth.

Studying the effects of condensed tannins using synthetic condensed tannins in the artificial diets is more informative than using condensed tannins in browse species. However, even condensed tannins in the artificial diets cannot explain the mechanisms which affect the body weight gain after consuming condensed tannin rich diets.

TABLE OF CONTENTS

DECLARATION	II
DEDICATION	III
ACKNOWLEDGEMENTS	IV
SUMMARY	VI
TABLE OF CONTENTS	VIII
LIST OF FIGURES	X
LIST OF TABLES	XII
APPENDICES	XII
CHAPTER 1	1
GENERAL INTRODUCTION	1
Background	1
Literature review	2
Aims and outline of the thesis	5
CHAPTER 2	7
THE RESPONSE OF BODY WEIGHT GAIN OF GOATS FED CONDENSED TANNIN-POOR AND CONDENSED TANNIN-RICH BROWSE	7
ABSTRACT	7
INTRODUCTION	9
MATERIALS AND METHODS	13
Study Area.....	13
Experimental Design	13
Chemical Analysis.....	15
Statistical Analysis	15
RESULTS	17
Response of body weight gain to condensed tannin concentrations	17
Response of feed intake to condensed tannin concentration.....	18
Response of protein intake, protein loss and protein retention to condensed tannin concentration	21
DISCUSSION	24
Response of body weight gain to condensed tannin concentrations	24
Response of feed intake to condensed tannin concentration.....	26
Response of protein intake, protein loss and protein retention to condensed tannin concentration	27
CONCLUSIONS.....	28
CHAPTER 3	29

THE EFFECT OF DIFFERENT CONDENSED TANNIN CONCENTRATIONS ON THE BODY	
WEIGHT GAIN OF GOATS	29
ABSTRACT	29
INTRODUCTION	30
MATERIALS AND METHODS	33
Study Area.....	33
Experimental Design.....	33
Chemical analysis.....	34
Statistical Analysis	34
RESULTS	35
Effect of condensed tannin concentrations on body weight gain.....	35
Effect of condensed tannin concentration on feed intake	37
Effect of condensed tannin concentration on protein intake, protein loss and protein retention by goats	39
DISCUSSION	42
Effect of condensed tannin concentrations on body weight gain.....	42
Effect of condensed tannin concentration on feed intake	43
Effect of condensed tannin concentration on protein intake, protein loss and protein retention by goats	44
CONCLUSIONS.....	45
CHAPTER 4.....	47
GENERAL CONCLUSIONS.....	47
Objectives of the study	47
Results and interpretation.....	48
Conclusions and future research	50
REFERENCES	52

LIST OF FIGURES

Figure	Page
1. Relationship between average body weight gain and condensed tannin concentration in goats (n=6 per diet) fed four browse species containing different amounts of tannin for three weeks	18
2. Relationship between the average browse intake and condensed tannin concentration in goats (n=6 per diet) fed four browse containing different amounts of tannin for three weeks	19
3. Relationship between the average pellet intake and condensed tannin concentration in goats (n=6 per diet) fed four browse containing different amounts of tannin for three weeks	20
4. Relationship between the average luscene intake and condensed tannin concentration in goats (n=6 per diet) fed four browse containing different amounts of tannin for three weeks	21
5. Relationship between the total protein intake by goats and condensed tannin concentration in goats (n=6 per diet) fed four browse containing different amounts of tannin for three weeks	22
6. Relationship between protein loss in faeces of goats and condensed tannin concentration in goats (n=6 per diet) fed four browse containing different amounts of tannin for three weeks	23

7. Relationship between net protein retention by goats and condensed tannin concentration in goats (n=6 per diet) fed four browse containing different amounts of tannin for three weeks 24
8. The effect of four different condensed tannin concentrations on the average body weight gain of goats (n=6 per diet) over the duration of four weeks 38
- 9 The effect of four different condensed tannin concentrations on the pellet intake of goats (n=6 per diet) 39
10. The effect of four different condensed tannin concentrations on the lucerne intake by goats (n=6 per diet) 40
11. The effect of four different condensed tannin concentrations on the total protein intake by goats (n=6 per diet) 41
12. The effect of four different condensed tannin concentrations on the protein faecal loss by goats (n=6 per diet) 42
13. The effect of four different condensed tannin concentrations on the protein retention by goats (n=6 per diet) 43

LIST OF TABLES

Table	Page
1. Chemical composition of the different dietary compounds	17
2. Chemical composition of the different dietary compounds	37

APPENDICES

Appendix	Page
1. Appendix 1	A

CHAPTER 1

General introduction

Background

Plants consumed by herbivores contain nutrients to meet animals' nutrient requirements, but they also contain chemical defences to discourage herbivory such as plant secondary compounds (PSCs). Plant secondary compounds are compounds that are not involved in the plant primary metabolism, thus they are produced by the plant but not used by the plant for its functions such as production and growth. However, PSCs can inhibit the growth of microbes and fungi and they are often referred to as defences (Forbey *et al.* 2009).

All woody species contain potential toxic or digestibility reducing PSCs, including tannins (Robbins *et al.* 1987; Baraza *et al.* 2009). Tannins are complex polyphenolic compounds with the molecular weights in range of 500-20 000 Daltons (Cheeke 1998; Nackz *et al.* 2001). The term "tannins" encompasses diverse oligomers and polymers. Tannins are widely distributed throughout the plant kingdom, especially in trees, shrubs and herbaceous leguminous plants (Cheeke 1998; Frutos *et al.* 2004; Nackz *et al.* 2001). These tropical trees and shrubs are important sources of proteins for goats. However, the limitation of trees and shrubs as a source of food is due to the widespread of tannins and other PSCs. This is more severe in tropical areas because trees contain high levels of PSCs such as tannins compared to temperate areas (Alonso-Diaz *et al.* 2010).

The use of tanniferous feeds will increase as the demand for foods such as maize and soyabean, traditionally used to supplement ruminants, increases with the human population increases. The competition for the same food resources between humans and ruminants will mean that the use of artificial diets as food for goats may cease as they become expensive because of the increasing demand. Therefore, there is a need to study more about the tanniferous feeds and their effects to ruminants especially goats in order to find ways to mitigate the effects of tannins and improve the nutritional value of various browse species.

Literature review

Tannins are divided into hydrolysable tannins (HT) and condensed tannins (CT). However, other tannins in plants occur as combinations of both CT and HT (McSweeney *et al.* 2001). Condensed tannins are also known as proanthocyanidins and are the most common type of tannins found in trees especially in legumes (Huang *et al.* 2010). Thus, CT are in feedstuffs such as fodder legumes and browse plants which are important sources of food for herbivores. The reactivity of CT with the molecules of biological significance has important nutritional and physiological consequences in animals.

Condensed tannins comprise a group of polyhydroxy-flavan-3-ol oligomers and polymers linked by carbon-carbon bonds (Schofield *et al.* 2001). Condensed tannins can have both beneficial and detrimental effects on ruminants. This depends on the vegetation type, nutritional background, experience of the animal and existence of

adaptation mechanisms (Alonso-Diaz *et al.* 2010). Positive effects on ruminants include preventing bloating and anthelmintic effects.

Negative effects of CT include the reduction of voluntary feed intake and digestion of nutrients. It is believed that CT reduce voluntary feed intake (Frutos *et al.* 2004). This reduces the nutritive value of forages. This remains controversial because goats have been observed selecting positively for the digestible material rather than avoiding tannins (Alonso-Diaz *et al.* 2010). Condensed tannins bind with proteins and carbohydrates rendering them undegradable (Frutos *et al.* 2004). Their multiple hydroxyl groups lead to the formation of complexes with proteins and carbohydrates (Schofield *et al.* 2001). The physiological activities of CT are attributed largely to their capacity to bind and precipitate proteins (Dawra *et al.* 1988). Condensed tannins type, form and concentration affect digestion in ruminants (Kariuk and Norton 2008). Condensed tannins affect the digestibility in ruminants by altering the rumen fermentation characteristics, inhibiting the growth of microorganisms and change the intestinal digestibility (McSweeney *et al.* 2001; Frutos *et al.* 2004). Proteins are the good indicators of feed nutritive value (Baraza *et al.* 2009), thus CT reduce the nutritive value of feeds by binding proteins.

Herbivores use both behavioural and physiological adaptation to counteract the effects of PSCs such as tannins (Alonso-Diaz *et al.* 2010). This includes feeding in a manner to avoid or reduce the cumulative intake of PSCs. Some PSCs are able to neutralize each other's effects, thus animals ingest certain compounds together in order to

neutralize each other's effects (Shrader *et al.* 2008). Animals learn to avoid PSCs and limit the ingestion of PSCs within the limits that are physiologically tolerable (Iason and Villalba 2006). Herbivores may consume the small amounts of a variety of plants so that the toxic levels of any single PSC can be avoided (Marsh *et al.* 2006; Forbey *et al.* 2009). Furthermore, herbivores can regulate the time interval between the meals such that feeding will not resume until the concentration of PSCs is reduced from the previous meal (Torregrosa and Dearing 2009).

More research is required on the effect of the differences of the doses, type and sources of CT. The CT of different plant species have different physical, chemical and biological properties (Frutos *et al.* 2004). The relationship between CT and nutritive value of feeds may improve the intake and tolerance to CT (Alonso-Diaz *et al.* 2010). Thus, there is a need to test the effects of CT from the same source. Furthermore, there are mixed results from studies that have investigated the effects of CT on the body weight gain of ruminants (Frutos *et al.* 2004). This calls for a need to study more about the effects of CT on the body weight gain of ruminants, especially goats. Studying the effects of CT is complicated because plants contain a diversity of tannins and other PSCs. In addition to chemical composition, differences in plant morphology can affect intake of tanniniferous feeds by goats. Thus, a prepared artificial diet is necessary to study the effects of CT, which is done in the current study. However, the use of natural browses is also important in order to mimic the foraging in the natural environment which is also done in the current study.

The domestic goats (*Capra hircus*) spend most of their time browsing compared to sheep and cattle. Goats depend on browse (Dziba *et al.* 2003) and herbage produced by wooded rangelands during certain times of the year to meet their nutrients requirements (Papachristou *et al.* 2005). Therefore, goats are more exposed to feeds with CT compared to sheep and cattle and nutrition plays an essential role in goat farming (Morand-Fehr 2005). This calls for more research on the effects of CT on goats. Goats are important herbivores in agricultural production systems throughout the world, contribute to rural economies, nutrition, traditional festivities, and various religious ceremonies and play an important part in daily life especially in developing countries (Boyazoglu *et al.* 2005; Papachristou *et al.* 2005). Goats being important to the society and prominent browsers, this calls for more research on the effects on CT on goats' nutrition and growth. This is important because little is known about the effects of CT on goats' body weight gain and most studies on CT have been done from the *in vitro* and *in vivo* studies with sheep and cattle not goats (Alonso-Diaz *et al.* 2008).

Aims and outline of the thesis

The aim of this study is to determine the effects of different CT concentrations on goats' body weight gain. The study postulates a close relationship between growth, intake and digestion of nutrients especially proteins. Therefore, if CT affect the intake and digestion of proteins this will indirectly affect goats' body weight gain. Natural browses with different CT concentrations are used in the first experiment in order to imitate browsing in the natural environment. However, in the second experiment artificial diets with different CT concentrations are used in order to remove any confounding factors found in browses.

Chapter 2 explains the effect of different condensed tannins concentrations on the body weight gain of goats fed browse species.

Chapter 3 tries to remove many confounding factors found in browse species and to gain more control in feeding by trying to determine the effects of different condensed tannins concentrations in goats' body weight gain using artificial diets.

Chapter 4 is the summary and the interpretations of the whole study. Also suggestions for future studies are made. This chapter tries to interpret the findings of the study while comparing the two experiments in their ability to provide the informative answers and the best experiment is suggested.

CHAPTER 2

The response of body weight gain of goats fed condensed tannin-poor and condensed tannin-rich browse

Abstract

Plant secondary compounds (PSCs) including condensed tannins (CT) occur nearly in every browse species used by goats as a source of food. It is assumed that CTs act as plant's defence mechanism against herbivory. The effects of CT on the body weight gain or loss in goats are not well defined. What are the factors governing either increase or decrease in body weight of goats after consuming CT-rich diets? How does the difference in CT concentrations relate to the body weight? This research hypothesized that goats fed diets low in CT concentration will gain more body weight than those fed diets high in CT concentration. Twenty four goats were randomly assigned to *Acacia caffra*, *Euclea crispa* which both have high CT concentrations, *Rhus lancea* which has moderate CT concentrations and *Ziziphus mucronata* which has low CT concentrations. Goats were fed browse species for three weeks. A marginal negative relationship between CT concentration and body weight gain was observed. There was no significant relationship between CT concentration and browse intake, pellet intake and lucerne intake. There was no significant relationship between CT concentration and protein intake, protein retention and protein loss by goats. It was concluded that different CT concentrations potentially affect body weight gain. It was suggested that significant results may be clearly observed at intervals longer than three weeks. Since CT concentration did not relate to the overall feed intake, it was concluded that factors affecting body weight gain of goats are post-ingestive. In future, different CT concentrations should be from one browse species. More research is required to study

the post-ingestive factors affecting the body weight gain of goats fed different CT concentrations.

Keywords: astringency, feed intake, digestibility, Boer goats, plant secondary compounds

Introduction

Plant secondary compounds (PSCs) occur widely among plant species and they are the most abundant and diverse occurring toxins on earth (Provenza *et al.* 2003; Foley and Moore 2005; Torregrossa and Dearing 2009). Therefore avoiding toxin-containing foods is not an option for herbivores. Phenolic compounds are a major group of PSCs. Polyphenols are found in nearly every species of higher plants. Tannins are a group of polyphenols found in plants. Tannins are defined as a very complex group of plant secondary compounds soluble in polar solution (Silanikove *et al.* 2001). Tannins are divided into condensed tannins and hydrolysable tannins. However, condensed tannins are a major group found in wide range of browse species (Papachristou *et al.* 2005). Condensed tannins are oligomers and polymers of flavan-3-ol units which are linked either via C4-C6 or C4-C4 bonds that are water-soluble (Romani *et al.* 2006). Condensed tannins (CT) are also known as proanthocyanidins (Reed 1995; Waghorn 2008). Condensed tannins protect plants against herbivory, act as plant defence against pathogens and also conserve energy and nitrogen which is mobilised during the times of plants' needs (Waghorn 2008).

The effect of condensed tannins on herbivores is widely recognised. Type and total amount of condensed tannins influence nutritive value in forages (Kariuk and Norton 2008). Tannins affect the diet selection (Shrader *et al.* 2008; Glasser *et al.* 2009) and affect digestibility of other compounds (Papachristou *et al.* 2005; Waghorn 2008) by binding and precipitating dietary proteins (Silanikove *et al.* 2001). Condensed tannins bind not only with dietary proteins but also with salivary proteins, endogenous proteins and gut microbes including microbial enzymes (Waghorn 2008). High faecal nitrogen

relative to nitrogen intake from feed rich in condensed tannins suggests that tannins bind endogenous proteins such as enzyme and microbial proteins (Krebs *et al.* 2003).

The binding effect of condensed tannins to proteins can protect other nutrients such as carbohydrates from digestion by rumen microbes. Condensed tannins not bound to proteins can inhibit the fermentation of structural carbohydrates in the rumen by forming indigestible complexes with the cell wall carbohydrates (Krebs *et al.* 2003). This may partially explain the weight loss and slow growth rate observed in animals fed condensed tannins (Krebs *et al.* 2003). These effects are likely caused by reduced feed intake because of reduced palatability, poor digestibility and reduced absorption of nutrients.

Animals must balance the nutrient rewards offered by particular plants and the negative effects of plant defence chemicals contained within those plants when making decisions about what to eat (Duncan *et al.* 2006). An animal will eat to meet its nutrient requirement while avoiding the accumulation of toxic compounds in the body beyond their physiological threshold (Duncan *et al.* 2006). One strategy for diet selection is “eat the best and leave the rest” (Papachristou *et al.* 2005). However, because of factors such as tannins, animals are usually forced to eat the best first and also eat the rest later.

Torregrossa and Dearing (2009) better summarise the effect of PSCs in diet selection. If an animal encounters food it can choose whether to eat it or not. Sometimes an animal

has no option because of hunger. If an animal does not eat a certain plant, three regulating factors are involved in the decision, which are: (i) the plant is nutritionally unacceptable, (ii) the toxin concentration is too high or (ii) the response is conditioned. An animal will eat food until nutritional requirements are met (nutritional satiety is met) or the toxin physiological threshold is reached. If the physiological threshold is reached animals may either maintain constant PSC intake by decreasing daily food intake, or increase inter-meal-interval until PSCs remain below acceptable threshold levels, which are physiologically safe to resume feeding. For example, hydrolysable tannins can be toxic when large quantities are given to ruminants without sufficient time for rumen microbial adaptation (Waghorn 2008). This implies that when animal's ability to excrete and detoxify PSCs is exceeded, the animal suffers toxicity. A decrease in meal size to minimize the intake of PSCs results in low energy intake (Torregrossa and Dearing 2009). The rate at which toxins are detoxified and excreted from the body, should determine the rate of intake of food containing toxins. The sooner the toxins are eliminated in the body, the better for animals. The rate at which tannins are detoxified has been poorly studied.

The domestic goat (*Capra hircus*) depends on browse and herbage produced mainly in savannas to meet their nutrients requirements (Dziba *et al.* 2003; Papachristou *et al.* 2005). Goats, like many other herbivores, have the ability to detoxify or tolerate consumed toxins (Papachristou *et al.* 2005). Unlike cattle and sheep (Alonso-Diaz *et al.* 2008), goats tend to seek diversity in their diet. This enables them to better meet their nutrient requirements while regulating intake levels of PSCs from different plants (Provenza *et al.* 2003; Morand-Fehr 2005). Furthermore, some PSCs can be

complementary to each other and neutralize each other's effect. Plants having one specific compound can exceed herbivore physiological threshold for that compound if consumed exclusively and therefore reduce intake (Torregrossa and Dearing 2009).

Goats increase their preference for foods associated with positive effects; that is, foods that meet their nutrient requirements. It can be suggested that animals on a low plane of nutrition or experiencing hunger are expected to tolerate the negative effects of PSCs as long as their protein, energy and water requirements are met, because the detoxification process requires these dietary components (Baraza *et al.* 2009). Thus, detoxification uses energy and protein that otherwise would be available for growth and maintenance.

Goats tend to browse more when tannin content is low (Launchbaugh 2001; Papachristou *et al.* 2005) especially in spring when tannin concentration in plants is low. In addition, goats browsing blackbrush prefer previous year's growth to current year growth, because of lower tannin content in previous year's growth (Launchbaugh 2001). Shrader *et al.* (2008) observed that tannins in goat diets increased feed left-overs by 10%. However, as goats try to minimize intake of tannin rich diets weight gain is negatively affected. Therefore, growth, intake and metabolism cannot be studied as single factors.

This study aimed to establish how condensed tannins concentration in four browse species relate to body weight gain. The following hypotheses were tested. Firstly, it was

hypothesized that goats given browse species low in condensed tannins will have higher intake than those given browse species high in condensed tannins. Secondly, it was hypothesized that goats given browse species low in condensed tannins will excrete less protein because of low binding capacity. It was predicted that goats fed browse species low in condensed tannins will gain more body weight than those fed species high in condensed tannins.

Materials and Methods

Study Area

The study was conducted at the ARC's Roodeplaat Experiment Farm, in Pretoria, South Africa. Roodeplaat is situated approximately 30 km north east of Pretoria (Panagos *et al.* 1998), 25°20′-25°40′ S, 28°17′-28°25′ E. The average annual rainfall is 646 mm. The maximum and minimum temperatures are 29 °C and 20 °C and 16 °C and 2 °C in January and July, respectively. The area is described as a savanna biome characterised by marikana thornveld vegetation (Mucina *et al.* 2005).

Experimental Design

Twenty four, 13-month of age female Boer goats, *Capra hircus* (live weight 23.2±2.9 kg) were used during the experiment. They were randomly housed in individual holding pens (1x3 m²). All goats had free excess to water through water nipples installed at the back of the pens. Pens were installed in a well ventilated shed with one side open to natural light and roofing to protect goats against sun and rain.

Goats were conditioned for one week to familiarise them with the feeding and research protocol. To familiarise goats with species, fresh branches of four plant species, *Acacia caffra*, *Ziziphus mucronata*, *Rhus lancea* and *Euclea crispa*, were harvested every morning and provided to goats. In order to control effects of plant morphology as much as possible, the four plant species were selected such that they had similar morphology, except that two species (*A. caffra* and *Z. mucronata*) had small hooked thorns and two species did not have thorns. The four species were abundant in the study area to permit collection of sufficient browse for the duration of the experiment. *A. caffra* has pairs of small hooked thorns that are widely spaced, while *Z. mucronata* has small thorns in pairs one straight and one hooked. *R. lancea* and *E. crispa* have no thorns. The four species were also selected to represent different condensed tannin concentrations, ranging from low to high. *A. caffra* and *E. crispa* have high condensed tannin concentration (>10%) (van den Braak *et al.* unpublished data and Scogings *et al.* unpublished data), while *Z. mucronata* has low concentrations (<5%) and *R. lancea* has moderate concentrations (5-10%) (Aganga and Mosase 2001).

Six goats were randomly assigned to each tree species. Lamb and ewe pellets (Epol (Pty) Ltd) and lucerne (*Medicago sativa*) hay was provided. The amount of pellets and hay was determined based on the amount of intake by goats to meet daily nutrient requirements, which was 900g and 300g for pellets and hay respectively. This formed the basis for the maintenance diet during the experiment.

Branches of equal size (approximately 50 cm) were collected from each tree species at 7:30 every morning and offered to the goats for three weeks. Branches were tied in a vertical position on the front of the pens at 8:00 every morning. Goats were allowed to browse species until the whole branch was browsed and another branch was provided if finished within one hour time period. Branches were weighed before and after feeding to determine intake. The maintenance diet of pellets and lucerne hay was provided after browsing. Goats were weighed daily every morning before feeding to monitor daily body weight gain.

Chemical Analysis

Before feeding, after the prepared branches of the same species were pooled together, one sample of each browse species from the pooled branches was randomly collected from harvested branches twice a week on Wednesday and Friday. Samples of collected branches were oven dried at 60 °C for 48 hours. Faecal samples were collected twice a week on Wednesday and Friday from each goat and dried at 60 °C in the oven for 48 hours. All samples were milled through a 1 mm hammer mill sieve. All samples were analysed for condensed tannins (CT) using acid-butanol method (Makkar 1995), crude protein (CP) (Nitrogen x 6.25), neutral detergent fibre (NDF), acid detergent fibre (ADF), and acid detergent lignin (ADL) (AOAC, 1997) at the University of KwaZulu-Natal.

Statistical Analysis

Average body weight gain, browse intake, pellet intake and lucerne intake for the first week and the last week of the experiment for each goat in each treatment was calculated. Browse intake was calculated based on dry matter basis after subtracting the moisture content. Pellet and lucerne were provided on dry matter basis. The difference

in body weight, browse intake, pellet intake and lucerne intake between the first and the last week was calculated. Regression analysis was used to test relationships between body weight gain, browse intake, pellet intake and lucerne intake and condensed tannin concentration in the diet. Similar analysis were conducted to test the effect of CT on the body weight gain, browse intake, pellet intake and lucerne intake using the One-way Analysis of Variance (ANOVA) (Appendix 1). This was done in order to determine if there are any differences between the two methods of analysis. Average body weight, browse intake, pellet intake and lucerne intake were each pooled for the two treatments low in condensed tannin and for treatments high in condensed tannin. The t-test was used to determine the effect of high or low condensed tannin on body weight, browse intake, pellet intake and lucerne intake.

Regression analysis was done to determine the relationship between CT concentration and protein retention, protein loss and total protein intake by goats. One-way Analysis of Variance (ANOVA) was also used for similar analysis (Appendix 1). Protein retention, protein loss and total protein intake by goats was calculated as done by van den Braak *et al.* (unpublished data). The amount of protein from each feed was calculated by multiplying the daily intake of each feed by the percent of protein in each feed obtained from chemical analysis. Total protein intake by goats was calculated by adding all protein from browses, pellet and lucerne. Faecal protein was calculated by multiplying the amount of protein obtained from chemical analysis by the faecal weight from each goat. All statistical tests were done using SYSTAT. Significance was declared when $P < 0.05$, but trends were noted when $0.10 > P > 0.05$.

Results

Response of body weight gain to condensed tannin concentrations

The chemical composition of browse trees, pellets and hay are shown in Table 1. Goats in treatment groups with low condensed tannin concentration gained more body weight compared to those on diets high in condensed tannin (Fig. 1). However, the significance of the effect was marginal ($F_{1,20}=3.56$, $r^2=0.36$, $P=0.073$). A t-test showed no significant difference ($t=1.24$; $df=22$; $P=0.226$) in body weight gain between goats in pooled groups of low and high condensed tannin concentrations of browse.

Table 1. Chemical composition of the different dietary compounds (mean \pm SEM) (CT condensed tannin; CP crude protein; NDF neutral detergent fibre; ADF acid detergent fibre; ADL acid detergent lignin). One pooled sample of lucerne was analysed. Pellet nutritional information was provided by Epol®

Food type	CT (%)	CP (%)	NDF (%)	ADF (%)	ADL (%)	Urea (g/kg)	Fat (g/kg)	Calcium (g/kg)	Phosphorus (g/kg)
<i>Acacia caffra</i>	11.5 ± 0.26	13.04 ± 1.88	59.1 ± 4.29	26.1 ± 1.26	16.8 ± 1.04	-	-	-	-
<i>Euclea crispa</i>	14.3 ± 0.17	8.5 ± 0.65	44.6 ± 3.40	21.7 ± 0.96	14.8 ± 1.19	-	-	-	-
<i>Rhus lancea</i>	5.9 ± 0.07	11.7 ± 0.74	47.9 ± 3.46	20.4 ± 1.15	13.3 ± 1.07	-	-	-	-
<i>Ziziphus mucronata</i>	2.8 ± 0.07	11.7 ± 0.38	33.4 ± 1.79	12.5 ± 0.34	7.6 ± 0.57	-	-	-	-
Lucerne hay	-	16.1	41.3	22.6	6.8	-	-	-	-
Pellets	-	13.0	-	-	-	10	25	15	2

- Not analysed.

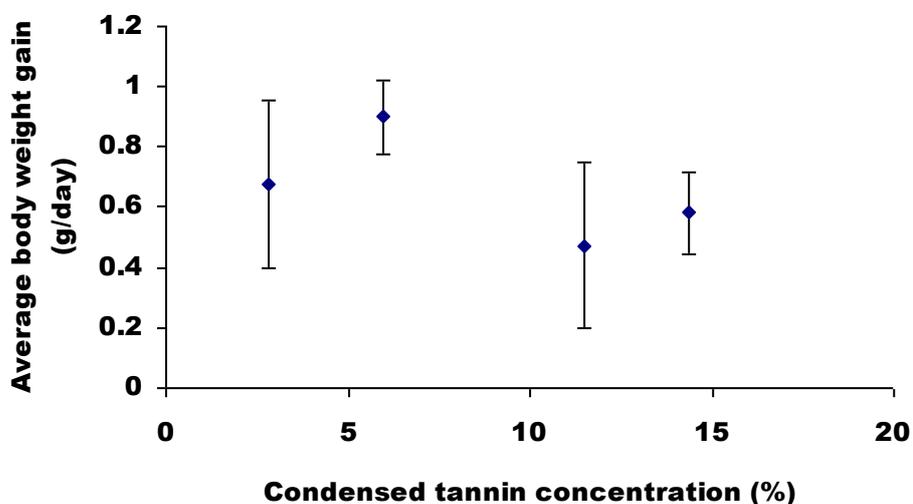


Figure 1. Relationship between average body weight gain and condensed tannin concentration in goats fed browse containing different amounts of condensed tannin (*Z. Mucronata* 2.8%, *R. lancea* 5.9%, *A. caffra* 11.5% and *E. crispa* 14.3%) for three weeks. Each point represents the mean of six goats and error bars represent the standard error of the mean.

Response of feed intake to condensed tannin concentration

There was no significant relationship between CT concentration and intake of browse species ($F_{1,20}=0.43$, $r^2=0.02$, $P=0.518$) (Fig. 2). There was no significant difference ($t=0.76$; $df=22$; $P=0.453$) in browse intake between goats in pooled groups of low and high CT concentrations.

There was no significant relationship between CT concentration and intake of pellets ($F_{1,20}=0.50$, $r^2=0.02$, $P=0.486$) (Fig.3). A t-test analysis showed no significant difference

in pellet intake between pooled treatments with low and high CT concentrations ($t=0.82$; $df=22$; $P=0.421$).

There was no significant relationship between CT concentration and intake of lucerne ($F_{1,20}=0.52$, $r^2=0.02$, $P=0.520$) (Fig. 4). A t-test analysis showed no significant difference in lucerne intake between pooled of treatments with low and high CT concentrations ($t=1.23$; $df=23$; $P=0.234$).

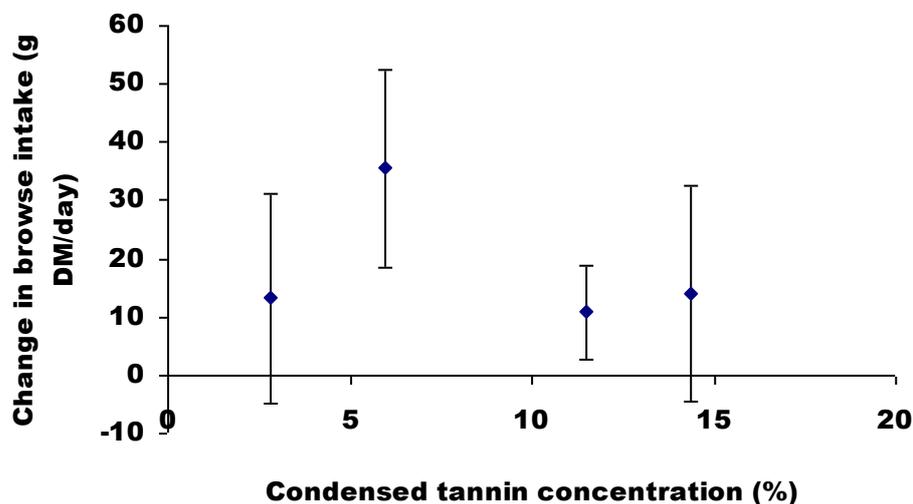


Figure 2. Relationship between the average browse intake and condensed tannin concentration in goats fed browse containing different amounts of condensed tannin (*Z. Mucronata* 2.8%, *R. lancea* 5.9%, *A. caffra* 11.5% and *E. crispa* 14.3%) for three weeks. Each point represents the mean of six goats and error bars represent the standard error of the mean.

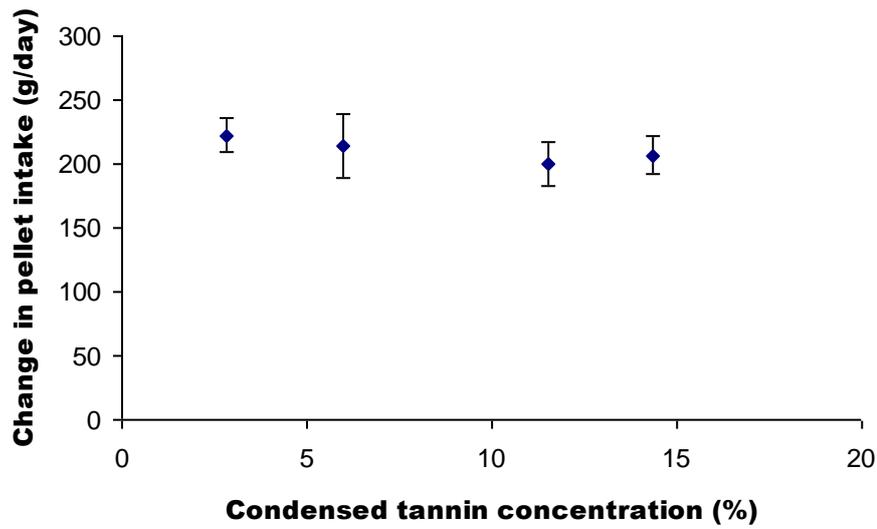


Figure 3. Relationship between the average pellet intake and condensed tannin concentration in goats fed browse containing different amounts of condensed tannin (*Z. Mucronata* 2.8%, *R. lancea* 5.9%, *A. caffra* 11.5% and *E. crispa* 14.3%) for three weeks. Each point represents the mean of six goats and error bars represent the standard error of the mean.

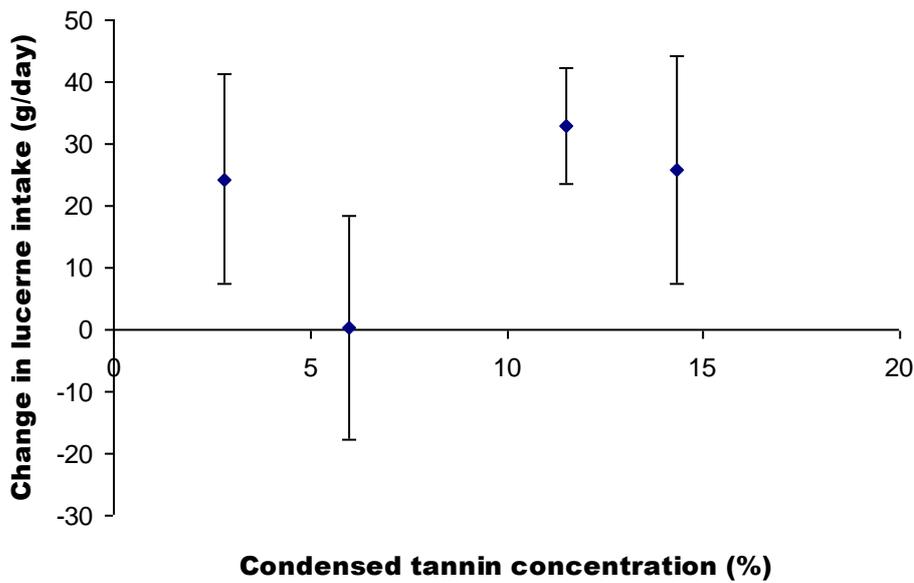


Figure 4. Relationship between the average lucerne intake and condensed tannin concentration in goats fed browse containing different amounts of condensed tannin (*Z. mucronata* 2.8%, *R. lancea* 5.9%, *A. caffra* 11.5% and *E. crispa* 14.3%) for three weeks. Each point represents the mean of six goats and error bars represent the standard error of the mean.

Response of protein intake, protein loss and protein retention to condensed tannin concentration

There was no significant relationship between total protein intake by goats and CT concentration ($F_{1,22}=0.09$, $r^2=0.05$, $P=0.858$) (Fig. 5). There was no significant relationship between protein loss in faeces of goats and CT concentration ($F_{1,22}=1.01$, $r^2=0.27$, $P=0.325$) (Fig. 6). There was no significant relationship between protein retention by goats and CT concentration ($F_{1,22}=0.02$, $r^2=0.22$, $P=0.984$) (Fig. 7).

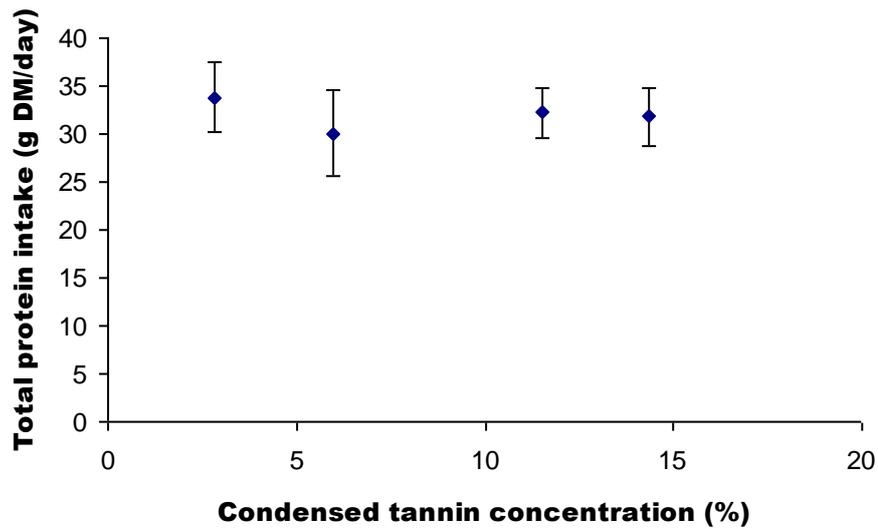


Figure 5. Relationship between the total protein intake by goats and condensed tannin concentration in goats fed browse containing different amounts of condensed tannin (*Z. mucronata* 2.8%, *R. lancea* 5.9%, *A. caffra* 11.5% and *E. crispa* 14.3%) for three weeks. Each point represents the mean of six goats and error bars represent the standard error of the mean.

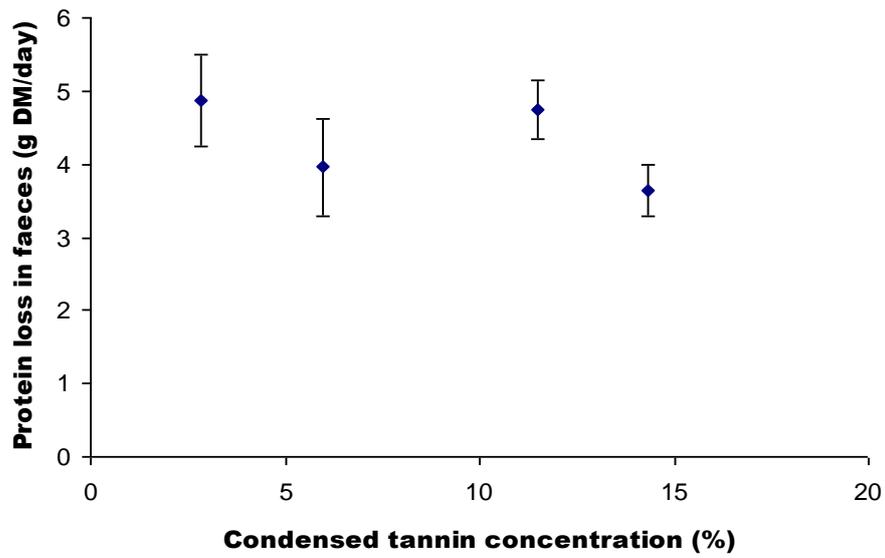


Figure 6. Relationship between protein loss in faeces of goats and condensed tannin concentration in goats fed browse containing different amounts of condensed tannin (*Z. mucronata* 2.8%, *R. lancea* 5.9%, *A. caffra* 11.5% and *E. crispa* 14.3%) for three weeks. Each point represents the mean of six goats and error bars represent the standard error of the mean.

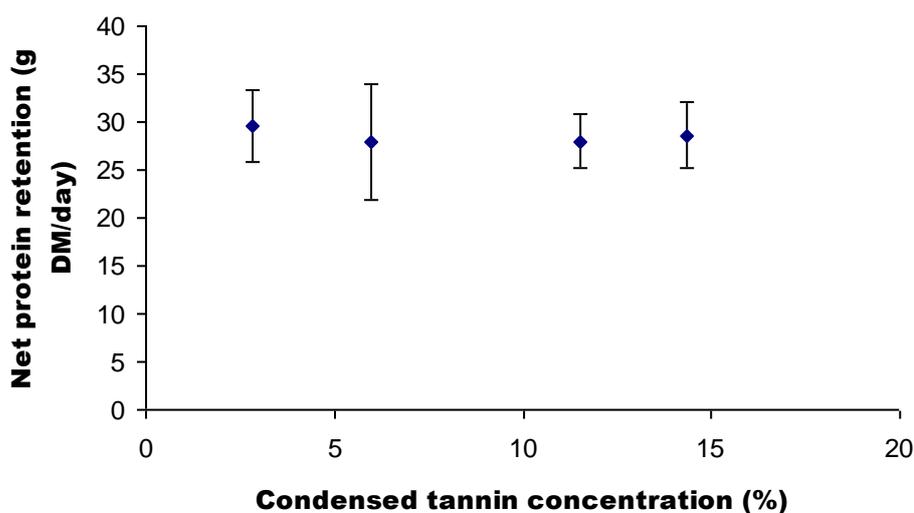


Figure 7. Relationship between net protein retention by goats and condensed tannin concentration in goats fed browse containing different amounts of condensed tannin (*Z. Mucronata* 2.8%, *R. lancea* 5.9%, *A. caffra* 11.5% and *E. crispa* 14.3%) for three weeks. Each point represents the mean of six goats and error bars represent the standard error of the mean.

Discussion

Response of body weight gain to condensed tannin concentrations

It was expected that goats given low CT concentration browse will gain more weight than those fed high CT concentration browse (Makkar 2003). Decline in body weight gain with increasing of CT concentrations can provide evidence that goats can tolerate a certain threshold level of condensed tannins. Concentrations of CT beyond 6% in the four browse species tended to relate negatively to body weight gain which supports the observations of reduced body weight gain in lambs fed feed with CT concentrations of 76-90 g/kg DM (Frutos *et al.* 2004). Stronger relationships may have been observed if

the experiment had run for several more weeks (Mbatha *et al.* 2002). Furthermore, CT concentrations exceeding 60 mg/g DM have been observed to depress voluntary feed intake, fibre and protein digestibility and subsequently reduce the growth rate of ruminants (Huang *et al.* 2010).

Increase in body weight gain of goats with the decreasing CT concentration can be attributed to the protection of protein from rumen degradation. This results in an increase in the influx of essential amino acids to the small intestines and increase in absorption of essential amino acids (Makkar 2003). Mbatha *et al.* (2002) observed a decrease of liver mass with increasing CT concentrations over 6 weeks, which signifies the negative effect of CT on goat body weight. However, after a long time the negative effects of CT can diminish as goats become adaptable to CT.

Polyethylene glycol (PEG) can counteract the negative effects of condensed tannins in goats. However, there was no difference in growth for goats fed cassava without or with PEG (Frutos *et al.* 2004). Therefore, it should not be assumed that body weight gain can only be affected by CT. Other factors can have an effect on body weight gain of goats, for example, crude protein and energy content in combination with PSCs can affect animal performance (Waghorn 2008). The nature of tannins in different fodder species and the species' stage of growth can have different effects on body weight gain. Condensed tannins from carob (*Ceratonia siliqua*) reduced lamb growth rate whilst sulla (*Hedysarum coronarium*) with double CT concentration did not reduce daily gain of lambs (Waghorn 2008). Studying the nutritional effects of tannins in plants is

therefore complicated because different plants contain a great diversity of tannins and other PSCs that have contrasting effects which changes with the plant age and season.

Response of feed intake to condensed tannin concentration

It was expected that high CT concentrations will reduce feed palatability, reduce feed intake, slow down digestion and develop conditioned aversions (Frutos *et al.* 2004). The absence of the significant relationship between CT concentration and intake of browses, pellets and lucerne signifies that CT concentration did not affect the intake. This contradicts the hypothesis that intake will decrease with the increasing CT concentrations. Condensed tannins affect feed intake but is not a reliable predictor of intake. Higher digestibility is the first predictor of feed intake while tannins and total phenols are second or third predictors of intake (Alonso-Diaz *et al.* 2010). This is of importance especially in this short term study because Alonso-Diaz *et al.* (2008) observed that in short term studies PSCs are the secondary signals of intake regulation. PEG did not increase the overall consumption of tannin rich diets and CT concentration of 1.5 and 4 % did not affect the total intake of cassava (Alonso-Diaz *et al.* 2010). Thus, CT cannot be used as the sole determining factor in intake especially of browses.

Lucerne and pellets had higher protein content compared to most browse species. Protein is the limiting nutrient in goats especially those exposed to CT. It can be expected that as CT limits the availability of proteins in goats, goats in high CT concentrations increased the intake of lucerne and pellets to replenish proteins bounded by CT. However, high protein content can be negatively correlated with dry matter

intake (Alonso-Diaz *et al.* 2010). This explains why there was no higher intake of lucerne and pellets for goats exposed to high CT concentration.

Reduction of feed intake but increase in live weight gain of sheep given tannin-rich feed has been observed (Frutos *et al.* 2004). This is contradictory to studies where CT affected intake of feed but not body weight gain (van den Braak *et al.* unpublished data). This explains why in the current study there was no relationship between browse CT concentration and feed intake but a marginal difference in body weight gain of goats. Thus, CT related to body weight gain of goats but not feed intake of goats. This means that although CT can affect body weight gain it did not affect intake in the current study. Therefore, from the results of this study it may be postulated that factors affecting the body weight gain of goats exposed to CT are post-ingestive factors not the intake. This agrees with what was observed by van den Braak *et al.* (unpublished data) where the intake of *Acacia caffra* did not affect the body weight gain of goats. Therefore, from this study there is no evidence that goats increase the intake of browse species based on the CT or protein content.

Response of protein intake, protein loss and protein retention to condensed tannin concentration

It was expected that high CT concentrations will bind more proteins in the rumen, reduce protein retention and increase faecal protein loss (Waghorn 2008). Also the opposite was expected with low CT concentrations. However, there was no significant relationship between CT concentration and protein intake, protein retention and faecal protein loss. CT decrease feed protein degradability in the rumen and increase the supply of non-ammonia nitrogen to the lower gut (Makkar 2003). This increases protein

availability to the abomasum and small intestines. However, this is only possible if CT-protein complexes dissociate in the abomasum and small intestines. CT-protein complexes can dissociate and proteins are digested in the abomasum and small intestines when the pH is below 3.5 or greater than 8, which is the normal pH in the abomasum and small intestines (Frutos *et al.* 2004). This means that proteins bound in CT from the rumen can be digested and absorbed in the lower gut. Thus, from the results of this study it can be postulated that irrespective of the concentration, CT binds proteins in the rumen but dissociates in the lower gut and allow digestion of proteins. This explains why there was no difference in protein retention and protein loss from goats fed different CT concentrations in the diet.

Conclusions

From the results presented above it can be concluded that CT can potentially affect average body weight gain of goats. However, body weight gain is not the function of feed intake because in the present study CT did not significantly relate to feed intake in all treatments. This can partially reject the theory that CT influences feed intake. It can then be suggested that CT has post-ingestive effects that affect body weight gain. This is still not clear because there was no relationship between CT concentration and protein retention and protein loss by goats. Digestibility in future studies based on different CT concentrations from the same source can be suggested. In future it will be important to consider all soluble and insoluble phenols in studies that include browses.

CHAPTER 3

The effect of different condensed tannin concentrations on the body weight gain of goats

Abstract

In African savannas, goats consume a diet that contains a large number of plants which contain condensed tannins (CT). Condensed tannins affect voluntary feed intake and digestibility of proteins which can affect the body weight gain. Mechanisms which affect the body weight gain when CT are consumed are not well defined. Does intake or digestibility or both factors affect the body weight? Are there other unknown mechanisms? Studies done with browse on the effects of CT are confounded by various factors which cannot be controlled. Thus, artificial diets were used in this study. It was hypothesized that high CT in diets will reduce the body weight gain of goats. Twenty four goats were randomly assigned to 0%, 3%, 5% and 10% CT concentration in the diets. Experiments were conducted for four weeks. There was, a significant negative effect of different CT concentrations on the body weight gain, pellet intake of goats as well as on the protein intake, faecal protein loss and protein retention of goats. With the exception of feed intake, protein retention and faecal protein loss were all negatively affected by CT concentrations and yet did not affect growth. The results suggest that other than feed intake these factors are not the mechanisms which affect body weight gain as influenced by CT. Future research on the effect of CT on the body weight gain of goats should focus on the digestibility of nutrients after the consumption of different CT concentrations.

Keywords: post-ruminal digestion, Boer goats, feed intake, condensed tannin-protein complexes

Introduction

Plants consumed by herbivores produce various plant secondary compounds (PSCs). In plants, PSCs inhibit the growth of microbes and fungi and have negative physiological and behavioural results in herbivores after ingestion (Forbey *et al.* 2009). Tannins are one of the most abundant PSCs (Cheeke 1998). Tannins are polyphenolic compounds with various molecular weights and a variable complexity (Makkar 2003). Tannins are divided into condensed tannins (CT) and hydrolysable tannins (HT). Condensed tannins are a group of polyhydroxy-flavan-3-ol oligomers and polymers linked by carbon-carbon bonds between flavanol subunits (Schofield *et al.* 2001). Condensed tannins affect the voluntary feed intake of herbivores (Tamir and Asefa 2009). The effect of CT on herbivores depends on their concentration, nature, animal species, animal's physiological state and composition of the diet (Makkar 2003). These factors also define the ability of herbivores to respond to CT. Salivary proteins in browsers such as goats affect digestion by binding dietary CT (Silanikove *et al.* 1996; Lamy *et al.* 2010). Proline rich protein (PRP) in most browsers have a high binding capacity and bind with CT, so there is less CT to bind with rumen proteins (Lamy *et al.* 2010). Therefore, goats should be more tolerant to CT because salivary PRP provides the first line of defence. However, voluntary intake is affected by constriction, dryness and roughness in the oral cavity caused by CT (Bengaly *et al.* 2007).

Condensed tannins bind to nutrients in the digestive tract. Condensed tannins have high affinity with proteins and to a lesser extent with metal ions and polysaccharides (Makkar 2003). Very high CT concentration limits the amount of dry matter available for digestion. However, this also depends on the source of CT. Condensed tannin

concentrations between 2% and 20% added in the animals' diet has been used to study the effects of CT to herbivores (Becker and Makkar 1999; Villalba and Provenza 2002; Mbatha *et al.* 2002; Ammar *et al.* 2009). However, calliandra tannins at a level of 2-3% in the diet reduce fibre digestibility, while *Leucaena leucocephala* tannins at 7.3 % have no effects on digestibility (Makkar 2003). A low digestion rate, especially under influence of a high fibre content, will slow the removal of feed residues from the rumen, which increases rumination and reduces voluntary feed intake (Waghorn 2008). Reduction of feed intake leads to a reduction in nutrient (protein and energy) intake and thus to a reduction in body weight gain. Studies on the effect of CT on body weight measured in browsers can provide incomplete information about the body weight.

Condensed tannins concentration can vary between different species, between plants of the same species or even between parts of the same plant (Baraza *et al.* 2009). Effects of tannins on goats' body weight and intake can be confounded by variation of physical and chemical characteristics in plant species offered to goats. Differences in chemical characteristics (minerals and proteins) and physical characteristics such as spinescence and pubescence can influence the intake and digestibility of nutrients in goats (Wilson and Kerley 2003). Nutrients and PSCs are confounded in plants, therefore obscuring diet selection (Jansen *et al.* 2007). Thus even species of the same genus vary in their nutrient, PSCs content and digestibility. Furthermore, CT are not the only PSCs found in browse. In trying to remove effects from other factors, a prepared artificial diet of known chemical composition should be used during experiments where greater control over a single or small set of variables is required.

It is not clear how CT affect the body weight of goat. Condensed tannins have the ability to provide proteins post-rationally (Mangan 1988; Reed 1995). If CT bind more proteins in the rumen, consequently more proteins flow into the intestines and rumen degradation of proteins is avoided (Deaville *et al.* 2010). These proteins can also be absorbed in the intestines and used for physiological requirements such as growth. Amino acids that are required for metabolism are absorbed from the small intestines. Dietary protein is broken down by rumen microbes to ammonia which is a poor protein source (Henning 1990). Therefore, CT can have a two-fold effect to goats' body weight, which can lead to either loss or gain of body weight. This depends on the amount of proteins that passes the rumen and the likelihood of tannins ending up in the lower gut (Bengaly *et al.* 2007).

In order to grow, goats must forage, and the greater the intake of quality nutrients the greater the quantity of nutrients to support growth. However, PSCs are found nearly in all dicotyledonous plant species. Herbivores must balance the nutrient rewards offered by plants and the negative balance effect of PSCs (Duncan *et al.* 2006). The specific objectives of this study were to determine the effects of CT on goats' body weight. Firstly, it was hypothesized that goats fed low CT concentration diets will have higher feed intake than goats fed high CT concentration diets. Secondly, it was hypothesized that goats fed low CT concentration diets will lose less faecal protein and retain more dietary protein than those fed high CT concentration diets. Lastly, it was hypothesised that goats given diets low in CT will lose less body weight than those fed high CT concentration diets.

Materials and Methods

Study Area

The study was conducted at the ARC's Roodeplaat Experiment Farm, in Pretoria, South Africa. Roodeplaat is situated approximately 30 km north east of Pretoria (Panagos *et al.* 1998), 25°20'-25°40' S, 28°17'-28°25' E. The average annual rainfall is 646 mm. The maximum and minimum temperatures are 29 °C and 20 °C and 16 °C and 2 °C in January and July, respectively. The area is described as a savanna biome characterised by marikana thornveld vegetation (Mucina *et al.* 2005).

Experimental Design

Twenty-four, 13-month of age female Boer goats, *Capra hircus* (live weights 23.2±2.9 kg) were used. They were randomly housed in individual holding pens (1x3 m²). All goats had free access to water through water nipples installed at the back of the pens. Pens were installed in a well ventilated shed with one side open to natural light and a roof to protect goats against sun and rain. Goats were conditioned for one week to familiarise them with the new feeding and research protocol.

Experiments were conducted for four weeks. Goats were randomly assigned to four groups of six goats in each group. The same standard diet was used in all four groups but with different CT concentrations of respectively 0 %, 3%, 5% and 10% CT concentration. Mimosa extract in powder form containing 66% CT (Mimosa Extract Company (Pty) Ltd, Pietermaritzburg) was dissolved in water (500 ml), enough to dissolve tannin without diluting it. Lamb and ewe pellets (Epol (Pty) Ltd) were thoroughly mixed with the CT solution. Pellets were oven dried at 30 °C overnight. Pellets (900 g) mixed with different CT concentrations were offered to goats in two

meals of 550 g in the mornings from 8:00 to 10:00 and 350 g in the evenings from 15:00 to 17:00. Twice a day, 150g of lucerne (*Medicago sativa*) hay was also provided to goats at the same time with the treated pellets. Feed was weighed before and after feeding to determine feed intake. Goats were weighed daily every morning to monitor daily body weight gain.

Chemical analysis

Feed samples were collected once a week and homogenised into one sample per CT concentration and dried at 60 °C in the oven for 48 h. Faecal samples were collected twice a week on Wednesday and Friday from each goat and dried at 60 °C in the oven for 48 h. All samples were milled through a 1 mm hammer mill sieve. All samples were analysed for condensed tannins using acid-butanol method (Makkar 1995), crude protein (CP) (Nitrogen x 6.25), neutral detergent fibre (NDF), acid detergent fibre (ADF) and acid detergent lignin (ADL) (AOAC, 1997) at the University of KwaZulu-Natal.

Statistical Analysis

Protein retention, protein loss and total protein intake by goats was calculated as done by van den Braak *et al.* (unpublished data). The amount of protein from each feed was calculated by multiplying the daily intake of each feed by the percent of protein in each feed obtained from chemical analysis. Total protein intake by goats was calculated by adding all protein from pellets and lucerne. Faecal protein was calculated by multiplying the amount of protein obtained from chemical analysis by the faecal weight from each goat.

We used a completely randomized block design. Average body weight gain was calculated by calculating the average body weight gain for each week per CT concentration. Pellet intake and lucerne intake for the first week and the last week of the experiment for each goat in each treatment was calculated in order to determine the changes due to different CT concentrations. Pellet and lucerne were provided on dry matter basis. The difference in pellet intake and lucerne intake between the first and the last week was calculated in order to determine the changes due to different CT concentrations.

One-way Analysis of Variance (ANOVA) was used to determine the effect of different CT concentrations on body weight gain, pellet intake, lucerne intake, total protein intake, faecal protein loss and protein retention of goats. The body weight gain was determined by the repeated measurements using the average body weight gain of four weeks per CT concentration. The pellet intake and lucerne intake were both used as the covariates for average body weight gain. All statistical tests were done using SYSTAT. Significance was declared when $P < 0.05$.

Results

Effect of condensed tannin concentrations on body weight gain

The chemical composition of various feeds is shown in Table 2. There was a significant increase in the body weight of goats fed low CT concentrations diets ($F_{3,20}=7.08$, $P=0.020$) (Fig. 8). As the covariates, there was no significant effect of different CT concentrations on the body weight gain of goats ($F_{3,20}=2.36$, $R^2=0.65$, $P=0.109$).

However, there was a significant effect of the pellet intake ($F_{3,20}=5.80$, $R^2=0.65$, $P=0.027$) and lucerne intake ($F_{3,20}=4.96$, $R^2=0.65$, $P=0.039$) on the body weight gain of goats.

Table 2. Chemical composition of the different dietary compounds (CT condensed tannin; CP crude protein, NDF neutral detergent fibre; ADF acid detergent fibre; ADL acid detergent lignin.). One homogenised sample was analysed from each feed type. One pooled sample of lucerne was analysed. Pellet nutritional information was provided by Epol®

Feed type	CT (%)	CP (%)	NDF (%)	ADF (%)	ADL (%)	Urea (g/kg)	Fat (g/kg)	Calcium (g/kg)	Phosphorus (g/kg)
0% CT	0	13	30.65	11.5	5.4	-	-	-	-
3% CT	2.8	15.97	27.05	9.69	4.53	-	-	-	-
5% CT	4.8	15.72	20.7	8.03	3.26	-	-	-	-
10% CT	9.54	14.87	20.48	7.66	3.56	-	-	-	-
Lucerne hay	-	16.48	41.33	21.7	6.95	-	-	-	-
Pellets	-	13.0	-	-	-	10	25	15	2

- Not analysed.

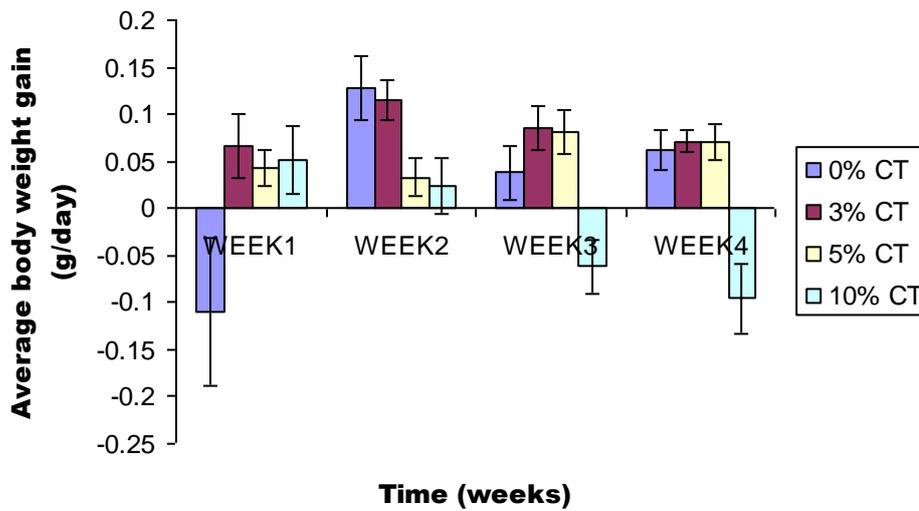


Figure 8. The effect of four different condensed tannin concentrations on the average body weight gain of goats (n=6 per diet) over the duration of four weeks. Each bar point represents the mean of six goats and error bars represent the standard error of the mean.

Effect of condensed tannin concentration on feed intake

An increase in the condensed tannin concentrations reduced the pellet intake of goats ($F_{3,20}=16.53$, $R^2=0.71$, $P<0.001$) (Fig. 9). There was no significant effect of different CT concentrations on lucerne intake of goats ($F_{3,20}=0.67$, $R^2=0.09$, $P=0.580$) (Fig. 10).

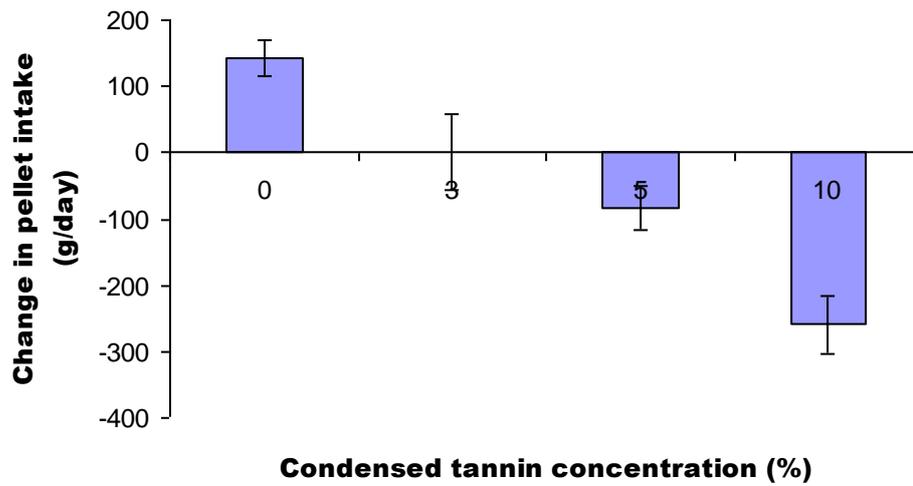


Figure 9. The effect of four different condensed tannin concentrations on the pellet intake of goats (n=6 per diet). Each bar point represents the mean of six goats and error bars represent the standard error of the mean. Each bar point represents the difference between the first and last week.

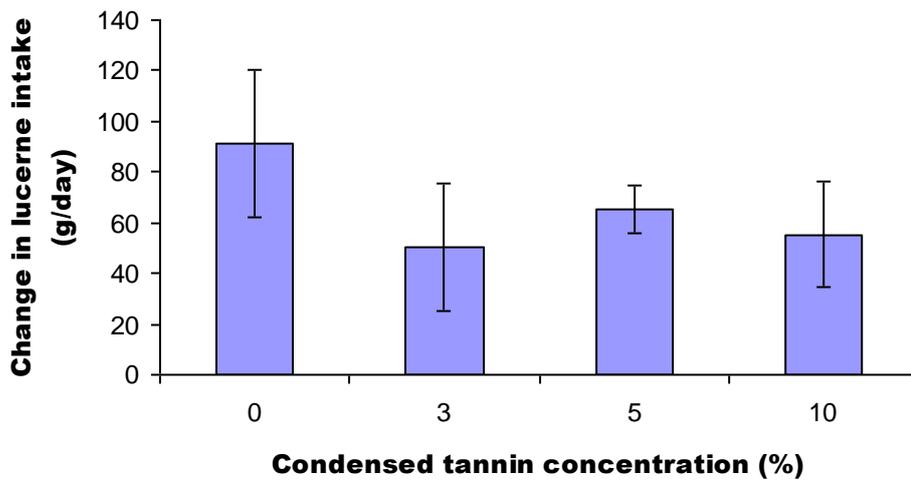


Figure 10. The effect of four different condensed tannin concentrations on the lucerne intake by goats ($n=6$ per diet). Each bar point represents the mean of six goats and error bars represent the standard error of a mean. Each bar point represents the difference between the first and last week.

Effect of condensed tannin concentration on protein intake, protein loss and protein retention by goats

Increase in CT concentration decreased the protein intake of goats ($F_{3,20}=12.38$, $R^2=0.65$, $P<0.001$) (Fig. 11). Increase in CT concentration increased the faecal protein loss of goats ($F_{3,20}=3.43$, $R^2=0.34$, $P=0.030$) (Fig. 12). Increase in CT concentration decreased the protein retention of goats ($F_{3,20}=11.24$, $R^2=0.62$, $P<0.001$) (Fig. 13).

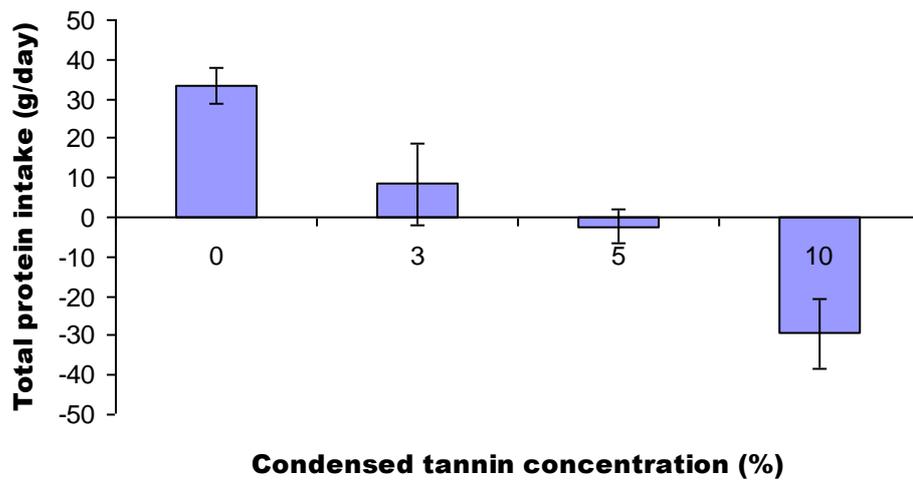


Figure 11. The effect of four different condensed tannin concentrations on the total protein intake by goats (n=6 per diet). Each bar point represents the mean of six goats and error bars represent the standard error of a mean. Each bar point represents the difference between the first and last week.

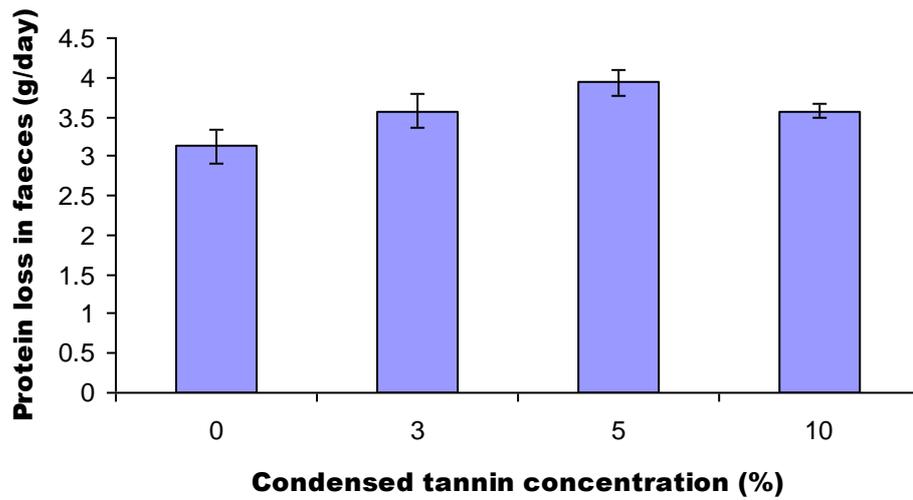


Figure 12. The effect of four different condensed tannin concentrations on the protein faecal loss by goats (n=6 per diet). Each bar point represents the mean of six goats and error bars represent the standard error of a mean of six goats. Each bar point represents the difference between the first and last week.

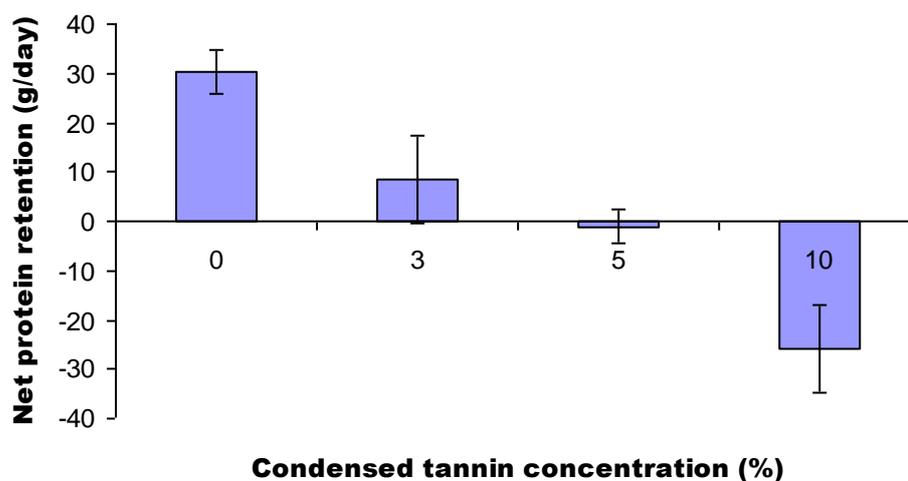


Figure 13. The effect of four different condensed tannin concentrations on the protein retention by goats (n=6 per diet). Each bar point represents the mean of six goats and error bars represent the standard error of a mean. Each bar point represents the difference between the first and last week.

Discussion

Effect of condensed tannin concentrations on body weight gain

It was expected that goats given low CT concentration diets would lose less body weight than those fed high CT concentration diets (Makkar 2003; Silanikove *et al.* 2006). The body weight gain decreased with the increasing CT concentrations in the diet which concur with the hypothesis. Condensed tannins reduce the body weight gain because of reduction in protein digestibility (Mole *et al.* 1993). Furthermore, the reduction in growth is the function of interaction of CT in the diet with food proteins, digestive enzymes and microbes (Silanikove *et al.* 2006). This suggests that post-ingestive effects affect the body weight gain of goats fed CT rich diets. However, the

repeated measurements with feed intake as the covariates signified that although CT affect the body weight gain but in the long term their effects are confounded by the intake. This also suggests that CT reduce the body weight through the reduction of feed intake. However, it is still not clear whether this happens through the reduction in palatability or digestibility. It has been observed that the low body weight gain of kids after feeding CT rich diets is because of the low protein, dry matter and organic matter digestibility (Silanikove *et al.* 2006). Thus, the results of this study suggest that pre- and post-ingestive effects affect the body weight gain of goats. It can be concluded that CT affect the body weight gain through different mechanisms depending on the duration of goats' exposure to CT.

Effect of condensed tannin concentration on feed intake

It was expected that high CT concentration would reduce the feed intake (Osborne and McNeill 2001; Waghorn 2008). The results showed a significant effect of CT on the pellet intake, and pellet intake decreased with increasing CT concentrations. The reduction of intake with increasing CT concentrations is attributed to poor palatability. Condensed tannins decrease palatability and palatability is measured in terms of dry matter intake (El-Rahman *et al.* 2006). Higher CT concentrations reduced the dry matter intake in other studies (Getachew *et al.* 2008). High CT concentrations (more than 50 g /kg DM) reduced feed intake while medium or low CT concentrations (less than 50g/kg DM) did not affect feed intake (Frutos *et al.* 2004). This concurs with what was observed in this study where goats fed lower CT concentration diets had a higher pellet intake compared to goats fed high CT concentration diets. The absence of significant effect on lucerne intake could indicate that any changes in the intake were due to CT in the pellets, especially since lucerne had no CT. From the results of this study it can be

concluded that an increase of CT concentration reduced palatability and thereby reduced intake from pellets. However, the intake of lucerne was the same for all treatments, independent of the CT concentration of the diet.

Effect of condensed tannin concentration on protein intake, protein loss and protein retention by goats

It was expected that high CT concentration would reduce protein retention and increase faecal protein concentration (Min and Hart 2003; Waghorn 2008). Faecal protein loss increased with increasing CT concentrations and protein retention decreased with increasing CT concentrations which concur with the hypothesis. Faecal protein losses were observed to be higher in silages with tannins (Deaville *et al.* 2010), which concurs with the results of the current study. There is no evidence that CT-protein complexes can dissociate in the lower gut. High faecal protein loss in high CT concentration diets is potentially attributed to the persistence of CT-protein complexes. There is a linear relationship between the amount of CT-protein complexes formed and the amount of CT consumed (Naczka *et al.* 2001). The lower the CT concentration, the lower the protein precipitating ability of CT (Osborne and McNeill 2001). Therefore, an increase in CT concentrations in the diet increases CT-protein complexes, which cannot be dissociated in the lower gut, resulting in high faecal protein loss. This is supported by a lower faecal protein loss and high protein retention in goats fed 0% CT.

Quebracho tannins reduce the protein absorption in the small intestines of sheep (Deaville *et al.* 2010). Higher CT concentrations reduce the dry matter degradability (Getachew *et al.* 2008). This is supported by low protein retention in goats fed high CT concentration diets. Low concentrations of CT protect the proteins from the rumen

degradation and increase the influx of amino acids to the small intestines (Min and Hart 2003). Condensed tannins up to 4% can have beneficial effects by binding to proteins in the rumen and allowing the dissociation of the CT-protein complexes post-ruminally (Makkar 2003). The difference can be explained by the type of bonds between CT and proteins, when different CT concentrations are consumed. Different bonds are formed under influence of the CT concentrations (Naczka *et al.* 2001). However, there are no data yet to support the hypothesis that different bonds are dissociated differently in the lower gut. The results of this study suggest, however, that there is no dissociation of CT-protein complexes and absorption of proteins in small intestines when high CT concentrations are consumed, probably because the effects of CT are distributed along the entire gastro-intestinal tract (Silanikove *et al.* 2006).

Conclusions

From the results of this study it can be concluded that different CT concentrations affected the body weight gain, pellet intake, protein retention and faecal protein loss. This shows that in this study, the body weight gain is to a certain extent the function of feed intake. The feed intake had an effect to the body weight gain but the underlying mechanisms were not found. Therefore, the pre- and post-ingestive effects were speculated as the mechanism that regulates the body weight gain of goats fed different CT concentrations. However, the digestibility studies were not done in this study. Thus, the mechanisms that affect body weight gain when CTs are consumed are still not clearly understood. Post-ruminal digestion of proteins when CTs are consumed was not proven in this study. It was concluded that the amount of CTs affect the formation of CT-protein complexes and the digestion of proteins. To decrease the bias in weight

measurements, in the future it is suggested to weigh goats before drinking water or excreting urine and faeces. In future, the digestibility studies are suggested in such studies.

Chapter 4

General Conclusions

Objectives of the study

The study was conducted in order to determine the effects of different CT concentrations on the body weight of goats. It was hypothesized that the goats fed low CT concentration diets will lose less body weight than those fed high CT concentration diets. It was also hypothesized that feed intake, protein intake and protein retention by goats will be affected differently by different CT concentrations in diets which will consequently affect the body weight gain of goats.

In order to try to mimic the feeding behaviour in the natural environment, browse species with different CT concentrations were fed to goats in the first experiments. Browse species have different chemical and physical characteristics. Furthermore, in browse species there are various phenols other than CT. All these factors act as confounding factors in determining the effects of CT on the body weight gain of goats. Therefore, the artificial prepared diets of the same chemical composition but with different CT concentration were prepared and fed to goats. This was done in the second experiments in order to have more control and eliminate the confounding factors. This was also done in order to make sure that any effects to the body weight gain are due to CT only.

Results and interpretation

Goats fed browses with low condensed tannin concentration gained more body weight compared to those on browses high in condensed tannin concentration, although the increase was marginal. In the second experiment, there was a significant increase in the body weight of goats fed low CT concentrations diets. This suggests that when all other confounding factors are eliminated the effects of CT are clearly defined. In the first experiment not only CT affected the body weight of goats but other phenolic compounds in browses may have influenced the results. Furthermore, browse species had various chemical and physical differences. This could have influenced the body weight of goats. Conducting the second experiment for a longer period than the first one can be an explanation of the observed minor differences on the effects of CT on goats' body weight gain. It is widely accepted that CT reduce the feed intake which can reduce the nutrient uptake by goats which can consequently affect the body weight gain. From this study, it can be assumed that the intake of feed also affect the body weight.

There was no significant difference in browse intake, pellet intake and lucerne intake between goats fed different browses with different CT concentrations. However, in the second experiment, an increase in the condensed tannin concentrations reduced the pellet intake of goats, with no significant effect of different CT concentrations on lucerne intake of goats. It can be expected that the goats in high CT diets will compensate by increasing the intake of CT free diets to increase the intake of nutrients. However, in the first experiment there was no relationship between CT and the intake. Whereas, in the second experiment it was expected that goats on diets with high concentration of CT will increase the intake of lucerne to compensate for high CT

concentration in pellets which did not happen. This concludes that CT is not the first determining factor on the intake of feeds. This suggests the differences in digestibility and the chemical composition in feeds other than differences in CT.

There was no difference on the total protein intake, protein loss in faeces and on protein retention by goats fed browses with different CT concentrations. However, an increase in CT concentration decreased the protein intake, increased the faecal protein loss and decreased the protein retention of goats in the second experiment. From the results of the second experiment it can be interpreted that CT binds proteins and prevents rumen degradation of proteins. However, this binding capacity does not guarantee that proteins will be digested in the lower gut. This explains that the CT remains effective throughout the digestive tract.

Goats that were used in both experiments were previously introduced to browses with CT at a young age. This can explain the observed non significant effects of CT mostly observed in goats fed browse species. Thus, before the start of the first experiment goats were already familiar to CT in browses. However, they were not familiar with CT which was added in the artificial diets. This explains why significant results were observed in goats in the second experiment. However, this does not ignore many confounding factors on browses explained above. The second experiment proved to be the best one to determine the effects of CT on the body weight of goats.

Conclusions and future research

For the future, studies that include the extraction of CT from the browses and added to the artificial diets will be more informative. This will allow the use of the CT from different browse species while removing the confounding factors. In this way the concentration of CT will be added in desirable concentrations. This will allow the use of CT from browses throughout the year without the limitations of seasons. This is important because the first experiment had to be stopped before the desired time due to shortage of leaf material in browses as the winter season was approaching. When the CT are extracted they can be stored and used in the different season from which were collected. This will allow testing the effect of CT extracted in different season to the body weight of goats in the different season.

Depending on the availability of leaf material, pelleting the browse species and provide them to goats as pellets can be suggested for future studies. This will not only allow the control on the amount of feed to be provided to goats but morphological differences such as thorns can be eliminated.

Digestibility studies are proposed for future studies. This will give the information on how nutrients are utilized after the intake of feeds rich in CT. This should include studies on the activity of rumen microbes after the consumption of CT rich diets. Digestibility studies will give more information about the flow of nutrients, CT and CT-protein complexes in the digestive tract. This can provide an idea about the expulsion

time for CT. This then suggests the use of markers in order to determine the flow and expulsion time of CT and nutrients after consumption of CT rich diets.

Presenting the different browse species with different CT concentration to goats will be more informative when studying the effect of CT on the intake of feed by goats. To make the conclusions on the effect of CT on the intake based on the single offered browse species is misleading. Presenting different browse species with different CT concentrations to the goats in a cafeteria style and allow the goats to choose what to eat can yield different results. Therefore the browse species with the overall intake over other browse species will be considered as the one with the highest intake. The intake will then be correlated to the CT concentration.

Extending the experiments for at least six weeks is proposed for future studies. This can provide the valuable information about the effects of CT to goats. However, the period should not be extended such that goats become familiar to CT.

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APPENDIX 1

Analysis of Variance (ANOVA) on the effect of CT concentration in different browse species on the body weight gain, browse intake, pellet intake, lucerne intake, protein intake, faecal protein loss and protein retention by goats

BODY WEIGHT GAIN

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.004	3	.001	.172	.914
Within Groups	.173	20	.009		
Total	.177	23			

BROWSE INTAKE

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	4303.009	3	1434.336	2.011	.145
Within Groups	14263.126	20	713.156		
Total	18566.135	23			

LUCERNE INTAKE

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	3142.578	3	1047.526	.872	.472
Within Groups	24030.610	20	1201.530		
Total	27173.188	23			

PELLET INTAKE

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	757.448	3	252.483	.345	.793
Within Groups	14656.853	20	732.843		
Total	15414.301	23			

PROTEIN INTAKE

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	10.292	3	3.431	1.761	.324
Within Groups	.000	20	.000		
Total	10.292	23			

FAECAL PROTEIN LOSS

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	10.829	3	3.610	1.674	.543
Within Groups	.000	20	.000		
Total	10.829	23			

PROTEIN RETAINED

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.007	3	.002	1.245	.754
Within Groups	.000	20	.000		
Total	.007	23			