

CHAPTER 1
INTRODUCTION

1.1 Background

The broiler chicken industry is an important source of animal protein in Limpopo province in comparison with cattle and pigs (Boer *et al.*, 2001). Small poultry holdings provide supplementary food, income and employment, and contribute to poverty alleviation in South Africa (Sonaiya, 1999).

Excess body fat deposition in broiler chickens is now of concern to both producers and consumers. The latter consideration is important because results of many human studies have related high dietary fat intake to the incidence of cardiovascular diseases and cancer (Lichtenstein, 1999). Due to increasing public demand for low fat and low cholesterol products, interest in manipulating the lipid composition of poultry meat via dietary means has become important (Hargis & Elswyk, 1993; Sacks, 2002).

1.2 Motivation

Rural poultry production can play an important role in poverty alleviation and in the supply of quality protein to rural people. Approximately 20 % of protein consumed in developing countries originates from chicken meat (Pedersen, 1998). Over the last century, the amount and proportion of animal fat in human diets have increased in many societies. These increases have been associated with the occurrence of cardiovascular diseases (Lichtenstein, 1999; Katan, 2000). In many societies, coronary heart diseases and arteriosclerosis are related to the dietary intake of cholesterol and saturated fatty acids, and are among the most important causes of human mortalities (Sacks, 2002).

It is widely acknowledged that there is a need for low intakes of cholesterol and saturated fats (Evans *et al.*, 2002). The control of lipid deposition in broiler chickens aimed at efficient lean poultry meat production is of current interest (Fisher & McNab, 1997). Tannins have been associated with reduced carcass fat content in grazing lambs, relative to lambs grazing white clover only (Purchase & Keogh, 1984). However, the effects of tannins on broiler chicken fat content are not known. Therefore, the present study was aimed at determining whether ingestion of tannins at finisher stage would reduce fat content in broiler chickens.

1.3 Aim and objectives

1.3.1 Aim

The aim of this study was to determine the effect of *Acacia karroo* leaf meal level of supplementation on fat deposition in broiler chickens.

1.3.2 Objectives

The objectives of this study were to:

1. Determine the effect of level of tanniferous *Acacia karroo* leaf meal supplementation at finisher stage on feed intake, digestibility, live weight, feed conversion ratio, mortality and carcass characteristics of male and female Ross 308 broiler chickens.
2. Determine the effect of dietary energy level at finisher stage on feed intake, digestibility, live weight, feed conversion ratio, mortality and carcass characteristics of male and female Ross 308 broiler chickens.

3. Determine interactive effects of level of tanniniferous *Acacia karroo* leaf meal supplementation and dietary energy level at finisher stage on feed intake, digestibility, live weight, feed conversion ratio, mortality and carcass characteristics of male and female Ross 308 broiler chickens.
4. Determine the relationships between *Acacia karroo* leaf meal level of supplementation and short-term biological responses of male and female Ross 308 broiler chickens.

1.4 Hypotheses

Hypotheses of the study were:

1. Tanniniferous *Acacia karroo* leaf meal level of supplementation at finisher stage has no effect on feed intake, digestibility, live weight, feed conversion ratio and mortality but affects carcass characteristics of male and female Ross 308 broiler chickens.
2. Dietary energy level at finisher stage has no effect on feed intake, digestibility, live weight, feed conversion ratio, mortality but affects carcass characteristics of male and female Ross 308 broiler chickens.
3. Tanniniferous *Acacia karroo* leaf meal level of supplementation and dietary energy level at finisher stage have no interactive effect on feed intake, digestibility, live weight, feed conversion ratio and mortality but affect carcass characteristics of male and female Ross 308 broiler chickens.
4. There are no relationships between *Acacia karroo* leaf meal level of supplementation and short-term biological responses of male and female Ross 308 broiler chickens.

CHAPTER 2
LITERATURE REVIEW

2.1 Introduction

In broiler chickens, extensive genetic selection towards a fast-growing chicken has led not only to a dramatic shortening of the growing period, but also to excessive carcass fatness, which consequently lowers meat yield and feed efficiency. In addition, fat deposition has become a serious threat in the breeder flocks since obesity also leads to infertility (Friedman-Einat *et al.*, 2003). Excessive fatness is one of the undesirable consequences of selection for increased growth of modern broiler chickens. Accumulation of fat in carcasses of broiler chickens represents waste product to consumers who are increasingly concerned about the nutritional and health aspects of their food (Mahmoud & Mihaly, 1998). Fat amount, fat quality and cholesterol content in food are important consideration when the relationships between fat and the risk of some cardiovascular diseases and cancer are evaluated (Ahn *et al.*, 1995; Cherian & Wolfe., 1996).

2.2 Tannins

Tannins are water-soluble phenolic metabolites of plants with a molecular weight of >500 and with the ability to precipitate gelatine and other proteins from aqueous solution (Mehansho *et al.*, 1987). Tannins are a very complex group of plant secondary metabolites, which are soluble in polar solution and are distinguished from other polyphenolic compounds by their ability to precipitate proteins (Silanikove *et al.*, 2001). Tannins are found in approximately 80% of woody and 15% of herbaceous dicotyledon species. They can occur at high levels in some forages and feeds (Bryant *et al.*, 1992).

Tannins, because of their protein-binding properties, are known to be strongly astringent. This astringency appears to be the major cause of reduced food intake in mammalian herbivores. There is some controversy, however, over whether reduced food intake is a result of the toxic nature of tannins. Singleton (1981) considers it unfair to consider the effects of tannins on feed intake as toxicity, since the result is due to a failure to consume, rather than to consumption itself. On the other hand, Provenza *et al.* (1991) suggested that mammals might reject tannin-containing plants because they cause internal malaise.

Severe growth depression can be a consequence of reduced feed intake, and has been shown to occur in rats and chicks when fed with tannin-containing diets (Alledredge, 1994). When tannins complex with protein in an animal's gut, they are believed to be responsible not only for growth depression, but also for low protein digestibility and increased faecal nitrogen concentrations. Thus, once they have been consumed, their adverse effects, again, seem to be related to their binding of dietary protein (Alldredge, 1994). There is evidence to suggest that enzymatic proteins, as well as other endogenous proteins, comprise a considerable portion of excreted nitrogen when animals are fed tannins (Alledredge, 1994). When endogenous proteins are lost in this manner, the animal may incur a deficiency in one or more essential amino acids. McKersie and Brown (1997) reported that the most widely recognized property of condensed tannins is their capacity to strongly and selectively bind to proteins and other macromolecules, such as cell wall carbohydrates and starch, due to their high level of phenolic hydroxyl groups.

During breakdown of foliage, such as chewing by animals, condensed tannins react with and precipitate plant proteins by hydrogen bonding to form complexes that are stable and insoluble in the rumen but unstable and release protein in the small intestines (McKersie and Brown, 1997).

2.2.1 Types of tannins

There are two types of soluble tannins present in a large number of plant species. These are the hydrolysable tannins (HTs) and the non-hydrolysable or condensed tannins (CTs). Hydrolysable tannins are characterized by a central carbohydrate core with a number of phenolic carboxylic acids bound by ester linkages. Condensed tannins have no carbohydrate core, but rather they are derived from the condensation of flavonoid precursors without participation of enzymes. Condensed tannins are more widely distributed in higher plant species than the hydrolysable variety and are thought to be more active in precipitating proteins. Condensed tannins vary from different multipurpose trees (Table 2.1) and the variation may be due to differences in the age of leaves and plants at harvest (Lege *et al.*, 1992, Wolfson *et al.*, 1993).

Table 2.1. Proanthocyanidins (CT) from acacia trees

Authors	Condensed tannins	Type of legume species
Balogun <i>et al.</i> (1998)	4.27 (% DM)	<i>Acacia currasavica</i>
Maasdorp <i>et al.</i> (1999)	11.35 (Au _{550nm} / g sample)	<i>Acacia boliviana</i>
Dube <i>et al.</i> (2001)	2.01 (A ₅₅₀ , g sample) 0.19 (A ₅₅₀ , g sample) 1.47 (A ₅₅₀ , g sample) 0.04 (A ₅₅₀ , g sample) 1.36 (A ₅₅₀ , g sample) 1.36 (A ₅₅₀ , g sample)	<i>Acacia karoo</i> <i>Acacia nilotica</i> <i>Acacia tortilis</i> <i>Acacia senegal</i> <i>Acacia erioloba</i> <i>Acacia albida</i>
Mashamaite (2004)	0.37 (% DM) 4.07 (% DM) 4.52 (% DM)	<i>Acacia nilotica</i> <i>Acacia tortilis</i> <i>Acacia karroo</i>

2.2.1.1 Condensed tannins

Condensed tannins are naturally occurring compounds found in a number of different plants, including some pasture species (Idso & Idso, 2002). Condensed tannins comprise a group of polyhydroxyflavan-3 oligomers and polymers linked by carbon-carbon bonds between flavanol subunits. The reactivity of proanthocyanidins with molecules of biological significance has important nutritional and physiological consequences. Their multiple phenolic hydroxyl groups lead to the formation of complexes with proteins (Hagerman *et al.*, 1998; Harbone, 1998). Proanthocyanidins in feeds for ruminants may interfere with intake and digestion of the feed in which they occur (Dube *et al.*, 2001). They are having a negative effect on protein metabolism and

decrease palatability of feeds at high levels (Barry & Manley, 1986) but at very low levels most are beneficial (Foo *et al.*, 1996). At moderate concentrations, however, condensed tannins can be beneficial to ruminant livestock production. Among some of the beneficial effects, condensed tannins complex with soluble proteins in the rumen and permit subsequent absorption of amino acids in the lower digestive tract (Barry & Manley, 1986), thereby facilitating ruminal escape protein utilization (Waghorn *et al.*, 1999). Condensed tannins can benefit ruminants by reducing protein losses to degradation in the rumen and improving the flow of protein to the small intestines, and some field trials have indicated an improved performance attributable to the condensed tannins in diets fed to sheep (Douglas *et al.*, 1995).

In Lotus species, condensed phenolic contents up to 25 g/kg DM appear to have little effect on rumen carbohydrate digestion but concentrations between 25 and 100 g/kg DM reduce carbohydrate digestion in the rumen in a dose dependent manner (Barry & Manley, 1986). Barry *et al.* (1986) observed increased levels of growth hormone and their results suggested an increase in the ratio of lipolysis to lipogenesis. The presence of condensed tannin has been associated with reduced carcass fat content in lambs grazing *L. pedunculatus* (Purchas & Keogh, 1984) and *H. coronarium* (Terrill *et al.*, 1992). However, in lambs grazing *L. corniculatus*, Wang *et al.* (1996) found no difference in carcass fatness. A possible explanation for this reduction of fatness has been suggested by Barry *et al.* (1986) who found a lower level of growth hormone (GH) in lambs when diets were sprayed with Polyethylene glycol. Growth hormone increase N retention and reduce fat deposition, with an increase in fat turn over. The reason for the higher level in

plasma GH has been explained with a possible inactivation of gut wall proteins by CT. However, Waghorn *et al.* (1994) did not find any difference in the GH titre in lambs fed *L.pedunculatus* with or without PEG. In trials conducted to compare two sorghum strains with different content of CT, lambs fed with the strain containing the higher level of CT showed a meat lighter in colour (Priolo & Salem, 2002).

2.2.2 Hydrolysable tannins

Hydrolysable tannins are esters of a sugar usually glucose and a phenolic acid such as gallic acid in gallotannins. They are known to be toxic to ruminants (Dollahite *et al.*, 1962; Shi, 1988). Because of their toxicity, they have received limited attention in animal nutrition studies. Recent studies show that even where they are not toxic, hydrolysable tannins may have significant effect on animal nutrition because they have inhibitory effect on various enzymes (Yoshida *et al.*, 2000). The amount and type of tannins synthesized by plants vary considerably depending on plant species, stage of development and environmental condition.

2.3 Mode of action of tannins in livestock

Tannins form soluble and insoluble and sometimes irreversible complexes with proteins, digestive enzymes and possibly starch in the digestive tract of pigs and poultry. Sorghum tannins may bind and precipitate at least 12 times their own weight of protein (Jansman, 1993). Formation of these complexes increases with molecular size of the tannins and inhibit enzymatic breakdown of protein and can increase endogenous amino acid loss. Results of *in vitro* enzyme assays with tannins do not necessarily mimic reactions in the digestive tract because of the special conditions in *in vivo* digestion (Butler, 1992).

Tannins can increase the size of the parotid glands and damage the mucosal lining of the gastro intestinal tract of chickens, but to a lesser extent in the laboratory rat (Ortiz *et al.*, 1994) and with much less evidence for pigs. Differences between pigs and poultry in their tolerance for foodstuffs rich in tannins may be due to the very few taste buds (twenty four) in the mouth of chickens compared to the high number (15 000) in the mouth of pigs (Moran, 1982).

Tannins are also found in many poultry foodstuffs such as sorghum, millet, barley and faba beans. Adverse effects of tannins on food palatability and consumption, feed efficiency, growth rate and digestibility of components such as proteins, carbohydrates, lipids and minerals have been repeatedly reported (Laurena *et al.*, 1984; Longstaff & McNab, 1991a; Makkar, 2003; Hassan *et al.*, 2003; Kim & Miller, 2005). There are also a number of reports on *in vitro* and/or *in vivo* effects of tannins on digestive enzymes (Van Der Poel *et al.*, 1992; Lizardo *et al.*, 1995; Helsper *et al.*, 1996). Although there are some indications on the influence of tannins on the increase in excretion of salivary and intestinal muco-proteins, bile acids, and hypersecretion of enzymes and endogenous loss of minerals in animals and poultry, report on the possible effect of these anti-nutrients on the endogenous losses of amino acids in poultry are uncommon (Horigome *et al.*, 1988; Karasov *et al.*, 1992; Mansoori & Acamovic, 1996, 1998 and 2000). Longstaff & McNab (1991b) reported that, chicks fed with diets containing faba beans hulls, that are high in condensed tannins had a poor apparent digestibility of amino acids, particularly

methionine and cystine, probably because of an increased excretion of inactivated enzymes and glycoproteins of the gastrointestinal mucosa. Studies on the effects of condensed tannins have given equivocal results. Flores *et al.* (1994) concluded that there was a negative effect of tannins on starch digestibility in three week old chickens. The extent of the depression depended on the quantity of tannins ingested. With young pigs there was no difference in starch digestibility on diets with faba beans of high and low condensed tannin contents.

Maize is the most commonly used grain source for monogastric animals in many countries due to its known nutritional value and stable composition. However, low tannin sorghums have the feeding values for monogastric animals similar to those of maize (Brand *et al.*, 1992; Douglas *et al.*, 1993). Leeson & Summers (1991) stated that low tannin feeds offer an excellent alternative in diets for the production of non-pigmented poultry products. Feeds high in tannins also have a potential to greatly reduce the speed of meat spoilage (Carpenter *et al.*, 2007; Vista *et al.*, 2007). The production of bird proof (high tannin) sorghum, however, poses nutritional problems when the grain is subsequently incorporated into monogastric feeds (NRC, 1994). Growth studies performed with pigs (sorghum was compared with maize as an alternative grain source) showed that pigs being fed with low tannin sorghum could perform as well as pigs being fed maize (Kemmer *et al.*, 1984; Brand *et al.*, 1992). Diao *et al.* (1990) indicated that broiler chicks can tolerate up to 0.48 % sorghum tannin in the diets and in the later four weeks could tolerate up to 0.64 % sorghum tannin without any adverse effect on weight

gain, feed efficiency, dressing percentage, total serum lipid, cholesterol and glutamatepyruvate transaminase levels.

Feeding a sorghum diet to chicks could reduce the yellow pigment on their beaks and legs. It has been demonstrated that when tannins or tannin containing materials are administered orally to chickens, endogenous losses are increased substantially, presumably a result of interaction between the tannin and epithelial tissue within the gastrointestinal tract (GIT) and also the microflora within the GIT (Muhammed *et al.*, 1994; Mansoori & Acamovic, 1998; Bento *et al.*, 2005). This interaction is likely to account, at least in part, for the invariable reduction in apparent digestibility coefficients of nitrogen and amino acids, and metabolisable energy found in animals that consume tannins. It is widely accepted that low tannin sorghums can be used in broiler chicken diets without any adverse effects on performance of the birds (Lucbert & Castaing, 1986). These authors stated that the nutritional value of sorghum with tannin content of lower than 10 g/kg was similar to that of maize. Pour-Reza & Edriss (1997) confirmed the results. These showed that all the dietary maize could be replaced by low tannin sorghum as indicated in Table 2.2

Table 2.2 Performance of chicks fed on diets with low or high tannin varieties at different inclusion levels (Pour-Reza and Edriss, 1997).

Variables	Dietary treatment				
	Maize	50/50 maize/ sorghum (low tannin)	Sorghum (low tannin)	50/50 maize and sorghum (high tannin)	Sorghum (high tannin)
Tannin content	0	1.16	2.32	2.61	5.22
Tannin intake (g)	0	6.9	13.5	19.5	29.3
Live weight gain (g)	1968	1988	1913	1973	1866
Feed intake (g) (7-49 days)	3713	3983	3918	3767	3792
Feed conversion ratio (g/g)	1.89	2.0	2.04	1.96	2.04

Armstrong *et al.* (1974) reported that the presence of tannins in some cultivars of sorghum has been associated with depression of growth rate, feed intake, metabolisable energy, protein digestibility and also with leg abnormalities in chicks. Even though tannins are known as anti-nutritional factors, in some instances they tend to be useful because they can also act as anti-microbial factors. They have been shown to increase nitrogen utilization in sheep and to have antihelminthic effects in sheep (Waghorn *et al.*, 1994a). Tannins added in ruminant food lower body mass (Buchsbaum *et al.*, 1984), reduce protein availability (Robbins *et al.*, 1987) and increase excreta nitrogen concentration (Bernays & Butler, 1989).

The leaves and stems of *Lotus pedunculatus* contain condensed tannins, which on disintegration of the plant material, such as during chewing, render the forage proteins insoluble (Ross & Jones, 1974). The presence of condensed tannins therefore makes lotus a non-bloating legume (Ross & Jones, 1974) and at 15 g/kg DM increases duodenal protein flow by reducing plant-protein degradation in the rumen (John & Lancashire, 1982). Condensed tannins seem to have different effects on wool growth depending on the concentration. Wool growth has a direct correlation with protein utilization. At low concentrations, condensed tannins seem to increase wool growth (Terrill *et al.*, 1992; Douglas *et al.*, 1995). Barry (1985) found that oral polyethylene glycol (PEG) administration in sheep fed *Lotus pedunculatus* tended to increase wool growth. This was due to higher level and activity of tannins of *L. pedunculatus*. On addition of PEG, the adverse effects of these tannins were alleviated, leading to the increased voluntary feed intake and to a possible increase of growth hormone (GH) titre. In ewes rearing twin lambs, Wang *et al.* (1996) found that CT from *L. corniculatus* increased milk yield, protein and lactose percentage, reducing fat percentage. An experiment designed to evaluate the specific effect of carob pulp CT on lamb growth and meat quality, showed that when the effects of CT from carob pulp are eliminated by PEG supply, Comisana lamb longissimus muscle was significantly darker.

2.4 Dietary energy levels on performance and carcass composition of broiler

chickens

Energy in broiler chickens is needed for maintenance and growth of body tissues, vital metabolic activities and maintenance of normal body temperature (Scott *et al.*, 1982).

Broiler chickens eat primarily to satisfy their energy requirements (Scott *et al.*, 1982; Reddy, 2000). Therefore, diets with higher energy concentration will have lower intake and those with lower energy concentration will have higher feed intake (Macleod, 1991; Leeson, 1996). Yolsin *et al.* (1990) and Holsheimer & Veerkamp (1992) reported that high energy diets significantly increased absolute carcass weight and yield of abdominal fat, however, carcass part weights were not influenced by dietary energy. Also, relative abdominal fat weight increased linearly with increments in dietary energy. Summers *et al.* (1992) found that increasing dietary energy from 11.02 to 12.75 MJ ME/kg DM diet resulted in male broiler chickens having a significantly higher percentage of fat and a lower percentage of protein. In addition, Waldroup *et al.* (1990) found that even male chickens fed high energy diet series had significantly higher dressing percentages than females fed the low energy diet series.

Since carcass fat deposition can be altered through modifying the energy intake of the broiler chickens (Summers & Leeson, 1984; Leeson *et al.* 1996) it seems reasonable that some positive effects may be obtained by reducing the energy level in broiler diets fed during the growing and finishing periods when the birds consume the major portion of their overall feed consumption.

2.5 Summary

Excessive fatness is one of the undesirable consequences of selection for increased growth of modern broiler chickens. In addition to optimizing growth rate and feed utilization, there is ongoing demand to maximize growth of lean tissue and minimize the undesirable fat accumulation in broiler chickens at marketing age. Tanniniferous feeds are widely used in animal feeding and there is some indication that they can reduce fat content in animals. However, no such studies have been done in chickens.

CHAPTER 3
MATERIALS AND METHODS

3.1 Study area

This experiment was conducted at the University of Limpopo Experimental farm, Limpopo Province, South Africa. The farm is located 10 km northwest of the Turfloop Campus. The ambient temperatures around this area are above 32 °C during summer and around 25 °C or lower during the winter season. Average annual rainfall is between 446.8 and 468.4 mm. This study was conducted between October and December, 2006.

3.2 The grower feeds

The experimental diets were purchased from Zet_B Feeds, Louis Trichardt. The company had been asked to formulate two grower diets, a low energy diet (13.2 MJ ME /kg DM) and a high energy diet (13.8 MJ ME /kg DM). Both diets were formulated to contain 190 g CP per kg DM.

3.3 Foliage material

Acacia karroo leaves were used as a supplement in the experiment. Leaves were hand-harvested early each morning for one week at the University of Limpopo main campus in May, 2006. The leaves were then shade dried and stored indoors for 14 days prior to grinding. The dried leaves were ground, using a 2 mm screen, and stored in air-tight bags until needed for feeding.

3.4 Experimental procedure, dietary treatments and designs

Three hundred and sixty, 21-day old Ross 308 broiler chickens were used in the experiment. The chickens had been on a commercial starter feed (NTK, Polokwane) before commencement of the experiment. The chickens were assigned to twelve dietary treatments, each with three replications and each replication having ten birds. Thus, a total of 36 pens were used. The birds were offered *ad libitum* feed and fresh water throughout the experiment. A 2 (Sex) x 2 (Energy levels) x 3 (Tanniferous acacia leaf meal levels) factorial arrangement in a complete randomized design (SAS, 1998) was used. The experimental treatments were as follows:

S₁E₁T₀: Male broiler chickens fed low energy (13.2 MJ ME /kg DM) diet without tanniferous *Acacia karroo* leaf meal supplementation.

S₁E₁T₁: Male broiler chickens fed a low energy (13.2 MJ ME /kg DM) diet supplemented with 9 g tanniferous *Acacia karroo* leaf meal per kg diet.

S₁E₁T₂: Male broiler chickens fed a low energy (13.2 MJ ME /kg DM) diet supplemented with 12 g tanniferous *Acacia karroo* leaf meal per kg diet.

S₁E₂T₀: Male broiler chickens fed a high energy (13.8 MJ ME /kg DM) diet without tanniferous *Acacia karroo* leaf meal supplementation.

S₁E₂T₁: Male broiler chickens fed a high energy (13.8 MJ ME /kg DM) diet supplemented with 9 g tanniferous *Acacia karroo* leaf meal per kg diet.

S₁E₂T₂: Male broiler chickens fed a high energy (13.8 MJ ME /kg DM) diet supplemented with 12 g tanniferous *Acacia karroo* leaf meal per kg diet.

S₂E₁T₀: Female broiler chickens fed a low energy (13.2 MJ ME /kg DM) diet without tanniferous *Acacia karroo* leaf meal supplementation.

S₂E₁T₁: Female broiler chickens fed a low energy (13.2 MJ ME /kg DM) diet

supplemented with 12 g tanniferous *Acacia karroo* leaf meal per kg diet.

S₂E₁T₂: Female broiler chickens fed a low energy (13.2 MJ ME /kg DM) diet

supplemented with 12 g tanniferous *Acacia karroo* leaf meal per kg diet.

S₂E₂T₀: Female broiler chickens fed a high energy (13.8 MJ ME /kg DM) diet without

tanniferous *Acacia karroo* leaf meal supplementation.

S₂E₂T₁: Female broiler chickens fed a high energy (13.8 MJ ME /kg DM) diet

supplemented with 9 g tanniferous *Acacia karroo* leaf meal per kg diet.

S₂E₂T₂: Female broiler chickens fed a high energy (13.8 MJ ME /kg DM) diet

supplemented with 9 g tanniferous *Acacia karroo* leaf meal per kg diet.

3.5 Data collection

The mean feed intake was measured daily by subtracting the weight of feed refusals from the feed offered per day, and the difference was divided by the total number of birds in each pen. Initial live weights of the chickens were measured at the start of the experiment. Mean live weight of the chickens was taken daily by weighing birds in each pen and the total weight was then divided by the total number of birds in the pen. These live weights were used to calculate growth rate. Feed conversion ratio was calculated as the total amount of feed consumed divided by the weight gain of live birds.

Digestibility measurements were carried out when the birds were between 35 and 42 days of age. This was done in specially designed metabolic cages fitted with excreta collection trays, separate watering and feeding troughs. Two birds were randomly selected from each replicate and transferred to metabolic cages. Birds were allowed to adapt for a period of three days in their crates prior to collection of excreta and feed refusals. After that period excreta was collected from each cage and stored at -15 °C during the collection period. Apparent digestibility (AD) of nutrients was calculated as follows:

$$AD (\%) = \frac{(\text{Amount of nutrient ingested} - \text{amount of nutrient excreted}) \times 100}{(\text{Amount of nutrient ingested})}$$

At 42 days old, the remaining birds per pen were weighed on an electronic weighing scale and then slaughtered. After slaughtering, carcass weight of an individual bird was measured. Dressing percentage was determined. Dressing percentage was equal to carcass weight divided by live weight and then multiplied by one hundred. The dressed

carcasses were further cut into parts to obtain the weights of fat pad, breast and thigh. Breast meat samples from each slaughtered bird were taken and stored for further analysis of dry matter and nitrogen.

3.6 Chemical analyses

3.6.1 Determination of dry matter (AOAC, 1990)

A clean crucible was placed in the oven set at 105 °C for 30 minutes. The crucible was then removed using metal tongs and allowed to cool for 20 minutes. It was then weighed to four decimal places [W_0]. Two grams of meat sample, feed refusals, feed or faeces were weighed into crucible [W_1], and crucible plus the sample were placed in the oven set at 105 °C for overnight. The crucible plus its contents were removed from the oven and allowed to cool to room temperature. After cooling, crucible plus dry sample [W_2] were then weighed. The dry matter content was then calculated using the formula:

$$\text{DM (\%)} = \{(W_2 - W_0)\} / \{(W_1 - W_0)\} \times 100$$

3.6.2. Determination of nitrogen and crude protein (AOAC, 1990)

Two grams of ground meat samples, feed refusals, faeces or feed were weighed on tarred ashless filter papers. The filter papers were then folded and placed in digestion tubes. Twenty-five milliliters of concentrated sulphuric acid and two tablets of a catalyst were added and tubes were then placed in the digestion unit. Water tubing was connected and tap water connected to the scrubber was turned on, and the power was also switched on. The tubes were gently heated at first and then the heating was increased after frothing ceased. The samples were digested until the solution was clear. The heat was turned off

and the digestion tubes were put in fumehood until cooled. The tubes were placed in a distillation unit for further distillation. Two drops of the indicator were added in an Erlenmeyer flask for each sample and the flasks were placed under the spout to receive ammonia. Titration of ammonia borate with hydrochloric acid was used to estimate the amount of nitrogen as follows:

$$N (\% \text{ of sample}) = (\text{Volume of acid used in sample titration} - \text{Volume of acid used in blank titration}) \times (\text{acid molarity} \times 0.014 \times 100) / (\text{weight of sample in gram} \times 1000).$$

$$\text{Crude protein} (\%) = N \% \times 6.25.$$

$$\text{Therefore, Crude protein (on \% DM basis)} = (\text{CP \%} / \text{DM \%}) \times 100$$

3.6.3 Determination of gross energy (AOAC, 1990)

The gross energies (GE) of the diet and excreta samples were determined using an adiabatic bomb calorimeter (Gallenkamp, University of Pretoria, South Africa). The apparent metabolisable energy (AME) content of the diets was calculated as follows:

$$\text{AME} = \text{Energy in feed consumed} - \text{energy excreted in the faeces.}$$

3.7 Tannin analyses

3.7.1 Extraction of polyphenols (FAO/IAEA, 2000)

A finely ground sample of 0.200 g dried plant material was weighed in a glass beaker of approximately 25 ml capacity. Ten milliliters of aqueous acetone 70 % or 50 % methanol was added, and the beaker was suspended in an ultrasonic water bath for 20 minutes at room temperature (25 °C). Contents of the beaker were centrifuged for 10 minutes at

approximately 3000 g using an ordinary clinic centrifuge. The supernatant was transferred to large test tubes and the solid was left in the small centrifuge tube.

3.7.2 Determination of total phenolics and tannin using Folin- Ciocalteu method

(Makkar *et al*, 1993)

Suitable aliquots of the tannin-containing extract of 0.02 ml was pipetted into the test tubes. The volumes of 0.48 ml distilled water, 0.25 ml of the Folin-Cicalteu reagent and 1.25 ml of sodium carbonate solution were added. The tubes were vortexed and then allowed to stand for 40 minutes at room temperature. The absorbance at 725 nm was recorded. The amount of total phenolics was calculated as tannic acid equivalent using calibration curve. Total phenolics were expressed on a dry matter basis (% DM tannic acid equivalent).

3.7.3 Determination of simple phenolics using polyvinylpolypyrrolidone (PVPP)

(Makkar *et al*, 1993)

A hundred milligram of PVPP was weighed into a 100 x 12 mm test tube, 1.0 ml distilled water and 1.0 ml of the tannin-containing extract was added. A hundred milligram of PVPP is sufficient to bind 2 mg of total phenols. If total phenolic content of the feed was more than 10 % on a dry matter basis, the extract was diluted appropriately. The tubes were vortexed and kept at 4 °C for 15 minutes. They were vortexed again before they were centrifuged at 300 g for 10 minutes and the supernatant was collected. This supernatant had only simple phenolics other than tannins. The tannins had been precipitated along with the PVPP. The phenolic content of the supernatant was measured

by taking three times the volume used for total phenol estimation, because the extract was already diluted two-fold and was expected to lose tannin-phenols through binding with PVPP. The absorbance was recorded at 725 nm after 40 minutes. The content of non-tannin phenols was expressed on a dry matter basis (% DM tannic acid equivalent).

3.7.4 Determination of condensed tannins (Waterman & Mole, 1994)

3.7.4.1 Extracted condensed tannins

A sample of 0.2 ml tannin extract diluted with 0.3 ml of acetone was pipetted into a 100 x 12 mm test tube. And 3.0 ml of butanol-HCL reagent and 0.1 ml of ferric acid were added. The tube was vortexed and then the mouth of the tube was covered with a glass marble and put in the heating block at 97 to 100 °C for 60 minutes. The tube was then allowed to cool and absorbance was recorded at 550 nm. The formula for calculating percentage of condensed tannins as leucoanthocyanidin equivalent is (absorbance 550 nm x 78.26 x dilution factor) / (% DM).

3.7.4.2 Unextracted condensed tannins

A 0.01 g of the pellet from condensed tannins extract, 3.0 ml of butanol HCL reagent and 0.1 ml of ferric acid were added into a 100 x 12 mm glass test tube. The tube was vortexed with the mouth of the tube covered with a glass marble and put in the heating block adjusted at 97 to 100 °C for 60 minutes. The tube was cooled and absorbance was recorded at 550 nm. For the blank, 0.5 ml of the extract, 3 ml of butanol and 0.1 of ferric

reagents were added. The formula for calculating percentage of condensed tannins in a gram of sample was: (Absorbance 550 nm/ weight of sample used) x (1000 mg) / (% DM).

3.7.5 Radial diffusion assay (Hagerman, 1987)

3.7.5.1 Preparation of plates

A 2.5 g agarose was weighed into 250 ml of the acetate buffer. The solution was heated to boil for 15 minutes with continuous stirring on a magnetic stirrer until agarose dissolved. The solution was allowed to cool to 45 °C by keeping the vessel containing the agarose solution into a water bath. A 250 mg BSA was added and dissolved in the agarose solution without allowing the solution to cool lower than 45 °C. A glass pipette of 10 ml with a large tip opening was used and approximately 10 ml of the solution was dispensed into each petri dish kept on a flat surface. The solution covered all the surfaces of the petri dish and allowed to be hardened. All the petri dishes were covered and sealed with strip of parafilm in order to prevent drying and cracking of the agarose layer. The petri dishes were stored for 4 days in a refrigerator.

3.7.5.2 Assay procedure

On the day of performing the assay, the petri dishes were taken out from the refrigerator, brought to room temperature and then opened. A puncher was used to punch four wells, far apart, in the solidified agarose in petri dishes. In each well 15, 30, 45 and 65 µl of the extract were pipetted. The petri dishes were covered and sealed again using parafilm. The

plates were placed in an oven adjusted at 30 °C . After 96 hours the petri dishes were removed from the oven, uncovered and the diameter of the ring was measured, if present, using a transparent millimeter ruler.

3.7.6 Protein binding capacity by filter paper assays (Dawra *et al.*, 1988)

The extraction was done using 70 % of acetone. A Whatman paper chromatography sheet of 1mm was cut into an appropriate size of 60 cm x 15 cm. The squares of approximately 3 cm² were drawn using a light lead pencil on the chromatography sheet. Different aliquot was done in triplicate (on three different squares). Amounts of 50 µl of plant extract were applied on the middle of the squares of the chromatography sheet. The spots were allowed to dry and immediately BSA was used to spray the paper until it was wet. The paper was washed with acetate buffer (pH 5; 0.05 M) with three 10 minutes changes with slight shaking to remove the unbound BSA. The paper was stained with 0.2 % Ponceau S dye solution by keeping the strips dipped for 10 minutes in the stain solution. The stain was washed in 0.2 % acetic acid solution until no more colour was eluted from the strips.

The strips were air dried and the stained areas were cut in small pieces and put in the test tubes where the colour was eluted by adding 3 ml of 0.1N sodium hydroxide solution and it was vortexed, followed by addition of 0.3 ml of 10 % acetic acid and centrifugation at approximately 2500 g. The absorbance of the colour was recorded at 525 nm against corresponding blank, which was done in the following way: a plain chromatography sheet was stained simultaneously as the sample chromatography and was washed in the

same manner to the samples. The absorbances were converted to protein content by using a standard curve. The standard curve was prepared by applying different concentrations of bovine serum albumin (BSA) (5 to 50 μ l of 1 mg/ml BSA solution in the acetate buffer). This was applied as separate spots in triplicates for each concentration on a chromatography sheet and cut into strips. These strips were stained with the dye solution for 10 minutes, washed, dried and cut, and the absorbance was recorded the same way as for the samples. The calculations were done as tannic acid equivalent from the calibration curve and expressed as μ g.

3.7.7 Reaction of polyethylene glycol (PEG) with tannins (Silanikove *et al.*, 1994)

A stock solution containing 100 g/l PEG in a 0.5 M buffer Tris-BASE, pH 7.1 was prepared. A working solution was prepared by mixing 1 part of the stock solution and two parts of distilled water. The ratio between the plant sample weight and the working solution was 1:15. One gram of the sample was used. The reaction was carried out in 50 ml centrifuge tubes. After the samples had been mixed with the solution of distilled water (in the case of those untreated or control), the tubes were left for 24 hours in a horizontal position, with occasional mixing. The tubes were then centrifuged for 30 minutes at 2 500 g and the supernatant was collected. Crucibles were dried in an oven to constant weights and then transferred into desiccators to cool down before weighing. A sample of 10 ml was poured into the crucible and then dried in the oven and then weighed after drying. The procedure of weighing and drying was repeated three times after every 30 minutes and weights were recorded for each period. This was done for treated and untreated feed samples.

3.8 Statistical analysis

Statistical analyses were conducted using the general linear model (GLM) procedure of the Statistical Analysis System (SAS, 1998) package. Analysis of variance was used to determine the effect of sex, dietary energy level and level of *Acacia karroo* leaf meal supplementation on diet intake, growth rate, feed conversion ratio, digestibility, nitrogen retention, and carcass characteristics. Duncan's Multiple Range Test was used to determine the significance of differences among the means (Duncan, 1955). Correlation analyses were used to relate tanniniferous feed supplementation level to animal performance indices (fat pad, digestibility and nitrogen retention).

CHAPTER 4
RESULTS

The high and low energy diets contained 11.05 and 11.98 ME DM, respectively, and 180 g crude protein per kilogram DM diet are shown in Table 4.1. Results of the nutrient composition of *Acacia karroo* leaf meal are presented in Table 4.2. *Acacia karroo* contained 120 g crude protein per kg DM, 1.5 % DM total phenolics, 4.5 % DM extracted condensed tannins and 3.72 % DM unextracted condensed tannins. The analysis by polyvinylpyrrolidone, radial diffusion, polyethylene glycol and precipitable phenolics by filter paper showed that *A. karroo* leaf meal had 0.57 % DM, 4.00 mm², 039 mg/g and 0.24 µg, respectively.

The effects of dietary energy level and tanniniferous *A. karroo* leaf meal level of supplementation and their interactions on feed intake, growth rate and feed conversion ratio of male and female Ross 308 broiler chickens from 22 to 42 days of age are presented in Table 4.3. Dietary energy level, tanniniferous *A. karroo* leaf meal level of supplementation, sex and their interactions had no effect ($P > 0.05$) on growth rates and feed conversion ratio of broiler chickens. Within the same sex, dietary energy level and tanniniferous *A. karroo* leaf meal level of supplementation had no effect ($P > 0.05$) on feed intake of broiler chickens. However, when compared on the same diet, male broiler chickens had higher ($P < 0.05$) feed intake than female chickens.

Table 4.1 Nutrient composition of the grower diets (units are g/kg for dry matter, g/kg DM for protein, and ME/kg DM).

Nutrient	Dietary treatments					
	E ₁ To	E ₁ T ₁	E ₁ T ₂	E ₂ To	E ₂ T ₁	T ₂ T ₂
Dry Matter	917	919	916	918	918	919
ME	11.05	10.12	10.33	11.98	10.05	10.72
Protein	180	181	180	182	180	182

E₁To: Low energy diet without tanniferous *Acacia karroo* leaf meal level supplementation.

E₁T₁: Low energy diet with 9 g of tanniferous *Acacia karroo* leaf meal level of supplementation/kg DM.

E₁T₂: Low energy diet with 12 g of tanniferous *Acacia karroo* leaf meal level of supplementation/kg DM

E₂To: High energy diet without tanniferous *Acacia karroo* leaf meal level of supplementation

E₂T₁: High energy diet with 9 g of tanniferous *Acacia karroo* leaf meal level of supplementation/kg DM

E₂T₂: High energy diet with 12 g of tanniferous *Acacia karroo* leaf meal level of supplementation/kg DM

Table 4.2. Tannin analysis of *Acacia karroo* leaf meal by total phenolics (TP), polyvinylpyrrolidone (PVPP), radial diffusion (RD), extracted condensed tannins (ExCT), unextracted condensed tannins (UNExCT), polyethylene glycol (PEG) and precipitable phenolics by filter paper method (PPFP), dry matter and crude protein.

<i>Acacia karroo</i>	
Dry matter (g/kg)	90.7
Crude protein (g/kg DM)	120.0
Tannin contents by method of:	
TP (% DM)*	1.51
PVPP (% DM)	0.57
RD (mm ²)	4.00
ExCT (% DM) **	4.52
UnExCT (% DM) **	3.72
PEG (mg/g)	0.39
PPFP (µg)	0.24

* percentage DM tannic acid equivalent

** percentage DM Leucocyanidin equivalent

Table 4.3. Effect of dietary energy level and tanniniferous *Acacia karroo* leaf meal level of supplementation on feed intake (g DM/bird/ day), growth rate (g/ bird/ day) and feed conversion ratio (FCR) (g feed/g live weight gain) of male and female Ross 308 broiler chickens from 22 to 42 days of age.

Treatment	Feed intake	Growth rate	FCR
ME ₁ T ₀	119.7 ^{ab}	57.1	2.0
ME ₁ T ₁	117.6 ^{abc}	54.3	2.1
ME ₁ T ₂	116.0 ^{abc}	46.2	2.5
ME ₂ T ₀	122.8 ^a	51.9	2.3
ME ₂ T ₁	120.7 ^{ab}	48.0	2.5
ME ₂ T ₂	117.8 ^{abc}	43.9	2.5
FE ₁ T ₀	111.8 ^{cde}	51.1	2.1
FE ₁ T ₁	110.6 ^{de}	46.6	2.3
FE ₁ T ₂	107.8 ^e	44.8	2.4
FE ₂ T ₀	111.8 ^{cde}	42.6	2.5
FE ₂ T ₁	113.2 ^{cde}	46.7	2.4
FE ₂ T ₂	107.9 ^e	47.7	2.1
SE	2.194	2.957	0.119

a, b, c, d, e: Means in the same column not sharing a common superscript are significantly different (P<0.05)

SE : Standard error

Results of the effect of dietary energy level and tanniniferous *Acacia karroo* leaf meal level of supplementation and their interactions on dry matter digestibility, crude protein digestibility, metabolisable energy and nitrogen retention of male and female broiler chickens between 38 and 42 days of age are presented in Table 4.4. Dietary energy level, tanniniferous *A. karroo* leaf meal level of supplementation and sex had no effect ($P > 0.05$) on dry matter and CP digestibilities, metabolisable energy and nitrogen retention in broiler chickens.

Dietary energy level, tanniniferous *A. karroo* leaf meal level of supplementation and sex had no effect ($P > 0.05$) on carcass weight, dressing percentage, breast meat and thigh weights of broiler chickens (Table 4.5). Tanniniferous *A. karroo* leaf meal supplementation had effects on fat pad weights. Broiler chickens supplemented with *A. karroo* leaf meal had lower ($P < 0.05$) fat pad weights than those not supplemented. However, sex of the chickens had no effect ($P > 0.05$) on fat pad weights. Dietary energy level, *A. karroo* leaf meal level of supplementation and sex had no effect ($P > 0.05$) on crude protein content of breast meat samples of male and female broiler chickens (Table 4.6).

Table 4.4 Effect of dietary energy level and tanniniferous *Acacia karroo* leaf meal level of supplementation at finisher stage on dry matter digestibility, crude protein digestibility, metabolisable energy (ME) and nitrogen-retention of male and female Ross 308 broiler chickens between 38 and 42 days of age.

Treatment	DM digestibility	CP Digestibility (decimal)	ME (MJ/kg DM)	N-retention (g/bird/day)
ME ₁ To	0.58	0.88	10.88	3.20
ME ₁ T ₁	0.51	0.87	9.88	3.00
ME ₁ T ₂	0.49	0.87	10.15	3.03
ME ₂ To	0.58	0.88	11.55	3.20
ME ₂ T ₁	0.47	0.87	9.46	2.80
ME ₂ T ₂	0.50	0.87	10.05	2.73
FE ₁ To	0.60	0.90	11.22	3.20
FE ₁ T ₁	0.53	0.88	10.37	3.10
FE ₁ T ₂	0.56	0.87	10.51	2.80
FE ₂ To	0.61	0.90	12.40	3.20
FE ₂ T ₁	0.55	0.87	10.64	3.10
FE ₂ T ₂	0.54	0.87	11.39	3.10
SE	0.022	0.01	0.408	0.160

^{abcd} : Means in the same column not sharing a common superscript are significantly different ($P < 0.05$)

SE : Standard error

Table 4.5 Effect of dietary energy level and tanniferous *A.karoo* leaf meal level of supplementation on carcass parts (g) and dressing percentage (%) of male and female Ross 308 broiler chickens at 42 days of age.

Treatment	Carcass weight	Dressing %	Fat pad	Breast	Thigh
ME ₁ To	1579	80	38 ^a	362	120
ME ₁ T ₁	1642	77	28 ^{bc}	355	119
ME ₁ T ₂	1704	79	27 ^c	366	126
ME ₂ To	1634	78	39 ^a	366	120
ME ₂ T ₁	1549	79	29 ^{bc}	337	121
ME ₂ T ₂	1495	75	28 ^{bc}	322	120
FE ₁ To	1474	79	38 ^a	352	117
FE ₁ T ₁	1498	78	28 ^{bc}	366	116
FE ₁ T ₂	1501	81	28 ^{bc}	333	113
FE ₂ To	1376	76	38 ^a	322	107
FE ₂ T ₁	1454	77	28 ^{bc}	317	111
FE ₂ T ₂	1236	66	28 ^{bc}	319	107
SE	85.978	4.095	2.747	19.5	5.028

a, b, c : Means in the same column not sharing a common superscript are significantly different ($P < 0.05$)

SE : Standard error

Table 4.6. Effect of dietary energy level and tanniniferous *A.karoo* leaf meal level of supplementation on crude protein contents (% DM) of breast meat samples of male and female Ross 308 broiler chickens at 42 days of age.

Treatment	Crude Protein %
ME ₁ To	26.6
ME ₁ T ₁	20.5
ME ₁ T ₂	22.0
ME ₂ To	20.9
ME ₂ T ₁	20.8
ME ₂ T ₂	21.0
FE ₁ To	20.3
FE ₁ T ₁	21.5
FE ₁ T ₂	20.5
FE ₂ To	22.3
FE ₂ T ₁	21.3
FE ₂ T ₂	21.4
SE	0.516

SE: Standard error

A series of linear regressions that predict fat pad content, crude protein retention and dry matter digestibility in male and female Ross 308 broiler chickens from tanniniferous *Acacia karroo* leaf meal level of supplementation are presented in Table 4.7. *Acacia karroo* leaf meal level of supplementation was poorly correlated with fat pad of male ($r^2 = 0.329$) and female ($r^2 = 0.071$) broiler chickens fed a low energy diet. Moderate relationships ($r^2 = 0.689$) were observed between *Acacia karroo* leaf meal level of supplementation in broiler chickens fed a low energy diet. A similar trend was observed when the chickens were fed a high energy diet. Similarly, poor correlations were observed between *A. karroo* leaf meal level of supplementation and crude protein retention in broiler chickens.

Table 4.7 Prediction of diet dry matter digestibility (decimal), fat pad content (g/bird) and CP retention (g/bird/day) in male and female Ross 308 broiler chickens from tanniniferous *Acacia karroo* leaf meal level of supplementation.

Factor	Y-variable	Formulae	r ²	P
10.30 MJ/kg DM Feed				
<i>A. karroo</i>	male fat pad	$y = -1.80x + 38.3$	0.329	0.120
<i>A. karroo</i>	male CP retention	$y = -0.24x + 22.5$	0.024	0.237
<i>A. karroo</i>	DM digestibility (males)	$y = 1.8x - 2$	0.689	0.027
<i>A. karroo</i>	female fat pad	$y = -7.55x + 40.7$	0.071	0.493
<i>A. karroo</i>	female CP retention	$y = -0.61x + 23.3$	0.332	0.003
<i>A. karroo</i>	DM digestibility (females)	$y = 1.8x - 2$	0.689	0.007
13.00 MJ/kg DM Feed				
<i>A. karroo</i>	male fat pad	$y = -1.26x + 42.4$	0.097	0.493
<i>A. karroo</i>	male CP retention	$y = -0.32x + 20.5$	0.062	0.062
<i>A. karroo</i>	DM digestibility (males)	$y = 1.8x - 2$	0.689	0.037
<i>A. karroo</i>	female fat pad	$y = -1.40x + 47$	0.456	0.018
<i>A. karroo</i>	female CP retention	$y = -0.53x + 23$	0.222	0.770
<i>A. karroo</i>	DM digestibility (females)	$y = 1.8x - 2$	0.689	0.044

r² : Correlation co-efficient

x : *A. karroo*

CHAPTER 5
DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

5.1 Discussion

This experiment was designed to include high and low energy diets. The analyzed experimental grower diets had ME levels of 10.5 and 11.0 MJ/kg DM for Low and High diets, respectively. The *Acacia karroo* used in this study contained 120 g of crude protein per kg DM. This is quite high and ideal for supplementation in animal feeds (Makkar, 2003). Similar results have been reported elsewhere (Dube and Ndlovu, 1993, Kahiya *et al.*, 2004; Mokoboki, 2005). However, *Acacia karroo* contained high concentrations of tannins, particularly condensed tannins. Dube and Ndlovu (1993) reported similar concentrations. Condensed tannins bind with diet protein and other nutrients, hence they tend to lower diet intake and digestibility in animals (Dube, 1993; Makkar, 2003). Thus, the performance of animals on high tanniferous feeds is usually low (Makkar, 2003).

The present results showed that dietary energy levels had no effect on feed intake, growth rate, FCR, digestibility, nitrogen retention, carcass weight, fat pad, carcass parts and dressing percentage of broiler chickens. Metabolisable energy levels of the grower diets were not very different. Thus, lack of differences in intakes may have been expected. This is because broiler chickens eat primarily to satisfy their energy requirements (Scott *et al.*, 1982), and hence feeds of similar energy levels will give similar intakes.

Acacia karroo leaf meal level of supplementation had no effect on growth rate, feed conversion ratio, carcass parts, dressing percentage and crude protein contents of breast meat of broiler chickens at 42 days of age. These results could be explained in terms of

similar intakes, digestibilities and nitrogen retention, irrespective of the treatment. Similar results were obtained by Al-Mamary *et al.* (2001) who found that addition of sorghum grains low in tannins to diets of rabbits did not change growth rate, feed intake and feed conversion ratio. Similar results were also reported by Diao *et al.* (1990). These findings are contrary to the findings of Laurena *et al.* (1984), Makkar (2003) and Hassan *et al.* (2003) who found adverse effects of tannins on feed efficiency, growth rate and protein digestibility.

Male broiler chickens ate more feeds than female chickens. These results are similar to those of Dozier *et al.* (2008) who found that male broiler chickens had higher feed intake than female chickens when both sexes were fed *ad libitum*. The differences were explained in terms of female chickens requiring on average 13 % less feed for maintenance per kg metabolic body weight than males. However, Gous *et al.* (1999) suggested that genetic potential influences broiler chicken growth responses because it affects their nutritional requirements. Thus, male broiler chickens have a pronounced genetic advantage of feed intake compared to female broiler chickens. However, there were no differences between sexes in growth rate, carcass weights, carcass parts and breast meat nitrogen content. These results may be explained in terms of similarities in digestibility values. These results are similar to the findings of Leeson and Summers (1991) who found no differences between male and female growth rates and carcass weights. Similarly, Acar *et al.* (1993) reported that sex had similar effects on carcass weight and nitrogen content of breast meat of broiler chickens at 42 days of age. However, Lipens *et al.* (2000) reported that female broiler chickens yielded smaller carcass weights than male chickens. Han and Baker (1993), also, reported that sex had an

effect on carcass weight and nitrogen content of breast meat of broiler chickens. The differences were explained in terms of higher feed intake in male compared with female chickens. It was, additionally, suggested that the differences between sexes probably arise from metabolic differences and also from the differences in the onset of fattening of broiler chickens. *Acacia karroo* leaf meal supplementation had an effect on fat pad weights of broiler chickens. Supplementation with 9 and 12 g of *Acacia karroo* leaf meal per kg DM of feed reduced fat pad weights in male broiler chickens by 26 and 29 percentage points, respectively. Similarly, supplementation with 9 and 12 g of *Acacia karroo* leaf meal per kg DM feed reduced fat pad weights in female chickens by 26 percentage points. These reductions were achieved without any significant reduction in feed intake and or digestibility. The physiological explanation for this effect is not clear and it, thus, merits further investigation. However, it is known that *A. karroo* leaves contain high contents of condensed tannins which tend to bind with feed and endogenous proteins, and other nutrients, thus lowering diet intake and digestibility (Makkar, 2003). The presence of condensed tannins has been associated with reduced carcass fat in ruminant animals (Purchase and Keogh, 1984; Terril *et al.*, 1992). However, no physiological explanations were given in their studies. No similar studies in chickens were found.

Low but positive correlations were found between *Acacia karroo* leaf meal level of supplementation and diet DM digestibility, fat pad weights and crude protein retention in broiler chickens. Mashimaite (2004) observed similar results in rabbits. No similar studies in chickens were found.

5.2 Conclusion and recommendations

Acacia karroo contained high amounts of condensed tannins. Supplementation with *Acacia karroo* leaf meal had no effect on diet intake, digestibility and live weight of broiler chickens. However, supplementation with 9 and 12 g of *Acacia karroo* leaf meal per kg DM feed reduced fat pad weights in male broiler chickens by 26 and 29 percentage points, respectively. Similarly, supplementation with 9 and 12 g of *Acacia karroo* leaf meal per kg DM feed reduced fat pad weights in female chickens by 26 percentage points. These reductions were achieved without any significant reduction in feed intake and digestibility. The physiological explanation for this effect is not clear and it, thus, merits further investigation.

CHAPTER 6
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6.0 References

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ABSTRACT

The study was conducted to determine the effect of dietary energy level and tanniniferous *Acacia karroo* leaf meal level of supplementation at finisher stage on performance and carcass characteristics of male and female Ross 308 broiler chickens. Three hundred and sixty, 21-day old male and female broiler chickens were assigned to twelve treatments with three replications of ten birds in a 2 (sex) x 3 (dietary energy level) x 3 (tanniniferous *Acacia karroo* leaf meal level) factorial, complete randomized design. Supplementation with *Acacia karroo* leaf meal had no effect on diet intake, digestibility and live weight of broiler chickens. However, supplementation with 9 and 12 g of *Acacia karroo* leaf meal per kg DM feed reduced fat pad weights in male broiler chickens by 26 and 29 percentage points, respectively. Similarly, supplementation with 9 and 12 g of *Acacia karroo* leaf meal per kg DM feed reduced fat pad weights in female chickens by 26 percentage points. These reductions were achieved without any significant reduction in feed intake and digestibility. However, the physiological explanation for this effect is not clear and it, thus, merits further investigation.