Nutritive value of *Acacia* species and response of pigs fed on incremental levels of *Acacia tortilis* leaf meal-based diets

By

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Declaration

I, Mbongeni Khanyile, declare that this dissertation has not been submitted to any University and that it is my original work conducted under the supervision of Prof. M. Chimonyo. All assistance towards the production of this work and all references contained herein have been duly acknowledged

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List of Abbreviations

ADF	acid detergent fibre
ADFI	average daily feed intake
ADG	average daily gain
ALP	alkaline phosphatase
ALT	alanine aminotransferase
AOAC	Association of Official Agricultural Chemists
CP	crude protein
DM	dry matter
EDTA	ethylene diamine tetra acetic acid
Fe	iron
GLM	generalized linear model
HSI	Hepatosomatic index
NDF	neutral detergent fibre
P	phosphorus
SAS	Statistical Analysis Systems
SHW	Scaled heart weight
SLW	Scaled lungs weight
SKW	Scaled kidneys weight
TP	total protein
UKZN	University of KwaZulu-Natal
WHC	water holding capacity

Abstract

Nutritive value of Acacia species and response of pigs fed on incremental levels of *Acacia tortilis* leaf meal-based diets

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M Khanyile

The broad objective of the current study was to determine the response of pigs fed on incremental levels of *Acacia tortilis* leaf meal-based diet. Eight trees of each of the following five dominant leguminous leaf meals; *A. tortilis, A. robusta, A. nilotica, A. nigrescens* and *A. xanthophloea*, were individually hand harvested from the same grazing camp at Makhathini Research Station, Jozini, South Africa. The leaf meals were harvested between April and May 2013 after the rainy season at advance stage of maturity. Following the nutritive evaluation of *Acacia* species, *A. tortilis* was selected for the feeding experiment. Thirty finishing male F_1 hybrid (Landrace × Large White) pigs with an initial weight of 60.6 (s.d. = 0.94) kg were randomly allotted to six dietary treatments containing 0, 50, 100, 150, 200, 250 g/kg DM inclusion levels of *A. tortilis* leaf meal. Each treatment diet was offered *ad libitum* to five pigs in individual pens for 21 days.

Average daily feed intake (ADFI), body weight, average daily gain (ADG) and gain: feed ratio was measured every week. Blood was collected at the end of the experimental period for the determination of nutritionally-related blood metabolites and activity of Aspartate aminotransferase (AST), alanine amino transferase (ALT) and alkaline phosphatase (ALP). Following a feed withdrawal period of 12 hours, pigs with a mean body weight of 80 ± 15 kg were slaughtered, eviscerated for the collection of livers, kidneys, lungs and heart from each pig and weighed individually using a digital scale.

Acacia tortilis and *A. xanthophloea* leaf meals had the highest CP and fat content ($P < 0.01$) among all the *Acacia* species. The NDF and ADF varied significantly across *Acacia* species ($P < 0.05$). *Acacia robusta* had significantly the highest levels of non-structural carbohydrates, whilst *A. tortilis* had the lowest. The concentration of proanthocynidins varied across the species. *Acacia tortilis* had significantly high levels (23 g/kg DM) of phosphorus compared to the other species. *Acacia nilotica* had the highest ($P < 0.001$) concentration of iron, but it had the lowest ($P < 0.01$) level of manganese.

There was a quadratic decrease in both ADFI and ADG ($P < 0.001$) with as *A. tortilis* leaf meal increased. The gain: feed ratio was linearly reduced ($P < 0.001$) with incremental levels of *A. tortilis* leaf meal in the diets. Serum concentrations of Fe, AST and ALP increased quadratically (P < 0.01) as *A. tortilis* leaf meal increased. There was a linear increase in ALT activity with increased leaf meal inclusion. Hepatosomatic index (HSI: liver weight/ body weight x 100), scaled kidney weight and scaled heart weight increased linearly (P < 0.001) as *A. tortilis* leaf meal increased. There was, however, a quadratic increase in the relative weight of lungs (P < 0.001) as leaf meal increased. In conclusion, the piecewise regression (brocken-stick) NLIN procedure (SAS, 2008; SASA institute Inc.) showed that *A. tortilis* leaf meal can be included in finisher pig diet up to 150 g/kg DM of feed without negatively affecting growth performance, nutritionally-related blood metabolites, liver enzymes and internal organs of pigs.

Keywords: feed intake; tannins; weight gain; blood metabolites; internal organs.

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CHAPTER 1: General Introduction

1.1 Background

Pigs are an important source of meat, income, employment and a source of livelihoods in Africa (Halimani *et al.,* 2012; Madzimure *et al.,* 2011). The South African pig sector, for example, produces about 120 000 tons per annum with per capita consumption of 2.6 kg (FAO, 2012). To increase the contribution of pigs to the national economy, it is vital to explore and characterize alternative feeding strategies that reduce competition of feed resources with humans. Feed accounts for over 60 % of the variable costs in most pig enterprises. Leguminous leaf meals can be used as a protein source in non-ruminants diets to replace expensive ingredients such as soybean and fish meals (D'Mello, 1995).

While more than half of the world's human population is starving, the issue of feeding livestock on high quality raw materials, such as maize and oil seed is increasingly being debated (Cole *et al.,* 1993; D'Mello, 1995). Non-conventional feed resources for livestock needs to be identified and characterised (Ly, 1990). Leguminous leaf meals are widely recognized to have a huge potential due to their relatively high crude protein (Dube *et al.,* 2001; Halimani *et al.,* 2005; Ng'ambi *et al.*, 2009). In addition, to their high nutritional composition, most legumes thrive in and tolerate adverse climatic and soil conditions (Heuzé and Tran, 2011). Common species available in South Africa include *Acacia tortilis, Acacia nilotica, Acacia robusta, Acacia nigrescens and Acacia xanthophloea.*

Acacia species are invasive, and have been identified as one of the dominant woody species that exacerbates the problem of bush encroachment in Southern Africa (Nyamukanza and Scogings, 2008). Effective control of *Acacia* species include chemicals and the use of simple low cost techniques such as lopping using homemade tools (Mapiye *et al.,* 2011). Leaves from the lopped trees can be used as feed for livestock, while branches and tree trunks can be utilized for fencing and firewood (Mapiye *et al.,* 2011). Seedling emergence can be discouraged by grinding the pods, thereby enhancing protein availability for livestock (Mlambo *et al.*, 2007).

Use of leguminous leaf meal in animal diets is, however, constrained by the abundance of fibre and tannins (D'Mello, 1995; Dube *et al.,* 2001; Halimani *et al.,* 2005). Feed intake, body weight gain, feed conversion efficiency and metabolic response are important parameters that need to be assessed to ascertain the suitability of leaf meal as an alternative source of protein in pigs. The extent to which pigs can consume *A. tortilis* leaf meal digest amino acids and the effect that leaf meal can have on liver enzyme secretion is not known. Although some studies have investigated the performance of pigs fed on *Acacia* leaves (e.g. Halimani *et al.,* 2005), few if any dose-response trials have been conducted. Dose-response trials can accurately determine the optimum inclusion levels for each of the parameters assessed

1.2. Justification

Little information is available on the nutritive value, content of anti-nutritional factors. Assessment of nutritional-related blood metabolites, liver enzymes and internal organs can be used to determine the extent of deleterious effect of polyphenolic compounds in finishing pigs. Determination of optimum inclusion will make it possible not to compromise the growth rate and health status of pigs. Response of pigs to *Acacia* leaf meal inclusion assists feed compounders in exploring use of non-conventional feed ingredients in pig feeding. If successful, inclusion of *Acacia* leaves reduce the requirement for soybean, thereby reducing feed costs and increasing the utilization of locally available resources for livestock feeding.

1.3. Objectives

The broad objective of the study was to determine the response of pigs fed on incremental levels of *Acacia tortilis* leaf meal. The specific objectives are to:

1. Assess the nutritive value of *Acacia* species;
2. Determine the effects of feeding incremental levels of *A. tortilis* leaf meal on performance parameters in finishing pigs;
3. Determine the effects of incremental levels of *A. tortilis* leaf meal on selected nutritional related blood metabolites, liver enzyme and internal organs in finishing pigs.

1.5. Hypotheses

1. The nutritive value of *Acacia* species vary with species;

2. As the inclusion levels of *A. tortilis* leaf meal increases, feed intake, performance parameters, increases linearly up to a point when it becomes constant or starts to decrease

3. *Acacia tortilis* leaf has no effect on nutritionally-related blood metabolites and organ weights.

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CHAPTER 2: Literature review

2.1. Introduction

Smallholder pig production is constrained by inadequate supply of quality feed in Southern Africa (Halimani et al., 2005). There is little information on the utilization of *Acacia tortilis* on the pig nutrition and its effect thereof on nutritionally-related blood metabolites and internal organs. This review of literature discusses the chemical composition of *Acacia* leaf meal, constraints to utilization of *Acacia* leaf meal in pig diet. The effect of Acacia leaf meal inclusion in pig performance, physiological adaptation of pigs to tanniferous diets and effect of *Acacia tortilis* leaf meal on nutritionally- related blood metabolites and internal organs is also reviewed.

2.2. Pig production systems

Preston and Leng (1987) suggested that livestock production systems in developing countries should be matched with local available feed resources in a way that aims at economic optimization instead of biological maximization. According to Mashatise *et al.* (2005), the most commonly production systems are the backyard system and free ranging system. In the backyard production system, pigs are kept inside fenced yard and provided with supplementary feeds such as kitchen wastes, vegetables, rotten maize, hominy chops, maize meal, maize husks, green maize, grasses and brewer wastes (Kanengoni *et al.*, 2002; Chimonyo, 2005). In free range systems, pigs are not confined during the day sometimes penned or un-penned at night but are allowed to roam around in the community (Mashatise *et al*., 2005). Although free range system is not labour intensive, however it is one of the major contributing factors in spread of diseases and the outbreak of diseases (Lekule and Kyvsagaard, 2003). The number of pigs per household is influenced by the limitation of feed resources (Chiduwa *et al*., 2008). It is imperative to evaluate non-conventional feed resources such as leguminous tree leaf meal in pig diets to reduce feed deficit in tropics.

2.2.1. Role of leaf meals in pig production

Feed cost is the major expenditure in livestock and pig farming and accounts for between 65 and 75 % of variable costs (Wiseman and Garnsworthy, 2001). The cost of production can be reduced if alternative source of protein such as leguminous leaf meals is supplemented in pig diets (Ly, 1990 and D'Mello, 1995). One of the perceived merits of leaf meal is their high levels of crude protein (CP) and amino acids. Leaf meal can be included in pig diets at 100g/kg of overall feed without compromising performance (Halimani *et al.,* 2005). Mueller-Harvey (2006) pointed out that tannins found in feeds such as fodder, legumes, browse leaves, fruits and *Acacia* species can improve animal welfare and health by acting as an anthelmintic. Proanthocyanidins may have direct effects on gastrointestinal parasites by reducing the number of eggs hatching, reduce the rate of larval development and decreased the mobility of larvae (Min and Hart, 2003). The indirect effect on resistance could be caused by changes in the supply of protein thus reduce the immunity (Barry and McNabb, 1999; Min and Hart, 2003). Few if any studies have been conducted doe response trial using indigenous leguminous leaf meal in pigs.

2.3 Influence of leaf meal on pig performance

2.3.1 Feed intake

High levels of leaf meal in pig diets resulted in an increase fibre contents of the ration (Huy *et al.,* 2006). Phuc *et al*. (2001) reported a reduction of feed intake where high levels of forages were included in pig diet, in accordance with recently report (Halimani *et al.*, 2005) who reported a decrease in feed intake of pigs fed 300 g/kg of feed inclusion levels of *Acacia karroo* (Table 2.1). Malavanh and Preston (2006), also demonstrated a reduction on feed intake by pigs fed different levels of sweet potato and water spinach leaves. High fiber content of forages and the present of anti-nutritioonal factors are the cause of low feed intake and since most soluble phenolics have astringent taste which contribute to low feed intake (D'Mello 1995). In most studies conducted on utilization of leaf in pig feeding (Huy and Phuc 2006; Phuc and Lindberg, 2000, Halimani *et al.*, 2005), increased feed intake has been demonstrated with low inclusion levels of leaf meal. Pigs eat more in an attempt to compensate limiting nutrients as a result of antinutrient effects (McDonald *et al*., 1995).

2.4.2 Nutrient digestibility

High inclusions levels of leguminous leaf meal can render feed constituents less digestible (D'Mello, 1995). Tannins present in most leaf meal are known to bind nutrients thereby reducing their digestibility (Mlambo *et al.*, 2007). Protein digestibility tends to be reduced most, but carbohydrates, starch and cell wall can also be affected.

Muller-Harvey (2006), pointed out that low protein digestibility and low dry matter digestibility could be also attributed to inhibition of digestive enzymes or intestinal

Table 2.1: Feed intake on pigs fed diets supplemented with incremental levels of *Acacia karroo* and *Acacia nilotica*

Diet	Inclusion levels (g/kg)	Average feed intake (kg)	SEM
Control	0	1.1	0.04
Acacia karroo	100	1.6	0.10
Acacia karroo	200	1.7	0.10
Acacia karroo	300	1.3	0.10
Acacia nilotica	100	1.4	0.06
Acacia nilotica	200	1.2	0.06
Acacia nilotica	300	1.3	0.06

Source: Halimani *et al.* (2005).

microorganisms. High nitrogen excretions have been reported in some leaf meal containing tannins (D'Mello, 1995).

Phuc and Lindberg (2000) reported that inclusion of forages in pig diet decreases the digestibility of organic matter, crude protein ether extract, neutral detergent fibre, acid detergent fibre and energy. These authors also demonstrated that ileal digestibility of fibre remained unchanged with the inclusion of foliages in the diet. These findings suggest that pigs possess the ability to digest a substantial part of the fibre that is present in forages. Amino acid digestibility of *A. tortilis* leaf meal by pigs is not known. Tannins, which are present in substantial proportions in these legume tree leaves have been reported to form insoluble complexes with macro nutrient and metals, such as copper (Cu) and iron (Fe), making them less available for absorption. They are, therefore, known to have anti-oxidant action in scavenging free radicals, binding metals and inhibiting lipid peroxidation (Bravo *et al.,* 1998). Lee *et al.* (2010) demonstrated an increase in faecal iron concentration in weanling pigs fed on diet supplemented with graded levels of tannic acids (Table 2.2).

2.3.3 Feed conversion ratio and average daily gain

Numerous studies on the inclusion of leaf meal in pig diet have shown that leaf meal can be included in the diet between 40, 80 and 120g/kg of feed without negative affecting growth rate (Figure 2.1) (D'Mello, 1995; Phuc *et al.,* 2000; Halimani *et al.,* 2007). For example, cassava leaf meal (Figure 2.1; Phuc *et al.,* 2000).

Table 2.2: Effect of tannic acid supplementation on the concentration faecal mineral (mg/kg) in weanling pigs (expressed on DM basis)

Item	TA mg/kg					
	0	125	250	500	1000	S.E.M
Manganese	380	375	372	376	393	9.7
Iron	902	976	976	1047	1131	26.8
Copper	640	635	703	695	710	40.1
Zinc	1178	1178	1189	470	1358	36.7

Source: Lee *et al*. (2010)

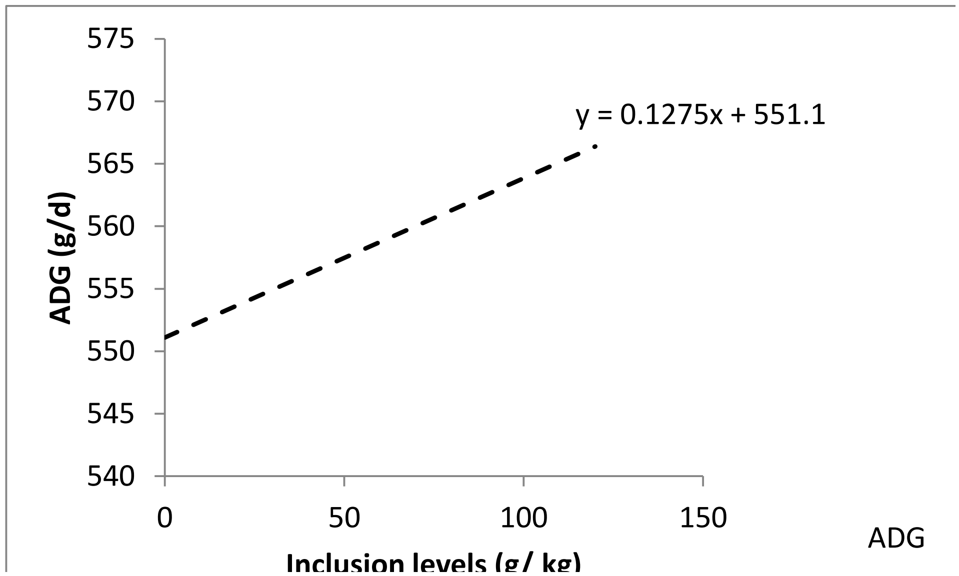

Figure 2.1: Effect of cassava leaf meal inclusion on average daily gain of growing pigs

Source: Phuc and Lindburg (2000).

High inclusion levels of leaf meal (more than 300g/kg of feed) in the diet of pigs are associated with depressed growth rate (D'Mello, 1995). The depressed growth rate is usually caused by low feed intake which is more prominent in high inclusion levels of (Halimani *et al.*, 2005). The complexing effect of tannins on nutrients is also one factor constraining the growth rate (Makkar, 2003). Phuc *et al*. (2001) demonstrated an increase average daily gain of pigs fed graded levels of cassava leaf meal (Figure 2.1).

2.4 Effect of *Acacia* leaf meal on nutritionally-related blood metabolites

Blood parameters have been shown to be major indices of physiological, pathological and nutritional status of an organism and changes in the constituent compounds of blood when compared to normal values could be used to interpret the metabolic state of an animal as well as quality of feed. The blood biochemistry parameters such as protein, iron, and cholesterol are important in determining the nutrients profile of animal (Ndlovu *et al.*, 2009). The effect of *Acacia* leaf meal on blood parameters in growing pigs has not been studied. Such information is crucial when developing appropriate feeding strategies and prevention of diseases. Polyphenolics in pig diets are known to reduce blood cholesterol concentration (Jansman, 1993). Reduction in Fe and Cu bioavailability in pigs fed condensed tannins from faba beans (Bravo *et al*., 2008). Although chelating action of condensed tannins on Fe and Cu can reduce their bioavailability, this can be beneficial in some instances (Stukelj *et al.*, 2010). Iron and copper are known as the main triggers of hydroxyl radical production (Jansman, 1993). Lee *et al*. (2010) reported a reduced Fe plasma concentration with the increase tannic acid supplementation in pig diet.

2.5 Description and distribution of *Acacia tortilis*

Acacia tortilis (Forssk) Hyne is a thorny legume tree, usually about 4 to 8m high but can grow up to 20m (Orwa *et al.,* 2009). The crawn is dense, umbrella-like and flat-topped; hence it is called Umbrella tree in some areas. Leaves are compound and the leaflets (6-22 pairs) are very small (1- 4 mm long x 0.6 – 1 mm broad), glabrous to pubescent. Flowers are white, cream or yellow and highly aromatic. Fruits are distinguished by twisted shape; hence the epithet "tortilis" (Ecocrop, 2009). *Acacia tortilis* is native to semi-arid areas of Africa and Middle-East. It is found between 15 and 30ºN and between Sea level and 1000m altitude (Araya *et al.,* 2003). *Acacia tortilis* is tolerant to severe drought due its deep taproot system. *Acacia tortilis* is nitrogen fixing species and thus a soil improver (FAO, 1985). The tree is used in controlling soil erosion since it can grow fast and stabilize shifting dunes or hill slopes. If introduced in humid areas where fire wood and grazing land are inadequate, the specie can grow out of control and become nuisance (Ecocrop, 2009).

2.6 Chemical composition of *Acacia tortilis* leaves

Acacia tortilis is considered as one of the valuable sources of protein for herbivorous (Dube *et al.,* 2001; Ng'ambi *et al.,* 2009). Its leaves contain high levels of crude protein (CP), ranging from 140 to180 g/kg DM. It has moderate levels of detergent fibre (Table 2.3). The levels of minerals in *A. tortilis* leaves are also favorable (Table 2.4) (Abdulrazak *et al.,* 1999). Nutrient composition depends on various factors such as soil condition, season and stage of leaf growth (Nyamukanza and Scogings, 2008).

Table 2.3: Chemical composition (g/kg DM) of *Acacia tortilis* leaves

Chemical composition	Mean	SEM
Dry matter	955	24.2
Ash	41	16.7
Crude protein	189	2.4
Crude fibre	184	6.8
Neutral detergent fibre	195	94.3
Acid detergent fibre	169	80.4
Acid detergent lignin	82	1.3
Hemicellulose	45	1.6
Crude fat	43	1.4
Gross energy (MJ/kg DM)	18	-
Digestible energy (MJ/kg DM)	12	-

Sources: Abdulrazak *et al*. (2000); Dube *et al.* (2001); Rubanza *et al.* (2005) and Heuzé and Tran, (2011).

Table 2.4 Mineral content of *Acacia tortilis* leaves

Mineral	Concentration (g/kg DM)
Calcium	6.1
Phosphorus	1.8
Magnesium	1.5
Potassium	11.4
Sodium	0.5
Zinc	21.6
Copper	17.2
Manganese	12.3
Iron	223

Source**:** Abdulrazak *et al.* (1999); Heuzé and Tran (2011).

2.7 Constraints to utilization of *Acacia* leaf meals

Despite high nutritional composition of A. tortilis leaves, its utilization in livestock feeding constrained by the presence of thorns, high fibre content and polyphenolics, such as tannins (Rubanza *et al.,* 2005).

2.7.1 Presence of thorns

The presence of thorns in most *Acacia* species is one of the factors restricting their utilization in animal feeding (Dube *et al.,* 2001; Ng'ambi *et al.,* 2009; Mapiye *et al.,* 2011). Thorns reduce the surface area for leaf biting by animals thus lowering the nutrients intake (Mapiye *et al.,* 2011). thorns can be avoided by cutting the small branches and drying it there by collecting only leaves which can be store and used in animal feeding (Halimani *et al.,* 2005; Ng'ambi *et al.,* 2009).

2.7.2 High fibre content

The utilization of leguminous leaf meal is affected by its high fibre content (D'Mello, 1995). The chemical composition and structure of plant fibre differ with the fibre source (Van Soest, 1978). Fibre sources with lignified cell walls are more resistant to microbial activity, and effective on increasing the faecal volume and decrease the overall transit time in piglets (Nguyen, 2001; Wiseman *et al.,* 2001). The extent of fibre digestibility depends predominantly on the source of fibre and to a lesser extent of amount of fibre in the diet (Staganogias and Pearce, 1985).

2.7.3 Presence of tannins

Tannins are phenolic compounds of moderately high molecular weight containing sufficient phenolic hydroxyls and other suitable groups to effectively form strong complexes with proteins and other macromolecules (Van Soest *et al.,* 1987). Tannins are found in approximately 80 % of woody and 15 % of herbaceous dicotyledonous species. They can occur at high levels in some forage and feed (Bryant *et al.,* 1992; D'Mello 1995; Dube *et al.,* 2001). In tree leaves tannins are present in NDF and ADF in significant amounts which are tightly bound to the cell wall and cell protein and believed to be involved in decreasing digestibility (Makkar, 2003). Tannins are classified into hydrolysable and condensed tannins (Van Soest *et al.*, 1987; Mueller-Harvey, 2006). Hydrolysable tannins are classified by a central carbohydrate core with a number of phenolic carboxylic acids bound by ester linkages (Mueller-Harvey, 2006).

Condensed tannins (proanthocyanidins) are the most common type of tannins found in legumes (Table 2.5), trees and shrubs (Makkar, 2003). Condensed tannins have no carbohydrate core; instead they are derived from the condensation of flavonoid precursors without the enzymes being involved (Hagerman and Butler, 1981). Different strategies have been use to mitigate the deleterious effects of tannins in legumes and sorghum grains, with heating, drying and soaking in water, acid and alkali (sodium hydroxide) and the use of polyvinyl-pyrrolidine (PVP), polyethylene glycol (PEG) and ferric salts (Barry *et al.,* 1999; Dube *et al.,* 2001). The major constraints to alkali, oxidizing agents and metal ions, is that it can results in huge loses of soluble nutrients and if improper managed, can be poisonous to animals (Vitti *et al.,* 2005).

Table 2.5: Content of total extractable phenolics, total extractable tannins and total condensed tannins, soluble and bound tannins in *Acacia tortilis* leaves

Phenolics	Concentration g/kg DM	SEM
Total extractable phenolics	241	8.8
Total extractable tannins	226	8.7
Total condensed tannins	77.8	1.46
Soluble condensed tannins	18.9	1.46
Protein bound condensed tannins	37.5	1.60
Fibre bound condensed tannins	21.5	1.55

Sources: Dube *et al.* (2001); Rubanza *et al.* (2005)

2.8 Detrimental nutritional effects of tannins in pigs

Tannins-rich diet is associated with negative impact in pig production, reduction in feed intake, dry matter and protein digestibility and daily live weight gain (D'Mello, 1995; Mueller-Havey, 2006; Halimani *et al.,* 2007). Condensed tannins complex with various molecules, including proteins, carbohydrates, nucleic acids and minerals (Min *et al.,* 2003). Complexes of nutrients mainly occurred through hydrophobic / hydrogen interaction (Hagerman and Butler, 1981). Tannins may reduce intake of forage legumes by decreasing palatability or by negatively reducing digestibility of nutrients (Barry *et al.,* 1999; Reed, 2003). Astringency is the sensation caused by the formation of complexes between tannins and salivary glycoprotein. Astringency may increase salivation and reduce palatability (Makkar, 2003).

2.9 Physiological adaptation of pigs to *Acacia* leaf meal diets

2.9.1 Production of tannin-binding salivary proteins

Two families of salivary proline rich protein (PRPs) and histatins are widely recognized due to their ability to bind tannins (Shimada, 2006). These PRPs vary in terms of molecular size, amino acid composition structure, and taxonomic occurrences in mammals (Mehansho *et al.,* 1987; Shimada, 2006). Animals normally consuming tannin-rich feeds appear to develop defensive mechanisms against tannins (Makkar, 2003). According to Mueller-Harvey (2006) in some animals, the salivary PRPs are thought to be the first line of defense against tannins, moreover these protein tend to precipitate only those tannins that are usually available in their diet, which suggest evolutionary link between the specificity of these salivary protein and dietary tannin structures. Proline-rich protein is known to be the prevalent group of proteins in the saliva of mammals (Makkar, 2003; Shimada, 2006). The molecular weight of PRP may range from 5000 to 25000 (Bennick, 1982). As their name describes, the amino acids composition of PRP is unique, proline being the highest constituents of PRP (Mehansho *et al.*, 1987).

Histatins are a group of relatively small proteins with high affinity to tannins, and the molecular weight is very small less than 5000. Histatins are characterized by high levels of histadine (Shimada, 2006). The significance of histatins in adaptation to tannins by livestock is not well understood (Makkar, 2003). Apart from the production of PRP, high fibre content in pigs may induce the development of gut capacity to consume fibrous diet (Phuch and Lindberg. 2000).

2.9.2 Production of liver enzymes

Tannins can be absorbed in the small intestine and in turn liver will secrete enzyme uridine diphoshpate glucoronyl transferase (UDPGTA) and glutathionine s-transferase which detoxifies tannins poisons (Muller-Harvey *et al.,* 1986 and Makkar, 2003). Halimani *et al*. (2005) demonstrated a high positive correlation between ytterbium-precipitable phenolic content of *Acacia nilotica* diet with the activity of hepatic microsomal on pigs.

Alanine transferase, ALP and aspartate aminotransferase (AST) are known to increase in the blood with a severe liver damage (Silanikove and Tomkin, 1996). The effect of *Acacia* leaf meal on the concentration of alanine aminotransferase (ALT), AST and ALP in pigs has never been studied. Previous report (Ganti, 1979) stated that ALP activity can be utilized to assess the health of the liver as it owes its origin to the osteoblasts and some of it is normally excreted in the bile. The liver enzymes such as ALT and AST are an indicator of hepatic degeneration and necrosis, with the presence of inflammatory cells in the tissues (Nworgu *et al.,* 2007). Increased in the level of AST, ALT and ALP has been reported in pigs fed of varying levels up to 300 g/kg of *Tithonia diversifolia* leaf meal (Fasuyi *et al.,* 2013). There is a wide range of literature on reference range of serum blood metabolites. However, the concentration also varies with laboratory. Table 2.6 shows selected liver enzymes and nutritionally-related blood metabolites.

2.10 Effect of *Acacia* leaf meal-based diet on internal organs

Few, if any, information is available on the effect of *Acacia* leaf meal-based diet on relatively weight of internal. Leaf meal-based diet is known to depressed feed intake and growth rate (D'Mello, 1995), thus resulting in more lean body. Lean body weight is known for high blood circulation which induces greater heart muscular development. Increased heart weight has been reported in chickens fed on leaf meal-based diet (Kerr *et al,* 1995). Pancreatic hypertrophy has been reported in chickens fed high levels of sorghum containing tannins (Nyachoti *et al.,* 1996). The increased in size of pancreas is induced by the need to secrete enzymes which are bind by tannins (Nyachoti *et al.,* 2006). Tannin rich diets may result in hepatotoxicity (Wells *et al.,* 1942; Korpassy, 1981).

Table 2.6: Reference range for liver enzymes and nutritionally- related blood metabolites in pigs

Property	Concentration
Alanine aminotransferase (U/L)	22-47
Aspartate aminotransferase (U/L)	15-55
Alkaline phosphatases (U/L)	41-176
Serum total protein (mg/ dL)	5.8-8.3
Serum phosphorus (mg/ dL)	5.5-9.3
Total cholesterol (mg/ dL)	81-134
Urea nitrogen (mg/ dL)	8.2-25

Source: Latimer *et al*. (2003).

Kidney weight has been reported to increase with elevated inclusion levels of leaf meal in pig diets, probably due to higher nitrogen content of leaf meal, increasing the nitrogen circulation in the body and the catabolism of nitrogenous compounds, therefore increasing the work load of the kidneys leading to an increase in size (Fasuyi *et al*., 2013). Theoretically, changes in organs sizes can have a profound implication on energy metabolism and possibly, therefore, on body composition (Agyekum *et al.,* 2012).

2.11. Summary

Inadequate feed supply is an important constraint in small-holder pig production. Information regarding *A. tortilis* as an alternative source of protein in pig diet is limited. There is a need, therefore, to determine the feed intake, performance parameters, nutritional related metabolites and metabolic response of pigs fed *A. tortilis* leaf meal diet. The broad objective of the present study is to determine the feed intake, growth performance, nutritional related blood metabolites and the liver enzymes secretion of pigs fed on incremental levels of *A. tortilis* leaf meal diet.

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Chapter 3: Nutritive value of *Acacia* species and response in growth performance of pigs fed on incremental levels of *Acacia tortilis* leaf meal-based diets

Abstract

The objectives of the current study were to evaluate nutritive value of *Acacia* leaf meals and to determine the response in growth performance of finishing pigs fed on incremental levels of *Acacia tortilis* leaf meal. Thirty finishing male F_1 hybrid (Landrace × Large White) pigs with an initial weight of 60.6 (s.d. = 0.95) kg were randomly allotted to six dietary treatments containing 0, 50, 100, 150, 200 and 250 g/kg DM inclusion levels of *A. tortilis* leaf meal. Each treatment diet was offered *ad libitum* to five pigs in individual pens for 21 days. Average daily feed intake (ADFI), average daily gain (ADG) and gain: feed ratio (G:F) was measured every week. There was an increase in both ADFI and ADG ($P < 0.001$) as *A. tortilis* leaf meal increased, before they started to decrease. The G:F ratio was linearly reduced ($P < 0.001$) with incremental levels of *A. tortilis* leaf meal in the diets. Using piecewise regression (broken-stick analyses), it was observed that *A. tortilis* leaf meal can be included up to 125, 129 and 137 g/kg DM in finisher pig feeds without negatively affecting ADFI, G:F and ADG, respectively. The ability with which pigs utilize leaf meal-based diets improves with duration of exposure to such diets.

Keywords: feed intake, tannins, bulk content, weight gain, optimum inclusion levels

3.1. Introduction

A majority of livestock production in tropical countries comes from small scale mixed farming systems in which there is a close association between local animal breeds and local feed resources (Régnier *et al.*, 2013). Grain seeds such as maize and soya beans are used as food for humans in Southern Africa which poses a serious competition between pigs and humans (Halimani *et al.,* 2005). The demand for cereals to feed the ever-growing human population in Southern Africa makes it imperative to identify alternative feedstuffs for feeding pigs. Leguminous leaf meal inclusion in pig diets can reduce the soybean proportion in conventional pig diets (Zakayo *et al.*, 2000; Huy and Phuc, 2006). *Acacia* leaves, for example, have a relatively high crude protein (CP), favorable mineral concentration (Palmer, 1977; Abdulrazak *et al.*, 2000; Rubanza *et al.*, 2005) and thrive in adverse soil and climatic conditions (Palmer, 1979; Heuzé and Tran, 2011). *Acacia* species are a serious encroacher threatening the productivity of grazing land (Nyamukanza and Scogings, 2008), however recently this perception has change due to its contribution as supplementary protein source in tropics (Mapiye *et al.*, 2011).

Despite the high nutritive value of *Acacia* leaves, their utilization in pigs is limited by high levels of fibre and the presence of anti-nutritious factors such as proanthocynidins (Dube *et al.*, 2001; Halimani *et al.*, 2005). Tannins are polyphenolic substances with various molecular weights and of variable complexity (Makkar, 2003). They have several negative post-ingestive effects in animals (Muellar-Harvey, 2006). These effects include depressing feed intake and reducing nutrient digestibility, depending on their quantities and types of tannins consumed.

Condensed tannins concentration in *Acacia* leaves varies with species, stage of maturity and soil type (Abdulrazak *et al.,* 2000; Rubanza *et al.,* 2005). Although some studies have investigated the performance of pigs fed on *Acacia* leaves, (e.g. Halimani *et al.,* 2005), few if any dose-response trials have, however, been conducted to accurately determine the optimum inclusion levels without depressing feed intake and growth performance of pigs. The first objective of this study was, therefore, to evaluate nutritive value of *Acacia* leaf meals. The second objective was to determine the effect of graded *Acacia tortilis* leaf meal on pig performance. It was hypothesized that *Acacia* species have different chemical composition and that the incremental levels of *A. tortilis* leaf meal in pig diets increases performance linearly up to the point where it starts decreasing.

3.2. Materials and methods

3.2.1. Foliage sampling, collection, processing and diet formulation

Eight trees of each of the following five dominant leguminous leaf meals; *Acacia tortilis, A. robusta, A. nilotica, A. nigrescens* and *A. xanthophloea*, were individually hand harvested from the same grazing camp at Makhathini Research Station, Jozini, South Africa. The leaf meals were harvested between April and May, 2013 after the rainy season at advance stage of maturity. The leaf meals were dried separately under shade for three days and passed through a 2 mm sieve to get rid of thorns, pods and twigs. After drying, the leaf meals were immediately bagged and stored in well-ventilated dry room pending analyses of their chemical and physical properties at the Animal and Poultry Science laboratory, University of KwaZulu-Natal (UKZN), Pietermaritzburg, South Africa (RSA).

Following evaluation of nutritional contents and bulking capacities of the leaf meals of *Acacia* species, *A. tortilis* was selected and included in experimental diets at 0, 50, 100, 150, 200 and 250 g/kg (dry matter (DM) basis). Maximum inclusion level was determined by the digestibility estimates of energy and amino acids in *A. tortilis*. Additional vitamins and trace minerals were added to each of the treatment diets, to ensure that these nutrients were non-limiting. Diets were formulated using Winfeed Feed Formulation Software and were rendered isonitrogenous and isoenergetic. The diet that had no leaf-meal was used as the basal control diet. The diets were not supplemented with any antibiotics or growth promoters. The ingredient compositions of the diets are shown in Table 3.1.

Table 3.1: Ingredients composition (g/kg as fed) of the finisher diets containing *Acacia tortilis* leaf meal-based diets

Ingredient	*Acacia tortilis* inclusion level (g/kg DM)					
	0	50	100	150	200	250
Maize	458	416.	374	333	292	250
Wheat bran	356	340	324	308	292	276
Soybean 46	86.0	94.7	103	112	121	129
$_{L}$-Lysine HCL	1.64	1.31	0.98	0.65	0.33	0
$_{DL}$-Methionine	0.38	0.45	0.51	0.58	0.64	0.70
$_{L}$-Threonine	0.97	0.79	0.60	0.42	0.23	0.05
Vitamin-mineral premix	1.50	1.50	1.50	1.50	1.50	1.50
Limestone	20.9	20.1	19.3	18.4	17.6	16.8
Salt	4.98	5.00	5.02	5.05	5.07	5.09
Monocalcium phosphate	9.61	10.1	10.6	11.1	11.5	12.0
Oil-sunflower	60.2	61.7	63.2	64.8	66.3	67.8

3.2.2 Pigs, study site, and housing

The pig performance trial was conducted at Ukulinga Research Farm, UKZN, Pietermaritzburg, RSA. The farm is located in a subtropical hinterland at 29º24'E and 30º24'S with elevation of 775 m. The vegetation around the area is characterized of various tree and grass species that are dominated by *Acacia karroo, Acacia nilotica* and *Acacia sieberiana*. The climate is characterised by mean annual maximum and minimum temperature of 25.7 and 8.9 ^{o}C respectively. During summer there is a mean annual rainfall of 735 mm, whilst light to moderate frost occurs in winter.

The experimental procedures were performed according to the ethical guidelines specified by the Certification of Authorization to Experiment on Living Animals provided by the UKZN Animal Ethics Committee (Reference No: 004/13/Animal). (see Appendix 1).

Thirty pigs with a mean body weight of 60.6 (s.d. = 0.95) kg were used in a completely randomized design. Each pig, representing the experimental unit, was individually penned and randomly allocated *ad libitum* to each of the six dietary treatments for 21 d following an adaptation period of 10 d. All pens used were within a single experimental house that had a single automated heating, lighting and ventilation system. The ambient temperature and relative humidity were maintained at 21. 2 (s.d. = 2.14) ^{o}C and 42.4 (s.d. = 4.13) %, respectively. Each pen was fitted with a pre-weighed bin feeder (Big Dutchman Lean Machine©, Postfach). Drinking water was provided *ad libitum* through low pressure nipple drinkers that were fitted on the opposite side to the feeder.

3.2.3. Chemical analyses of *Acacia* leaves and diets

Following milling through a 1 mm sieve, experimental diets and leaf meal samples from each of the eight trees of the same species were pooled as unique samples and analyzed in triplicates for their chemical and physical properties. The dry matter, ash, crude protein and ether extract were determined according to the following method of AOAC (1995); 945.15, 942.05, 979.09 and 920.39, respectively. The nitrogen contents of the samples were determined using the Dumas Combustion method in a Leco Truspec Nitrogen Analyser, St Joseph MI, USA. The neutral detergent fibre (NDF) and acid detergent fibre (ADF) were analysed using ANKOM Fibre Analyser (Ankom Macedon, NY, USA) according to Van Soest *et al.*, (1991) The NDF was assayed using heat stable α-amylase (Sigma A3306; Sigma Chemical Co., St. Louis, MO, USA). Both ADF and NDF were expressed with residual ash content. Samples for mineral analyses were ashed at 550 ^{o}C for 6 hours and the ash was dissolved in 1 M HCl (Abdou *et al.*, 2011). Mineral contents were detected using Varian 720 Inductively Coupled Plasma Emission Spectrometer (ICP-OES, Frankfurt, Germany). Condensed tannins were estimated by calorimetrically by the butanol-HCL method (Reed *et al.*, 1982). Water holding capacity (WHC) was determined according to Whittemore *et al*. (2003). The swelling capacity was determined according to the method described by Canibe and Bach-Knudsen (2002). The chemical and physical properties, and mineral contents of the diets are shown in Table 3.2 and Table 3.3, respectively.

Table 3.2: Physicochemical properties of the experimental diets

Item	*Acacia tortilis* inclusion level (g/kg DM)					
	0	50	100	150	200	250
DM	956	957	966	964	966	969
GE	17.4	17.3	17.3	17.2	17.3	17.2
Ash	68.6	106	124	152	175	194
CP	162	158	157	161	165	182
EE	109	90.3	92.8	91.9	98.2	89.1
ADF	137	138	125	127	166	171
NDF	346	344	307	334	337	323
ADIN	14.3	12.6	13.0	15.0	15.9	17.8
NDIN	16.5	19.1	19.4	21.3	24.4	22.6
Lysine (g/kg DM)	2.86	3.06	1.83	2.93	2.38	2.81
Threonine	3.28	1.99	1.80	2.23	1.93	2.03
Methionine	1.00	0.65	1.06	0.55	0.61	0.61
CT	0	2.61	5.22	7.71	10.3	12.9
SWC	2.78	2.76	2.81	2.74	2.76	2.91
WHC	3.26	3.33	3.47	3.71	4.05	4.39

DM - dry matter (g/kg); GE- gross energy; Ash (g/kg DM); CP-crude protein (g/kg DM); EE-ether extract (g/kg DM); ADF- acid detergent fibre (g/kg DM) NDF- neutral detergent fibre (g/kg DM);; ADIN - acid detergent insoluble nitrogen (g/kg DM), NDIN - neutral detergent insoluble nitrogen (g/kg DM), CT - condensed tannins (mg/kg DM);; SWC = swelling capacity (ml/g DM); WHC- water holding capacity (g_{water}/g_{feed} DM)

Table 3.3: Mineral composition of the experimental diets

Item	*Acacia tortilis* inclusion level (g/kg DM)					
	0	50	100	150	200	250
Ca	10.9	16.0	16.7	16.3	23.0	26.8
P	7.41	10.9	12.7	10.6	19.5	22.6
Ca:P	1.52	1.51	1.33	1.53	1.21	1.21
Mg	2.73	2.31	2.6	2.52	3.01	3.01
K	9.42	8.35	10.3	10.2	11.4	11.1
Na	1.92	5.64	10.1	13.1	15.5	17.8
Zn (mg/kg)	91.1	73.2	81.2	85.2	70.0	74.2
Cu (mg/kg)	9.12	7.24	9.12	8.14	7.00	7.23
Mn (mg/kg)	124	110	123	127	125	132
Fe (mg/kg)	140	386	369	473	540	590

3.2.4. Growth performance

Performance variables were determined every week for an experimental period of 21 d following an adaptation period of 10 d. Weekly feed intakes (WFI) for each pig were determined by determining the difference between the weight of the feeder bin at the beginning and end of each week. Weekly feed intake was divided by seven to determine average daily feed intake (ADFI) for each week. Pigs were weighed every week. Average daily gain (ADG) was determined by dividing the differences between body weight at the beginning and the end of each week by seven. The gain: feed ratio (G: F) for each pig was determined by dividing ADG by ADFI.

3.2.5. Statistical analyses

Data on chemical and physical properties of the leaf meals individually harvested from each of the eight *Acacia* species were analysed using the General Linear Model (GLM) procedure of SAS (2008) that accounted for the effects of the species as the main factor. Each tree was regarded as the experimental unit. Comparison of the means was performed using the PDIFF procedure (SAS, 2008). Differences among means were considered significant when $P < 0.05$.

Performance variables were analysed using a mixed model procedure to consider repeated measures done every week. The first-order autoregressive correlation (AR [1]) was fitted to model on the week of successive feeding. The model countered for the effects of inclusion level of *A. tortilis* leaf meals, week of feeding and their interactions on ADFI, ADG and G:F. The pig was the experimental unit. The initial body weight of each pig was used as a covariate and excluded from the model. The regression model (PROC REG) procedure of SAS (2008) was used to determine the relationships between each of performance parameters with inclusion level of *A. tortilis* leaf meal during each week of successive feeding. The piecewise regression (broken-stick) analysis was conducted using NLIN procedure (SAS, 2008) to estimate the threshold value at which the inclusion level of *A. tortilis* leaf meal causes the ADFI, ADG and G:F ratio to be constant or to decrease as the incremental levels increased. The model used was as follows:

$Y_i = \gamma_o + \gamma_1 + \gamma_2 (Ix_c) (x_i - x_c) + \varepsilon_i$,

Using parameters (γ_o, γ_1, γ_2) and the x_c, the two segmented simple regression functions where;

$Y_j = \gamma_o + \gamma_1 (x_i)$, for $x_i \leq x_c$; and

$Y_k = Y_o + (\gamma_1 + \gamma_2) x_i$, for $x_i \geq x_c$

Where;

Y_i is the response variable when *A. tortilis* leaf meal inclusion level is constraining performance;

Y_j is the response variable before *A. tortilis* leaf meal inclusion level constrains performance;

Y_k is the response variable when *A. tortilis* leaf meal inclusion level exceeds the optimum inclusion level;

$Y_o = \gamma_o - \gamma_2 x_c$; when $x_i = 0$;

γ_o is the intercept or minimum Y_i when $x_c < 0$;

γ_1 is the rate of change of Y_i when $x_i < x_c$;

γ_2 is the rate of increase in Y_i when $x_i > x_c$;

x_i is the inclusion level of *A. tortilis* in the diet;

x_c is the optimum level of inclusion beyond which performance is constrained by increase in *A. tortilis* leaf meal in the diets; and

Ix_c is a dummy variable with value 0 when $x_i < x_c$ and 1 when $x_i \geq x_c$. All measurements were considered significant at $P < 0.05$.

3.3. Results

3.3.1. Nutritional composition of *Acacia* species

The physicochemical properties and mineral concentrations in five common *Acacia* species are shown in Tables 3.4 and 3.5, respectively. *Acacia tortilis* and *A. xanthophloea* leaf meals had the highest CP and *A. tortilis* had the highest fat content ($P < 0.01$) among all the *Acacia* species. The NDF and ADF varied significantly across *Acacia* species ($P < 0.05$). *Acacia robusta* had significantly the highest levels of non-structural carbohydrates, whilst *A. tortilis* had the lowest. *Acacia nigrescens* had high levels of ADIN and lower levels of NDIN whilst *A. nilotica* and *A. tortilis* had significantly high levels of NDIN. *Acacia nilotica* had significantly highest levels of proanthocyanidins, followed by *A. robusta*, *A. xanthophloea*, and then *A. tortilis* and lastly *A. negriscens*. *Acacia tortilis* had the lowest WHC whilst *A. xanthophloea* and *A. nigrescens* had the highest capacity to bind water ($P < 0.01$). High SWC (Table 3.5) was observed in *A. xanthophloea*, followed by *A. nigriscens*, *A. robusta*, *A. tortilis* and then *A. nilotica* ($P < 0.05$). *Acacia tortilis* had significantly high levels (23 g/kg DM) of P.

Table 3.4: Physicochemical properties of five common *Acacia* species

Acacia species	DM	Ash	CP	EE	NDF	ADF	ADIN	NDIN	CT	WHC	SWC
A. nilotica	947^{ab}	56.5^{b}	198^{b}	32.7^{bc}	399^{d}	227^{d}	18.5^{c}	32.4^{a}	67.7^{a}	6.44^{b}	4.57^{a}
A. robusta	950^{a}	82.7^{a}	160^{d}	30.5^{dc}	455^{c}	279^{c}	20.4^{b}	27.9^{b}	64.4^{a}	6.49^{b}	5.59^{c}
A. xanthophloea	943^{c}	87.6^{c}	216^{a}	33.8^{b}	471^{c}	304^{b}	20.1^{b}	32.0^{a}	59.5^{a}	7.99^{d}	6.30^{d}
A. tortilis	944^{b}	65.0^{d}	218^{a}	40.1^{a}	494^{b}	298^{b}	18.3^{c}	27.4^{b}	51.5^{b}	6.06^{a}	4.78^{b}
A. nigrescens	938^{c}	78.3^{e}	178^{c}	30.0^{d}	630^{a}	477^{a}	23.7^{a}	27.0^{b}	40.5^{c}	6.44^{d}	5.68^{c}
SEM	1.22	1.5	8.0	7.7	58.6	54.7	3.4	9.1	1.54	0.08	0.04
Significance level	***	***	***	***	***	***	***	**	***	**	*

^{abc} Values in the same column with the same superscript are not significantly different ($P > 0.005$); SEM = standard error of means;

Level of significance (* = $P < 0.05$; ** = $P < 0.01$; *** = $P < 0.001$);

DM - dry matter (g/kg); Ash (g/kg DM); CP-crude protein (g/kg DM); EE-ether extract (g/kg DM); NDF- neutral detergent fibre (g/kg DM); ADF- acid detergent fibre (g/kg DM); ADIN -acid detergent insoluble nitrogen (g/kg DM), NDIN - neutral detergent insoluble nitrogen (g/kg DM), CT - condensed tannins (mg/kg DM); WHC- water holding capacity (g_{water}/g_{feed} DM); SWC = swelling capacity (ml/g DM).

Table 3.5: Contents of minerals of five common *Acacia* species

Acacia species	Macro-elements (g/kg DM)					Micro-elements (mg/kg DM)			
	Ca	Mg	K	Na	P	Zn	Cu	Mn	Fe
A. nilotica	14.9^b	4.9^a	16.0c	1.1^b	15.0^b	12.0^b	4.0^c	30.3^d	261^a
A. robusta	21.4^a	3.4^b	13.0^d	0.9^c	13.0^c	12.0^b	5.0^b	49.0^b	158^d
A. xanthophloea	12.0^c	3.1^c	18.0^a	3.7^a	22.0^a	19.3^a	4.0^c	49.0^b	255^a
A. tortilis	9.6^e	3.0^d	17.3^b	0.4^d	23.0a	19.0^a	2.0^d	35.0^c	178^c
A. nigrescens	11.5^d	3.5^b	10.6^e	0.1^e	15.0^b	12.7^b	7.0^a	61.7^a	242^b
SE	8.0	3.0	9.0	5.0	2.0	3.33	1.1	3.33	28.67
Sig. level	***	**	***	*	***	**	***	*	***

Ca = calcium, Mg = Magnesium, K = potassium, Na = sodium, P = Phosphorus, Zn = Zinc, Cu = Copper, Mn = Manganese, Fe = Iron.

SE = standard error; sig. level = level of significance, * = $P < 0.05$; ** = $P < 0.01$; *** = $P < 0.001$

[abc] Values in the same column with the same superscript are not significantly different (P > 0.005).

Acacia nilotica had the highest ($P < 0.001$) concentration of Fe, but it had the lowest ($P < 0.01$) level of Mn.

3.3.2. Pig performance

The responses of performance to incremental levels of *A. tortilis* leaf meals in the diets during each week of successive feeding are illustrated in Table 3.6. During the first week of feeding, quadratic decreases in ADFI ($P < 0.006$) and ADG ($P < 0.0279$) were observed with increased levels of *A. tortilis* leaf meal in the diets. As the inclusion levels were increased they caused linear decreases ($P < 0.0119$) in ADFI during Week 2 as well as for ADG in Week 2 and 3, respectively. However, during the whole experimental period, increases in ADFI ($P < 0.0007$) and ADG ($P < 0.001$) were observed before they started decreasing with further increase in *A. tortilis* leaf meals in the diets. Although incremental levels of the leaf meals linearly decreased feed efficiency during Week 1 and 2, a quadratic decrease in G:F was observed during the third week of feeding. An increase in *A. tortilis* leaf meal levels in the diets caused a quadratic decrease ($P < 0.01$) in gain: feed ratio during the experimental period.

Table 3.7 shows broken stick analyses indicating the optimum inclusion levels of *A. tortilis* leaf meal beyond which the maximum performance parameters were attained. The maximum ADFI and ADG were reached at 125 ± 19.4 and 137 ± 12.1 g/kg DM of *A. tortilis* inclusion level, respectively ($P < 0.05$). The maximum G: F ratio was attained at 129 ± 10.7 ($P < 0.01$). The maximum inclusion levels of 125, 129 and 137 g/kg contain 6.4, 6.6 and 7.1 g/kg DM of condensed tannins, respectively.

Table 3.6: Performance of pigs fed on incremental levels of *A. tortilis* leaf meal-based diet

Variable		*Acacia tortilis* inclusion level (g/kg DM)							*P*-value			Regression coefficient		
	Week	0	50	100	150	200	250	SEM	Diet	Week	D×W	Linear	Quadratic	*P*-value
ADFI	1	2.02	3.18	2.54	2.64	1.85	1.35	0.212	0.008	0.001	0.005	0.207	-0.0351	<0.006
	2	2.91	3.22	3.06	2.99	2.42	2.22	0.212	0.011	<0.001	0.004	-0.169		0.0119
	3	3.22	2.84	2.73	3.45	2.78	2.52	0.212	0.001	0.045	0.015	0.083	-0.012	*NS*
	Overall	2.72	3.08	2.78	3.02	2.35	2.03	0.151	<0.0001	<0.0001	0.021	0.460	-0.088	<0.0007
ADG	1	0.626	0.709	0.737	0.523	0.406	0.336	0.059	0.001	0.002	0.016	0.122	-0.018	<0.0279
	2	0.720	0.844	0.826	0.623	0.429	0.387	0.059	0.028	0.035	0.011	-0.111		<0.0001
	3	0.845	0.921	0.903	0.708	0.497	0.414	0.059	0.002	0.046	0.035	-0.107		<0.0001
	Overall	0.714	0.860	0.853	0.559	0.442	0.387	0.051	<0.0001	<0.0001	0.003	0.113	-0.020	<0.0001
G:F	1	0.461	0.342	0.336	0.383	0.266	0.362	0.039	0.0333	0.00159	0.001	-0.019		<0.0001
	2	0.415	0.333	0.307	0.288	0.334	0.286	0.039	0.0456	0.0487	0.015	-0.019		0.0213
	3	0.452	0.430	0.381	0.315	0.334	0.323	0.039	0.0125	0.0365	0.017	-0029	-0.004	<0.001
	Overall	0.443	0.368	0.341	0.329	0.308	0.324	0.023	0.0008	0.0189	0.049	-0.023	-0.003	<0.0007

ADFI - average daily feed intake (kg/d DM); ADG - average daily gain (g BW/d); BW - Live body weight; G:F- gain to feed ratio

SEM - standard error of mean; D×W – interaction effect of the diet and week of feeding.

Table 3.7: Optimum *A. tortilis* inclusion level marking threshold values when performance variables are constrained

Variable	γ_o		γ_1		γ_2		x_c	
	Estimates	*P-value*	Estimates	*P-value*	Estimates	*P-value*	Estimates	*P-value*
ADFI	2.78 (±0.108)	*	0.003(±0.001)	**	-0.016(±0.004)	**	125 (±19.4)	***
ADG	0.84(±0.041)	***	0.001(±0.001)	*	-0.007(±0.001)	***	137(±12.1)	**
G: F	0.49(±0.022)	**	0.001(±0.001)	**	-0.004(±0.0004)	*	129(±10.7)	*

ADFI = average daily feed intake (kg/d DM); ADG = average daily gain (g BW/d); BW = Live body weight; SEM = standard error of mean; *S*ignificance level (* = $P < 0.05$; ** = $P < 0.01$; *** = $P < 0.001$).

$Y_i = \gamma_o + \gamma_1 + \gamma_2 (Ix_c)(x_i - x_c) + \varepsilon_i$, when $(x_i > x_c) = 1$.

Where;

Y_i is the response variable when *A. tortilis* leaf meal inclusion level is constraining performance;

γ_o is the intercept or minimum Y_i when $x_c < 0$;

γ_1 is the rate of change of Y_i when $x_i < x_c$;

γ_2 is the rate of increase in Y_i when $x_i > x_c$;

x_c is the optimum level of inclusion beyond which performance is constrained by increase in *A. tortilis* leaf meal in the diets; and

Ix_c is a dummy variable with value 0 when $x_i < x_c$ and 1 when $x_{i \geq} x_c$. All measurements were considered significant at $P < 0.05$.

3.4 Discussion

Feed compounders are continuously searching for alternative feed formulation strategies that improve the nutrition and welfare status of pigs. The need for alternative feed ingredients is exacerbated by the ever increasing growing feed prices. Furthermore, cconventional diets predispose pigs to gut health problems, impair animal well-being and escape the gastrointestinal tract (GIT), thereby causing nutrient losses to the environment (Aarnink and Verstegen, 2007; Bindelle *et al.*, 2008; Jorgensen *et al.*, 2010). Leguminous leaf meals obtained from *Acacia* species namely *A. nilotica*, *A. robusta, A. tortilis, A. xanthophloea* and *A. nigrescens* are potential substitutes for protein ingredients and they can also improve the dietary fibre content in pig feeds. The level of inclusion of these tannin-rich leaf meals should not be such that the pig is prevented from consuming sufficient nutrients that are required for it to attain its potential growth. Little, if any information on the nutritive value, proanthocyanidins concentrations and bulking properties of leguminous leaf meals from above-named species is available.

Characterisation of chemical composition and physical properties of *Acacia* leaf meals is vital in designing intervention formulation strategies to ensure that anti-nutritional factors namely proanthocyanidins and bulking properties (NDF, ADF, SWC and WHC) does not constrain performance. Although the leaf meals in the current study were harvested from the same grazing camp during the same season, the observation that *A. tortilis* had the highest protein content could be attributed to genotypic difference. The difference in WHC and SWC of the leaf meals could be ascribed to the fact that exposure of hydrophilic binding sites within the fibre matrix is unique for each species principally because of the difference in fibre types and the arrangement of polysaccharide building block forming the structure of those feedstuffs (Elleuch *et al.*, 2011).

The finding that *A. tortilis* had comparatively lower bulking properties such as WHC and SWC therefore, suggest that it is likely to constrain intake of sufficient nutrients required for growth at a low severity than other leafs such as *A. robusta, A. xanthophloea and A. nigrescens*. The high CP content, moderate ADF, NDF and proanthocyanidins composition of the leguminous forages in the current study concur with earlier reports (Abdulrazak *et al.,* 2000; Dube *et al.,* 2001; Rubanza *et al.*, 2005). Dietary inclusions of leaf meals with high protein levels and lower bulking capacities implies that intake will not be suppressed and pigs are likely to consume more of the nutrient required for potential growth with minimum constrain on the gut capacity. All the forages were harvested in the same environment at their advanced stage of maturity after the rainy season. The differences indicate that the nutritive value of *Acacia* species has a huge influence on inclusion levels in pig diets. The observed concentrations of proanthocyanidins were expected to limit the utilization of *Acacia* leaf meal.

The characterization of these leaf meals as sources of proteins opens way for exploration in feeding pigs. *Acacia tortilis* was the most favourable for its highest CP content, moderate NDF and ADF as well as its lowest levels of proanthocyanidins compare to all species. Although leaf meals from *A. nilotica* and *A. negrescens* had lower bulking properties in terms of their SWC and NDF content and CT content, respectively, *A. tortilis* was selected for use during feed formulation due to its wide distribution (abundance) and easiness of harvesting, which makes it an ideal ingredient to potentially decrease feed costs. Feed costs constitute the major part of the production costs of pork (Hermesch *et al.*, 2003). An optimum inclusion level of *A. tortilis* leaf meal in pig diet has not been reported. Therefore, *A. tortilis* leaf meal was included at varying inclusion level up to 250 g/kg, through a dose-response trial such that the break point at which performance parameters are constrained can be identified. The maximum inclusion level of *A. tortilis* in the current study was determined using the formulation program which accounted for the proximate analysis results of the leaf meals as well as the digestibility of amino acids. Therefore, the treatment diets were formulated to have similar levels of CP and energy, however, an unexpected slightly increase in protein content at higher inclusion of *A. tortilis* (250 g/kg) could be attributed to the fact that CP contribution of the *A. tortilis* was ignored during formulation. As expected, there was an increase in ADF, Ca, P, Mg and Fe content as the inclusion level of the leaf meals were increased.

All pigs were generally clinically health throughout the trial. The initial increase in overall ADFI of *A. tortilis* leaf-based diets suggest that pigs increased consumption to complement limiting nutrients due to the binding of nutrients by the tannins (MacDonald *et al*., 1995; Lee *et al.*, 2010; Stukelj *et al.*, 2010). Increase in overall ADFI as *A. tortilis* leaf meal inclusion level increased could also be explained by the increase in bulkiness of the diets. Increase in feed bulk prompts the pig to fail to meet its nutritional requirements for growth (Ndou *et al.*, 2013). Therefore, the consumption of more feed is an attempt to compensate for limiting effects of bulking properties such as ADF, WHC and SWC (MacDonald *et al.*, 1995). These observations concurs with findings from previous studies by D'Mello (1995) and Cappai *et al.* (2010), that low levels of leaf meal diets (100 g/kg DM) may not depress intake. The observation that there was a general increase in intake during each week of successive feeding could have been caused by increase in body weight and concurs with previous reports by Whittemore *et al.* (2003). For pigs with a weight less than 120 kg live weight, intake is directly proportional to body weight as the gut will be developing to have a capacity to accommodate more feed to meet the requirements for potential growth (Tsaras *et al.*, 1998; Whittemore *et al.*, 2003; Ndou *et al.*, 2013).

Further increase in leaf meal inclusion in diets reduced ADFI suggesting that pigs could not to cope with the deleterious effect of the tannins and the bulking capacity. Using the broken-stick model, the observation that the optimum inclusion level before consumption of feed is constrained at 125 ± 19.4 g/kg DM indicates that if *A. tortilis* leaf meal is included beyond these levels, they have potential to reduce feed intake. Negative effects of leaf meals above 100 g/kg DM inclusion level in ADFI of pigs fed on leaf meal-based diets in pigs has been demonstrated by many authors (Reed *et al.,* 1982; Ben Salem *et al*., 2001; Halimani *et al*., 2007). Tannin-rich diets cause astringent sensation in the mouth thereby inducing negative feedback prompting pigs to reduce ingestion (Reed, 1986). Furthermore, continuous increase in *A. tortilis* leaf meal elevates the capacity of the feed to hold water and swell, thereby reducing the gut capacity of the pigs to consume more feed (Bindelle *et al.,* 2008; Ndou *et al.,* 2013). The finding that as leaf meal inclusion levels exceeded optimum levels, the rate of change of intake decreases at slower rates with each week of successive feeding indicates that pigs were developing mechanisms to cope with the anti-nutritional factors in the diets. Although these were not measured in the current study, it has been repeatedly mentioned that pigs develops internal organs to cope with anti-nutritional factors namely condensed tannins and bulking properties (ADF, NDF, SWC and WHC) (Nyachoti *et al.*, 2004; Halimani *et al.*, 2005; Cappai *et al.*, (2010).

The observed initial weight gains followed by reduced ADG with inclusion level of *A. tortilis* suggest that pigs tolerate diets with moderate proportions of leaf meal. Apart from that these findings also indicates that at low inclusion levels, tannins did not complex protein required for metabolism in order to meet potential growth. The bulking capacities of the diets in terms of their fibre content, WHC and SWC at low inclusion levels of the leaf meals below the optimum could have permitted consumption of adequate nutrients required for potential growth. Furthermore, moderate inclusion levels of leguminous leaf meals in pig diets induce desirable levels of phenolic compounds that increase feed intake as well as acting as growth promoters (D'Mello, 1995; Halimani *et al.,* 2005; Štukelj *et al.*, 2010).

The differences in rates of weight gain during each week of successive feeding in the present study could be attributed to the fact that as animals grows their ability to utilize tanniferous and fibrous diets is enhanced with continuous exposure to such feeds (Maasdorp *et al.,* 1999). In particular, there was an initial increase in weight gain and then a quadratic reduction as more leaf meal was added but in Week 1 weight gain decrease linearly in Week 2 and 3, which suggests that the adaptation of pigs to leaf meal based diets needs to be prolonged. Our suggestions are supported by postulations by that adaptation of steers to *A. karroo* leaf meal based diets should be lengthened from 21 to 35 days (Kaitho *et al.*, 1997; Mapiye *et al.*, 2009). In addition, the observations that rate of decrease in weight gain was lower in Week 3 than in Week 2 could also be ascribed to the fact that the gut capacity of growing pigs and their ability to digest dietary fibre improves with age. The finding that the rates of decrease in weight gain were more pronounced in Week 2 than in Week 3 further confirms the ability of pigs to utilize tannin-rich diets more efficiently with age (Makkar, 2003; Mapiye *et al*., 2011). Apart from that, the increases in weight gain during each week of successive feeding could be also attributed to the increase in that sizes of the internal organs, another possible mechanism with which pig adapt to as a way of improving their capacity to metabolize anti-nutritional factors in the diets (Nyachoti *et al.*, 2004; Bindelle *et al.*, 2008). Future research should investigate the chances of compensatory growth of pigs following diet sequencing from a high to low tannin-rich diets.

There inevitably came a point when ADG was depressed with incremental levels of the leaf meals. The ADG could have decreased due to reduction in digesta transit time and total tract excretion of nutrients induced by higher insoluble fibre content of the leguminous leaf meals (Phuc and Lindberg, 2000). Another plausible explanation for the decrease in ADG as more *A. tortilis* were included pig diets could be the unavailability of proteins as some of it is bound by polyphenols and fibre or by physically entrapped by fibre in the leaf meals (Phuc and Lindberg, 2000). In current study the maximum inclusion level of *A. tortilis* leaf meal for optimum ADG was 137 ± 12.1 g/kg, which contrast findings by D'Mello (1995), Laswai *et al.* (1997) and Halimani *et al.* (2005) who demonstrated a reduced ADG in pigs fed on diet containing more than 100 g/kg DM of leaf meals. In the current study, the inclusion levels were widely distributed to permit dose-response, which is more accurate in predicting maximum amount of leaf meal to be incorporated in finisher pig diet.

The observed increase in the gain: feed ratio before a point when it started to decrease could be ascribed to that the concentration of tannins at low inclusion levels did not bind nutrients. As more *A. tortilis* leaf meal was included in the diets, a break point was attained at 129 ± 10.7, marking the point at which the optimum G: F ratio. Inclusion of *A. tortilis* leaf meal beyond this break point depressed the efficiency at which dietary nutrients are converted to body proteins suggesting that incorporation of leaf meals to excess induced low nitrogen retention (Cheverria *et al.*, 2002). In addition, the decrease in G:F beyond the optimum inclusion level identified in the current study can also be explained by the fact that pigs utilise the fibre fractions of the leaf meals less efficiently through hindgut fermentation (D'Mello, 1995). The rates of decrease in feed efficiency were observed to be pronounced more with the age (week of feeding) of the pigs due to that as animals are exposed to diets with tannins, they develop mechanism either through development of the gut organs or through secretion of enzymes or proteins that promote the process of converting feed to muscle (Halimani *et al.*, 2005; Mapiye *et al.*, 2011). The finding that G:F decreased agrees with previous studies by Halimani *et al*. (2005) and Stukelj *et al*. (2010) and can also be ascribed to masking effect of the fibrous components of the leaf meals that could have hindered exposure of non-fibrous nutrients to enzymatic digestion and absorption (Glisto *et al.*, 1998; Mikkelsen *et al.*, 2004). The lower body weight gain and lower feed intake observed when leaf meal was included above 150 g /kg correspond to the decrease in G:F.

Although the rate of change of ADFI, ADG and G:F followed almost similar trends, they were constrained at different break points and decreased at different rates. The observed quadratic relationships indicate the existence of the potential of finishing pigs to tolerate low levels of tannin contents in the diet. On the other hand, the differences in these break points suggest the existence of different mechanisms with which physicochemical properties of leaf meal-based diets influence each one of the parameter. The best parameter to be considered during feed formulation largely depends on the circumstances of the farm. Therefore, there is need for future research to determine the most appropriate dietary properties influencing growth performance by simultaneous use of leguminous leaf meal sources with a wide range of physicochemical properties.

3.5 Conclusions

Leaves harvested from *A. tortilis, A. robusta, A. nilotica, A. nigrescens* and *A. xanthophloea* could substitute soybean protein source ingredients in pig diets. The concentration of condensed tannins and fibrous properties could limit the optimal exploration of *Acacia* leaves in pig diets. Incorporation of *A. tortilis* leaf meal in pigs' diets improves performance intake up to a point were incremental levels beyond threshold proportions inevitably suppress growth performance. The extent with which pigs efficiently utilize leaf meal based diets improves with continuous exposure to such feeds. The incorporation of moderate levels of *A. tortilis* leaf improves growth performance of pigs. However it is crucial to determine the effect of *A. tortilis* leaf meal-based diet on nutritionally-related blood metabolites, liver enzymes and internal organs of pigs to efficiently explore *A. tortilis* leaf meal without constraining healthy status of pigs.

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Chapter 4: Influence of *Acacia tortilis* leaves on nutritionally-related blood metabolites, liver enzymes and internal organs in pigs

Abstract

The objective of the current study was to determine the effect of incremental levels of *Acacia tortilis* leaf meal on nutritionally-related blood metabolites, liver enzymes and internal organs of pigs. Thirty Large White x Landrace pigs of 60.6 ± 0.94 kg were randomly allotted to six dietary treatments containing 0, 50, 100, 150, 200 and 250 g/kg DM of *A. tortilis* leaf meal. Blood was collected at the end of the experimental period of 21 days for the determination of nutritionally-related blood metabolites and activity of aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatases (ALP). Serum concentrations of Fe, AST and ALP increased quadratically ($P < 0.001$) as *A. tortilis* leaf meal increased. There was a linear increase in ALT activity with increased leaf meal inclusion. Hepatosomatic index (HSI: liver weight/ body weight x100), scaled kidney weight and scaled heart weight increased linearly ($P < 0.001$) as *A. tortilis* leaf meal increased. There was, however, a quadratic increase in the relative weight of lungs ($P < 0.001$) as leaf meal increased. It was concluded that *A. tortilis* leaf meal could be incorporated up to 150 g/ kg without compromising nutritional related blood metabolites, liver enzymes and relatively of internal organs. Elevated inclusion levels of *A. tortilis* leaf meal above 150 g /kg was associated with increased Fe cholesterol, uric acid, enzymes activity and the size of internal organs.

Key words: liver enzymes, tannins, *A. tortilis,* internal organs.

4.1 Introduction

In Southern Africa, indigenous legume trees can be used as protein sources for animals (Dube *et al.,* 2001; Rubanza *et al.,* 2005; Mlambo *et al.,* 2007). There have been numerous work on the use of *Acacia* species as protein supplements for livestock in the tropics (Halimani *et al.*, 2005; Ndlovu *et al.,* 2009; Mapiye *et al.,* 2011), however little, if any, information is available on the use of *A. tortilis* leaf meals as an alternative protein source for pigs. Due to their abundance and the ability to thrive under harsh conditions in the sub-tropics, *A. tortilis* cause bush encroachment. Therefore, if their leaves are incorporated in pig diets, they can assist in increasing grazing capacity as well as being a reliable substitute for the expensive soybean.

The utilization of *Acacia* leaf meal in pig diets is constrained by the presence of condensed tannins and high fiber content. Tannins are polyphenolic substances with relatively high molecular weights (Makkar, 2003). They damage the internal mucosal membranes, compromising the absorption of nutrients from the gut (McLeod, 1974). It is not clear how pigs cope with increased tannins in the feed. Furthermore, inclusion of leaf meals inevitably elevates the dietary fibre content in the diet. Together with tannins, dietary fibre is also likely to impose some concomitant effects on physiological mechanisms with which pigs adjust to changes dietary contents. It has been suggested that internal organs change in response to the dietary tannin and fibre content (Nyachoti *et al.*, 1996; Mashatise *et al.*, 2005; Agyekum *et al.*, 2012). Few, if any, studies have used dose-response experiments to determine the effect of leaf meals on nutritionally-related blood metabolites, liver enzymes activity and the relative weights of internal organs.

Increase of certain blood metabolites, liver enzymes and decrease or increase size of internal organs are conventionally used for diagnosing hepatic damage (Silanikove *et al.*, 1996; Ndlovu *et al.*, 2009). Understanding metabolic response of pigs when fed on tanniferous diets is, therefore, useful in determining the appropriate inclusion levels of tannin-rich leaf meal in finisher pig diet. The objective of the current study was to determine the effect of incremental levels of *A. tortilis* leaf meal on blood metabolites, liver enzyme activity and internal organs in pigs. The hypothesis tested was that as the *A. tortilis* leaf meal increased in the diets, nutritional related blood metabolites, liver enzymes activity and size of internal organs increased.

4.2 Materials and methods

4.2.1 Study site

The study site has been described in detail in Chapter 3.

4.2.2 Pigs and diets

Pigs and diet has been fully described in previous chapter.

4.2.3 Blood sample collection and analyses

At least 10 ml blood was collected through jugular venepuncture in non-coagulated vacutainer tubes from each pig at the end of a feeding experiment (day 21) between 0700 to 0900 h. Blood was allowed to coagulate at room temperature (25 ˚C) and centrifuged for 10 minutes at 1000 x g within two hours of collection. Serum was the stored in polypropylene tubes and kept at -20 ^{0}C, pending analyses. The serum was analysed spectrophotometrically for total protein (TP) (Doumas and Biggs, 1972); phosphorous (P), alkaline phosphatases (ALP) were assayed using the colometric method (Tietz *et al*., 1993). The serum was analysed spectrophotometrically for iron (Fe) (Tietz, 1976). Enzymatic method was used for the determination of cholesterol (Allain *et al*., 1974). Enzymatic method was used for the determination of uric acid (UA) (Tietz, 1995). The ultraviolet method was used for aspartate aminotransferase (AST) (Bergmeyer *et al*., 1986) and alanine aminotransferase (ALT) (Horder *et al*., 1991). The analysis of nutritionally-related blood metabolites and liver enzymes is fully described in Appendix 2.

4.2.4 Collection of internal organs

Following a feed withdrawal period of 12 hours after an experimental period of 21 d, five pigs fed on each of the diets containing 0, 50, 100, 150, 200 and 250 g/kg of *A. tortilis* leaf meal were slaughtered at 89.9, 89.2, 83.4, 83.7, 72.5 and 68.8 (sd. 2.91) kg live weight, respectively. Pigs were electrically stunned (300 V for 3 seconds) with a pair of tongs, shackled by the right leg and exsanguinated while hanging. Carcasses were placed in a dehearer at 62 ºC for 5 min. Remaining hairs were removed using a knife and flame. Carcasses were eviscerated and the livers, kidneys, lungs and heart were removed from each pig and weighed individually using a digital scale. The hepatosomatic index (HSI) was determined by dividing liver weight by body weight at slaughter and expressed as a percentage (Liu *et al.*, 2009). The scaled kidneys weight (SKW), lungs weight (SLW) and heart weight (SHW) were determined by dividing the weights of the kidneys, lungs and heart with slaughter body weight.

4.2.5 Chemical analyses of the diets

Chemical analyses of the diets have been described in Chapter 3.

4.2.6 Statistical analysis

A polynomial regression (PROC REG) procedure of (SAS, 2008) was used to determine the relationship between the measured parameter with inclusion level of *A. tortilis* leaf meal.

4.3 Results

4.3.1 Chemical composition of the diets

The chemical composition and mineral concentrations of diets is shown in Tables 3.2 and 3.3 (Chapter 3). Crude protein ranged from 162.6 to 182.1 g /kg DM. Phosphorus varied from 7.4 to 22.6 g /kg of feed. Iron concentration varied in the diets from 140 to 590 mg /kg of feed.

4.3.2 Nutritionally-related blood metabolites

The influence of feeding graded level of *A. tortilis* leaf meal on nutritionally-related blood metabolites is shown in Table 4.1. There were quadratic increases ($P < 0.001$) in serum Fe and cholesterol level with increase in inclusion levels of *A. tortilis* leaf meals in the diets. As the incremental levels of *A. tortilis* leaf meal were elevated, there was a quadratic decrease (*P* > 0.05) in uric acid level. Total protein and serum phosphorus were not affected by incremental levels of *A. tortilis* leaf meal inclusion.

4.3.3 Liver enzymes activity

Figure 4.1 depicts changes in liver enzymes with increase in leaf meal inclusion. Feeding incremental levels of *A. tortilis* leaf meal in pigs caused quadratic increases in AST (*P* < 0.05) and ALP (*P*< 0.001) activity, respectively, however, the rates of increase were pronounced more in the latter than the former. The ALT increased linearly (*P* > 0.05) with increase of leaf meal inclusion.

4.4.4 Relative weights of liver, kidneys and heart

Effects of inclusion levels of *A. tortilis* leaf meals on scaled kidney weight (SKW), scaled heart weight (SHW), scaled lung weight (SLW) and Hepatosomatic index (HSI) are shown in Figure 4.2. The HIS, SHW and SKW increased linearly (*P*< 0.001) with increased inclusion levels of leaf meal. Corresponding rates of increase for HSI, SKW and SHW were 0.06, 0.09 and 0.17, respectively. A quadratic increase was observed (*P* < 0.001) in scaled lungs weight with incremental levels of *A. tortilis* leaf meal.

Table 4.1: Nutritionally-related blood metabolites of finishing pigs fed on graded level of *Acacia tortilis* leaf meal

Item	*A. tortilis* leaf meal inclusion level (g/kg DM)							Regression coefficient		
	0	50	100	150	200	250	S.E.M	Linear	Quadratic	Sig. Level
Fe (ug/dl)	231	230	235	252	263	288	3.56	-7.65 ± 4.07	2.72 ± 0.57	***
P (mg/dl)	12.9.	12.8	13.1	13.3	13.0	13.1	0.151	0.251 ± 0.08	-0.031 ± 0.01	
UA (mg/dl)	1.11	1.39	1.64	1.47	0.744	0.684	0.032	0.579 ± 0.09	-0.10 ± 0.013	***
TC (mg/dl)	60.8	63.2	65.6	72.4	81.2	85.0	0.821	1.12 ± 1.16	0.58 ± 0.16	***
TP (g/dl)	6.32	6.35	6.35	6.56	6.87	6.36	0.014	0.24 ± 0.09	-0.03 ± 0.01	

Fe = iron, P = phosphorus; UA = uric acid, TC = total cholesterol, TP = total protein, ALP = alkaline phosphatase; SEM = standard error mean; Sig .level = Level of significance.

[abc] Values in the same row with the same superscript are not significantly different (*P*> 0.005).

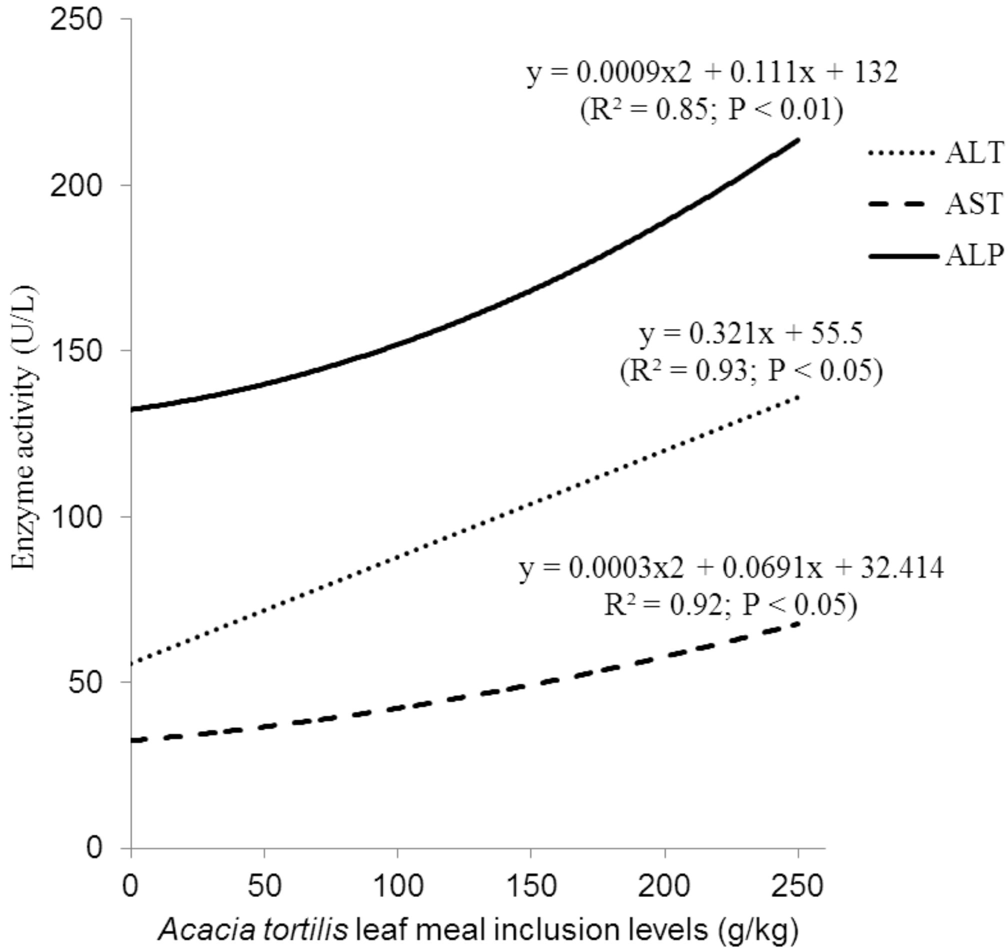

Figure 4.1: Effect of *A. tortilis* leaf-based diet on liver enzymes concentration in finishing pigs

ALT: alanine amino transferase; AST: aspartate aminotransferase; ALP: alkaline phosphatase

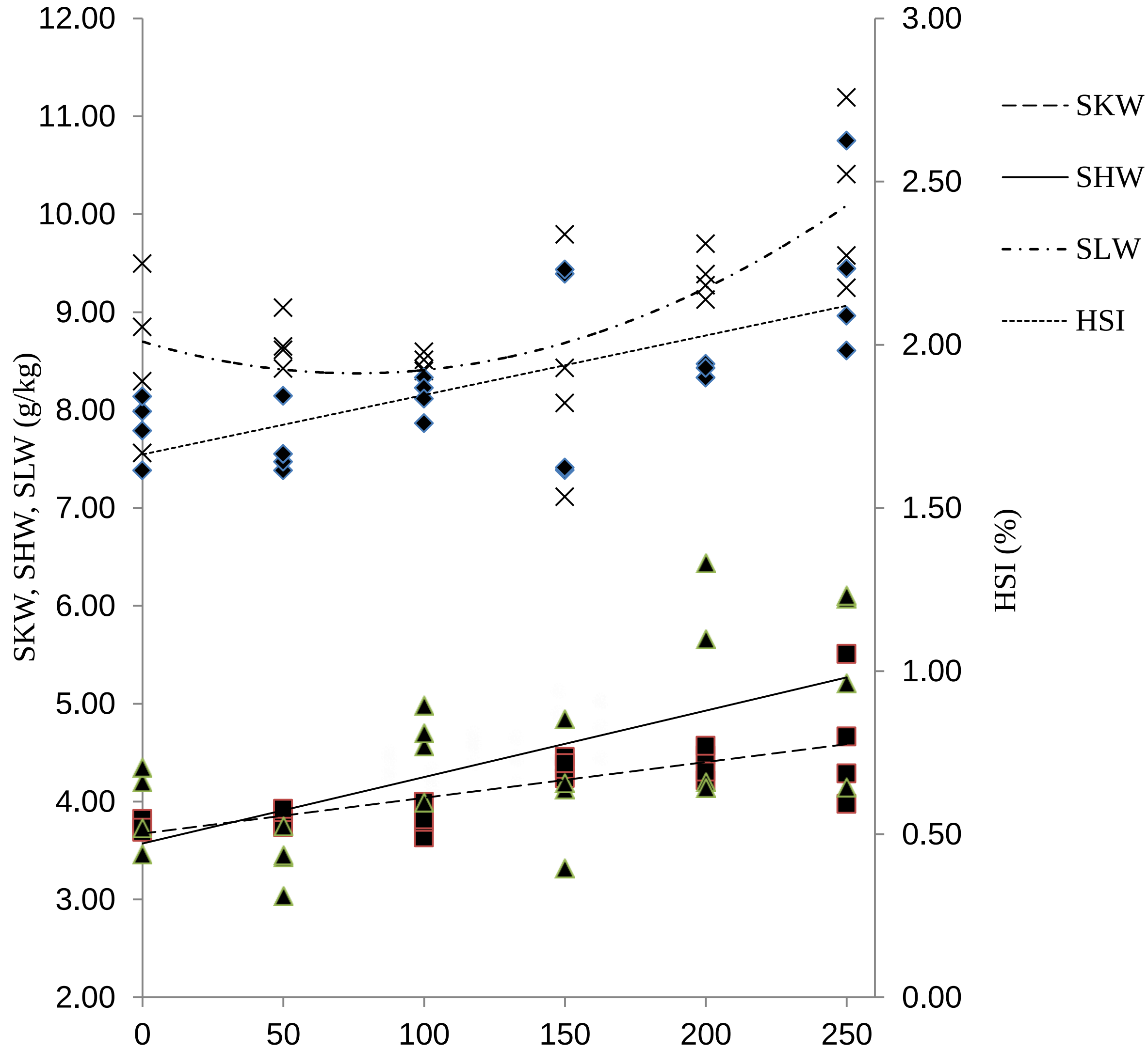

Figure 4.2: Effects of inclusion levels of A. tortilis leaf meals on the weight of internal organs in finishing pigs

Scaled kidney weight (SKW), (SKW:Y = 6E-05x^2 – 0.0085x + 8.70 (R^2 =0.47; P < 0.01), scaled heart weight (SHW) (SHW: Y = 0.0037 + 3.67 (R^2 = 0.56; P < 0.001), scaled lung weight (SLW) (SLW: Y = 0.0018x + 1.66 (R^2 = 0.41; P < 0.05) and hepatosomatic index (HSI) (HSI: Y = 0.0068x + 3.57 (R^2 = 0.41; P < 0.001)

4.5 Discussion

The diets were formulated to have similar levels of crude protein and energy. However, ADF, NDF, mineral concentrations and proanthocynadins varied across all dietary treatments. As expected, levels of ADF, NDF and proanthocynadins increased with leaf meal inclusion. An increase in *A. tortilis* inclusion level elevated the Ca, P and Fe content in the diet. With nutritionist searching for alternative feed ingredients, the use of *A. tortilis* leaf meal is vital in that it is rich in protein and mineral contents. In spite of its high tannin and fibre contents, if it is included at moderate levels, therefore, can improve growth performance, gut health as well as promoting animal welfare. The current dose-response trial was designed to allow identification of optimum inclusion level of *A. tortilis* before nutritionally-related blood constituents and internal organs are compromised. Understanding changes in blood metabolites and internal organs with increase in dietary levels of *A. tortilis* leaf meal reflects healthy status of pigs when subjected to varying internal and external environment, which include feed and feeding.

The observation that serum Fe increased with incremental levels of *A. tortilis* in the diets is a reflection of the increase in dietary Fe content. As the inclusion level of *A. tortilis* leaf meal in the diets increased, the Fe content also increased. This finding contradicts reports by Lee *et al*. (2010) who reported a decrease in serum iron concentration with the increase of dietary tannic acids.

Inclusion of *A. tortilis* leaf meal did not influence the serum TP. Serum total protein is an indirect index that indicates the nutritional protein adequacy. The serum protein levels were within the normal range of 5.8 and 8.3 g/ dl (Latimer *et al.*, 2003), indicating that the quality of protein in the diets were not compromised with the inclusion of leaf meal. Despite the adequacy of protein in the diet depressed growth rate was observed in the experiment (Chapter 3). Similar findings have been reported on pigs given diets with varying dietary levels of wild sunflower leaf meal by Fasuyi *et al*. (2013) who suggested that the lack of differences indicates that the nutritional quality of the leaf meal as protein sources may be acceptable if included at moderate level. The changes in blood uric acid concentrations are cardinal in diagnosis of kidney toxicosis, and kidney malformation and massive tissue destruction cause hyper-uricemia (Chandra *et al*., 1983; Silanikove *et al.*, 1996). On the other hand, uric acid is a primary catabolic product of protein, non-protein nitrogen and purines is also a good indicator of the quality of protein fed to pigs (Aderolu *et al*., 2007). The finding that uric acid concentration followed a pattern of initial increase and gradual decrease when inclusion levels of the leaf meal exceeded 100 g/kg indicates the efficiency of the kidney in excreting the nitrogenous compounds.

Cholesterol has crucial functions as a component of the cell membrane of mammals and as a precursor of the bile acid (Krieger, 1999). These functions are often neglected by the association of cholesterol levels to atherosclerotic diseases. The observation that cholesterol concentrations in pigs fed on graded levels of *Acacia* leaf meal containing condensed tannins increased contrasts the report by Štukelj *et al*. (2010) who reported decreases in total cholesterol of pigs supplemented with different levels of tannic acids. These findings suggest that there could have been obstruction of bile due to condensed tannins which indicate a mild and progressive damage to the livers (Silanikove *et al.*, 1996). Another plausible explanation could be the amount of sunflower oil used (67.8 g/kg) in the diets which was at high proportion especially when the leaf meal was incorporated at high inclusion levels in the diets. Despite the increases in total cholesterol in the serum, all the levels were within the reference range (81-134 mg/dl) in the blood (Latimer *et al.,* 2003).

Activities of AST, ALP and ALT indicate liver damage and can be used to assess the homeostatic response of the liver to metabolites that pass through the portal blood. The observed increase in ALT activity concentration as leaf meal content increased in the diet could suggest that there was hepatic degeneration and mild necrosis (Silanikove *et al.,* 1996). Alanine aminotransferase levels were above the normal range (15–55 U/L) in all dietary treatments (Latimer *et al.*, 2003). The observed increase in the activity of both AST and ALP concurs with Halimani *et al*. (2005), who reported that the activity of hepatic microsomal urine diphosphate glucoronyl tranferase increased in pigs supplemented with leaf meal diets. The ALP activity can be used to assess the health of the liver as it originates from the osteoblast and some of it are excreted in the bile (Ganti, 1979). The increase in the activity of AST and ALP above the normal range of 15–55 and 41–176 U/L, respectively (Radostits *et al.,* 2000), demonstrated that high levels of *A. tortilis* leaf meal could have had negative effects on the liver function of pigs. The elevated ALP concentrations observed with an increase in *A. tortilis* leaf meal agrees with Adesehinwa *et al.* (2011), and could be attributed to the obstruction of bile flow (Silanikove *et al.,* 1996).

The liver is one of the body's most sensitive visceral organs that respond to toxic factors or protein deficiency (Marzo *et al.*, 2002). The observed increase in HSI with an increase dietary inclusion of *A. tortilis* leaf meal could have been caused by the need for the liver to secrete ALP and AST enzymes as they showed increased with increased inclusion level of *A. tortilis* leaf meal. Another possible reason for increased size of liver could be the toxic effects of tannins as it is reported that condensed tannins may be absorbed once the hepatic portal system is damaged dietary tannins that triggered an increase in liver size to meet the need for secretion of liver enzymes for detoxifying the polyphenolic compounds. Marzo *et al.* (2002) in chicks and Marzoni *et al.* (2005) in pheasants observed a reduced liver weight at 2.03 % tannin content. The reduced liver weight was due to proteolysis of the liver. The maximum level of tannins in the current study was lower than 2.03 %, suggesting a possible coping strategy in which pigs metabolize considerable proportions of phenolic compounds that escape through the portal blood are annihilated by increase in observed ALT, ALP and AST activities. In addition, necrosis of the liver could have increased liver weight as more of these enzymes were secreted to suppress it. However, biochemical changes in liver enzyme or proteolytic activities in response to dietary tannin content have not been previously studied. Nyachoti *et al.* (1996) observed pancreatic hypertrophy due to increased enzyme secretion to compensate for inhibition caused by tannins in chickens.

The observed increase in SKW with incremental levels of *A. tortilis* could have been caused by the need to excrete excess nitrogenous compounds that could have increased due to consumption of protein rich leaf meal-based diets. Catabolism of nitrogenous compounds increases the concentration of wastes in blood, thereby increasing the work load of the kidneys, and causing them to increase in size. This was supported by the observed decrease in uric acid, indicating that more of it was excreted. Malavahn and Preston (2006) demonstrated high urinary nitrogen excretion in pigs fed on diets containing high levels of leaf meal. Although water intake was not measured in the current study, pigs on high levels leaf meals based diets were observed to frequently visit the drinkers, partly explaining the increase in kidney weight, as more of the water was excreted. An increase in water intake is an attempt by pigs to counter the astringent taste of the tannin rich ingredients.

The observed linear increase in SHW concurs with Amata and Bratte (2008), who reported heavier hearts of rabbits fed on *Gliricidia* leaf meal-based diet. The increase in SHW could have been due to the attempt to supply oxygen and increased rate of blood circulation. The observed increase in lung weight could have been caused by the demand of oxygen required for various organs as they showed hypertrophy. According to Yen (1997), visceral organs constitute at most 15 % or less of the total body mass and account for a high proportion of whole-body energy expenditure in growing pigs. Therefore, feeding pigs using diets that increase visceral organ mass will, consequently, lead to an increase in energy expenditure (Agyekum *et al*., 2012).

4.5 Conclusions

Inclusion of *A. tortilis* leaf meal was efficacious in increasing the iron concentration, cholesterol level and weight of liver, kidneys, heart and lungs. The inclusion of *A. tortilis* did not compromise serum protein concentrations. *Acacia tortilis* leaf meal could be included in pig diets up to 150 g/ kg without negatively affecting serum iron, phosphorus, total cholesterol, uric acid, total protein, ALP, AST. ALT, weight of liver, kidneys, heart and lungs. However excessive inclusion levels could negatively affect the healthy status of pigs.

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Chapter 5: General discussion, conclusions and recommendations

5.1 General discussion

The broad objective of the current study was to evaluate the response of pigs fed on incremental levels of *A. tortilis* leaf meal-based diet in the KwaZulu-Natal province South Africa. The main hypothesis tested was that the inclusion level of *A. tortilis* leaf meal in the diet increases the ADFI, ADG and feed: gain up to a point when it start to decrease and that the incremental levels of *A. tortilis* leaf meal has no effect on nutritional related blood metabolites, but increases the secretion of ALP, ALT, AST, relatively weight of liver, kidneys, lungs and heart.

The nutritive evaluation of *Acacia* species was conducted to test the hypothesis that there is no difference in nutrients composition among *Acacia* species. The chemical composition of *Acacia* species varied across species. *Acacia tortilis* had highest crude protein content among all species and moderate bulking properties and total condensed tannins. The observed differences in chemical composition was ascribed to genotypic differences since all *Acacia* specie evaluated were harvested in the same grazing camp and at the same age after rainy season. The results thus reject the hypothesis.

The dose-response trial was conducted in Chapter 3 to test the hypothesis that incremental levels of *A. tortilis* leaf meal in the diet increases ADFI, ADG and G: F up to a certain point where it starts constrain or decrease. The incremental levels of *A. tortilis* leaf meal in the diet increases ADFI up to 125 ± 19.4 g/ kg of feed DM. The inclusion of leaf meal above 125 g/kg resulted in decreased feed intake due to astringency effect of tannins present in leaf meal. Reduced ADG was observed when *A. tortilis* leaf was included above 137 ± 12.1 g /kg DM of feed. Feed conversion efficiency was constrained when *A. tortilis* leaf meal was included above 129 ± 10.7 g/ kg DM of feed. The results obtained from the current study are in accordance with the hypothesis. Given that high levels of *A. tortilis* depressed feed intake and growth rate as a results of tannins present in leaf meal, it was therefore, important to assess the concentration of protein, iron, phosphorus, cholesterol, activity of ALP, AST, ALT and to determine the relatively weight of liver, kidneys, lungs and heart.

In chapter 4, it was hypothesised that *A. tortilis* leaf meal has no effect on serum total protein, iron, phosphorus and total cholesterol, but increases the activity of ALP, AST, and ALT in the blood, relatively weight of liver, kidneys, lungs and heart of pigs. Total protein and phosphorus were not affected by diets containing leaf meal. There was an increase in the level of iron and cholesterol in the blood of pigs fed on incremental levels of *A. tortilis* leaf meal diets. Feeding incremental levels of *A. tortilis* leaf meal in pigs resulted in a quadratically increased AST and ALP activity. The ALT increased linearly with increased of leaf meal inclusion. *Acacia tortilis* leaf meal could therefore, be included in optimum rate in the pig diets. The increased in the concentration of liver enzymes in the present study indicates that the incremental levels resulted in mild damage of the liver.

The HSI, SKW and SHW all increased linearly with increased inclusion levels of leaf meal. A quadratic relationship was observed in scaled lungs weight. The hypertrophy of liver was attributed to the necrosis and the need for secretion of AST, ALT and ALP. The increased SKW was attributed to the high nitrogen content of the diets which was caused by complexing of protein by tannins as leaf meal was increased in the diet. The binding of protein could have cause increased catabolism of nitrogenous compounds which induce the enlargement of kidneys. The increased in weight of SLW and SHW observed in this study was attributed to the increased blood circulation and oxygen associated with lean body which was evidence in groups of pigs fed more than 150 g/ kg DM inclusion levels of leaf meal.

The findings on the nutritional related metabolites agree with the hypothesis that *A. tortilis* leaf meal has no effect on the nutritional related blood metabolites. These finding suggest that the inclusion of *A. tortilis* did not compromise the quality and adequacy of nutrients. The increase in weight of internal organs and the concentration of liver enzymes support the hypothesis that *A. tortilis* influence the physiological processes in the body. Leaf meal inclusion beyond 150 g/ kg can have negative effect on the size and functioning of various internal organs, but the increase in size of lungs and heart suggest that animal can develop some organs to cope with leaf meal inclusion in their diet.

5.2 Conclusions

Acacia species could substitute soybean protein source ingredients in pig diets. The concentration of proanthocyanidins and fibrous properties could limit the optimal exploration of *Acacia* leaf in pig diets. Incorporation of *A. tortilis* leaf meal in pigs' diets increases feed intake and ADG up to a point were incremental levels beyond threshold proportions inevitably suppress both feed intake and growth ADG. The extent with which pigs efficiently utilize leaf meal based diets improves with continuous exposure to such feeds. The inclusion of *A. tortilis* leaf meal did not compromise serum protein and phosphorus status. The incremental levels of leaf meal were related to increases in iron concentration, cholesterol level, enzyme activity and weight of internal organs. The utilization of *A. tortilis* leaf meal could reduce the proportion of expensive ingredients thus reduce the cost of feed in smallholder pig production.

5.3 Recommendations and further research

It can be advised that farmers should use *Acacia* leaf meal to reduce the cost of protein in pig diets. The choice of species would largely dependent on the chemical composition and the abundance of the species in the area. For the efficiently utilization and conservation of *Acacia* species, farmers need a comprehensive training on the sustainable harvesting and processing of leaf. With the context that adaptation to leaf meal diets improves with time it can be advised that high levels of leaf meal should be used to feed the sows.

Aspects that require further research include the following:

1. Determination of the meat quality of pigs fed on *Acacia* leaf meal-based diet.
2. Determine the effect of breeds on the utilization of *Acacia* leaf meal.
3. Evaluate the effect of *Acacia* leaf meal on sow and litter performance
4. Evaluate the potential of *Acacia* leaf meal as iron supplementation in suckling piglets
5. Characterize milk composition of sow fed *Acacia* leaf meal

Appendix 1: Ethical approval of research project.

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Research Office
Animal Ethics Research Committee

Govan Mbeki Centre, Westville Campus,
University Road, Chiltern Hills, Westville, 3629, South Africa
Telephone 27 (031) 260-2273/35 Fax (031) 260-2384
Email: animalethics@ukzn.ac.za

2 November 2012

Reference: 004/13/Animal

Mr M Khanyile
School of Agricultural, Earth and
Environmental Sciences
University of KwaZulu-Natal
PIETERMARITZBURG Campus

Dear Mr Khanyile

Ethical Approval of Research Projects on Animals

I have pleasure in informing you that the Animal Ethics Sub-committee of the University Ethics Committee has granted ethical approval for **2012/2013** on the following project:

"Feed intake and metabolic response of Large White pigs fed on incremental levels of *Acacia tortilis*."

Yours sincerely

Professor Theresa HT Coetzer
Chairperson: Animal Ethics Sub-committee

Cc Registrar – Prof. J Meyerowitz
Research Office – Dr N Singh
Supervisor: Prof. M Chimonyo
Head of School – Prof. A Modi
Mrs M Manjoo

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Appendix 2: Methods for determining serum concentration of serum nutritionally-related blood metabolites and liver enzymes

1. **Determination of serum Iron (Fe)**

Iron (Fe) concentration in the blood was determined by a colorimetric method described by Tietz, (1976) that uses ferrozine as the chromogen. During the measurement of serum Fe, ferric Fe is disassociated from its carrier protein (transferrin) by action of guanidine in an acid medium and simultaneously reduced to the ferrous form by hydroxylamine. The ferrous iron is then complexed with the chromogen to produce a blue chromophore. Iron concentration in the sample is directly proportional to the chromophore colour intensity measured at 560 nm.

2. **Determination of serum phosphorus (P)**

Serum phosphorus was determined colorimetrically according to the method of Young (1990). The method is based the reaction between ammonium molybdate and P in the sample under acidic conditions to form phosphomolybdate . In the completion of the reaction, the absorbance of the sample reagent mixture is read bichromatically at 340 nm/378nm. The difference between these two absorbance values is proportional to the amount of SIP in the sample.

3. **Determination of uric acid concentration**

Uric acid determination was done using urease kinetic ultraviolet method described by Tietz (1995).The procedure involves the hydrolysis of uric acid to produce ammonia and carbon dioxide. The ammonia produced in the first reaction combines with α oxoglutarate and Nicotinamide adenine dinucleotide (NADH) in the presence of glutamate-dehydrogenase to yield glutamate and NAD^+. The conversion of NADH chromophore to NAD^+ product, measured at 340 nm/647 nm is propotional uric acid in the sample.

4. Determination of serum total protein

Serum total protein was estimated by the Biuret method described by Weicheselbaum (1946). In this method, biuret reagent was allowed to complex with the peptide bonds of protein from the sample under alkaline condition to form a violet-coloured compound. Sodium potassium titrate was used as an alkaline stabilizer, and potassium iodine was used to prevent auto-reduction of the copper sulfate. The amount of the violet complex formed was proportional to the increase in absorbance when measured bichromatically at 544 nm/692 nm.

5. Determination of total cholesterol concentration

Total cholesterol was determined using enzymatic method described by Allain *et al*. (1974). The method involves complete hydrolysis of cholesterol esters in the serum to free fatty acids by pancreatic cholesterol esterase. Thereafter, cholesterol liberated by esterase, plus any free cholesterol originally present in the serum, are both oxidized by cholesterol oxidase. The liberated peroxide reacts with phenol and 4 aminoantipyrine in a peroxide catalyzed reaction to form a quinoeimine dye, which absorbs at 500 nm. The change in absorbance is measured bichromatically at 505 nm/692 nm and is directly propotional to amount of cholesterol present in the sample.

6. Determination of enzyme concentration

Alkaline phosphatase (ALP) was determined by the spectrophotometric nitrophenol method of Tietz *et al.* (1993). In this method, ALP in the serum catalyzes the hydrolysis of colourless p-nitorphenyl phosphate to p-nitrophenol and inorganic phosphate. In alkaline solution of pH = 10.5, p-nitrophenol is in the phenoxide form and has a strong absorbance at 408 nm. Zinc and magnesium act as activators for this reaction while 2-amino-2menthly-l-prdopanol buffer acts as an acceptor for the phosphate ions, which prevent the enzyme. The rate of increase in absorbance, monitored bichromatically at 408 nm/486 nm, is directly proportional to the ALP concentration in the sample.

Aspartate and alinine amino transaminases were determined by spectrophotometric method of Bergmeyer *et al.* (1986). The Alinine transaminase (ALT) enzymatic assay kit uses a coupled enzymatic reaction scheme: alinine and α-oxoglucotarate are first converted by ALT to L- glutamate and pyruvate, which is converted by lactate and NAD^+. For aspartate transaminase (AST) determination, L-aspartate reacts with α-oxoglucotarate in the presence of AST to produce L-glutamate and oxaloacetate. Oxaloacetate is then converted by malate dehydrogenase to L-malate and NAD^+. The conversion of NADH chromophore to NAD^+ product, measured at 340 nm, is proportional to the level of enzyme (ALT or AST) concentration in the sample.