EVALUATION OF MIXTURES OF *ACACIA KARROO* LEAF MEAL AND *SETARIA VERTICILLATA* GRASS HAY FOR INDIGENOUS PEDI GOAT PRODUCTION

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by

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A THESIS SUBMITTED IN FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY (ANIMAL NUTRITION) IN THE DEPARTMENT OF AGRICULTURAL ECONOMICS AND ANIMAL PRODUCTION, SCHOOL OF AGRICULTURAL AND ENVIRONMENTAL SCIENCES, FACULTY OF SCIENCE AND AGRICULTURE, UNIVERSITY OF LIMPOPO, SOUTH AFRICA

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DECLARATION

I declare that the thesis hereby submitted to the University of Limpopo for the degree of Doctor of Philosophy (Animal Nutrition) has not previously been submitted by me for a degree at this or any other university, that it is my work in design and execution and that all material contained therein has been duly acknowledged.

Signature……………………………………… Date…………………………………

Brown, D.A (Mr)

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DEDICATION

This thesis is dedicated to my firstborn son, Melchisedec Adeiye Brown, you are the reason why we embarked on this journey.

ABSTRACT

Acacia browse utilization by ruminant animals is reviewed. The effects of Acacia browse inclusion on intake, digestibility and body weight of ruminants and the large variability in response are noted. A series of experiments were carried out to determine the effects of *Acacia karroo* leaf meal inclusion level on performance of indigenous Pedi goats. The first experiment determined the effect of *A. karroo* leaf meal inclusion level on intake and relative palatability indices of male Pedi goats fed *Setaria verticillata* grass hay-based diets. The results of this study indicated that *A. karroo* leaves had high protein contents, ranging from 106 to 147 g/kg. Diets with higher *A*. *karroo* inclusion levels had higher (P<0.05) intakes and relative palatability rankings by Pedi goats, regardless of the higher condensed tannin and phenolic levels.

The second experiment determined the effect of *A. karroo* leaf meal inclusion level on feed intake, digestibility, body weight change and blood profile of male Pedi goats fed a *S. verticillata* grass hay-based diet. The study also determined inclusion levels for optimal performance of the goats. Inclusion of *A. karroo* leaf meals improved (P<0.05) nutrient digestibility and growth rate of male Pedi goats but not (P>0.05) intake. Dry matter (DM), crude protein (CP) and neutral detergent fibre (NDF) digestibilities were optimized at different *A. karroo* leaf meal inclusion levels of 69.4, 48.3 and 42.7 %, respectively. However, higher *A. karroo* inclusion levels, like 50 %, resulted in higher (P<0.05) dietary condensed tannin contents which exerted negative effects on serum total proteins.

The third experiment determined the effect of polyethylene glycol (PEG) 4000 supplementation on diet intake, body weight change and blood profiles of male Pedi goats fed different mixture levels of *A. karroo* leaf meal and *S. verticillata* grass hay. It also determined PEG 4000 supplementation levels for optimal performance of the goats. Supplementation with 23 g of PEG 4000 improved (P<0.05) dry matter, organic matter (OM), NDF and acid detergent fibre (ADF) intakes of male Pedi goats fed a dietary mixture of 20 % *A. karroo* leaf meal and 80 % *S. verticillata* grass hay. A supplementation level of about 20 g of PEG 4000 per goat per day optimized DM, OM, NDF and ADF intakes, respectively. Supplementation with either 23 or 30 g of PEG

4000 improved DM, OM, CP and NDF digestibilities by goats fed a dietary mixture of 20 % *A. karroo* leaf meal and 80 % *S. verticillata* grass hay. Crude protein digestibility was optimized at a PEG 4000 supplementation level of 15.78 g/goat/day. Supplementation with either 23 or 30 g of PEG 4000/goat/day did not (P>0.05) improve the performance of the goats when fed a dietary mixture of 50 % *A. karroo* leaf meal and 50 % *S. verticillata* grass hay.

The last experiment determined the effect of *A. karroo* leaf meal inclusion level on carcass characteristics, pyhsico-chemical and sensory attributes, and histological parameters of male Pedi goats fed *S. verticillata* grass hay-based diets. *Acacia karroo* leaf meal inclusion level did not (P>0.05) affect intake, weight gain, carcass weight and carcass component weights of male Pedi goats. However, all the parameters were at moderate levels for Pedi goats. *Acacia karroo* leaf meal inclusion level did not (P>0.05) adversely affect goat meat tenderness, juiciness, flavour, taste, aroma and overall acceptability. *Acacia karroo* inclusion levels of 20, 25 or 30 % did not have adverse effects on livers and kidneys of male Pedi goats. However, increased hepatocyte degeneration and dilalation of uriniferous spaces were noticed in goats fed diets with *A. karroo* leaf meal inclusion levels of 40 or 50 %. Possible reasons for variable responses to *A. karroo* inclusion level are discussed. It is concluded that indigenous Pedi goat optimal productivity responses to dietary *Acacia karroo* leaf meal inclusion were variable, depending on the production parameter in question. This has practical management implications when formulating feeding strategies aimed at improving productivity of Pedi goats.

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CHAPTER ONE

GENERAL INTRODUCTION

1.1 Background

Goat production contributes immensely to the economy and food security of many smallholder farmers, particularly in the rural areas (Ng'ambi *et al*., 2013). Indigenous Pedi goats are important domestic animals in Limpopo Province of South Africa. These goats are nutritionally, economically and culturally important to rural households (Aziz, 2010). However, their productivity is constrained by a shortage of good quality feed, especially during the long dry season (Matlebyane *et al*., 2010). Available fodder during this period is fibrous and low in protein. Poor nutrition results in poor reproductive performance and productivity. Undernourished animals are, also, predisposed to diseases and parasites and may die in extreme cases (Matlebyane *et al*., 2010).

Tree leaves from Acacia species are an important component of the diets of goats in Limpopo province (Mokoboki *et al*., 2005). *Acacia karroo* Hayne is an important leguminous tree in communal rangelands of South Africa and is able to thrive in severe and dry conditions. The foliage is evergreen and it's consumed by goats during periods of drought. The leaves, fruits and pods are highly palatable and excellent fodder for goats (Nyamukanza & Scogings, 2008). *Acacia karroo* leaf meal contains between 100 – 160 g crude protein (CP) per kg dry matter (Chepape *et al*., 2011; Halimani *et al.,* 2005), and has potential as a protein supplement. Despite its importance in goat production, the species contains phenolic compounds, especially condensed tannins or proanthocyanidins (Mokoboki *et al*., 2005; Dube *et al*., 2001). Condensed tannins have been reported to be beneficial and or detrimental in ruminant nutrition depending on their concentration and nature, amount ingested, animal species, physiological state of the animal and composition of the diet (Mueller-Harvey, 2006; Makkar, 2003). Recommendations on the use of *Acacia karroo* leaf meal are complicated due to the presence of condensed tannins in the plant. The effects of condensed tannins in *A*cacia *karroo* on productivity of indigenous Pedi goats have not been extensively explored and available reports are not conclusive. Additionally, there is dearth of information on *Acacia karroo* leaf meal inclusion levels for optimal productivity of Pedi goats.

The knowledge of these secondary plant metabolites is essential to achieve an optimal use of these resources as fodder for goats. Understanding such detrimental biological effects and devising feeding strategies that enhance the positive effects caused by tanniniferous feeds is essential for improving productivity of indigenous Pedi goats.

1.2 Motivation of the study

This study will generate information on the utilization of tanniniferous *Acacia karroo* leaf meal by indigenous Pedi goats. Data on such information will help in formulating feeding strategies that enhance the productivity of the goats. Optimization of productivity of the goats may improve the economic, nutritional and cultural status of indigenous goat farmers in South Africa.

1.3 Objective

The broad objective of this study was to determine optimal production responses of indigenous male Pedi goats to different *Acacia karroo* leaf meal inclusion levels.

The specific objectives of this study were as follows:

i To determine nutrient composition of dietary mixtures of *Acacia karroo* leaf meal and *Setaria verticillata* (L.) P.Beauv. grass hay-based diets.

ii To determine the effect of *Acacia karroo* leaf meal inclusion level on intake and relative palatability indices of male Pedi goats fed *Setaria verticillata* grass hay-based diets.

iii To determine the effect of *Acacia karroo* leaf meal inclusion level on feed intake, digestibility, body weight gain and blood profiles of male Pedi goats fed *Setaria verticillata* grass hay-based diets.

iv To determine the effects of polyethylene glycol 4000 supplementation level on the performance of indigenous male Pedi goats fed different mixtures of *Acacia karroo* leaf meal and *Setaria verticillata* grass hay.

v To determine the effects of *Acacia karroo* leaf meal inclusion level on carcass characteristics, physico-chemical attributes, sensory evaluation and histological parameters of male Pedi goats fed a *Setaria verticillata* grass hay-based diet.

CHAPTER TWO

LITERATURE REVIEW

2.1 Introduction

Domestic goats (*Capra hircus*) were among the first farm animals to be domesticated (Aziz, 2010). They have played a central role in the Neolithic agricultural revolution (Fernandez *et al*., 2006; Luikart *et al*., 2001). The origins of domestic goats remain uncertain and controversial, but archaeological evidence suggests that they were probably first domesticated in the Fertile Crescent region of the Near East about 10,000 years ago (Zeder & Hesse, 2000). Other authors hint that a second domestication in Pakistan could have given rise to the cashmere breeds (Meadow, 1996; Porter, 1996), while others suggest that at least two wild species of *capra* could have contributed to the gene pool of domestic goats (Shackleton, 1997; Clutton-Brock, 1981). There has been rapid spread of domestic goats worldwide due to different activities, such as commercial trade, pilfering, warfare, or the migration of people with their livestock (Clutton-Brock, 2000).

2.2 Indigenous goat production

Indigenous goats are generally owned by smallholder farmers in the communal areas (Lebbie & Ramsay, 1999). They represent the principal economic output, contributing a large proportion of income to the resource-poor farmers (Ben Salem & Smith, 2008). The contribution of indigenous goats to the nutritional and economic status of the rural dwellers is well recognized (Ng'ambi *et al.* 2013; Iniguez, 2011; Rumosa Gwaze *et al.* 2009). Reports from a number of development projects in the last 20 years indicate the significant role of indigenous goats in poverty alleviation and household food security (ICG, 2010; Morand-Fehr, 2005). Despite the important role that indigenous goats play in the lives of the rural poor, not much has been done in terms of improving their productivity (Peacock, 1996). One of the main constraints to increasing goat production in the communal areas is limited forage availability, particularly during the dry season (Alemu *et al*., 2014). Inadequate feeding of goats is a major limiting factor facing the smallscale goat producers in the communal areas of Southern Africa as the vast majority of goats in these areas are kept under extensive system of management

(Rumosa Gwaze, 2009). This problem is more pronounced during the dry season due to erratic rainfall patterns. The quality and quantity of available fodder decline during this period, and goats utilize feedstuff from low quality natural pasture which is low in protein (Tolera *et al*., 2000). Poor nutrition results in low productivity and body weight loss (Aw-Hassan, 2010). There is a need to identify alternate feed resources to boost the feeding values of low quality roughages.

2.3 Tree fodder in ruminant nutrition

Browse plants are constituents of rangeland systems that act as shade, windbreakers, nitrogen fixers and soil stabilizers. The use of browse trees as fodder for ruminants is increasingly becoming important in many parts of the tropics (Njidda, 2010). Tree fodders maintain higher protein and mineral contents during growth than grasses, which decline rapidly in quality with progress to maturity (Shelton, 2004). These browse trees, thus, represent an important source of high quality feed for ruminants and as supplements to improve the productivity of herbivores fed on low quality feed (Njidda, 2010).

The introduction of non-native herbaceous and browse legume species into the natural rangeland community in the early 80’s and 90’s such as *Stylosanthes guianensis* (Graham stylo), *S*. *hippocampoides* (Oxley fine-stem stylo), *S*. *scabra* (Shrubby stylo), *Macroptilium atropurpureum* (Siratro), *Neonotonia wightii* (Glycine), *Chamaecrista rotundifolia* (cv. Archer) and *Lotononis bainesii* (Belt lotononis) has been unsuccessful due to the scarcity and high cost of agronomic inputs, difficulties associated with their establishment and their competition for land with food crops (Mapiye *et al*., 2011; Mapiye *et al*., 2006). These exotic herbaceous species also pose a threat to the local forage legume genetic resources (Clatworthy, 1991). Hence, there is need to explore the potentials of the indigenous legume species on smallholder and communal rangelands.

2.4 Potential of *Acacia karroo* trees

Acacia genus is widely distributed throughout the arid and semi-arid regions of Sub-Saharan Africa, and *Acacia karroo* is the most widespread among the acacias in Southern Africa (Barnes *et al*., 1996). This browse specie is abundant in most communal rangelands, the foliage is ever green and is preferred by goats (Nyamukanza & Scogings, 2008). *Acacia karroo* was considered an ecological threat to natural rangelands and tends to be an invasive species (Mapiye *et al*., 2011). It is the second on the Alert List for Environmental Weeds and has been targeted for eradication (CRS Weed Management, 2003). It can invade rangelands and open grasslands, and reduce agricultural productivity by suppressing the growth of grasses, preventing stock movement and restricting watering (CRS Weed Management, 2003). Several methods have been devised to eradicate encroaching *Acacia karroo* with little or no success (Trollope *et al*., 1989; Strang 1974; Trollope 1974; Du Toit 1972). It has been suggested that browsing animals like goats are best suited in the defoliation studies of *Acacia karroo* (Teague, 1988a). Focus has now shifted from its eradication as a weed to its utilization as a protein supplement for livestock feed, particularly for goats (Mapiye *et al*., 2011). Adoption of this resource browse tree as a protein supplement during the dry season amongst the smallholder and communal farmers in Southern Africa is limited due to poor understanding of its nutritional benefits (Mapiye *et al*., 2011).

2.5 Description of *Acacia karroo* trees

Acacia karroo Hayne is synonymous with karroo thorn or sweet thorn, belonging to the family called Fabaceae (International Legume Database and Information Service, 2010). It is one of the fastest growing acacias in Southern Africa, and produces high-density wood between 800 and 890 kg/m^3 (Barnes *et al*., 1996). Historically, this woody plant is named after the Karroo region of the Cape Province of South Africa, where it is common, and often the only tree found (Barnes *et al.,* 1996). It is referred to as sweet thorn due to the sweet aroma of the flowers, and the species can be found in sweetveld due to its nutritious vegetation. Ross (1979) presented the modified botanical description of *Acacia karroo* leaves as being typically glabrous but occasionally densely pubescent. More detailed reviews on the botanical description of *Acacia karroo* have been described by Barnes *et al*. (1996), Palgrave (1996) and Ross (1979). *Acacia karroo* contains thorns which are long (2 - 5 cm), straight and whitish with brown tips and paired at the nodes (Teague, 1989). The presence of thorns obstructs efficient browsing by goats. The tree is habitually evergreen under auspicious conditions (Pooley, 1998), and can tolerate severe and frequent defoliations (Teague & Walker, 1988). These positive attributes contribute to its use as a sustainable protein supplement for goats during periods of drought. The plant has a long tap root which enables it to use water and nutrients from deep within the soil profile (van Wyk *et al.,* 2000) and can grow on acidic infertile soils with large temperature variations (Barnes *et al.,* 1996). Information regarding the root morphology of *Acacia karroo* is limited. *Acacia karroo* varies in height from a shrub to a tree (Figure 2.01), generally between 1 and 15 m tall, but can grow up to 25 m tall (Archibald & Bond, 2003).

Figure 2.01 *Acacia karroo* tree (Source: Barnes *et al*., 1996)

2.6 Nutritive value of *Acacia karroo* leaves

The feeding value of forages depends on the balance between the nutritive components of the plants, the digestibility of such nutrients, the metabolism of absorbed nutrients and the quantity of nutrients ingested by the animal (Adesogan *et al.,* 2006). Feeds of high nutritive value promote high levels of production. Performance of ruminants is greatly influenced by the amount of nutrients consumed. Tropical forages such as hays, straw and stovers are low in nitrogen (N), which is the critical element in the dry season (Lazzarini *et al*., 2013; Dube, 1993). The crude protein content of these forages (20 - 50 g crude protein/kg DM) does not meet the minimum crude protein (CP) requirement of 80 g CP/kg DM needed for optimal rumen microbial function (Annison & Bryden, 1998). *Acacia karroo* is two to three times richer in CP than grasses and cereal grains (Chepape *et al*., 2011; Goodchild & McMeniman, 1994). The use of *Acacia karroo* as dry season protein supplements has been extensively reported in literature (Ngambu *et al*., 2013; Marume *et al*., 2012; Mapiye *et al*., 2011). *Acacia karroo* leaves contain high levels of CP, minerals and fatty acids (Halimani, 2002; Ngwa *et al*., 2002) (Tables 2.01 and 2.02). The CP values for *Acacia karroo* are within the optimal range of 120 - 230 g/kg DM required for body weight gain, maintenance and production requirements in growing goats (Negesse *et al*., 2001; Meissner 1997; Goodchild & McMeniman 1994). *Acacia karroo* leaves, also, have moderate levels of detergent fibres which are an indication of high feeding values (Mapiye *et al*., 2011; Mokoboki *et al*., 2005). The review of Mapiye *et al.* (2011) on the amino acid, mineral and fatty acids profiles of *Acacia karroo* is an indication of the significant importance of this browse specie in the diets of ruminants, particularly goats.

Table 2.01 Nutritive value (g/kg DM) of *Acacia karroo* leaves

Composition	Mean	SD
Dry matter (DM)	922.0	22.51
Crude protein (CP)	154.0	46.00
Neutral detergent fibre (NDF)	450.0	150.01
Acid detergent fibre (ADF)	300.0	100.10
Fat	20.0	2.11
In vitro DM degradability	462.5	2.51
Organic Matter degradability	439.0	11.03
Apparent digestibility coefficient of DM	520.0	130.21
Apparent digestibility coefficient of CP	350.0	50.31
Apparent digestibility coefficient of NDF	435.0	105.12
Apparent digestibility coefficient of ADF	350.0	50.22
Total phenolics	25.7	5.63
Simple phenolics	9.1	0.15
Extractable phenolics	13.5	0.51
Extracted condensed tannin	82.5	27.52
Condensed tannin in neutral detergent fibre	39.0	0.13
Condensed tannin in acid detergent fibre	24.5	0.51
Crude protein-bound proanthocyanidins	2.1	0.14

Adapted from Mapiye *et al*. (2011).

SD: Standard deviation

Table 2.02 Mineral (g/kg DM) and fatty acid (g fatty acid/kg DM) profiles of *Acacia karroo* leaves

Composition	Mean	SD
Potassium	1.4	0.45
Calcium	27.4	10.35
Magnesium	1.5	0.35
Fe (mg/kg)	3.6	0.35
Lauric acid	11.5	1.07
Myristic acid	38.5	0.38
Palmitic acid	287.4	0.19
Stearic acid	91.6	1.07
Oleic acid	58.0	0.95
Linoleic acid	169.4	1.59
α-Linolenic acid	343.6	0.61
Total saturated fatty acids (SFA)	429.1	2.32
Total monounsaturated fatty acids (MUFA)	58.6	1.11
Total polyunsaturated fatty acids (PUFA)	512.9	2.16
Total omega-6 fatty acids (n-6)	169.4	1.60
Total omega-3 fatty acids (n-3)	343.6	0.60
PUFA/SFA	1.2	0.02
n-6/n-3	0.5	2.15

Adapted from Mapiye *et al*. (2011).

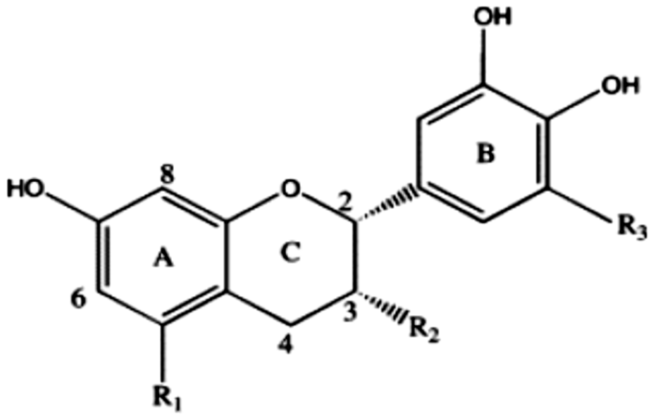
SD: Standard deviation

The variation in the nutrient content of *Acacia karroo* leaves in Tables 2.01 and 2.02 can be attributed to differences in populations, soils, climate, season, stage of growth, browsing pressure, assay methods and presence of secondary plant metabolites (Mapiye *et al*., 2011; Rubanza *et al*., 2005b). Supplementation of goats with *Acacia karroo* leaves could benefit smallholder farmers of Southern Africa during the critical fodder scarcity. However, recommendations on the use of *Acacia karroo* leaves are complicated owing to the effect of the anti-nutritional factors in the plant. The knowledge of these secondary plant metabolites is essential to achieve an optimal use of these resources as fodder for goats.

2.7 Anti-nutritional factors and their adverse effects on animal production

2.7.1 Condensed tannins

Condensed tannins (CT), also known as proanthocyanidins, are phenolic plant secondary compounds that are found in plants, leaves, bark, fruit, wood and roots (Hassanpour *et al*., 2011). Condensed tannins are the most common type of tannins found in forage legumes, trees and shrubs such as *Lotus corniculatus* and in several Acacia species (Min *et al.,* 2005). They are more copious in the parts of the plants which are more likely to be consumed by herbivores (Alvarez del Pino *et al*., 2001). There have been several notions regarding the basis for CTs synthesis which include protection against herbivory, plant defence against pathogens, nitrogen conservation, etc. (Waghorn, 2008). Structurally, CTs are complexes of oligomers and polymers of flavonoid units (i.e. flavan-3-ols and flavan-3, 4,-diols) linked by carbon-carbon bonds (Figures 2.02 and 2.03) that are not susceptible to anaerobic enzyme degradation (Tharayil *et al*., 2011; Monagas *et al*., 2010).



R_1	R_3	Class
OH	H	Proanthocyanidin
OH	OH	Prodelphinidin
H	H	Profisetinidin
H	OH	Prorobinetinidin

Figure 2.02 The basic repeating unit in condensed tannins. If R_1=R_2=OH, R_3 = H, then the structure is that for (-)-epicatecchin. The groups at R_1 and R_3 for other compounds below the structure. R_2 = O-galloyl in the catechingallates.

Source: Schofield et al. (2001)

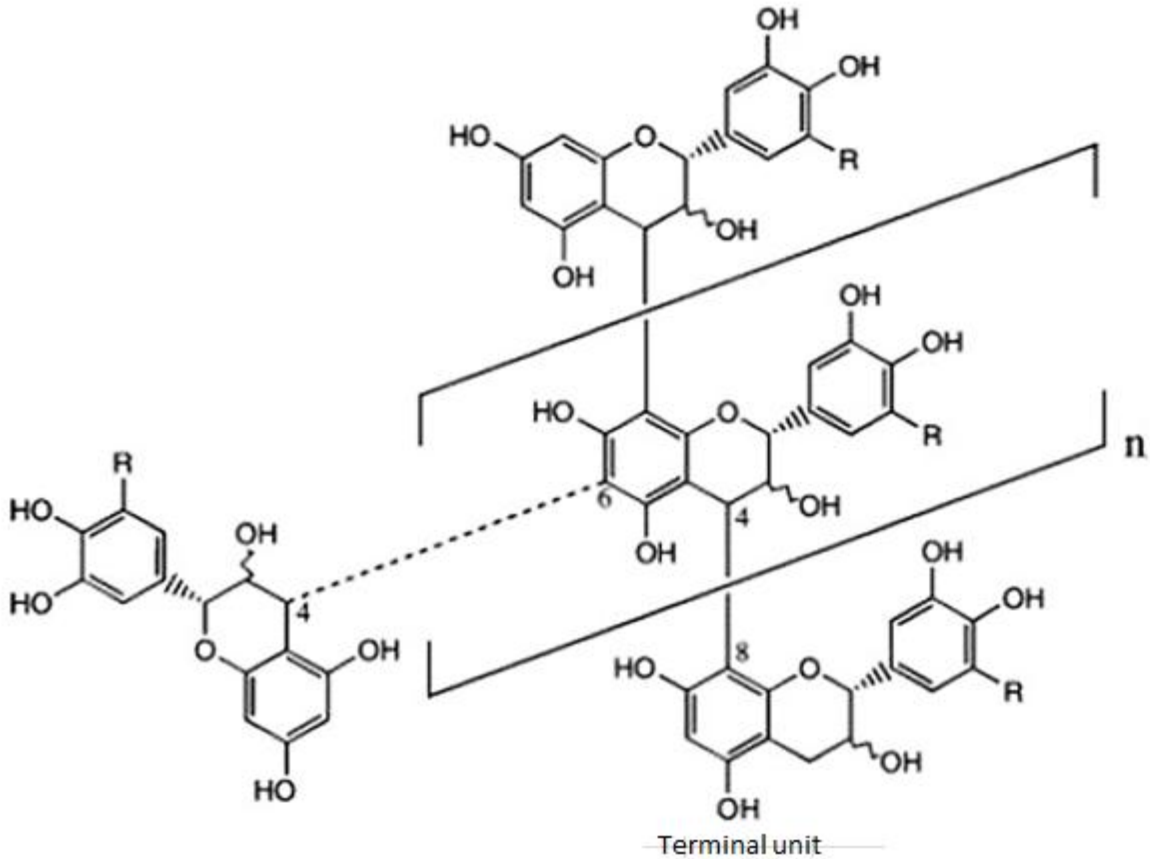


Figure 2.03. Model structure for condensed tannin. If R = H or OH then the structure represents a procyanidin or prodelphinidin. The 4→6 linkage (dotted line) is an alternative interflavan bond. The terminal unit is at the bottom of such a multi-unit structure.

Source: Schofield et al. (2001)

Condensed tannins possess high molecular weights ranging from 500 to 20,000 Dalton (Da) (Okuda & Ito, 2011). These high molecular weights cause them to react with and precipitate most proteins (Huang *et al*., 2010). They form soluble and insoluble complexes with proteins which may be responsible for the anti-nutritional effects in ruminants (Makkar, 2003). Furthermore, the anti-nutritive effects of tannins are also linked with their ability to combine with polymers such as cellulose, hemicelluloses and pectins, and minerals, thus retarding their digestion (Naumann *et al.,* 2013). Tannins affect the nutritive value of ruminant feeds by reducing voluntary feed intake and digestibility (Gxasheka *et al*., 2015). Condensed tannins are also astringent, apparent to ruminants as bitterness, thus affecting forage palatability (Lamy *et al*., 2011). High levels of condensed tannins in leaves restrict the nutrient utilization and nutrient digestibility and N retention (Kamalak, 2006). In addition, CTs may bind to digestive enzymes, thus reducing their activities (Christopher, 2011). There is, also, evidence in literature that high intake of tannins in the diet can cause liver necrosis, kidney damage with proximal tubular necrosis, lesions associated with haemorrhagic gastroenteritis and mortality (Karimi *et al*., 2014; Reed, 1995). The anti-nutritional effects of condensed tannins present in tree leaves are summarised in Table 2.03.

Tannins also play a positive significant role in the nutrition of animals. The major benefit of tannins in ruminant nutrition is the protection of plant protein from digestion in the rumen, making it available for digestion and utilization in the abomasum and small intestine (Alipour & Rouzbehan, 2010). Tannins bind with proteins at common rumen pH of 5.5 to 7.0, thereby slowing down microbial degradation of proteins (Makkar, 2003). The tannin protein complexes are dissociated in the acidic pH of 2.5 to 3.5 in the abomasum. Consumption of tannin-rich plants can have both adverse and beneficial effects depending on how much is ingested, their concentration, molecular weight, physiological state of the animal and composition of the diet (Amlan & Jyotisna, 2010a; Waghorn, 2008; Makkar, 2003). Information on the inclusion rates at which its effects on digestion and goat performance move from being beneficial to detrimental are inconclusive. Additionally, the response of ruminants to diets containing CTs is inconclusive. Hervas *et al.* (2003c) reported that high intake of tannins can produce toxicity and even cause death in animals, while others found no clear evidence (Krueger *et al*., 2010). Attention, therefore, need to be paid to inclusion ratios so as to avoid lower dry matter intake and subsequent reduction in animal performance (Theodoridou *et al*., 2010).

Table 2.03 Examples of anti-nutritional effects of condensed tannins in shrub and tree forages

Fodder tree/shrub	ANF	Animal	Nutritional effect
Acacia aneura	CT	Sheep	Reduction in N digestibility, decreased wool yield and growth
Acacia cyanophylla	CT	Sheep	Reduced feed intake, negative N digestibility, loss in weight
Acacia nilotica (pods)	CT	Sheep	Low growth rate, reduced N and NDF digestibility
L. *leucocephala*	CT	Poultry	Poor N retention, low apparent metabolisable energy value
Albizia chinensis	CT	Goat	Reduced *in sacco* N digestibility
Prosopis cineraria	CT	Sheep	Reduction in feed intake, protein digestibility, decreased wool yield and growth
Ziziphus nummularia	CT	Sheep	Reduction in feed intake, protein and DM digestibility, decrease weight loss

Source: Kumar (2003). ANF: Anti-nutritional factor, CT: Condensed tannins; L: *Leuceana*, N: Nitrogen; NDF: Neutral detergent fibre, DM: Dry matter.

2.7.2 Effects of condensed tannins in *Acacia karroo* on goat performance

The presence of CTs in *Acacia karroo* has been documented by several authors (Gxasheka *et al*., 2015; Ngambu *et al.,* 2012; Mokoboki *et al*., 2005). *Acacia karroo* contains high levels of extracted CTs ranging from 55 - 110 g/kg DM (Mokoboki *et al*., 2005; Dube *et al*., 2001). Goats can tolerate CTs better than cattle and sheep (Aganga & Tshwenyane, 2003). It has been hypothesized that browsing animals, such as goats, secrete proline-rich proteins (PRPs) in their saliva which are considered to be the first line of defence against dietary tannins (Lamy *et al*., 2011; Mueller-Harvey 2006). Condensed tannins in *Acacia karroo* have been implicated in increasing faecal N and negative N retention in goats (Mapiye *et al*., 2011; Dube & Ndlovu, 1995). The negative nitrogen balance is as a result of complexation between tannins and endogenous proteins (Barbehenn & Constabel, 2011; Silanikove *et al.,* 1996). Faecal excretion of N is a clear proof that tannins reduce the digestibility of feed (Frutos *et al.,* 2004). *Acacia karroo* supplementation has been reported to improve growth performance, meat quality and reduction in the establishment of *Haemonchus contortus* in indigenous Xhosa lop-eared goats (Ngambu *et al*., 2013; Marume *et al*., 2012; Ngambu *et al*., 2012; Nyamukanza & Scogings, 2008). However, there is lack of information on the effects of CTs in *Acacia karroo* on productivity of Pedi goats. Additionally, no studies have reported inclusion levels of *Acacia karroo* leaf meal for optimal productivity of goats.

2.8 Strategies to overcome detrimental effects of tannins

Several methods have been developed to reduce the anti-nutritional effects of dietary CTs in livestock production. A more promising mitigation is through the provision of tannin-binding compounds such as polyethylene glycol (PEG) ((Tshabalala *et al*., 2013). Polyethylene glycol is an inert and unabsorbed molecule that can form a stable complex with tannins, preventing the binding between tannins and proteins (Makkar, 2003). Additionally, PEG contains numerous oxygen atoms which are able to form hydrogen bonds with the phenolic groups in tannins and to precipitate them (Decandia *et al*., 2000). Due to its stronger hydrophobic property, PEG may release protein from tannin-protein complex (Makkar, 2003). Polyethylene glycol has been reported to prevent the formation of tannin-protein complexes and even displace protein from a pre-formed tannin-protein complex (Yisehak *et al*., 2013). Thus, supplementation with PEG has been used to alleviate the negative effects of tannins in ruminants (Landau *et al.,* 2000). Inactivation of tannins through PEG supplementation increased availability of nutrients and decreased microbial inhibition. This, in turn, increased degradability of nutrients leading to higher animal performance (Makkar, 2003).

Polyethylene glycol 4000 has the potential to neutralize negative effects of CTs present in forage legumes. Several studies have used goats in evaluating the efficacy of PEG 4000 (Tshabalala *et al*., 2013; Motubatse *et al*., 2008; Decandia *et al*., 2000). According to Motubatse *et al*. (2008), supplementation with PEG 4000 improved nutrient digestibility and live weight gain of goats. However, other authors found no clear responses (Tshabalala *et al*., 2013; Mlambo, 2002). Results from PEG 4000 supplementation are contrasting and inconclusive. Additionally, studies on *in vivo* optimal tannin- PEG ratio are limited.

2.9 Conclusion

Shortage of good quality feed, especially during the long dry season in tropical and subtropical areas has increased the need to provide supplementary feed to sustain livestock production, particularly goats. Tree fodders, such as *Acacia karroo,* are adapted to harsh environmental conditions and remain green long into the dry season. The leaves are rich in most principal nutrients, such as proteins and minerals, and tend to be more digestible than tropical grasses and crop residues (Yisehak & Janssens, 2013). Increased goat productivity could be achieved by incorporating *Acacia karroo* leaves in the diets of goats fed low quality roughages. It is, therefore, important to evaluate this fodder tree in terms of its effects on diet intake and productivity of goats.

The major constraint to the use of *Acacia karroo* is the presence of phenolic compounds, such as condensed tannins. Condensed tannins may lower dry matter intake and reduce digestion of protein and fibre. Alternatively, consumption of tannin-rich plant materials may increase the efficiency of protein utilization by ruminants. The effects of such compounds in *Acacia karroo* on intake and animal performance have not been extensively studied. Additionally, the inclusion levels of *Acacia karroo* for optimal productivity in goats need to be determined. The objective of the study was, therefore, to determine optimal productivity responses of Pedi goats to different mixtures of *Acacia karroo* leaf meal and *Setaria verticillata* grass hay.

CHAPTER THREE

VOLUNTARY FEED INTAKE AND PALATABILITY INDICES OF PEDI GOATS FED ON DIFFERENT MIXTURES OF *ACACIA KARROO* LEAF MEAL AND *SETARIA VERTICILLATA* GRASS HAY

Abstract

A study was conducted to determine preference intake and relative palatability indices of tanniniferous *Acacia karroo* leaf meal fed to five growing male Pedi goats with an average body weight of 19.81 ± 1.83 kg. Five feeding troughs were provided to each goat and each animal was exposed to all the experimental diets. A cafeteria feeding approach was used, thus, permitting goats' free access to the diet of their choice. *Acacia karroo* (A) was offered in a mixture with *Setaria verticillata* (S) hay at five different levels. The diet contained *Acacia karroo* leaf meal inclusion levels of 20 % ($S_{80}A_{20}$), 25 % ($S_{75}A_{25}$), 30 % ($S_{70}A_{30}$), 40 % ($S_{60}A_{40}$) and 50 % ($S_{50}A_{50}$). The experiment was conducted for a total period of 23 days, consisting of a 15-day adaptation period to confinement feeding followed by eight days of data collection. The daily relative palatability index (RPI) obtained for each diet was subjected to analysis of variance with feeds as treatments and individual goats as replicates in a completely randomized design. Pedi goats on diets containing 40 or 50 % *Acacia karroo* leaf meal inclusion level had higher ($P<0.05$) dry matter intake per metabolic weight than those on other dietary treatments. Similarly, goats on 30 % inclusion level had higher ($P<0.05$) dry matter intake per metabolic weight than those on 20 % inclusion level. Goats on 20 or 25 % inclusion level had similar ($P>0.05$) dry matter intake per metabolic weight. Relative palatability index of the experimental diets followed the same trend. First preference among the treatments was diet containing 50 % *Acacia karroo* leaf meal inclusion level as indicated by higher DMI, while diet containing 20 % *Acacia karroo* leaf meal inclusion level was least preferred by goats. Palatability indices were positively and significantly ($P<0.05$) predicted from dry matter intake of goats ($r^2 = 0.71$). Similarly, intake and palatability indices of the diets related positively ($P<0.05$) with the nutrient and tannin contents. Results of this study indicated that tanniniferous *Acacia karroo* leaves when fed in a mixed diet could influence preference and intake by Pedi goats. Palatability studies could be used in designing supplemental feeding programs for ruminant livestock in the tropics.

3.1 Introduction

Inadequate feed resources are the main constraints to animal production in the tropics, particularly during the dry season. Fodder trees and shrubs are increasingly recognized as important components of animal feeding and form part of the natural diets of goats (Jamala *et al*., 2013). These trees represent an enormous potential source for ruminants in the tropics (Njidda, 2010). *Acacia karroo* is an important leguminous tree in communal rangelands of Southern Africa and is able to thrive in severe and dry conditions when available forage is fibrous and low in protein (Mapiye *et al*., 2011). Studies indicate that *Acacia karroo* contains high concentrations of crude protein (100 - 250 g/kg DM) and minerals and can provide enough nutrients to goats (Mokoboki *et al.,* 2005; Abdulrazak *et al.,* 2000; Aganga *et al*., 2000). The only limitation to the use of this browse tree is the presence of secondary plant metabolites which include condensed tannins. These tend to reduce intake and palatability of the feed (Ngambu *et al.,* 2012; Mokoboki *et al.,* 2005).

Palatability of a feed has been defined by several authors. According to Jarrige (1988), palatability of a feed is defined as the physical (plant bearing and spines) and chemical (odour, taste, etc.) characteristics of the feed that act on appetite. Condensed tannins lower feed intake by reducing palatability (Muir, 2011). It has been hypothesized that the reduction in palatability and intake is associated with the astringency in the mouth of the animals (Muir, 2011; Breslin *et al*., 1993). Astringency is a puckering or drying sensation in the mouth that typically develops and dissipates slowly (Lamy *et al*., 2011).

Tamir & Asefa (2009) observed that dry matter intake (DMI) is influenced by palatability, chemical composition and physical attributes of the diet. However, information on DMI and palatability of tannin-rich diets in goats are equivocal. Some authors reported higher DMI in goats fed tannin-rich diets at different levels (Gwanzura *et al*., 2011; Ramirez *et al*., 1992; Nunez-Hernandez *et al*., 1989) while others observed significant reduction in DMI (Ngwa *et al*., 2003). Preference and selection studies present a useful tool in evaluating tannin rich plants such as *Acacia karroo*. Information on the inclusion level of *Acacia karroo* in grass-based diets on DMI and palatability is limited and not conclusive.

3.2 Objective

The objective of the current study was to determine the effects of *Acacia karroo* leaf meal inclusion level on voluntary intake, palatability and preference rankings of diets fed to indigenous male Pedi goats.

3.3 Hypothesis

The hypothesis of the current study was that there is no effect of *Acacia karroo* leaf meal inclusion level on voluntary intake, palatability and preference rankings of diets fed to indigenous male Pedi goats.

3.4 Materials and methods

3.4.1 Study site

The study was conducted at the University of Limpopo Experimental farm. The farm is located about 10 km northwest of the Turfloop campus. The ambient temperatures at the study site range between 20 and 36 ºC during summer (November to January) and between 5 and 25 ºC during winter (May to July). University of Limpopo lies at latitude 27.55 ºS and longitude 24.77 ºE. It receives a mean annual rainfall of less than 400 mm (Kutu & Asiwe, 2010). The vegetation is a mixture of shrubs, trees and grass species. The herbaceous layer is dominated by grasses such as *Digitaria eriantha, Schmidtia pappophoroides*, *Aristida, Egagrostis* and *Acacia* species (Low & Rebelo, 1996).

3.4.2 Feeds

Fresh leaves *of Acacia karroo* were hand-harvested at the University of Limpopo Experimental farm using a pair of long scissors in summer (November to January). The leaves were air-dried under the shade to minimize nutrient loses to ultra violet rays (Dzowela *et al*., 1995). After drying, the leaves were collected from the branches by shaking them off gently with a long stick, leaving the thorns behind. The leaf meal was stored until feeding time. *Setaria verticillata* (Bur bristle grass/Klits grass) hay was bought from the local farmer (Fourie Boerdery, Polokwane, South Africa). The grass and *Acacia karroo* leaves were passed through a hammer mill (13 mm screen) to reduce diet selection by the animals when fed.

3.4.3 Animals, housing and feeding

Five growing male Pedi goats, with an average body weight of 19.81 ± 1.83 kg were used in the experiment. The goats were individually housed in a well ventilated roofed pen. Five feeding troughs were provided to each goat and each animal was exposed to all the experimental diets. A cafeteria feeding approach described by Larbi *et al*. (1993) was used, thus, permitting free access to the diet of their choice. The feed composition is presented in Table 3.01. The position of the troughs was randomized each day to avoid "habit reflex". The experiment was conducted for a total of 23 days, consisting of a 15-day adaptation period to confinement feeding followed by eight days of data collection. The goats were fed *ad libitum,* allowing a 15 % refusal of each diet as suggested by Kaitho *et al*. (1996). Water and a mineral lick (Table 3.02) were provided *ad libitum.* The feeds offered and refusals were weighed and recorded daily and dry matter determined for each animal. The intake data was used to determine the relative palatability of the forage mixture. A daily relative palatability index (RPI) was calculated for each diet by dividing the amount consumed by that of the highest value, and multiplying the result by 100 as described by Larbi *et al*. (1993). These daily RPI values obtained for each diet were then subjected to analysis of variance with feeds as treatments and individual animals as replicates in a completely randomized design. The diets were then ranked based on these calculations with the highest consumption value being the most preferred and vice versa.

Table 3.01 Feed composition of the experimental diets

Diet code	Diet description
$S_{80}A_{20}$	A mixture of 80 % *Setaria verticillata* hay and 20 % *Acacia karroo* leaves
$S_{75}A_{25}$	A mixture of 75 % *Setaria verticillata* hay and 25 % *Acacia karroo* leaves
$S_{70}A_{30}$	A mixture of 70 % *Setaria verticillata* hay and 30 % *Acacia karroo* leaves
$S_{60}A_{40}$	A mixture of 60 % *Setaria verticillata* hay and 40 % *Acacia karroo* leaves
$S_{50}A_{50}$	A mixture of 50 % *Setaria verticillata* hay and 50 % *Acacia karroo* leaves

Table 3.02 Nutritional composition of the mineral block offered to the experimental animals

Nutrient	Quantity
Calcium	120 g/kg (max)
Phosphorus	60 g/kg (min)
Manganese	1200 mg/kg
Copper	200 mg/kg
Cobalt	3 mg/kg
Iron	750 mg/kg
Iodine	15 mg/kg
Zinc	1200 mg/kg
Selenium	3 mg/kg

Source: Voermol Feeds (Pty) Ltd, South Africa.

3.4.4 Chemical analysis

Dry matter determination (AOAC, 2005): Dry matter of feeds was determined according to the AOAC (2005). Thoroughly cleaned crucibles were placed in an oven at 105 ºC for 30 minutes and then transferred to a desiccator and cooled to room temperature (25 ºC). The crucibles were then weighed. Samples were weighed and placed into crucibles and placed in the oven overnight at 105 ºC. The crucibles and contents were weighed as soon as possible to prevent moisture absorption. Dry matter was calculated as follows:

DM (%) = Weight of the sample before drying/Weight of the sample after drying x 100

Determination of ash and organic matter (AOAC, 2005): Air-dried plant samples (2 g) were weighed and placed in pre-weighed clean-labelled beakers. The sample plus the beaker were placed in the muffle furnace at 550 ºC overnight. The beaker and content were weighed as soon as possible to prevent moisture absorption. Ash determination was as follows:

Ash weight = (Weight of beaker + ash) - (Weight of beaker)

Ash (%, DM basis) = (Ash weight/Dry sample weight) x 100

Organic matter (OM) was calculated as:

OM (%) = 100 - Ash %

Determination of nitrogen content (AOAC, 2005): Nitrogen contents of the feed samples were determined using the Kjeldahl procedure (AOAC, 2005).

The formula for nitrogen content was as follows:

N (%) = (ml acid titrated - ml blank titrated) x (Acid N x 0.014 x 100)/Weight of sample in grams (g).

Determination of neutral detergent fibre content (Van Soest, 1994): Neutral detergent fire (NDF) was determined by weighing 1.0 g of the sample into digestion tube, and 100 ml neutral detergent solution was added and heated to boil. Heat was reduced as boiling commenced to prevent foaming. From the onset of boiling, the mixing was refluxed for 60 minutes. Sintered glass crucibles (porosity number 1) were weighed and placed on filtering apparatus (Buchner flask). Contents of digestion tubes were transferred to the crucibles and filtered with a low vacuum initially, and then with a gradual increase of the vacuum. Samples were rinsed into the crucibles with a minimum of distilled hot water. The vacuum was then shut off, residues broken up and washed with hot water followed by two washes with acetone. The crucibles were dried at 105 ºC overnight and weighed. Recovered cell wall residues were reported as neutral detergent fibre. The formula used for neutral detergent fibre determination was as follows:

NDF (%) = [(Weight of crucible + residue) – (Weight of crucible/Weight of sample] x 100

NDF (%, on a DM basis) = [NDF % on as fed basis/DM % of sample] x 100

Determination of acid detergent fibre (Van Soest, 1994): Acid detergent fibre (ADF) was determined by accurately weighing 1.0 g of the sample into a digestion tube; 100 ml acid detergent was added and heated to boil. From onset to boiling, the mixture was refluxed for 60 minutes. The light solutions were filtered through sintered glass crucibles (porosity number 1). Residues were broken up carefully and washed with hot water, rinsing sides of crucibles. The residues were then washed again twice with acetone and finally with hexane. The residue was filtered and dried in an oven at 105 ºC overnight. The residues were then cooled in a desiccator and weighed. The formula for acid detergent fibre determination was as follows:

ADF (%) = [(Weight of crucible + residues) – weight of crucible/Weight of sample] x 100

ADF (%, on a DM basis) = [ADF % on as fed basis/DM %] x 100

Determination of total polyphenols (Waterman & Mole, 1994; Hagerman & Butler, 1991): Air-dried and finely ground plant material was placed in conical flasks of approximately 25 ml capacity. To each flask, 20 ml of methanol was added and vortexed every 10 minutes for 2 hours. Separation was achieved by sedimentation. After removing the supernatants, another 20 ml of methanol was added to the residue and vortexed every five minutes for 20 minutes. After repeating the separation and vortexing, the supernatants were collected and stored in the fridge at 10 °C until ready for analysis.

Different standard concentrations (0, 0.2, 0.4, 0.6, 0.8 ml) were prepared from a stock solution of gallic acid (1 mg in 10 ml) by diluting with sodium carbonate. Approximately 10 ml of distilled water, 0.5 ml of the extract or standard was added to the volumetric flasks. After allowing to react for 1 - 8 minutes, 7.5 ml of sodium carbonate was also added and the flasks were filled with distilled water. Finally, after mixing and allowing to react for 2 hours, folin reagent was added and the absorbance was read at 760 nm.

Determination of extracted condensed tannins (Porter *et al*., 1986): A sample of 0.2 ml of tannin diluted with 0.3 ml of 70 % acetone was pipetted into a 100 x 12 glass test tube and 3.0 ml of Butanol-HCI reagent and 0.1 of ferric reagent were added. The tubes were vortexed and then the mouths of the tubes were covered with glass marbles placed in a heating block adjusted to 97 to 100 ºC for 60 minutes. The tubes were swirled gently every two minutes. The tubes were then cooled and absorbance was recorded at 550 nm against a suitable blank (unheated mixture). The formula for calculating percentage of condensed tannins as leucocyanidin equivalent was = (absorbance 550 nm x 78.26 dilution factor) / (% DM).

3.4.5 Statistical analysis

Data on chemical composition and tannin contents were subjected to analysis of variance (SAS, 2010). Treatment means were compared using a least significant difference (LSD) test at the 5 % level of probability. General linear model procedures (GLM) of SAS (2010) were used to compare effects of diets on feed intake and relative palatability indices (RPI) of goats. Linear regression analysis was used to establish associations between chemical characteristics and RPI and intake.

3.5 Results

Nutrient composition and tannin contents of *Acacia karroo* and *Setaria verticillata* are presented in Table 3.03. *Acacia karroo* had a higher crude protein content than *Seteria verticillata* grass hay. However, NDF and ADF contents were higher in *Setaria verticillata* grass hay. *Acacia karroo* contained moderate levels of condensed tannins and total phenolics. Tannins were not detected in *Setaria verticillata* grass hay.

The nutritive values of dietary mixtures of *Setaria verticillata* and *Acacia karroo* are presented in Table 3.04. There were differences ($P<0.05$) in the chemical composition of the dietary mixtures of *Seteria verticillata* and *Acacia karroo* leaves. The organic matter, crude protein, fat, condensed tannins and total phenolic contents were higher ($P<0.05$) in the diet containing a 50 % *Acacia karroo* leaf meal inclusion level, followed by 40, 30, 25 and 20 % inclusion levels, respectively. The ADF and NDF contents of the diets increased ($P<0.05$) as the proportion of *Seteria verticillata* grass hay increased in the diets. All the diets had similar ($P>0.05$) ash content values.

Results of dry matter intake (DMI) per metabolic weight, relative palatability index (RPI) and preference ranking (PR) of the experimental diets are presented in Table 3.05. Pedi goats on diets containing 40 or 50 % *Acacia karroo* leaf meal inclusion level had higher ($P<0.05$) dry matter intake per metabolic weight than those on other dietary treatments.

Similarly, goats on a 30 % inclusion level had a higher (P<0.05) dry matter intake per metabolic weight than those on a 20 % inclusion level. Goats on a 20 or 25 % inclusion level had similar (P>0.05) dry matter intakes per metabolic weight. Relative palatability index of the experimental diets followed the same trend. Most preferred among the treatments was a diet containing a 50 % *Acacia karroo* leaf meal inclusion level as indicated by a higher DMI, while a diet containing 20 % *Acacia karroo* leaf meal inclusion level was the least preferred by goats.

Series of linear regression equations that predict dry matter intake and relative palatability index of goats from nutrient composition of the five diets fed to Pedi goats are presented in Table 3.06. There were significant (P<0.05) relationships between nutrients of the diets and their intake and palatability indices by goats.

Table 3.03 Chemical composition (mean ± SD) of the experimental forages

Nutrient (% DM)	*Acacia karroo* leaves	*Setaria verticillata* hay
Dry matter	97.1 ± 2.01	96.2 ± 0.40
Organic matter	92.1 ± 0.21	91.4 ± 0.12
Crude protein	12.7 ± 2.02	7.9 ± 1.12
Fat	2.4 ± 0.10	0.8 ± 0.01
Ash	7.9 ± 0.40	8.6 ± 0.31
Acid detergent fibre	32.5 ± 3.02	50.7 ± 4.01
Neutral detergent fibre	38.0 ± 4.01	77.9 ± 3.02
Condensed tannins[#]	2.0 ± 0.01	ND
Total Phenolics[##]	1.95 ± 0.001	ND

[#]: Condensed tannins as percentage DM leucocyanidin equivalent [##]: Expressed as tannic acid equivalent (%); ND: Not detected

Table 3.04 The nutrient composition of experimental diets (% DM basis) offered to Pedi goats for palatability assessment

Nutrient	Treatment					SEM
	$S_{80}A_{20}$	$S_{75}A_{25}$	$S_{70}A_{30}$	$S_{60}A_{40}$	$S_{50}A_{50}$	
Dry matter	95.24^{c}	95.86^{b}	94.05^{e}	95.21^{d}	97.01^{a}	0.000
Organic matter	91.52^{e}	91.56^{d}	91.60^{c}	91.67^{b}	91.75^{a}	0.000
Ash	8.47	8.43	8.39	8.32	8.24	0.110
Fat	1.12^{e}	1.20^{d}	1.28^{c}	1.45^{b}	1.61^{a}	0.041
Crude Protein	8.90^{e}	9.16^{d}	9.34^{c}	9.84^{b}	10.37^{a}	0.132
ADF	47.03^{a}	46.12^{b}	45.21^{c}	43.39^{d}	41.57^{e}	0.671
NDF	69.90^{a}	67.91^{b}	65.91^{c}	61.93^{b}	57.94^{e}	0.421
Condensed tannins[#]	0.41^{e}	0.51^{d}	0.61^{c}	0.82^{b}	1.02^{a}	0.017
Total Phenolics[##]	0.39^{e}	0.49^{d}	0.58^{c}	0.78^{b}	0.98^{a}	0.003

[a,b,c]: Means in the same row with different superscripts are significantly different (P<0.05)

SEM: Standard error of the means

ADF: Acid detergent fibre

NDF: Neutral detergent fibre

[#]: Condensed tannins as percentage DM leucocyanidin equivalent

[##]: Expressed as tannic acid equivalent (%)

Table 3.05 Relative palatability index (RPI) rankings and average daily intake (g/kg $W^{-0.75}$) by Pedi goats offered the experimental diets by a cafeteria feeding method

Diet	DM intake (g/kg $W^{-0.75}$)	RPI %	Preference ranking
$S_{20}A_{80}$	14.59^{c}	23.65^{c}	5
$S_{25}A_{75}$	20.00^{bc}	31.97^{c}	4
$S_{30}A_{70}$	30.14^{b}	53.07^{b}	3
$S_{40}A_{60}$	44.16^{a}	81.83^{a}	2
$S_{50}A_{50}$	52.38^{a}	96.91^{a}	1
SEM	4.413	5.725	

^{a,b,c}: Means in the same column with different superscripts are significantly different ($P<0.05$)

SEM: Standard error of the means

Table 3.06 Prediction of dry matter intake (DMI) and relative palatability index (RPI) of Pedi goats offered mixtures of *Setaria verticillata* and *Acacia karroo* from intake, crude protein (CP), neutral detergent fibre (NDF), acid detergent fibre (ADF), condensed tannins (CT), and total polyphenol (TP) contents

Factor	Y-variable	Formulae	r^2	P
DM (%)	DMI	Y = 0.222x + 7.057	0.99	<0.0001
OM (%)	DMI	Y = 1.070x - 0.041	1.00	<0.0001
CP (%)	DMI	Y = 5.131x + 4.908	0.97	<0.0001
NDF (%)	DMI	Y = 1.442x – 2.073	0.98	<0.0001
ADF (%)	DMI	Y = 1.777x – 1.311	0.99	<0.0001
CT*	DMI	Y = 20.703x + 14.717	0.92	<0.0001
TP**	DMI	Y = 21.171x + 15.480	0.89	<0.0001
OM (%)	RPI	Y = 1.821x + 2.601	0.71	<0.0001
CP (%)	RPI	Y = 8.913x + 9.983	0.72	<0.0001
NDF (%)	RPI	Y = 2.463x – 1.135	0.71	<0.0001
ADF (%)	RPI	Y = 3.021x + 0.412	0.71	<0.0001
CT[#]	RPI	Y = 34.047x + 28.643	0.61	<0.0001
TP[##]	RPI	Y = 34.353x + 30.266	0.58	<0.0001

r^2: Coefficient of determination

P: Probability

[#]: CT as percentage DM luecocyanidin equivalent

[##]: Expressed as tannic acid equivalent (%)

3.6 Discussion

The diets containing 40 and 50 % *Acacia karroo* inclusion levels provided 9.84 and 10.37 % CP contents, respectively. The high CP contents in these diets were due to higher *Acacia karroo* inclusion levels. *Acacia karroo* leaves contain high levels of CP and essential amino acids (Halimani, 2002; Ngwa *et al*., 2002). The CP contents of all the experimental diets were above the minimum level of 8 % required for optimal microbial function in the rumen (Norton, 2003; Annison & Bryden, 1998), suggesting that all the diets could support maintenance requirements and some production levels in ruminants (Van Soest, 1994). *Acacia karroo* leaf meal contains between 100 and 250 g of crude protein per kg dry matter (Marume *et al.,* 2012; Halimani *et al.,* 2005; Mokoboki *et al.,* 2005). Leaves of fodder trees have been used as supplements to grass hay-based diets, especially during the critical dry periods of the years (Tshabalala *et al*., 2013). These tree fodders have been incorporated into the rations of ruminants as substitutes for more expensive processed protein sources (Norton, 1994).

Detergent fibre contents in the experimental diets decreased as the supplementation levels of *Acacia karroo* increased in the diets. This observation is similar to the findings of Olafadehan (2011). According to Tamir & Asefa (2009), feeds with higher fibre contents limit intake and digestibility. Low levels of the detergent fibres are associated with high voluntary DM intakes as observed in this study. Goats consumed more of a diet with 50 % *Acacia karroo* leaf meal inclusion level.

A diet containing 50 % *Acacia karroo* leaf meal inclusion level had the highest condensed tannins and polyphenolic contents. Relative palatability indexes (RPI) were highest in diets containing 40 and 50 % *Acacia karroo* leaf meal inclusion level. First preference among the treatments was a diet containing 50 % *Acacia karroo* leaf meal inclusion as indicated by the DMI of 52.38 g/kg $W^{-0.75}$ followed by a diet containing 40 % inclusion level, with a DMI of 44.16 g/kg $W^{-0.75}$. Tannins have been associated with low palatability values, however, medium to low levels may be tolerated by ruminants (Lamy *et al.,* 2011). The high level of voluntary intake by goats in the present study suggests that tannin-rich diets do not always depress intake. Gwanzura *et al.* (2011) observed that Pedi goats consumed more mucuna hay with high condensed tannin concentrations when offered different legume species. Ramírez & Ledezma-Torres (1997) reported that inclusion of *Acacia rigidula* and *Acacia farnesiana* did not adversely affect forage intake of goats. Similarly, Ramírez & Coello (1990) found that goats fed diets low in condensed tannins (CT) had similar nutrient intakes as goats fed low levels of alfalfa hay. Nuňez-Hernandez *et al.* (1989) observed similar DM intakes for Angora goats on a diet containing a high tannin shrub (*Juniperus monosperma*) compared with goats fed an alfalfa hay diet. In contrast, Holechek *et al.* (1990) stated that diets high in CTs reduced intake of goats. Other studies have indicated that plant secondary metabolites (PSM) such as tannins may reduce dry matter intake of forage legumes by decreasing palatability (Barry & McNabb, 1999; Reed, 1995). Reduced palatability in tannin-rich plants may be related to the type than the amount of tannins present in browse species. Generally, animals consuming tannin-rich feeds develop defensive mechanisms against tannins (Makkar, 2003). These animals secrete proline-rich proteins (PRPs) in their saliva as a defense mechanism against dietary tannins (Lamy *et al.,* 2011). The presence of microorganisms resistant to high level of condensed tannins has also been reported in ruminants consuming tannin-rich feeds (Muir, 2011).

Nutrient composition related positively with RPI of the examined diets. Positive relationships of chemical composition of forages with animal preference have been reported in previous studies (Lambert *et al.,* 1989; Marten, 1970). The assertions of Minson (1990) and Thompson & Poppi (1990) that a strong correlation exists between many of the chemical constituents of plants and palatability indices were fully supported by the findings of this study. As opposed to the findings of Gwanzura *et al.* (2011), nutrient content could be a definitive predictor of intake and palatability of forage-rich diets.

3.7 Conclusion

Results of this study indicate that *Acacia karroo* leaves have high protein contents, ranging from 10.65 to 14.65 %. Thus, *Acacia karroo* leaves have a potential of being a protein feed for ruminant animals. Indeed, diets with higher *Acacia karroo* levels had higher intakes and relative palatability rankings by Pedi goats, regardless of the higher condensed tannin and total phenolic levels. It is, thus, important to determine the effects of these secondary plant metabolites on diet digestibility, body weight and blood profiles of Pedi goats offered such diets.

CHAPTER FOUR

EFFECT OF *ACACIA KARROO* LEAF MEAL INCLUSION LEVEL ON FEED INTAKE, DIGESTIBILITY, BODY WEIGHT CHANGE AND BLOOD PROFILE OF PEDI GOATS FED A *SETARIA VERTICILLATA* GRASS HAY-BASED DIET

Abstract

An experiment was carried out to determine the effects of *Acacia karroo* leaf meal inclusion level on feed intake, digestibility, body weight gain and blood profiles of indigenous Pedi goats fed on a *Seteria verticillata* grass hay-based diet. Twenty indigenous male Pedi goats with an average initial body weight of 17.44 ± 2 kg were allocated, in a completely randomized design, to five dietary treatments containing *Acacia karroo* leaf meal inclusion levels of 20 % ($S_{80}A_{20}$), 25 % ($S_{75}A_{25}$), 30 % ($S_{70}A_{30}$), 40 % ($S_{60}A_{40}$) and 50 % ($S_{50}A_{50}$). Daily dry matter intakes were similar (P>0.05) across the treatments, ranging from 617 to 679 g per goat per day. *Acacia karroo* leaf meal inclusion improved (P<0.05) dry matter, organic matter, crude protein, neutral detergent fibre and acid detergent fibre digestibility coefficients. The crude protein (CP) digestibility values of goats consuming diets having 30, 40 or 50 % *Acacia karroo* leaf meal inclusion levels were higher (P<0.05) than those of goats on diets having 20 or 25 % inclusion levels, while goats on diets 20 or 30 % had similar (P<0.05) CP digestibility values. Dietary treatments had no effect on initial and final body weights of goats. However, body weight gains were higher (P<0.05) in goats fed a diet containing 50 % *Acacia karroo* leaf meal inclusion level as compared to other treatment groups. Similarly, goats on diets having 30 or 40 % *Acacia karroo* leaf meal inclusion levels had higher (P<0.05) body weight gains than those on diets having 20 or 25 % inclusion levels. However, goats on diets having 30 or 40 % *Acacia karroo* leaf meal inclusion levels had similar (P>0.05) body weight gains. Similarly, goats on diets having 20 or 25 % *Acacia karroo* leaf meal inclusion levels had the same (P>0.05) body weight gains. Dry matter, CP and neutral detergent fibre digestibility coefficients were optimized at *Acacia karroo* leaf meal inclusion levels of 69.4, 48.3 and 42.7 %, respectively. Haematology and serum biochemical parameters of male Pedi goats revealed no differences between the treatments at the beginning of the experiment. These results served as a baseline to monitor the changes in the blood profile of the goats as affected by the treatments. At the end of the trial, goats consuming diets with 40 or 50 % *Acacia karroo* leaf meal inclusion levels had lower (P<0.05) serum TP than those on other treatments.

It is concluded that *Acacia karroo* leaf meal inclusion improved nutrient digestibility and body weight gain of the goats and thus has potential to be utilized as a protein supplement when low quality roughage is used as a basal diet.

4.1 Introduction

Indigenous goats play multiple roles in livelihood of households of South Africa (Ng'ambi *et al.,* 2013). They provide benefits in the form of meat, milk, manure, hide and skins, and cash (Peacock, 2005). However, the productivity of these goats is constrained by shortage of good quality feed, especially during the long dry season. Available feed resources during this period are, generally, deficient in nutrients such as protein, energy, minerals and vitamins, and hence cannot adequately meet the nutritional requirements for maintenance (Manaye *et al*., 2009). Earlier studies have shown that indigenous Pedi goats lose body weight and condition during the dry season (Gwanzura, 2011; Ravhuhali *et al*., 2011). There is need to identify more nutritious feed to alleviate the prevailing nutritional problems of indigenous goats in the communal areas during the dry season.

Browse tree legumes and shrub foliage have been identified as important sources of fodder for livestock in communal rangelands of southern Africa (Solomon *et al*., 2008; Silanikove *et al*., 1996). An important attribute of browse trees is their positive effect on intake and digestibility when supplemented with fibrous basal feeds (Umunna *et al*., 1995). *Acacia karroo* is an important ecological component of the bushveld vegetation and is abundant in the Limpopo Province of South Africa (Mokoboki *et al*., 2005). The browse tree retains its green leaves throughout the year (Barnes *et al*., 1996) and has potential as a crude protein supplement in ruminant diets (Mapiye *et al*., 2011). However, the utilization of *Acacia karroo* leaves is restricted by the presence of secondary plant compounds such as condensed tannins (Mokoboki *et al*., 2005; Dube *et al.,* 2001). Intake of condensed tannins by ruminants may depress feed intake and digestibility of diets, and hence adversely affect productivity of the animals (Waghorn, 2008; Frutos *et al*., 2004). Mixing of shrubs with grass hay-based diets has been hypothesized as an efficient way of diluting the negative effects of undesirable secondary compounds, such as tannins (Bhat *et al.,* 2013; Ben Salem & Smith, 2008). According to Topps (1992), the most practical alternative to dilute the effect of secondary plant metabolites is to use them as supplements at low levels in a suitable mixture, or to feed them as a combination of two or more rather than one browse species. Ondiek *et al*. (2013) observed higher feed intake, average daily gain and nutrient digestibility in goats fed maize stover supplemented with taninniferous *Acacia tortilis* or *Balanites aegyptiaca* leaf meals. Earlier studies have also reported improved body weight gains in meat and dairy goats fed low N roughage-based diets supplemented with shrub legumes (Ondiek *et al.,* 1999; Abdulrazak *et al*., 1997).

Additionally, adverse effect of tannin-rich *Pterocarpus erinaceus* in the blood profile of goats have been reported (Olafadehan, 2011). Changes in haematological and biochemical indices are indication of various disease conditions, even at sub-clinical levels (Taiwo & Anosa, 1995). It is, therefore, important to check physiological and biochemical indicators of health and sub-lethal toxicant effects on livestock consuming feeds that contain tannins (Olafadehan *et al*., 2014). However, the effects of tannin-rich *Acacia karroo* on intake, performance and blood profile of indigenous Pedi goats are not conclusive and extensive. Additionally, information on the *Acacia karroo* leaf meal inclusion level for optimal productivity of Pedi goats is not available.

4.2 Objectives

The objectives of the study were to determine:

a. the effect of *Acacia karroo* leaf meal inclusion level on feed intake, digestibility, body weight changes and blood profile of indigenous Pedi goats fed a *Setaria verticillata* grass hay-based diet.

b. *Acacia karroo* leaf meal inclusion levels for optimal diet intake, digestibility and body weight gain of Pedi goats fed a *Setaria verticillata* grass hay-based diet.

4.3 Hypotheses

a. There is no effect of *Acacia karroo* leaf meal inclusion level on feed intake, digestibility, body weight changes and blood profile of indigenous Pedi goats fed a *Setaria verticillata* grass hay-based diet.

b. There are no *Acacia karroo* leaf meal inclusion levels for optimal diet intake, digestibility and body weight gain of Pedi goats fed a *Setaria verticillata* grass hay-based diet.

4.4 Materials and methods

4.4.1 Study site

The study was conducted at the University of Limpopo Experimental farm. The site is described in Section 3.4.1.

4.4.2 Experimental diets and feeding

Fresh leaves *of Acacia karroo* were hand-harvested at the University of Limpopo Experimental farm using a pair of long scissors in summer (November to January) as described in Section 3.4.2. The chopped forage was thoroughly mixed with *Acacia karroo* leaves according to the dietary treatments described in Section 3.4.3 and presented in Table 3.01. The goats were fed *ad libitum*, allowing a 15 % refusal of each diet, as suggested by Kaitho *et al.* (1996). Clean fresh water was offered *ad libitum* and each goat had access to a mineral lick (Section 3.4.3). There was a preliminary period of 14 days to familiarize the goats with the feeds and research protocol. The goats were individually offered feed once a day at 0800 h. Feed offered was weighed and recorded daily and feed refusals were weighed every morning before fresh feed was offered. Dry matter values of the feeds and feed refusals were determined. The feeding trial lasted for 21 days, the last 7 days being for data collection.

4.4.3 Animals, housing and experimental design

Twenty yearling indigenous male Pedi goats with an average initial live weight of 17.44 ± 2 kg were used in the study. The goats were randomly divided into five groups consisting of four animals per group. The animals in each group were randomly allocated to the five treatments in a completely randomized design (CRD). Before the commencement of the experiment, the pens and the surrounding environment were thoroughly cleaned and disinfected with an antiseptic (Jeyes fluid disinfectant and cleanser manufactured by Tiger Consumer (Pty) Ltd, South Africa). The goats were given prophylactic treatments consisting of an intramuscular injection of an antibiotic (Oxytetracycline LA) at the rate of 1 ml/10 kg BW (Inversa, Spain), drenching with anthelmintic (Valbazen® broad spectrum dewormer; Pfizer Animal NY, USA) and spraying against ectoparasites with diazintol (Pfizer Animal, NY, USA). The goats were housed in individual holding pens (1 x 3 m^2). The pens were installed in a well-ventilated shed with one side open to natural light and roofed to protect goats against the sun and rain. The goats were weighed three times, at the start of the experiment, on the 14^{th} day when data collection commenced and on the 21^{st} day when data collection ended. The weighing of the goats was carried out before morning feeding to avoid feed effect (Sarwatt *et al*., 2003). Average daily body weight gain was determined by difference between initial and final weight divided by the total number of the experimental days.

4.4.4 Digestibility trial

On day 21, male Pedi goats were transferred to metabolic crates and each animal was fitted with a faecal bag for *in vivo* digestibility. The goats were adapted for three days to the carrying of faecal collection bag which was followed by total faecal collection for a period of seven successive days for each animal. At the end of the collection period the faeces of individual animals were thoroughly mixed and a sample taken for chemical analysis. From the food consumed and faecal matter secreted, apparent digestibility of the nutrients was calculated (McDonald *et al*., 2011).

4.4.5 Chemical analysis

The procedure for the determination of dry matter, fat, organic matter, crude protein, acid detergent fibre, neutral detergent fibre, condensed tannins and total polyphenols were as described in Section 3.4.4.

Blood samples were collected from each goat via the jugular vein puncture using hypodermic syringes at the start and end of the experiment. A 5 ml blood sample was collected into labelled sterile bottles containing ethylene diamine tetra acetic acid (EDTA) as an anticoagulant for the determination of haematological parameters. Blood samples for serum analysis were collected into anticoagulant free bottles, allowed to coagulate at room temperature and centrifuged at 1500 x g for 10 minutes. The supernatant sera were collected and stored at a temperature of -20 ºC for biochemical analysis. A 2 ml blood sample was collected in sodium oxalate fluoride bottle for serum glucose analysis.

4.4.6 Statistical analysis

All data on feed intake, *in vivo* digestibility and body weight gain of male goats were analysed using the General Linear Model procedures of SAS (SAS, 2010). Fisher's least significant difference (LSD) test was applied for mean separation where there were significant differences (P<0.05). The responses in optimal intake, digestibility and body weight change to the level of browse inclusion were modelled using the following quadratic equation:

$$Y = a + b_1x + b_2x^2$$

Where Y = feed intake, digestibility or body weight change; a = intercept; b_1 and b_2 = coefficients of the quadratic equation; x = level of inclusion and $-b_1/2b_2$ = x value for optimal response. The quadratic model was used because it gave the best fit.

The linear relationships between browse inclusion level and responses in feed intake, digestibility and body weight changes were modelled using the following linear equation:

$$Y = a + bx$$

Where Y = feed intake, digestibility or body weight change; a = intercept; b = coefficient of the linear equation; x = level of inclusion.

For blood profile, data were analysed by analysis of covariance (ANCOVA) using blood baseline values as the covariate in order to statistically control for differences in baseline values. Where the covariate showed no significant effect (P>0.05), the data were analysed using analysis of variance (ANOVA) in a completely randomized design at 5 % level of significance (SAS, 2010). Where significant treatment effects were detected, means were separated by Fisher's least significant difference.

4.5 Results

4.5.1 Nutrient composition

Results of the nutritive values of the diets were similar to those presented in Section 3.5. Higher *Acacia karroo* leaf meal inclusions resulted in higher (P<0.05) crude protein and condensed tannin contents of the diet.

4.5.2 Effect of *Acacia karroo* leaf meal inclusion level on feed intake, digestibility and body weight change of Pedi goats fed a *Setaria verticillata* grass hay-based diet

The results of the effect of *Acacia karroo* leaf meal inclusion level on feed intake, digestibility and body weight change of male Pedi goats fed *Setaria verticillata* grass hay-based diets are presented in Table 4.02. Daily dry matter intakes were similar (P>0.05) across the treatments, ranging from 617 to 679 g per goat per day. Similarly, goats consumed the same (P>0.05) amounts of dietary organic matter (OM), crude protein (CP), neutral detergent fibre (NDF) and acid detergent fibre (ADF) contents. Intakes per metabolic weight were similar (P>0.05) across the diets.

Diet DM, OM, CP, NDF and ADF digestibility values were different (P<0.05) across the treatments. Diet DM digestibility values were higher (P<0.05) in goats consuming a diet having a 50 % *Acacia karroo* leaf meal inclusion level as compared to diets containing 20, 25 or 30 % *Acacia karroo* leaf meal inclusion levels. Similarly, DM digestibility values were higher (P<0.05) in goats fed diets having 30 or 40 % Ac*acia karroo* leaf inclusion levels than those on 20 or 25 % inclusion levels. Goats consuming diets having 20 or 25 % *Acacia karroo* leaf meal inclusion levels had similar (P>0.05) DM digestibility values. Organic matter digestibility values were higher (P<0.05) in goats consuming diets containing 40 or 50 % *Acacia karroo* inclusion levels than those on 20, 25 or 30 % inclusion levels. Male goats fed 20, 25 or 30 % inclusion levels had similar (P>0.05) OM digestibility values. Goats consuming diets having 30, 40 or 50 % *Acacia karroo* leaf meal inclusion levels had higher (P<0.05) CP digestibility than those on diets having 20 or 25 % inclusion levels. Male goats consuming diets containing 20 or 25 % *Acacia karroo* leaf meal inclusion levels had similar (P>0.05) CP digestibility values.

Male goats consuming diets containing 25, 30 or 50 % *Acacia karroo* leaf meal inclusion levels had higher (P<0.05) NDF digestibility values than those of goats on diets with 20 or 40 % *Acacia karroo* leaf meal inclusion levels. Goats fed diets containing 20 or 40 % *Acacia karroo* leaf meal inclusion levels had similar (P<0.05) NDF digestibility values. Acid detergent fibre digestibility was higher (P<0.05) in goats consuming a diet having a 50 % *Acacia karroo* leaf meal inclusion level than those on diets having 20, 25 or 30 % inclusion levels. Goats fed diets containing 40 or 50 % inclusion levels had similar (P>0.05) ADF digestibility values. Similarly, goats on diets having 20, 25, 30 or 40 % *Acacia karroo* leaf meal inclusion levels had similar (P>0.05) ADF digestibility values.

Male Pedi goats had similar (P>0.05) final body weights. However, body weight gains (BWG) were higher (P<0.05) in goats fed a diet having a 50 % *Acacia karroo* leaf meal inclusion level as compared to other treatment groups. Similarly, goats on diets having 30 or 40 % *Acacia karroo* leaf meal inclusion levels had higher (P<0.05) body weight gains than those on diets having 20 or 25 % *Acacia karroo* leaf meal inclusion levels. However, goats on diets having 30 or 40 % *Acacia karroo* leaf meal inclusion levels had similar (P>0.05) live weight gains. Similarly, goats on diets having 20 or 25 % *Acacia karroo* leaf meal inclusion levels had the same (P>0.05) live weight gains.

Dry matter, crude protein and neutral detergent fibre digestibilities were optimized at *Acacia karroo* leaf meal inclusion levels of 69.4, 48.3 and 42.7 %, respectively (Figures 4.01, 4.02 and 4.03, respectively and Table 4.03). Organic matter (r^2 = 0.88) and acid detergent fibre (r^2 = 0.92) digestibilities, and body weight gain (r^2 = 0.91) increased linearly with increased inclusion levels of *Acacia karroo* leaves in the diet (Figures 4.04, 4.05 and 4.06, respectively and Table 4.04).

Table 4.01 Effect of different inclusion levels of *Acacia karroo* on diet intake, digestibility and live weight change of male Pedi goats fed a basal diet of *Setaria verticillata* hay

Variable	Treatment					SEM
	$S_{80}A_{20}$	$S_{75}A_{25}$	$S_{70}A_{30}$	$S_{60}A_{40}$	$S_{50}A_{50}$	
Intake (g/goat/day)						
DM	679	633	642	633	617	56.6
OM	621	580	588	581	566	51.9
CP	60	58	60	62	64	5.38
NDF	475	430	423	392	358	36.6
ADF	319	292	290	274	256	25.1
Intake ($g/kgW^{-0.75}$)						
DM	68.2	72.3	69.7	64.8	61.1	6.92
OM	62.4	66.2	63.8	59.4	56.0	6.35
CP	6.07	6.60	6.50	6.30	6.30	0.70
NDF	47.7	49.1	45.9	40.1	34.4	4.20
ADF	32.1	33.3	31.5	28.0	24.6	2.97
Digestibility (coefficient)						
DM	0.57^{c}	0.58^{c}	0.67^{b}	0.72^{ab}	0.77^{a}	0.024
OM	0.56^{b}	0.56^{b}	0.57^{b}	0.73^{a}	0.75^{a}	0.030
CP	0.40^{b}	0.41^{b}	0.55^{a}	0.56^{a}	0.60^{a}	0.042
NDF	0.48^{b}	0.63^{a}	0.66^{a}	0.56^{ab}	0.66^{a}	0.038
ADF	0.36^{b}	0.36^{b}	0.37^{b}	0.49^{ab}	0.61^{a}	0.048
Initial BW (kg)	17.32	18.28	16.52	18.49	16.57	4.841
Final BW (kg)	17.74	18.77	17.24	19.25	17.83	4.610
ADG (g/goat/day)	20^{c}	23^{c}	34^{b}	36^{b}	60^{a}	10.37

^{a,b,c}: Means with different superscripts in the same row are significantly different ($P<0.05$)

SEM: Standard error of the means

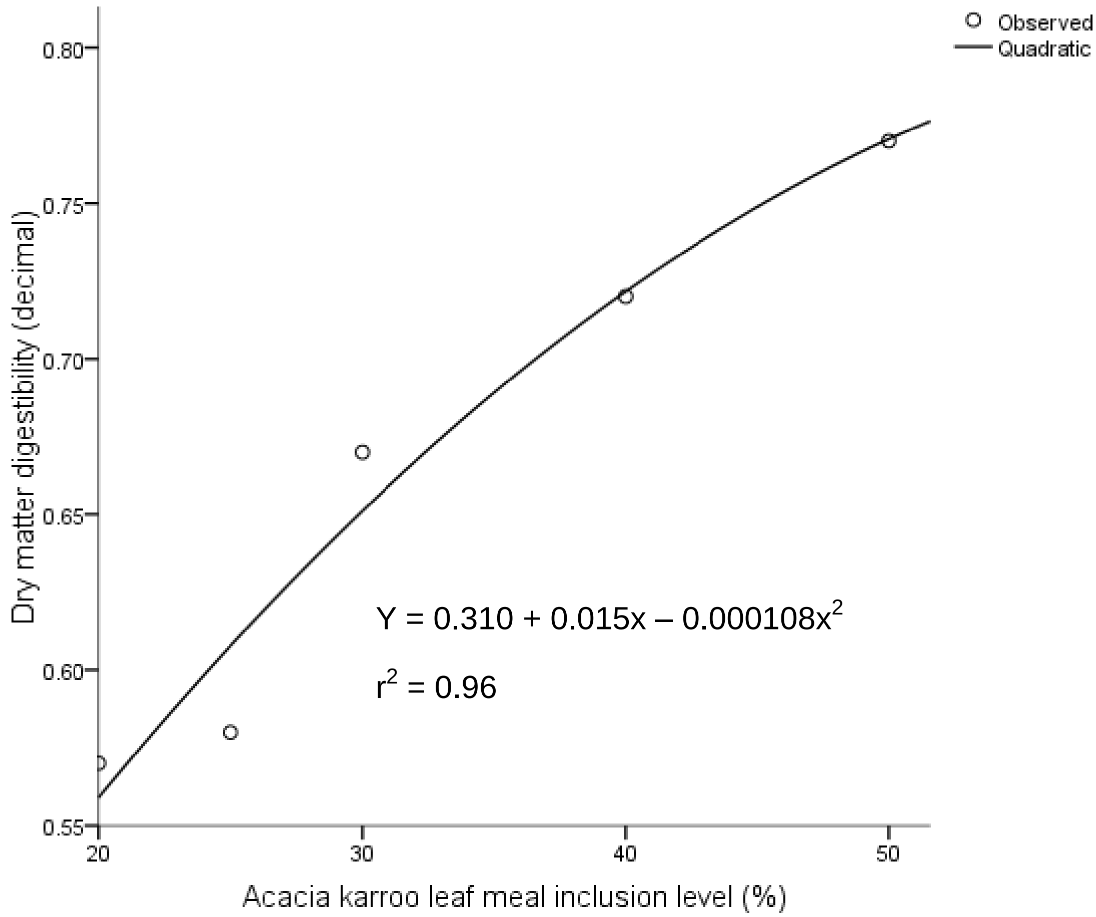


Figure 4.01 Effect of *Acacia karroo* leaf meal inclusion level on dry matter digestibility of indigenous male Pedi goats fed a *Setaria verticillata* grass hay-based diet

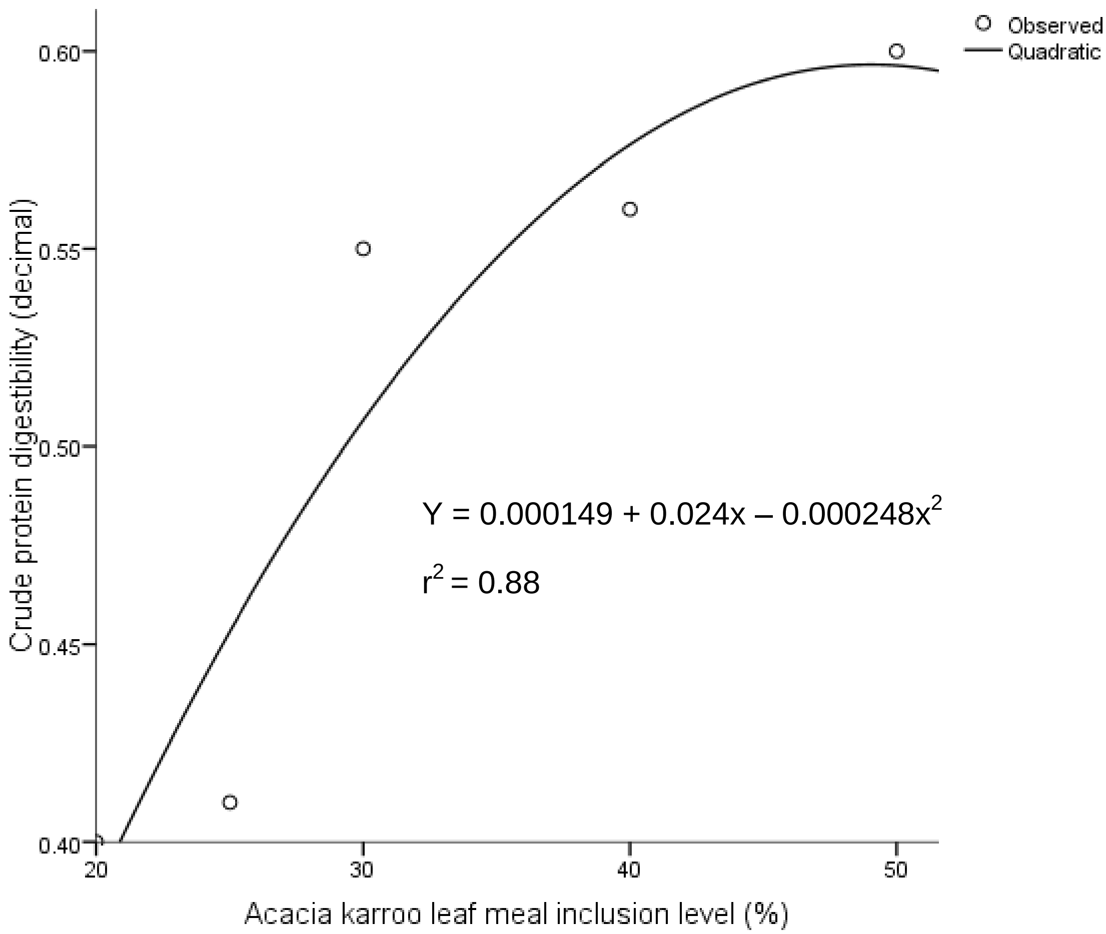


Figure 4.02 Effect of *Acacia karroo* leaf meal inclusion level on crude protein digestibility of indigenous male Pedi goats fed a *Setaria verticillata* grass hay-based diet

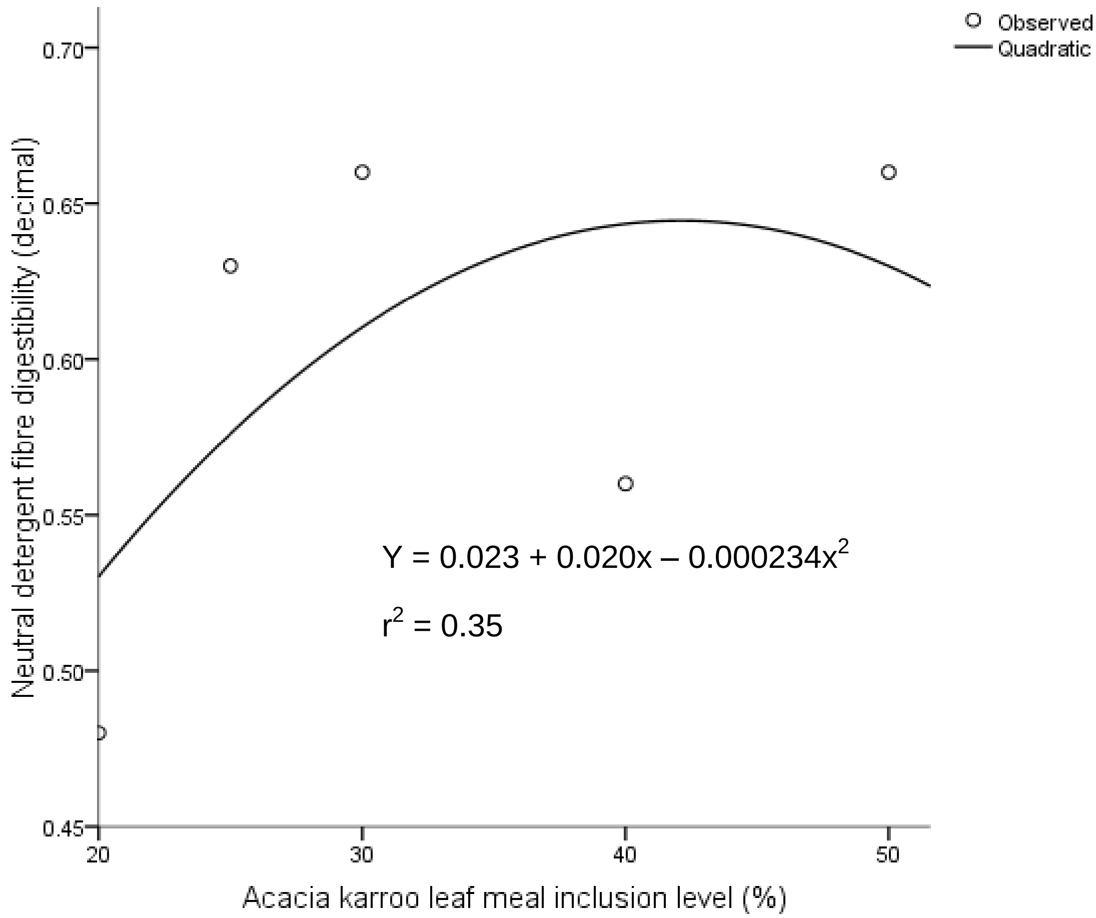


Figure 4.03 Effect of *Acacia karroo* leaf meal inclusion level on neutral detergent fibre digestibility of indigenous male Pedi goats fed a *Setaria verticillata* grass hay-based diet

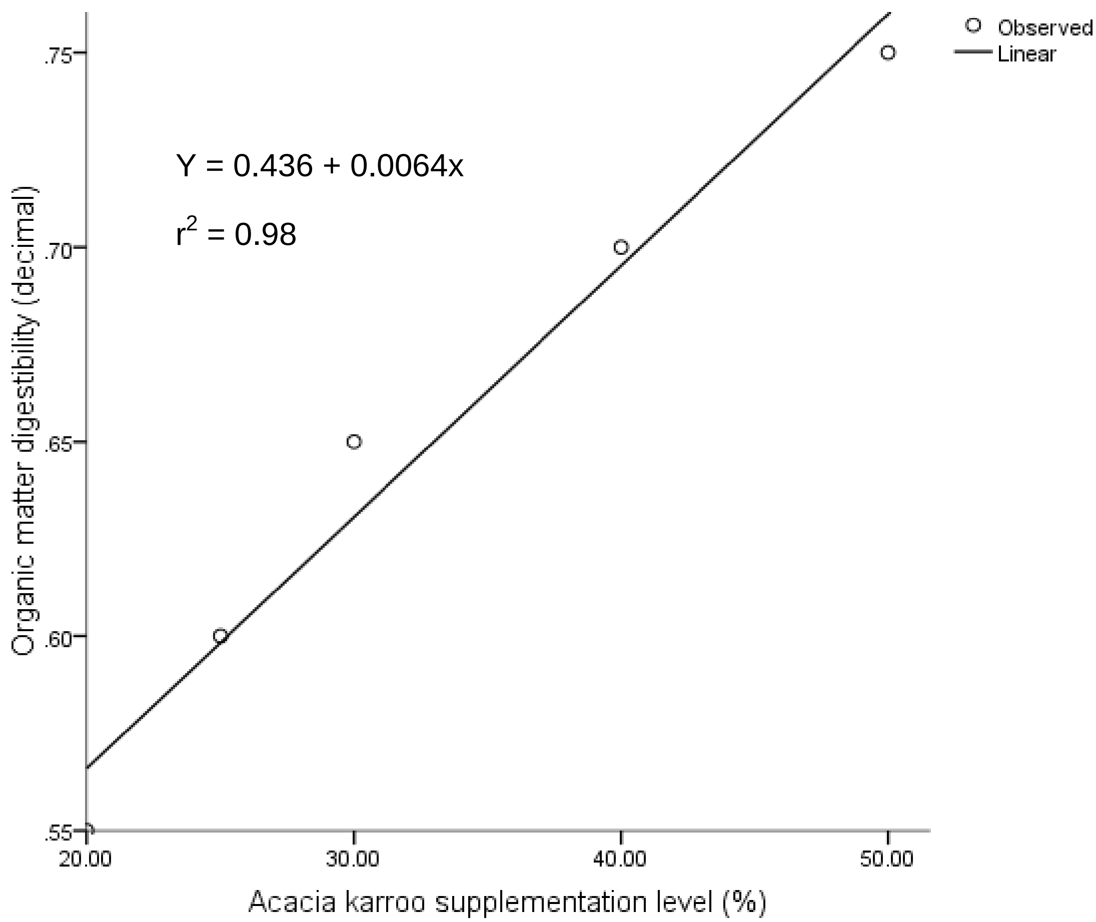


Figure 4.04 Relationship between *Acacia karroo* leaf meal inclusion level on organic matter digestibility of indigenous male Pedi goats fed a *Setaria verticillata* grass hay-based diet

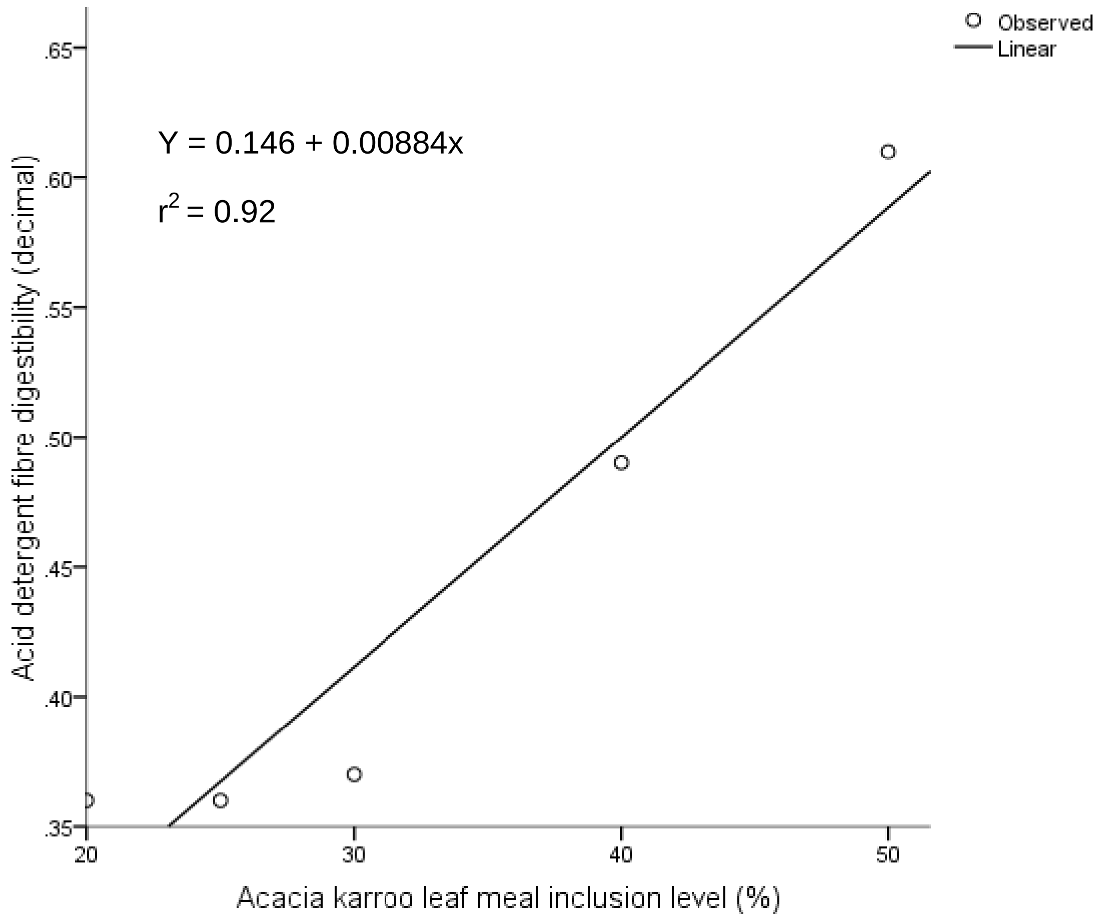


Figure 4.05 Relationship between *Acacia karroo* leaf meal inclusion level and acid detergent fibre digestibility of indigenous male Pedi goats fed a *Seteria verticillata* grass hay-based diet

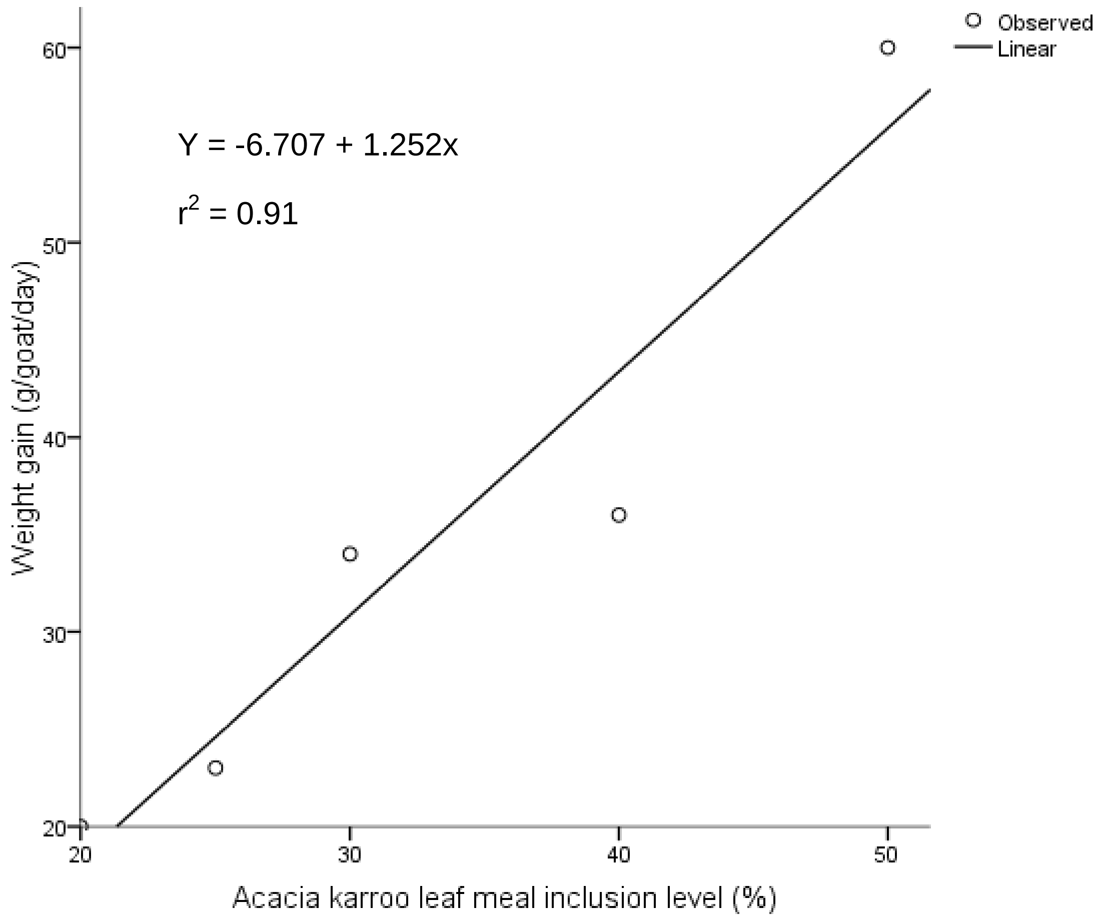


Figure 4.06 Relationship between *Acacia karroo* leaf meal inclusion level and body weight gain of indigenous male Pedi goats fed a *Setaria verticillata* grass hay-based diet

Table 4.02 *Acacia karroo* leaf meal inclusion levels for optimal dietary dry matter (DM), crude protein (CP), neutral detergent fibre (NDF) and acid detergent fibre (ADF) digestibility (decimal) in male Pedi goats on a basal diet of *Setaria verticillata* grass hay

Factor	Formula	AK level	Optimal Y-Level	r^2
DM digestibility	$Y = 0.310 + 0.015x + -0.000108x^2$	69.4	0.830	0.96
CP digestibility	$Y = 0.000149 + 0.024x + -0.000248x^2$	48.3	0.580	0.88
NDF digestibility	$Y = 0.230 + 0.020x + -0.000234x^2$	42.7	0.657	0.35

AK level: *Acacia karroo* leaf meal inclusion level for optimal Y-value

r^2: Coefficient of determination

Table 4.03 Relationships between *Acacia karroo* leaf meal inclusion level (%) and organic matter and acid detergent digestibilities (decimal) and live weight change (g/goat/day) in male Pedi goats on a basal diet of *Setaria verticillata* grass hay

Factor	Formula	r^2	Probability
Organic matter digestibility	$Y = 0.436 + 0.0064x$	0.97	0.002
ADF digestibility	$Y = 0.671 – 0.000474x$	0.03	0.795
Live weight change	$Y = -6.707 + 1.252x$	0.91	0.011

r^2: Coefficient of determination

ADF digestibility: Acid detergent fibre digestibility

4.5.3 Effect of *Acacia karroo* leaf meal inclusion level on haematology and blood biochemical components of male Pedi goats fed a *Setaria verticillata* grass hay-based diet

The results of the effect of *Acacia karroo* leaf meal inclusion level on haematology and blood biochemical components of male Pedi goats fed a *Setaria verticillata* grass hay-based diet are presented in Tables 4.05, 4.06, 4.07 and 4.08, respectively.

Table 4.04 Haematological parameters (mean ± SD) of male Pedi goats at the beginning of the experiment

Parameter	Treatment				
	$S_{80}A_{20}$	$S_{75}A_{25}$	$S_{70}A_{30}$	$S_{60}A_{40}$	$S_{50}A_{50}$
WBC $(x10)^3$ µL	22.65±3.45	28.36±6.86	26.70±6.54	26.78±9.50	26.13±3.52
RBC $(x10)^6$ µL	16.38±2.44	18.13±2.40	19.26±2.40	17.77±3.84	20.00±5.03
HB (g/dL)	8.67±0.79	14.50±5.74	10.07±0.81	9.95±2.44	9.80±2.20
HCT (L/L)	0.62±0.12	0.73±0.15	0.76±0.14	0.68±0.12	0.78±0.19
MCV (fL)	37.75±2.62	40.80±3.78	39.37±3.55	38.83±1.97	39.37±1.61
MCH (pg)	5.35±0.62	5.46±0.17	5.27±0.43	5.57±0.40	4.95±0.50
MCHC (g/dL)	14.27±2.33	13.63±1.76	13.50±2.05	14.45±1.55	12.60±1.58

WBC: White blood cell; RBD: Red blood cell; HB: Haemoglobin; HCT: Haematocrit; MCV: Mean corpuscular volume; MCH: Mean corpuscular haemoglobin; MCHC: Mean corpuscular haemoglobin concentration.

Table 4.05 Effect of experimental diets on haematological parameters (mean ± SD) of male Pedi goats at the end of the experiment

Parameter	Treatment				
	$S_{80}A_{20}$	$S_{75}A_{25}$	$S_{70}A_{30}$	$S_{60}A_{40}$	$S_{50}A_{50}$
WBC $(x10)^3$ μL	17.77±3.88	24.67±5.40	17.16±8.60	21.59±7.66	17.16±2.03
RBC $(x10)^6$ μL	17.11±2.74	17.74±1.78	18.36±3.06	17.09±2.61	20.05±4.49
HB (g/dL)	9.10±0.60	9.55±0.77	9.60±1.20	9.45±1.47	9.82±1.70
HCT (L/L)	0.66±0.21	0.68±0.08	0.68±0.13	0.65±0.12	1.01±0.52
MCV (fL)	38.40±5.97	38.45±1.25	37.00±2.36	38.27±3.96	48.70±16.4
MCH (pg)	5.40±0.63	5.40±0.14	5.27±0.25	5.55±0.34	4.97±0.54
MCHC (g/dL)	14.40±3.35	14.02±0.69	14.30±1.53	14.60±1.76	11.20±3.95

WBC: White blood cell; RBD: Red blood cell; HB: Haemoglobin; HCT: Haematocrit; MCV: Mean corpuscular volume; MCH: Mean corpuscular haemoglobin; MCHC: Mean corpuscular haemoglobin concentration.

Table 4.06 Serum chemistry (mean ± SD) of male Pedi goats at the beginning of the experiment

Parameter	Treatment				
	$S_{80}A_{20}$	$S_{75}A_{25}$	$S_{70}A_{30}$	$S_{60}A_{40}$	$S_{50}A_{50}$
Glucose (mmol/L)	3.45±0.23	3.12±0.09	3.90±0.80	3.75±0.47	3.37±0.17
TP (g/L)	74.50±5.25	81.75±6.23	77.25±5.90	71.25±13.81	64.25±9.53
Albumin (g/dL)	15.25±0.50	16.75±1.50	17.75±1.50	17.00±0.81	15.25±2.06
Chol (mmol/L)	1.53±0.10	2.22±0.62	1.71±0.29	2.05±0.84	1.75±0.54
ALT (IU/L)	23.25±2.75	26.00±6.05	24.00±5.59	28.50±10.34	26.25±5.79
AST (IU/L)	68.00±12.78	73.75±4.57	75.25±11.35	78.00±8.36	50.00±7.87
K(mmol/L)	4.89±0.31	5.12±0.11	5.01±0.63	4.96±0.42	4.89±0.28
Ca (mmol/L)	2.22±0.02	2.28±0.71	2.34±0.03	2.22±0.06	2.14±0.13
Mg (mmol/L)	0.98±0.86	0.93±0.06	0.94±0.13	0.95±0.05	1.01±0.12
Na (mmol/L)	143.47±2.57	143.72±1.18	144.67±1.50	144.75±1.19	143.45±1.93

TP: Total protein; Chol: Cholesterol; ALT: Alanine aminotransferase; AST: Aspartate Aminotransferase; K: Potassium; Ca: Calcium; Mg: Magnesium; Na: Sodium

Table 4.07 Effect of experimental diets on serum chemistry (mean ± SD) of male Pedi goats at the end of the experiment

Parameter	Treatment				
	$S_{80}A_{20}$	$S_{75}A_{25}$	$S_{70}A_{30}$	$S_{60}A_{40}$	$S_{50}A_{50}$
Glucose (mmol/L)	2.92±0.30	2.77±0.26	2.82±0.15	2.86±0.16	2.92±0.27
TP (g/L)	76.50±1.29^{ab}	80.00±2.94^{a}	73.50±1.29^{b}	64.00±4.08^{c}	58.00±0.81^{d}
Albumin (g/dL)	15.75±0.95	16.50±1.00	17.50±1.91	16.50±1.91	15.25±1.70
Chol (mmol/L)	0.86±0.23	1.28±0.63	1.02±0.14	0.72±0.36	0.98±0.94
ALT (IU/L)	43.00±10.16	25.75±5.12	34.50±5.80	34.50±5.50	36.75±18.5
AST (IU/L)	84.00±13.73	83.00±9.76	79.00±9.45	101.75±18.87	85.25±15.2
K (mmol/L)	9.75±1.64	11.28±1.31	10.41±2.80	10.63±2.62	10.28±1.87
Ca (mmol/L)	2.22±0.06	2.30±0.03	2.32±0.10	2.32±0.02	2.23±0.09
Mg (mmol/L)	1.13±0.09	1.09±0.11	1.15±0.04	1.13±0.05	1.18±0.08
Na (mmol/L)	138.40±1.68	136.65±0.61	140.47±3.17	139.55±3.31	138.35±0.59

^{a,b,c,d}: Means in the same row with different superscripts are significantly different ($P<0.05$).

TP: Total protein; Chol: Cholesterol; ALT: Alanine aminotransferase; AST: Aspartate Aminotransferase; K: Potassium; Ca: Calcium; Mg: Magnesium; Na: Sodium

Haematology and serum biochemical parameters of male Pedi goats revealed no differences between the treatments at the beginning of the experiment. These results served as a baseline to monitor the changes in the blood profile of the goats as affected by the treatments. At the end of the trial, haematology and blood serum chemistry parameters of the goats were not affected by the dietary treatment except for serum total proteins (TP). Goats consuming diets with 40 or 50 % *Acacia karroo* leaf meal inclusion levels had lower ($P<0.05$) serum TP than those on other treatments. Furthermore, goats consuming diets with 20 or 30 % *Acacia karroo* leaf meal inclusion levels had similar serum TP values.

4.6 Discussion

The compositions of the diets were as reported in Section 3.6. The condensed tannin contents of the experimental diets varied from 4.1 g/kg DM for the diet containing a 20 % *Acacia karroo* inclusion level to 10.2 g/kg DM for the diet containing 50 % *Acacia karroo*. Mokoboki *et al.(*2005) and Dube *et al*. (2001) indicated that A*cacia karroo* leaves contain high levels of extracted condensed tannins (CT), ranging from 55 to 110 g/kg DM. It has been reported that CTs values above 50 g/kg DM of the diet can produce adverse effects when fed to ruminant animals (Frutos *et al.,* 2004). The CT contents in the experimental diets were below 50 g/kg DM, the level that could be considered detrimental to the animal. According to Ben Salem & Smith (2008), intake of mixed diets (shrubs and grass hay) is an efficient way of diluting the adverse effects of secondary compounds in plants, like tannins.

The threshold NDF content beyond which feed intake and digestibility is adversely affected by ruminants is 600 g NDF/kg DM (Meissner & Paulsmeier, 1995). The diet containing a 50 % *Acacia karroo* inclusion level had 579 g NDF/kg DM, still lower than 600 g NDF/kg DM, indicating its high feeding-value potential. However, Riaz *et al*. (2014) observed that goats appeared less responsive to increases in dietary fibre fractions (NDF and ADF) than other animal species, and that these feed fractions had a less negative impact on their dry matter intake.

All the experimental goats had similar feed intakes. This might be because all the diets had CP levels above the minimum level of 8 % required for optimal ruminal microbial functioning, (Norton, 2003) and hence optimal diet intake. This result is similar to the findings of Dludla (2010) who reported non-significant differences in total intake when goats were fed *Acacia caffra* and *Euclea crispa* (with high CT concentrations), *Rhus lancea* (with moderate CT concentrations) and Ziziphus mucronata (with low CT concentrations). Animut *et al.* (2008) observed that intakes of DM and OM were unaffected by goats consuming diets with different levels of condensed tannins from lespedeza. According to Reed (1995) and Frutos *et al*. (2004), condensed tannin concentration in the diet will depress feed palatability, reduce feed intake, slow down digestion and develop conditioned aversions. The reduction in palatability and intake is associated with the astringency in the mouth of the animals (Breslin *et al.,* 1993). A decrease in feed intake has, also, been reported with higher CT concentration (Bhatta *et al*., 2002). In the present study, an increase in inclusion of *Acacia karroo* leaf meal in the diet did not adversely affect forage intake of goats.

Diets containing 30, 40 or 50 % *Acacia karroo* leaf meal inclusion levels improved crude protein digestibility, despite the relatively higher tannin contents. *Acacia karroo* leaf meal inclusion level of 48.3 % optimized crude protein digestibility. *Acacia karroo* leaf meal inclusion increased ruminal protein levels, resulting in improved conditions for microbial growth and multiplication (Makkar, 2003). Increased microbial population results in higher digestibility and intake of the diet. The nutritional benefit of CT in a ruminant's diet is also due, primarily, to the protection of plant proteins from microbial degradation in the rumen and the resultant increase in protein flow to the intestines (McNabb *et al.,* 1996; Waghorn *et al*., 1994b). Tannins in forages may enhance protein utilization and improve amino acid absorption in ruminants (Waghorn *et al.,* 1987). These reports support the findings in the present study. Makkar (2003) observed that tannins bind with proteins at pH of 5.5 to 7.0, thereby slowing down microbial degradation of the proteins. The slower rate of microbial protein digestion in the rumen prevents nitrogen loss as urea, facilitating the absorption of amino acids in the small intestines, and improving overall nitrogen utilization (Mueller-Harvey, 2006). It has, also, been documented that condensed tannins exert their inhibitory effect on protein degradation in the rumen by protecting plant protein from cleavage by proteases or directly inhibiting the proteases themselves (Aerts *et al.,* 1999). The precise mechanism by which tannins reduce ruminal protein digestion and hence improve its overall utilization merits further study.

Diets containing a 50 % *Acacia karroo* leaf meal inclusion level improved fibre digestibility. However, the level of inclusion for optimal NDF digestibility was 42.7 %. Usually, tannins tend to reduce fibre digestibility by binding bacterial enzymes and forming indigestible complexes with carbohydrates, particularly hemicellulose, cellulose, starch and pectins, thus, rendering these nutrients inaccessible to microorganisms (Schofield *et al*., 2001). However, the present results indicate that fibre degradation in the rumen can be increased in animals consuming forage tannins. Waghorn *et al.* (1987), also, reported a 13 % increase in fibre digestion in the rumen of sheep consuming *Lotus pedunculatus* containing 5.5 % CT.

The increase in the inclusion level of *Acacia karroo* leaf meal resulted in increased daily weight gains of the goats. A significantly higher average daily gain was observed in goats consuming a diet with a 50 % *Acacia karroo* leaf meal inclusion level. This might have been due to increase in diet digestibility with an increase in *Acacia karroo* inclusion level. Ash & Norton (1987) observed that body weight gain in goats is sensitive to protein and energy contents of the diet consumed. The improvement in live weight gain of Pedi goats might, also, be associated with improved protein utilization by goats on diets with higher *Acacia karroo* inclusion levels (Solaiman *et al*., 2010). This result is similar to the findings of Gwazura (2011) who reported higher live weight gains in Pedi goats fed taninniferous Mucuna hay. Forages containing from 20 to 40 g of CT kg^{-1} DM increase rumen escape value of herbage (Terril *et al*., 1992) and enhance weight gains in ruminants (Turner *et al.,* 2005). Other authors have reported improved weight gain in goats and lambs fed forages high in CT (Solaiman *et al*., 2010; Douglass *et al.,* 1995). Results of Njidda & Ikhimioya (2010) with tannin-rich plants revealed that some rumen microorganisms are able to metabolize tannins or remain active in a high tannin environment and overcome their detrimental effects, which in turn, improves animal performance. Higher concentrations of total polyphenols in a diet tend to reduce nutrient availability to the animals, thus adversely affecting their body weight gains (Tanner *et al.,* 1990). However, in the present study, the high total polyphenolic content present in the diets having a 50 % *Acacia karroo* inclusion level diet did not adversely affect the live weight of the goats.

Blood metabolite levels provide an immediate indication of an animal's nutritional status, and their analyses are, therefore, recommended when assessing the effects of a given diet on performance of animals in the short to medium-terms (Pambu-Gollah *et al*., 2000). In the present study, the experimental goats did not show any distress or clinical signs of ill health or signs of tannin toxicity such as head pressing, generalized depression, grinding of teeth, foaming at the mouth and twitching and jerking of the body (Odenyo *et al*., 1997). The mean WBC counts obtained in this study were within the normal physiological range of 6.8-20.1 x 10^6/μL reported for clinically healthy goats (Daramola *et al*., 2005). The results are similar to the findings of Olafadehan *et al*. (2014) who reported that WBC count in goats on tannin-rich Ficus leaf meal mixed with concentrate diet (700:300) were within the normal range value for healthy goats. In another study, goats fed a sole diet of tannin-rich *Pterocarpus erinaceus* forage had lower WBC count which implies that the animals must have ingested a considerable amount of tannins in their diets (Olafadehan, 2011). The concentration of CT in the present study was below the level that could cause adverse effects in the animals. The RBC values obtained in the present study, also, fell within the range of 8-17 x 10^{12}/ μL reported by Sirois (1995). These results are similar to the findings of Solaiman *et al*. (2010) who reported non-significant differences in red blood cell numbers when Kiko crossbred male kids were fed a *Sericea lespedeza* –based diet containing varying levels of CT. Red blood cells indices help in the characterization of anaemia, thus, the RBC values obtained in the present study indicate the absence of haemolytic anaemia and depression of erythrogenesis (Olafadehan, 2011). Haemoglobin concentration is an indication of the oxygen carrying capacity of the blood. The values reported in this study were within the range of 7 - 15 g/dL for clinically healthy goats (Daramola *et al*., 2005). Previous research also reported that goats fed *Ficus polita* with varying CT levels in their diets had similar Hb concentrations (Olafadehan *et al*., 2014). The implication of the values obtained in the present study is that the experimental diets were capable of supporting high oxygen carrying capacity without depressing respiratory capability of the goats. The results also indicate the absence of microcytic hypochromic anaemia due to iron deficiency and improper utilization of Hb (Olafadehan *et al.,* 2014). Haematocrit (HCT), also, known as packed cell volume (PCV) is the percent of blood volume filled by erythrocytes and, thus, a measure of oxygen carrying capacity of the blood. The HCT results in the present study are in agreement with the findings of Solaiman *et al*. (2010) who reported non-significant differences in haematocrit values when goats were fed various levels of tannin-rich *Sericea lespedeza* diets. This implies that RBC production was adequate and there was no loss or breakdown of the cells.

Erythrocytic indices such as MCV, MCH and MCHC are useful in the diagnosis of certain anaemias (Pratt, 1985). These values were similar with the baseline results at the beginning of the experiment. This further elucidates that the experimental goats were not anaemic. In a study reported by Olafedehan (2011), goats that were fed different levels of tannin-rich *Pterocarpus erinaceus* forage diets had similar erythrocytic values with the control group. Similar results were observed by Solaiman *et al*. (2010) on goats fed tannin-rich *Sericea lespedeza* diets.

Serum biochemistry parameters indicate pathophysiological states which help in identification of pathogenesis and causes of disease (Solaiman *et al*., 2010). Serum glucose levels for all the diets in the present study were within the normal range of 2.78 - 4.16 mmol/l reported for healthy goats (Kaneko, 1997). Previous studies indicated that serum glucose was unaffected as tannin-rich *Sericea lespedeza* inclusion level increased in the diets of Kiko crossbred male kids (Solaiman *et al*., 2010). However, Olafadehan (2011) observed that goats fed a sole diet of tannin-rich *Pterocarpus erinaceus* or *Andropogon gayanus* grass had depressed serum glucose as compared to animals on mixed forage diets. Serum total protein values of goats consuming diets with a 50 % *Acacia karroo* leaf meal inclusion level were outside the normal range of 64 - 70 g/L (Kaneko, 1997) despite the fact that the diet had the highest crude protein content. This observation may be an indication of underutilization of the protein and carbohydrates due to the high tannin level in the diet. Tannins are functionally defined by their capacity to bind with proteins and carbohydrates (Makkar, 1993). Frutos *et al*.

(2004) reported that tannins have detrimental effects which include dietary and endogenous protein binding and direct toxic effect. Adverse effects of tannins in ruminant nutrition have also been reported elsewhere (Zhu *et al*., 1992).

Albumin is a protein made by the liver and serum albumin gives an indication of the nutritional status of the animals (Solaiman *et al*., 2010). The serum albumin concentrations in the present study were within the baseline values recorded before the commencement of the experiment. In contrast to the present observation, Olafedehan (2011) reported significantly higher serum total protein and albumin levels when goats were fed mixed forage diets consisting of tannin-rich *Pterocarpus erinaceus* and *Andropogon gayanus*. The discrepancies observed could be due to the amount of tannins ingested, chemical structure or molecular weight of the tannins, and the physiology of the animals consuming the diet (Frutos *et al.,* 2004). Cholesterol is used for diagnosing hepatic damage of domestic animals (Kaneko, 1997). Increased cholesterol levels are a risk factor for heart diseases while a reduction in serum cholesterol indicates improper liver function, malnutrition, stress, decreased nutrient intake and hormonal insufficiency (Kaneko, 1997). Similar cholesterol levels of the experimental animals in the present study indicate absence of adverse effects with increase in tannin levels (Olafadehan *et al*., 2014). Similar findings have been reported by Olafedehan (2011) and Olafadehan *et al.* (2014) when goats were fed tannin-rich forages.

Serum enzymes alanine transaminase (ALT) and aspartate transaminase (AST) of the experimental goats were similar with the baseline values. Earlier studies have reported non-significant differences in goats fed tannin-rich forages (Olafadehan *et al*., 2014; Olafadehan 2011). Ruminant animals, like goats, consuming tannin-rich plants develop defensive mechanisms to tolerate the negative effects of condensed tannins without showing any signs of tannin toxicity (Lamy *et al.,* 2011). Some of these mechanisms are secretion of proline rich protein (PRP) in the parotid saliva, increased number of tannin-resistant organisms in the rumen and increased intestinal mucus production as a defense against intestinal damage (Lamy *et al*., 2011; Makkar, 1993).

Blood serum mineral and electrolyte values obtained in the present study were not affected by the dietary treatments and were similar to the baseline values. This report is similar to the findings of Olafadehan *et al*. (2014) who reported non-significant differences in goat's serum minerals and electrolyte when fed tannin-containing forages in varying proportions. These results indicate the absence of mineral depletion and that their absorption from the gastro-intestinal tracts was not hindered by the dietary tanniniferous compounds.

4.7 Conclusion

Inclusion of *Acacia karroo* leaves improved nutrient digestibility and growth rate of indigenous Pedi goats. Dry matter, crude protein and neutral detergent fibre digestibilities were optimized at different *Acacia karroo* leaf meal inclusion levels of 69.4, 48.3 and 42.7 %, respectively. This may indicate that levels for optimal productivity will depend on the particular parameter in question. *Acacia karroo* leaf meal, therefore, has the potential of being utilized as a protein feed during the dry season when goats depend on low quality roughages.

Higher *Acacia karroo* inclusion levels, like 50 %, resulted in higher dietary tannin contents which exerted negative effects on serum total proteins. It is, therefore, important to find compounds that will reduce or neutralize the adverse effects of tanniniferous feeds in Pedi goats.

CHAPTER FIVE

EFFECT OF POLYETHYLENE GLYCOL 4000 SUPPLEMENTATION ON THE PERFORMANCE OF INDIGENOUS PEDI GOATS FED DIETS WITH DIFFERENT MIXTURES LEVELS OF *ACACIA KARROO* LEAF MEAL AND *SETARIA VERTICILLATA* GRASS HAY

Abstract

An experiment was carried out to determine the effect of PEG 4000 supplementation on diet intake, digestibility, body weight change and blood profiles of indigenous Pedi goats fed different mixture levels of *Acacia karroo* leaf meal and *Setaria verticillata* grass hay. Eighteen yearling male indigenous Pedi goats with mean live weight of 21.76 ± 3.1 kg were randomly allocated to six treatments in a 2 (*Acacia karroo* leaf meal levels) x 3 (levels of PEG 4000) factorial arrangement in a completely randomised design. Each treatment had three replicates with one goat per replicate. Each goat was supplemented with 0, 23 or 30 g of PEG/litre of water per day in addition to dietary mixture of *Acacia karroo* and *Setaria verticillata* hay. The experiment lasted for 21 days, with the first 14 days as a preliminary period designed to allow goats adjust to experimental diets and feeding regimes, and the last seven days for data collection. Goats supplemented with 23 g of PEG 4000/goat/day and fed a dietary mixture of 20 % *Acacia karroo* leaf meal and 80 % *Setaria verticillata* grass hay had higher (P<0.05) DM, OM, NDF and ADF intakes than those on a mixture of 50 % *Acacia karroo* leaf meal and 50 % *Setaria verticillata* grass hay and supplemented with either 23 or 30 g of PEG 4000/goat/day. Similar trend was observed for intake per metabolic weight of goats. Dry matter, OM, NDF and ADF intakes per goat were optimized at PEG 4000 supplementation levels of 19.62, 19.62, 19.61 and 19.53 g/goat/day, respectively, for diets with a mixture of 20 % *Acacia karroo* and 80 % *Setaria verticillata* grass hay. Dietary mixture level and PEG 4000 supplementation did not affect (P>0.05) daily CP intake by goats. Dry matter, OM, NDF and ADF intakes per goat metabolic weight were optimized at PEG 4000 supplementation levels of 20.34, 20.03, 20.56 and 20.56 g/goat/day, respectively, for goats on a mixture of 20 % *Acacia karroo* leaf meal and 80 % *Setaria verticillata* grass hay. Supplementation with either 23 or 30 g of PEG 4000/goat/day did not improve (P>0.05) DM, OM, NDF or ADF intakes per goat metabolic weight for the animals on a mixture of 50 % *Acacia karroo* leaf and 50 % *Setaria verticillata* grass hay. Dietary mixture level and PEG 4000 supplementation did not affect (P>0.05) daily CP intake per metabolic weight of goats. Goats supplemented with either 23 or 30 g of PEG 4000/goat/day and fed 20 % *Acacia karroo* leaf meal and 80 % *Setaria verticillata* grass hay had higher (P<0.05) DM, OM, CP and NDF digestibility values than those on a

mixture of 50 % *Acacia karroo* leaf meal and 50 % *Setaria verticillata* grass hay and supplemented with either 23 g of PEG 4000/goat/day. Crude protein digestibility was optimized at a PEG 4000 supplementation level of 15.78 g/goat/day. Additionally, dietary mixture level and PEG 4000 supplementation did not affect ($P>0.05$) initial and final weight of Pedi goats. Similarly, dietary mixture level and PEG 4000 supplementation did not affect ($P>0.05$) daily live weight, serum glucose and urea concentrations of Pedi goats. It is concluded that PEG 4000 has potential to improve the feeding value of *Acacia karroo* leaf meal and can, therefore, be used in the feeding systems for ruminant animals.

5.1 Introduction

Livestock production is the key to food security for many smallholder farmers in the semi-arid areas of Africa (Aziz, 2010). The major problem to increasing productivity of ruminant livestock is the scarcity of good quality feeds, especially during the dry or winter seasons. Fibrous feedstuffs constitute the main feed given to animals during these periods and these feed sources are limiting in protein contents (Basha *et al*., 2015). Thus, animals on such feeds tend to lose body weight and are prone to disease infection (Matlebyane *et al*., 2010). Alternative feed sources must be provided to alleviate the nutritional problem.

Browse plants have the potential to alleviate feed shortages and nutritional deficiencies experienced during the critical dry period of the year (Belachew *et al*., 2013). Indigenous legume trees such as *Colophospermum mopane*, *Brachystegia spiciformis* and Acacia genus are gaining importance as protein sources for livestock during the dry season (Mapiye *et al*., 2011; Dube, 2000). Of particular importance to animal nutritionists in Southern Africa is *Acacia karroo*. *Acacia karroo* is widely spread and abundantly available during the dry season (Ngambu *et al*., 2012; Barnes *et al*., 1996). The tree contains high concentrations of crude protein (100 - 250 g/kg DM) and minerals (Mokoboki, 2005), and has the potential to increase productivity of goats feeding on low quality roughages. However, their use as protein supplements is restricted by the high content of tannins in their foliage. High levels of tannins have adverse effects on the feeding value of the diet (Ammara *et al*., 2011). Additionally, condensed tannins (CT) affect the digestibility and availability of nutrients such as protein, carbohydrates and minerals (Etuk *et al*., 2012). The negative effects posed by high tannin contents must be overcome to improve the feeding value of such feeds.

Tannin-binding agents such as polyethylene glycol (PEG) offer a viable technique to enhance the nutritive value of tannin-rich trees and shrubs (Nsahlai *et al*., 2011; Singh *et al*., 2005). Polyethylene glycol is an inert unabsorbed molecule that can form stable complexes with tannins, preventing the binding between tannins and proteins and can even displace protein from a pre-formed tannin-protein complex (Frutos *et al*., 2004; Makkar, 2003). Polyethylene glycol 4000 has been used to alleviate negative effects of

CT, consequently improving the performance of animals on tanniniferous-based diets (Motubatse *et al*., 2008; Decandia *et al*., 2000). However, other authors have found that PEG 4000 supplementation does not improve performance of animals on tannin-rich diets (Bhatta *et al*., 2002; Barry *et al*., 2001). Responses to PEG 4000 supplementation may depend on the Acacia species (amount and type of tannins present) and animal breed. It is, therefore, important to ascertain the effects of PEG 4000 on the performance of indigenous male Pedi goat breeds fed on diets supplemented with tanniniferous *Acacia karroo* leaf meal.

5.2 Objectives

The objectives of the study were to determine:

a. the effect of PEG 4000 supplementation on diet intake, digestibility, live weight change and blood profiles of indigenous male Pedi goats fed different mixture levels of *Acacia karroo* leaf meal and *Setaria verticillata* grass hay.

b. polyethylene glycol 4000 supplementation levels for optimal diet intake, digestibility, live weight change and blood profiles of male Pedi goats fed different mixture levels of *Acacia karroo* leaf meal and *Setaria verticillata* grass hay.

5.3 Hypotheses

a. There is no effect of PEG 4000 supplementation on diet intake, digestibility live weight change and blood profiles of indigenous male Pedi goats fed different mixture levels of *Acacia karroo* leaf meal and *Setaria verticillata* grass.

b. There are no PEG 4000 supplementation levels for optimal diet intake, digestibility, live weight change and blood profiles of male Pedi goats fed different mixture levels of *Acacia karroo* leaf meal and *Setaria verticillata* grass hay.

5.4 Materials and methods

5.4.1 Study site

The experiment was conducted at the Goat Unit of the University of Limpopo Experimental farm. The site is described in Section 3.4.1.

5.4.2 Animals, management, diets and experimental design

Eighteen yearling male indigenous Pedi goats with a mean live weight of 21.76 ± 3.1 kg were randomly allocated to six treatments in a 2 (*Acacia karroo* leaf meal levels) x 3 (levels of PEG 4000) factorial arrangement (SAS 2010), in a completely randomised design. Each treatment had three replicates with one goat per replicate. The six treatments are indicated in Table 5.01. The animals were housed in individual holding pens (1 x 3 m^2) and given the experimental diets during the study period. The pens were installed in a well-ventilated shed with one side open to natural light and roofing to protect goats against the sun and rain. The experiment lasted for 21 days, with the first 14 days as a preliminary period designed to allow goats adjust to experimental diets and feeding regimes, and the last 7 days for data collection. Feed intake was recorded on a daily basis during the experimental period. Intake was calculated by subtracting leftovers from the feed given. Water and a mineral lick were provided *ad libitum*. The PEG 4000 was dissolved in water (23 or 30 g of PEG 4000/half litre of water per animal per day) and the contents were given to the animal through dosing daily at 8.00 hours before morning feeding (Decandia *et al*., 2000). Animals not receiving PEG 4000 were dosed with half litre of water. The goats were weighed prior to being assigned to a treatment and body weights for goats were recorded weekly, thereafter.

Table 5.01 Feed composition of the experimental diets

Diet code	Diet description
$A_{20}S_{80}P_0$	A diet having a mixture of 20 % *Acacia karroo* leaf meal and 80 % *Setaria verticillata* grass and not supplemented with PEG 4000
$A_{20}S_{80}P_{23}$	A diet having a mixture of 20 % *Acacia karroo* leaf meal and 80 % *Setaria verticillata* grass and supplemented with 23 g of PEG 4000/goat/day
$A_{20}S_{80}P_{30}$	A diet having a mixture of 20 % *Acacia karroo* leaf meal and 80 % *Setaria verticillata* grass and supplemented with 30 g of PEG 4000/goat/day
$A_{50}S_{50}P_0$	A diet having a mixture of 50 % *Acacia karroo* leaf meal and 50 % *Setaria verticillata* grass and not supplemented with PEG 4000
$A_{50}S_{50}P_{23}$	A diet having a mixture of 50 % *Acacia karroo* leaf meal and 50 % *Setaria verticillata* grass and supplemented with 23 g of PEG 4000/goat/day
$A_{50}S_{50}P_{30}$	A diet having a mixture of 50 % *Acacia karroo* leaf meal and 50 % *Setaria verticillata* grass and supplemented with 30 g of PEG 4000/goat/day

5.4.3 Blood sample collection

A 5 ml blood sample was taken from each goat via the jugular vein just before the morning feeding on the first and the last day of the experiment. The blood samples were collected into anticoagulant free bottles, allowed to coagulate at room temperature and centrifuged at 1500 × g for 10 minutes. The supernatant sera were then harvested and stored in a freezer for subsequent biochemical analysis (Olafadehan *et al*., 2014).

5.4.4 Chemical analysis

Dry matter, organic matter, nitrogen, neutral detergent fibre, acid detergent fibre, condensed tannins and total polyphenol contents of the feeds were determined as described in Section 3.4.4. Sodium oxalate fluoride bottles were used for glucose preservation while the blood glucose was determined by enzymatic colorimetric test (Quimica Clinica Applicada, S.A Kit). Serum urea was obtained by the method of Valley *et al*. (1980).

5.4.5 Statistical analysis

The effects of PEG 4000 supplementation, level of *Acacia karroo* leaf meal inclusion and their interactions on intake, growth rate, serum urea and blood glucose of indigenous Pedi goats were analysed using GLM Procedures of SAS (2010). Where significant treatment effects were detected, means were separated by Fisher's least significant difference. The responses in optimal intake, digestibility and body weight change to the level of browse inclusion were modelled as described in Section 4.4.6.

5.5 Results

The effects of PEG 4000 supplementation and *Acacia karroo* leaf meal inclusions and their interactions on diet intake, digestibility and body weight change of indigenous Pedi goats fed a basal diet of *Setaria verticillata* grass hay are presented in Table 5.02. Goats supplemented with 23 g of PEG 4000/goat/day and fed a dietary mixture of 20 % *Acacia karroo* leaf meal and 80 % *Setaria verticillata* grass hay had higher (P<0.05) DM, OM, NDF and ADF intakes than those on a mixture of 50 % *Acacia karroo* leaf meal and 50 % *Setaria verticillata* grass hay and supplemented with either 23 or 30 g of PEG 4000/goat/day. Dry matter, OM, NDF and ADF intakes per goat were optimized at PEG 4000 supplementation levels of 19.62, 19.62, 19.61 and 19.53 g/goat/day, respectively (Figures 5.01, 5.02, 5.03 and 5.04, respectively and Table 5.03) for diets with a mixture of 20 % *Acacia karroo* and 80 % *Setaria verticillata* grass hay. However, different results were observed when goats were fed a dietary mixture of 50 % *Acacia karroo* and 50 % *Setaria verticillata* grass hay. Supplementation with either 23 or 30 g of PEG 4000/goat/day did not improve (P>0.05) DM, OM, NDF or ADF intake per goat per day. Dietary mixture level and PEG 4000 supplementation did not affect (P>0.05) daily CP intake by goats.

Goats supplemented with 23 g of PEG 4000/goat/day and fed a dietary mixture of 20 % *Acacia karroo* leaf meal and 80 % *Setaria verticillata* grass hay had higher (P<0.05) DM, OM, NDF and ADF intakes per metabolic weight than those on 50 % *Acacia karroo* leaf meal and 50 % *Setaria verticillata* grass hay supplemented with either 23 or 30 g PEG 4000/goat/day. Similarly, goats supplemented with 30 g of PEG 4000/goat/day and fed a dietary mixture of 20 % *Acacia karroo* leaf meal and 80 % *Setaria verticillata* grass hay had higher (P<0.05) NDF and ADF intakes per metabolic weight than those on 50 % *Acacia karroo* leaf meal and 50 % *Setaria verticillata* grass hay supplemented with either 23 or 30 g PEG 4000/goat/day. Dry matter, OM, NDF and ADF intakes per goat metabolic weight were optimized at PEG 4000 supplementation levels of 20.34, 20.03, 20.56 and 20.56 g/goat/day, respectively, for goats on a mixture of 20 % *Acacia karroo* leaf meal and 80 % *Setaria verticillata* grass hay (Figures 5.05, 5.06, 5.07 and 5.08, respectively and Table 5.03). Supplementation with either 23 or 30 g of PEG 4000/goat/day did not improve (P>0.05) daily DM, OM, NDF or ADF intakes per goat metabolic weight for the animals on a mixture of 50 % *Acacia karroo* leaf meal and 50 % *Setaria verticillata* grass hay. Dietary mixture level and PEG 4000 supplementation did not affect (P>0.05) daily CP intake per metabolic weight of goats.

Goats supplemented with either 23 or 30 g of PEG 4000/goat/day and fed a dietary mixture of 20 % *Acacia karroo* leaf meal and 80 % *Setaria verticillata* grass hay had higher (P<0.05) DM, OM, CP and NDF digestibility values than those on a mixture of 50 % *Acacia karroo* leaf meal and 50 % *Setaria verticillata* grass hay and supplemented with either 23 g of PEG 4000/goat/day. Crude protein digestibility was optimized at a PEG 4000 supplementation level of 15.78 g/goat/day (Figure 5.09 and Table 5.03). Similarly, goats supplemented with 30 g of PEG 4000/goat/day and fed a dietary mixture of 20 % *Acacia karroo* leaf meal and 80 % *Setaria verticillata* grass hay had higher (P<0.05) DM, OM and NDF digestibility values than those on a mixture of 50 % *Acacia karroo* leaf meal and 50 % *Setaria verticillata* grass hay and supplemented with 30 g of PEG 4000/goat/day. Dry matter, OM and NDF digestibilities increased linearly with increasing levels of PEG 4000 supplementation (Figures 5.10, 5.11 and 5.12, respectively and Table 5.04). Dietary mixture level and PEG 4000 supplementation did not affect (P>0.05) ADF digestibility values of goats. Dietary mixture level and PEG 4000 supplementation did not affect (P>0.05) initial and final weights of Pedi goats. Similarly, dietary mixture level and PEG 4000 supplementation did not affect (P>0.05) daily live weight gains of the goats.

Table 5.02 Effects of polyethylene glycol 4000 supplementation and *Acacia karroo* leaf meal inclusion on diet intake, digestibility and live weight change of indigenous Pedi goats fed a *Setaria verticillata* grass hay-based diet

Treatment							
Variable	$A_{20}S_{80}P_0$	$A_{20}S_{80}P_{23}$	$A_{20}S_{80}P_{30}$	$A_{50}S_{50}P_0$	$A_{50}S_{50}P_{23}$	$A_{20}S_{80}P_{30}$	SEM
Intake (g/goat/day)							
DM	495^{bc}	766^{a}	696^{ab}	593^{abc}	394^{c}	486^{bc}	82.54
OM	453^{bc}	701^{a}	637^{ab}	544^{abc}	362^{c}	445^{bc}	75.59
CP	44	68	62	61	40	50	7.885
NDF	346^{bc}	535^{a}	486^{ab}	343^{bc}	228^{c}	282^{c}	54.12
ADF	233^{bc}	361^{a}	327^{ab}	246^{abc}	164^{c}	202^{c}	37.11
Intake (g/kg $W^{-0.75}$)							
DM	49^{bc}	71^{a}	66^{ab}	50^{abc}	41^{c}	46^{bc}	6.912
OM	45^{bc}	65^{a}	60^{ab}	46^{abc}	37^{c}	42^{bc}	6.322
CP	4.3	6.3	5.8	5.2	4.2	4.7	0.654
NDF	34^{bc}	49^{a}	46^{ab}	29^{c}	24^{c}	28^{c}	4.590
ADF	23^{bc}	33^{a}	31^{ab}	21^{c}	17^{c}	19^{c}	3.130
Digestibility (decimal)							
DM	0.72^{bc}	0.74^{ab}	0.76^{a}	0.71^{c}	0.70^{c}	0.71^{bc}	0.009
OM	0.73^{bc}	0.75^{ab}	0.77^{a}	0.73^{bc}	0.70^{c}	0.71^{bc}	0.011
CP	0.66^{ab}	0.71^{a}	0.67^{ab}	0.59^{c}	0.55^{c}	0.62^{bc}	0.023
NDF	0.68^{b}	0.72^{ab}	0.73^{a}	0.60^{c}	0.61^{c}	0.61^{c}	0.012
ADF	0.68	0.70	0.71	0.57	0.61	0.67	0.042
Initial W (kg)	20.7	23.4	22.5	21.8	20.4	21.9	1.544
Final W (kg)	21.5	24.2	23.1	22.8	21.4	22.9	1.625
Gain (g/goat/day)	40.0	38.0	30.3	49.6	46.3	49.3	12.35

^{a,b,c}: Means with different letters in the same row are significantly different (P<0.05)

SEM: Standard error of the means

W: Body weight

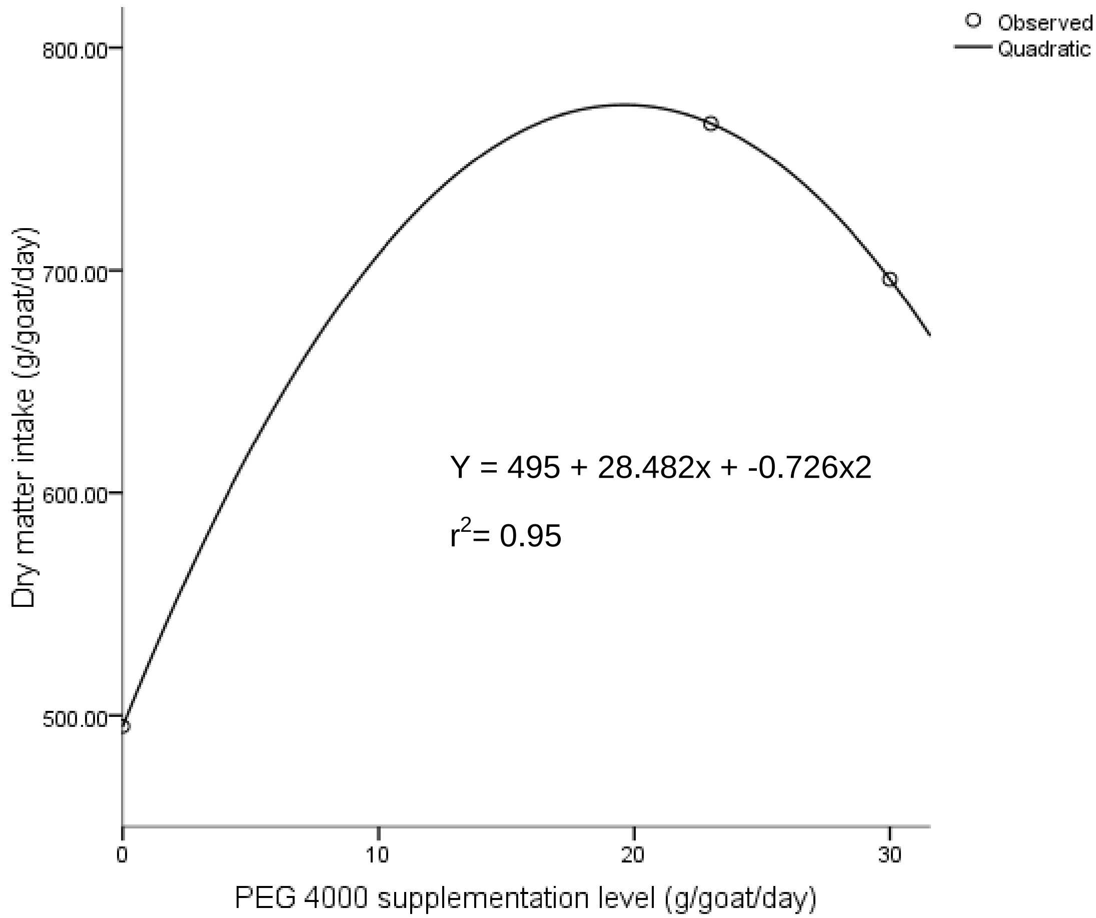


Fig 5.01 Effect of PEG 4000 supplementation level on dry matter intake in male Pedi goats fed diets with a 20 % *Acacia karroo* leaf meal inclusion level

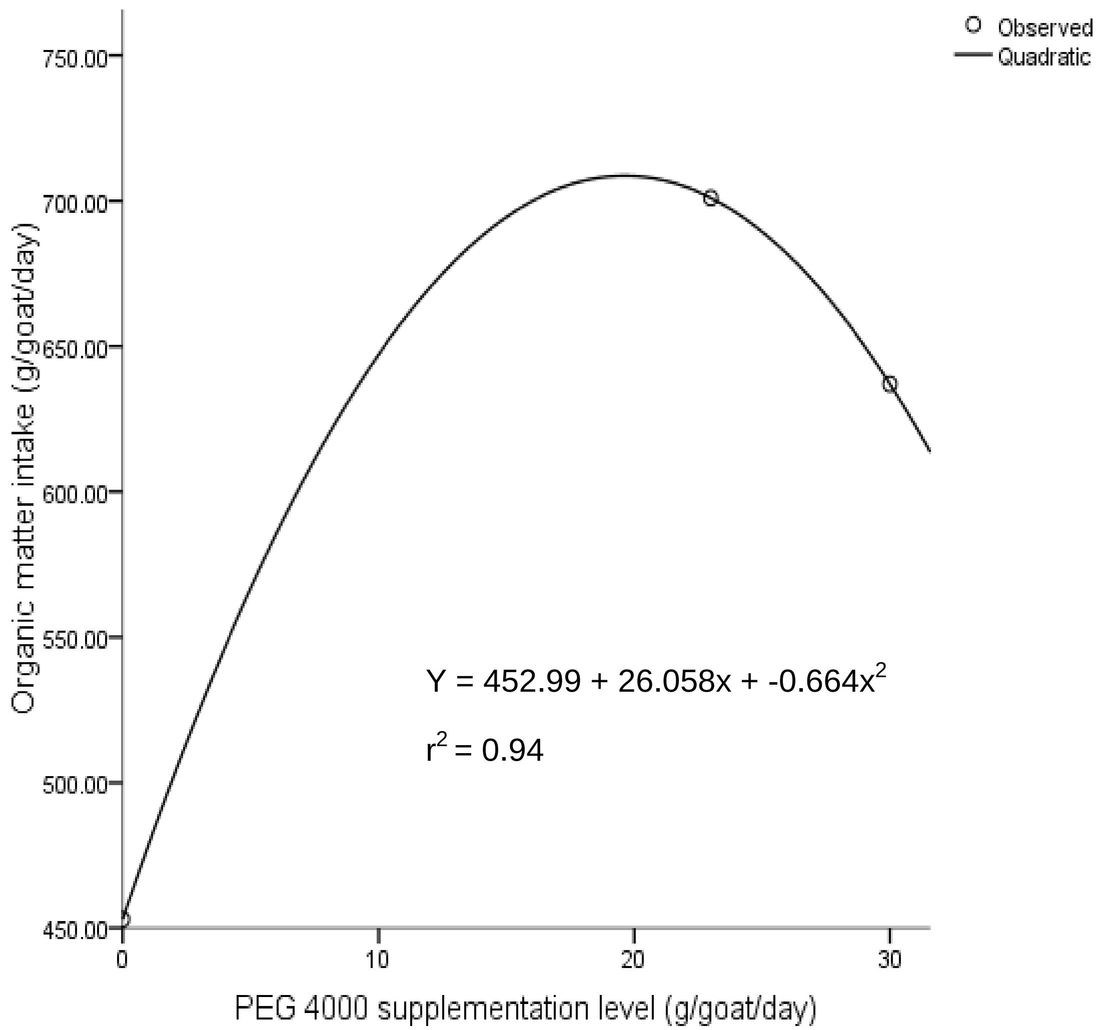


Fig 5.02 Effect of PEG 4000 supplementation level on organic matter intake in male Pedi goats fed diets with a 20 % *Acacia karroo* leaf meal inclusion level

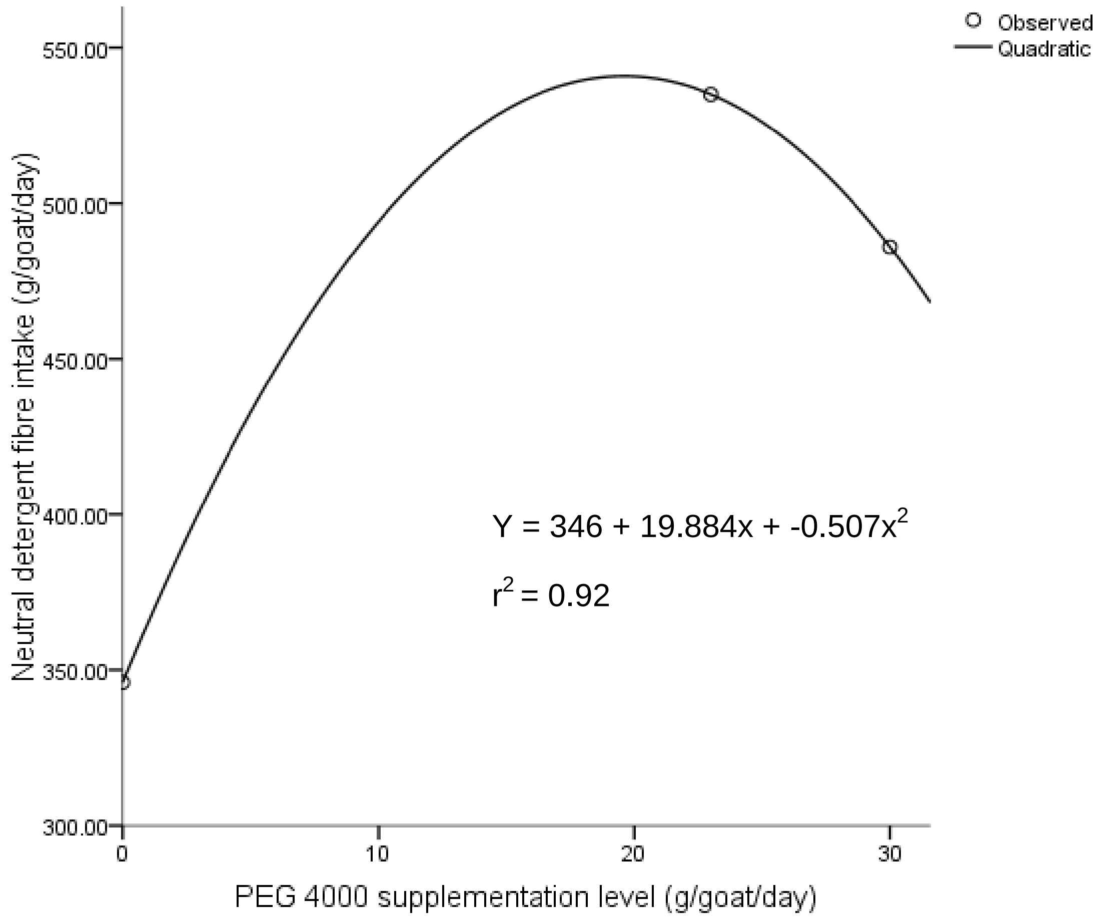


Fig 5.03 Effect of PEG 4000 supplementation level on neutral detergent fibre intake in male Pedi goats fed diets with a 20 % *Acacia karroo* leaf meal inclusion level

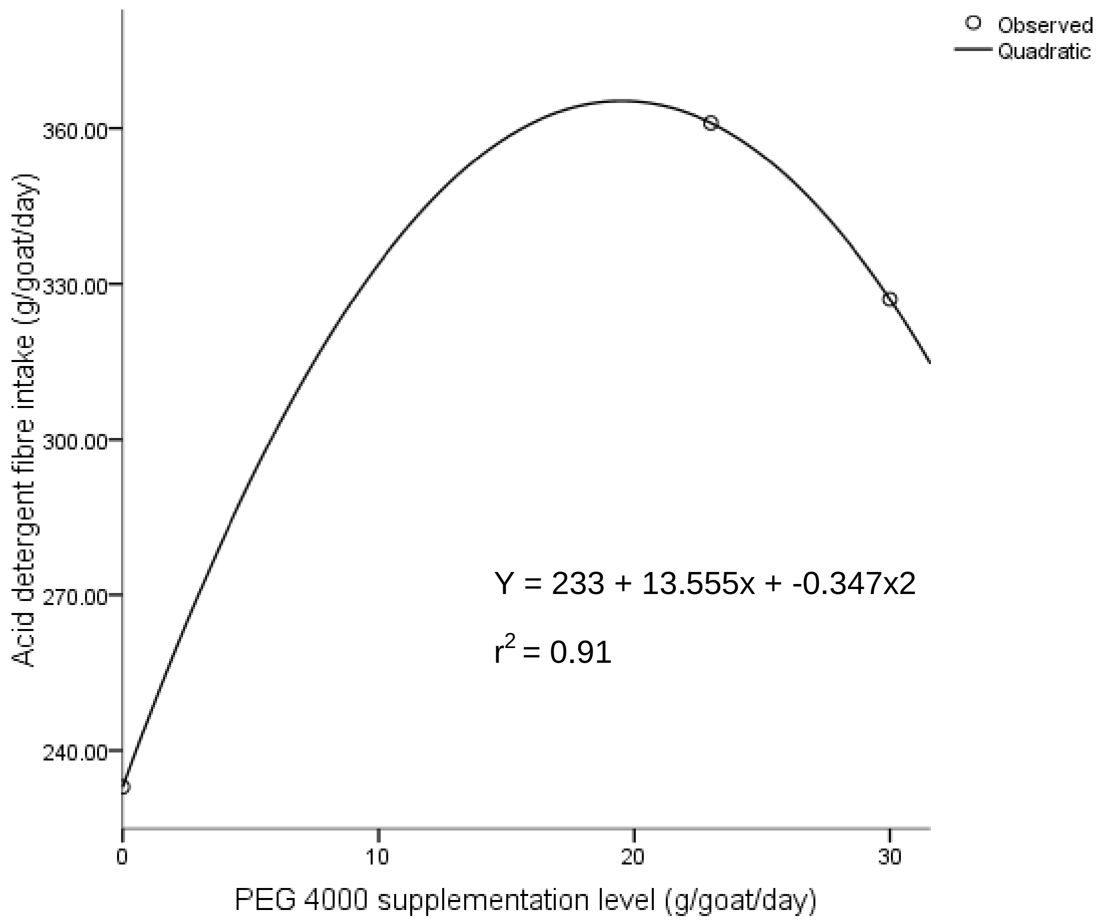


Fig 5.04 Effect of PEG 4000 supplementation level on acid detergent fibre intake in male Pedi goats fed diets with a 20 % *Acacia karroo* leaf meal inclusion level

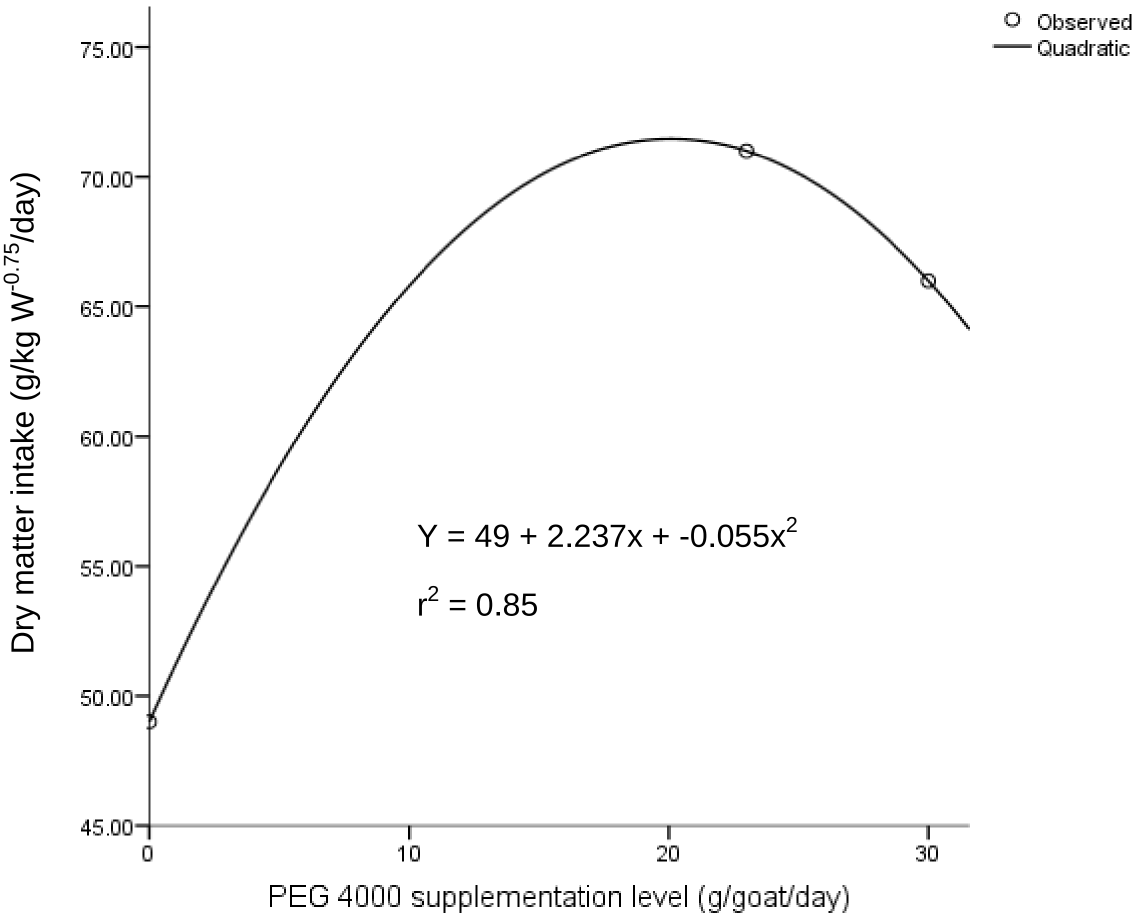


Fig 5.05 Effect of PEG 4000 supplementation level on dry matter intake per metabolic weight in male Pedi goats fed diets with a 20 % *Acacia karroo* leaf meal inclusion level

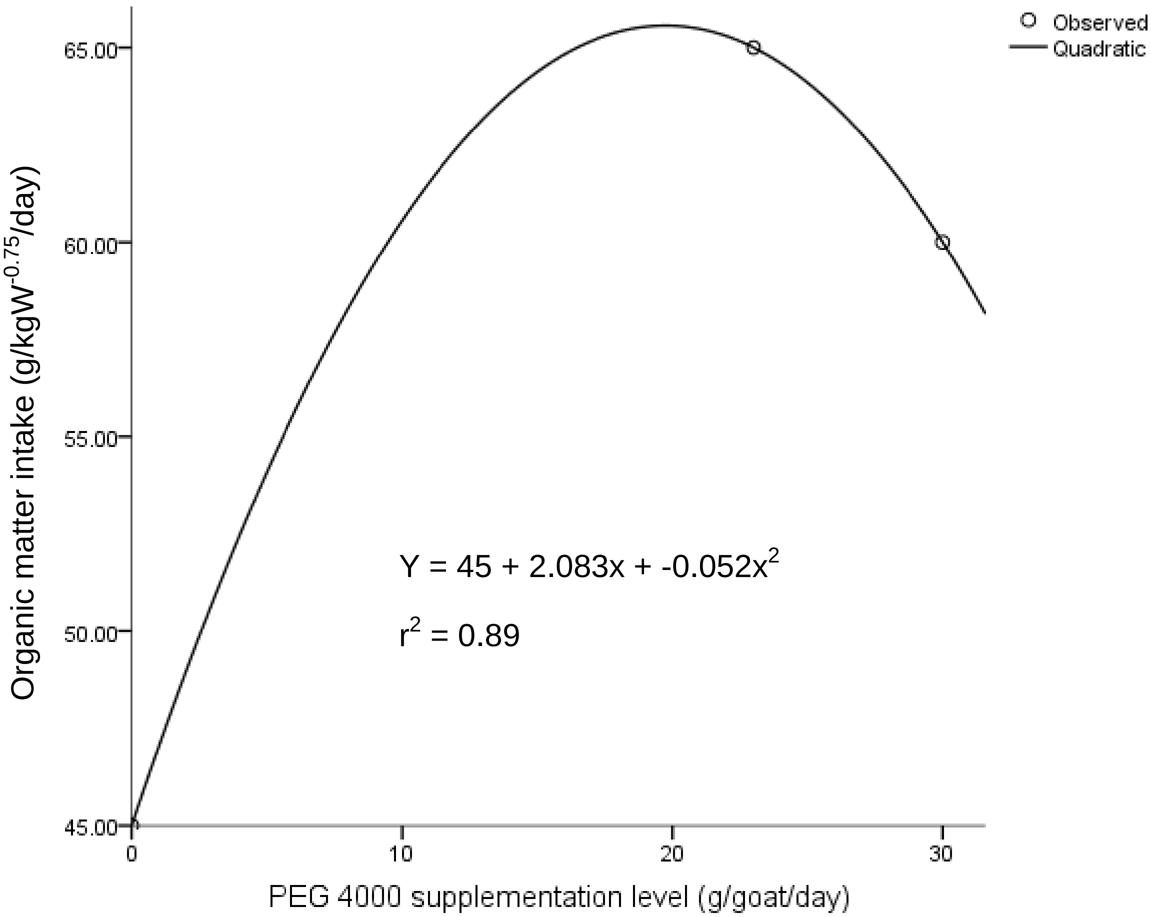


Fig 5.06 Effect of PEG 4000 supplementation level on organic matter intake per metabolic weight in male Pedi goats fed diets with a 20 % *Acacia karroo* leaf meal inclusion level

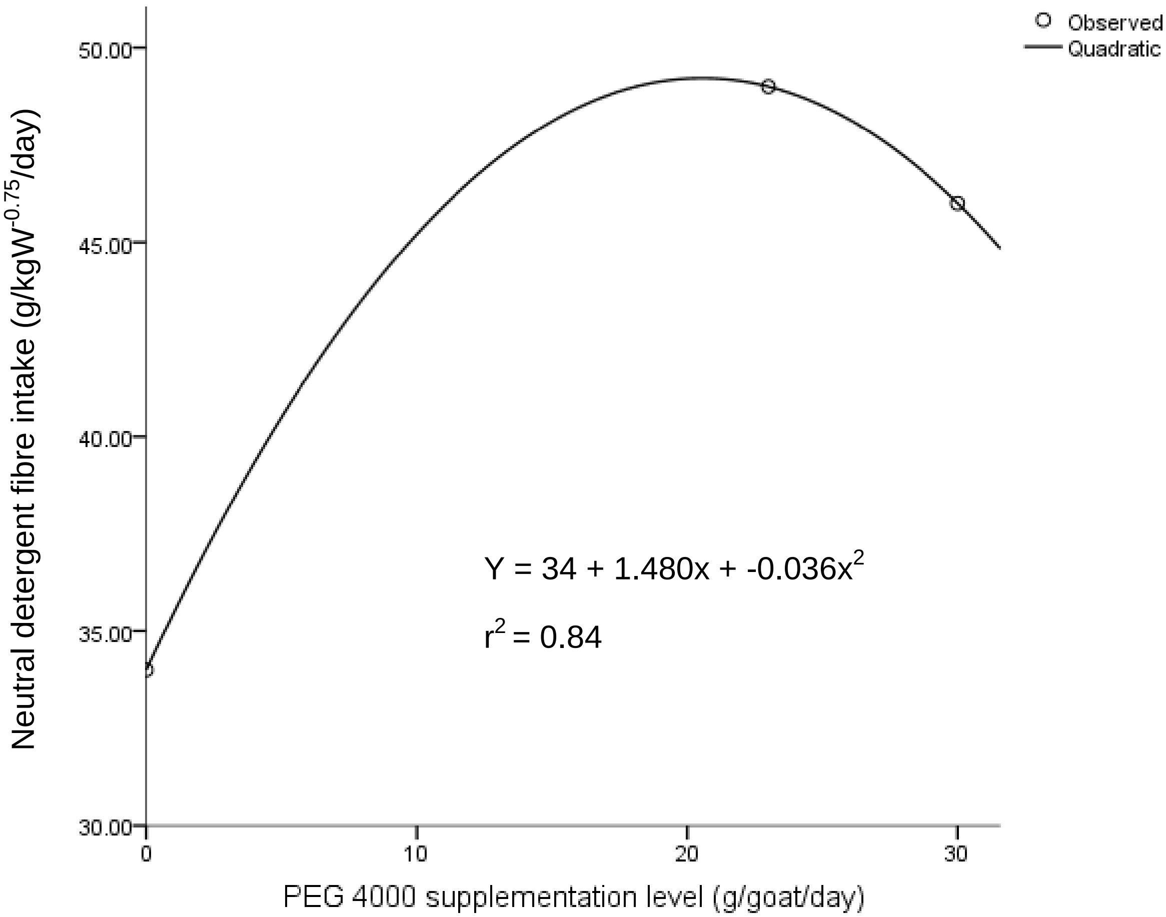


Fig 5.07 Effect of PEG 4000 supplementation level on neutral detergent fibre intake per metabolic weight in male Pedi goats fed diets with a 20 % *Acacia karroo* leaf meal inclusion level

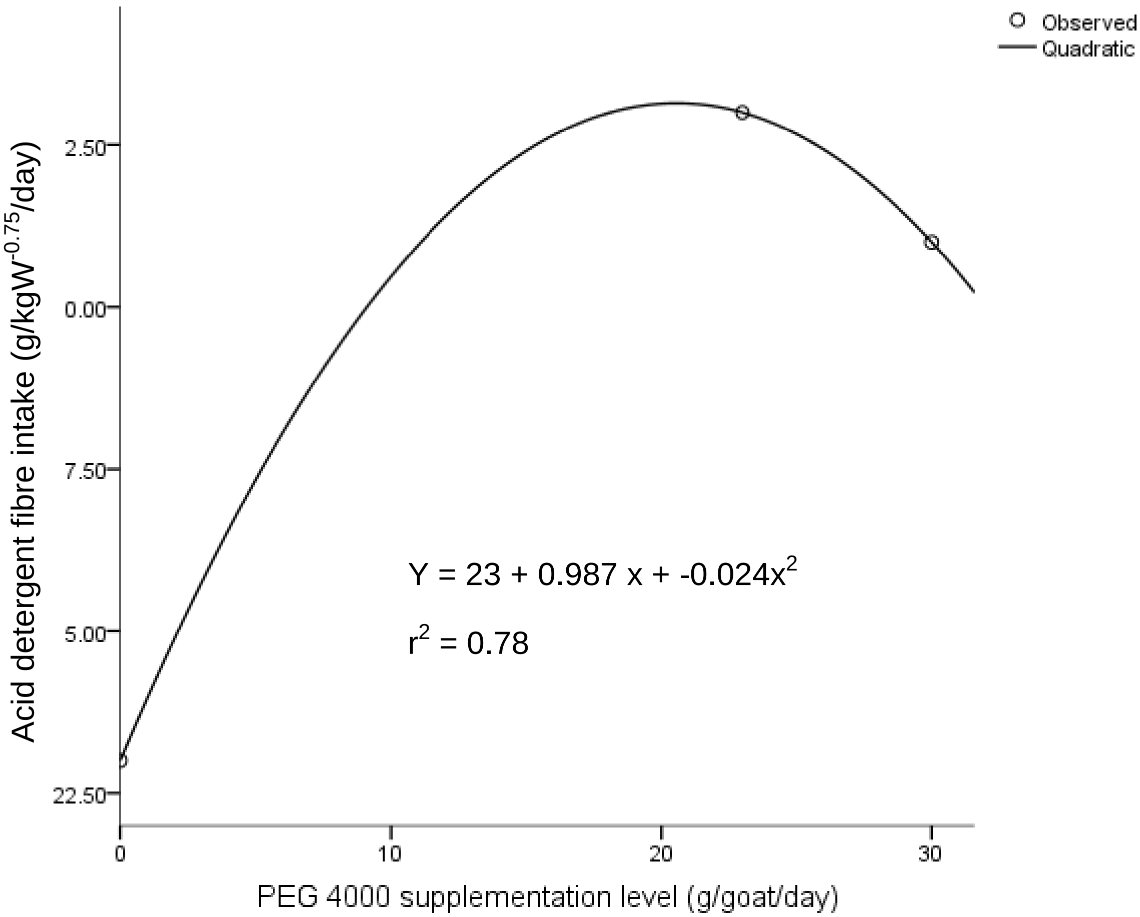


Fig 5.08 Effect of PEG 4000 supplementation level on acid detergent fibre intake per metabolic weight in male Pedi goats fed diets with a 20 % *Acacia karroo* leaf meal inclusion level

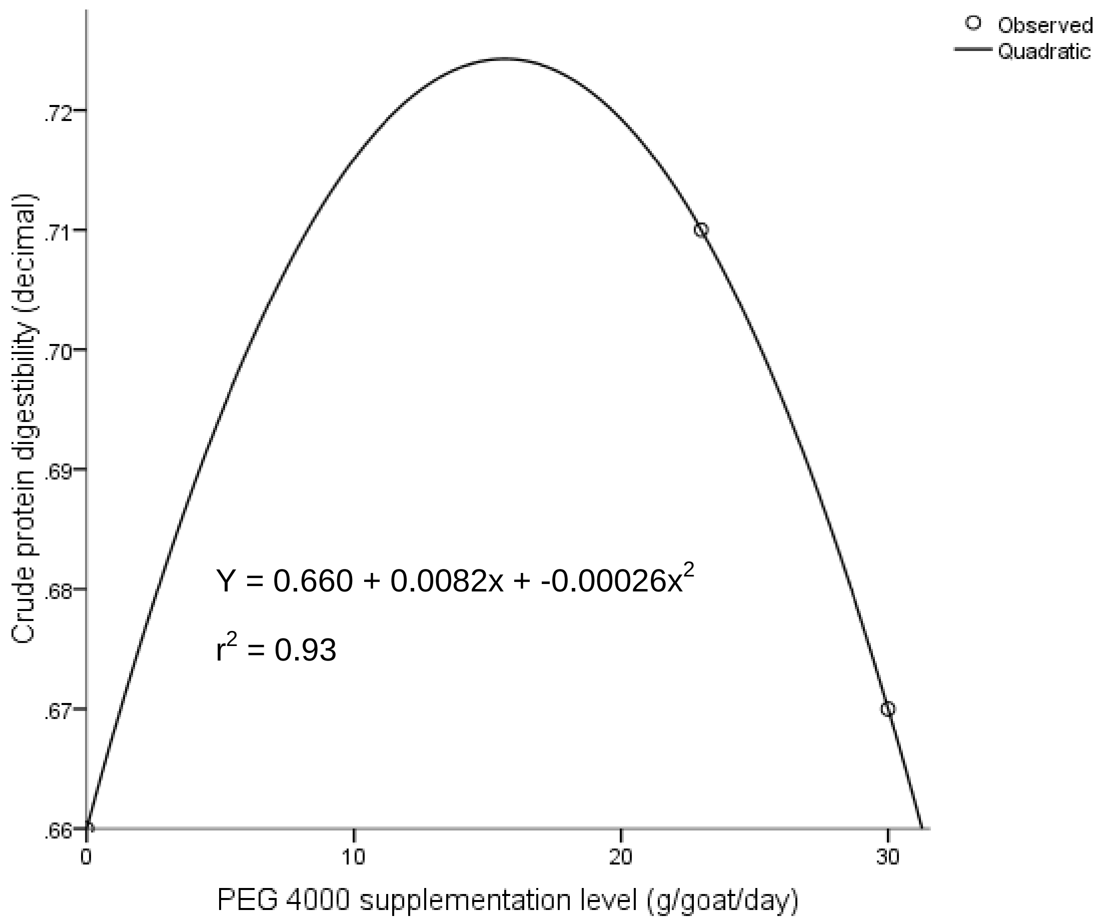


Fig 5.09 Effect of PEG 4000 supplementation level on crude protein digestibility in male Pedi goats fed diets with a 20 % *Acacia karroo* leaf meal inclusion level

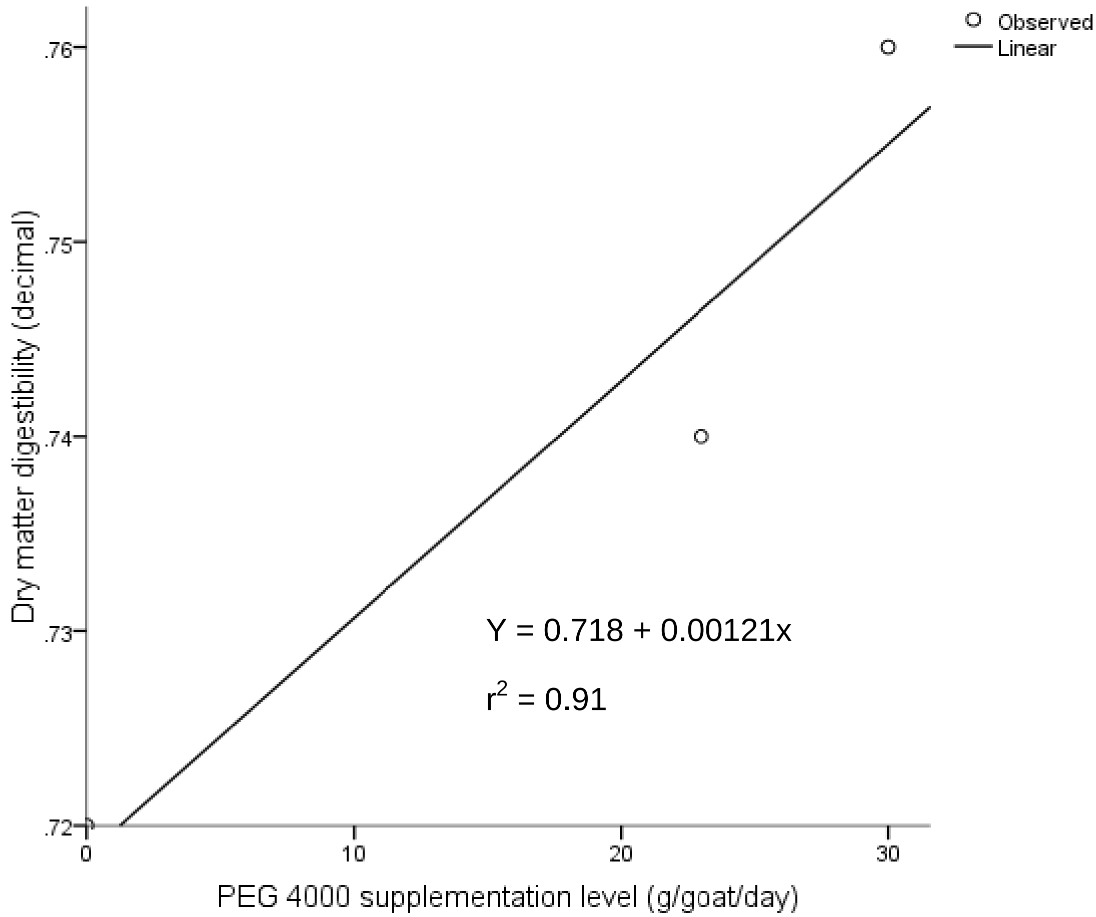


Fig 5.10 Relationship between PEG 4000 supplementation level and dry matter digestibility in male Pedi goats fed diets with a 20 % *Acacia karroo* leaf meal inclusion level

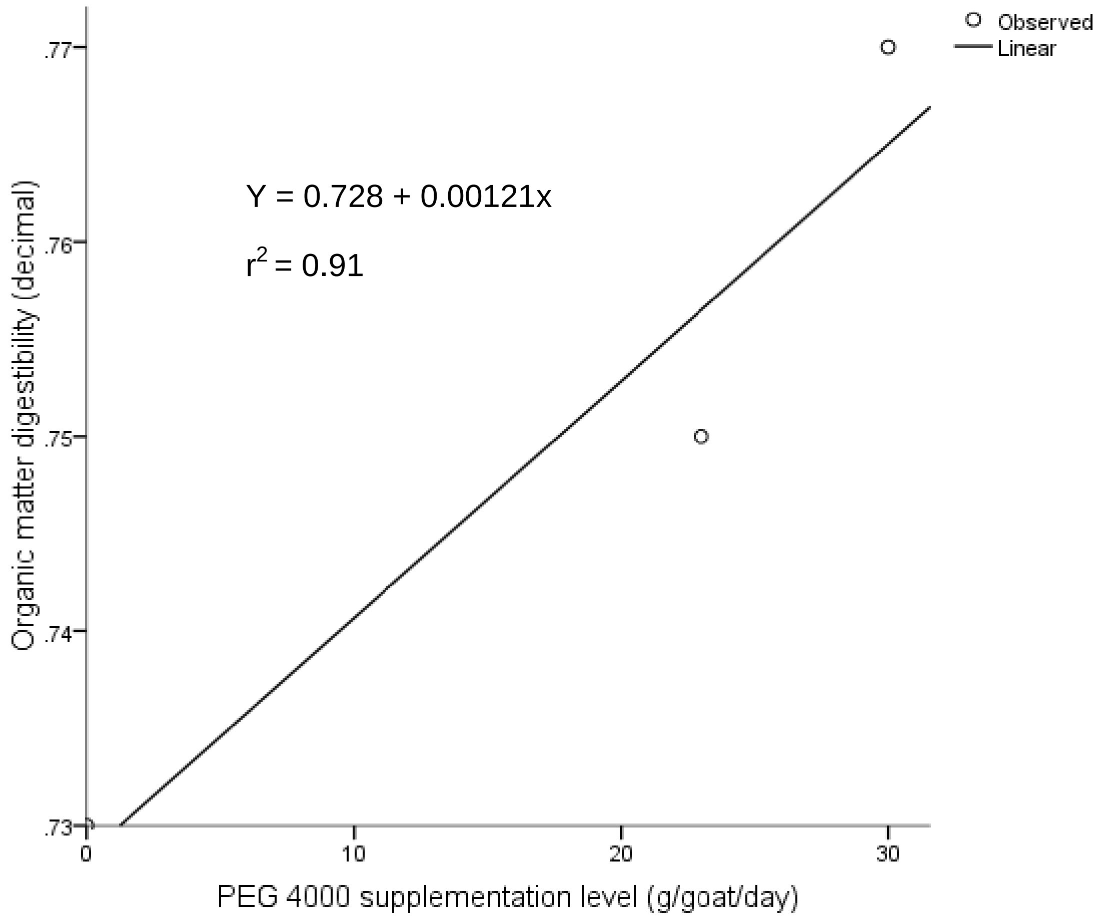


Fig 5.11 Relationship between PEG 4000 supplementation level and organic matter digestibility in male Pedi goats fed diets with a 20 % *Acacia karroo* leaf meal inclusion level

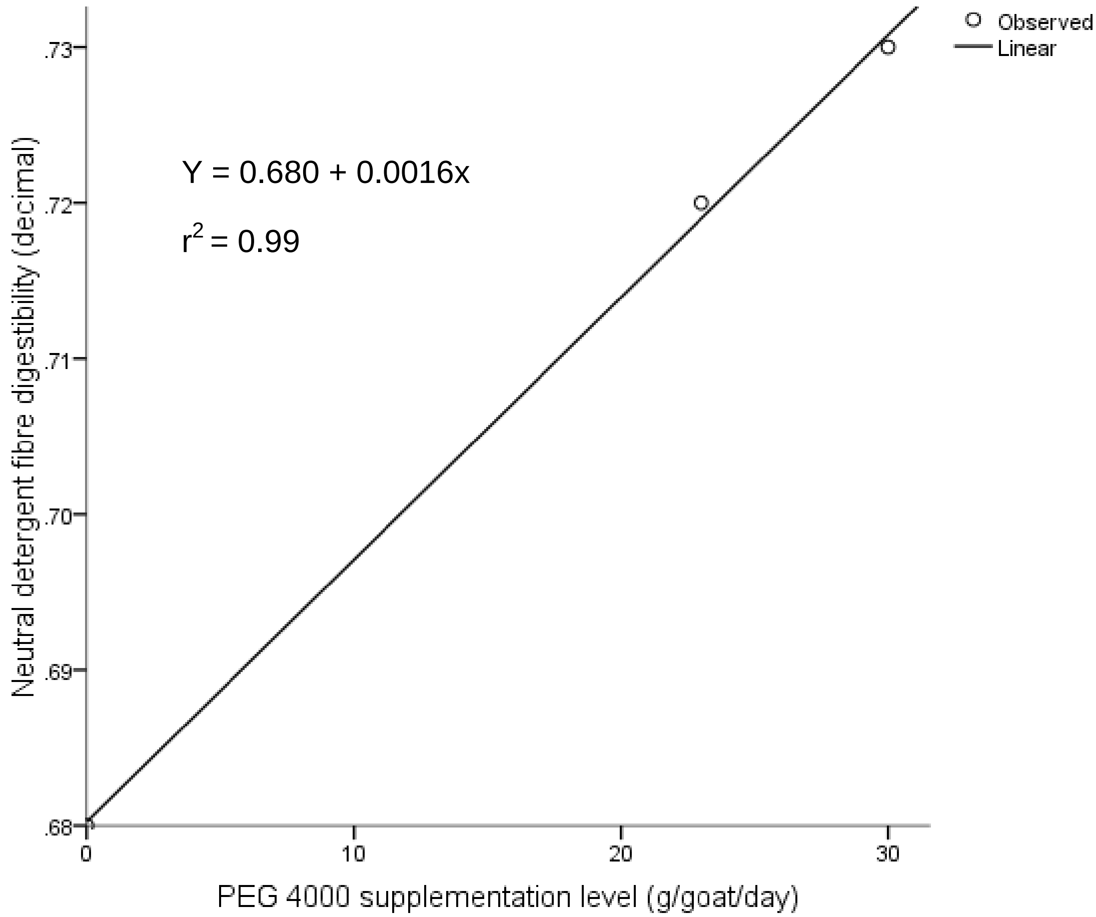


Fig 5.12 Relationship between PEG 4000 supplementation level and neutral detergent fibre in male Pedi goats fed diets with a 20 % *Acacia karroo* leaf meal inclusion level

Table 5.03 PEG 4000 supplementation levels for optimal dietary intakes in male Pedi goats fed diets with a mixture of 20 % *Acacia karroo* leaf meal and 80 % *Setaria verticillata* grass hay

Variable	Formula	PEG supplementation level	Optimal Y-Level	r^2
Intake (g/goat/day)				
DM	$Y = 495 + 28.482x + -0.726x^2$	19.62	774.75	0.95
OM	$Y = 452.99 + 26.058x + -0.664x^2$	19.62	708.67	0.94
NDF	$Y = 346 + 19.884x + -0.507x^2$	19.61	540.95	0.92
ADF	$Y = 233 + 13.555x + -0.347x^2$	19.53	365.36	0.91
Intake ($g/kgW^{-0.75}$)				
DM	$Y = 49 + 2.237x + -0.055x^2$	20.34	71.75	0.85
OM	$Y = 45 + 2.083x + -0.052x^2$	20.03	65.86	0.89
NDF	$Y = 34 + 1.480x + -0.036x^2$	20.56	49.21	0.84
ADF	$Y = 23 + 0.987x + -0.024x^2$	20.56	33.14	0.78
Digestibility (decimal)				
CP	$Y = 0.660 + 0.0082x + -0.0002x^2$	15.78	0.72	0.93

r^2 : Coefficient of determination

Table 5.04 Relationships between PEG 4000 supplementation level and nutrient digestibility (decimal) in male Pedi goats fed diets with a mixture of 20 % *Acacia karroo* leaf meal and 80 % *Setaria verticillata* grass hay

Variable	Formula	r^2	Probability
DM	Y = 0.718 + 0.0012x	0.91	0.190
OM	Y = 0.728 + 0.0012x	0.91	0.008
NDF	Y = 0.680 + 0.0016x	0.99	0.022

r^2 : Coefficient of determination

The results of the effects of PEG 4000 supplementation and *Acacia karroo* leaf meal inclusions and their interaction on serum urea and glucose concentrations of indigenous Pedi goats fed a basal diet of *Setaria verticillata* grass hay are presented in Table 5.06. Dietary mixture level and PEG 4000 supplementation did not affect (P>0.05) serum glucose and urea concentrations of Pedi goats.

Table 5.05 Effect of polyethylene glycol 4000 supplementation and *Acacia karroo* leaf meal inclusion on serum urea and glucose (mmol/L) of indigenous male Pedi goats fed *Setaria verticillata* grass hay-based diet

	Treatment						
Variable	$A_{20}S_{80}P_0$	$A_{20}S_{80}P_{23}$	$A_{20}S_{80}P_{30}$	$A_{50}S_{50}P_0$	$A_{50}S_{50}P_{23}$	$A_{50}S_{50}P_{30}$	SEM
Urea	3.0	2.6	2.5	2.8	3.3	2.5	0.450
Glucose	2.6	2.8	2.9	2.8	2.8	2.8	0.082

SEM: Standard error of the means

5.6 Discussion

Polyethylene glycol 4000 supplementation increased diet DM, OM, NDF and ADF intakes and intake per metabolic weight of goats on a dietary mixture of 20 % *Acacia karroo* leaf meal and 80 % *Setaria verticillata* grass hay. Dry matter, OM, NDF and ADF intakes were optimized at PEG 4000 supplementation levels of 19.62, 19.62, 19.61 and 19.53 g/goat, respectively. Similarly, DM, OM, NDF and ADF intakes per metabolic weight of the goats were optimized at PEG 4000 supplementation levels of 20.34, 20.03, 20.56 and 20.56 g/goat, respectively. This finding is similar to those of Silanikove *et al*. (1996) who reported higher DM intakes in goats fed tannin-rich *Pistacia lentiscus* and supplemented with 20 g of PEG 4000 per goat per day. The incorporation of PEG in the diet breaks tannin-fibre complexes, which consequently allows their digestion by microbial enzymes (Yisehak *et al*., 2013, *Frutos et al*., 2004). Inactivation of tannins through PEG increased availability of nutrients to the animal (Makkar, 2003). Other authors have, also, reported that PEG supplementation increases intake of tanniniferous browse foliage by sheep and goats (Titus *et al*., 2001; Pritchard *et al*., 1998; Barahona *et al*., 1997; Silanikove *et al*., 1994). In the present study, supplementation with PEG 4000 did not have effect on intake of Pedi goats on a dietary mixture of 50 % *Acacia karroo* leaf meal and 50 % *Setaria verticillata* grass hay. Similarly, increasing the *Acacia karroo* inclusion level from 20 % to 50 % did not improve intake of the diets. The effect of PEG depends on the level of protein in the diet. The higher the level of protein in a diet, the lower is the effect of PEG (Alonso-Diaz *et al*., 2010; Makkar & Becker, 1996; Austin *et al*., 1989). The higher level of protein in the present dietary mixtures of 50 % *Acacia karroo* leaf meal and 50 % *Setaria verticillata* grass hay may account for this non-significant effect. Contrary to this result, Motubatse *et al*. (2008) found that PEG 4000 supplementation increased diet CP intake as the level of *Acacia nilotica* leaf meal increased from 80 to 120 g/goat/day. The differences in responses may be due to differences in chemical composition of the two Acacia species. *Acacia karroo* contains more CT than *Acacia nilotica* (Mokoboki *et al*., 2005)

Polyethylene glycol 4000 supplementation to a mixture of 50 % *Acacia karroo* leaf meal and 50 % *Setaria verticillata* grass hay did not affect digestibility of the diets. However, supplementation with PEG 4000 to the dietary mixtures of 20 % *Acacia karroo* leaf meal and 80 % *Setaria verticillata* grass hay improved DM, OM, CP and NDF digestibilities. Crude protein digestibility was optimized at a PEG 4000 supplementation level of 15.78 g per goat per day. This result is similar to the findings of Motubatse *et al*. (2008) who reported that supplementation with 23 g of PEG 4000 increased diet OM and CP digestibilities in Pedi goats fed *Acacia nilotica* leaf meal. The positive effect of nutrient digestibility with PEG supplementation confirmed other previous literature reports on Acacia species such as *Acacia aneura* (Pritchard *et al*., 1992) and *Acacia cyanophylla* (Moujahed *et al*., 2000). Improvement in nutrient digestibility may be attributed to the positive effect of PEG supplementation on rumen microbial activity. Condensed tannins bind to dietary constituents, intestinal enzymes and bacteria (Makkar, 2003). This in turn decreases the availability of these nutrients, particularly protein, for microbial digestion. However, PEG 4000 binds with tannins and this increases the availability of proteins and other nutrients for digestion. Additionally, PEG 4000 may prevent tannins from binding to intestinal enzymes which in turn increase the digestibility of tannin-rich feeds (Makkar, 2003).

Acid detergent fibre digestion was unaffected by dietary mixture level and PEG 4000 supplementation. A Similar effect was reported by Motubatse *et al*. (2008) when Pedi goats were fed different levels of *Acacia nilotica* and supplemented with PEG 4000 supplementation. According to Makkar (2003), tannin-protein complexes appear as NDF or ADF in faeces which compromise their digestibilites. Silanikove *et al*. (1996) reported a negative NDF digestibility in goats fed on Pistacia foliage with PEG 4000 supplementation. The authors attributed this to the tannin-protein complexes as artefacts in faeces.

Average daily gains and final body weight of goats were not improved with dietary mixture level and PEG supplementation. These findings are similar to those of Ben Salem *et al*. (2005), who reported non-significant effects on the final and daily weight gains of goats fed tannin-rich oak (*Quercus coccifera* L.) and supplemented with PEG 4000. In contrast, Motubatse *et al*. (2008) reported improved average daily gains and final body weights in Pedi goats fed different levels of *Acacia nilotica* leaf meal and supplemented with PEG 4000. Similarly, Silanikove *et al*. (1996) found a significant increase in weight gain of goats fed Pistacia and supplemented with 20 g PEG 4000 per day. The difference in responses may be related to differences in the chemical composition of the browse species and animal breeds (Madibela *et al*., 2006). However, such discrepancies in body weight change may also be due to changes in rumen fill, which might not represent changes occurring in the body tissues (Motubatse *et al*. 2008).

Dietary *Acacia karroo* supplementation and PEG 4000 supplementation levels had no effect on serum urea and glucose concentrations of the goats. However, serum glucose was within the normal range of 2.78 - 4.16 mmol/l reported for healthy goats (Kaneko, 1997). These findings are similar to those of Ben Salem (2005). Serum urea level was below the normal established range of 3.5 – 10.7 mmol/l (Sirois, 1995). The reason for depressed serum urea in the present experiment merits further study.

Supplementation with 23 or 30 g of PEG 4000 per goat per day for animals fed a mixture of 50 % *Acacia karroo* leaf meal and 50 % *Setaria verticillata* grass hay had no effect on the performance of the animals in the present study. All the measured parameters tended to decrease linearly with increasing levels of PEG 4000 supplementation.

5.7 Conclusion

Acacia karroo leaf meal contained 126.5 g CP per kg DM, indicating that it has potential as a protein feed for ruminants. However, it also contained relatively high amounts of condensed tannins, which can deter intake and digestion in goats.

Supplementation with 23 g of PEG 4000 improved DM, OM, NDF and ADF intakes of Pedi goats fed a dietary mixture of 20 % *Acacia karroo* leaf meal and 80 % *Setaria verticillata* grass hay. Dry matter, OM, NDF and ADF intakes were optimized at PEG 4000 supplementation levels of 19.62, 19.62, 19.61 and 19.53 g/goat/day, respectively. Similarly, DM, OM, NDF and ADF intakes per metabolic weight were optimized at supplementation levels of 20.34, 20.03, 20.56 and 20.56 g of PEG per goat per day, respectively. Thus, the supplementation level of about 20 g of PEG 4000 per goat per day optimized DM, OM, NDF and ADF intakes. This level of supplementation is recommended if intake is the parameter of interest. Supplementation with either 23 or

30 g of PEG 4000 improved DM, OM, CP and NDF digestibility by goats fed a dietary mixture of 20 % *Acacia karroo* leaf meal and 80 % *Setaria verticillata* grass hay. Crude protein digestibility was optimized at a PEG 4000 supplementation level of 15.78 g per goat per day. This supplementation level for optimal CP digestibility is lower than the 20 % for intake. Thus, the supplementation level for optimal productivity depended on the production parameters of interest. This has implications on supplementation recommendations. Thus, further studies are recommended to ascertain these levels of supplementation. Supplementation with either 23 or 30 g of PEG 4000 per goat per day did not improve the performance of the animals when fed a dietary mixture of 50 % *Acacia karroo* leaf meal and 50 % *Setaria verticillata* grass hay. It is possible that PEG 4000 was not enough to have effect on the performance of the goats. Similarly, the high amounts of condensed tannins may have exerted adverse effects on the performance of the goats. All this requires further studies.

CHAPTER SIX

EFFECT OF *ACACIA KARROO* LEAF MEAL INCLUSION LEVEL ON CARCASS CHARACTERISTICS, PHYSICO-CHEMICAL AND SENSORY ATTRIBUTES, AND HISTOLOGICAL PARAMETERS OF PEDI GOATS FED ON A *SETARIA VERTICILLATA* GRASS HAY-BASED DIET

Abstract

The current study was carried out to evaluate the effect of *Acacia karroo* leaf meal inclusion level on carcass characteristics, physico-chemical and sensory attributes and histological parameters of Pedi goats fed a *Setaria verticillata* grass hay-based diet. Fifteen indigenous male Pedi goats with an average initial body weight of 16.7 ± 3.26 kg were allocated in a completely randomized design to five dietary treatments containing *Acacia karroo* leaf meal inclusion levels at 20 % ($S_{80}A_{20}$), 25 % ($S_{75}A_{25}$), 30 % ($S_{70}A_{30}$), 40 % ($S_{60}A_{40}$) and 50 % ($S_{50}A_{50}$), respectively, for 60 days. Daily dry matter intakes were similar ($P>0.05$) across the dietary treatments, ranging from 638 to 786 g per goat. Dietary treatments had no effect ($P>0.05$) on the final live weights and average daily gains of the goats. Similarly, yields of different carcass components and meat sensory attributes were not affected ($P>0.05$) by the treatments. *Acacia karroo* leaf meal inclusion level had no effect ($P>0.05$) on the physico-chemical attributes of goats, except for the meat colour at 24 h post-mortem. Goats on a diet having 25 % *Acacia karroo* leaf meal inclusion level had higher ($P<0.05$) values for meat colour lightness than those on diets having 30 or 40 % inclusion levels. Histological photomicrographs of the liver of Pedi goats on diets containing 20, 25 or 30 % *Acacia karroo* leaf meal inclusion levels showed moderate hepatocellular hydropic degeneration, dilation of central veins and proliferation of sinusoidal kupfer cells. The hepatocytes of goats fed diets containing 40 or 50 % *Acacia karroo* leaf meal inclusion levels showed severe hepatocellular hydropic degenerations with multifocal single cell and clustered cell necrosis. The kidneys of goats on diets containing 20, 25 or 30 % *Acacia karroo* leaf meal inclusion levels revealed early dilatation of uriniferous spaces and mild renal tubular nephrosis while those on diets having 40 or 50 % *Acacia karroo* inclusion levels had dilatation of uriniferous spaces and ectasia of some proximal convoluted tubules. Moderate renal nephrosis with intra-tubular proteinaceous fluid and protein cast formation accumulation were also noticed. It is concluded that *Acacia karroo* leaf meal inclusion levels of 20 to 30 % supported moderate productivity of the goats and did not cause any adverse effects. These levels are, thus, recommended.

6.1 Introduction

Pedi goats in communal areas of Limpopo province depend on *Acacia karroo* leaves, particularly during the dry season (Jamala *et al*., 2013; Mokoboki *et al*., 2005). *Acacia karroo* leaves contain high levels of condensed tannins and have potent antioxidant properties (Moon *et al*., 2014; Hagerman *et al*., 1998). Antioxidant properties of condensed tannins have positive effect on meat quality and oxidative stability (Qwele *et al*., 2013; Moyo *et al*., 2012). On the other hand, high condensed tannin contents in the diet negatively affect nutrient availability (Gxasheka *et al*., 2015). However, reports on the effects of tannin-rich diets on small ruminant productivity and meat quality are not conclusive. Some authors have found that condensed tannin-rich diets positively influence chevon quality (Ngambu *et al*., 2013; Ngambu *et al*., 2012; Bakare & Chimonyo 2011; Marume, 2010; Mapiye *et al*., 2009; Priolo & Vasta, 2007), whereas, others have not found any clear evidence of that (Mapiye *et al.,* 2010). Additionally, *Acacia karroo* leaf meal inclusion levels for optimal Pedi goat meat quality were not found in the literature.

Condensed tannins in browse leaves have, also, been implicated in toxic degenerative damages in the intestines, livers, spleens and kidneys of ruminants (Mbatha *et al*., 2002; Kumar & Singh, 1984). Similarly, large intakes of tannins may cause kidney and liver damages (Karimi *et al*., 2014). It is, therefore, important to determine the effect of *Acacia karroo* leaf meal inclusion level on meat characteristics and histology of livers and kidneys of indigenous male Pedi goats.

6.2 Objectives

The objectives of the study were to determine:

a. the effect of *Acacia karroo* leaf meal inclusion level on carcass characteristics, physico-chemical and sensory attributes of indigenous male Pedi goats fed *Setaria verticillata* grass hay-based diets.

b. the effects of *Acacia karroo* leaf meal inclusion level on the histology of livers and kidneys of male Pedi goats fed *Setaria verticillata* grass hay-based diets.

6.3 Hypotheses

a. *Acacia karroo* leaf meal inclusion level has no effect on carcass characteristics, physico-chemical and sensory attributes of indigenous male Pedi goats fed *Setaria verticillata* grass hay-based diets.

b. *Acacia karroo* leaf meal inclusion level has no effect on the livers and kidneys of male Pedi goats fed *Setaria verticillata* grass hay-based diets.

6.4 Materials and methods

6.4.1 Study site

The study was conducted at the University of Limpopo Experimental farm. The site is described in Section 3.4.1.

6.4.2 Experimental diets, goats and experimental design

Fresh young leaves *of Acacia karroo* were hand-harvested at the University of Limpopo Experimental farm using a pair of long scissors in summer (November to January, 2014) as described in Section 3.4.2. Fifteen growing male Pedi goats with a mean initial body weight of 16.7 ± 3.26 kg were used in the study. The goats were randomly assigned in a completely randomized design to five treatments consisting of three goats (replicates) per treatment. Each group had one goat per replicate. All goats were drenched with an anthelmintic (Valbazen® broad spectrum dewormer, manufactured by Pfizer Animal NY, USA) and sprayed with Diazintol® (Alfasan International, Holland) before the start of the experiment. The goats were housed in individual holding pens (1 x 3 m^2) that were installed in a well ventilated shed with one side open to natural light and roofed to protect animals against the sun and rain. There was a preliminary period of 14 days to familiarize the goats with the feeds. The goats were individually offered the experimental diets (Table 6.01) once a day at 0800 h. Feed offered was weighed and recorded daily and feed refusals were weighed every morning before fresh feed was offered. The goats were weighed three times, at the start of the experiment, on the 54th

day when data collection commenced and on the 60th day when data collection ended. The weighing of the goats was carried out before morning feeding to avoid feed effect (Sarwatt *et al*., 2003). Dry matter values of the feeds and feed refusals were determined. The feeding trial lasted for 60 days, the last 7 days being for data collection.

Table 6.01 Feed composition of the experimental diets

Experimental code	Diet description
$S_{80}K_{20}$	A mixture of 80 % *Setaria verticillata* hay and 20 % *Acacia karroo* leaves
$S_{75}K_{25}$	A mixture of 75 % *Setaria verticillata* hay and 25 % *Acacia karroo* leaves
$S_{70}K_{30}$	A mixture of 70 % *Setaria verticillata* hay and 30 % *Acacia karroo* leaves
$S_{60}K_{40}$	A mixture of 60 % *Setaria verticillata* hay and 40 % *Acacia karroo* leaves
$S_{50}K_{50}$	A mixture of 50 % *Setaria verticillata* hay and 50 % *Acacia karroo* leaves

6.4.3 Slaughter and carcass evaluation

At the completion of the study, the goats were fasted for 16h with free access to drinking water (Hozza *et al*., 2014). The animals were then weighed to obtain pre-slaughter weight, and thereafter, they were slaughtered humanely by electrical stunning and bled immediately. Carcass components such as head, skin, feet, kidney, liver, lung, heart and genital scrotum were eviscerated and weighed. Full and empty alimentary canal, reticulo-rumen and intestines were also weighed. Warm carcass weights were recorded 1 h after slaughter. The carcasses were chilled overnight at 3 °C and weighed again the following day to obtain cold carcass weights. At the end of the first 24 h post slaughter, samples were taken from the *longissimus dorsi* muscle for instrumental colour and pH readings. Muscle pH was measured 1 h and 24 h (pH_1 and pH_{24}, respectively) post-mortem using a digital pH metre (Crison pH25, CRISON instruments S.A., Alella, Spain) equipped with a penetrating electrode. The pH meter was calibrated with pH4, pH7 and pH9 standard solutions before each measurement. The pH measurements and colour were taken from the *longissimus dorci* muscle. Meat colour

(L* = lightness, a* = redness and b* = yellowness) was measured with a 45/0 BYK-Gardener instrument (BYK-Gardener, GmbH, Germany), with a 20 mm diameter measurement area and illuminant D65-day light, 10º standard observer, 1 and 24 h post-mortem. Three readings were taken by rotating the instrument 90º between measurements, in order to obtain a representative average value of the colour, and avoiding connective tissues and intramuscular fat. The instrument was calibrated before measurement using the green standard (Priolo *et al*., 2001).

Blocks of *longissimus dorsi* muscle, measuring approximately 7 x 4 x 4 cm, were used to determine cooking loss (Babikerm *et al*., 1990) and shear force values (Chrystall *et al*., 1994). The muscle was weighed, placed in a water tight PVC plastic bag and cooked in a water bath at 85 ºC for 45 minutes, until an internal temperature of 70 ºC was attained. The samples were cooled and re-weighed. Cooking loss (CL) was calculated using the following formula: Cooking loss % = [(weight before cooking – weight after cooking) / weight before cooking] × 100 as described by Ding *et al*. (2010). After measurement of cooking loss, cooked samples were used to determine meat Warner Bratzler shear force. Three sub-samples (cut parallel to the muscle fibres with a cross-section of 1 x 1 cm and at least 3 cm long) were removed from each cooked muscle. The sub-samples were sheared perpendicular to the fibre direction with an Instron Universal Testing Machine (Model 3344, Instron Industrial Products, GC, USA) equipped with a Warner-Bratzler (WB) shear force apparatus (crosshead speed at 400 mm/min, one shear in the centre of each core). The measurements were read in Newtons. Dressing percentage was calculated as the ratio of hot carcass weight divided by slaughter body weight and the result multiplied by 100. Water holding capacity (WHC) of the meat was measured as the amount of water expressed from a fresh meat sample (1 g) held under pressure (60 kg) using the filter-paper press method developed by Grau & Hamm (1957).

6.4.4 Meat sensory evaluation

Meat samples that were used for consumer sensory evaluation were obtained from each carcass and were cut 24 h post-slaughter. The meat samples were cut into cubes (about 2 x 2 cm), which were placed in watertight PVC plastic bags and cooked in a boiling water bath at a temperature of 85 ºC for 45 minutes (Babikerm *et al*., 1990). Salt was added to taste. Twenty trained consumer panelists from the University of Limpopo were used for the consumer sensory assessment of meat. Panelists were screened and selected following guidelines from Cross *et al*. (1978). The panelists were taught how to infer and record scores for each variable. The waiting period between meat sample tastings was 10 minutes. Distilled water was served to panelists to freshen their mouths between sub-sample assessments to avoid crossover effects. Five-point descriptive scales (Table 6.02) were used to evaluate tenderness (1 = too tough to 5 = extremely tender), juiciness (1 = too dry to 5 = extremely juicy), flavour (1 = very bad flavour to 5 = very good flavour), taste (1 = dislike extremely to 5 = like extremely), aroma (1 = dislike extremely to 5 = like extremely), overall acceptability (1= dislike extremely to 5 = like extremely).

6.4.5 Histological analyses

Liver and kidney samples from each goat were collected and preserved in 10 % neutral buffered formalin for 24 hours. Subsequently, liver and kidney tissues were dehydrated using standard histological techniques in graded ethanol series and embedded in paraffin wax for histopathology examination (Sanchez-Chardi *et al*., 2008). From each sample, 60 - 65 µm sections were cut and mounted on glass slides before staining with haematoxylin and eosin. Slides were examined under light trinocular microscopy at 400X (Leica Microsystems model DM750, Leica, Bannockburn, IL, USA). Each slide was photographed with a DVC digital camera (Digital Video Camera Company, Austin, TX) mounted on a BH-2. The process was as described by Sanchez-Chardi *et al.* (2009).

Table 6.02 Evaluation scores used by the sensory panel

Sensory attribute						
Score	Tenderness	Juiciness	Flavour	Taste	Aroma	Overall acceptability
1	Too tough	Too dry	Very bad flavour	Dislike extremely	Dislike extremely	Dislike extremely
2	Tough	Dry	Poor flavour	Dislike	Dislike	Dislike
3	Neither tough nor tender	Neither dry nor juicy	Neither bad nor good	Neither like nor dislike	Neither like nor dislike	Neither like nor dislike
4	Tender	Juicy	Good flavour	Like	Like	Like
5	Extremely tender	Extremely juicy	Very good flavour	Like extremely	Like extremely	Like extremely

6.4.6 Chemical analysis

The procedures for the determination of dry matter, fat, organic matter, crude protein, acid detergent fibre, neutral detergent fibre, condensed tannins and total polyphenols were as described in Section 3.4.4.

6.4.7 Statistical analyses

All data on feed intake, live weight change, carcass characteristics, physico-chemical attributes and meat sensory scores were analysed using the General Linear Model procedures of SAS (SAS, 2010). Fisher's least significant difference (LSD) test was applied for mean separation where there were significant differences ($P<0.05$).

6.5 Results

Results of the nutritive values of the diets were similar to those presented in Section 3.5. Higher *Acacia karroo* leaf meal inclusions resulted in higher ($P<0.05$) crude protein and condensed tannin contents. The results of the effect of *Acacia karroo* leaf meal inclusion level on diet intake and body weight change of Pedi goats fed *Seteria verticillata* grass hay-based diets are presented in Table 6.03. Daily dry matter intakes were similar ($P>0.05$) across the dietary treatments, ranging from 638 to 786 g per goat. Similarly, goats consumed the same ($P>0.05$) daily amounts of organic matter (OM), crude protein (CP), neutral detergent fibre (NDF) and acid detergent fibre (ADF) contents. *Acacia karroo* inclusion level did not ($P>0.05$) affect final body weights and average daily gains of the goats.

The yields of different quantitative carcass components are presented in Table 6.04. *Acacia karroo* leaf meal inclusion level did not ($P>0.05$) affect hot and cold carcass weights of goats. Similarly, the head, skin, feet, kidney, liver, lung, heart and genital scrotum of the goats were not affected ($P>0.05$) by the dietary treatments. The same effects were observed for empty alimentary canals, empty reticulo-rumens and empty intestines.

Acacia karroo leaf meal inclusion level had no effect ($P>0.05$) on pH, 1 and 24 h post-mortem (Table 6.05). Similarly, dietary treatment had no effect ($P>0.05$) on meat colour at 1 h post-mortem; however, there was treatment effect ($P<0.05$) on meat colour at 24 h post-mortem. Goats on a diet having 25 % *Acacia karroo* leaf meal inclusion level had higher ($P<0.05$) values for lightness (L^*) than those on diets with 30 or 40 % inclusion levels. Goats on diets having 20, 30, 40 or 50 % *Acacia karroo* leaf meal inclusion levels had similar ($P>0.05$) values for L^*. *Acacia karroo* leaf meal inclusion level had no effect ($P>0.05$) on water-holding capacity, shear force, cooking loss and dressing percentage of the meat of Pedi goats. Similarly, dietary treatments had no effect ($P>0.05$) on chevon sensory attributes (Table 6.06).

Histological photomicrographs of the livers of Pedi goats on diets containing 20, 25 or 30 % *Acacia karroo* leaf meal inclusion levels showed moderate hepatocellular hydropic degeneration, dilation of central veins and proliferation of sinusoidal kupfer cells (Figures 6.01, 6.02 and 6.03, respectively). The hepatocytes of goats fed diets containing 40 or 50 % *Acacia karroo* inclusion levels showed severe hepatocellular hydropic degeneration with multifocal single cell and clustered cell necrosis (Figures 6.04 and 6.05, respectively). The kidneys of goats on diets containing 20, 25 or 30 % *Acacia karroo* leaf meal inclusion levels revealed early dilatation of glomerular uriniferous spaces and mild renal tubular nephrosis (Figures 6.06, 6.07 and 6.08, respectively), while those on diets having 40 or 50 % *Acacia karroo* leaf meal inclusion levels had dilatation of uriniferous spaces and ectasia of some proximal convoluted tubules (Figures 6.09 and 6.10, respectively). Moderate renal nephrosis with intra-tubular proteinaceous fluid and protein cast formation accumulation were also noticed in the kidney of goats on diets 40 or 50 % *Acacia karroo* leaf meal inclusion levels (Figures 6.09 and 6.10, respectively).

Table 6.03 Effect of *Acacia karroo* leaf meal inclusion level on diet intake and live weight of male Pedi goats fed a *Setaria verticillata* grass hay-based diet

	Treatment					
Variable	$S_{80}A_{20}$	$S_{75}A_{25}$	$S_{70}A_{30}$	$S_{60}A_{40}$	$S_{50}A_{50}$	SEM
Intake (g/goat/day)						
Dry matter	752	786	638	661	686	70.3
Organic matter	688	720	585	606	630	64.5
Crude protein	66	72	59	64	70	6.83
NDF	525	533	421	409	397	45.9
ADF	353	362	288	286	285	31.5
Initial BWT (kg)	16.0	17.3	13.7	16.1	15.2	2.55
Final BWT (kg)	22.8	24.1	21.4	23.8	24.6	2.61
ADG (g/goat/day)	113	114	129	129	157	23.8

SEM: Standard error of the means, NDF: Neutral detergent fibre, ADF: Acid detergent fibre, BWT: Body weight, ADG: Average daily gain

Table 6.04 Effect of *Acacia karroo* leaf meal inclusion level on carcass components of male Pedi goats fed a *Setaria verticillata* grass hay-based diet

	Treatment					
Variable	$S_{80}A_{20}$	$S_{75}A_{25}$	$S_{70}A_{30}$	$S_{60}A_{40}$	$S_{50}A_{50}$	SEM
Pre-slaughter weight (kg)	22.8	24.1	21.4	23.8	24.6	1.61
Hot carcass weight (kg)	7.8	8.3	6.9	8.2	8.9	0.64
Cold carcass weight (kg)	7.4	7.8	6.1	7.1	8.0	0.57
Head (g)	1882.3	1888.9	1772.8	1706.8	1987.3	141.8
Skin (g)	2092.2	2459.9	2328.3	2399.1	2486.3	210.8
Feet (g)	635.1	640.7	636.9	636.2	667.2	34.1
Kidney (g)	71.9	76.4	60.9	65.8	66.6	5.36
Liver (g)	310.0	334.8	275.8	286.8	284.8	24.87
Lung (g)	177.5	226.9	230.3	227.7	219.9	17.78
Heart (g)	106.6	96.8	96.0	96.4	119.3	8.01
Genital scrotum (g)	177.8	215.2	165.1	179.6	143.9	27.2
Empty alimentary canal (g)	1722.8	1449.7	1432.5	1792.7	1403.3	139.7
Empty reticulo-rumen (g)	585.0	543.8	458.8	555.8	446.6	52.6
Empty intestine (g)	790.8	561.2	636.6	862.9	544.0	92.5

SEM: Standard error of the means

Table 6.05 Effect of *Acacia karroo* leaf meal inclusion level on physico-chemical properties of meat from male Pedi goats fed a *Setaria verticillata* grass hay-based diet

	Treatment					
Variable	$S_{80}A_{20}$	$S_{75}A_{25}$	$S_{70}A_{30}$	$S_{60}A_{40}$	$S_{50}A_{50}$	SEM
Muscle pH						
pH_1	6.9	6.9	6.9	6.7	6.6	0.202
pH_{24}	5.9	5.9	5.7	5.8	5.9	0.156
Meat colour 1 hr post-mortem						
L*	34.4	27.6	29.7	28.0	25.0	2.244
a*	14.8	17.4	15.3	15.9	18.1	1.555
b*	6.9	6.9	6.5	5.7	5.7	0.613
Meat colour 24 hr post-mortem						
L*	34.2^{ab}	37.3^{a}	27.2^{b}	29.2^{b}	33.6^{ab}	2.295
a*	16.4	15.6	20.1	17.6	15.1	2.071
b*	8.3	6.7	10.9	6.5	7.9	1.730
Water-holding capacity (g)						
Initial	13.6	13.2	12.9	12.1	12.1	1.993
Final	13.1	12.8	12.3	11.7	11.5	1.963
Shear force (N)	36.6	35.1	31.4	31.8	33.3	4.729
Cooking loss (%)	38.2	38.0	35.7	37.7	40.3	1.799
Dressing percentage	35.5	36.8	35.9	34.6	39.7	1.675

^{a,b,c}: Means with different superscripts in the same row are significantly different ($P<0.05$)

SEM: Standard error of the means

pH_1: Muscle pH measured 1 h post-mortem

pH_{24}: Muscle pH measured 24 h post-mortem

L*: Lightness, a*: Redness; b*: Yellowness

Table 6.06 Effect of *Acacia karroo* leaf meal inclusion level on meat sensory traits of male Pedi goats fed a *Setaria verticillata* grass hay-based diet

Sensory characteristic	Treatment					SEM
	$S_{80}A_{20}$	$S_{75}A_{25}$	$S_{70}A_{30}$	$S_{60}A_{40}$	$S_{50}A_{50}$	
Tenderness	3.18	3.22	3.83	3.09	3.41	0.345
Juiciness	3.01	3.25	3.46	2.77	3.07	0.358
Flavour	3.15	3.29	3.59	3.46	3.51	0.305
Taste	3.21	3.34	3.55	3.64	3.51	0.305
Aroma	3.16	3.20	3.55	3.49	3.35	0.286
Overall acceptability	3.26	3.49	3.84	3.63	3.40	0.322

SEM: Standard error of the means

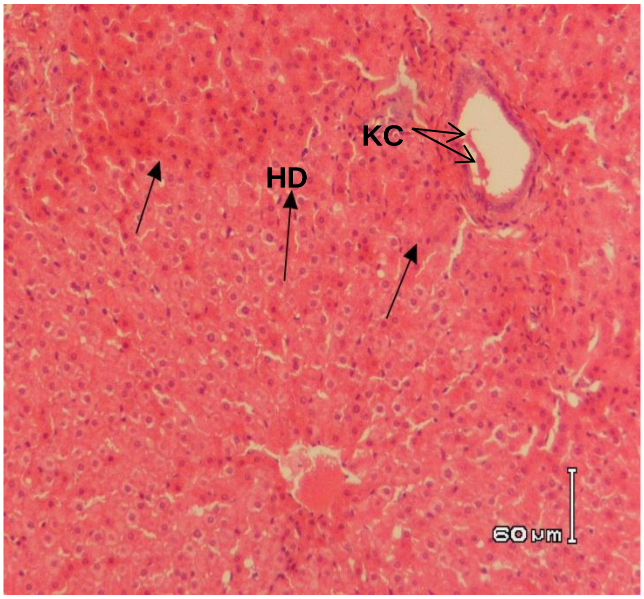


Fig 6.01 Moderate hepatocellular hydropic degeneration and proliferation of sinusoidal kupfer cells

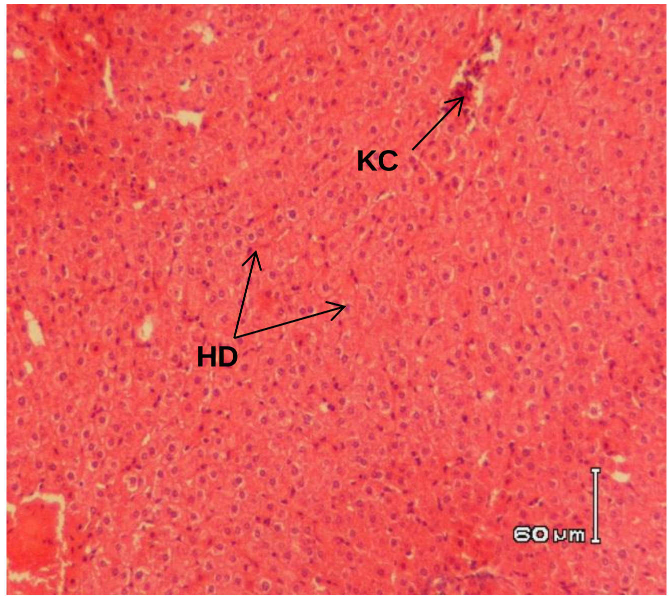


Fig 6.02 Moderate hepatocellular hydropic degeneration and proliferation of sinusoidal kupfer cells

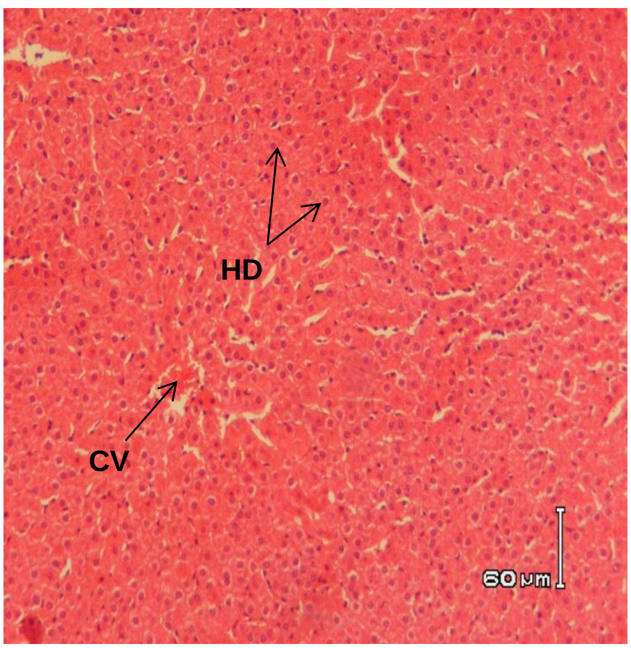


Fig 6.03 Moderate hepatocellular hydropic dilatation of central veins

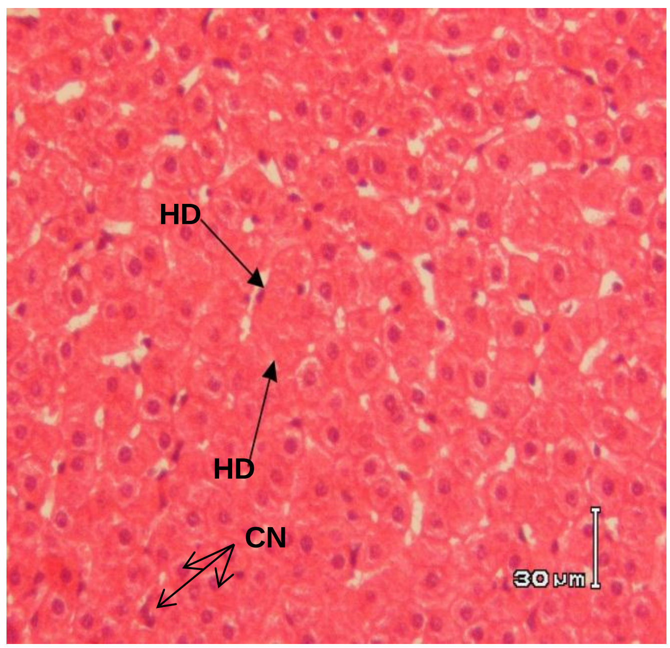


Fig 6.04 Severe hepatocyte degeneration and multifocal single cells

Effect of *Acacia karroo* leaf meal inclusion levels on liver histology of Pedi goats. Arrows point to hepatocellular hydropic degeneration (HD), kupfer cells (KC), central veins (CV) and cell necrosis (CN).

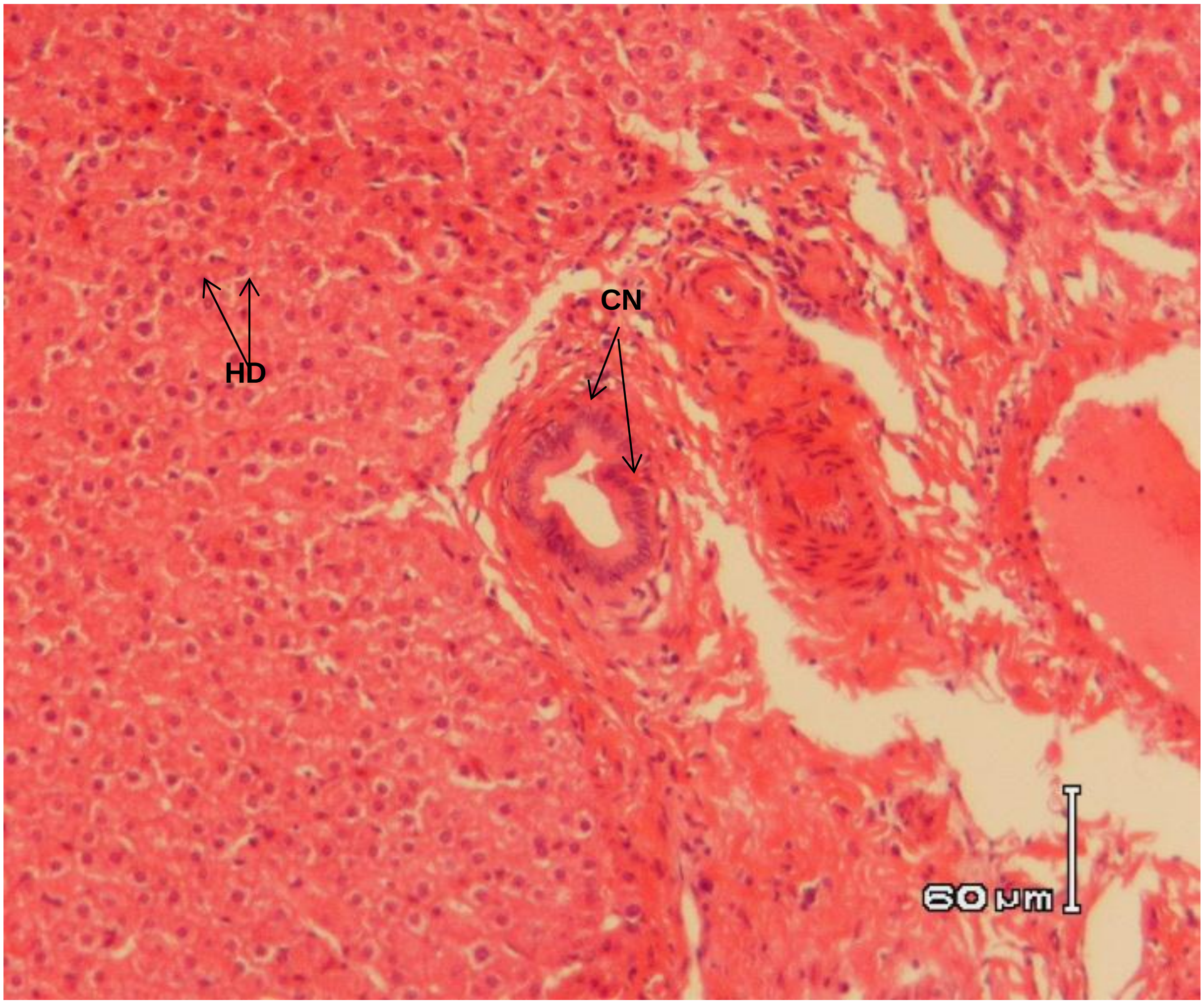


Fig 6.05 Effect of *Acacia karroo* leaf meal inclusion levels on liver histology of Pedi goats: Severe hepatocyte degeneration, multifocal single cell and clustered cell necrosis. Arrows point to hepatocellular hydropic degeneration (HD) and cell necrosis (CN).

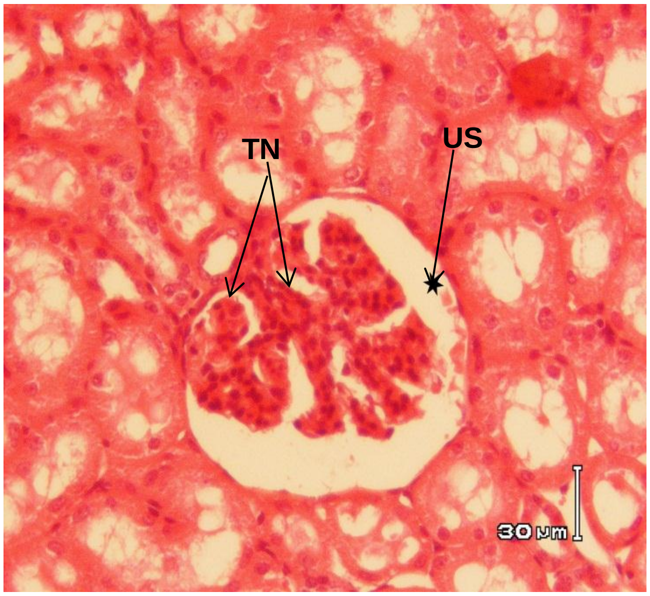


Fig 6.06 Early dilatation of glomerular uriniferous space and mild renal tubular nephrosis

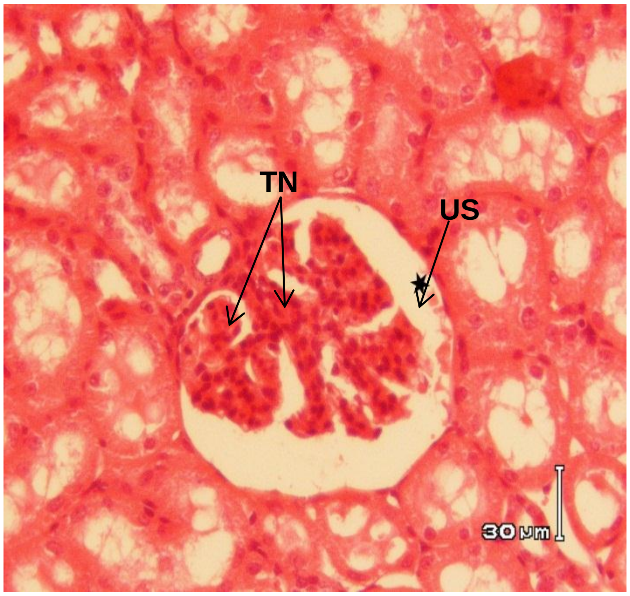


Fig. 6.07 Dilatation of glomerular uriniferious spaces and mild to moderate renal tubular nephrosis

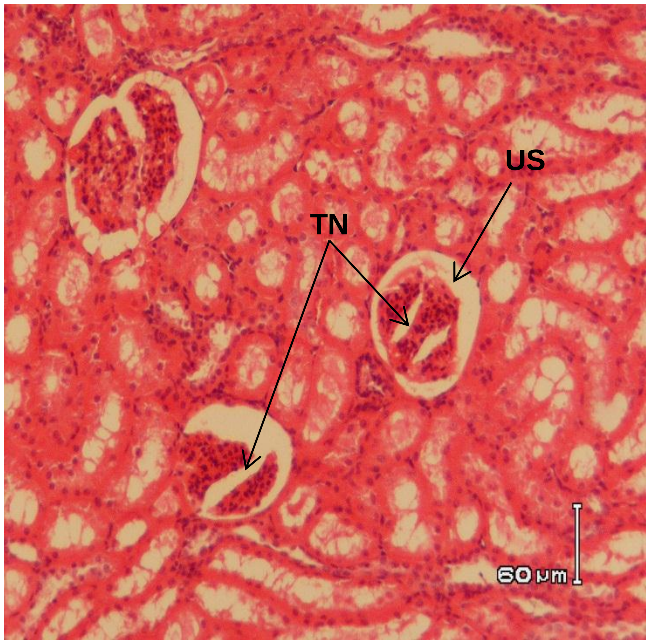


Fig 6.08 Dilatation of uriniferous spaces, moderate renal tubular nephrosis

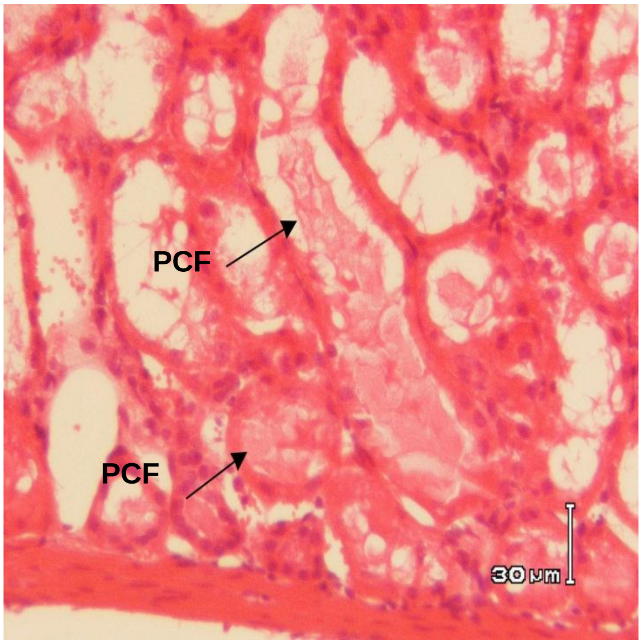


Fig 6.09 Dilatation of uriniferous spaces, intra-tubular proteinaceous fluid with protein cast formation

Effect of *Acacia karroo* leaf meal inclusion levels on kidney histology of Pedi goats. Arrows points to tubular nephrosis (TN), uriniferous space (US) and protein cast formation (PCF)

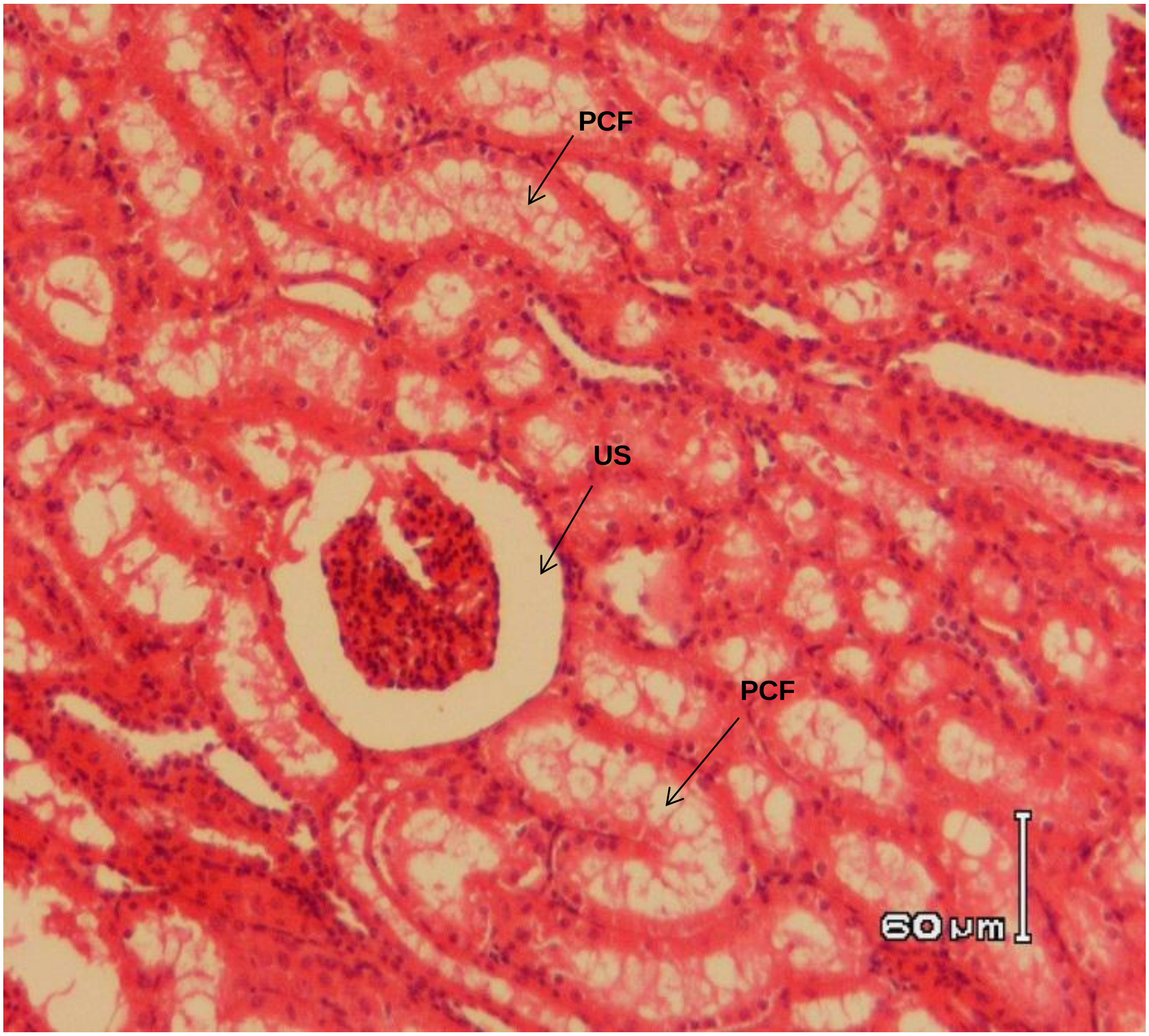


Fig 6.10 Effect of *Acacia karroo* leaf meal inclusion levels on kidney histology of Pedi goats: Dilatation of uriniferous space, intra-tubular proteinaceous fluid with protein cast formation. Arrows points to uriniferous space (US) and protein cast formation (PCF).

6.6 Discussion

Acacia karroo inclusion level did not affect diet intake by Pedi goats. Similar results were reported for kids fed tannin-rich *Ageritum conyzoides*, *Eupotarium odoratum* and *Crystellina* parasitica by Das *et al*. (2011). It has also been reported elsewhere that diets high in CT reduce intake of goats (Holechek *et al.*, 1990). However, others have reported increased intake when tannin-rich supplements are given to animals (Solaiman *et al*., 2010). Condensed tannins in forage legumes adversely affect feed intake through astringency in the oral cavity (Lamy *et al*., 2011). However, in the present study, diets with higher levels of CT were well-tolerated by goats. This might be because the goats developed mitigatory systems against tannin toxicity such as secretion of proline-rich proteins in their saliva or proliferation of microorganisms resistant to condensed tannins in their digestive tracts (Lamy *et al.,* 2011; Muir, 2011).

Increasing *Acacia karroo* leaf meal did not improve growth performance of Pedi goats in the present study. This is similar to the findings of Dludla (2010) who reported no differences in body weight gain of goats fed forages with different tannin concentrations. Wright (2015) observed similar results in Kiko-cross goats fed tannin-rich *Sericea lespedeza*, pine bark powder and combination of *Sericea lespedeza* and pine bark powder. However, Ngambu *et al*. (2013) reported improved growth performance when Xhosa lop-eared goats were supplemented with 200 g of fresh *Acacia karroo* leaves. The lack of significant differences in body weight gain in the present study could be due to the fact that all the diets met the nutrient requirements for a growing goat and hence nutrient supplies were not limiting growth (McDonald *et al.,* 2011). Thus, the growth rates were quite reasonable for the Pedi goats.

In the present study, *Acacia karroo* leaf meal inclusion level had no effect on the carcass weight and yields of Pedi goats. This is similar to the finding of Wright (2015) who reported no significant differences in carcass characteristics of Kiko-cross goats fed diets rich in condensed tannins. This finding was expected since the dietary treatments did not influence average daily weight gains of goats.

Meat pH values can be influenced by dietary treatments (Priolo *et al*., 2001). In the present study, *Acacia karroo* leaf meal inclusion level had no effect on meat pH values. This result is similar to the findings of Priolo *et al*. (2002) who reported that the consumption of tanniniferous feeds by goats did not affect chevon pH. However, Ngambu *et al*. (2013) observed significant differences in chevon pH when goats were supplemented with *Acacia karroo* leaf meal. The discrepancies in the results could be due to the amount of dietary tannins ingested by the animals in each study. The ultimate pH (pH_u) of a carcass usually varies from 5.3 to 6.8 (Mostert, 2007). Under-nutrition is a primary cause of high pH_u in meats since animals do not have the possibility to accumulate sufficient glycogen reserve in their muscles (Bray *et al*., 1989). However, the experimental diets in the present study met the nutrient requirements for a growing goat and hence differences in meat pH values were not expected. Additionally, animals stressed prior to death are more likely to have a high pH_u. This is due to depletion of muscle glycogen resulting in lower lactic acid production. The animals in this study were not stressed prior to slaughter.

The present study indicates that a diet containing 25 % *Acacia karroo* leaf meal inclusion level influenced the L* value of Pedi goat meat at 24 h post-mortem. Verna *et al*. (1989) found a similar result when lambs were fed two sorghum varieties with different tannin contents. Effect of tanniniferous feed on meat lightness have, also, been reported by other authors (Priolo *et al*., 2001; 2000). It has been hypothesized that tannins present in forages are responsible for the differences found in meat colour (Priolo *et al*., 2005). The effect of tannins on meat colour is attributed to the reduced microbial biosynthesis of vitamin B_{12} (Priolo & Vasta, 2007). An increase in intramuscular and marbling fat is another factor that contributes to meat lightness (Baublits *et al*., 2004). However, the mechanism of action of forage tannins on meat colour is not clear and merits further studies.

Dietary treatments did not have effect on water holding capacity, shear force, cooking loss and dressing percentage of chevon meat. These findings are similar to the report of Mapiye *et al*. (2010) who observed that beef from steers supplemented with *Acacia* *karroo* leaves had no effect on shear force, WHC, and cooking loss. Further research is needed to deepen the knowledge in this area.

In the present study, meat sensory attribute values were similar across the dietary treatments. Ngambu et al. (2012), however, reported significant differences in consumer sensory attributes of meat from indigenous Xhosa lop-eared goat breed supplemented with different amounts of *Acacia karroo* leaves. It is not clear how dietary tannins affect the sensory attributes of goat meat and this may require further studies.

The liver is a primary site of detoxification and is generally the major site of intensive metabolism, hence, it is prone to various disorders as a consequence of exposure to toxins (Ganong, 2005). In the present study, goats that were exposed to 20, 25 or 30 % *Acacia karroo* leaf meal inclusion levels had mild hepatocyte degeneration, dilation of central veins and proliferation of sinusoidal kupfer cells. This is similar to the findings of Karimi *et al*. (2014), who reported mild congestion in central veins and sinusoidal abnormality when Merino lambs were fed tannin-rich sorghum-based diets. Hervas *et al*. (2003c) reported moderate hydropic degeneration in the hepatocytes of sheep dosed intra-ruminally with 3.0 g quebracho tannin extracts. The results indicate that the livers of the goats on diets with *Acacia karroo* leaf meal inclusion levels of 20, 25 or 30 % were not adversely affected. However, increased hepatocyte degeneration was noticed in the liver of goats fed diets with 40 or 50 % *Acacia karroo* leaf meal inclusion levels. Lesions associated with natural oak toxicosis characterised by hepatic and renal damage have been reported in cattle (Spier *et al*., 1987). Histological changes such as shrinkage of hepatocyte, degeneration and necrosis of hepatocytes around the central veins and degeneration of some hepatic sinusoids have been reported in lambs fed high tanniniferous sorghum grain diets (Karimi *et al*., 2014). The present results showed that high *Acacia karroo* leaf meal inclusion levels of 40 or 50 % in the ration may cause damages to the liver of Pedi goats, and hence such inclusion levels are not recommended.

The kidney is also an important organ for detoxification and elimination of waste or toxic materials in the body. Exposure of the kidney to toxins may cause serious damages to this organ (Junqueira, 2003). However, the present study showed that the experimental diets did not cause any observable serious damage to the kidneys of the goats. This agrees with the findings of Silanikove *et al*. (1996) who reported no damage to the kidneys of goats fed tannin-containing leaves. On the contrary, Karimi *et al*. (2014) observed that high levels of sorghum grains in the diets induced histological changes in the kidneys of sheep. Some ruminants on tannin-rich browse tend to develop adapted rumen microorganisms that detoxify plant secondary metabolites (Alonso-Diaz *et al*., 2010). The detoxification process may, however, cause adverse effects in ruminants as a consequence of increased enzymatic demand in the liver, kidney, gut mucosa and other tissues (Van Soest, 1994).

6.7 Conclusions

Acacia karroo leaf meal inclusion level did not affect intake, daily weight gain, live weight, carcass weight and carcass components of male Pedi goats. The parameters were at moderate levels. This may be an indication that all the diets were supplying adequate nutrients for growth of the goats.

Acacia karroo leaf meal inclusion to the diets of male Pedi goats did not adversely affect meat tenderness, juiciness, flavour, taste, aroma and overall acceptability. The values for these parameters were moderate but on the higher side. This is good for goat meat consumers, and requires further studies to confirm the responses. Pedi goat meat pH, colour, water-holding capacity, shear force, cooking loss and dressing percentage were not affected by high and low *Acacia karroo* leaf meal inclusion levels. This is an indication that the diets had no adverse effects on these parameters. However, the effect of *Acacia karroo* on meat lightness was variable, depending on the inclusion level but without any recognizable pattern.

Acacia karroo leaf meal inclusion levels of 20, 25 or 30 % did not have adverse effects on livers and kidneys of Pedi goats. However, increased hepatocyte degeneration was noticed in goats fed diets with *Acacia karroo* leaf meal inclusion levels of 40 or 50 %. Thus, *Acacia karroo* leaf meal inclusion levels of 20 to 30 % are suggested. However, more detailed studies are recommended to confirm these results.

CHAPTER SEVEN

GENERAL DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

Inadequate feed resources are the major constraints of animal production in the tropics, particularly during the dry season. Fodder trees and shrubs are increasingly recognized as important components of ruminant feeding and form part of the natural diets of goats in South Africa. These trees represent an enormous potential feed resource for ruminants in South Africa and other tropical countries. *Acacia karroo,* one of these browses, is an important leguminous tree in communal rangelands of southern Africa. It is able to thrive in severe and dry conditions when other forages are fibrous and low in protein. *Acacia karroo* browse contains 100 to 250 g of crude protein/kg DM and can provide enough nutrients to goats during the dry season when food is scarce (Aganga *et al.,* 2000; Mokoboki *et al.,* 2005). The only limitation to the use of this browse tree is the presence of secondary plant metabolites, including condensed tannins, which when taken in large amounts tend to have adverse effects on productivity of the animals (Mokoboki *et al.,* 2005). Thus, the aim of this study was to evaluate the effects of dietary *Acacia karroo* leaf meal inclusion on intake, digestibility, palatability, growth, live weight, blood, kidney, liver and carcass characteristics of Pedi goats on a basal diet of *Setaria verticillata.*

Acacia karroo leaves have high protein contents, ranging from 106.5 to 146.5 g/kg. The CP contents of all the experimental diets were above the minimum level of 8 % required for optimal microbial function in the rumen (Norton, 2003; Annison & Bryden, 1998), suggesting that all the diets could support maintenance requirements and some production levels in ruminant animals (Van Soest, 1994). Thus, *Acacia karroo* leaves have a potential of being a protein feed for ruminant animals. However, diets having high *A. karroo* inclusion rates contained high amounts of condensed tannins. Condensed tannin contents of the experimental diets varied from 4.1 g/kg DM for the diet containing a 20 % *Acacia karroo* inclusion level to 10.2 g/kg DM for the diet containing 50 % *Acacia karroo*. Thus, all the diets had condensed tannin values below 50 g/kg DM, the level that could be considered detrimental to the animal (Mokoboki *et al.*, 2005; Frutos *et al.,* 2004; Dube *et al*., 2001).

The objective of the palatability study was to determine the effects of dietary level of *Acacia karroo* leaf meal on voluntary intake, palatability and preference rankings of diets fed to Pedi goats. Diets with higher *A. karroo* levels of inclusion had higher intakes and relative palatability rankings by Pedi goats, regardless of the higher condensed tannin and total phenolic contents. Results of this study indicated that tannin-rich *A. karroo* leaves when fed in a mixed diet can influence preference and intake by Pedi goats. Palatability indices were positively and significantly predicted from dry matter intake of goats. Similarly, intake and palatability indices of the diets related positively with the nutrient contents. Palatability studies could be used in designing supplemental feeding programs for goats during the dry season when feed is scarce.

Inclusion of *A. karroo* leaves improved nutrient digestibility and growth rate but not intake of indigenous Pedi goats. Dry matter, crude protein and neutral detergent fibre digestibilities were optimized at different *A. karroo* leaf meal inclusion levels of 69.4, 48.3 and 42.7 %, respectively, possibly indicating that *A. karroo* inclusion levels for optimal productivity will depend on the particular parameter of interest. *Acacia karroo* leaf meal, therefore, has the potential of being utilized as a protein feed during the dry season when goats depend on low quality roughages. However, higher *A. karroo* inclusion levels, like 50 %, resulted in higher dietary tannin contents which exerted negative effects on serum total proteins. This led to the next study which determined the effects of supplementing the diets with PEG 4000, a product that reduces or neutralizes the adverse effects of tannins, on productivity of Pedi goats.

Acacia karroo leaf meal contained relatively high amounts of condensed tannins, which can have adverse effects on diet intake and digestion in goats. Thus, a study was conducted to determine the effect of PEG 4000 supplementation on intake, digestibility and productivity of Pedi goats. Supplementation with 23 g of PEG 4000/goat/day improved DM, OM, NDF and ADF intakes of Pedi goats fed a dietary mixture of 20 % *Acacia karroo* leaf meal and 80 % *Setaria verticillata* grass hay. A supplementation level of about 20 g of PEG 4000 per goat per day optimized DM, OM, NDF and ADF intakes. This level of supplementation is recommended if intake is the parameter of interest. Supplementation with either 23 or 30 g of PEG 4000 improved DM, OM, CP and NDF digestibility by goats fed a dietary mixture of 20 % *Acacia karroo* leaf meal and 80 % *Setaria verticillata* grass hay. Crude protein digestibility was optimized at a PEG 4000 supplementation level of 15.78 g per goat per day. This supplementation level for optimal CP digestibility is lower than the 20 % for intake. Thus, the supplementation level for optimal productivity depended on the production parameter of interest. Supplementation with either 23 or 30 g of PEG 4000 per goat per day did not improve the performance of the animals when fed a dietary mixture of 50 % *Acacia karroo* leaf meal and 50 % *Setaria verticillata* grass hay. It is possible that PEG 4000 was not enough to have effect on the performance of the goats. Similarly, it might have been that the high amounts of condensed tannins may have exerted adverse effects on the performance of the goats. All this requires further studies.

In Chapter 6, a study was conducted to determine the effect of *A. karroo* leaf meal inclusion on carcass characteristics and histological parameters of Pedi goats. *Acacia karroo* leaf meal inclusion levels of 20, 25, 30, 40 or 50 % did not affect intake, daily weight gain, live weight and carcass components of Pedi goats. However, all the measured parameters were at moderate levels, possibly indicating that all the diets were supplying adequate nutrients for growth of the goats.

Acacia karroo inclusion, including low and high levels, did not adversely affect meat tenderness, juiciness, flavour, taste, aroma and overall acceptability. All the measured values for these parameters were of moderate to high levels, possibly indicating good acceptability of meat from goats raised on diets containing *A. karroo* leaf meal. However, higher *A. karroo* leaf meal inclusion levels of 40 or 50 % caused increased hepatocyte degeneration in Pedi goats. Thus, *A. karroo* leaf meal inclusion levels of above 30 % are not recommended because they may adversely affect livers and kidneys of the goats. However, further studies are recommended to explore biological reasons for these adverse effects.

It is concluded that indigenous Pedi goat optimal productivity responses to dietary *Acacia karroo* leaf meal inclusion were variable, depending on the production parameter in question. This has practical management implications when formulating feeding strategies aimed at improving productivity of Pedi goats.

CHAPTER EIGHT

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APPENDIX A

David Brown, Jones W. Ng'ambi and David Norris 2016. Voluntary intake and palatability indices of Pedi goats fed different levels of *Acacia karroo* leaf meal by cafeteria method. *Indian Journal of Animal Research* 50(1): 41-47.

APPENDIX B

D. Brown, J.W Ng'ambi and D. Norris 2015. Feed potential of *Acacia karroo* leaf meal for communal goat production in Southern Africa: A review. *The Journal of Animal and Plant Sciences.*

APPENDIX C

David Brown, Jones W. Ng'ambi and David Norris 2016. Effect of tanniniferous *Acacia karroo* leaf meal inclusion on feed intake, digestibility and live weight gain of Pedi goats fed a *Setaria verticillata* grass hay-based diet. *Journal of Applied Animal Research.*

APPENDIX D

D. Brown, J.W Ng'ambi, D. Norris and E.F Mbajiorgu 2015. Influence of tanniniferous *Acacia karroo* leaf meal feeding on blood profiles of indigenous Pedi goats. *South African Journal of Animal Science*.