THE EFFECTS OF TANNIN INGESTION ON THE PHYSIOLOGY OF BOER-GOATS

KHANYISILE REBECCA MBATHA

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PREFACE

This study was carried out in School Botany and Zoology, University of Natal, Pietermaritzburg, from February 1999 to January 2001, under the supervision of Dr Colleen T. Downs and Dr Ignatius V. Nsahlai.

This study represents original work of the author and has not been submitted in any form for any diploma or degree to another university. Where the work of others was referred to it has been duly acknowledged in the text.

This thesis is written in the format of manuscripts and most in accordance of the required format for submission to the journal *Animal Science* (Britain) or otherwise stated.

Khanyisile R. Mbatha

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Abstract

This study was conducted to determine the effects of different levels of dietary tannin on the physiology of Boer goats. In particular, dietary intake, digestibility, gastrointestinal tract (GIT) histology and presence of bacteria along the GIT were measured. In addition the ability of goats to detoxify tannins by measuring liver and kidney mass; and urinary glucuronic acid concentration was investigated.

Commercialized tannin was used because of wide variation of tannin levels, which can be affected by season, species, and part of the plant. Thirty adult, male goats were fed one of five diet treatments of different tannin levels (0%, 5%, 10%, 15% and 20%) for 6 weeks following which they were kept in metabolic crates for data collection for a further 10 days. Dietary intake of feed decreased significantly as tannin levels increased between the diets. Digestibility of dry matter (DM) tended to decrease with increasing tannin levels. However, digestibility of crude proteins (CP), organic matter, neutral detergent fibre and acid neutral detergent fibre decreased significantly with increasing tannin levels. Faecal CP increased while urinary CP decreased with increasing tannin levels. There was no tannin present in the faeces. It appears that goats cope with low levels of tannin ingestion. There appears to be a threshold above which greater tannin ingestion has detrimental effects. The linear decreased dietary intake with increased tannin level may indicate that goats limit their intake of tannin below some threshold as a defence strategy.

Differences in the histopathology of the oesophagus, reticulum, rumen, abomasum and duodenum were evaluated. Animals on the control diet had more protozoa present in the GIT

than the other diets. Number and types of bacteria observed in the reticulum and rumen increased with tannin level in the diet. These may be responsible for tannin-protein complex degradation. Few bacteria were observed in the abomasum.

There was a loss of epithelial cells and erosion of microvilli in duodenum with increased tannin levels, which would impair absorption of nutrients. The width of the keratinized GIT epithelial layer increased and villi height decreased as tannin levels increased which could further reduce nutrient absorption.

Goats in the present study did not show detoxification abilities because the liver and kidney masses, and urinary glucuronic acid concentration did not increase with increased dietary tannin levels

In summary, condensed tannins as large compounds appear to be metabolized and absorbed from the GIT. However, it is not clear if they are detoxified at the epithelial mucosa interface. The main detrimental effect of tannin on goats appears to be the reduction of feed intake and increased faecal CP.

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CONTENTS

Chapter 1

Introduction

Plant defences against herbivory are physical (for example thorns) and chemical (for example secondary metabolites). Woody plants with chemical defences are common on nutrient deficient soils whereas, plants with physical defences are common on fertile soils (Cooper and Owen-Smith, 1985). This pattern is also evident in grasses (Ellis, 1990). Secondary metabolites produced by some plants apparently play no role in normal pathways of plant biochemistry, and appear to have evolved purely in response to herbivore pressures (Mehansho *et al.*, 1987; Begon *et al.*, 1996). There are three main classes of secondary metabolites: phenolics, nitrogen-containing compounds and terpenoids. Physiological effects of secondary metabolites have been classified into two groups: toxic (qualitative) chemicals that are poisonous even in small quantities; and digestion reducing (quantitative) chemicals which act in proportion to their concentration (Feeny, 1976; Rhoades and Cates, 1976; Rhoades, 1979; 1985; Begon *et al.*, 1996). Alkaloids and cyanogenic glycosides are toxins (Rhoades, 1985). Tannins (Feeny, 1976; Rhoades and Cates, 1976) and creosote resins (Rhoades and Cates, 1976) are presented as examples of digestive reducing secondary metabolites that can be indirectly toxic (Rhoades, 1985). Tannins, a diverse group of polyphenolic substances of no single biogenic origin, are common factors affecting food taste and protein availability (Van Soest 1994).

Tannin

The term tannin was introduced at the end of eighteenth century to define the organic substances present in water extracts of the leaves, barks, woods, fruit or galls of certain ferns,

gymnosperms and angiosperms (Swain, 1979; Haslam, 1979). Tannins are kept in special organs in the leaves of dicotyledonous plants to prevent their interference with the plant's own metabolic apparatus (Mehansho *et al.*, 1987; Ellis, 1990). Monocotyledons, on the other hand, are notably poor in tannins and have tannin like substances (Ellis, 1990). Tannin levels and related tannin types vary with plant age (Bryant *et al.*, 1985) plant part and species (Williams, 1930; Burns and Cope, 1974; Stock *et al.*, 1993), genotype, geography (Lester, 1974) and season (Butler, 1982). Moreover, tannin levels in plants respond to diseases, stress and attack by fungi (Van Soest, 1994).

Plant tannins affect many aspects of the consumer's physiology, nutrition and metabolism including dietary intake, dietary protein availability, digestive enzyme activity, endogenous protein excretion, integrity of intestinal mucosa detoxification activity, integrity of the kidney and liver postabsorptive metabolism, growth and performance (McLeod, 1974; Price and Butler, 1980; Salunkhe *et al.*, 1990). The ability of tannins to form strong complexes with proteins is the most important aspect of their nutritional and toxicological effects (Hagerman and Butler, 1981). They are water soluble with a wide range of molecular weight of 500 to 3000, or more and are able to precipitate gelatine and other proteins from aqueous solutions (Swain, 1979; Gupta and Haslam, 1980; Kumar and Singh, 1984; Bryant *et al.*, 1985). This definition, however, excludes certain of those phenolics that bind strongly to protein without precipitation. Tannins also bind to most soluble protein, causing insoluble copolymers at normal pH and ionic strength (Hagerman and Butler, 1991). Tannins have a harsh, astringent taste and produce a feeling of constriction, dryness and roughness in the mouth (Haslam 1979).

For mammals, a herbivorous diet is relatively low in quality because it requires large intake

volumes (Cork and Foley, 1991; Robbins, 1993), and then micro-organisms to digest the cellulose so energy assimilation is slow and transit times are slow (Parker *et al.*, 1996). Each plant has evolved a unique set of defensive metabolites that deter attack from most herbivores except for few species that have broken through the defences by counter-adaptation (Rhoades, 1985). Presence of tannins in the plant has been characterised as a deterrent against herbivory (Freeland and Janzen, 1974; Swain, 1979; Zucker, 1983; Kumar and Singh, 1984; Rhoades 1985; Robbins *et al.*, 1987a). For example, the astringency that tannins contribute to unripen fruit, results in their avoidance by herbivores until seeds are mature and ready for dispersal (Mehansho *et al.*, 1987). Ingestion of phenolics is common in mammal herbivores with browsing and mixed feeding foraging strategies (McArthur *et al.*, 1991).

Terrestrial tannins can be divided into two main classes hydrolyzable tannin (ellagitannins and gallotannins) and condensed tannin (pro-anthocyanidins) (Zucker, 1983; Mehansho *et al.*, 1987). These can be differentiated by their structures and reactivity towards hydrolytic agents (Church, 1988). The third class of tannin is phlorotannins that are found only in marine algae (Hagerman *et al.*, 1998). Less information is available about the latter class of tannins and ruminants are not exposed to it.

Hydrolyzable tannins are composed of gallic acid or its condensation product ellagic acid esterified to the hydroxyl group of glucose (Mehansho *et al.*, 1987; Haslam, 1979). The hydrolyzable tannins can also undergo condensation reactions (Reed, 1995). They are reliant on one major phenolic building block of gallic acid and its derivatives. The other feature of hydrolyzable tannins is the presence of a sugar (hexose) core on to which gallic acid can be linked by esterification. One glucose unit can accommodate esterification with five gallic

acids if it is in the cyclic form (Waterman and Mole, 1994). The simplest hydrolyzable tannin is therefore pentagalloyl glucose, but it is usually more complex with the gallic groups often bonding to each other to give dimeric forms. Hydrolyzable tannins split easily into sugars and phenolic carboxylic acids (Hagerman and Butler, 1989; Van Soest, 1994) because ester bonds are acid-, base-, and enzyme-labile (Hagerman and Butler, 1989). They also can be cleaved by hot water and tannases, and consist of a carbohydrate core with phenolic carboxylic acid bound by ester linkages (Van Soest, 1994). Hydrolyzable tannins are further classified according to the products of hydrolysis; gallotannins yield gallic acid and glucose and ellagitannins yield ellagic acid and glucose (Haslam, 1979). Digestibility-inhibiting effects are reduced, but toxicity increases if the hydrolyzable tannins are degraded by hydrolysis and then absorbed (Reed, 1995; Foley *et al.*, 1999). Hydrolyzable tannins cause hypersecretion of gastric mucous and histopathological lesions of the gastrointestinal tract (Mitjavila *et al.*, 1977; Sell *et al.*, 1985).

Condensed tannins are the most widely distributed group of tannins (Waterman and Mole, 1994). They are the principal forage tannin and are found in sorghum grains, legumes, tree parts and in certain agricultural waste products (Kumar and Singh, 1984). They are flavonoid polymers, with carbon-carbon bonds joining the individual flavonoid monomers (Hagerman and Butler, 1989). When condensed tannins are heated with strong acids, which act as catalysts, the interflavan bond is cleaved oxidatively but it is not susceptible to hydrolysis (Porter *et al.*, 1986). Also condensed tannins diminish the permeability of the gut wall by reacting with the epithelium reducing the absorption of nutrients (Fahey and Berger, 1988). In large mammalian herbivores, condensed tannins may influence digestion by limiting microbial fermentation of plant cell walls while in the digestive tract (Ellis, 1990). These tannins may therefore, act as anti-feedants because 1, they complex with food proteins; 2,

they bind to microbial enzymes; reducing fermentation and degradation of fibrous tissue; 3, they bind to digestive enzymes in general reducing their activity, and 4, they have an astringent taste that can reduce palatability (Swain, 1979).

Benefits of tannins on ruminants

Beneficial effects of polyphenols on ruminant animals are bloat prevention (Jones and Mangan, 1977; Salunkhe *et al.*, 1990; Lees, 1992), increased nitrogen retention, and live weight gain (Nsahlai *et al.*, 1998). Tannins also reduce the effects of intestinal nematodes on productivity (Niezen *et al.*, 1993) and prevent excessive degradation of high-quality leaf protein in the rumen, all of which can lead to an improvement in animal nutritional status (Kumar and Singh, 1984; Mehansho *et al.,* 1987). They can protect intact cell walls against microbial or fungal attack (Zucker, 1983; Jachman, 1989) by attaching to the cellulose and fibre-bound proteins of the cell walls. The protein-tannin complexes formed may dissociate in the post rumen providing additional amino acids for digestion, absorption and utilization by the animal (Barry, 1989; McNabb *et al.*, 1996; Karchi, 1998).

Faeces and urine

When plants are ingested by animals, many substances other than nutrients are absorbed from the gut, transported through the circulatory system to various organs and tissues (Smith, 1992). These are metabolized from parent compounds to various metabolites and then excreted in some form. Metabolic wastes are excreted in the urine and faeces or as methane gas (Van Soest, 1994).

Faeces consist of materials of dietary, metabolic and endogenous origin (Church, 1988). Although tannin ingestion increases faecal nitrogen excretion in a variety of mammals

(Lindroth *et al.*, 1986; Robbins *et al.*, 1987b; Iason and Palo, 1991), the mechanism responsible for decreased growth rates appear to be more intricate than simple tannin binding (Rhoades and Cates, 1976; Mole *et al.*, 1990; Hagerman and Butler, 1991). Tannins also have been implicated in reducing fibre digestion in mammalian herbivores. The tannins bind to microbial enzymes, especially cellulase, which has been suggested as the mechanism responsible for decreasing fibre digestion (Barry *et al.*, 1986). Reduction in forage digestibility and protein availability to the animal have serious implications to the overall nutrition. The fitness of the animal is also affected because many mammalian herbivores need energy from the fermentation of fibre through gut symbionts (Foley, 1992).

Browsers produce the greatest loss of urinary energy because of the necessary excretion of absorbed terpenoids, phenols and many other secondary plant compounds (Robbins, 1993). Indigestible and unmetabolized substances of low molecular weight such as dietary phenols and essential oils may be absorbed and excreted in urine with little or no alteration (Van Soest, 1994). Thus, both urine and faeces can contain unmetabolized substances, although those that did not appear in faeces would seem to have been digested when in reality they might have been passed on to urine without energy being extracted.

Uronic acid excretion can be a useful index of the detoxification load of a wild mammal (Lindroth and Batzli, 1983). It may be valuable also as an indication of xenobiotic levels in diets of natural populations of herbivores. Glucuronic acid is used as conjugate to enhance water solubility and excretion of ingested secondary metabolites (Foley *et* al., 1999). Uronic acid excretion increased greatly with increase of dietary-phenolic concentrations, but not as a simple linear function of phenolic concentration (Lindroth and Batzli, 1983). Phenolics bound to protein would not have been assimilated and hence would not have to contribute to

an increase in uronic acid excretion.

Kidney and liver

Non-toxic substances in plants can be converted in animal tissues to metabolites that cause poisoning (Smith, 1992). The principle of toxicology emphasizes the role of both dose and physiology of the animal ingesting the toxin (Klaassen and Rozman, 1993). Animals vary widely in their capacities to biotransform absorbed non-nutritive substances in plants, and differences among species have been emphasized (Smith, 1992). When the defences in the mouth and gut fail, the secondary compound or its degradation products are absorbed and the second line of defence, in the liver and kidneys must be used (MacArthur *et al.*, 1993). Kidney and liver organs are the primary sites of detoxification and excretion of toxins by mammals (Schuster, 1964; Klaassen and Rozman, 1993). Also, absorbed compounds are transported through the hepatic portal venous system to the liver, which acts as a screen and protects other organs.

Kidney and liver organs can concentrate more toxins than all other organs combined (Klaassen and Rozman, 1993). Their sizes have been shown to change in response to plant toxins in the diet (Jung and Batzli, 1981). Voles (*Microtus ochrogaster*) (Lindroth and Batzli, 1983) and snowshoe hares (*Lepus americanus*) (Bryant *et al.*, 1985) possess enzyme systems that detoxify phenolic compounds through conjugation with glucuronic acid. Such enzymes are allocated primarily in the kidneys and liver (Freeland and Janzen, 1974) but also in the visceral tissue such as the rumen wall (Distel and Provenza, 1991).

Bacteria and histology

Ruminants often graze or lightly browsed on potentially toxic plants. Adapted rumen

organisms detoxify many, but not all secondary metabolites (Carrlson and Breeze, 1984; Cheeke, 1988). This detoxification, however, can cause adversity in ruminants by enzymatic change in the liver, kidney and gut mucosa and other tissues (Van Soest, 1994). Some chemical changes increase the toxicity of plant compounds and some cause detoxification.

Microbial populations in the gastrointestinal tract can be a significant avenue for detoxification (Freeland and Janzen, 1974). Tannins can bind with intestinal bacteria (Jones *et al.*, 1994) inhibiting their growth and digestion of various dietary components or proteins (Foley *et al.*, 1999). However, some bacteria tolerate and grow in condensed tannin media in-vitro and their protein enzymes are not affected (Jones *et al.*, 1994; Nelson *et al.* 1997). Therefore binding with tannins does not always represent a loss in activity of bacterial enzymes.

Experimental animals

Goats are selective and agile feeders (Lambert, 1990; Steele, 1996). They change their feeding habits between seasons (Steele, 1996) and can graze up to 2 m in height by standing on their hind legs (Lambert, 1990). They forage on a range of vegetation including trees, shrubs and grasses. Therefore, they consume significant amounts of tanniferous forages (Robbins *et al.*, 1987b). During dry seasons, they forage on bushes and trees which in wetter periods they ignore preferring grasses and legumes (Steele, 1990). Goats appear to be able to tolerate a wide range of tastes, show tolerance to bitter and salty tastes (Lambert, 1990; Steele, 1996), and can use a wider range of feeds than cattle and sheep. Bitter tastes are often associated with toxins (Provenza *et al.*, 1992). Animals can be conditioned to prefer bitter taste foods if they are paired with nutrients (Mehiel 1991). Goats are able to extract almost all the water from ingested dietary levels. They are less subject to high temperatures than

other species of domestic livestock, so they can survive in more arid regions (Lambert, 1990; Steele, 1996). Moreover, goats can prune bushy shrubs and increase the availability of nutritious forage for other domestic animals.

Domestic browsers, such as goats have greater tolerance of tannins than other domestic livestock species (Owen-Smith and Cooper, 1985; Kaitho *et al.*, 1997). The reduced cell wall digestion due to tannins observed in domestic sheep and cattle (Barry and Manley, 1984; Barry *et al.*, 1986) may not apply to browsing ruminants. Goats appear to defend against tannins to a better extent than do sheep by being able to detoxify tannins or their degraded products (Distel and Provenza, 1991), and having active tannase in the rumen (Begovic *et al.*, 1978). The grass diet and behavioural avoidance of tanniferous forage by domestic sheep and cattle would not require development of physiological defences against tannins. Provenza and Malechek, (1984) suggested that the salivary or plant protein consumed by domestic goats might bind with as much as 50% of the dietary tannins during ingestion.

The aims of the study were:

1) To determine the effects of tannins on the physiology of goats by studying intake of basal diet and digestibility.

2) To investigate the effects of tannins on the epithelial tissue of the gastrointestinal tract (GIT) and the presence of bacteria in various regions of the GIT of goats. Histological changes were studied using scanning electron microscopy and light microscopy (SEM) and the presence of bacteria in the GIT was studied using SEM.

3) To determine the ability of goats to detoxify dietary tannins by measuring the mass of liver and kidney; and the concentration of the urinary glucuronic acid. It was expected that the mass of the kidney and liver would increase and the urinary glucuronic acid concentration

would increase as dietary-tannin content increased as a sign of detoxification of plant phenolics by goats.

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Chapter 2

The effects of tannin ingestion on dietary parameters of Boer goats

Khanyisile R. Mbatha*, M.L.K. Bonsi, Colleen T. Downs*, I.V.Nsahlai and D. Ward

*School of Botany and Zoology and School of Agricultural Sciences and Agribusiness, University of Natal, P/ Bag X01, Scottsville, 3209, South Africa.

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Abstract

Tannins are principal forage, secondary metabolites, and are found in sorghum grains, legumes, tree parts and agricultural waste. Tannins may have positive and negative effects on ruminant digestion.

The effects of feeding diets containing different levels of condensed tannin on intake and digestibility were investigated in Boer goats. Commercialized tannin was used because of wide variation of tannin levels, which can be affected by season, species, and part of the plant. Thirty adult, male goats were fed one of five diet treatments of different tannin levels (0%, 5%, 10%, 15% and 20%) for 6 weeks following which they were kept in metabolic crates for data collection for 10 days. Dietary intake of feed decreased significantly as tannin levels increased between the diets. Digestibility of dry matter (DM) tended to decrease while the digestibility of crude proteins (CP), organic matter, neutral detergent fibre and acid detergent fibre decreased significantly with increasing tannin levels. Faecal CP increased while urinary CP decreased with increasing tannin levels. CP retention was similar in all diets, but with the same trend as DM and CP intake. The importance of these is discussed with respect to Boer goat nutrition and foraging.

Keywords: *boer goats, dietary tannin, digestibility, proteins, tannin levels*

Introduction

Anti-herbivory defences have evolved in plants. These include plant chemical secondary metabolites, that may have anti-nutrient and toxic effects on mammals (Freeland and Janzen, 1974; Rhoades, 1985; Lowry *et al.*, 1996). These secondary plant metabolites, apparently play no role in the normal pathways of plant biochemistry, and appear to have evolved purely in response to herbivore pressures (Mehansho *et al.*, 1987; Begon *et al.*, 1996). The three main classes of secondary plant metabolites are phenolics, nitrogen-containing compounds and terpenoids.

Although plants have evolved a unique set of defensive metabolites that deter attack from most herbivores, some animal species have broken through the defences by counter-adaptation especially to tannin ingestion (Rhoades, 1985). Ingestion of phenolics is common in mammalian herbivores with browsing and mixed feeding foraging strategies (McArthur *et al.*, 1991). Some mammals have mechanisms to counter attack high levels of dietary tannins (McArthur *et al.* 1991). These include production of proline rich salivary proteins that neutralize the effects of tannins (Mehansho *et al.*, 1987; Hagerman and Robbins, 1993; McArthur *et al.*, 1995).

Tannins are a group of polyphenolic substances of no single biogenic origin (Van Soest, 1994). The term tannin is used to include any naturally occurring plant polyphenolic compound of molecular weight 500-3000 with a large number of free phenolic hydroxylic groups that form strong hydrogen bonds with proteins and carbohydrates (Haslam, 1979; Swain, 1979). They may exist in mixtures with other classes of plant phenolic compounds (Reed, 1995). Tannins are widely distributed in plants including those for food, feeds and medicines (Hagerman *et al.*, 1998). They are also important in plant defence mechanisms against herbivores especially in areas with high herbivory, poor soil and dry conditions (Cooper and Owen-Smith, 1985; Zimmer

and Cordesse, 1996; Nsahlai *et al.*, 1998b).

Many aspects of the consumer's physiology, nutrition and metabolism including dietary intake, dietary protein availability, digestive enzyme activity, endogenous protein excretion, integrity of intestinal mucosa, detoxification activity, integrity of the kidney and liver post-absorptive metabolism, growth and performance are affected by plant tannin levels in ingesta (McLeod, 1974; Price and Butler, 1980; Salunke *et al.*, 1990; Van Soest, 1994). The most important aspect of tannin nutritional and toxicological effects is their ability to form complexes with proteins (Hagerman and Butler, 1981). Strength of these complexes depends on characteristics of both tannin and protein. Examinations of tannin-protein interactions clearly demonstrated that protein structure plays an important role in tannin complex formation (Hagerman *et al.*, 1998).

Interaction of tannin with protein is influenced by reaction conditions including temperature, pH, solvent composition and tannin:protein ratio; (Lees, 1992) chemistry of tannin, levels at which of ingestion, and characteristics of the consumer (Lindroth and Batzli, 1984; Hagerman *et al.*, 1992). Moreover, interaction of tannins with protein influences the appearances, and tastes of feeds (Hagerman *et al.*, 1998), the functional ecological systems (Hagerman and Butler, 1991) and agricultural systems (Kumar and Singh, 1984).

Pro-anthocyanidin (condensed tannin) and hydrozable tannin (ellagitannin and gallotannin) are the two main tannin classes (Zucker, 1983; Mehansho *et al.*, 1987). These are differentiated by their structures and reactivity towards hydrolytic agents (Church, 1988).

Condensed tannins are the most widely distributed group of tannins (Waterman and Mole, 1994).

When condensed tannins are heated with strong acids that act as catalysts, the interflavan bond is cleaved oxidatively, but is not susceptible to hydrolysis (Porter *et al.,* 1986). Also condensed tannins diminish the permeability of the gut wall by reacting with the epithelium reducing the absorption of nutrients (Fahey and Berger, 1988). These tannins may therefore act as anti-feedants because 1, they complex with food proteins; 2, they bind to microbial enzymes, reducing fermentation and degradation of fibrous tissue; 3, in general reducing their activity, and 4, they have an astringent taste that can reduce palatability (Swain, 1979).

Despite the negative effects of tannins, they also have beneficial effects on ruminants. These include bloat prevention (Jones and Mangan, 1977; Salunkhe *et al.*, 1990; Lees, 1992), increased nitrogen retention, and live weight gain (Nsahlai *et al.*, 1998a). Tannins also reduce the effects of intestinal nematodes on productivity (Niezen *et al.*, 1993) and prevent excessive degradation of high-quality leaf protein in the rumen, all of which can lead to an improvement in animal nutritional status (Kumar and Singh, 1984; Mehansho *et al.,* 1987). The protein-tannin complexes formed may dissociate in the post-rumen providing additional amino acids for digestion, absorption and utilization by the animal (Barry, 1989; McNabb *et al.*, 1996).

Faeces consist of materials of dietary, metabolic and endogenous origin (Church, 1988). Increased tannin ingestion has increases faecal nitrogen excretion in many mammals (Lindroth *et al.*, 1986; Robbins *et al.*, 1987; Iason and Palo, 1991). Tannins also have been implicated in reducing fibre digestion in mammalian herbivores (Church 1988). Reduction in forage digestibility and protein availability to the animal has serious implications on the overall nutrition.

Goats forage on a range of vegetation including trees, shrubs and grasses, therefore they consume significant amounts of tanniferous forages (Robbins *et al.*, 1987). Although they may vary their food preference seasonally (Steele, 1996) they appear to be able to tolerate a wide range of tastes, including tolerance to bitter and salty tastes (Lambert, 1990; Steele, 1996) and can use a wider range of feeds than cattle and sheep.

The aim of this study was to assess the effects of ingestion of varying levels of commercial tannins by goats on dietary intake and digestibility with the view of identifying a tolerable limit.

Materials and methods

Experimental animals

Thirty male adult, uncastrated Boer goats (*Capri hircus)*, (South African breed), aged between 3 - 4 years of age with live weight ranging from 25 to 40 kg (32 ± 1.6; mean ± SE) were used in the experiments. Goats were purchased from a local farm (Jeneve Goat Farm) near Pietermaritzburg. Experimental animals were kept at Ukulinga Research Farm, University of Natal, Pietermaritzburg (30°24'S, 29°24'E) and 700m altitude.

Composition of experimental diet

Dietary treatments consisted of the following components: commercialised condensed tannin (Wattle Bark Industry of South Africa) which was of wattle extract composition, alfalfa (*Medicago sativa*) (NCD, Pietermaritzburg), hay K11 (coast-cross) from Ukulinga Research Farm and molasses meal (NCD, Pietermaritzburg). Since the side effects of tannins were

reduced when protein content is increased, and when animals are fed low protein diets the toxic effects occur because less protein is available (Lindroth and Batzli 1984), the percentage of alfalfa, was kept constant in all experimental treatments. This was done to control the protein content in diets because alfalfa had the highest protein content compared to other feed ingredients. Experimental treatments consisted of differing percentages of condensed tannin and hay (Table 2.1). Molasses was added to all diets to increase palatability.

Experimental procedure

Goats were blocked by weight into 6 groups of five animals each. Within each group, goats were randomly assigned to the five dietary treatments. Goats were selected randomly and divided into 5 groups, and fed respective experimental diet treatments (Table 2.1) for 6 weeks acclimatization while housed in outdoor pens. Each pen was provided with a feed trough and a water container. As goats like to foul feed by direct defaecation and urination and refuse to eat contaminated feed with urine and faeces, three times daily feed troughs were cleaned and new feed offered. Initial body mass of goats obtained prior to the 6 weeks feeding regime was measured.

Three animals from each of the 5 treatments initially were moved from outside pens used during acclimatization and placed in individual metabolic crates situated in a well-ventilated animal house. To prevent the goats from jumping out, the tops of metabolic crates were fitted with fibre thread nets. Each goat continued to receive the respective diet treatment *ad-libitium*. Food quantity given to each animal was 3% of its body mass. Goats were allowed to adapt to metabolic crates for 3 days before data collection, which continued for a further 7 days. Quantity of experimental feed given and the remnants were recorded every morning during the 7-day data

collection period. Water was offered *ad libitum* each day. The second batch of goats was moved into the metabolism crates three days after finishing collection of data from the first batch. The procedure followed was as in the first batch.

Faeces

To prevent contamination of faeces with urine, faeces were collected using a bag harnessed around the body of each goat and emptied daily. Total mass of faeces voided daily was recorded every morning. Ten percent of the daily faeces voided was sampled, pooled over days on an animal basis and stored at -20°C. The pooled samples were later dried in a forced air oven at 60°C for 48 hours, ground through a 1-mm screen and stored in sealed plastic containers at room temperature pending analysis. For faecal dry matter determination, 100 g of the daily voided faeces were dried in a forced air oven at 110°C for 48 hours and reweighed.

Urine

The mesh floor of each metabolic cage had a metal tray beneath, into which goat's urine was voided and drained, into a plastic collection bucket. To prevent bacterial growth (Lindroth and Batzli, 1983) and ammonia volatilisation (Chung-MacCoubrey *et al.*, 1997) 50 ml of 10% sulphuric acid was added to cleaned plastic buckets each morning. Total daily volume of urine produced by each goat was measured every morning, 10% subsampled, pooled over days on animal basis and stored at -20°C. Urine was later thawed and subsampled for later analyses of protein, ammonia and urea contents.

Chemical analyses

Feed samples were analysed for dry matter, protein, ash, tannin, neutral detergent fibre and acid detergent fibre content as well as digestibility. Faecal samples were analysed for tannin, protein, and dry matter. All samples were ground through a 1-mm screen.

Samples of feed and faeces were analyzed for dry matter (DM), gross energy (GE) and crude protein (CP), (AOAC, 1990), neutral detergent fibre (NDF) and acid detergent fibre (ADF) (Van Soest *et al.*, 1991) on a dry matter basis. The faecal and feed condensed tannin analyses were conducted at the Center for Desert Biodiversity, Ben Gurion University of the Negev Desert, in Israel using the proanthocyanidin method for condensed tannins (Hagerman, 1995 unpublished laboratory manual). Results of condensed tannins analyses were expressed in percentage quebracho equivalent (Hagerman and Butler, 1982; Martin and Martin, 1982; Wisdom *et al.* 1987).

Urinary protein measure was determined by Bradford dye-binding assay (Bradford, 1976) (Appendix 1). Urinary crude protein was determined using the Kjeldahl method (Soil Science Department, Cedara Agricultural College).

Urinary urea concentrations were determined by an enzymatic test with salicytate using a commercial Urea S kit (Boehringer, Mannheim). Urine samples were diluted by 1:100 ml with distilled water. All absorbancies were measured in Spectronic® 20 Genesys™ Spectrophotometer (Spectronic Instruments, Inc. USA). Absorbancies were read at 600 nm using 1 cm glass cuvettes.

Urinary ammonia concentrations were determined using a chemical kit for the ultra violet method (Boehringer, Mannheim). Urine was centrifuged and diluted 1:19 ml with distilled water. Absorbance was read at 340 nm using 1 cm glass cuvettes.

Statistical analysis

Analysis of variance of data from the feeding trial was conducted using the general linear model (GLM) procedure of the Statistical Analysis Systems (1987) (SAS, version 6) for completely randomized design. The initial body mass was used as co-variate for all variables. Contrasts among the levels of tannin were done by applying the probability of difference PDIFF option of the LSMEANS (least square means) statement available in the GLM. The treatment sums of squares were further partitioned into the linear and quadratic orthogonal contrast by applying the contrast statement of the GLM.

Results

Chemical composition of the diet

Composition of the diets used in the experiment is shown in Table 2.2. The dietary range of NDF was narrow (102 -108 g/kg). This was because of increased hay content when commercial soluble tannin levels were low. Gross energy (GE) levels were similar for all dietary treatments with a difference of 1.4 % between the highest (20% tannin level) and least (0% tannin level). Similarly crude protein (CP) composition varied little between diet treatments, with 4.6% difference between diet with the least and the highest tannin content.

Intake and digestibility during the metabolism study

Dry matter (DM) intake of goats declined ($P < 0.0001$) linearly by 19 g/ % increase in tannin (Table 2.3). The linear decrease in DM intake was consequently reflected in the intake of organic matter (OM), CP, GE, NDF and ADF. The quadratic effect of dietary tannin content was not significant ($P > 0.05$) for the intake of DM, OM, CP, GE, NDF and ADF.

Digestibility of DM tended ($P = 0.054$) to vary among the different tannin treatments (Table 2.3). Tannins linearly reduced the digestibilities of CP ($P < 0.006$; 8.1 kg^{-1} /% increase in tannin) and NDF ($P < 0.0001$; 14 kg^{-1}/ % tannin). However, both the linear and quadratic effects of dietary tannin level were significant for OM, GE and ADF (Table 3) with minimum occurring at 13.41%, 13.94% and 18.76% dietary tannin contents, respectively, as these equations:

$$OM = 710.0 - 18.5\ Tannin + 0.691\ Tannin^2 \quad (2.1)$$

$$GE = 701.3 - 17.57\ Tannin + 0.63\ Tannin^2 \quad (2.2)$$

$$ADF = 656.37 - 32.65\ Tannin + 0.87\ Tannin^2 \quad (2.3)$$

For ADF there was a decrease to the minimum value and then a slight increase while the increase after the minimum value for OM and GE was bigger.

Faecal and urinary excretions

Faecal CP output (FCP) decreased ($P < 0.001$) significantly with increasing dietary tannin content (Table 2.4). The quadratic effect of dietary tannin level was significant as equation 4, with the maximum excretion occurring at tannin level of 5.83%. However, when faecal CP output was expressed per unit of CP intake it increased significantly linearly ($P < 0.001$) with increased tannin levels. Negligible tannin output was observed in faecal samples irrespective of

the dietary treatment.

$$FCP = 34.6 - 0.63\ Tannin + 0.054\ Tannin^2 \qquad (2.4)$$

The volume of urine produced decreased significantly linearly ($P < 0.007$; 17 ml/ % increase in tannin) with increasing dietary tannin levels (Table 2.4). Effect of body mass on urine excretion was not significant ($P > 0.05$). Urinary urea output tended to decrease ($P < 0.06$) linearly (6 kg^{-1}/ % increase in tannin) with increasing tannin level. When urinary urea output was expressed per digestible CP there was no significant difference ($P > 0.05$) among dietary treatments. With increased dietary tannin levels, there was a linearly decrease ($P < 0.03$; 0.4) in urine ammonia outputs. The effect of tannin levels on urinary ammonia output disappeared when ammonia output was expressed per gram digestible CP. Urinary CP output declined ($P < 0.04$) among the diets as tannin levels increased. The quadratic effect of dietary tannin level, was not significant ($P > 0.05$) for the urinary volume, CP, urea and ammonia outputs.

Discussion

The linear decline in dietary intake may be attributed to the effect of high tannin levels and the chemistry of tannin on palatability. The general decrease in the intake of all nutrients is a reflection that these are constituents of DM. Goats instinctively avoid plants and plant parts with different kinds and concentrations of tannin (Provenza *et al.*, 1990). This linearly decreased dietary intake with increased tannin level may indicate that goats limit their intake of tannin below some threshold as a defence strategy. Cooper and Owen-Smith, (1985) reported a threshold effect with plants species containing more than 5% condensed tannin while Waghorn, (1990) reported above 6% condensed tannin. In the present study, dietary intake of goats was

similar on the 0% and 5% tannin levels supporting the previous findings of a threshold. This may indicate that goats tolerate bitter or astringent tastes to a certain degree. Sometimes animals can be conditioned to prefer bitter taste of foods if they are paired with nutrients (Mehiel, 1991).

The adverse linear effect of tannins on DM digestibility could be explained by the fact that condensed tannins associate with cell walls and form detergent insoluble complexes in the digestive tract (Robbins *et al.*, 1987; Hagerman, 1989; Makkar *et al.*, 1993; Bonsi *et al.*, 1996). The decline of DM and CP digestibilities with increasing levels of tannin has been related to complexing of fibre with tannins and incapability to utilize CP post-ruminally (Van Soest, 1981), resulting in high faecal CP output (Bonsi *et al.*, 1996; Bonsi and Osuji 1997). It is expected that the ruminal indigestible tannin-CP complex is dissociated in the abomasum because of its low pH level (Jones and Mangan, 1977; Perez-Maldonado *et al.* 1995), resulting in increased available dietary proteins (Barry and Manley, 1984; Barry *et al.* 1986; Wang *et al.* 1996).

In this study, goats on low tannin level consumed more fibre, but were able to digest it better than the goats fed high tannin levels. It is known that NDF/ADF (fibre) depresses digestibility of feed in view of the need for fibre breakdown (Van Soest, 1994). However, this may be an artefact of the detergent system in the presence of tannins as tannins increase the residue determined as fibre (Reed, 1995). Based on fibre content, it was expected that goats on low tannin levels would have low digestibility as they had higher fibre intake compared to the goats fed high tannin levels. This did not accord with the present results suggesting that high tannin contents have a negative effect on fibre digestibility. The digestible energy decreases to a

minimum value of approximately 13.94% tannin level, which is similar to the trend of OM.

Faecal DM did not differ among the diets. This is probably because the animals on the control diet had relapses of diarrhoea where there was no effect of tannin. This may explain the effect of tannin on controlling the diarrhoea. However, herbivores that often feed on plants with high tannin levels have developed protective mechanisms (Zimmer and Cordesse 1996). Some of the animals synthesize salivary proteins (some rich in proline), active tannase (that is capable of breaking down ingested tannins) and/or possess increased population of bacteria that degrade protein tannin complex. Condensed tannin may be lost through faeces as a chemically measured component (McArthur and Sanson, 1991) and/or degraded and/or absorbed from the digestive tract in sheep and goats (Degen, 1995; Perez-Maldonado and Norton, 1996). This may indicate that condensed tannin in the present study had low molecular weight and was absorbed and lost from the gut without being degraded first as noticed by (Hagerman *et al.*, 1992). This could be an explanation why we observed negligible tannin in faecal samples. Similar observations have been made in another studies (Degen, 1995; Perez-Maldonado and Norton, 1996; Kiringe *et al.*, 1999). In addition, the methods used to detect tannin in faeces may have failed to cleave tannin-protein and/or tannin-fibre complexes that could have been formed during transit through the GIT.

Faecal CP decreased as tannin increased between the diets, which corresponded to CP and DM intake. When faecal CP was expressed per unit CP intake, the faecal CP increased significantly with the increase of dietary tannin showing the effect of tannins on faecal CP. Elevated faecal CP, especially in animals without functional proline rich proteins, may be the result of intestinal

proteins (Foley *et al.*, 1999), which could be the result of the loss of the brush border (Chapter 3). In addition it may be a consequence of increased production of microbe-generated proteins and high supply of dietary protein post-ruminally for animals fed higher tannin levels (Barry and Manley, 1984; Barry *et al.*, 1986; Wang *et al.*, 1996). Furthermore, where the complex is only partially dissociated, the increased flow may passed undigested through the lower part of GIT and be completely lost as excretion in faeces (Perez- Maldonado and Norton 1996).

With increasing tannin levels, the urine production decreased. Reduced urine excretion may be the result of depressed intake of feed and consequently water. Urinary urea tended to decrease with increasing tannin levels because urea excretion is possibly correlated with digestible CP intake (Van Soest, 1994). Rapid fermentation in low tannin diets, result in excessive ammonia production resulting in loss of excess ammonia as urea in urine (Dube and Ndlovu, 1998). Thus decreased urinary urea as tannin levels increased is the result of decreased protein breakdown in the rumen. The low CP excretion in urine is associated with increased levels of dietary tannin as observed by Nastis and Malechek, (1981) possibly reflecting requirement of protein by animals.

Tannin levels used in the present study, cover the range of tannin levels that goats are normally exposed to in the wild (Williams, 1930). Consequently it is not expected that the tannin levels influenced direction and extent of response.

Conclusion

Increased tannins adversely affected feed intake. The wastage of CP through microbial degradation in the rumen is reduced possibly resulting in the low urinary urea or ammonia. High

dietary tannin levels have a negative effect on fibre digestibility. Elevated faecal CP could be the result of the loss of the brushed border of the GIT.

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Table 2.1 *Physical composition of experimental diet treatments (fresh weights)*

Diet	Alfalfa (%)	Hay (%)	Molasses (%)	Tannin (%)
1	50	40	10	0
2	50	35	10	5
3	50	30	10	10
4	50	25	10	15
5	50	20	10	20

Table 2.2 Chemical *composition of experimental feeds on dry matter bases (NDF = neutral detergent fibre; ADF = acid detergent fibre; GE = gross energy; CP = crude protein. Tannin is in quebracho equivalent and not on a dry mass basis)*

Parameters	Diet				
	1 (n=6)	2 (n=6)	3 (n=6)	4 (n=5)	5 (n=6)
NDF (g/kg)	638	596	554	512	470
ADF (g/kg)	441	416	391	366	341
GE (MJ/kg/day)	17446	17507	17568	17630	17691
Ash (g/kg)	101	97	93	89	67
CP (g/kg)	108	107	105	103	102
Tannin (quebracho)	0.38	21.11	41.88	62.69	83.53

Table 2.3 *Intakes and digestibilities (mean ± SE) of Boer goats fed five respective levels of tannin content (dry matter; NDF = neutral detergent fibre; ADF = acid detergent fibre; GE = gross energy)*

	Diet					Significance							Optimal point
Parameter	1 (0%) (n=6)	2 (5%) (n=6)	3 (10%) (n=6)	4 (15%) (n=5)	5 (20%) (n=6)	Diet P-value	Linear P-value	Quadratic P-value	1 vs. 2 P-value	1 vs. 3 P-value	1 vs. 4 P-value	1 vs. 5 P-value	Min or Max
Intake													
Dry matter (g/day)	854 ± 37.7	857 ± 38.8	671 ± 37.7	559 ±41.4	528 ± 41	0.0001	0.0001	0.97	0.95	0.002	0.0001	.0001	
Organic matter (g/day)	768 ± 34.5	774 ± 35.4	609 ± 34.5	509 ± 37.8	492 ± 37.5	0.0001	0.0001	0.9	0.9	0.003	0.0001	0.0001	
Crude protein (g/day)	93 ± 3.9	91 ± 4	70 ± 3.9	58 ± 4.3	53 ± 4.3	0.0001	0.0001	0.9	0.85	0.0007	0.0001	0.0001	
GE (MJ/day)	14.9 ± 0.7	15 ± 0.7	11.8 ± 0.7	9.9 ± 0.7	9.3 ± 0.7	0.0001	0.0001	0.99	0.91	0.003	0.0001	0.0001	
NDF (g/day)	545 ± 21.2	512 ± 22.7	372 ± 21.2	286 ± 23.3	251 ± 23.1	0.0001	0.0001	0.6	0.28	0.0001	0.0001	0.0001	
ADF (g/day)	377 ± 14.9	357 ± 15.3	263 ± 14.9	204 ± 16.4	182 ± 16.2	0.0001	0.0001	0.6	0.37	0.0001	0.0001	0.0001	
Digestibility													
Dry matter (g/kg)	666 ± 22.0	620 ± 22.6	596 ± 22	562 ± 24.1	607 ± 23.9	0.054	0.03	0.06	0.16	0.03	0.004	0.08	
Organic matter (g/kg)	707 ± 22.0	638 ± 22.6	607 ± 22	570 ± 24.2	625 ± 23.9	0.005	0.005	0.010	0.03	0.004	0.0003	0.2	13.41
GE (kJ/MJ)	699 ± 22.4	629 ±22.9	604 ± 22.4	561 ± 24.6	611 ± 24	0.006	0.004	0.02	0.04	0.006	0.0004	0.0147	13.94
Crude protein (g/kg)	649 ± 31.8	538 ± 32.6	540 ± 31.8	467 ± 34.9	483 ± 34.6	0.006	0.001	0.2	0.02	0.2	0.0008	0.002	
NDF (g/kg)	732 ± 36.9	619 ± 37.9	537 ± 36.9	447 ± 40.5	462 ± 40.2	0.0001	0.0001	0.104	0.04	0.001	0.0001	0.0001	
ADF (g/kg)	655 ± 36.7	509 ± 37.7	442 ± 36.7	333 ± 40.0	359 ± 39.9	0.0001	0.0001	0.05	0.01	0.0004	0.0001	0.0001	

Table 2.4 *Excretion of CP products of Boer goats fed five respective levels of tannin content (mean ± SE)*

	Diet treatment (% tannin)					Significance							Optimal point
Parameter	1 (0%) (n=6)	2 (5%) (n=6)	3 (10%) (n=6)	4 (15%) (n=5)	5 (20%) (n=6)	P-value	Linear F-value	Quadratic F-value	1 vs. 2	1 vs. 3	1 vs.4	1 vs.5	Min. or Max.
Faeces													
CP (g/day)	32.4±2	41.7± 2.1	32.3±20	30.5± 2.2	26.5± 2.2	0.001	0.003	0.023	0.004	0.98	0.54	0.07	5.83
CP/CP intake (g/g CP intake)	0.35± 0.03	0.46± 0.03	0.46±0.03	0.53±0.03	0.52±0.04	0.006	0.001	0.2	0.023	0.024	0.001	0.002	
Urine:													
Volume (ml/day)	736 ± 60	727± 61	569± 60	554± 66	399± 65	0.007	0.0003	0.5	0.91	0.06	0.05	0.001	
CP (g/day)	40 ± 4.4	39 ±4.5	36 ± 4.4	29 ± 4.8	20 ±4.8	0.04	0.003	0.3	0.88	0.58	0.10	0.007	
urea (g/day)	264 ± 28	196 ± 29	186 ± 28	158 ± 31	144 ± 30	0.06	0.007	0.4	0.1	0.06	0.0.2	0.008	
urea/ digestible CP (g/day/kg)	4.5 ± 1.2	4.3 ± 1.2	5.3 ± 1.2	6.3 ± 1.3	6.8 ± 1.3	0.6	0.1	0.8	0.93	0.63	0.30	0.19	
Ammonia (g/day)	7.8 ± 1.3	6.6 ± 1.3	5.2 ± 1.3	2.9 ± 1.4	2.1 ± 1.4	0.03	0.002	0.9	0.51	0.15	0.01	0.006	
Ammonia/ digestible CP (g/day/kg)	0.13 ± 0.03	0.14 ± 0.03	0.14 ± 0.03	0.14 ± .03	0.11 ± 0.03	0.9	0.4	0.7	0.85	0.85	0.55	0.57	

Chapter 3

The effects of feeding different levels of tannin on the gastrointestinal tract tissues of Boer goats

Khanyisile R. Mbatha*, Colleen T. Downs* and Ignatius V. Nsahlai

*School of Botany and Zoology and School of Agricultural Sciences and Agribusiness,

University of Natal, P/Bag X01, Scottsville, 3209, South Africa.

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Abstract

This study was conducted to determine the effects of different levels of dietary tannin on the gastrointestinal tract (GIT) histology and the presence of bacteria along the GIT. Five groups of Boer goats were fed diets containing 0, 5, 10, 15 and 20% tannin levels for 6 weeks before data collection. Differences in the histopathology of the oesophagus, reticulum, rumen, abomasum and duodenum were evaluated. Animals on the control diet had more protozoa present in the GIT than the other diets. Number and types of bacteria observed in the reticulum and rumen increased with tannin level in the diet. These may be responsible for the degradation of tannin protein complexes. Few bacteria were observed in the abomasum. This was possibly a consequence of the harsh environment (low pH level) of the abomasum. There was a loss of epithelial cells and erosion of microvilli in the duodenum with increased tannin levels, which would impair absorption of nutrients. The width of the keratinized GIT epithelial layer increased and villi height decreased as tannin levels increased which could further reduce nutrient absorption. Consequently, condensed tannins have a negative effect on the histopathology of the Boer goats.

Keywords: *bacteria, gastrointestinal tract, goats, histology, tannin*

Introduction

Ruminants that graze or browse on toxic plants have adapted rumen organisms that detoxify many, but not all secondary metabolites (Carrlson and Breeze, 1984; Cheeke, 1988). However, the detoxification process may cause adverse effects in ruminants as a consequence of enzymatic change in the liver, kidney and gut mucosa and other tissues (Van Soest, 1994). Some chemical changes increase the toxicity of plant compounds and some cause detoxification.

Microbial populations in the gastrointestinal tract (GIT) are a significant avenue for detoxification (Freeland and Janzen, 1974). Tannins can bind with intestinal bacteria inhibiting their growth and the digestion of various dietary components or proteins (Jones *et al.*, 1994; Nelson *et al.*, 1997; Foley *et al.*, 1999). However, some bacteria tolerate and grow in condensed tannin media and their protein enzymes are not affected (Jones *et al.*, 1994; Brooker *et al.*, 1994; Nelson *et al.*, 1997). Therefore, binding with tannins does not always represent a loss in activity of bacterial enzymes. Previous studies have isolated intestinal bacteria from many herbivores that degrade tannin protein complexes (Osawa, 1990, 1992; Osawa and Sly, 1992; Brooker *et al.*, 1994; Nelson *et al.*, 1994). These include bacteria from goats that prevent or minimize the digestibility reducing effects of tannin (Brooker *et al.*, 1994). Foley *et al.* (1999) proposed that if the condensed tannins do not reduce protein digestion, then there is probably a specialised bacterial population that can degrade tannin protein complexes. Even though bacteria can degrade the complexes, the digestion is still reduced by tannin in some of the species. The bacterial effect cannot be effective if the passage rate is fast and bacterial numbers are low to speed up the net dissociation (Foley *et*

al., 1999). For example, bacterial dissociation of condensed tannin protein complexes occurred in ringtail possums (O'Brien *et al.*, 1986; McArthur and Sanson, 1991) which have a slow rate of digestion for their size.

Increased dietary tannin content may result in variable damage of the epithelial tissue along the GIT resulting in reduced nutrient absorption and may affect the presence of microbes that could dissociate tannin protein complexes. There have been few investigations of this.

This study investigated the effects of tannins on the epithelial tissue of the GIT and presence of bacteria in the various regions of the GIT of the goats fed different tannin levels (0 - 20%). The presence of bacteria in the GIT was studied using scanning electron microscopy (SEM) and histological changes were studied using SEM and light microscopy.

Materials and methods

Animals and diets

Animals and experimental design are presented in detail in Chapter 2. Goats were blocked by weight into 6 groups of five animals each. Within each group, goats were randomly assigned to the five dietary treatments. Goats were selected randomly and divided into 5 groups, and fed respective experimental diet treatments (Table 2.1) for 6 weeks acclimatization while housed in outdoor pens. Each pen was provided with a feed trough and a water container. As goats like to foul feed by direct defaecation and urination and refuse to eat urine and faeces contaminated feed, three times daily feed troughs were cleaned and new feed offered.

Animals were removed from the outside pens to a well-ventilated animal house. Goats were kept in metabolic crates for 3 days to acclimitize before 7 days of data collection for metabolic studies. On the seventh day, goats were slaughtered by electrical stunning followed by exsanguination in the abattoir facility at Ukulinga Research Farm, University of Natal, Pietermaritzburg.

Scanning Electron Microscopy (SEM)

Immediately after slaughter of each goat (n = 29), tissue samples were obtained for SEM study from the oesophagus, rumen, reticulum, abomasum and duodenum. Samples were fixed in 3 % buffered glutaraldehyde (0.1 M; pH 7.2) for 24 h and thoroughly washed in 0.05 M sodium cacodylate buffer twice for 30 min. Samples were post-fixed in 2% osmium tetroxide for 2 h, buffer rinsed for 30 min, dehydrated in a graded ethanol series and transferred to the critical point drying (CPD) baskets under 100% alcohol. The Hitachi HCP-2 critical point dryer was used to dry the samples. Samples were sputter coated with gold palladium and viewed in a Philips XL30 Environmental Scanning Electron Microscope (ESEM) at 15 - 20 kV and 6500X magnification.

Light microscopy

Small cross-sections of the rumen, reticulum, abomasum duodenum, kidney and liver were excised, from the goats (n = 29) at the same time as the SEM sampling for light microscope preparation and examination. These samples were placed in small vials and fixed in Bouin's solution for 24 h. They were removed and placed in 70% alcohol until dehydration. Cross sections were cut perpendicularly to the long axis and stained with haematoxylin and eosin

for light microscopy viewing. Sample slides were examined for each section of the GIT for histological tissue changes. Villi height was measured from the crypt opening to the tip of the villus (Makinde *et al.*, 1996). From the base of the crypt to the level of the crypt opening was measured as crypt depth. The villi height and crypt depth were measured in triplicate specimens by the same individual at 10X magnification with a binocular light microscope using an ocular micrometer. Epithelial tissue changes were examined in all the samples. Degree of keratinization of the epithelium was measured.

Parasites

Faecal samples collected during the metabolic trials were subsampled. These were taken for microscopic examination at Allerton Provincial Veterinary Laboratory (Pietermaritzburg). Levels of parasite in the faeces were investigated because one goat that died had 60800 coccidial oocysts per gram although the cause of the death was cachexia.

Statistical analysis

Data were analysed using analysis of variance of STATISTICA (release 6, 1998; Statsoft, USA). Post hoc Scheffe tests were made. Mean values with their standard error are presented. The analysis of variance of coccidial oocysts was conducted using the general linear model (GLM) procedure of the Statistical Analysis Systems (1987) (SAS, version 6) for completely randomized design. The treatment sum of squares was further partitioned into the linear and quadratic orthogonal contrast by applying the contrast statement of the GLM.

Results

SEM

Few bacteria were observed in the oesophagus irrespective of the dietary tannin level (Figure 3.1a). Only one goat fed 5% tannin had numerous bacteria in the oesophagus (Figure 3.1b). Compared with the other regions of the GIT, all the oesophagus samples showed nodule like lesions in the epithelial tissue (Figure 3.1c). Flat appearance and erosion were observed in the oesophageal epithelial tissue of animals fed high tannin levels (Figure 3.1d). In addition, few mucus and goblet cells were observed in these samples.

In the rumen and reticulem, goats fed low tannin levels had little or no epithelial erosion, few goblet cells and bacteria compared to goats fed high tannin levels (Figure 3.2a, b and c). Protozoa were observed in goats fed the control diet (Figure 3.2d and e). As tannin level increased mucus secretion increased (Figure 3.3a, b, and c). Bacteria of different morphotypes were observed in the rumen and reticulum (Figure 3.4a, b, and c) irrespective of diet. There was flatness of the surface of reticulum and rumen and the erosion of the epithelial tissue with increased tannin levels (Figure 3.4e, f and g).

Generally the abomasum had few or no bacteria (Figure 3.5a), more mucus, goblet cells and pits than the rest of the gastrointestinal tract irrespective of the tannin level (Figure 3.5b). Although the bacteria were few, they had different shapes and sizes. (Figure 3.5c and d). Samples obtained from goats fed control diet showed less mucus, goblet cells and pits compared to other diets (Figure 3.5c, d and e).

In the duodenum, the length of the microvilli decreased as tannin level increased (Figure 3.6a, b and c). The control samples had microvilli on the surface epithelial cells which were tightly packed forming a dense smooth surface (Figure 3.6a). However, as tannin level increased, less densely packed microvilli and more goblet cells were observed (Figure 3.6b and c; 3.7 a). In addition, partial to total denudation of microvilli occurred in certain patches of the epithelium (Figure 3.6b and 3.8a). Sometimes the microvilli cells were sunken resulting in partial loss of the brushed border (Figure 3.7a b, c and d) and/or a prominence of mucus (Figure 3.8a, b, c, and d).

Light microscopy

Histological studies revealed some structural changes in the GIT epithelial tissue. No significant difference ($P > 0.05$) was observed in the width of the epithelial layer of the reticulum and omasum among dietary tannin levels (Table 3.1; Figure 3.9a and c). However, the width of epithelial layer of the rumen and abomasum increased significantly with tannin levels ($P < 0.007$; $P < 0.021$; Figure 3.9b and d), respectively. The abomasal epithelial width, was significantly different between control diet and 15% tannin-containing diet ($P < 0.03$). Villi height in the duodenum differed significantly among the diets ($P < 0.011$, Figure 3.9e), whereas the cryptic depth was not affected ($P > 0.05$, Figure 3.9f). Degree of keratinization of the epithelium increased with tannin level in reticulum ($P < 0.004$), rumen ($P < 0.006$), omasum ($P < 0.012$) and abomasum ($P < 0.00001$).

Parasites

The coccidial oocysts (CO) count differed significant among the diets ($P < 0.026$, Table 3.2).

The coccidial oocysts decreased as dietary tannin increase to 10.5% tannin level, then increased slightly. The quadratic effect of the coccidial oocysts count can be represented by the equation (3.1). Minimum number of coccidial oocysts count was observed at the 10% tannin level.

$$CO = 5.079 - 0.343\ \text{Tannin} + 0.017\ \text{Tannin}^2 \qquad (3.1)$$

Discussion

The presence of low numbers of bacteria in the oesophagus and abomasum contrast the reticulum and rumen that both had multiple bacteria irrespective of tannin level. The latter generally provides a favourable environment for bacterial growth (Church, 1988). In general, different microbial groups have preference for characteristic pH level, although most bacteria are neutrophiles (Prescott *et al.*, 1993). Therefore, decreased number of bacteria in the abomasum may be a result of the harshness of the low pH level of the abomasum, which affects bacteria survival.

Microbial presence can be an important route for detoxification (Freeland and Janzen, 1974). Tannins can bind with intestinal bacteria inhibiting their growth and digestion of various dietary components and proteins (Jones *et al.*, 1994; Nelson *et al.*, 1997). However, some bacteria tolerate and grow in condensed tannin media and their protein enzymes are not affected because binding with tannins, does not always represent a loss in activity of bacterial enzymes (Jones *et al.*, 1994; Nelson *et al.*, 1997). Previously, it was reported that there is less interaction between tannin and gram-negative bacteria because of the complex structure of their cell walls due to high lipid content and phospholipids that can prevent tannin

penetrating the bacterial wall (Sotohy *et al.*, 1995; 1997). *Streptococcus caprinus*, are the ruminal bacteria observed in feral goats that can degrade tannin protein complexes, although not observed in domestic goats and sheep (Brooker *et al.*, 1994). Contrary to Sotohy *et al.* (1995, 1997), these bacteria are gram-positive, occurring in short chains, able to grow and form clear zones on complex media containing at least 2.5% (w/v) condensed tannins derived from *Acacia aneura.* Because goats are not highly affected by tannin compared to cattle and sheep (Domingue *et al.*, 1991; Degen *et al.* 1995; Perez-Maldonado and Norton, 1996), it may be possible that among the increased population of bacteria observed in reticulum and rumen, there are specialized tannin- protein degrading bacteria (Foley *et al.*, 1999).

Increased dietary tannin levels resulted in damage of epithelial tissue along the GIT. This probably resulted in reduced nutrient absorption. The increased mucus, goblet cells and the pits are indicative of the physiological changes of the tissues possibly during the process of negating the tannin effects. Pits among the cells corresponded to goblet cells whose mucus has been extruded

Nodule like lesions observed in the oesophagus may not indicate the effect of tannins on the oesophageal epithelium because these were observed in all oesophageal samples. The effect of tannin on the epithelial tissue of the oesophagus was not observed because the flatness of the epithelial tissue was on the entire oesophageal samples irrespective of the diet. Duodenal surfaces that consisted of less tightly packed, shorter and denuded microvilli were observed as dietary tannin levels increased, showing the effects of tannin on the epithelial tissue of the small intestine. Furthermore, when goats were slaughtered it was observed that the colour of

the small intestines darkened with increased dietary tannin levels. This may suggest that the small intestine of goats is playing a role in the absorption and detoxification of tannins, as there was no tannin in the faeces (Chapter 2). However, it is possible that the methods used to detect tannin in faeces may have failed to cleave tannin-protein and/or tannin-fibre complexes that could have been formed during transit through the GIT (Chapter 2). As a second line of defence against secondary metabolites and their degradation products, detoxification may occur in the liver and kidney provided defences in the mouth and gut have failed (Smith, 1992). In the present study, it appeared that tannins and their degradation products are absorbed and detoxified in the GIT before they reach the liver and kidney (Chapter 4). This possibly explains why it is claimed that the kidney and liver of goats do not detoxify tannins (Silanikove *et al.*, 1996).

Observations using a light microscopy, showed that condensed tannin did not have adverse effects on the morphology of the intestinal tract of rats (Sell *et al.*, 1985) as opposed to tannic acid (Mitjavila *et al.*, 1977). In the present study, histological studies revealed some structural changes in the GIT epithelial tissue. The epithelial keratinized layer increased as tannin levels increased. Epithelial tissue of the small intestine has to be simple and thin to permit maximum absorption of nutrients. Thus as the keratinized epithelial tissue increased, it is expected that absorption of nutrients would reduce leading to a lower health status of goats. Furthermore, duodenal villi height decreased as tannin levels increased, supporting that nutrient absorption was affected with increased tannin intake. Therefore, tannin may have detrimental effect on the absorption of nutrients in the GIT. It is interesting to note that some regions of the GIT did not have the thick layer of the epithelium (reticulum and

omasum), indicating that some areas of the GIT may have normal absorption; as such the effect of tannin might not be highly pronounced in goats. With increased tannin the short, denuded and mucus-covered microvilli will have a detrimental impact on the absorption of the nutrients by goats resulting in chronic health changes e.g. cachexia. Furthermore, duodenal villi height decreased as tannin levels increased, supporting the view that nutrient absorption could be affected by increased tannin intake.

It was observed that lambs grazing on sulla (*Hedysarum coronarium)* with high tannin level (10-20%) were healthier with no signs of diarrhoea compared to lambs grazing on lucerne alfalfa (*Medicago* sativa) which contained low condensed tannin (0.1 - 0.2 %) (Niezen *et al.*, 1993). In the present study most of the animals in the control group had diarrhoea during metablic study suggesting that tannin have an effect on controlling the diarrhoea. One animal fed 15% tannin content died. Cachexia was the cause of the death according to the post mortem, while the animal haboured 60800 coccidial oocysts per gram faeces. Faecal examination of goats for parasite presence showed that tannin reduces parasite load in sheep (Niezen *et al.*, 1993; 1995; Butter *et al.*, 2000; Athanasiadou *et al.*, 2000; Houdijk *et al.*, 2000).

Coccidiosis is a protozoal infection of the intestines of domestic animals caused by *Eimeria* and *Isospora* species (Hunter, 1994). These organisms are host specific (Hunter, 1994) although a number of different *Eimeria* species may infect a specific host (Hall, 1982). Some animals retain low-grade infections throughout life without showing symptoms. Symptoms, depending on the severity, can be loss of appetite, weakness, loss of weight, and death (Hall,

1982). Usually the younger animals become ill, whereas older hosts are generally healthy. The coccidia attack and destroy the mucosa of the intestine, which results in enteritis and diarrhoea (Mönnig and Veldman, 1982).

The coccidial oocysts count decrease with increasing tannin levels suggesting that dietary tannins has an effect to reduce the parasites as observed previously (Niezen *et al.*, 1993; 1995; Butter *et al.*, 2000; Athanasiadou *et al.*, 2000; Houdijk *et al.*, 2000). Usually animals carry a large number of non-pathogenic coccidial oocysts. It was observed that coccidial oocysts decreased to a minimum at tannin level of approximately 10%. This implies that the dietary tannin may act in conjuction with the immune system to reduce the population of this class of parasites. Probably, as tannin level increased above 10% the immune system may have failed to control the coccidial oocysts.

Conclusion

Detoxification of tannin and its degradation products appears to partly occur in the GIT. This explains the negligible tannin content observed in faecal samples (Chapter 2). Keratinization of epithelial tissue probably contributed to the reduced dietary intake observed as tannin levels increased (Chapter 2) reducing the passage of nutrients. In particular, increased epithelial thickness, flat appearance and erosion of the epithelial surface, and shorter microvilli; and shorter villi as tannin levels increased, may explain that reduced feed intake was a result of negative post-ingestion effects of tannins in the GIT. Overall, the increased tannin levels appear to affect nutrient absorption because of the effects on the histology of the GIT.

Dietary tannin may act in conjunction with the immune system to reduce the population of coccidial oocyst parasites. It was observed that coccidial oocysts decreased to a minimum at tannin level of approximately 10%.

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Table 3.1 *Effects of feeding different tannin levels on the gut physiology of Boer goats ($\mu m \pm SE$)*

	Diet treatments (Tannin %)				
	0 (n = 6)	5 (n = 6)	10 (n = 6)	15 (n = 5)	20 (n = 6)
Reticulum					
Epithelium layer	139.8 ±16.9	110.9 ±12.6	119.2 ± 8.4	127.5 ± 6.7	103.8 ±8.1
Keratinized layer	22.1 ± 1.8	37.6 ± 4.2	36.6 ± 4.8	43.4 ± 2.9	34.5 ± 2.7
Rumen					
Epithelium layer	146 ± 9.6	97.5 ± 9.3	118.3 ± 12.2	99.0 ±8.6	111.7 ± 4.4
Keratinized layer	21.5 ± 1.8	32.0 ± 5.4	37.2 ± 3.6	39.1 ± 3.4	42.6 ± 3.5
Omasum					
Epithelium layer	173.5 ± 22.6	141.8± 13.1	140.8 ± 15.1	119.5 ± 6.6	112.1 ± 9.0
Keratinized layer	17.4 ± 2.3	30.8 ± 7.7	41.5 ± 4.9	38.5 ± 3.8	43.9 ± 4.3
Abomasum					
Epithelium layer	441.7 ± 47.7	471.0 ± 88.9	594.2 ± 76.9	831.9 ± 49.6	571.7 ± 84.4
Keratinized layer	12.5 ± 1.1	87.6 ± 15.3	181 ± 30.1	271.6 ± 14.2	223.5 ± 34.8
Duodenum					
Villus height	680 ± 8.5	476.0 ± 83.3	426.5 ± 104.8	365.3 ± 90.3	240.5 ± 65.7
Crypt height	262 ± 99.1	263.8 ± 99.7	290.5 ± 69.7	365.0± 30.2	484.0 ± 18.0

Table 3.2 *Coccidia oocysts per gram (log transformation ± SE) in Boer goats fed different levels of tannin*

Tannin level (%)	Coccidia oocyst
0	9.98 ± 0.54
50	8.94 ± 0.55
10	7.91 ± 0.54
15	8.29 ± 0.59
20	10.34 ± 0.59
P-value	0.026
Linear effect	0.97
Quadratic effect	0.002
Optimal value	10.5

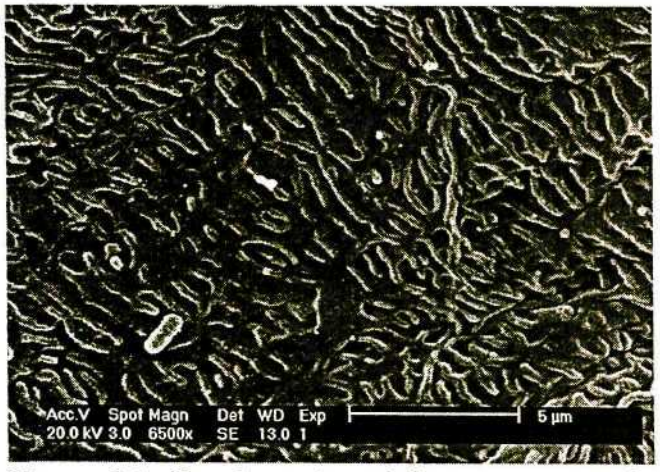


Figure 3.1a Few bacteria and flat appearance of the epithelial layer in the oesophagus

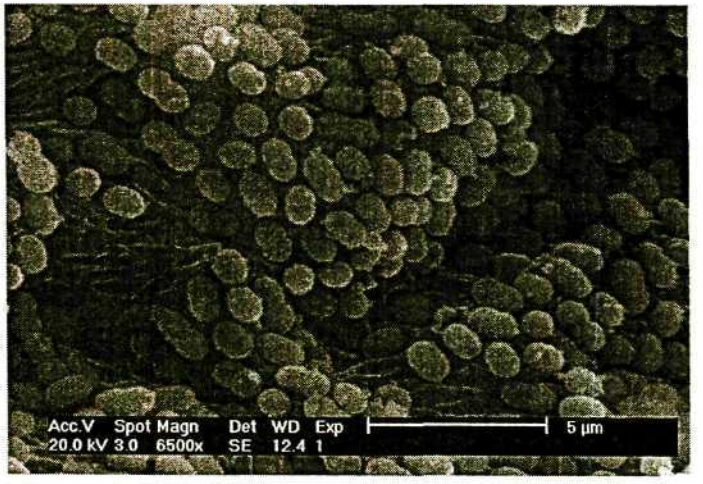


Figure 3.1b Only one goat (5% tannin) had numerous bacteria in the oesophagus

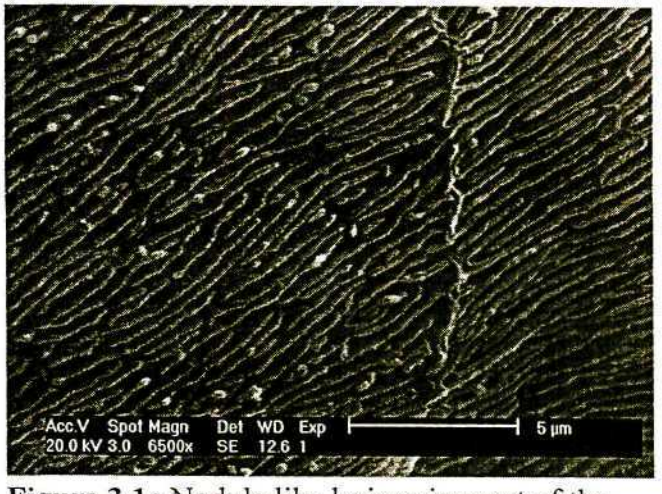


Figure 3.1c Nodule like lesions in most of the oesophageal samples

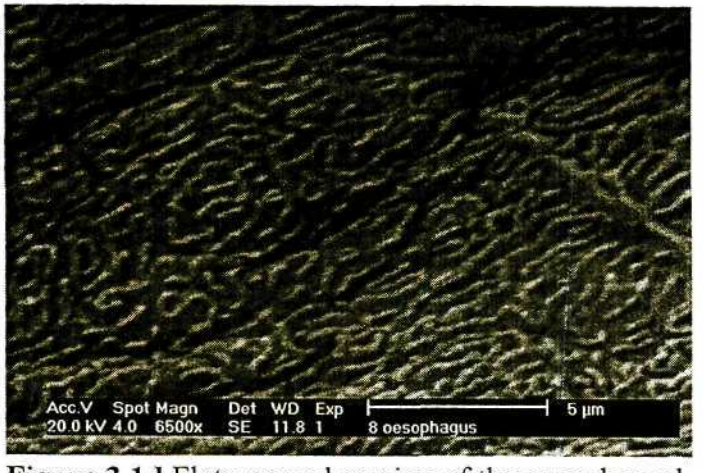


Figure 3.1d Flatness and erosion of the oesophageal epithelial layer

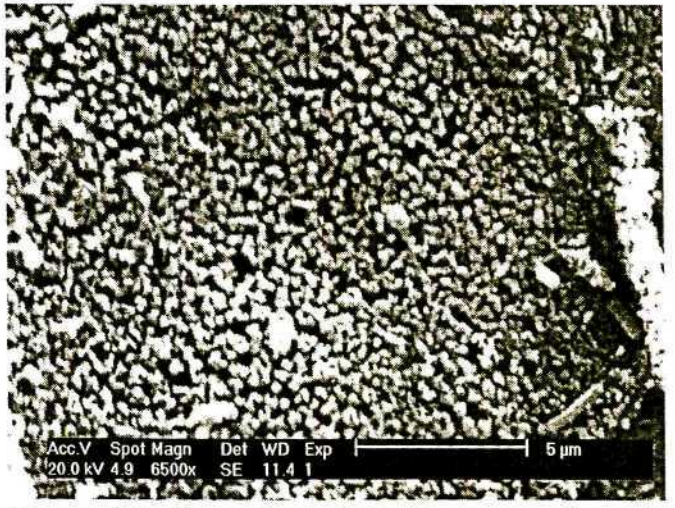


Figure 3.2a Fewer bacteria in the rumen of a goat fed control diet

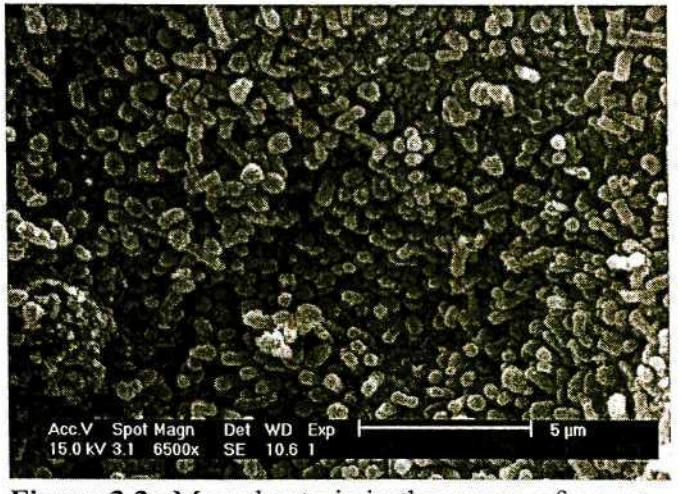


Figure 3.2c Many bacteria in the rumen of a goat fed the 20% tannin

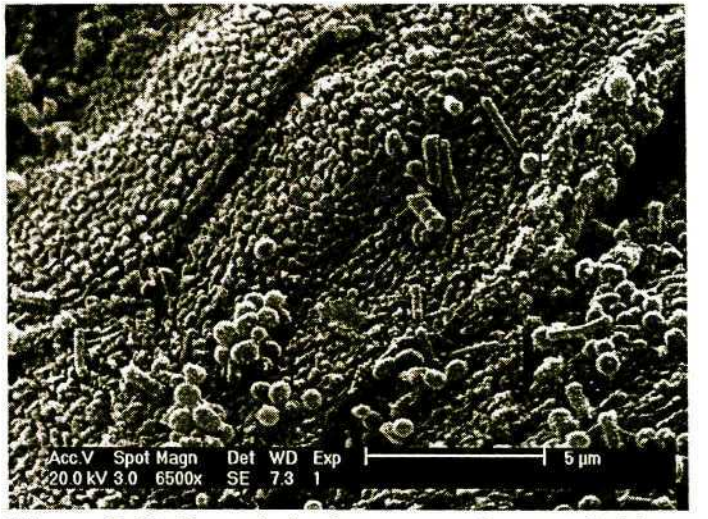


Figure 3.2b Bacteria in the rumen of a goat fed the 5% tannin

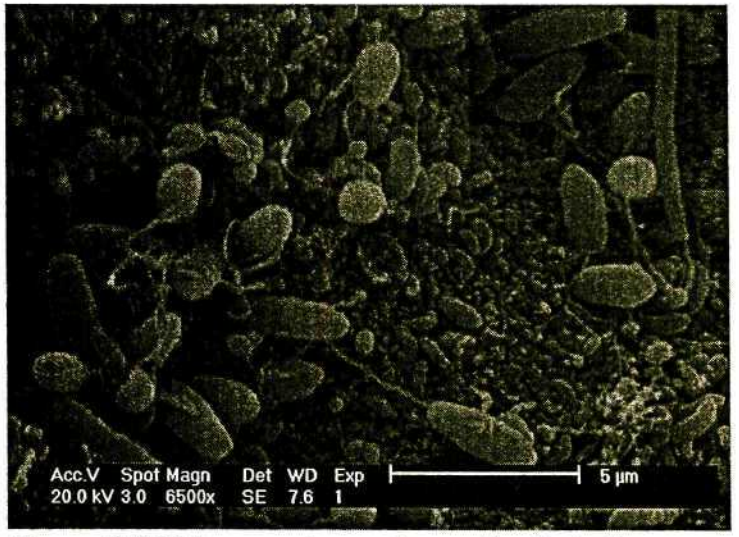


Figure 3.2d Many protozoa observed only in the control group

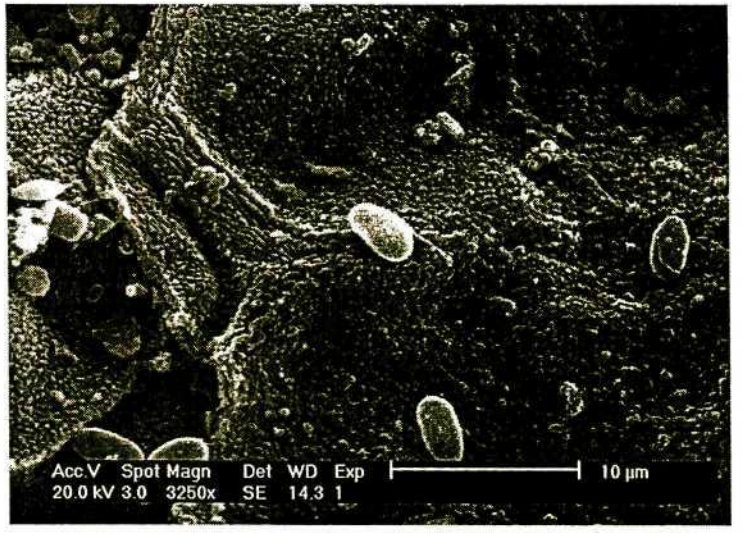


Figure 3.2e Few protozoa observed in goats fed 5% tannin

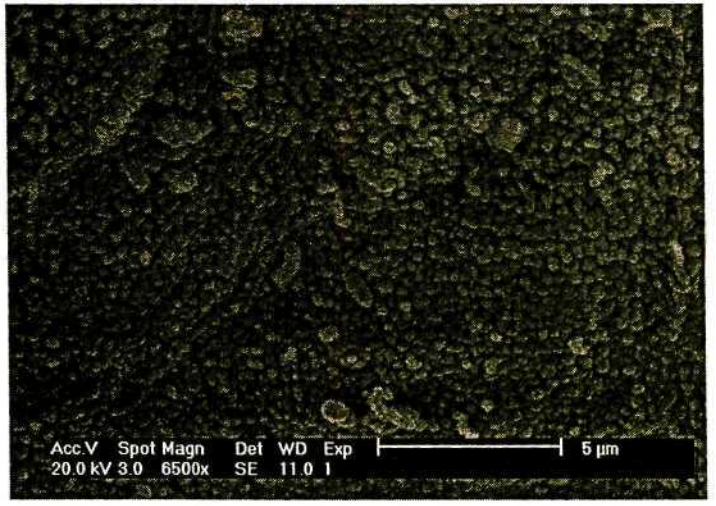


Figure 3.3a Little secretion of mucus showing surface tissue in the rumen of a goat fed the 5% tannin

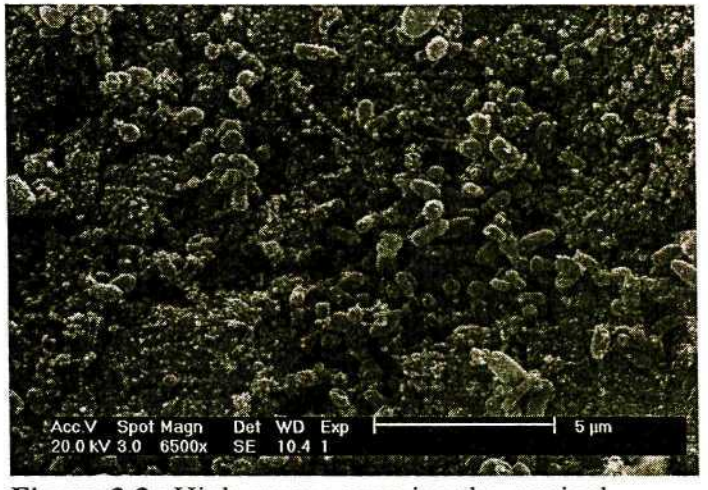


Figure 3.3c High mucus covering the ruminal surface tissue of the 20% tannin

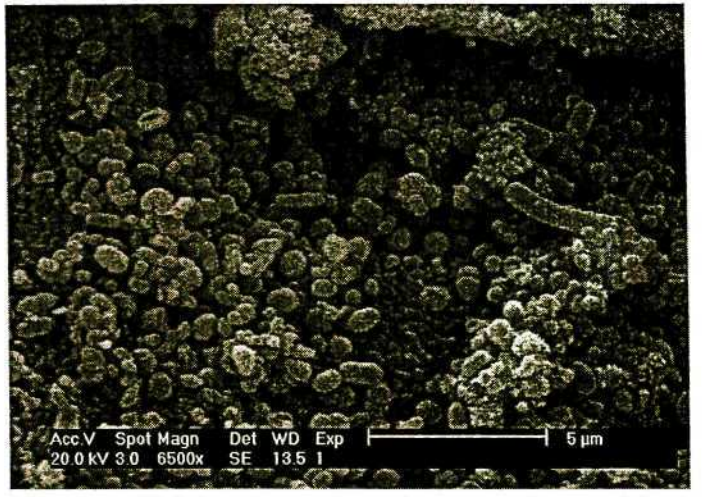


Figure 3.3b More bacteria and mucus observed in the rumen of a goat fed the 15% tannin

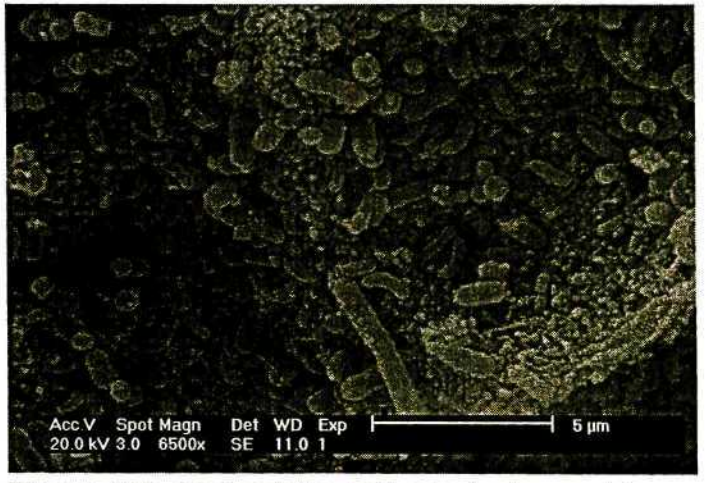


Figure 3.4a Chained shaped bacteria observed in rumen of a goat fed 0%

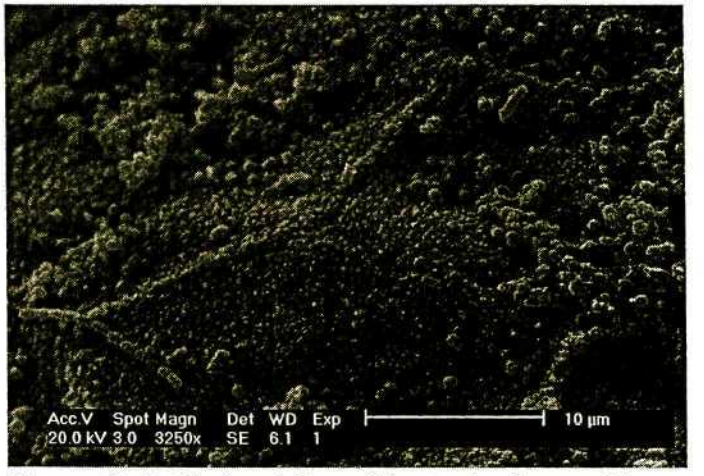


Figure 3.4b Chained shaped bacteria observed in rumen of a goat fed 5% tannin

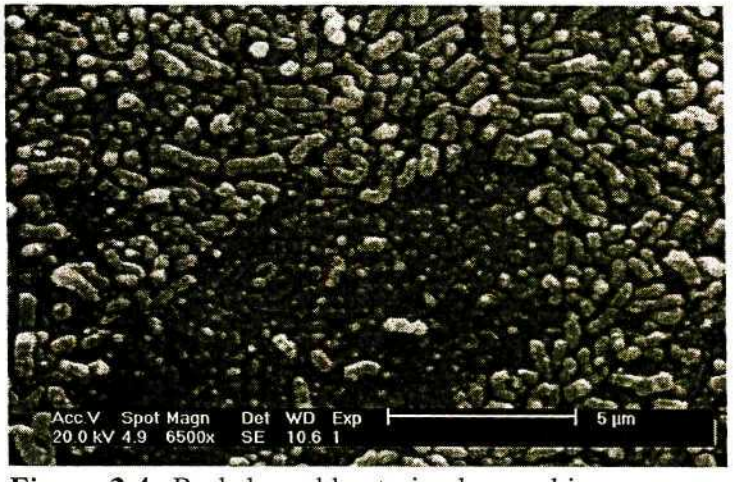


Figure 3.4c Rod shaped bacteria observed in reticulum of a goat fed 10% tannin

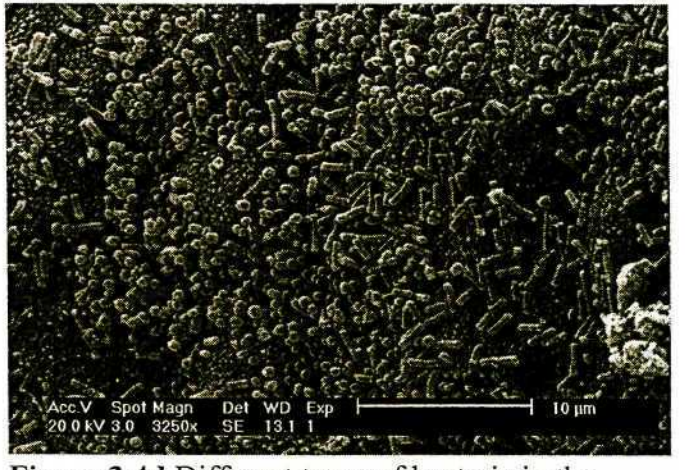


Figure 3.4d Different types of bacteria in the reticulum of a goat fed 15% tannin

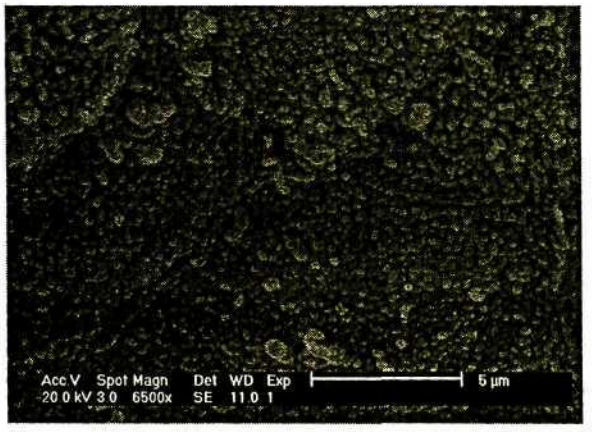


Figure 3.4e Surface appearance in the rumen of a goat fed 5% tannin

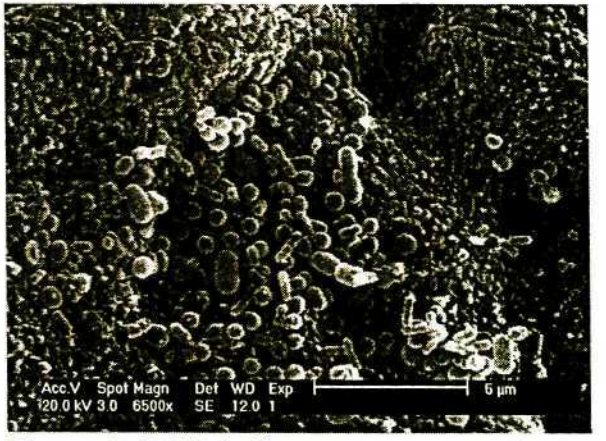


Figure 3.4f Slight flatness appearance in the rumen of a goat fed 10% tannin

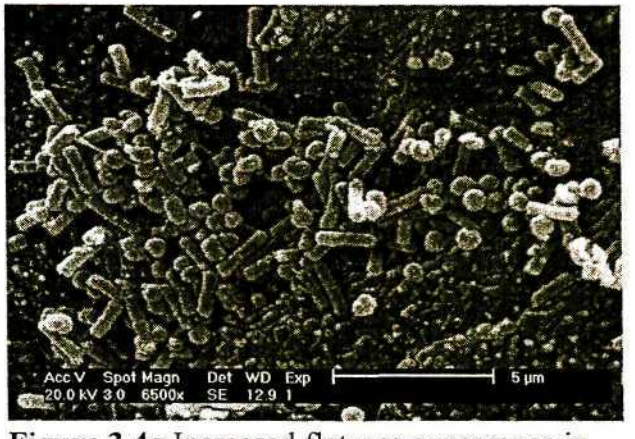


Figure 3.4g Increased flatness appearance in the reticulum of a goat the fed 15% tannin

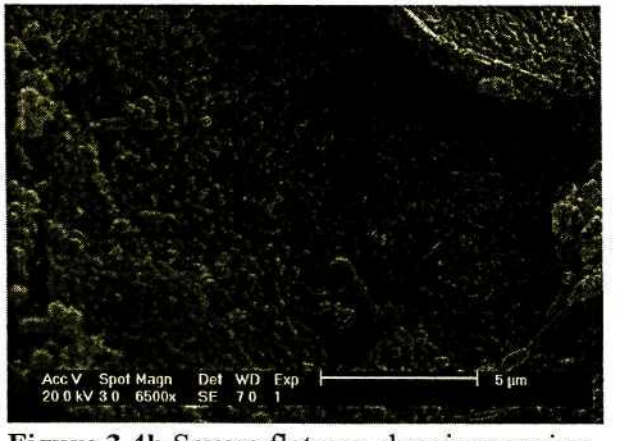


Figure 3.4h Severe flatness showing erosion of the surface tissue in the reticulum of a goat fed 20% tannin

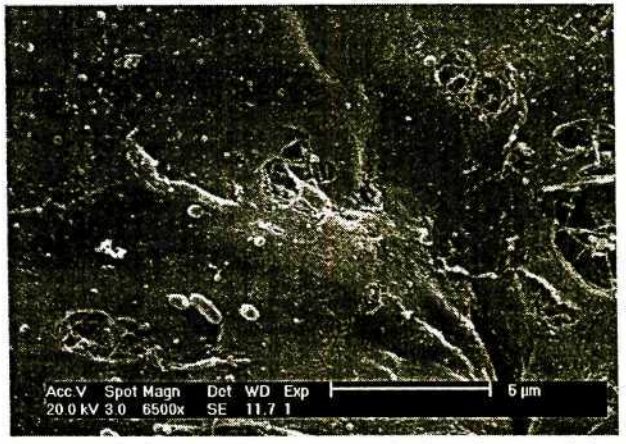


Figure 3.5a Few bacteria and pits observed in the abomasum (0% tannin)

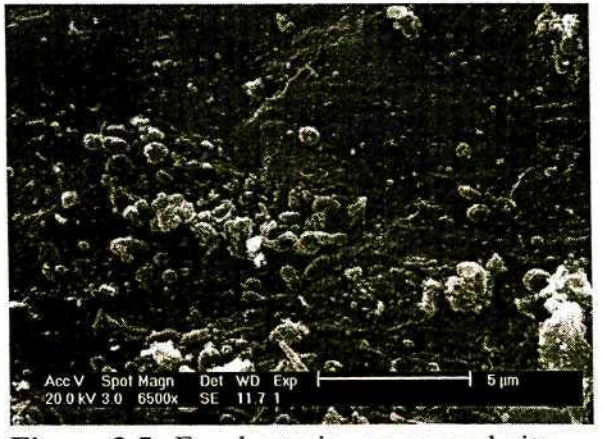


Figure 3.5c Few bacteria, mucus and pits observed in the abomasum (15% tannin)

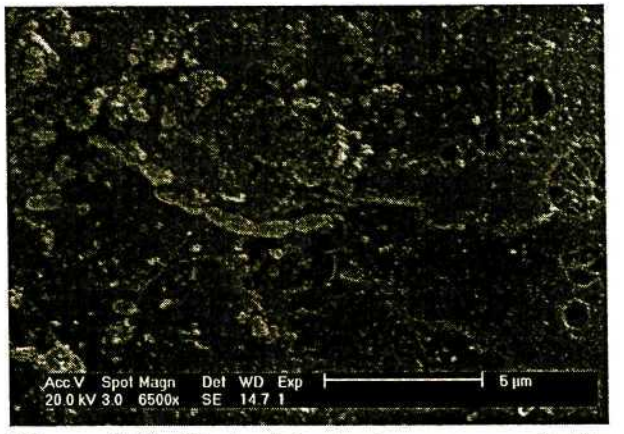


Figure 3.5b Chained bacteria, mucus and pits observed in the abomasum (5% tannin)

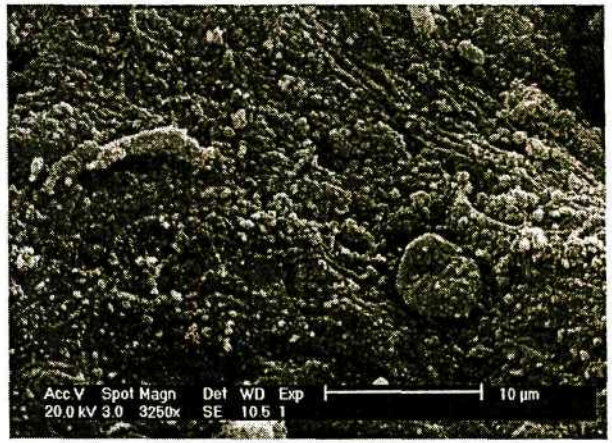


Figure 3.5d Mucus, goblet cells covering the surface of the abomasum of a goat fed 15% tannin

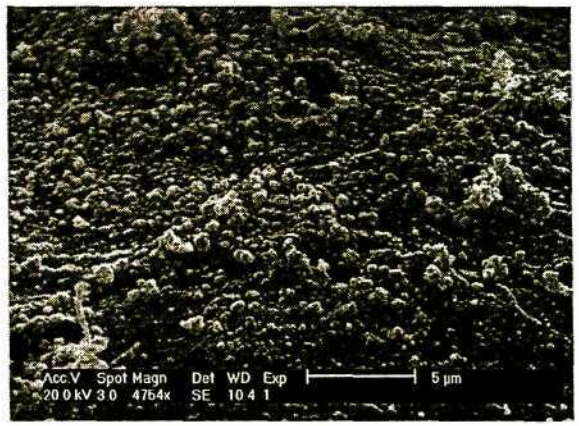


Figure 3.5e Mucus, pits and goblet cells covering the abomasal epithelial layer (20% tannin)

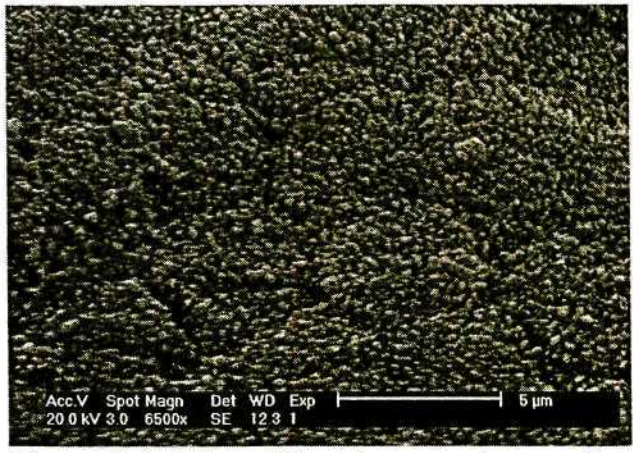


Figure 3.6a Microvilli of the control group that are tightly packed with mucus in the duodenum

Figure 3.6b Microvilli that are less tightly packed and showing denudation of microvilli in the duodenum (10% tannin)

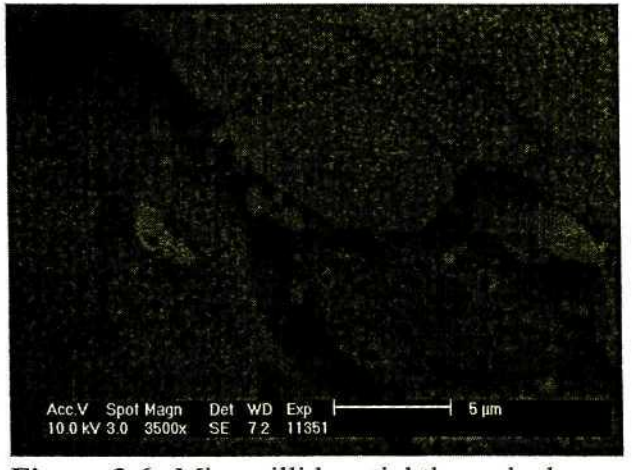


Figure 3.6c Microvilli less tightly packed, shorter, mucus and goblet cells in the duodenum(15% tannin)

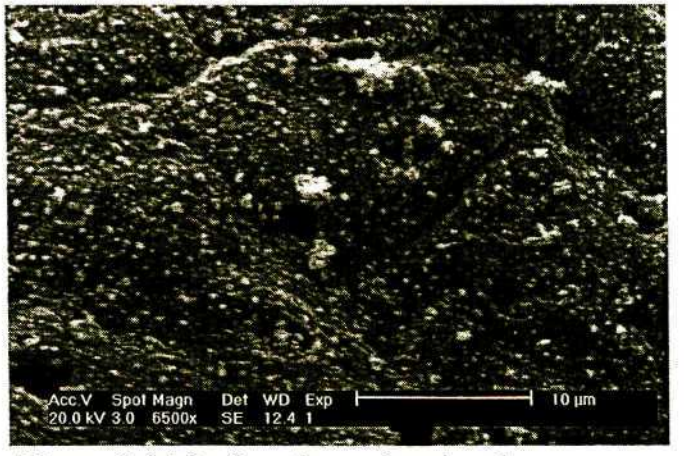


Figure 3.6d Surface tissue showing the appearance of total denudation of the microvilli in the duodenum (20% tannin)

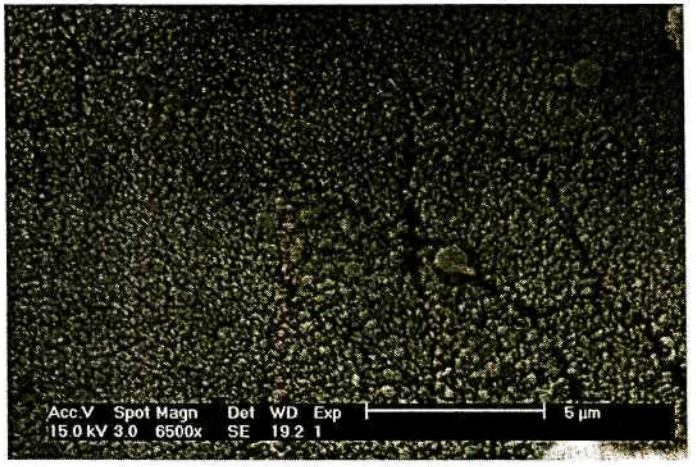


Figure 3.7a Brushed border with less mucus secretion present on the tightly packed microvilli in the duodenum (0% tannin)

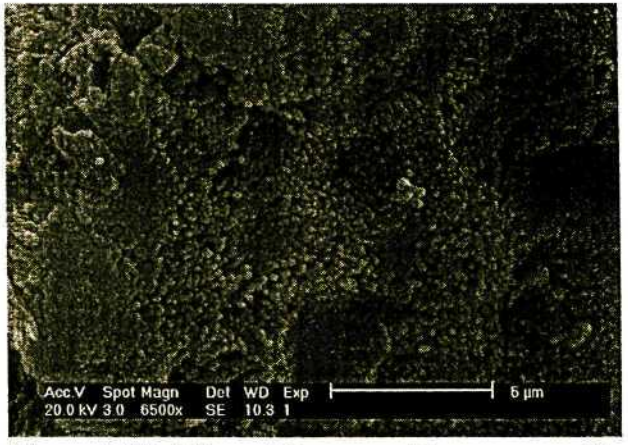


Figure 3.7b Microvilli covered by mucus with flattened/sunken cells of the microvilli in the duodenum (10% tannin)

Figure 3.7c Less tightly packed, different lengths of microvilli, and goblet cells in the duodenum (15% tannin)

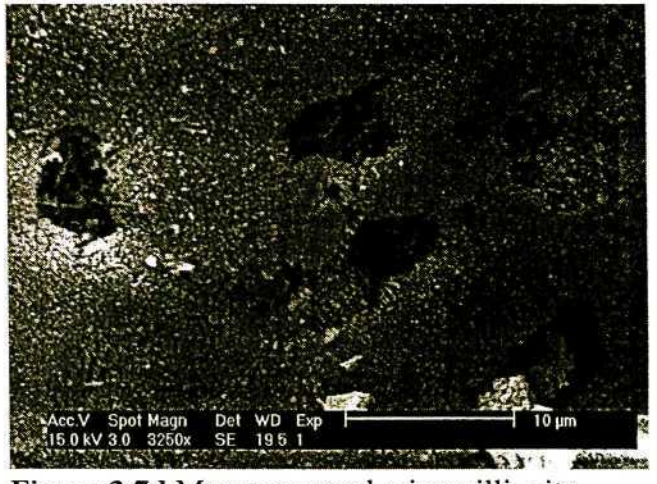


Figure 3.7d Mucus covered microvilli, pits, goblet cells and sunken microvilli cells in the duodenum (20% tannin)

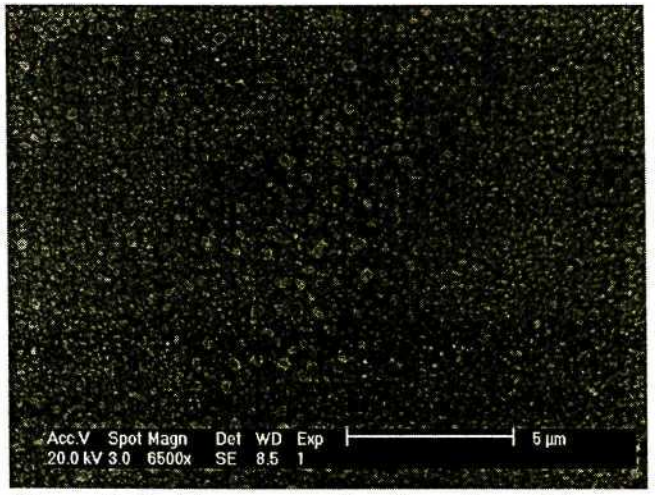


Figure 3.8a Mucus covering the microvilli showing the flatness appearance (5%)

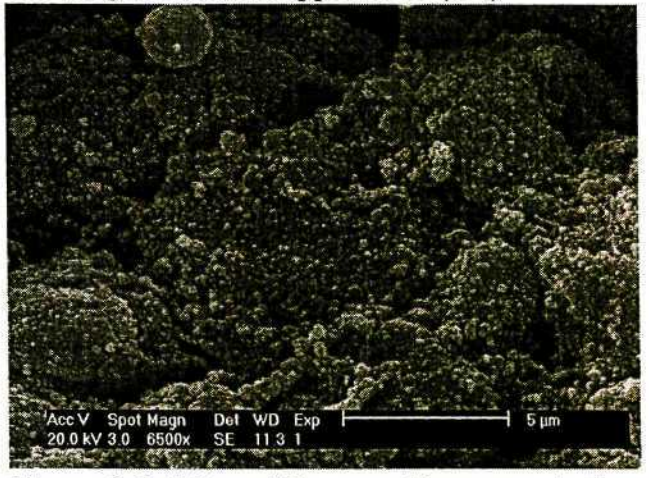


Figure 3.8c Microvilli covered by mucus in the duodenum (20% tannin)

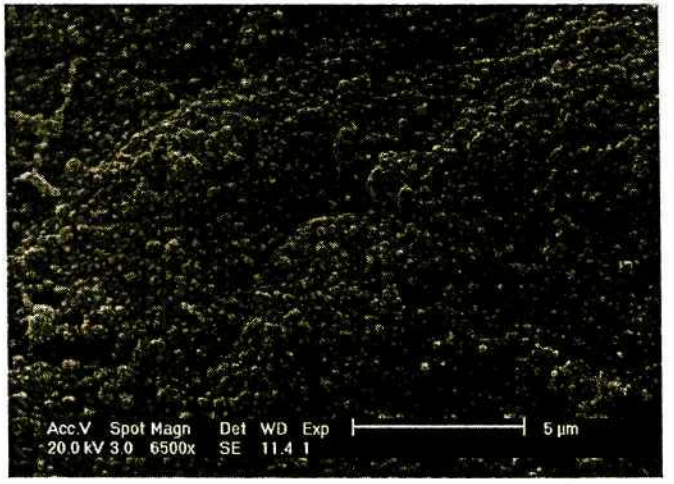


Figure 3.8b Mucus covering the surface of the microvilli (10% tannin)

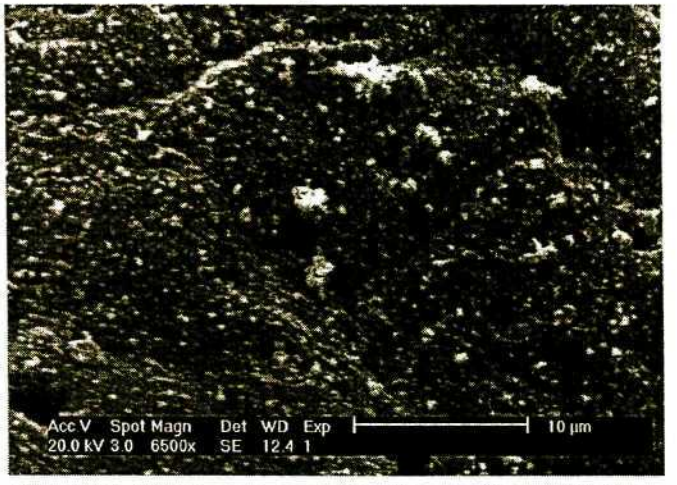
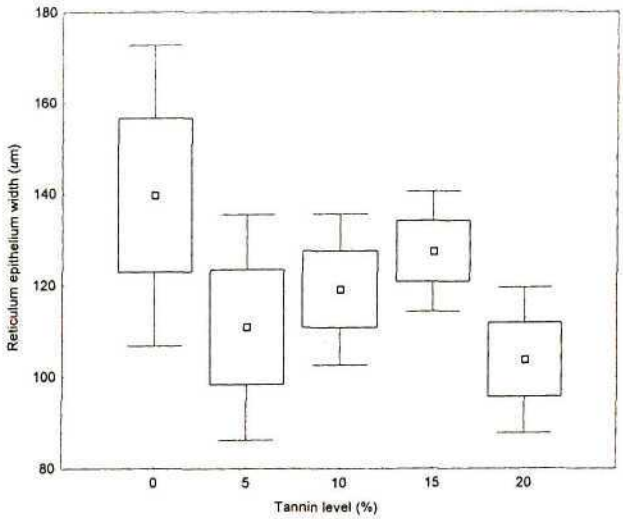
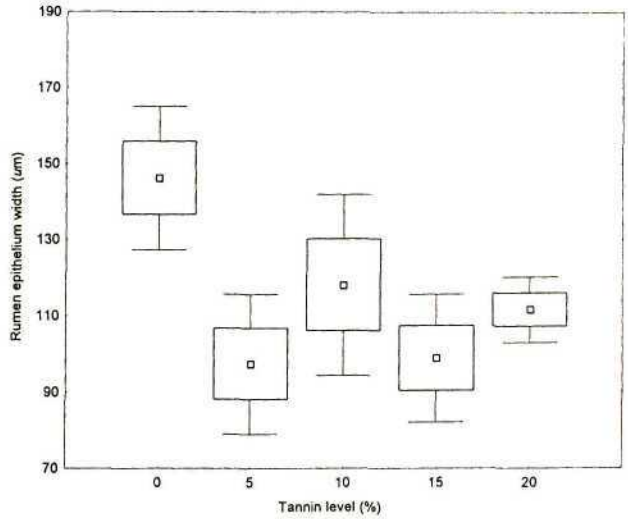


Figure 3.8d Mucus covering the surface with the appearance of total denudation of the microvilli in the duodenum (20% tannin)

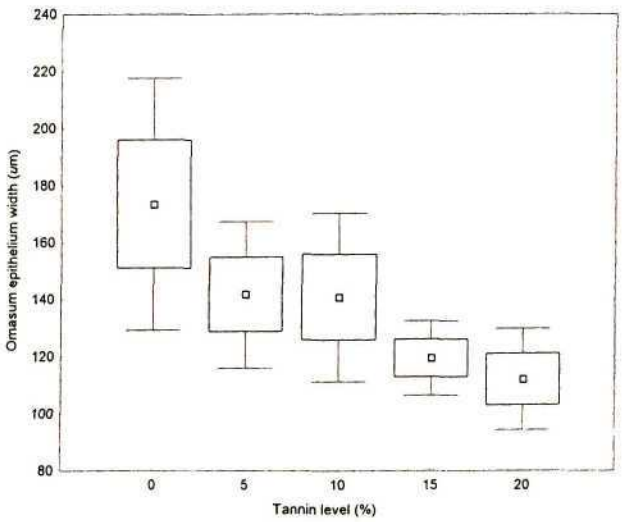
Figure 3.9. Changes in the epithelial width of a. reticulum, b. rumen, c. omasum, d. abomasum as well as e. duodenal villi height and f. duodenal crypt height in response to increased tannin levels.

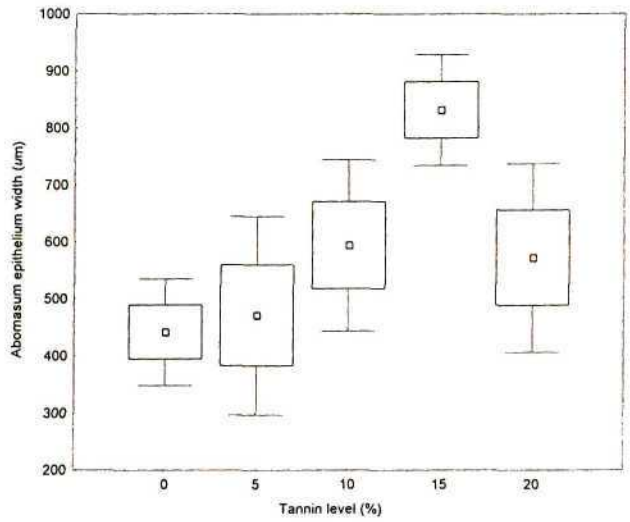
b.


Reticulum epithelium width (um)
180
160
140
120
100
80
0
5
10
15
20
Tannin level (%)

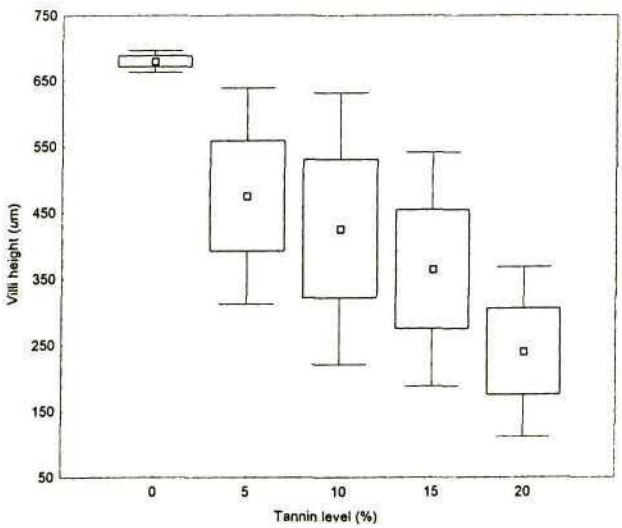

Rumen epithelium width (um)
190
170
150
130
110
90
70
0
5
10
15
20
Tannin level (%)

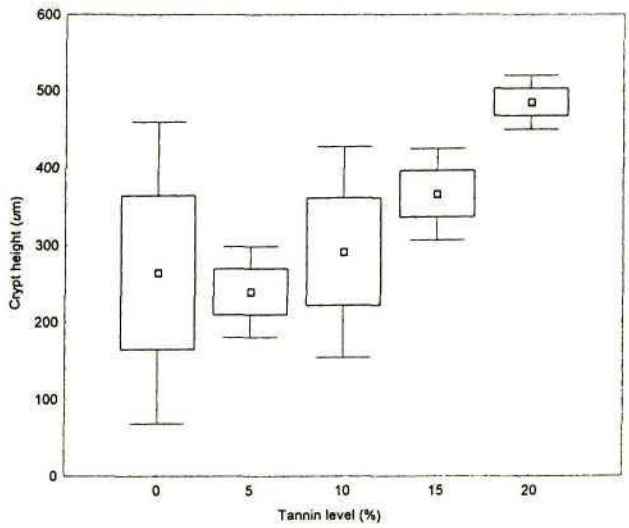
d.


Omasum epithelium width (um)
240
220
200
180
160
140
120
100
80
0
5
10
15
20
Tannin level (%)


Abomasum epithelium width (um)
1000
900
800
700
600
500
400
300
200
0
5
10
15
20
Tannin level (%)

f.


Villi height (um)
750
650
550
450
350
250
150
50
0
5
10
15
20
Tannin level (%)


Crypt height (um)
600
500
400
300
200
100
0
0
5
10
15
20
Tannin level (%)

Chapter 4

The effects of dietary tannin ingestion on the urine, liver and kidney of Boer goats

Khanyisile R. Mbatha*, Colleen T. Downs* and Ignatius V. Nsahlai

*School of Botany and Zoology and School of Agricultural Sciences and Agribusiness, University of Natal, P/Bag X01, Scottsville, 3209, South Africa.

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Abstract

This study was conducted to determine the ability of goats to detoxify tannins by measuring liver and kidney mass, and urinary glucuronic acid concentration. Goats were fed five diets containing incremental levels of tannin (0 - 20%). The effects of feeding diets containing different levels of condensed tannin on intake and digestibility were investigated in Boer goats. Commercialized tannin was used because of wide variation of tannin levels, which can be affected by season, species, and part of the plant. Thirty adult, male goats were fed respective experimental diets for 6 weeks following which they were kept in metabolic crates for data collection for 10 days. Liver and kidney mass did not increase with increased dietary tannin levels. In addition urinary glucuronic acid concentration did not increase as dietary tannin increased. Thus it appears that goats used in this did not show detoxification of tannin in the liver and kidneys.

Keywords: *goats, tannin, detoxification*

Introduction

When the defences in the mouth and gut fail, the secondary compound or its degradation products are absorbed and the second line of defence in the liver and kidneys must be used (Smith, 1992). The kidney and liver organs concentrate more toxic compounds than all other organs combined (Klaassen and Rozman, 1993). Their sizes have been shown to change in response to plant toxins in their diet (Jung and Batzli, 1981). Larger size is a response to detoxification of absorbed compounds. The kidney and liver organs are the primary sites of detoxification and excretion of toxins by mammals (Schuster, 1964; Klaassen and Rozman, 1993).

Substances absorbed from the gut into blood usually pass to the liver but what reaches the liver is rarely what was consumed (Smith, 1992). This is the result of biotransformational enzyme and microbial activities in gut tissues that may be more important in some cases than the activities of enzymes in the liver. Moreover, liver enzymes are under genetic control, whereas microbes are not heritable (Cheeke, 1994; Cheeke and Palo, 1995). The liver has a major role in mediating the effects of potentially toxic metabolites (Freeland and Janzen, 1974). This is because the liver removes toxic agents from the blood after absorption from the gastrointestinal tract before they reach the general circulation (Klaassen and Rozman, 1993).

Voles (Lindroth and Batzli, 1983) and snowshoe hares (Bryant *et al.*, 1985) possess enzyme systems that detoxify phenolic compounds through conjugation with glucuronic acid. Such enzymes are allocated primarily in the kidneys and liver (Freeland and Janzen, 1974) but also in the visceral tissue such as rumen wall (Smith, 1986). Urinary excretion of glucuronic acid has been used to describe the metabolic load in voles containing tannic acid and alkaloids

(Lindroth and Batzli, 1983); in common ringtail possums to compare between different diets of *Eucalyptus* leaves (Foley, 1992); and in pikas to estimate the physiological consequences of phenolic rich diets (Dearing, 1997). Phenolics that bind to protein would not have been assimilated and hence would not have contributed to an increase in glucuronic acid excretion. Protein deficiency has also been shown to increase glucuronic activity (Chung-MacCoubrey *et al.*, 1997).

Goats as browsers or intermediate feeders are able to ingest significant amounts of dietary tannins. It was expected that the mass of the kidneys and liver would increase in animals fed increased tannin levels as a result of detoxification or degradation of tannins. Urinary glucuronic acid excretion as an index of detoxification of plant phenolic by goats was expected to increase with increased dietary tannin levels.

Materials and Methods

Experimental diet composition

Dietary treatments consisted of the following components: commercialised condensed tannin (Wattle Bark Industry of South Africa) which was of wattle extract composition, alfalfa (*Medicago sativa*) (NCD, Pietermaritzburg), hay K11 (coast-cross) from Ukulinga Research Farm and molasses meal (NCD, Pietermaritzburg) (Chapter 2, Table 2.1). Feed mixture was not pelleted because feed with tannin can be converted to toxic form if pelleted (Dietz *et al.*1994).

Animals and diets

The experimental procedure and the diets of animals are presented in Chapters 2 and 3.

Liver and kidney

After each trial the animals were slaughtered. Immediately after the slaughtering of each goat the kidneys and livers were removed and weighed. Liver and kidney masses were expressed as g/kg of body weight. Final body weight was used.

Urine

The mesh floor of each metabolic cage had a metal tray beneath, into which goat's urine was voided before draining into a plastic collection bucket. To prevent bacterial growth (Lindroth and Batzli, 1983) and ammonia volatilisation (Chung-MacCoubrey *et al.*, 1997) 50 ml of 10% sulphuric acid was added to cleaned plastic collecting buckets each morning. Total daily volume of urine produced by each goat was measured every morning, 10% subsampled, pooled together on animal basis and stored at -20°C. Urine was later thawed and subsampled for analysis of glucuronic acid. To measure the concentration of total glucuronic acids in goats' urine, the method of Lindroth and Batzli, (1983) was used (Appendix 2)

Statistical analyses

Analysis of variance of data from the feeding trial was conducted using the general linear model (GLM) procedure of the Statistical Analysis Systems (1987) (SAS, version 6) for completely randomized design. The initial body mass was used as a co-variate for all variables. Contrasts among the levels of tannin were done by applying the probability of

different PDIFF options of the Lsmeans Statement available in the GLM. The treatment sum of squares was further partitioned into the linear and quadratic orthogonal contrast by applying the contrast statement of the GLM.

RESULTS

Liver mass (LM) decreased ($P < 0.0001$) with increasing tannin levels ($P > 0.05$) (Table 4.1). Both the linear and quadratic effects of tannin on liver mass were significant. The liver mass decreased to a minimum mass at 15% tannin level and then increased slightly. The quadratic pattern can be represented by the equation (1), of which the minimum liver mass is estimated at 15.5% tannin level. Although the effect of tannin level on kidney mass only tended towards significance ($P = 0.11$), the pattern was such that the kidney mass peaked at 5% tannin level from where it decreased linearly. The linear, but not the quadratic effect level was significant ($P < 0.03$).

$$LM = 16.21 - 0.527\ Tannin + 0.017\ Tannin^2 \qquad (4.1)$$

Glucuronic acid concentration did not differ ($P > 0.05$) significantly between dietary tannin levels (Table 4.1). Both the quadratic and linear effects of dietary tannin content had no *effect ($P > 0.05$) on glucuronic acid concentration.*

Discussion

Due to the anterior position of the major digestive fermentation organs, partial microbial modification of toxins may take place before absorption in ruminant herbivores (Freeland and Janzen 1974). This can potentially affect the toxicity of compounds in ruminants. Greater

secretion of salivary proteins (Domingue *et al.*, 1991) and parotid saliva rich in proline, glutamine and glycine (Domingue *et al.*, 1991), that enhance the affinity of proteins to tannins (Mehansho *et al.*, 1987) are the first attempt by goats to negate the tannin effects. In addition, using oesophageal fistula, Provenza and Malechek, (1984) observed in goats that tannin level was reduced before swallowing. Furthermore, goats have active tannase to neutralize tannins (Begovic *et al.*, 1978). The increased number of bacteria present and the gastrointestinal tract epithelial changes observed as tannin levels increased (Chapter 3) may be able to dissociate the tannin-protein complexes, reduce absorption to a certain level and possibly detoxify dietary tannins, thus reducing detoxification processes required in the liver and kidney. In the present study, negligible tannin was present in the faeces (Chapter 2), indicating that condensed tannin was either degraded prior to absorption or absorbed from the GIT. This would have resulted in little tannin left to be detoxified by the liver and kidneys. If tannin is not degraded in the GIT, detoxification in the liver and the kidney is supposed to be the next stage where by tannins are metabolized by conjugation to glucuronic acid.

The fact that both the liver and kidney mass did not increase with increased dietary tannin levels is contrary to our expectations. Generally, liver and kidney mass of most animals increase, with the ingestion of high levels of tannins as a response to detoxify the plant secondary compounds (Baudinette *et al.*, 1980; Hofmann, 1989; McArthur *et al.*, 1991). However, the present study supports the observation by Distel and Provenza, (1991) that tannin-rich diets have no effect on either liver or kidney mass. Goats consumed less energy and protein as dietary tannin levels increased (Chapter 2). In addition, increased tannin intake

could have increased energy demand because both energy and amino acids are required in the detoxification process. Consequently, it is possible that when animals are not meeting their energy requirements, ingestion of increasing quantities of tannin could result in decreased body mass of the whole body and of organs. So, it is difficult to conclude that goats do not detoxify dietary tannin by observing mass of liver and kidney.

Increased glucuronic acid concentration in urine was observed as a sign of detoxification of plant secondary compounds (Lindroth and Batzli, 1983; 1984; Dearing, 1997). On the contrary, in the present study, concentration of urinary glucuronic acid did not respond to increased tannin levels. Lack of toxicity in goats could support the hypothesis that mammalian herbivores (Freeland and Janzen, 1974), consume toxins in amount that they can detoxify (Provenza *et al.*, 1990; Silanikove *et al.*, 1996).

Conclusion

It appears that there was little detoxification of tannin in the kidneys and liver of goats with increased dietary tannin intake if increased mass of these organs reflects these processes. However, as goats decreased dietary intake with increased tannin levels, the lack of increase in mass of these organs may reflect decreased body mass. As there was no increase in glucuronic acid, however, it appears that Boer goats were not detoxifying tannin in their liver nor kidneys.

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Table 4.1 *Mass of liver and kidney (g/kg final body weight) and glucuronic acid concentration (μg/ml) in Boer goats fed different levels of tannin ($\bar{X} \pm SE$)*

	Mass (g/kg body weight)				
Diet Treatment	Initial body mass	Final Body mass	Liver	Kidney	Glucuronic acid
Tannin level (%)					
0 (n = 6)	37.83 ± 2.83	33.67 ± 0.45	533 ± 15.2	98 ± 3.6	0.41 ± 0.040
5 (n = 6)	37.08 ± 1.54	35.17 ± 1.29	493 ± 15.7	104 ± 3.8	0.48 ± 0.041
10 (n = 6)	37.00 ± 1.31	33.75 ± 1.37	405 ± 15.2	93 ± 3.7	0.45 ± 0.040
15 (n = 5)	37.25 ± 1.23	33.20 ± 1.6	400 ± 16.7	92 ± 4.0	0.45 ± 0.044
20 (n = 6)	38.58 ± 2.11	30.08 ± 2.16	422 ± 16.6	84 ± 3.9	0.47 ± 0.044
Significance			0.0001	0.109	0.28
Linear effect			0.0001	0.03	0.57
Quadratic effect			0.0024	0.33	0.63
Optimal value			15.5 %		

Chapter 5

General conclusion

Few studies have been conducted to determine the various strategies concurrently that can be observed in the counter adaptation to secondary metabolites by herbivorous mammals (Foley *et al.*, 1999). In the present study the following were determined:

1) the effects of tannins on the physiology of goats by studying intake of basal diet and digestibility.

2) the effects of tannins on the epithelial tissue of the gastrointestinal tract (GIT) and the presence of bacteria in various regions of the GIT of goats. Histological changes were studied using scanning electron microscopy and light microscopy (SEM) and the presence of bacteria in the GIT was studied using SEM.

3) the ability of goats to detoxify dietary tannins by measuring the mass of liver and kidneys; and the concentration of the urinary glucuronic acid. It was expected that the mass of the kidney and liver would increase and the urinary glucuronic acid concentration would increase as dietary-tannin content increased as a sign of detoxification of plant phenolics by goats.

The various avenues that tannins may affect a mammalian herbivore are summarised in Figure 5.1 (Foley *et al*, 1999). Goats were used as models to determine the effects of tannin ingestion and explore these various avenues. There have been few investigations that have examined more than two avenues simultaneously. This study attempted to investigate most of the avenues concurrently. Presence of proline rich compounds in saliva is presently being researched in a corollary study.

The results of this study showed that dietary tannin has negative and positive effects on Boer goats. The main detrimental effect of increased tannin ingestion on goats appears to be the reduction of feed intake, which could be caused by, reduced passage of feed resulting from keranitinization of the epithelial or the harsh, astringent taste. It appears that goats cope with low levels of tannin ingestion. There appears to be a threshold above which greater tannin ingestion has detrimental effects. The linear decreased dietary intake with increased tannin level may indicate that goats limit their intake of tannin below some threshold as a defence strategy (Chapter 2). Dietary intake of goats was similar on both the 0% and 5% tannin levels supporting the previous findings of a threshold in amount of tannin that is not detrimental.

Tannin ingestion had a negative effect on fibre digestibility (Chapter 2). Goats that fed on low tannin diets had higher fibre digestibility compared to the ones fed the high tannin diet although the former consumed more fibre (Chapter 2). Digestibility of dry matter (DM) tended to decrease with increasing tannin levels. However, digestibility of crude proteins (CP), organic matter, neutral detergent fibre and acid neutral detergent fibre decreased significantly with increasing tannin levels. Faecal CP increased while urinary CP decreased with increasing tannin levels. There was no tannin present in the faeces. Elevated faecal CP could be the result of the loss of the microvilli observed.

Number and types of bacteria observed in the reticulum and rumen increased with tannin level in the diet. These may be responsible for tannin-protein complex degradation. Few bacteria were observed in the abomasum.

Tannin ingestion affected the histology of the GIT (Chapter 3). There were differences in the histopathology of the GIT with increased dietary tannin. In particular the reticulum, rumen, abomasum and duodenum were affected. Animals on the control diet had more protozoa present in the GIT than the other diets. The flat appearance of the epithelial tissue, erosion of the epithelial surface, and shorter villi and microvilli as tannin levels increased may explain that reduced feed intake was the result of negative post-ingestion of tannins in the GIT (Chapter 3). Keratinization of epithelial tissue probably contributed indirectly to the reduced dietary intake observed as tannin levels increased (Chapter 2) as it possibly reduced the passage of nutrients. This would eventually affect the nutrient absorption and health status of the animal.

Detoxification of tannin and its degradation products appear to partly occur in GIT possibly explaining the negligible tannin observed in the faecal samples (Chapter 2). Goats did not show the ability to detoxify tannins as the mass of the kidney and liver as well as urinary glucuronic acid concentration did not increase as the tannin content increased (Chapter 4).

The positive aspect of tannin ingestion observed in the present study was the apparent ability to reduce parasite load. In particular, the number of coccidial oocysts was reduced.

It was observed that coccidial oocysts decreased to a minimum at tannin level of approximately 10% (Chapter 3). The dietary tannin may act in conjuction with the immune system to reduce the population of coccidial oocyst parasites. Tannin ingestion also appears to reduce diarrheoa. The goats fed the control diet had diarrheoa relapses whereas the goats on the other diets had no diarrhoea (Chapter 2).

Further investigations of tannin detoxification, in particular, the role of the epithelial mucosa and bacteria in the GIT , are necessary. A study similar to the present one should be initiated using juvenile goats and a second generation of adult goats that have not been exposed to tannin to determine the adaptations that these animals develop in response to tannin ingestion. Another aspect that was not investigated in the present study is the effect of tannin ingestion on fecundity which needs to be investigated.

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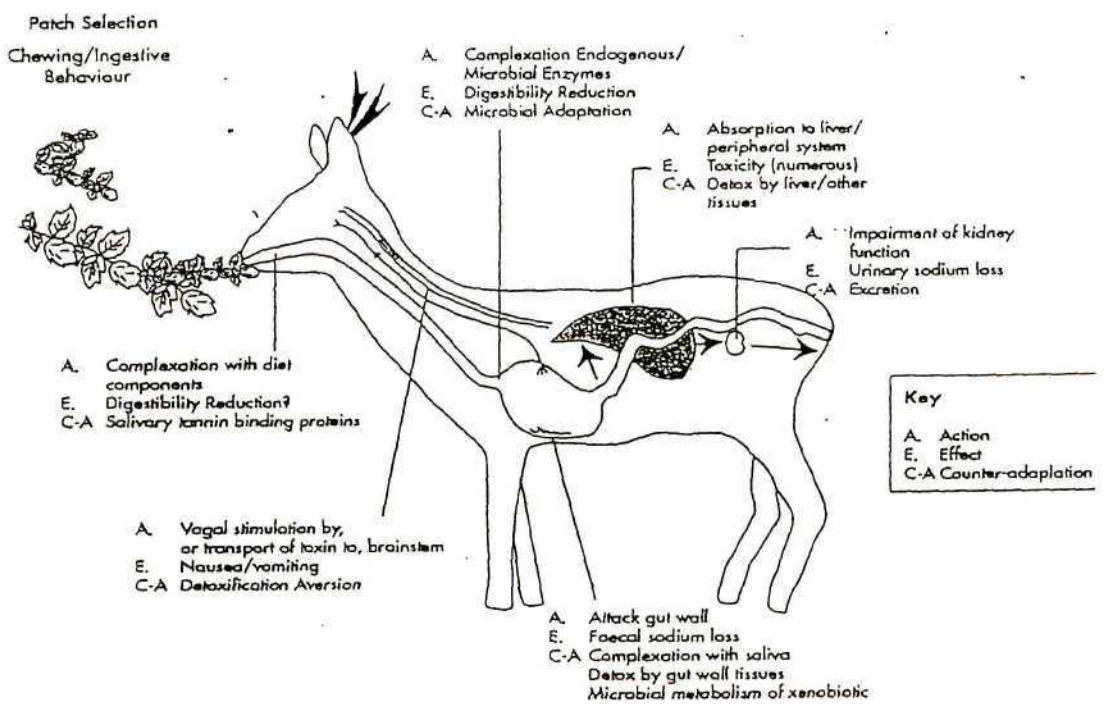

Figure 5.1 A summary of actions, effects, and counter-adaptations of plant secondary metabolites in mammalian herbivores (after Foley *et al.* 1999).

Appendix 1.

The Bradford dye-binding assay (Bradford, 1976) used for urinary protein concentrations determinations of goats in the present study.

This procedure provides a sensitive and quantitative method

Reagents

Dye Reagent: Brilliant blue G dye (50 mg) was dissolved in 50 ml of 88% phosphoric acid and 23.5 ml of 99.5 % ethanol. The solution was made up to 500 ml with distilled water and stirred for 30 minutes on a magnetic stirrer. The resulting solution was filtered through Whatman No. 1 filter paper and stored in a brown bottle. This solution could be stored for up to 6 months, although visual checks for precipitation were made before use. If precipitation was visible, the reagent was filtered and re-calibrated before use.

Standard protein solution: The 1 mg/ml ovalbumin solution was made up in distilled water.

Procedure

The standard ovalbumin solution (0-25 µl) or sample protein, was diluted to final volume of 100 µl with distilled water to give the desired protein concentration (0-25 µg). Then Bradford dye reagent (5 ml) was added and the mixture was vortexed. The colour was allowed to develop for 2 minutes before the absorbance at 595 nm was read in 1 cm cuvettes. All absorbencies were read using a Spectronic 20 Genesys spectrophotometer

(Spectronic Instruments). The cuvettes were cleaned with a detergent solution. The assays for a standard curve were carried out in quintuplicate at five protein concentrations of ovalbumin.

The results for the above assays were calculated from equations generated by linear regression analysis of the standard curves, for each assay type, developed for each batch of dye reagent made up.

Appendix 2

The Lindroth and Batzli method (1983) used to measure the concentration of total uronic acids in goats' urine.

Reagents

Sulphuric acid reagent: A 0.025M sodium tetraborate $10H_2O$ (analytical grade) in sulphuric acid, sp.gr. 1.84 (analytical grade).

Carbazole reagent: A 0.125% carbazole in absolute ethanol. This solution is stable for up to 12 weeks at 4° C in the dark.

Standard glucuronolactone: Glucuronolactone standards of 4-40 μg/ml were prepared by glass distilled water saturated with benzoic acid from a stock standard in water saturated with benzoic acid.

Procedure

Test tubes were placed in crushed ice for ±30 minutes before the experiment. Five ml of sulphuric acid reagent were placed in these, then allowed to cool to room temperature. Urine samples were diluted by 1+25 with distilled water. 1 ml of each diluted urine sample was carefully layered on to the acid. Test tubes were closed with Teflon stoppers and shaken at first gently, then vigorously with constant cooling. The temperature of the mixture did not exceed room temperature. The tubes were then heated for 10 min in a boiling distilled water bath before being cooled to room temperature. Carbazole reagent (0.2 ml) was added. Test tubes were shaken again. They were heated in the boiling water

bath for 15 min, and cooled to room temperature. The absorbance of each sample was read at 530 nm in a 1 cm glass cuvette.

The results for the above assays were calculated from equations generated by linear regression analysis of the standard curves.