Influence of polyethylene glycol inclusion on growth performance and serum biochemistry of growing pigs fed on *Acacia tortilis* leaf meal

By

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Declaration

I, Vuyisa Andries Hlatini, declare that **Influence of polyethylene glycol inclusion on growth performance and serum biochemistry of growing pigs fed on *Acacia tortilis* leaf meal** is my own work, except where indicated through referencing. This work has not been submitted for a degree in any other institution of higher learning other than University of KwaZulu-Natal.

Candidate's signature................................ Date......................

Supervisor's signature................................ Date......................

List of abbreviations

Abbreviation	Definition
ADF	acid detergent fibre
ADFI	average daily feed intake
ADG	average daily gain
ALP	alkaline phosphatase
ALT	alkaline transaminase
AST	aspartate amino transaminase
BWT	body weight
CP	crude protein
CT	condensed tannins
G: F ratio	gain: feed ratio
HD	hydrolysable tannins
N	nitrogen
NDF	neutral detergent fibre
PEG	polyethylene glycol
s.d.	standard deviation
SFI	scaled feed intake
TP	total protein

Abstract

The broad objective of the study was to determine the influence of polyethylene glycol (PEG) in leaf meal diets on growth performance and serum biochemistry of growing pigs. Forty eight clinically healthy male F1 hybrid (Landrace x Large White, IPC group) pigs were randomly allotted to individually pens, in a complete randomized design. There were eight pigs with an initial body weight of 15.8 (s. d. 0.032) kg per treatment. The *Acacia tortilis* leaf meal was treated with six incremental levels (0, 5, 10, 15, 20 and 25 g/kg) of PEG. Each of the six diets was offered *ad libitum* to pigs for four weeks. Clean water was available at all times.

Average daily feed intake (ADFI) and average daily gain (ADG) were determined every week. Scaled feed intake (SFI) and gain: feed ratio (G: F) were also calculated. Blood was collected at the end of the experiment to determine total protein (TP), albumin, globulin, creatinine, cholesterol, uric acid and activities of alkaline phosphatase (ALP), alanine aminotransferase (ALT) and aspartate aminotransferase (AST).

There was a quadratic increase in SFI ($P < 0.01$) and G: F ratio ($P > 0.05$) with PEG and ADF inclusion. There was a linear relationship between ADG and PEG inclusion ($P < 0.01$). There was linear relationship between PEG inclusion level and TP and globulin concentrations ($P < 0.01$). A quadratic increase on albumin with PEG inclusion was observed ($P < 0.01$). Cholesterol, creatinine and uric acid concentrations were not influenced by PEG inclusion ($P > 0.05$). There was a linear decrease in the activity of AST and ALT ($P < 0.01$). An increasing quadratic relationship on ALP enzyme to PEG inclusion was also observed ($P > 0.05$).

In conclusion, PEG up to 25 g/kg increase most of the response variables quadratically. It can be concluded that the relationship between PEG inclusion and performance of growing pigs fed on *A. tortilis* is exponential, rather than linear.

Keywords: albumin; alkaline phosphatase; alkaline transaminase; aspartate amino transaminase; average daily gain; gain: feed ratio; globulins; polyphenolic compounds; scaled feed intake; total proteins.

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Dedication

This theses is highly dedicated to my late Father: Hlatini Tandikaya, Mother: James-Hlatini Nomachule D, brothers (Xolisa Hlatini, Asonele Hlatini) and sisters (Weziwe Hlatini, Asanda Hlatini, Noxolo Hlatini, Sive Hlatini and Onela Hlatini) for believing in me and offering the opportunity with support to further my studies.

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CHAPTER 1: General Introduction

1.1 Background

The dominant pig breeds are Landrace, Large White, Duroc and Pietrian (Visser, 2004). Pork is a widely consumed meat due to high demand for protein. This has generated additional pressure on feed ingredients and increased competition of the same products (e.g. maize and soyabean) between human and pigs. The Farmer's Weekly (2013) reported that between 2010 and 2012, feed costs in South Africa increased by 60 % while the producer price of pork increased by only 29 %. Therefore, emerging farmers might struggle to meet nutrient requirements to maximize the production of pigs (Martens *et al.*, 2012).

There is a need to identify alternative non-conventional feed resources which meet the nutrient requirements for livestock (Woyengo *et al*., 2014). Various agro-industrial by-products or agroforestry-based feeds can be used as ingredients for pig feeds (D'Mello, 1995). Legume leaves have a huge potential to be included into feeds to provide protein in pigs (Halimani *et al.*, 2005b). *Acacia tortilis, A. robusta, A. karroo, A. nilotica, A. nigrescens* and *A. xanthophoea,* cassava, duckweed, *Carica papaya, Faba beans, Colophospermum mopane, Xanthosomas agittifolium, Leucaena leucocephala* and *Moringa oleifera* are common examples of leaf meals. Most of these legumes are even available at the village level. Leaves could be used to feed animals while branches and tree trunks could be used for making kraals, fencing, and roofing, building animal drawn carts and for firewood. In addition, this could be used as simple method to alleviate encroaching species.

Generally, there are six dominant woody plants in Southern Africa (O'Connor *et al*., 2014). Of these, *Acacia tortilis* has been the most important encroaching species in South Africa (Munyati *et al*., 2013). *Acacia* species contain a relatively high crude protein (above 100 g/kg DM), and amino acid contents, as well as favourable mineral concentration (Khanyile *et al*., 2014). Khanyile *et al*. (2014) reported that high inclusion levels of *Acacia tortilis* (beyond 137 g/kg) leaf meal in pig diets may reduce pig performance. The utilization of *Acacia* leaf meals in pig diet is limited by anti-nutritional factors, thorns and high fibre content (Halimani *et al*., 2005a). Polyphenolic compounds have anti-nutritional and toxic effects, including reduced feed intake, growth rate, and gain: feed ratio and biochemical parameters to pigs when included at high levels in the diet (Makkar, 2003; Phuc & Lindberg, 2001; Khanyile *et al*., 2014). Polyphenolic compounds bind to proteins, reduce nutritive value and palatability, which, in turn, reduce feed intake (Barbehenn & Constabel, 2011).

Feed intake is one of the fundamental aspects which affect productivity, nutrient intake, body weight gain, feed conversion ratio and profitability of pig enterprises. Polyphenolic compounds could also be harmful to the lining of the small intestines, thus disturbing normal absorptive function of the gut (Silverstein *et al*., 1996; Priolo *et al*., 2000). This, in turn, negatively affects performance of pigs (Pluske *et al*., 1997; Barnabas *et al*., 2011). Despite its anti-nutritional and toxic effects, polyphenolic compounds can be beneficial on growth performance. They also have anthelmintic properties. Therefore, there is a need to block these polyphenolic compounds to reduce the adverse effects of leaf meals.

There are many possible ways to neutralize the negative effect of polyphenolic compounds. A number of authors (e.g. Silanikove *et al.,* 2001; Makkar, 2003; Ben Salem *et al*., 2005; Nsahlai *et al*., 2011) have explored the use of tannin-binding agents, heating, soaking in water and drying, wood ash, chopping and storage, urea, solid-state fermentation and enzymes. One such way is the use of polyethylene glycol (PEG) supplements (Njidda & Ikhimioya, 2012). The PEG has a high affinity for tannins. The most important property of all is its solubility in water, which makes them ideally suitable for inclusion in leaf meals (Salem *et al*., 2002). Adding PEG to tannin-rich leaf meal increases feed utilization in small ruminants (Makkar *et al*., 1995; Silanikove *et al*., 2001; Villalba & Provenza, 2002; Priolo *et al*., 2005; Nsahlai *et al*., 2011). Inclusion of PEG has been determined to have a potential to improve feed intake in goats and sheep (Villalba *et al*., 2002), rats (Horigome *et al*., 1988) and cattle (Landau *et al*., 2000). Improved growth and metabolism from growing pigs fed on rapeseed meal treated with PEG and copper, was attained at 17 g/kg PEG (Rowan and Lawrence, 1986). Savage *et al*. (1980) also reported positive results when PEG was added to brown sorghum fed to growing pigs. There is little information on use of PEG to deactivate tannins from *Acacia* leaf meals on pigs. Role of PEG on serum metabolite concentrations have not been reported. Prvulovic *et al*. (2007) reported that blood metabolites reflect an animal's physiological and health response.

Increasing PEG levels is expected to produce a linear relationship with digestibility, nitrogen retention and performance of animals. Since PEG is relatively expensive, in relation to other physical and chemical methods, it is important to accurately predict performance of animals, even at low inclusion levels. The theory that the relationship is linear needs to be tested so that farmers include appropriate levels. It is likely that, since, PEG binds to all compounds that possess the phenol compounds, including alkaloids and lignin, the relationship between PEG inclusion and animal performance may not be linear.

1.2 Justification

Inclusion of PEG on tannin- rich leaf meals assist feed compounders to compose diets which ensure the optimum feed intake, without limiting pig performance. Leaf meals that are rich in crude protein can provide alternative sources of proteins for feed compounders. Determining the optimal levels of PEG enhances sustainable use of leaf meals ingredients for pig diets. Tree and shrub legume plants are under-utilized (Halimani *et al*., 2005b) and cause bush encroachment, thus reducing carrying capacities of rangelands. Use of legume leaves is likely to reduce competition between pigs and humans for soya-bean meal and other conventional protein sources. Therefore, there is a need to determine the influence of PEG to tannin containing feeds on growth performance, nitrogen retention and serum biochemistry in growing pigs.

1.3 Objectives

The broad objective of the study was to investigate the influence of PEG-treated leaf meal on growth performance and serum biochemistry in growing pigs.

The specific objectives are to:

1. Determine influence of PEG in *Acacia tortilis* leaf meal-based diets on feed intake and growth performance of growing pigs; and
2. Establish the relationship of PEG treated *Acacia tortilis* leaf meal diet on nutritionally-related metabolites and liver enzymes of pigs.

1.4 Hypotheses

The hypotheses tested were that:

1. Inclusion of PEG in *Acacia* leaf meal diet increases linearly with pig performance; and
2. There is a positive linear relationship between PEG inclusion level in *Acacia tortilis* leaf meal and blood metabolites.

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CHAPTER 2: Review of Literature

2.1 Introduction

The ever-increasing population of humans and animals put a strain on cereal and legume grains. This calls for the need to come up with alternative non-conventional feeds for feeding livestock. Leguminous trees and shrubs have been documented as the potential alternative feeds due to their nutritive value. The use of the leaf meals is, however, limited by the presence of polyphenolic compounds, presence of thorns and high fibre content. Ways of neutralizing the effects of the polyphenolic compounds, such as using polyethylene glycol (PEG) need to be explored. The current chapter discusses the role of leaf meals, description and distribution of leaf meals, polyphenolic compounds, and the potential of using PEG in pig feeds to increase growth performance, nitrogen retention in pigs.

2.2 Distribution of indigenous legume plants

In Africa, at least 75 % of the trees and shrubs are leguminous (Abule *et al*., 2007). The composition and distribution of native fodder trees and shrubs vary according to place of origin and environmental conditions the plants are subjected to. Ward *et al.* (2014) reported that an increase of woody vegetation is associated with increasing ambient temperatures. These species are widely distributed and dominant throughout Southern Africa in diverse habitats. Table 2.1 shows examples of the dominant legume species, in some countries of Southern Africa. In Southern Africa, there are six legume species that are the main encroaching species (Munyati *et al*., 2013). These legume tree leaves can be a potentially exploited to provide protein for sustainable animal production systems. Acacia species are one of the most dominant legume trees.

Table 2.1: Distribution of species across selected countries in Southern Africa

Country	Dominant/encroaching species
Tanzania	*Acacia tortilis, Cappeins tomentosa*
Namibia	*Sengelia mellifera, Sengelia. erubscen, Acacia tortilis, Dichrostachys cinerea*
Swaziland	*Dichrostachys cinerea*
Botswana	*Acacia tortilis, Sengelia erbubsen, Acacia fleckii, Salvia mellifera, Dichrostachys cinerea, Grewia flawa, Termina sericea, Arisaema erubscens*
Zimbabwe	*Acacia borleae, Acacia karroo, Colophospermum mopane, Acacia nilotica*
South Africa	*Colophospermum mopane, Acacia tortilis, Acacia nilotica, Sengelia mellifera, Acacia karroo, Dichrostachys cinerea, Acacia ataxacauntha, Scutia myrtina, Euclea crispa, Ataxaxautha sieberiana*

Sources: O'Connor *et al.* (2014); Frost (1999)

2.2.1 Description and distribution of Acacia tortilis species

Acacia species is the most abundant woody plant species in Southern African rangeland (Solomon *et al*., 2007). Currently, there is a growing in interest of using Acacia species as an affordable source of protein for pigs (Abdurazak *et al*., 2000; Mashamaite *et al*., 2009). *Acacia tortilis* is a thorny legume tree containing high amounts of condensed tannins and high fibre content. It normally grows up to 6 m high, but it can reach 20 m (Orwa *et al*., 2009) (Figure 2.1). It is fairly slow-growing tree and relying on environmental conditions, flowering occurs from November to January. Flowers are white pale yellow fruits forming in July and the highly nutritious pods are twisted into a spiral. Leaves are compound and the leaflets (22 pairs) are very small (1-4 mm long x 0.6 – 1mm broad), glabrous to pubescent (Foden & Potter, 2005). There are usually 2 to 6 leaves per node. In any such group there are one or two main leaves, but most of them are of the same size (Figure 2.1).

Figure 1.1: *Acacia tortilis* species

Source: Foden & Potter (2005)

Acacia tortilis is a widely dispersed species (throughout the savannah and dry zones of Africa), indigenous to semi-arid regions of Africa and Middle-East (Wahbi *et al*., 2013). An average tree yields 6 kg pods of which 2.6 kg is seed and one tree yields about 4 to 6 kg dry leaf meal and between 10 and 12 kg of pods per year (Orwa *et al*., 2009). Yields from young plantations could harvest about 2.5 kg per tree per year at 400 trees/ha (NFTA, 1991). Over 90 % of the flowers abort and drop to the ground, providing additional important forage. As a nitrogen-fixing tree, *Acacia tortilis* is important in maintaining the health status of soil. It survives where rainfall ranges from 100 to 1000 mm, temperatures regularly falls to near freezing at night and it tolerates dry season conditions. *Acacia tortilis* occurs in deciduous woodland, thornveld and bushveld.

2.2.2 Functional uses of Acacia tortilis

Acacia tortilis is spiny and is used for different activities such as supplying staple browse specifically for goat and other animals (Mokoboki *et al*., 2011). It is used as source of feed (Anderse & Krzywinski, 2007). It can improve soil fertility, positively affect grass status and provides shade for animals. It also plays a vital role in holding soil particles together and has the ability to fix atmospheric nitrogen. The plant is capable of improving herbaceous plant productivity, diversity and encourages palatable species to grow beneath its canopy (Mugunga & Mugumo, 2013). By so doing, the carrying capacity of the rangelands improves. The species is also documented for sand dune stabilization and shelterbelts in Africa (FAO, 1993). The increase in density of Acacia plants in rangelands is an ecological problem under pasture management (Solomon & Mlambo, 2010). Use of *Acacia* species as protein source would decrease dominance of bush over grass species. Due to its wide range of benefits such as being the main source of feed for cattle, sheep and goats, fodder, browse, wood, timber, fencing, fuel and medicinal uses, the species is generally accepted by local people. Currently, *Acacia tortilis* leaves are used as a source of feed for pigs due to their nutritive value (Halimani *et al*., 2005b; Khanyile *et al*., 2014).

2.2.3 Nutritive value of **Acacia tortilis**

The chemical composition (g/kg DM) and mineral content of *Acacia tortilis* are shown in Tables 2.2 and 2.3, respectively. *Acacia tortilis* contains high levels of crude protein (CP), ranging from 140 to180 g/kg DM. Crude protein and digestibility coefficients of *Acacia tortilis* are between 180 and 462 g/kg, respectively (Orwa *et al*., 2009). Nutrient composition of leaf meals is determined by soil condition, season and stage of leaf growth (Nyamukanza & Scogings, 2008). The concentration of polyphenolic compounds also depend on soil fertility and environmental conditions (Manfield *et al*., 1999; Kraus *et al*., 2004). These anti-nutritional are, perhaps, the biggest challenge to using *Acacia tortilis* as pig feed.

Table 2.2: Chemical composition (g/kg) of *Acacia tortilis* leaves

Component	Mean	Source
Organic matter	940.0	Abdulrazak *et al*. (2000)
Crude protein	189.0	Abdulrazak *et al*. (2000)
Dry matter	947.7	Mokoboki *et al*. (2005)
Neutral detergent fibre	494.0	Khanyile *et al*. (2014)
Acid detergent fibre	298.0	Khanyile *et al*. (2014)
Ether extracts	40.1	Khanyile *et al*. (2014)
Total phenolics	89.7	Mokoboki *et al*. (2005)
Simple phenolics	13.9	Mokoboki *et al*. (2005)
Extracted phenolics	241.0	Dube *et al*. (2001)
Extracted condensed tannins	100.0	Abdulrazak *et al*. (2000)
Total condensed tannins	77.8	Rubanza *et al*. (2005)
Protein-bound condensed tannins	37.5	Rubanza *et al*. (2005)
Condensed tannins in acid detergent fibre	16.3	Mokoboki *et al*. (2005)
CT in neutral detergent fibre	19.8	Mokoboki *et al*. (2005)
Energy digestibility	0.62	Heuze & Trana, (2011)

Table 2.3: Mineral profile of *Acacia tortilis* leaves

Mineral	**Concentration (g/kg DM)**	**Source**
Potassium	9.1	Tefera *et al*. (2008)
Calcium	1.0	Mtengeti & Mhelelo (2006)
Protein	1.6	Heuze and Trana (2011)
Magnesium	1.5	Abdulrazak *et al*. (2000)
Iron	178	Khanyile *et al*. (2014)
Sodium	0.5	Khanyile *et al*., (2014)
Zinc	0.2	Tefera *et al*. (2008)
Copper	2.0	Khanyile *et al*. (2014)

2.3 Potential of using leaf meals on pig productivity

Legume leaves could be used to feed pigs. The leaves and pods could be incorporated into pig diets to provide protein (Ben Salem *et al*., 2004; Martens *et al*., 2013). One of the perceived merits of leaf meals is their high levels of crude protein, essential amino acids and energy (Halimani *et al*., 2005b; Mapiye *et al*., 2011). Leaf meals from legume foliage and fodder are also abundant locally and easy to harvest on-farm even at village level. Nevertheless, the concentration of tannins and high fibre contents are the major challenges. Khanyile *et al*. (2014) reported that inclusions of leaf meals improves intake up to a point where increment levels beyond threshold proportions inevitably reduce performance. Thus, excessive inclusions of tannin-rich leaf meals reduce intake, digestibility, absorption capacity and growth performance (Phuc *et al*., 2001; Halimani *et al*., 2005b).

There are both positive and negative effects in tannin containing feedstuffs. It has been observed that mammals fed tannin-rich leaf meals demonstrated a reduced gastrointestinal parasites and slowed distribution of intestinal parasites and viruses causing diarrhoea in piglets due to the antibacterial properties (Halimani *et al*., 2005a). Tannins are also responsible for clearing of poisonous substances in the body of animals (Halimani *et al*., 2005a). Mueller-Harvey (2006) reported that from the tannins found in feeds such as fodder, legumes browse leaves, and fruits, *Acacia* species can improve animal welfare and health status by acting as an anthelmintic. Min *et al.* (2003) also reported that tannins also reduce the number of eggs hatching, reduce the rate of larval development and decreased mobility of larvae. Leaf meals with tannins carry antibacterial properties that are used as a defence mechanism against gastrointestinal parasites in mammals (Brus *et al*., 2013). Leaf meals are also rich in fibre, which improves microbial populations in the hindgut, where fermentation of undigested dry matter from the small intestines occurs. On the other hand, tannin containing leaf meals are associated with poor feed intake which is directly proportional to growth performance.

2.3.1 Performance of pigs fed to leaf meals

Consumption of tannin-containing feed reduce feed intake (generally > 100 g/kg DM), while medium or low consumption (≤ 100 g/kg DM and 137 g/kg DM) seems not to affect it (Halimani *et al*., 2005a; Khanyile *et al*., 2014; Figure 2.2). Feed intake is one of the important factors limiting productivity because it determines nutrient intake levels, daily weight gain, feed conversion ratio and growth rate. Thus, has a great impact on the efficiency of pig production and economic loss. High intake of polyphenolic compounds make dietary protein unavailable for absorption and decrease palatability of feeds (Foo *et al*., 1996). Condensed tannins can also help return the gastrointestinal flora to a state of balance in pigs. Figure 2.2 shows the effect of *Acacia tortilis* leaf meals on the performance of finishing pigs.

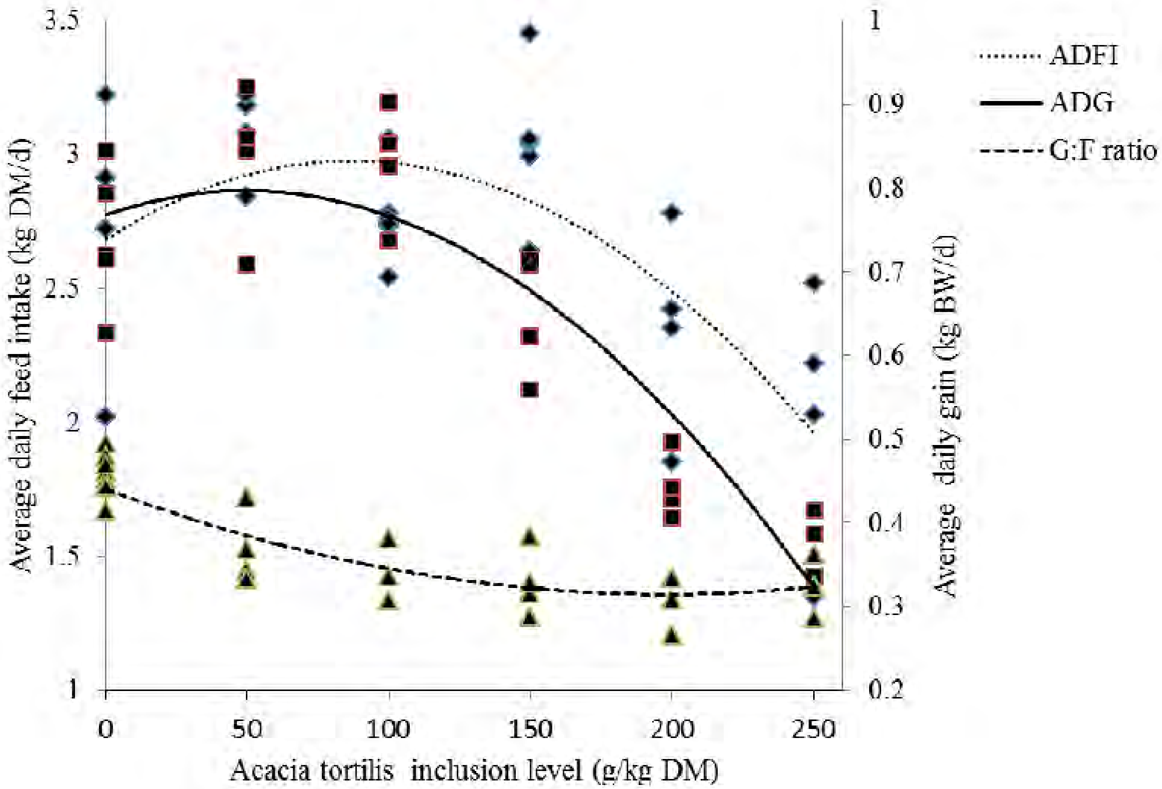

Figure 2.2: Performance of pigs fed graded levels of *Acacia tortilis* leaf meal

Source: Khanyile *et al*. (2014)

2.4 Polyphenolic compounds

Polyphenolics (e.g. tannins) are natural compounds found in plants and are made up of an aromatic benzene ring substituted with hydroxyl groups (Kim *et al*., 2003; Rubanza *et al*., 2005). Tannins are naturally occurring plant polyphenols with a molecular weight greater than 500 and have the ability to bind and precipitate protein (Makkar, 2003). The amount and type of tannins synthesized by plants vary with stage of growth and environmental conditions. Tannins form complexes with proteins by covalent, hydrogen and ionic bonding (Figure 2.3), and these complexes are mostly broken under high acidity or high alkalinity conditions (Salem *et al*., 2005). The binding strength of tannins-protein complexes is determined by the molecular weight, tertiary structure, isoelectric point and compatibility of binding sites (Silanikove *et al*., 2001). Tannins are found in almost any part of the plant and in different plant tissues. They are present in the seed, roots, stem, leaf and bud and are associated with defence role against disease, microbial infestation and predation. Polyphenols reduce nutrient intake and digestibility (Scogings *et al*., 2004). Tannins are divided into hydrolysable and condensed forms (Figure 2.3).

Hydrolysable tannins are characterized by a central carbohydrate core containing various phenolic carboxylic acids such as gallic acid, ellagic acid and hexahydroxydiohenic acid (Ashok & Upadhayaya, 2012; Figure 2.3). These tannins are easily hydrolyzed by acids, alkali or some enzymes. Hydrolysable tannins are not present in cereals and/or legumes. As a result, they are associated with positive impact on animal nutrition (Benrick, 2002). When these tannins are hydrolyzed, gallic or epigallic and sugar are formed. On the other hand, condensed tannins are dimers or oligomers of catechin, epicatechin or similar units (Catherine, 2009). The structure of these units is joined by carbon-carbon bonds, which can be broken by hydrolysis (Halimani *et* *al*., 2007). Condensed tannins have been identified as a major factor affecting productivity of pigs fed leaf meals from legume trees and shrubs.

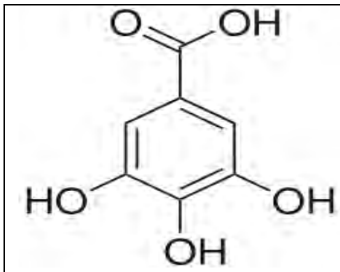	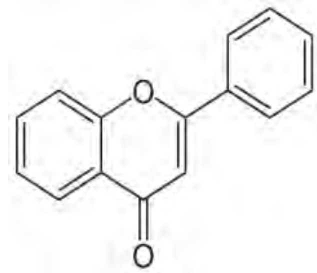
Gallic acid	Flavone
hydrolysable tannins	condensed tannins

Figure 2.3: Structure of condensed and hydrolysable tannins

Source: Ashok and Upadhayaya (2012)

Although Acacia species contain high amounts of polyhenolic compounds, there is need to develop sustainable strategies to ameliorate their effects on pig performance.

2.5 Ways of inactivating tannins-protein complexes

A number of methods have been used to inactivate detrimental and toxic effects of tannins to improve their nutritive value of feedstuffs. These methods include the use of binding agent (polyethylene glycol, polyvinylpyrrolidone, polyvinyl polypyrrolidone, acetic acid and sodium hydroxide), heating, soaking in water and drying, wood ash, chopping and storage, urea, solid-state fermentation and use of enzymes (Ben Salem *et al*., 2000; 2005a; Silanikove *et al*., 2001; Makkar, 2003). The methods are commonly grouped into physical and chemical methods.

2.5.1 Physical methods

Drying reduces levels of condensed tannins in *Acacia* foliage (Ben Salem *et al*., 2000; Makkar, 2003). Drying cassava and *Leucaena* leaves at 90 °C for 24 hours decreased tannin content. Tannin content can be reduced by storing fresh (40 % moisture leaves) chopped leaves at 37 °C prior feeding (Ben Salem *et al*., 2000; 2005ab; Makkar, 2003). Grinding and chopping also decrease the negative effects of tannins in leaf meals (Wina *et al*., 2005). Soaking is amongst the recommended methods in reducing the detrimental effects of tannins (Medunga *et al*., 2012). Some methods of physical treatments do not seem to be economically viable and are less effective due to intensive labour requirements and being time consuming. However, chopping of fresh leaves, water soaking and then storage can be of practical use to the farmer as it requires only minor changes in normal farm practices (Bhat *et al*., 2013). Moreover, these methods can block effects of tannins in *Acacia* foliage (Ben Salem *et al*., 2005b).

2.5.2 Chemical methods

Several chemical methods from urea to tannin-binding agents for inactivating tannins have been tested (Alipour & Rouzbehan, 2007; Ben Salem, 2005ab; Kyarisiima *et al*., 2004). Kyraissima *et al* (2004) reported that wood ash treated sorghum reduce level of tannins in tannin rich feed resources. The use of urea to neutralize tannin-rich leaf meals is recommended and also gives extra nitrogen to the animals (Sahnoune *et al*., 1991). Moreover, addition of urea is effective due to the higher pH caused by evolution of urea-ammoniation.

Tannin-binding agents and enzymes are also used to neutralize anti-nutritive factors (Schons *et al*., 2012; Tshabalala *et al*., 2013). Polyethylene glycol, polyvinylpyrrolidone, polyvinyl polypyrrolidone, charcoal and sodium hydroxide are common examples of tannin-binding agents. Challenges of chemical methods include the loss of soluble nutrients, analyses and laboratory work and costly to resource-poor farmers. There is little information on the use of tannin-binding agent such polyethylene glycol to overcome problems associated with reduced feed nutrient value, toxic effect and utilization on pigs fed *Acacia* leaf meal diets. Therefore, there is a need to explore the influence of polyethylene glycol in growing pigs fed *Acacia tortilis* leaf meals diets. Animal performance is expected to increase linearly as PEG levels increase, until a point when the performance starts to decrease. The linear increase at low to moderate inclusion levels need to be investigated, for farmers to determine cost-effective and sustainable levels to optimize pig growth.

2.5.3 Structure of polyethylene glycol

Polyethylene glycol is a non-nutritive synthetic polymer, an oligomer or polymer of ethylene oxide with a molecular mass below 20 000 g/mol (Makkar, 2003; Makkar *et al*., 1995). Polyethylene glycols of different molecular weight are available commercially, and their molecular weight ranges from as little as 200 upwards. The chemical formula for PEG is $(C_2H_4O)_{n+1}$ H_2O (Henning, 2002) where "n" represents the average number of oxethylene groups, and below 55 °C it is a free-flowing white powder freely soluble in water. Molecular weight of PEG from 200 to 600 occurs as a slight vapour, colourless, slightly hygroscopic liquids with a slight characteristics odour (Henning, 2002). While PEG with higher molecular weight are available as free-flowing white powder (1500 and 4000) and creamy with flakes for the range 4000 to 8000 (Gao, 1993). Makkar *et al.* (1995) reported that PEG 4000 and 6000 has more capacity to bind with tannins than PEG of various other molecular weights. Therefore, it might be preferred to others, because of its ability to bind tannins at near neutral pH. It is preferred because it makes plant proteins to be more available for digestion and absorption by binding to tannins. Ferraz de Oliveira *et al.* (2008) used PEG 6000 to determine its effect on protein output of free range pigs. Crude protein concentration from faeces was low indicating availability of dietary protein (Ferraz de Oliveira *et al*., 2008). Polyethylene glycol has the ability to dissolve many substances and the higher molecular weight PEG is capable to solubilize water-insoluble compounds (Gao, 1993).

The most important property of PEG is its solubility in water, making it ideal for inclusion in leaf meals (Henning, 2002; Ben Salem *et al*., 2005b). Polyethylene glycol has numerous properties such a viscosity, melting range, hygroscopicity, solubility in organic solvents, PEG solvency, additional property and blended PEG compounds (Turner *et al*., 2011). Salem *et al*. (2000) reported that PEG has been used for fractional precipitation of properties. It is usually used as a substance to make proteins from ingested tannin containing feed to be available for utilization by livestock.

2.5.4 Effects of PEG on tannins

Polyethylene glycol blocks the formation of tannin-protein complex. Besharati & Taghizadeh (2011) reported that PEG breaks already formed tannin-protein complexes, due to its high affinity (together with larger number of atoms). It deactivates tannins over a wide pH range of 2 to 8.5. Adequate oxygen molecules from water-soluble PEG form hydrogen bonds together with phenolic and hydroxyl group in tannins (Silanikove *et al*., 2001). This phenomenon makes protein and other nutrients available for utilization, and also increases the voluntary intake of leaf meals (Mantz, 2008). A minimum of 1.8 g PEG can completely reverse the binding effect of 1 g of tannins (Barry & Foss, 1983). Therefore, addition of PEG in diets containing high levels of tannins improves the feed value of such diets. In addition, supplementing PEG to zero tannin-containing feed source has no effect on feed utilization, because it does not provide nutrients.

2.5.5 PEG treated leaf meal diet on pig performance and metabolism

The effect of tannins on eating activity of pigs, and interactions with PEG supplementation has not been reported. The present study is done to determine the amount of PEG required for optimum voluntary feed intake and growth performance in pigs. Sodium hydroxide (NaOH) has been used to treat high levels of tannins in leaves which decrease voluntary feed intake and nutrient absorption in the small intestines (Liu *et al*., 2002; Silanikove *et al*., 2001). In poultry, effect of PEG treated rapeseed meal on the performance of broiler chickens has been done (Karunajeewa *et al*., 1990). Oduguwa *et al.* (2007) reported that PEG (1 g and 10 g /kg DM + 30 g of malted sorghum with 140 g/kg tannin) had no improvement on the apparent amino acid digestibility and true amino acid digestibility. This could be associated with tannin bound by other components such as fibre. While Mansoori & Acamovic (2009) determined that PEG improved protein digestibility, utilization and reduced negative effect of tannins in the gastrointestinal tract of birds fed on high tannins feeds. Feeding fermented liquid compound diet to weaned piglets improved daily gains and also modified the gastrointestinal environment (Russel *et al*., 1996).

Yu *et al.* (1996) revealed that the addition of PEG to the diets significantly increased the apparent ileal digestibility of nitrogen and some amino acids for the pigs and rats. The PEG also increases the palatability of high tannin forages thereby increasing their intake (Silanikove *et al*., 1996; Nsahlai *et al*., 2011). Yu *et al*. (1995) added PEG at a rate of 2 mg/g total condensed tannins to make most of protein available for utilization. Detrimental effects in nutrition caused by tannins may not be completely reversed by application of PEG. However, the presence of PEG may increase astringency and appetite when low-tannin alternatives offer fewer nutrients than the high-tannin forage (Titus *et al*., 2001). Unfortunately, the information on effect of polyethylene glycol supplementation on the performance of pigs fed leaf meal diet is scarce and generally limited.

The adaptation of pigs to different types of diet and levels of nutrition is important in pig production. Hence, blood metabolites concentrations are commonly used in motoring health (functioning of liver, heart and kidney) and nutritional problems of pigs. Blood metabolites are undoubtedly the best practical predictor and it could be useful in preventing metabolic shortages together with damages in the animal (Marzo *et al*., 2002; Khanyile, 2013). Changes in the constituent compounds of blood when compared to normal values could be used to determine metabolic state together with feed quality. Moreover, blood indicators alongside body weight condition of pigs could detect status of energy, protein, glucose and early sign of metabolic disorders (Lim *et al*., 2013). Serum protein, iron, and cholesterol are vital when identifying the nutrients profile of animals (Ndlovu *et al*., 2009). Activities of aspartate aminotransferase, alakaline phosphate and alanine aminotransferase which are slightly dependent on cholesterol indicate liver damage and can be used to describe the quality of feed given to pigs.

Rowan & Lawrence (1986) studied growth, tissue deposition and metabolism from growing pigs fed on rapeseed meal treated with different levels of PEG and copper, and they determined that

PEG at 17 g/kg DM significantly improved growth rate. Savage *et al.* (1980) reported the influence of micronization and polyethylene glycol on the nutritional value of brown sorghum for growing pigs. The effect of PEG treated Acacia leaf meal diets on blood parameters in growing pigs has not been studied. Therefore, evaluation of nutritional status of pigs is vital because information is fundamental in the formulation of their quality diets. Blood cholesterol can be obtained from the diet (Washington & Van Hoosier, 2012) and acceptable level indicates the healthy safely of the leaf meal with the pigs. Blood cholesterol concentration could be reduced by the presence of tannins in pig diets (Jansman, 1993), and reduction in iron and copper bioavailability was observed in pigs given tannin rich feeds (Bravo *et al*., 2008). Khanyile (2013) observed that *Acacia tortilis* leaf meal diet has no influence on the serum phosphorus and total protein. There is little information concerning the effect of PEG treated leaf meal on blood metabolites from growing pigs. There are few challenges of treating leaf meals with PEG compared to other methods. Thus, there is a need to determine effect of PEG treated leaf meal on blood lipid profiles that may be of interest to human nutritionists and vital to pigs health.

2.5.6 Constraints to utilization of PEG

The PEG must have a large number of oxygen atoms and it should contain sufficient oxygen molecules in a chain to form strong bonds with phenolics and hydroxyl groups of tannins (Silanikove *et al*., 2000). It has the disadvantage, however, of requiring tissue samples. Tunner *et al*. (2011) discovered that the potentially unfavourable effects that might be caused by PEG can be divided into several groups; adverse side effects in the body can be motivated by the polymer itself or by side products formed during synthesis that lead to hypersensitivity. When excess amount is given, unexpected changes in the body of animal can occur with PEG-based carries.

The PEG requires some skills and knowledge to use without compromising health of pigs. The PEG might be administered to animals in different ways such as spraying leaves, oral dosage and mixed with diet (Ben Salem *et al*., 2000). Application of PEG by spraying the standing plants prior utilization requires a lot of labour and its time consuming, while mixing with diets is preferred. Oral dosage may include a slower onset of action, irritating and stressing to animals (Turner *et al*., 2011). Otherwise, spraying and oral dosage both requires a moderate technical skill accompanied with confidence. The use of PEG needs to be performed accurately, rapidly, and humanely in animals (Gao, 1993). Application of PEG has been recommended however, the methods are either uneconomical and/or impractical under farmer management. There is little on the literature concerning the supplementation of PEG on leaf meal diets of pigs.

2.6 Summary

Tannin-containing feed sources such as trees and shrubs foliage and agro-industrial by-products could be used to partially replace expensive commercial protein source, because they are not directly consumed by humans. For example, *Acacia* leaves and pods could be used as an alternative source to provide protein for pigs. However, these feed resources contain anti-nutritive factors that reduce utilization. Therefore, the response of pigs to low and moderate levels of polyethylene glycol on pig growth and metabolism needs to be explored. Use of PEG may assist in increasing protein and amino acid supply to meet nutrient requirements. The response and optimum inclusion level for pig performance of PEG treated *Acacia tortilis* effect on pigs is, however, not yet known. The objective of the study is, therefore, to determine growth performance and serum biochemistry of growing pigs fed on *Acacia tortilis* leaf meal-based diet treated with low to moderate levels of PEG, with the purpose of accurately predicting response at levels before the plateau is reached.

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CHAPTER 3: Feed intake and growth performance of growing pigs fed on *Acacia tortilis* leaf meal treated with polyethylene glycol

Abstract

The objective of the study was to determine the response in feed intake and performance of pigs fed on incremental levels of polyethylene glycol (PEG). Forty-eighty clinically healthy male F1 hybrid pigs were randomly allotted to six diets containing 0, 5, 10, 15, 20 and 25 g/kg of PEG, respectively. *Acacia tortilis* leaf meal was included at a rate of 150 g/kg. Each diet was offered *ad libitum* to eight pigs in individual pens. Average daily feed intake (ADFI), scaled feed intake (SFI), average daily gain (ADG), body weight (BWT), and gain to feed ratio (G: F) were determined weekly. The ADG showed a linear response to PEG ($P < 0.01$). The linear regression equation was $y = 0.0061x + 0.6052$ ($R^2 = 0.64$). There was a quadratic response to PEG on ADFI, BWT and SFI ($P < 0.01$) and G: F ($P > 0.05$). The regression equations and R^2 values were: ADFI $y = 0.0008x^2 – 0.0086x + 1.2339$ ($R^2 = 0.96$); SFI $y = 0.0147x^2 – 0.2349x + 40.096$ ($R^2 = 0.95$) and G: F ratio $y = 0.0002x^2 – 0.017x + 0.5168$ ($R^2 =0.52$). The ADFI, SFI and ADG increased as weeks of feeding progressed ($P < 0.01$), but the G: F ratio decreased as weeks increased. The response of average daily feed intake, scaled feed intake, average daily gain, and gain: feed ration were related to PEG inclusion.

Keywords: gain: feed ratio; quadratic relationship; scaled feed intake; polyphenolic compounds.

3.1 Introduction

Protein-rich tree legumes are highly abundant in the tropics, yet protein is one of the most limiting nutrients in livestock productivity (Leterme *et al*., 2005). Locally available fruits, legume foliage, tree leaves and pods can be utilized as protein sources for pigs (D'Mello, 1995; Makkar, 2003; Woyengo *et al*., 2014). Growth rates of up to 350 g/day have been reported in pigs fed on Acacia leaf meal-based diets (Stur *et al*., 2007; Phensavanh & Phommaly, 2010). Leguminous tree leaves contain high crude protein and amino acids. *Acacia* species have high crude protein (up to 218 g/kg DM), making one of the forages with a huge potential for feeding animals (Dube *et al*., 2001; Mlambo *et al*., 2007; Khanyile *et al*., 2014).

These forages, however, contain polyphenolic compounds and high fibre content. The polyphenolic compounds for Acacia species range between 11 and 90 g/kg DM (Mokoboki *et al*., 2005). Polyphenolic compounds cause astringent or bitter taste (Khanyile *et al*., 2014). Low levels of tannin in pig diets may not lower feed intake and growth performance of pigs (Cappai *et al*., 2013). When high levels of tannin-rich leaf meals are fed to pigs, feed intake and growth performance is suppressed (Halimani *et al*., 2005; Materns *et al*., 2012; Khanyile *et al*., 2014). Khanyile *et al*. (2014) estimated that the optimum *A. tortilis* inclusion level should not exceed 137 g/kg DM. Otherwise feed intake and growth performance is compromised.

Polyphenolic compounds form complexes with nutrients which are difficult to digest and utilize. Khanyile *et al*. (2014) estimated that inclusion level of *A. tortilis* leaf meal beyond 137 g/kg DM suppresses feed intake, growth rate and gain: feed ration of pigs. Therefore, to increase digestibility, utilization and absorption of nutrients in the leguminous leaf meals, there is a need to explore the benefits of incorporating tannin-binding agents to pig diets. Examples of potential compounds include polyethylene glycol (PEG), polyvinyl pyrrolidone (PVP), and polyvinyl polypyrrolidone (PVPP). Of these examples, PEG has been found to be more effective in reducing the detrimental effects of polyphenolic compounds on animal performance (Silanikove *et al*., 2001; Makkar 2003; Ben Salem *et al*., 2005a). The PEG does not affect digestion processes, intake and growth performance of animals fed on diets without polyphenolic compounds (Getachew *et al*., 2000; Provenza *et al*., 2000). The PEG bounds all the polyphenolic compounds including flavone, lignin and tannins, though it has high affinity for tannins (Makkar, 2003). Studies have been focusing on determining the optimum inclusion level of PEG in *Acacia tortilis*. Mokoboki *et al*. (2013) found that, to maximize pig performance, pigs should be fed on an *Acacia tortilis* diet with an inclusion level of 25 g per kg. Considering the high cost of PEG, it is, however, expensive for pig producers to adopt a PEG inclusion level of 25 g per kg. Predicting pig performance at PEG inclusion levels below 25 g/kg becomes important. Little, if any, information is available on the response in feed intake and growth performance of pigs fed on *A. tortilis* leaf meal-based diet treated with incremental levels of PEG at low to moderate levels. Acacia leaf meals are rich in protein and amino acids that are required for growth and maintenance of pigs. The objective of the study was, therefore, to determine the response in feed intake and growth performance of pigs fed on *A. tortilis* leaf meal-based diet treated with incremental levels of PEG at low to moderate inclusion levels. It was hypothesized that there is a linear relationship between PEG inclusion levels and pig performance.

3.2 Materials and Methods

3.2.1 Study site

The study was conducted at Ukulinga Research farm at the University of KwaZulu-Natal in Pietermaritzburg, South Africa. The farm is located at 30° 24’S, 29° 24’E and altitude ranges from 700 to 775 m above sea level. The mean annual rainfall is 735 mm, most of which occurs between October and April. The mean maximum and minimum temperatures are 25.7 °C and 8.9 °C, respectively. Light to moderate frost occurs in winter.

3.2.2 Leaf meal collection

Acacia tortilis leaves were harvested at Makhathini Research Station, Jozini, South Africa. *Acacia tortilis* was selected for its superior nutritive value (high crude protein and amino acids), wide distribution in addition to it being one of the main encroaching species in Southern Africa. The leaves were harvested during the post rainy season at an advanced stage of maturity. Tree branches were clipped from the growing points and air-dried for three days in a shade to prevent damage of heat sensitive nutrients. Polyethylene sheets were used during drying to prevent contamination with soil. During drying, the leaves were often turned to prevent accumulation of moulds. The branches were then carefully beaten by a stick to release leaves. To remove thorns and pods, leaves were passed through 2 mm sieve, bagged and kept in well-ventilated dry room until required for dietary formulation.

3.2.3 Pigs and housing

Forty-eight clinically healthy male F_1 hybrid (Landrace x Large White, PIC Group) pigs were randomly allotted into individual pens and fed a leaf meal diet for 31 days. Pigs with an initial body weight of about 15.8 ± 0.032 kg were used in the experiment. The pigs were purchased from Kanhym farm, about 60 km from Ukulinga farm, KwaZulu-Natal Province, South Africa. They were then transported on a tarred road in a truck. The pigs were ear tagged and housed in a room with artificial heating, lighting, and proper ventilation systems. Individual pens measuring in 1.5 x 1 m^2 were arranged in two rows (24 each side). The ambient temperature and relative humidity were recorded throughout the experiment at 15 minute intervals using a HOBO TEMPERATURE, RH©, 1996 ONSET logger (Onset Computer Corporation). The house conditions were kept at a temperature of 21.9 ± 2.24 ^{o}C, 45.2 ± 6.85 % relative humidity and a 12 h dark-12 h artificial light cycle. Each pen was provided with water through a low-pressure nipple drinker and feed was supplied in a plastic self-feeder trough (Big Dutchman Lean Machine®, Postfach). The pigs were not given any antibiotics or growth promoters. The care and use of the pigs was performed following the Certificate of Authorization to experiment on Living Animals from UKZN Animal Ethics Committee (Ref: 076/14/Animal).

3.2.4 Experimental design, diets and feeding

Experimental pigs were blocked based on body weight and used in a completely randomized design. Each pig, representing an experimental unit, was individually penned and randomly allocated to each of the treatments. The *A. tortilis* leaf meal was treated with six increment levels (0, 5, 10, 15, 20 and 25 g/kg) of PEG. Tannin binding agent, PEG 4000 molecular weight (MW) was supplied by Old Mill Industrial Park, Durban, South Africa. The pigs were purchased at around 10 kg body weight and fed on a conventional weaner diet (from Meadow feed) until they weighed an average of 15.8 ± 0.032 kg. They were then allowed a 7 day of adaptation to the experimental treatments. Pigs were weighed prior to the start of the experiment. Six experimental diets were formulated using Winfeed Feed formulation Software programme (Table 3.1). All the eight pigs per treatment were fed a diet containing 150 g/kg of *A. tortilis* (DM basis) treated with six increment levels (0, 5, 10, 15, 20 and 25 g/kg) of PEG. Clean water was available at all times. Feeding was provided *ad libitum* for the 31 days trial period.

3.2.5 Chemical composition

Analysis of the chemical composition was determined in the Animal and Poultry Science Laboratory at the University of KwaZulu-Natal, Pietermaritzburg, South Africa. The methods described by AOAC (1990) were followed to determine dry matter (945.15), ash (942.05), crude protein (979.09) and ether extract (920.39). Crude protein was calculated using N x 6.25, where N contents were analysed following Dumas Combustion method in a Leco Truspec Nitrogen Analyser, St Joseph MI, USA. The neutral detergent fiber (NDF) and acid detergent fiber (ADF) were determined using Ankom Fiber Analyser (ANkom Macedon, NY, USA) following methods by Van Soest *et al.* (1991) and Van Soest (1973), respectively. Acid hydrolysis were used for amino acids preparation (AOAC, 1984; method 982.30) before analyses using an amino acid analyser (SY-KAM, Erising, Germany), Modifications by Mills *et al*. (1989) were followed. Condensed tannins were estimated calorimetrically by the butanol-HCL method (Reed et al., 1982). Water holding and swelling capacity were determined following methods described by Whittemore *et al*. (2003) and Canibe and Bach-Knueben (2002). Samples for minerals were ashed at 450 °C and 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) using atomic absorption. Table 3.2 shows the chemical compositions of *A. tortilis* leaves and diet containing PEG treated *A. tortilis* leaf meal.

Table 3.1: Ingredient composition of the diet used in the study

Ingredient	DM basis (g/kg)
Acacia tortilis leaf meal	150.0
Maize	345.8
Wheat bran	194.0
Soybean meal	220.1
Sunflower oil	50.0
Limestone	17.6
Monocalcium phosphate	13.3
Salt	3.3
Lysine	2.0
Vitamin + Mineral premix	1.5
DL- Methionine	1.4
L-Threonine	0.9

Table 3.2: Chemical composition of *Acacia tortilis* leaves and diet

Component	Leaf meal	Diet
Dry matter (g/kg)	992	894
Ash (g/kg DM)	65.1	72.2
Crude protein (g/kg DM)	218.4	215.2
Ether extract (g/kg DM	40.2	86.4
Neutral detergent fibre (g/k DM)	495	241.2
Acid detergent fibre (g/kg DM)	298.6	104.2
Neutral detergent insoluble nitrogen (g/kg DM)	27.5	ND
Acid detergent insoluble nitrogen (g/kg DM)	18.3	ND
Condensed tannins (mg/kg DM)	51.6	7.7
Water holding capacity (g_{water}/g_{feed} DM)	6.1	4.09
Swelling capacity (ml/g DM)	4.8	3.26
Lysine (g/kg DM)	ND	13.5
Threonine (g/kg DM)	ND	8.5
Methionine (g/kg DM)	ND	5.3
Calcium (g/kg DM)	ND	12.1
Phosphorus (g/kg DM)	ND	7.9
Iron (mg/kg)	ND	250

ND: not determined

3.2.6 Measurements

Weekly weight gain, amount of feed consumed, feed refusals and spillages were recorded. Weekly feed intake (WFI) was determined by weighing the feed trough at the beginning and end of the week. Plastic trays were placed underneath each cage to collect spillages, and spillages were collected with the aid of a hand broom and a duster. Dried feed spillages and refusals were subtracted from the total weight of feed given to pigs for that particular week. Average daily feed intake (ADFI) for each week was estimated by dividing feed consumed for that particular week by seven. A scaled daily feed intake (SFI) was calculated as g feed per kg body weight per day. Weighing was done every week from 0700h to 0830 h throughout the trial. Average daily gain (ADG) was determined by dividing the difference between body weight at the beginning and the end of each week by seven. The gain: feed ratio (G: F) for each pig was also calculated by dividing ADG by ADFI.

3.2.7 Statistical analyses

The General Linear Models procedure of SAS (2008) was used to test for significance of inclusion level of PEG and week on performance parameters. The initial body weight of pigs was included in the model as a covariate. PROC REG was used to determine the relationships between PEG inclusion level and BWT, ADFI, SFI, ADG and the G: F ratio.

The GLM model used were: $Y_{ijk} = \mu + P_i + W_j + (P \times W)_{ij} + E_{ijk}$

Where: Y_{ijk}– is the response variable

μ - is the overall mean common to all observations

P_i – is the effect of PEG inclusion level

W_i – is the effect of weeks of feeding

$(P \times W)_{ij}$– is the interaction between the PEG inclusion level and weeks

E_{ijk}– is the residual error.

The regression model used was: $Y = \beta_0 + \beta_1 L + \beta_2 + E$

Where: Y is the response variables (ADFI, SFI, ADG and G: F ratio)

$\beta_0 + \beta_1 + \beta_2$ linear regression component

L is the PEG inclusion levels

E is the residual error

3.3 Results

3.1 Influence of PEG inclusion levels on pig performance

The summary of statistics for the performance parameters of pigs fed *A. tortilis* meal treated with incremental levels of PEG are shown in Table 3.3. The initial body weight had no significant impact on the performance of pigs fed on *A. tortilis* leaf meal-based diet. Inclusion level of PEG in leaf meal diets affected BWT, ADFI, SFI, and ADG during the 4 week study period ($P < 0.01$). The G: F ratio was also affected by inclusion level of PEG ($P > 0.05$). The response of BWT, ADFI, SFI, ADG and G: F to PEG treated *A. tortilis* leaf meals during each of successive feeding were different ($P < 0.05$). There was a significant PEG inclusion level × week interaction on ADFI, SFI, ADG ($P < 0.01$) but did not affect BWT and G: F ($P > 0.05$).

Table 3.3: Summary statistics and level of significant of body weight, average daily feed intake, scaled feed intake, average daily gain, gain: feed ratio of growing pigs fed on polyethylene glycol (PEG) in *Acacia tortilis* leaf meal diets

Parameters	PEG level	Week	PEG × Week interaction
Body weight	***	***	NS
Average daily feed intake	***	***	***
Scale feed intake	***	**	**
Average daily gain	***	***	***
Gain: feed ratio	*	*	NS

*** P < 0.001; ** P < 0.01; * P < 0.05; NS; not significant (P > 0.05)

Figure 3.1 and Table 3.4 show the relationship between PEG level in *A. tortilis* leaf meals and ADFI, SFI, ADG and G: F in growing pigs fed on *A. tortilis* leaf meal diet treated with PEG. There was a quadratic PEG effect in ADFI (Figure 3.1; $P < 0.01$). As the PEG inclusion level increased, the ADFI increased at an increasing rate. The R^2 value was 0.96. The quadratic relationship between PEG and SFI was observed ($P < 0.01$). The SFI decreased at low PEG inclusion levels before it started to increase at an increasing rate. The R^2 value was 0.95 and then SFI is explained by the quadratic regression.

Increasing levels of PEG inclusion in *A. tortilis* leaf meal illustrated significant effect of PEG on ADG ($P < 0.05$). There was a linear increase in ADG as the PEG inclusion level increased (Figure 3.1, Table 3.4). The R^2 value was 0.64. There was a quadratic PEG effect on G: F ratio ($P > 0.05$, $R^2 = 0.52$). The gain: feed ratio was decreasing as PEG level increased before it gradually increased. The pigs fed on *A. tortilis* leaf meal diet with no PEG had high gain: feed ratio than pigs fed PEG treated leaf meal (Figure 3.1; Table 3.4). There was a decreasing quadratic response to PEG in BWT ($P < 0.05$) of growing pigs fed PEG treated *A. tortilis* leaf meals diets. The highest BWT of 33.6 ± 0.63 was observed when supplemented with the high level.

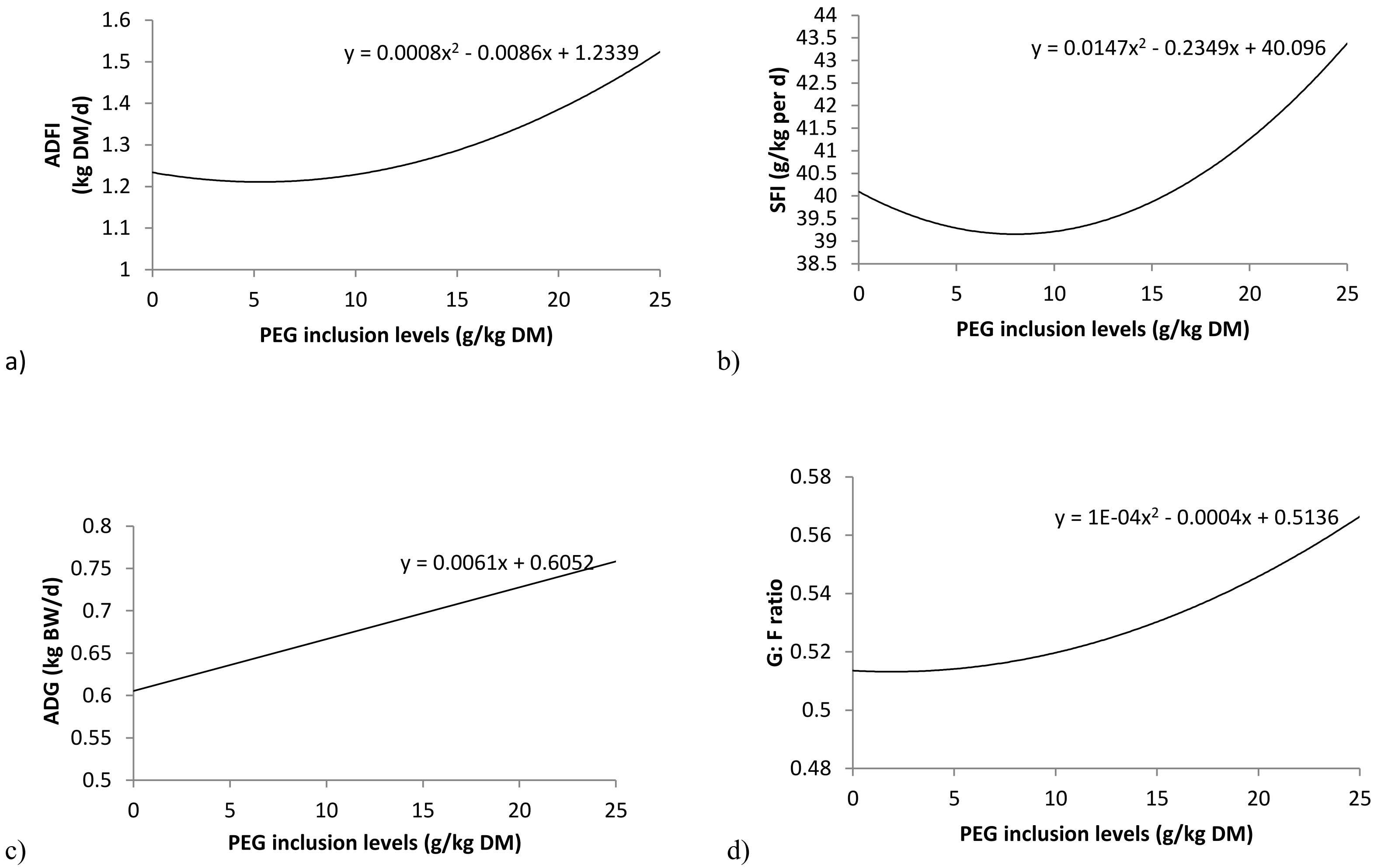

Figure 3.1: Response of PEG treated *Acacia tortilis* leaf meals on average daily feed intake (ADFI), scaled feed intake (SFI), average daily gain (ADG) and Gain: feed ratio (G: F) in growing pigs

Table 3.4: Relationship between polyethylene glycol (PEG) treated *Acacia tortilis* leaf meals and average daily feed intake (ADFI), scaled feed intake (SFI), average daily gain (ADG) and Gain: feed ratio (G: F) in growing pigs

	Inclusion level of PEG						SEM	Equations	R^2	Sig
Parameter	0	5	10	15	20	25				
ADFI (kg DM/d)	1.23	1.24	1.21	1.31	1.35	1.54	0.019	$y = 0.0008x^2 - 0.0086x + 1.234$	0.96	***
SFI (g/kg per d)	0.5	39.04	39.01	40.52	40.83	43.46	0.461	$0.0147x^2 - 0.2349x + 40.096$	0.95	***
ADG (kg BW/d)	0.65	0.58	0.65	0.74	0.69	0.78	0.02	$y = 0.0061x + 0.6052$	0.64	*
G:F ratio	0.53	0.49	0.5	0.57	0.54	0.55	0.019	$y = 0.0002x^2 - 0.0017x + 0.517$	0.56	*

SEM= mean standard error; R^2: R-square value; Sig: Significance level; *** $P < 0.001$; ** $P < 0.01$; * $P < 0.05$; NS; not significant ($P > 0.05$); n= 8

Table 3.5 shows least square means of the effect of PEG level for pig performance in a 4 week period. Body weight increased with increase in weeks of feeding. The pigs consumed more feed in Week 3 (1.55 ± 0.02) and decrease of intake was observed in Week 4 (1.34 ± 0.02), ($P < 0.001$). The ADFI, SFI and ADG increased as weeks of feeding progressed and decrease in feed intake was seen in Week 4. The gain: feed ratio decreased as number of weeks for feeding increased.

3.4 Discussion

All the pigs used in the current experiment remained clinically healthy throughout the trial. The PEG is a tannin binding agent which forms complexes with hydrolysable and condensed tannins (Jones, 1965). Polyethylene glycol has high ability to bind with tannins than protein, thus release protein from tannin-protein complex. Polyethylene glycol 4000 eliminates the negative effects of polyphenolic compounds, such as tannins in livestock and prevents formation of tannin-protein complex (Ben Salem *et al*., 2005; Motubase *et al*., 2008; Mlambo *et al*., 2009). The presence of PEG in the diet may increase astringency, appetite and palatability of high tannin forages (Titus *et al*., 2001). The *Acacia tortilis* leaf meal-based diet used in the current trial was formulated to meet the nutrient requirements for growing pigs (NRC, 2012). The recommended target levels of crude protein in diets for growing pigs is 180 to 220 g/kg DM (NRC, 1998) for potential development, growth and maintenance.

Increasing PEG level is expected to produce a linear relationship with digestibility, nitrogen retention and performance of animals. The linear relationship is expected to start diminishing until it reaches a plateau.

Table 3.5: Least square means for pig performance fed on polyethylene glycol in *Acacia tortilis* leaf meal-based diet in a four week period

Parameters	Weeks of successive feeding					Significance	
	1	2	3	4	SEM	Linear	Quadratic
BWT (kg)	23.51	28.47	33.98	38.50	0.51	***	**
ADFI (kg DM/d)	1.07	1.30	1.55	1.34	0.02	***	***
SFI (g/kg/ d)	36.47	45.67	45.00	34.95	0.38	**	***
ADG (kg BW/d)	0.63	0.71	0.76	0.63	0.02	NS	***
G: F	0.59	0.56	0.49	0.49	0.02	***	*

SEM= mean standard error; NS: not significant; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$

ADFI: average daily feed intake; SFI; scaled feed intake; ADG: average daily gain; G: F: Gain: feed ratio; BWT: body weight of pigs at four week; n= 8

Beyond that point, no further increase in animal performance is expected. Since PEG is relative expensive (1 kg = R 120.00), it is important to accurately predict performance of pigs at low to moderate inclusion levels. The hypothesis tested in the current study was to test whether the linear relationship expected would be obtained when *Acacia tortilis* was included at 150 g/kg inclusion level. The information enables farmers to utilize PEG sustainably and cost effectively. The findings in the current study highlighted that most growth parameters are not linearly related to PEG.

The ever-increasing human population puts a strain on cereals and legumes, leading to increased cost of feed ingredients for animals (FAO, 2011). In pig production, leaf meals can be a good source of crude protein, amino acids and energy (Woyengo *et al*., 2014). Therefore, better utilization of leaf meals is crucial for sustainable pig production systems and provision of cheap quality feed. The quadratic relationship between PEG inclusion and SFI suggests that PEG stimulates feed consumption of tannin-rich feeds. Pigs fed on *A. tortilis* leaf meal diet treated with PEG had an increased ADFI. The improved feed intake indicates that PEG has a positive influence on tannin-rich leaf meals. The increase in feed intake agrees with Titus *et al*. (2001) and Mantz (2008) who treated tannin-rich feedstuffs with PEG and observed increased feed intake. Pigs on high levels of PEG consumed more feed which is associated with the ability of PEG to bind with tannins and release protein from the complex. Tannins cause astringency or bitter taste that reduces palatability and digestibility (Alam *et al*., 2007; Materns *et al*., 2012). The supplementation of PEG 4000 to *A. tortilis* leaf meal diets could have prevented formation of tannin-protein complexes and improve nutritive value of the feed. Polyethylene glycol can improve digestibility and utilization of tannin feedstuffs, allowing animals to grow ability to utilize tanniferous feeds (Decandia *et al*., 2000; Mansoori & Acamovic, 2007).

As expected, there was a significant increase in ADFI and SFI as number of weeks increased. The increase in ADFI could be explained by increase in body size and the increasing gut capacity of the pigs. Moreover, as the number of feeding weeks progresses the pigs were adapting to PEG in *A. tortilis* leaf meal diet. These factors influence feed consumption to meet the requirements for potential growth (Nyachoti *et al*., 2004; Ndou *et al*., 2013). Feed intake determines nutrient intake levels, body weight gain, feed convention ratio and growth rate. The quadratic relationship between ADFI and PEG can be explained by benefits of PEG inclusion at different levels. Echeverria *et al*. (2002) results, showed that pigs offered acetic acid and/or sodium hydroxide treated *Leucaena leucocephala* leaf meal demonstrated a quadratic relationship. Palmer and Jones (2000) demonstrated that PEG binding capacity to tannins in leaves increase with PEG up to a point where it becomes partially constant *in vitro* digestion. Organic matter digestibility and metabolizable energy of tannin containing tree leaves increased with increasing levels of PEG (Kalamal *et al*., 2005; Rubanza *et al*., 2005).

As expected, pigs fed on high levels of PEG treated leaf meal diet had a higher ADG, possibly as a result of the increased ADFI. Increased ADG could be attributed to the pigs getting enough nutrients for increase in body weight. The response of the pigs given low levels of PEG may show that PEG given to them was not sufficient to eliminate deleterious effects of polyphenolic compounds on pigs. The low ADFI at low inclusion level of PEG could also be ascribed by the digestibility of the diet offered to the pigs (Whittemore *et al*., 2003). The *A. tortilis* has high fibre content with large indigestible components which influence digestibility of feed. Provenza *et al*. (2000) reported that animals could recognize the benefits of ingesting PEG if they first eat a meal of high tannin containing feed prior to PEG treated leaf meal-based diet. This might be the case in the current study where pigs offered low levels of PEG had low ADFI and ADG. The observed general increase in ADG as weeks of feeding increased could be due to increase in body weight, adaptation to feed and concur with previous findings (Whittemore *et al*., 2003). The observation that ADG linearly increased with PEG inclusion levels could be associated with the ability of different amounts of PEG to bind with tannins. The linear response of ADG while SFI is quadratic could not be explained.

The quadratic relationship observed between PEG inclusion levels and gain: ratio, suggest that pigs offered high amounts of PEG gas better conversion of feed into weight gain. These results suggest that pigs had enough PEG to neutralize the negative effects of tannins (Provenza *et al.,* 2000). Thus pigs at high PEG level are associated with better fed efficiency for a given rate of growth. The pigs fed on *A. tortilis* leaf meal treated with PEG requires 1.5 g less feed to gain a body mass.

A reduction in ADFI, ADG and G: F in pigs fed on diet containing more than 100 g/kg DM of leaf meal has been reported (D’Mello, 1995; Laswai *et al*., 1997; Halimani *et al*., 2005). In the current study, growing pigs fed on diet containing 150 g/kg *A. tortilis* leaf meal treated with PEG demonstrated improved feed intake and overall performance. These findings indicate that in a diet based on *A. tortilis* leaf meal (150 g/kg DM) PEG eliminates the negative effects of tannins. The observation that pigs fed on 150 g/kg *A. tortilis* leaf meal with no PEG had a low performance agrees with Khanyile *et al*. (2014) who showed a reduced ADFI and ADG when leaf meal was included above 137 g/kg. This indicates that the application of PEG positively affects astringency and bitter taste produced by high levels of ingested tannins. This could be attributed to improved feed nutritive value in presence of PEG as the result of the inactivation of tannins. Inclusion of PEG to tannin containing feed resources improves feed nutritive value due to PEG high ability to bind tannins (Makkar, 2003; Motubatse *et al.,* 2008).

The *Acacia tortilis* leaf meal diet could be useful to provide protein, because it is rich in protein, affordable and locally available. Although, the use of PEG offers an opportunity to improve ADF, ADG and G: F ratio it has been suggested it may not be economically feasible (Wina, 2010). In the current study, it was estimated that each gram of PEG costs about 0.12 R. From the equation y= 0.0061x + 0.6052, incorporating one gram of PEG in a diets containing 150 g/kg *Acacia tortilis* is expected to increase ADG by 6.1 g. As such, incorporating PEG reduces the time it would take to reach the target market weight for pigs. The economic benefit of using PEG would, therefore, depend on other factors affecting profitability of pig enterprises, such as labour costs, risk of pigs dying or being stolen, availability of Acacia leaves, costs of processing and storage and the acceptability of the pork produced from pigs fed on the indigenous leaf meals. All these factors need to be included in models that estimate the economic benefits of using leaf meals and PEG in pig rations.

3.5 Conclusions

The response of average daily feed intake, scaled feed intake, average daily gain, and gain: feed ration were related to PEG inclusion. Feeding *Acacia tortilis* leaf meals treated with PEG does not necessarily lead to linear increases in pig performance. It can be concluded that the relationship between PEG inclusion and performance of growing pigs fed on *Acacia tortilis* is exponential, rather than linear. This suggests that PEG (molecular weight 4000) is effective in preventing the binding of tannins and protein from *Acacia tortilis* leaves. The PEG could also be binding other polyphenolic compounds such as lignin. There is a need to assess the nitrogen retention and enzymes and serum concentrations in pigs fed *Acacia tortilis* leaf meal treated with PEG.

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CHAPTER 4: Response of nutritionally-related blood parameters and liver enzymes in pigs fed on *Acacia tortilis* treated with polyethylene glycol

Abstract

The relationship between nutritionally-related blood metabolites and liver enzymes of pigs fed on *Acacia tortilis* leaf meal treated with polyethylene glycol (PEG) were estimated. In a completely randomized design, 48 clinically healthy male growing pigs were random allotted to individual pens. There were eight pigs per treatment. The diet contained 150 g/kg *A. tortilis* which had been treated with six increasing levels (0, 5, 10, 15, 20 and 25 g/kg) of PEG for 31 days. The pigs were allowed *ad libitum* access to the diets. Inclusion of PEG had no effect on cholesterol, creatinine and uric acid concentration ($P > 0.05$). Inclusion of PEG showed a linear response on total protein (TP) and globulin, but quadratic to albumin ($P < 0.01$). There was linear relationship between PEG inclusion and cholesterol, creatinine and uric acid ($P > 0.05$). The concentration of aspartate aminotransferase (AST) ($P < 0.01$) and alanine aminotransferase (ALT) ($P < 0.05$) decreased linearly as PEG inclusion increased. There was a quadratic increase in alkaline phosphatase (ALP) as the PEG inclusion level increased ($P > 0.05$). The findings of the current study suggest that PEG can be included in leaf meal diet without any adverse effects on the health status and nutritional status of pigs. It could be concluded that inclusion of PEG levels in *A. tortilis* leaf meal up to 25 g/kg does not necessarily produce linear relationship with blood metabolites.

Keywords: albumin; alkaline phosphatase; alanine aminotransferase; aspartate aminotransferase; polyphenolic compounds; total protein.

4.1 Introduction

Halimani *et al*. (2005) reported that high intake of polyphenolic compounds could cause acute toxicosis and hepatocellular damage to pigs. Metabolite concentrations reflect the nutritional status and physiological responsiveness of the animal to its feed and feeding (Etim *et al*., 2014). Alkaline phosphatase (ALP), aspartate aminotransferase (AST) and alanine aminotransferase (ALT) are markers of hepatic disease and liver cytolysis in pigs (Jansman 1993; Kaplan *et al*., 2003).

In Chapter 3, it was concluded that feed intake, growth rate and the gain: feed ratio were related non-linearly to PEG inclusion. It was postulated that PEG could also be binding other polyphenolic compounds, calling for the need to assess concentrations of liver enzymes and serum metabolites concentrations in pigs fed on *Acacia tortilis* leaf meal-based diet treated with PEG. Inclusion of PEG in tannin-rich feeds has been shown to increase digestibility of nitrogen and amino acids for pigs and rats (Yu *et al*., 1996; Mariscal-landin *et al*., 2004). The objective of the study was to establish the influence of PEG inclusion in *A. tortilis* leaf meal diet on nutritionally-related metabolites and liver enzymes. It was hypothesized that PEG inclusion in leaf meals has a linear relationship with serum metabolites.

4.2 Materials and Methods

4.2.1 Study site

The study site is as described in section 3.2.1

4.2.2 Leaf meal collection

The preparation of leaf meal was conducted as described in section 3.2.2

4.2.3 Pigs and housing

Details on the management of pigs are provided in section 3.2.3

4.2.4 Experimental design, diets and feeding

The experimental diets and feeding process was described in section 3.2.4

4.2.5 Chemical composition

The analyses of leaves and diet are explained in section 3.2.5

4.2.6 Collection of blood samples and analyses

A 10 ml blood was collected through jugular venipuncture in non-coagulated vacutainer tubes (Becton Dickinson, Franklin, NJ), from each pig. This was done on the last day (day 31) of the trial. Samples were collected between 0700 to 0900h. After collection, the blood samples were kept in cooler box with ice. After centrifugation (1000 × g 10 min at 25 °C) within two hours of collection, the serum were stored in polypropylene tubes and kept at - 20 °C, pending analyses. The serum was analyzed spectrophotometrically for total protein (TP) concentration (Doumas & Biggs, 1972). Alkaline phosphatases (ALP) were assayed using the colometric method (Tietz *et al*., 1993). The enzymatic method was used for the determination of both cholesterol and uric acid following a method described by Allain *et al*. (1974) and Tietz (1995). Activities of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were analyzed using the ultraviolet method (Bergmeyer *et al*., 1986; Horder *et al*., 1991).

4.2.7 Statistical analyses

The PROC REG of SAS (2008) was used to determine relationships between TP, albumin, globulin, creatinine, and cholesterol, uric acid, AST, ALT and ALP with PEG inclusion level.

The model used was: $Y = \beta_0 + \beta_1 P + E$

Where: Y is the response variables (TP, albumin, globulin, creatinine, and cholesterol, uric acid, AST, ALT and ALP)

$\beta_0 + \beta_1$ linear regression component

P is the PEG inclusion levels

E is the error

4.3 Results

4.3.1 Nutritionally-related metabolites

The response of selected nutritionally-related blood metabolites to PEG treated *A. tortilis* leaf meal diet in growing pigs is shown in Table 4.1. There was a significant difference in TP, globulin and albumin concentration across different levels of PEG in *A. tortilis* leaf meal diet ($P < 0.01$). There was no relationship between PEG inclusion level in *A. tortilis* leaf meal-based diet and cholesterol, creatinine and uric acid levels in the blood ($P > 0.05$). Feeding incremental levels of PEG in *A. tortilis* leaf meal diet did not affect cholesterol, creatinine and uric acid concentration in pigs. The PEG in *A. tortilis* leaf meal tended to reduce the cholesterol level concentration in the blood.

Table 4.1: Response of selected nutritionally-related blood metabolites to PEG treated *Acacia tortilis* leaf meal-based diet

Parameter	PEG inclusion level (g/kg DM)						SEM	Regression coefficient		
	0	5	10	15	20	25		linear	Quadratic	Significance
Total protein (g/dl)	6.47	6.77	7.00	7.24	7.16	7.65	0.12	0.10 (0.05)	-0.01 (0.01)	*
Globulin (g/dl)	3.26	3.62	3.80	3.99	3.76	4.51	0.15	0.23 (0.06)	-0.02 (0.01)	**
Albumin (g/dl)	3.21	3.14	3.20	3.24	3.40	3.14	0.08	-0.12 (0.03)	0.01 (0.03)	**
Cholesterol (mg/dl)	74	73.38	77.13	69.13	71.75	72.14	2.43	0.73 (0.94)	-0.10 (0.09)	NS
Creatinine (mg/dl)	2.56	1.50	1.47	2.10	0.70	1.24	0.40	-0.12 (0.15)	0.01 (0.02)	NS
Uric acid (mg/dl)	0.23	0.15	0.35	0.25	0.19	0.25	0.05	0.01 (0.02)	-0.001 (0.00)	NS

SEM: Standard error of mean; ** *P < 0.01*; * *P < 0.05*; NS- Not significant;

There was a linear relationship between PEG inclusion and TP ($P < 0.05$; Figure 4.1). Total protein concentration increased linearly as the PEG inclusion increased (b= 0.21; $R^2 = 0.92$). There was a linear relationship between PEG inclusion level and globulin ($P < 0.01$). Globulin concentration in the blood increased with PEG inclusion increased (b = 0.20; $R^2 = 0.78$). The quadratic increase on albumin with PEG inclusion was observed ($P < 0.01$; Figure 4.1). As the PEG inclusion level increased albumin increased at an increasing rate (b= 0.01; $R^2 = 0.77$).

4.3.2 Liver enzymes

The relationship of *A. tortilis* leaf meal diet treated with PEG inclusion on liver enzyme activities are depicted in Figures 4.2 and 4.3. The concentration of the enzyme ALP was quadratically affected by PEG inclusion in the diet ($P > 0.05$; Figure 2). As PEG inclusion increased, ALP activity increased at an increasing rate ($P < 0.05$). The activities of ALT and AST were influenced by inclusion of PEG in *A. tortilis* leaf meal diet of pigs ($P < 0.05$). A linear response on AST activity to PEG inclusion was observed ($P < 0.001$). The activity of AST (b = - 5.76) and ALT (b = - 2.32) decreased as PEG inclusion level increased ($P < 0.05$; Figure 4.3).

4.4 Discussion

Khanyile *et al*. (2014) indicated that the optimum inclusion level of *A. tortilis* in diets of growing pigs, when no tanning binding agents are incorporated, is 137 g/kg. It is essential to determine the relationship of metabolites and liver enzymes with inclusion level of PEG, a substance that eliminates polyphenolic compounds (tannins being the major) effects, which in turn, suppresses pig performance and health (Halimani *et al*., 2005; Alam *et al*., 2007; Khanyile *et al*., 2014).

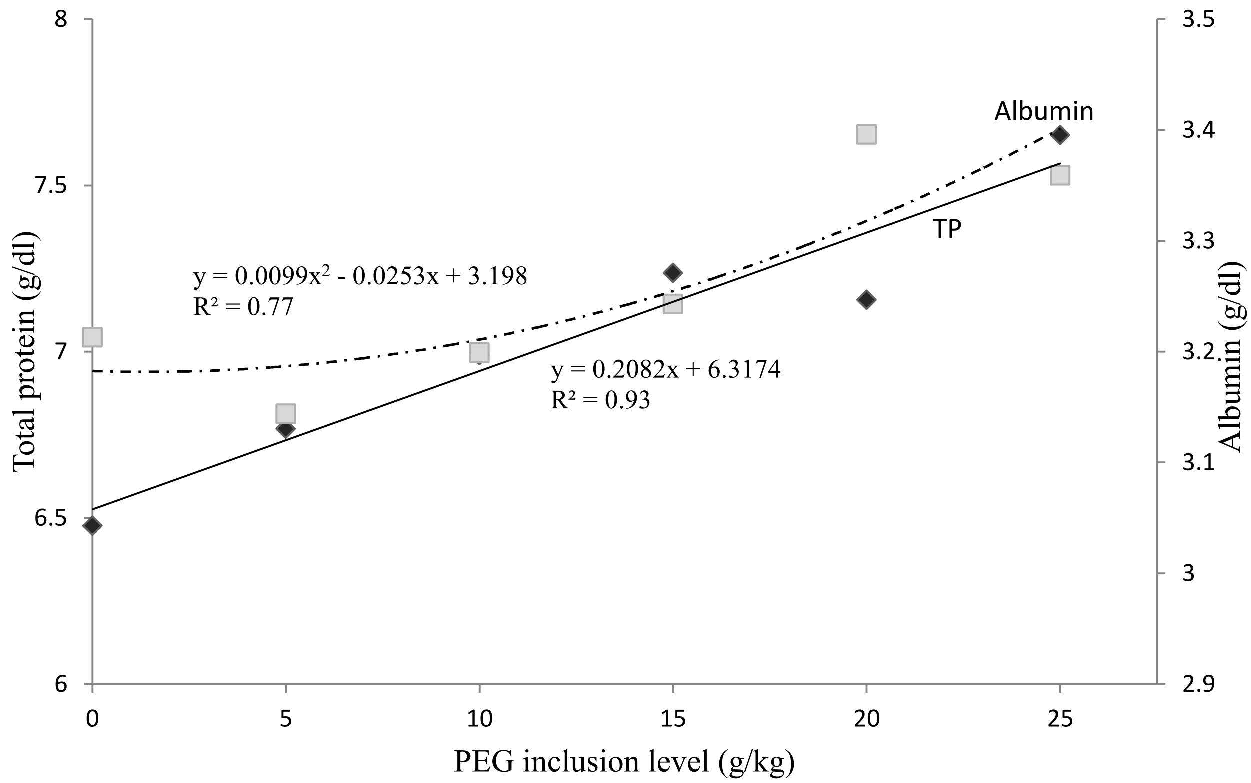

Figure 4.1: Relationship of polyethylene glycol and both total protein and albumin in pigs fed on *Acacia tortilis* leaf meal

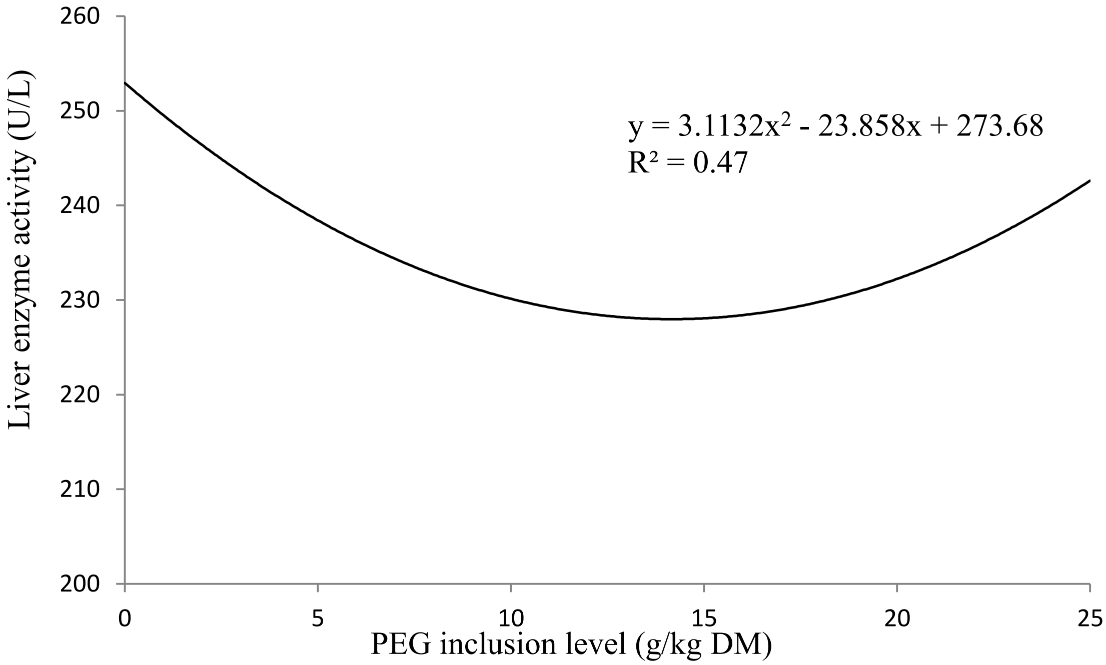

Figure 4.2: Relationship between polyethylene glycol in *Acacia tortilis* leaf meal and alkaline phosphatase

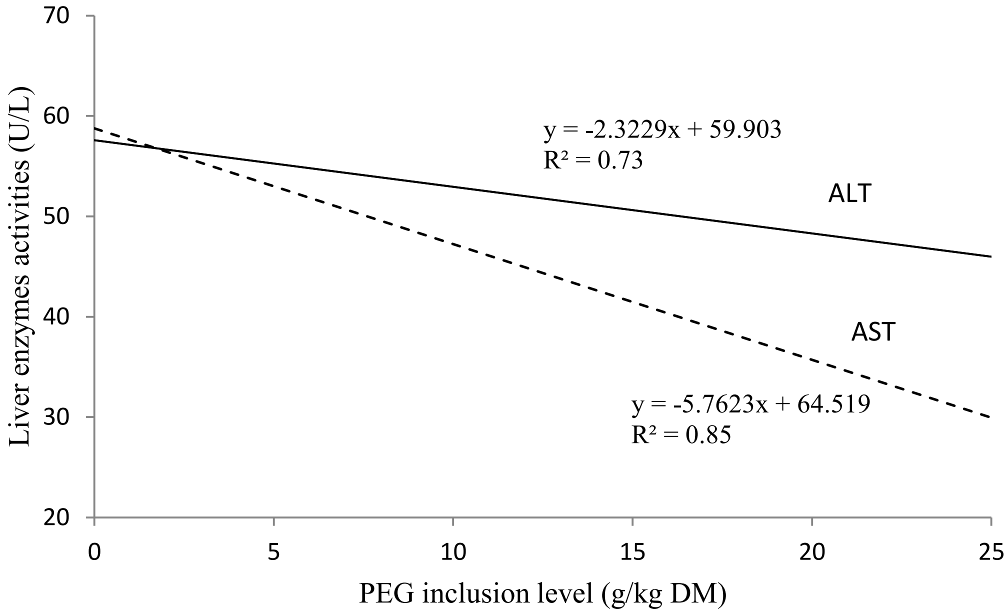

Figure 4.3: Relationship of polyethylene glycol and alanine aminotransferase (ALT) and aspartate aminotransferase (AST)

Understanding the relationship enhances our understanding of appropriate inclusion levels, and enables us to predict the effect of PEG inclusion on pig welfare.

Evaluation of blood parameters of pigs is fundamental in assessing the influence of PEG inclusion when formulating pig diets (Stukelj *et al*., 2010; Etim *et al*., 2014). Concentrations of TP, albumin, cholesterol and creatinine were within the normal reference range in the blood of pigs, but uric acid was below the expected range (10-30 mg/d1) (Radostits *et al*., 2000; Latimer *et al*., 2003). The positive relationship between PEG inclusion and the metabolites was, therefore, expected.

Ben Salem *et al*. (2005) reported that the inclusion of PEG in Acacia foliage is expected to unbind protein from polyphenolic compounds. The TP is an indirect indicator of protein adequacy in an animal. There was a linear increase in TP as PEG inclusion level in the Acacia-based diet, increased. Increasing PEG inclusion level in *A. tortilis* leaf meal-based diets resulted in an increased amount of protein available to the pigs. The linear relationship between TP and PEG inclusion could be explained by the ability of PEG to break the tannin-protein complexes and release protein for utilization (Mansoori & Acamovic, 2009). The linear increase in TP with PEG inclusion could be associated with dietary protein intake (Bhatti *et al*., 2009). Ferraz de Oliveira *et al*. (2008) reported the positive PEG response on protein output of pigs fed on diets containing polyphenolic compounds. The increased TP concentration with PEG inclusion also indirectly reflects the efficiency at which the liver and kidney are operating. Bergen and Potter (1975) reported that increase in TP is positively related to the amount of digestible protein provided to animals.

Albumin concentration was within the normal reference range (Hellwing *et al*., 2007; Mellesse *et al*., 2013). The quadratic relationship between PEG inclusion level and albumin could, suggest that the pigs were not absorbing equal protein (El-Daraway & Farghaly, 1999). This shows that PEG inclusion reduces the ability of tannins to bind to protein. Since albumin and globulin are generally influenced by protein utilization, their increased concentration from pigs offered PEG indicates nutritional sufficiency of the dietary protein (Nworgu *et al*., 2007). The observation that PEG inclusion is quadratically related to albumin but linearly to TP was not expected. Such relationships require further investigation. The quadratic response of albumin while TP is linearly related to PEG inclusion is also difficult to explain. Concentration of albumin is associated with protein accretion and is an important indicator of protein status (Ekenyem & Madubuike, 2007). An increase in albumin concentration with PEG inclusion might indicate liver synthesis function.

Concentrations of cholesterol that are within the reference indicate the healthy safely of using the leaf meals to feed pigs. Decreasing cholesterol concentration as PEG inclusion level increased is beneficial, because it reduces chances of pigs suffering from anorexia, liver problems and mal-absorption of fat (Njidda & Isidahomen, 2010). Too much cholesterol could block arteries, and lead to heart problems in humans. Krieger (1999) revealed that cholesterol has crucial functions as a component of the cell membrane of mammals and precursor of bile acid. The decreased serum total cholesterol with PEG inclusion may be associated with decline in lipid mobilization (Silanikove *et al*., 2001; Prvulovic *et al*., 2007). The observed cholesterol concentration ranged from 68 to 78 mg/dl. The expected blood cholesterol range in pigs is 81 to 134 mg/dl (Latimer *et* *al*., 2003). This fall in cholesterol concentration of pigs fed on *Acacia tortilis* leaf meal with or without PEG, suggests that Acacia utilization produce pig products with low cholesterol content. There is an increase in the demand for intake of low cholesterol products due to health concerns (Ng'ambi *et al*., 2009). It is important to highlight that Acacia leaf meal could be used to manipulate the lipid composition of pork.

Creatinine levels were not influenced by PEG inclusion, though creatinine from pigs fed on *A. tortilis* leaf meal diet tended to have high values than the PEG treated diet. The creatinine concentrations were within the normal reference range in the blood (Radostits *et al*., 2000). These findings suggest that the pigs had a ready source of energy for muscle and adequate quantity and quality of dietary protein (Esonu *et al*., 2001). The decreased creatinine with PEG inclusion level might be vital because too much creatinine have negative influence on meat quality. Young *et al*. (2007) reported that creatinine is associated with improvement of weight of growth of pigs, and could be used as indices of renal functions. Creatinine is a substance found in muscle and has antioxidant activity.

Uric acid concentration indicates protein catabolism. Pigs fed on *A. tortilis* leaf meal with or with no PEG inclusion levels showed no trend to uric acid concentration. The uric acid concentrations were lower than reference values given by Radostits *et al*. (2000) and Latimer *et al*. (2003). Iyayi and Tewe (1998) reported that high blood urea levels could be attributed to the presence of anti-nutritional factors in leaf meals. Decreased uric acid concentrations suggest sufficient protein consumption, because increased or decreased serum urea depends on the quality and quantity dietary protein. Silanikove and Tiomkin (1992) reported that blood uric acid concentration help to diagnose kidney disorder, liver disease and purines in feed.

The AST, ALP and ALT are most commonly used as markers of hepatic disease in pigs and poultry (Rani *et al*., 2011), and their relationship to PEG inclusion could be vital to feed compounders and farmers. These enzymes are localized within the cells of the liver, heart, gill, muscles and other organs. These enzymes are of major importance in assessing and monitoring liver cytolysis and damage to hepatocyte's plasma membrane. Moreover, the liver is the sensitive visceral organ that responds to toxic factors and/or protein deficiency (Marzoni *et al*., 2005). The PEG inclusion is relatively non-toxic to pigs. Serum concentrations of these liver enzymes were within the normal reference range (Radostits *et al*., 2000; Latimer *et al*., 2003). The observed quadratic relationship of ALP activities to PEG inclusion could reflect normal liver and intestinal functions of pigs (Kaplan *et al*., 2003). The ALP in pigs fed on *A. tortilis* with no PEG added was higher than the pigs that consumed diets treated with PEG. The decreased ALP activity shows the advantage of using PEG, because the increased ALP activities in control pigs are associated with liver cytolysis (Makkar, 2003). The ALP findings suggest that as PEG inclusion increased, tannins were neutralized in such a manner that causes no damage to the liver and kidney. Rani *et al*. (2011) reported that increased activities of liver enzymes in bird were associated with liver damage.

The linear relationship of AST and ALT activity with PEG in *A. tortilis* leaf meal was expected. Decreased in AST and ALT activity with PEG inclusion demonstrated a normal liver and organ function (Mellesse *et al*., 2013). Increased AST and ALT activities usually occur due to damaged liver cells (Kaplan *et al*., 2003). The decreased ALT and AST shows that including PEG in *A. tortilis* diet reduces tannin-induced heart and liver damage. Linear decreased activity of AST and ALT in growing pigs offered graded levels of PEG in *A. tortilis* leaf meal could suggest that no liver and intestinal damage. The high AST and ALT activities in pigs fed on *A. tortilis* leaf meal with no PEG could be associated with myocardial and liver damage. Similar observations were reported in birds (Rani *et al*., 2011).

4.5 Conclusions

Polyethylene glycol can be included in leaf meal diet successfully without any adverse relationship on the health and nutritional status of pigs. The inclusion of PEG in *A. tortilis* leaf meal showed a linear response on several metabolites, but a quadratic response to albumin. The PEG in *Acacia tortilis* demonstrated a linearly decreasing AST and ALT relationship and it was quadratically related to ALP activity. The relation future studies need to determine the relationship between the metabolites with digestibility and nitrogen balance of pigs offered PEG.

4.6 References

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CHAPTER 5: General Discussion, Conclusions and Recommendations

5.1 General Discussion

The hypothesis of the study was that pig performance increases linearly with PEG inclusion up to 25 g/kg and it has no adverse relationship with concentration of enzymes and serum metabolites at low to moderate levels. Pig producers are challenged to increase productivity to meet the ever increasing demands of human nutrition which includes pork. The use of leaf meal from legume trees and shrubs could provide the alternative feed to increase animal production with less supplementation. The use of Acacia leaf meal is, however, limited by the presence of polyphenolic compounds, high fiber content and thorns. Polyethylene glycol is a tannin binding-agent, with high affinity for tannins than protein, thus release protein from complex. As a result, it might help to reduce the effect of anti-nutritive factors such as tannins in leaf meals to promote its use at higher inclusion levels. The incorporation of PEG up to 25 g/kg in Acacia-based diet is expected to unbind protein from polyphenolic compounds. Thus the relationship between PEG inclusion and performance of pigs is expected to be linear. Understanding the relationship enhances our understanding of appropriate inclusion levels and enables us to predict the effect of PEG inclusion at low to moderate levels on pig welfare.

The response in average daily feed intake (ADFI), scaled feed intake (SFI), average daily gain (ADG), gain: feed ratio (G: F) and body weight gain (BWT) of pigs fed on *Acacia tortilis* leaf meal treated with polyethylene glycol (PEG) was discussed in Chapter 3. The hypothesis was that PEG inclusion increases linearly with pig performance. The increasing quadratic relationship between PEG inclusion and both ADFI and SFI, suggesting that inclusion levels of PEG in Acacia-based diet result in increased ADFI and SFI. This could be because PEG reduces astringency and improves appetite and palatability of leaf meals. The increase in ADFI and SFI from week 1 to week 4 was expected. This could be associated with increase in body size and adaptation to the *A. tortilis* leaf meal diet treated with graded levels of PEG. As expected, there was a linear increase in ADG with increase in inclusion levels of PEG. This could be explained by the fact that increasing PEG level eliminates the deleterious effects of tannins on pigs and allow utilization of nutrients such as protein, amino acids and some minerals. The observation that ADG linearly increased with increase in PEG inclusion levels could be because as PEG level increases more tannins are bound and, thus reducing the influence of tannins. The response of G: F ratio to PEG inclusion was increasing quadratically, suggesting that PEG levels are associated with better fed efficiency for a given rate of growth. Pigs fed on *A. tortilis* leaf meal diet supplemented with PEG require 1.5 g less feed to gain one kg body mass. There was a decrease in feed needed to be converted to a body weight gain. Therefore, the inclusion of PEG in *Acacia tortilis* leaf meal diet offered to pigs is effective. Determination of blood metabolites and liver enzymes concentration is necessary to explain the findings on chapter 3 and observe how PEG reflects on metabolites and liver enzymes activity.

The objective of Chapter 4 was to establish the influence of PEG inclusion in *A. tortilis* based diets on selected nutritionally-related metabolites and liver enzymes in growing pigs. It was hypothesized that there is a positive linear relationship between PEG inclusion level and blood metabolites. There was a linear increase in globulin and total protein levels (TP), as PEG level in acacia diet increased. This shows that increasing PEG inclusion level in *A. tortilis* based-diets increases the amount of protein in the feed which is available to the pigs. Serum total protein concentrations are influenced by the quality and quantity of the dietary protein. The significant positive linear response of TP to PEG inclusion could be explained by the ability of different PEG levels to prevent tannin-protein complex and release protein for utilization. As expected, presence of PEG in leaf meal released protein. The quadratic relationship between PEG and albumin concentration while TP was linear is difficult to explain. Decrease in creatinine concentration as PEG inclusion level increased might be vital because too much creatinine have negative impact on meat quality. On the other hand, decrease in cholesterol levels as PEG inclusion level increased is beneficial it reduces chances of pigs suffering from anorexia, live problems and mal-absorption of fat. Uric acid concentration was less than the reference range for all the pigs used in the study, this could be associated with hypouricemia in pigs. Concentration of albumin, total protein, cholesterol and creatinine were within the normal reference range in the blood, but advantage of PEG was observed.

Liver enzymes AST, ALP and ALT are most commonly used as markers of hepatic disease in pigs. The AST and ALT enzyme activity to linearly decreased with increase in PEG inclusion level up to 25 g/kg, suggesting normal liver and intestinal function. Pigs fed on *A. tortilis* diet with no PEG were associated with myocardial and liver damage. This shows that including PEG in *A. tortilis* diets reduces tannin-induced heart and liver damage. The ALP activity and PEG showed an increasing quadratic response up to 25 g/kg of PEG. The increased activity of ALP from pigs offered no PEG show the advantage of including PEG in *A. tortilis* leaf meal diet. The decreased enzyme activity of ALP to PEG in *A. tortilis* leaf meal indicates a positive relationship between PEG inclusion and ALP enzyme.

5.2 Conclusions

Increase in PEG level in *Acacia tortilis* meals resulted in improved average daily feed intake, average daily gain and gain: feed ratio. Polyethylene glycol can be included in the leaf meals successfully without adverse relationship on the performance, health and nutritional status of pigs. Decreased activity of AST, ALP and AST on growing pigs fed on *Acacia tortilis* leaf meal treated with PEG suggests no damage to liver and intestine.

5.3 Recommendations

The use of leaf meals from tree and shrubs legumes can be incorporated in the diet without any adverse effects and limits on pigs in presence of PEG. Therefore, farmers require some training in collection leaves and preparation of leaf meals together with application of PEG. Future studies need to be conducted to:

- Determine the digestibility and nitrogen balance of Acacia leaf meal treated with graded levels of PEG in pigs
- Assess the behaviour of growing pigs offered PEG in Acacia leaf meals diet.
- Evaluate water intake of pigs fed on Acacia leaf meals treated with PEG.
- Investigate the optimum inclusion level of PEG for performance of growing pigs.
- The effect of PEG treated Acacia leaf meals on Meat quality of pigs

Appendix 1: Methods that were used to measure serum biochemistry in pigs

Method used to measure total protein

Biuret method was followed to measure serum total protein (Weicheselbaum, 1946). A violet-coloured compound was formed by allowing biuret reagent to complex with the peptide bonds of protein from the sample under alkaline condition. Sodium potassium titrate was used as an alkaline stabilizer, and potassium iodine was used to prevent auto-reduction of the copper sulphate. The amount of the violet complex formed was proportional to the increase in absorbance when measured bichromatically at 544 nm/692 nm.

Method used to measure total cholesterol

Determination of total cholesterol was done according to enzymatic method (Allaib *et al*., 1974). This method includes complete hydrolysis of cholesterol esters in the serum to free fatty acids by pancreatic cholesterol esterase. Then, 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 proportional to amount of cholesterol present in the sample.

Method used to measure uric acid

Uric acid was determined with urease kinetic ultraviolet method (Tietz, 1995).The procedure involves the hydrolysis of uric acid to produce ammonia and 91 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 proportional uric acid in the sample.

Method used to determine liver enzyme activities

The spectrophotometric nitrophenol method fully described by Tietz *et al.* (1993) was followed to determine alkaline aspartate level in the blood. The 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 alanine amino transaminases were done according to the spectrophotometric method (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.

Appendix 2: Ethical Approval of Research Project on Animals

UNIVERSITY OF KWAZULU-NATAL
INYUVESI YAKWAZULU-NATALI

31 March 2014

Reference: 076/14/Animal

Mr V Hlatini
Animal Science
School of Agricultural, Earth & Environmental Sciences
University of KwaZulu-Natal
PIETERMARITZBURG Campus

Dear Mr Hlatini

Ethical Approval of Research Projects on Animals

I have pleasure in informing you that the Animal Research Ethics Committee has granted ethical approval for **2014** on the following project:

"Investigating the influence of polyethylene glycol (PEG) treated leaf meal on pig growth and productivity."

Yours sincerely

Professor Theresa HT Coetzer
Chairperson: Animal Ethics Sub-committee

Cc Registrar – Mr C Baloyi
Research Office – Dr N Singh
Supervisor – Prof. M Chimonyo
Head of School – Prof. A Modi
SAEES – Mrs M Manjoo

Animal Ethics Committee
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