Response in carcass yield, organ weights and the gut morphology to *Vachellia tortilis* inclusion in broilers

By

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Declaration

I, Angelique Miya, declare that this dissertation has not been submitted to any University and that it is my original work guided under supervision of Prof. M. Chimonyo. The production of this work and all references contained here have been accordingly acknowledged.

Candidate's Signature: …………………. Date: …………………….

Supervisor's signature: …………………... Date: ……………………

List of abbreviations

Abbreviation	Definition
ADF	Acid detergent fibre
AVSA	Apparent villus surface area
BW	Body weight
CD	Crypt depth
CP	Crude protein
CT	Condensed tannins
DAAF	Department of Agriculture, Forestry and Fisheries
DCW	Dressed carcass weight
DM	Dry matter
DP	Dressing percentage
DW	Drumstick weight
EE	Ether extracts
ME	Metabolizable energy
NDF	Neutral detergent fibre
NFE	Non-fibre extract
SAS	Statistical Analysis System
SW	Slaughter weight
TW	Thigh weight
UKZN	University of KwaZulu-Natal
VH	Villus height

Abstract

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A Miya

The broad objective of this study was to determine the response to incremental levels of *Vachellia tortilis* leaf meal on carcass yield, organ weights and gut morphology of broilers. Five-hundred unsexed cobb 500-day-old broiler chicks were fed on conventional starter mash for 14 days. The chicks were randomly allocated to six dietary treatments. These treatments contained 0, 30, 60, 90, 120 and 150 g/kg DM inclusion levels of *Vachellia tortilis* leaf meal. Each diet was offered *ad libitum* to 10 birds per pen for 17 days before they were slaughtered. Six pens received the same experimental diet.

There was a linear decrease in both slaughter weight (SW) and dressed carcass weight (DCW) ($P<0.05$) as *V. tortilis* leaf meal increased. The SW and DCW decreased by 0.77 and 0.94 g for each g/kg increase in *V. tortilis* inclusion. The scaled weights of thighs (TW) and drumstick (DW) showed no relationship with levels of leaf meal. The weight of gizzard, intestines and stomach, however, increased linearly ($P<0.05$) as levels of leaf meal increased. Relative gizzard, intestine and stomach weights increased by 0.0028, 0.0059 and 0.0008 g for each g/kg increase in *Vachellia tortilis* ($P<0.05$) inclusion. There was a linear increase in relative heart weight and kidney weight with increasing levels of *V. tortilis* leaf meal in the diet. The relative heart and kidney weight increased by 0.0009, 0.0006 g as *V. tortilis* increased ($P<0.05$). The relative weight of the liver was, however, not related to *V. tortilis* inclusion. It was concluded that carcass yield and organ weights responded differently to increase in *Vachellia tortilis* inclusion.

There was a linear increase in villus height (VH), apparent villus surface area (AVSA) and villus height: depth ratio ($P<0.05$) as *V. tortilis* leaf meal increased. The VH, AVSA and CD/VH ratio increased by 0.12, 1.08, and 1.96 g for each g/kg increase in *V. tortilis* inclusion. The crypt depth (CD) showed no relationship ($P>0.05$) with levels of leaf meal. The thickness of submucosa, muscularis and epithelial, however, increased linearly *($P<0.05$)* as levels of leaf meal increased. Submucosa, muscularis and epithelial thickness increased by 8.73, 1.15, and 0.38 g for each g/kg increased in *Vachellia tortilis* inclusion. Elevated inclusion levels of *V. tortilis* leaf meal was associated with increased villus height, apparent villus surface area, villus height and depth ratio, thickness of submucosa, muscularis and epithelial. In conclusion, increasing *V. tortilis* in the diet increase gut morphology parameters of broiers thus improve the digestibility and absorption with a negative effect on growth performance, carcass yield and internal organ weights.

Keywords: Dressed carcass weight, intestinal histomorphology, Jejunum, relative organ weight, Slaughter weight

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Dedication

This thesis is highly dedicated to my mother: Nokuzola Fransisca Miya, sister: Nobuhle Miya, brother: Lizwi Miya and my late brother: Innocent Miya for believing in me and offering the opportunity with support to my future study.

Table of Contents

List of Tables

List of Figures

CHAPTER 1: General Introduction

1.1 Background

The demand for broiler meat is increasing more especially in developing countries due to increasing human population. The fast-growing human population enables breeders to select birds that will grow fast, with high final weights and efficiency (Petracci and Cavani, 2012). These chickens reach about 1.8 kg in 31 to 35 days (Baeza *et al.*, 2002). The challenge of breeding for fast growing birds are likely to be aggravated by excessive carcass fatness, which lowers meat yield and quality. The increase in carcass fats resulting from selective breeding programs of broilers can decrease meat quality and have detrimental effects on the health of consumers (Ng'ambi *et al.,* 2009). For broiler producers, dressed carcass weight is a trait of economic importance, that affect amount of income generated (Wang *et al.,* 2009).

Carcass weight, also referred to as dressed weight, is the weight of bird after removing all the internal organs, legs and head. The dressing percentage is mostly used to evaluate meat yield. Different feeding systems, feeding periods and slaughter ages have an impact on carcass weight and quality (Mathivanan *et al.*, 2006). The carcass can be retailed as a whole, separated into different portions such as wings, whole breast, drumstick and thigh. Factors such as nutrition, age, fattening period before slaughter, and body weight the impact on carcass yield (Nikolova and Pavlovski, 2009).

The production of high protein conventional feed sources for broilers such as soybean and maize are affected by adverse climatic conditions. This reduces the availability of conventional feed sources for broilers since human beings also utilize these feed sources of broilers for consumption. Therefore, it is indispensable to identify other available protein sources that can be used in formulating broiler diets. Non-conventional protein sources such as leaf meals can be used in broilers diets due to their high crude protein and favourable proportions of minerals and vitamins. Leguminous trees have good growth potential under drought conditions due to their long penetrative roots and have the potential of increasing the utilization of locally available protein for livestock. Use of plant protein sources is, however, restricted by the presence of anti-nutritional factors. *Vachellia tortilis*, for example, is a tropical legume that can be possibly used for broiler diets (Ncube *et al*., 2017). It is, however, constrained by the abundance of polyphenolic compounds and fibre. Polyphenolic compounds reduce feed intake, nutrient digestibility and compromise functions of the liver, kidneys and intestines (Ndou *et al*., 2015).

The shape and structure of the gastrointestinal tract is well adapted to accomplish roles of digestion and absorption of nutrients. The intestinal wall comprises of four layers: mucosa, sub mucosa, muscularis and serosa. The mucosa of small intestines is structured to form villi, which are small projections on the surface of mucous membrane and upsurge the overall absorption surface area of the organ (Baily, 2010). The external epithelium of the villi is small with numerous goblet cells. Changes in morphology, particularly in its mucosal layer, can modify absorption rate of nutrients. and consequently, performance (Rezaian and Hamedi, 2014). The relationship between diet and intestinal mucosa should provide a theoretical basis in the formulation of diet for broilers. Feed compounders should formulate diets that allow optimum gut health in broilers. Very few, if any, studies have been conducted to determine the influence of *Vachellia* leaf meal on carcass yield, organ weight and gut morphology.

Determining the response in organ weights is important, since organs reflect the extent of metabolism and presence of anti-nutritional factors in the diet. For example, Khanyile *et al*. (2014) reported an increase in size of kidneys in pigs fed on *V. tortilis* leaf meal, this could be due to higher nitrogen content of leaf meal, thereby increasing the nitrogen circulation in the body and the catabolism of nitrogenous compounds, which resulting in increasing the work load of the kidney and an increase in size .In addition, the liver is a sensitive visceral organ that responds to toxic substances and protein deficiencies and imbalances (Forbes and Provenza, 2000). Determining organ weights is, therefore, useful when predicting broiler performance. There is lack of knowledge on the effect of leaf meal inclusion on carcass yield and gut morphology of broilers.

1.2 Justification

It is important for broiler producers to gain knowledge of using *Vachellia tortilis* as an alternative protein source to improve broiler performance, carcass yield and gut morphology. Using *Vachellia tortilis* has the potential of increasing the utilization of locally available protein sources to minimize the competition for consuming conventional raw materials such as soybean. Leaf meal characterization increases the number of raw materials used in diet formulation without little, if any, competition with humans. In addition, use of leaf meals decreases the proportion of other feed in the diet. Therefore, the demand for leaf meal is, currently, not as high as other protein sources. Leaf meals are a good substitute for protein sources such as cotton and sunflower meal. In addition, understanding the response of broilers to *Vachellia tortilis* leaf meal inclusion assist feed compounders to explore the use of non-conventional feed ingredients in broiler feeding. Broiler farmers are then able to make informed choices based on bird performance, carcass yield and health status of their birds. The knowledge of using *Vachellia tortilis* also mitigate the adverse impact of climate change on feed supply to chickens. The study also benefits both provincial and national governments for creating and growing job opportunities to decrease unemployment rates. Use of leaf meals require harvesting, processing, packaging and marketing, thereby creating sustainable jobs within the broiler industry. Exploring the use of leaf meals also creates research and innovations opportunities on developing technologies to reduce the negative impacts of leaf meal on birds.

1.3 Objectives

The broad objective of the study was to determine the response to the incremental levels of *Vachellia tortilis* leaf meal on carcass yield and organ weights and gut morphology of broilers.

The specific objectives were to:

1. Determine the effects of *Vachellia tortilis* inclusion on carcass yield and internal organ weights of broilers; and
2. Assess the histomorphological changes of small intestines of broilers fed on incremental levels *Vachellia tortilis* leaf meal.

1.4 Hypotheses

The alternative hypothesis tested were;

1. Carcass yield and internal organ weights respond to *Vachellia tortilis* inclusion levels in broiler chickens; and
2. *Vachellia tortilis* inclusion increase the histomorphology of small intestines in broiler chickens.

1.5 References

Baeza, E., Dessay, C., Wacrenier, N., Marche, G. and Listrat, A., 2002. Effect of selection for improved body weight and composition on muscle and meat characteristics in Muscovy duck. *British Poultry Science, 43*(4), pp.560-568.

Bailey, R., 2010. *Intestinal microbiota and the pathogenesis of dysbacteriosis in broiler chickens*. PhD dissertation, University of East Anglia, United Kingdom.

Forbes, J.M. and Provenza, F.D., 2000. Integration of learning and metabolic signals into a theory of dietary choice and food intake. *Ruminant Physiology: Digestion, Metabolism, Growth and Reproduction*, 32(9), pp.3-19.

Khanyile, M., Ndou, S.P. and Chimonyo, M., 2014. Influence of Acacia tortilis leaf meal-based diets on growth performance of pigs. *Livestock Science, 167 (2)*, pp.211-218.

Petracci, M., Laghi, L., Rocculi, P., Rimini, S., Panarese, V., Cremonini, M.A. and Cavani, C., 2012. The use of sodium bicarbonate for marination of broiler breast meat. *Poultry Science, 91*(2), pp.526-534.

Mathivanan, R., Edwin, S.C., Amutha, R. and Viswanathan, K., 2006. Panchagavya and Andrographis paniculata as alternatives to antibiotic growth promoter on broiler production and carcass characteristics. *International Journal of Poultry Science*, *5*(12), pp.1144-1150.

Ncube, S., Halimani, T.E., Mwale, M. and Saidi, P.T., 2017. Effect of Acacia angustissima leaf meal on the physiology of broiler intestines. *Journal of Agricultural Science*, *9*(2), pp.53-62.

Ndou, S.P., Khanyile, M. and Chimonyo, M., 2015. Growth performance and nutrition-related serum metabolites in growing pigs fed on Acacia tortilis leaf meal. *Livestock Science, 182 (3),* pp.22-27.

Ng'ambi, J.W., Nakalebe, P.M., Norris, D., Malatje, M.S. and Mbajiorgu, C.A., 2009. Effects of dietary energy level and tanniferous Acacia karroo leaf meal level of supplementation at finisher stage on performance and carcass characteristics of Ross 308 broiler chickens in South Africa. *International Journal of Poultry Science*, *8*(1), pp.40-46.

Nikolova, N. and Pavlovski, Z., 2009. Major carcass parts of broiler chicken from different genotype, sex, age and nutrition system. *Biotechnology in Animal Husbandry*, *25*(5-6-2), pp.1045-1054.

Rezaian, M. and Hamedi, S., 2014. Effect of different single feeding diets on small intestinal mucosa of cocks: a histomorphometrical study. *Comparative Clinical Pathology*, *23*(3), pp.519-522.

Wang, K.H., Shi, S.R., Dou, T.C. and Sun, H.J., 2009. Effect of a free-range raising system on growth performance, carcass yield, and meat quality of slow-growing chicken. *Poultry Science*, *88*(10), pp.2219-2223.

CHAPTER 2: Review of Literature

2.1 Introduction

Chickens are an essential source of animal protein. The meat is mostly consumed as individually quick-frozen portions and as a processed by-product (Le Bihan-Duval *et al.*, 2008). Soybean meal is the ingredient commonly utilized as a protein source with an ideal amino acid profile (Furuya *et al.*, 2004). Soybean meal represent about 25% and maize represent about 65% of poultry diets. The use of these grains as ingredients in poultry diets increased competition with other livestock and with humans (Abouelezz *et al.*, 2012). There is, therefore need to identify and characterize alternative sources, such as leaf meals to be taken into consideration in formulating poultry diets.

Legume trees such as *Leucaena leucocephala, Gliricidia sepium, Sesbania sesbaria* and *Manihot esculenta* has been tested in ruminants. *Vachellia* trees are leguminous plants that are abundant in numerous parts of arid and semi-arid areas of Africa and have wide range of uses. Furthermore, *V. tortilis* trees are well adapted to areas with low rainfall and these trees are predominant in Botswana (Aganga and Tshwenyane, 2003). The consumption of leaf meals is constrained by the high concentration of polyphenolic compounds. These compounds decrease feed intake and nutrient digestibility. There is lack of information based on the response of broilers to *Vachellia tortilis* leaf meal inclusion on carcass yield, the gut morphology and internal organ weights. The review, therefore, discuss the role of leguminous trees as alternative protein sources in chicken production.

2.2 Utilization of leaf meals in poultry production

Fodders and shrubs leguminous species are utilized to feed livestock as an alternative protein source. Over the past years there has been a major interest in the utilization of leaf meal ingredient of fodder and shrubs as a source of protein. Leguminous species has high crude protein contents (Nworgu and Fasogbon, 2007). Leaf meal do not only provide protein but also supply vitamins, minerals and oxycarotenoids. Common leaf meals are from *Leucaena leucephala, Gliricidia sepium, Robinia seudoacacia, Cajanus cajan,* and *Sesbania sesban.* These leaf meals are favorable to be used in the diet due to their nutrient compositions including proteins, minerals, vitamins and fibre. Minimal inclusions of these leaf meals are essential to maintain the functioning of gizzard and gastro-intestinal tract (Agbede and Aletor, 2003; Nkukwana *et al.*, 2014).

Precise inclusion levels of these leaf meals are, however, basically not available. Inclusion of leaves above optimum levels result to endogenous losses of minerals and amino acids, hepatotoxicity and toxic nephrosis (Ndou *et al.*, 2015). Most researchers have identified that leaf meal inclusion could be incorporated at levels up to 5 % in broiler diet and up to 10 % in layer diet to avoid straining performance due to high fibre content. Increase in bulkiness reduces digestibility of nutrients. The other constraint of leaf meals is the presence of anti-nutritional substances that reduce feed intake. The influence of incremental levels of leaf meal in broiler diets, therefore, need to be explored.

Indigenous and exotic species of tree legume vary in terms of environmental adaptation, disease resistance, biomass production, and presence of thorns and concentration of secondary compound such as proanthocyanins (Dube *et al.*, 2001). Leguminous tree leaves such as *Faidherbia albida, Colophospermum mopane, Brachystegia spieiforms* and *Vachellia* species are commonly utilized as protein sources (Dube *et al.*, 2001). Common *Vachellia* species are *nilotica, tortilis, karro, robusta, Senegal, erioloba, nigrescens* and *xanthophoea* (Mokoboki *et al.*, 2005; Mapiye *et al.*, 2011). Factors such as soil condition, season and stage of leaf growth influence nutrient composition on these leaf meals (Nyamukanza and Scogings, 2008). These leaf meals are rich in crude protein. These species have been recognized as dominant woody species that worsens the problem of bush encroachment in reducing grazing capacities of the veld (Nyamukanza and Scogings, 2008). The branches and tree trunks can be used for fencing and firewood. Leaf meal from these leguminous trees are also rich in polyphenolic compounds and dietary fibre.

Leucaena leucocephala is the commonly used leaf meal in non-ruminants, especially in poultry diet (Agbede, 2003). *Leucaena leucocephala* is identified as highly nutritious but contains of toxic substances such as mimosine and this is harmful for poultry when included above 7 % (Nworgu and Egbunike, 2013) recommended optimum inclusion level of up to 2 % of *Mimosa invisa* and *Puerairia phaseoloides* leaf meal in broilers' diets and 2.5 % of *Centro sema pubescens* leaf meal in broiler starter and finishers. Inclusion of 6 % *Trichantera gigantean* leaf meal had a positive influence on egg quality (Nworgu and Fasogbon, 2007).

Nutrient composition of leaf meals varies greatly in both quality and quantity. The composition depends on plant species. Age of plant, climate change and the proportional size of the leaves and stems influence nutrient composition of leaf meals (Mtuli *et at*., 2006). High inclusion levels of leaf meals, however, can impair nutrient digestion and absorption. *Vachellia tortilis* is one of the valuable sources of nutrients. *V. tortilis* leaves contains crude protein within the optimal range of 140 to 180 g/kg DM. These leaves contain moderate content of detergent fibre (Table 2.1) and favorable levels of minerals in *V. tortilis*.

Table 2.1: Chemical composition (g/kg DM) of *Vachellia tortilis*

Chemical composition	Mean
Dry matter	955
Ash	41
Crude protein	189
Crude fibre	84
Neutral detergent fibre	195
Acid detergent fibre	169
Acid detergent lignin	82
Hemicellulose	45
Crude fat	43
Gross energy (MJ/kgDM)	18
Digestible energy (MJ/kgDM)	12

DM= dry matter, MJ= megajoule. Source: (Khanyile, 2014)

Table 2.2: Mineral content of *Vachellia tortilis*

Mineral	Concentration (%)
Calcium	0.96
Magnesium	0.30
Potassium	1.73
Sodium	0.04
Phosphorus	2.30
Zinc	1.90
Copper	0.20
Manganese	3.50
Ion	17.80

Source: Khanyile (2014)

Table 2.3: Chemical composition (g/kg) dry matter basis of common *Vachellia species*

Species	DM	Ash	CP	EE	NDF	ADF
Vachellia nilotica	947	56.5	198	32.7	399	227
Vachellia robusta	950	82.7	160	30.5	455	279
Vachellia xanthophloea	943	87.6	216	33.8	471	304
Vachellia tortilis	944	65	218	40.1	494	298
Vachellia nigrescens	938	78.3	178	30	630	477

DM: dry matter, CP: crude protein, EE: ether extract, NDF: neutral detergent fibre, ADF: Acid detergent fibre. Source: (Khanyile. 2014)

2.3 Influence of leaf meal inclusion on broiler performance

Measurements such as feed intake, weight gain and conversion ratio are the main variables used when assessing growth performance in broilers. Leaf meals have huge potential in feeding broilers due to high crude protein content that can influence growth in broilers (Nkukwana *et al.*, 2014). Given that leaf meal may reduce digestibility due to high fibre content in the diets and consequently suppress feed intake and growth performance in broilers when there is large proportion available in the diet (Simol, 2012). In broilers, the bulk of fibre is not digestible, and thus contributes to gut fill, thereby decreasing feed intake.

2.3.1 Growth performance

Growth performance is the main factor used to evaluate the productivity of broiler chickens. Body weight gain and feed conversion ratio indicate the efficiency of feed utilization in broilers. Onyimonyi *et al.* (2009) reported average daily gain improved by 3.98 g as 5 g/kg of neem leaf meal was included in the diet and had a feed conversion ratio of 2.94, which was lower than 3.17 for birds on the control diet. The depression of growth performance might be due to the imbalance of nutrients which deprives rate of metabolism. The deficiency of nutrients could, possibly, be due to presence of anti-nutritional factors such as phytate and polyphenolic compounds in the leaf meal (Messel *et al.*, 2009), which might also reduce the bioavailability of minerals. Supplementation of leaf meals in broiler diets may have a positive influence on performance of chicks (Nkukwana *et al.*, 2014).

The decrease in growth rate is caused by low feed intake, which is more prominent at high inclusion levels of leaf meal (Halimani *et al.*, 2005). The effect of polyphenolic compounds on nutrients is also one factor limiting growth rate (Makkar, 2003). Also, the high fibre content of leaf meal reduce the digestibility of nutrients (Iheukwumere *et al.*, 2008). Esonu *et al.* (2003)

reported that high fibre content of leaf meal dilutes energy in the feed and increases in feed intake. Iheukwumere *et al*. (2008) demonstrated an increase daily feed intake, daily weight gain and fed conversion ratio of broiler fed graded levels of cassava leaf meal (Figure 2.1). High leaf meal inclusion decreases growth due to reduction in nutrients intake.

2.3.2 Carcass traits

Body weight at slaughter, slaughter weight, dressing weight, chilled carcass weight, organ weight and portion yield are the common measures of carcass yield in broilers (Agbede and Aletor, 2003; Gadzirayi *et al*., 2012). The possibility of increasing the proportion of prime parts in the carcass, especially breast meat and reduction of fat is important. Increasing inclusion levels of leaf meals also increases weights of slaughter, dressed carcass and breast (Melesse *et al*., 2013). Agbede and Aletor (2003) reported that feeding incremental levels of *Glyncidia sepium* leaf meal had no effect on carcass yield. Previous study reported that inclusion of leaf meal added to proportion of breast muscle by 1.2 % (Nkukwana *et al*., 2015). The inclusion at 200 g/kg DM dried leaves of sweet potato, however, decreased the weights of drumstick, thigh and breast meat of broilers, probably due to poor nutrient consumption of birds, due to insufficient intake of certain essential amino acids (Tami and Tsega, 2009).

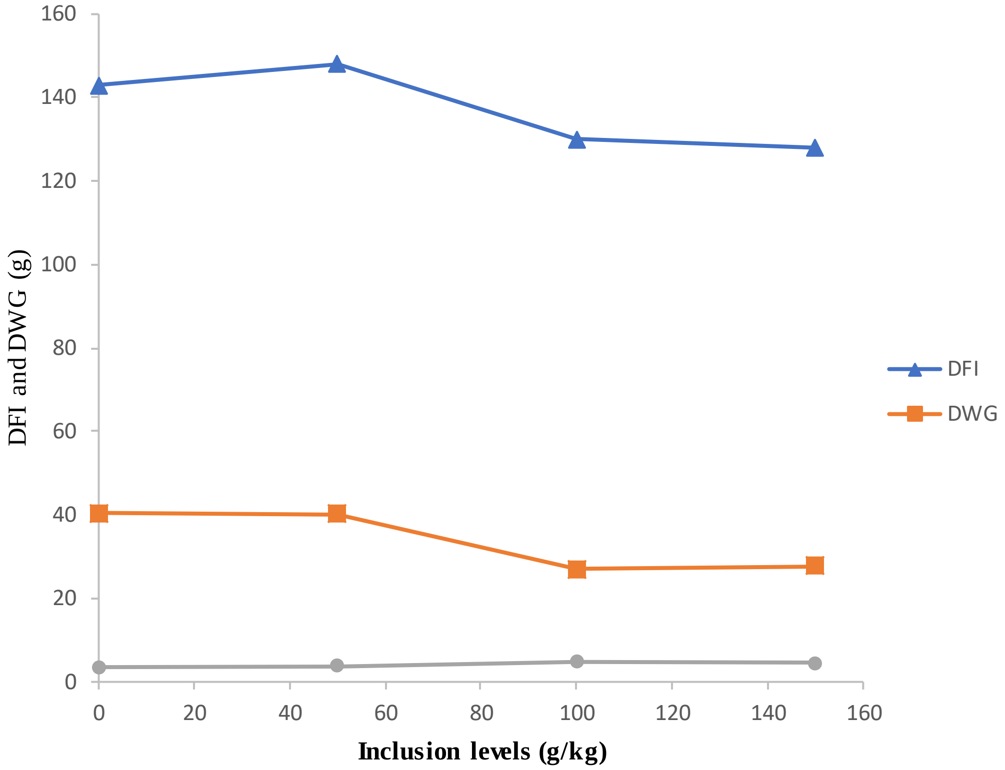


Figure 2.1: Effects of different dietary inclusion levels of cassava leaf meal on performance of broilers.

Source: (Iheukwumere *et al.*, 2008)

Iheukwumere *et al.* (2007) showed dressed weights of the broilers were similar as inclusion levels of Cassava leaf meal increased. Nwoche *et al.* (2006), on the other hand, observed that broiler finishers had the highest weight of the cut parts with increasing level of palm oil in the diet. These values decreased as the inclusion level of leaf meal increased. The decrease in weights of the carcass cut parts could be due to the reduced feed intake (Esonu *et al.*, 2002). On the other hand, Onyimonyi and Onu (2009) reported an increase in dressed weight and dressing percentage as levels of Pawpaw leaf meal increased. These different observations on influences of leaf meal on carcass yield warrant further investigation. Table 2.4 shows the effect of Cassava leaf meal inclusion on broiler carcass yield.

Table 2.4: Effect of different dietary inclusion levels of cassava leaf meal on the carcass yield of broilers

	Inclusion level (g/kg DM)			
Parameters	0	50	100	150
Slaughter weight (k/g)	1.43	1.05	0.93	0.6
Dressed weight (%)	79	69.5	69	67
Expressed as % body weight				
Breast	154.1	100.8	95.8	87.8
Drumstick	75	58.8	50.3	50
Thigh	76.1	58.3	50.8	50

Source: Iheukwumere *et al.* (2007)

2.3.3 Organ weights

The information on the effect of *Vachellia tortilis* leaf meal inclusion on weight of internal organs are scantily documented, if available, therefore further research is required. High inclusion levels of leaf meals are expected to stimulate gizzard activity, and this increases the volume of the gizzard substantially when leaf meals are added into the diet (Hetland and Svihus, 2001). Leaf meal inclusion is also associated with the increased secretion of bile salt and digestive enzymes due to changes in organ activity (Meteos *at al.*, 2012). Zanu *et al.* (2012) reported that increment levels for *Leucaena leucocephala* leaf meal increased internal organ weights. Agbede and Aletor (2003) reported that the increase in the inclusion level of *G. sepium* leaf meal influenced the relative weight of the heart, but not other organs. The size of the kidneys is expected to increase due to an increase in nitrogen circulation in the body and the increased catabolism of nitrogenous compounds, thus increasing the workload of kidneys (Fasuyi *et al.*, 2013). Since leaf meal inclusion reduces feed intake and growth rate (D'Mello, 1995), it, therefore, leads to lean body. A lean body weight increases blood circulation, which stimulate greater heart muscular development (Kerr *et al*, 1995). Figure 2.2 illustrates the response in internal organ weights to cassava leaf meal inclusion in broilers.

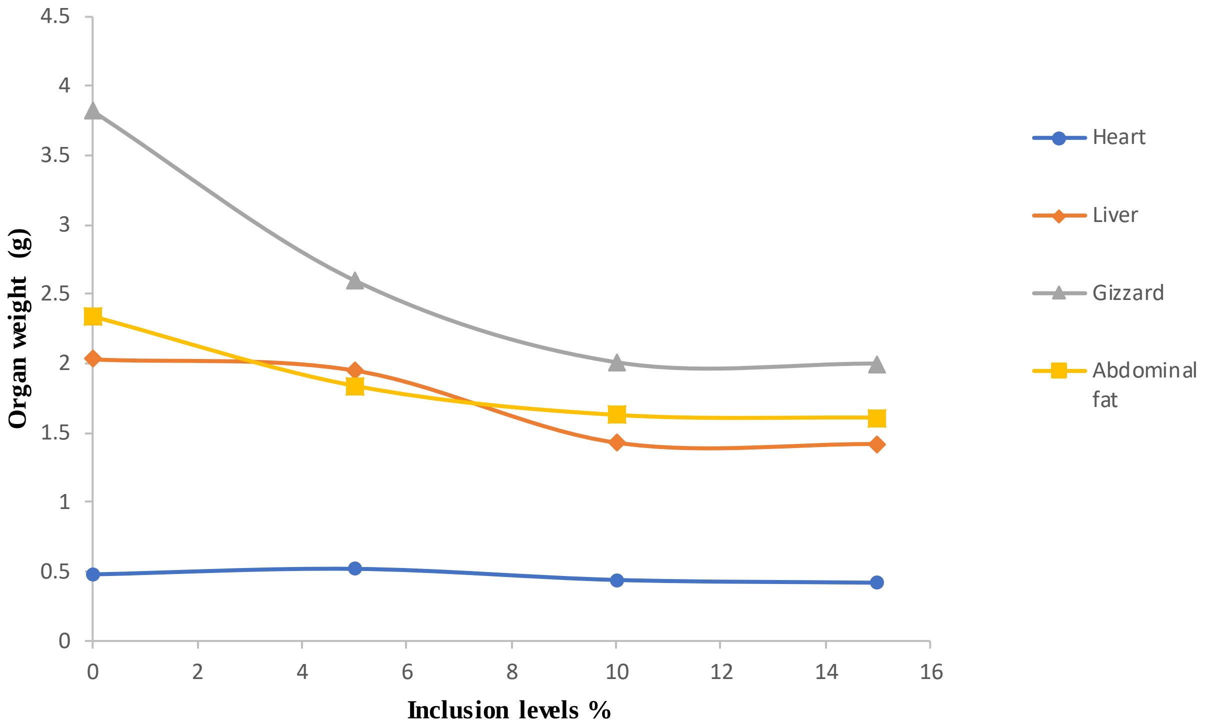


Figure 2.2: Effect of dietary levels of Cassava leaf meal on organ weights of broilers.

Source: (Iheukwumere *et al.*, 2008)

2.4 Challenges of using leaf meals

Regardless of high nutritional composition of crude protein content in *V. tortilis* leaves, consumption of such leaves is limited by the presence of thorns, high fibre content and polyphenolic compounds.

2.4.1 Presence of thorns

The presence of thorns in most *Vachellia* species is one of the factors that limiting their utilization of as animal feed in browsers. The presence of thorns decreases the surface area for leaf biting by animals and this leads in reducing the nutrient intake (Mapiye *et al.*, 2009). To avoid this anti-quality factor, small branches must be harvested and dried (Halimani *et al.*, 2005; Ng'ambi *et al.*, 2009). Separating leaves from thorns is a huge challenge.

2.4.2 High content of fibre

High fibre content in leguminous species limit the utilization of leaves in feeds. The plant fibre varies with fibre source due to chemical composition and structure of plant (D'Mello, 1995). Fibres with lignified cell walls are not easily exposed to microbial activity for access of nutrient digestibility. Therefore, digestibility of fibre depends on the source of fibre and the amount of fibre present in the diet (Wenk, 2001). In poultry, the bulk of fibre is not digestible, and thus contributes to gut fill, thereby reducing feed intake.

2.4.3 Polyphenolic compounds

Polyphenolic compounds are a class of chemicals that contain hydroxyl group joined directly to an aromatic hydrocarbon group. Phenolic acid, flavonoids, tannins and lignans are the major polyphenolic compounds in plants. Anti-nutritional factors are mechanisms for plants to defend themselves against herbivores. The concentration of polyphenolic compounds in leaf meals depends on the condition of plant species. Young leaves have high concentrations of polyphenolic compounds (Rubanza *et al.*, 2005). In addition, environmental factors to which plants are exposed also influence the content of polyphenolic compounds. Polyphenolic compounds in *Vachellia tortilis* reduce feed intake, nutrient digestibility and absorption of nutrients (Ndou *et al.*, 2015). Polyphenolic compounds also increase protein lost in faeces and reduce the secretion of gut enzymes. Compromise

Several studies have demonstrated changes in intestinal morphology with incremental levels of leaf meals (Viveros, *et al.* 2011). High concentrations of polyphenolic compounds have been shown to cause histological lesions in the ileum, loss of epithelial cells, erosion of microvilli and shortened villi height in the duodenum, which impairs nutrient absorption capacity. Polyphenolic compounds reduce crypt depth, intestinal wall thickness and increase secretion of mucus in the intestinal tract. The mucous layer plays a role of protection, lubrication and transportation of nutrients. High content of polyphenolic compounds, thus predisposes birds to infection, alters the properties of intestinal barrier, reduce nutrient absorption and, consequently, performance.

Increasing leaf meal inclusion reduce digestibility of nutrients due to an increase in the degree of lignification, which influences rate of feed passage through the GIT to maintain gizzard activity and gastrointestinal tract functionality of broilers require minimal fibre in the diet (Jimenez-Moreno *et al.*, 2010). Leaf meal inclusion levels increases secretion of hydrochloric acid, bile salt and digestive enzymes due to probable changes in organ activity (Meteos *at al.*, 2012). The liver is the most sensitive internal organ to poisonous substances, therefore the high level of polyphenolic compounds in the diet increases liver activity. Since digestion and absorption of nutrients depends on the structure and anatomy of intestines, it is important to discuss gut morphology for farmers to predict inclusion of *vachellia tortilis*. Table 2.4 shows the concentration of polyphenolic compounds present in *Vachellia tortilis* leaves.

2.4.4 Adaptation of broilers fed on leaf meals

The gastrointestinal tract health in broilers has been recognized to have a positive impact to their health and performance (Mount-Zouris *et al*., 2007). The gastrointestinal mucosa is the primary tissue that gets into contact with digesta. Mucosa is the microscopic structure which is a reliable indicator of the response of the intestinal tract to feed substances such as structural substances and polysaccharides. These nutrients affect the mucosa thickness and villi height (Jamroz *et al*., 1992). Incremental levels of dietary crude fibre intake enhanced total small intestinal length and surface area (Ncube *et al*., 2017). Such information is useful in creating strategies to determine appropriate inclusion levels of leaf meals. A healthy gut is of great importance to attain target performances of carcass yield and improved organs function.

Changes in the morphology in the duodenum and colon imply alteration in digestive and absorption capacity of the intestines (Svihus, 2014). The presence of *Acacia angustissima* leaf meal in the diet of broilers increased the length and weight of the intestinal segments. Ndou *et al*. (2015) also reported that the presence of leaf meal influences the development capacity and volume of the intestinal segments. The enhanced intestinal volume is a physiological adaptation to the increased volume of the digesta associated with incremental level of the leaf meal (Borin *et al*., 2006)

Table 2.5: Concentration of polyphenolic compounds in *Vachellia tortilis* leaves

Phenolic	Concentration (g/gk DM)
Total extractable phenolics	241
Total extractable tannins	226
Total condensed tannins	77.8
Soluble condensed tannins	18.9
Protein bond condensed tannins	37.5
Fibre bond condensed tannins	21.5

Source: Rubanza *et al*. (2005)

The extreme effort by the intestines in pushing digesta along the digestive tract could be reflected by the thickening of the duodenum and colon muscle layer with incremental levels of the leaf meal (Ncube *et al.,* 2017). The thickening of the mucosal layer along the digestive tract suggest a stronger movement that forces digesta transportation. In addition, thickening of layer reflects highly activated digestion and absorption function (Van der Klis and Jansman, 2002). Svihu (2014) reported that the muscle layer of the jejunum decreases in thickness with addition of leaf meal, but the explanation is still unclear. Decrease in thickness of the jejunum could be due to faster rate of digesta movement.

Dong *et al.* (2016) reported that leaf meals increase the height of villi in the intestine and decrease the presence of bacteria. The increase in surface area for absorption in the intestine was due to increase in the depth of crypt and height of villi. The increase in height of villi in the intestine influences feed utilization efficiency during the early stages of chicken life (Nkukwana, 2012). *Moringa oleifera* leaf meal contains high amounts of short-chain carbohydrates and glycosides, which increases the height of the jejuna villi (Nkukwana *et al.*, 2015). The increase of villus height of small intestine protects the bird from toxic substances such as polyphenolic compounds.

2.5 Summary

Inadequate feed supply is an important constrain in broiler production and increased competition for grains between livestock and humans. Information regarding *Vachellia tortilis* as an alternative source of protein in broiler diet is limited. There is, therefore, a need to identify and characterize alternative sources, such as leaf meals to be considered in formulating poultry diet. Exploring the use of *V. tortilis* leaf meal in determining measures of carcass yield, organ weight and gut morphology in broilers is important to expand the knowledge and innovations opportunities on developing technologies to improve leaf meal in birds. The broad objective of the study was to determine the response to the incremental levels of *Vachellia tortilis* leaf meal on carcass yield organ weights and gut morphology of broilers.

2.6 References

Abouelezz, F.M.K., Sarmiento-Franco, L., Santos-Ricalde, R. and Solorio-Sanchez, F., 2012. Outdoor egg production using local forages in the tropics. *World's Poultry Science Journal*, *68*(04), pp.679-692.

Aganga, A.A. and Tshwenyane, S.O., 2003. Feeding values and anti-nutritive factors of forage tree legumes. *Pakistan Journal of Nutrition*, *2*(3), pp.170-177.

Agbede, J.O. and Aletor, V.A., 2003. Evaluation of fish meal replaced with leaf protein concentrate from Glyricidia in diets for broiler-chicks: Effect on performance, muscle growth, haematology and serum metabolites. *International Journal of Poultry Science*, *2*(4), pp.242-250.

Dong, Z.L., Wang, Y.W., Song, D., Hou, Y.J., Wang, W.W., Qi, W.T., Yun, T.T. and Li, A.K., 2016. The effects of dietary supplementation of pre-microencapsulated Enterococcus fecalis and the extract of Camellia oleifera seed on growth performance, intestinal morphology, and intestinal mucosal immune functions in broiler chickens. *Animal Feed Science and Technology*, *212*, pp.42-51.

Dube, J.S., Reed, J.D. and Ndlovu, L.R., 2001. Proanthocyanidins and other phenolics in Acacia leaves of Southern Africa. *Animal Feed Science and Technology*, *91*(1-2), pp.59-67.

D'Mello JPF (1995) Leguminous leaf meals in non-ruminant nutrition. In: D'Mello JPF and Devendra C (eds) Tropical Legumes in Animal Nutrition, pp 247-282.

Esonu, B.O., Iheukwumere, F.C., Emenalom, O.O., Uchegbu, M.C. and Etuk, E.B., 2002. Performance, nutrient utilization and organ characteristics of broilers fed Microdesmis puberula leaf meal. *Livestock Research for Rural Development*, *14*(16), p.146.

Esonu, B.O., Iheukwumere, F.C., Iwuji, T.C., Akanu, N. and Nwugo, O.H., 2003. Evaluation of Microdesmis puberula leaf meal as feed ingredient in broiler starter diets. *Nigerian Journal of Animal Production*, *30*(1), pp.3-8.

Gadzirayi, C.T., Masamha, B., Mupangwa, J.F. and Washaya, S., 2012. Performance of broiler chickens fed on mature Moringa oleifera leaf meal as a protein supplement to soyabean meal. *International Journal of Poultry Science*, *11*(1), pp.5-10.

Tsega, W. and Tamir, B., 2009. The effect of increasing levels of dried leaves of sweet potato (Ipomoea batatas) on dry matter intake and body weight gain performance of broiler finisher chickens. *Livestock Research for Rural Development*, *21*, p.12.

Fasuyi, A.O., Ibitayo, F.J. and Alo, S.O., 2013. Histopathology, haematology and serum chemistry of growing pigs fed varying levels of wild sunflower (Tithonia diversifolia) leaf meal as protein supplements. *Journal of Agriculture and Veterinary Science*, *4*, pp.41-50.

Furuya, W.M., Pezzato, L.E., Barros, M.M., Pezzato, A.C., Furuya, V.R. and Miranda, E.C., 2004. Use of ideal protein concept for precision formulation of amino acid levels in fish-meal-free diets for juvenile Nile tilapia (Oreochromis niloticus L.). *Aquaculture Research*, *35*(12), pp.1110-1116.

Halimani, T.E., Ndlovu, L.R., Dzama, K., Chimonyo, M. and Miller, B.G., 2005. Metabolic response of pigs supplemented with incremental levels of leguminous Acacia karroo, Acacia nilotica and Colophospermum mopane leaf meals. *Animal Science*, *81*(1), pp.39-45.

Heuzé, V.Tran, G. Hassoun, P. Bastianelli, D. Lebas, F. *Moringa (Moringaleifera).*Available online: http://www.feedipedia.org/node/124.

Hetland, H. and Svihus, B., 2001. Effect of oat hulls on performance, gut capacity and feed passage time in broiler chickens. *British Poultry Science*, *42*(3), pp.354-361.

Iheukwumere, F.C., Ndubuisi, E.C., Mazi, E.A. and Onyekwere, M.U., 2007. Growth, blood chemistry and carcass yield of broilers fed cassava leaf meal (Manihot esculenta Crantz). *International Journal of Poultry Science*, *6*(8), pp.555-559.

Iheukwumere, F.C., Ndubuisi, E.C., Mazi, E.A., Onyekwere M.U. 2008. Performance, Nutrient Utilization and Organ Characterstics of Broilers Fed Cassava Leaf Meal (Manihot esculenta Crantz). *Pakistan Journal of Nutrition*, 7(1), pp. 13- 16.

Jamroz, D., Wiliczkiewicz, A. and Skorupinska, J., 1992. The effect of diets containing different levels of structural substances on morphological changes in the intestinal walls and the digestibility of the crude fibre fractions in geese (Part III). *Journal of Animal Feed Research*, *1*(3), pp.37-50.

Jiménez-Moreno, E., González-Alvarado, J.M., González-Sánchez, D., Lázaro, R. and Mateos, G.G., 2010. Effects of type and particle size of dietary fiber on growth performance and digestive traits of broilers from 1 to 21 days of age 1. *Poultry Science*, *89*(10), pp.2197-2212.

Karthivashan, G., Arulselvan, P., Alimon, A.R., Safinar Ismail, I. and Fakurazi, S., 2015. Competing role of bioactive constituents in Moringa oleifera extract and conventional nutrition feed on the performance of cobb 500 broilers. *BioMed Research International*, *2015*, 13

Kerr, B.J., McKeith, F.K. and Easter, R.A., 1995. Effect on performance and carcass characteristics of nursery to finisher pigs fed reduced crude protein, amino acid-supplemented diets. *Journal of Animal Science*, *73*(2), pp.433-440.

Le Bihan-Duval, E., Debut, M., Berri, C.M., Sellier, N., Santé-Lhoutellier, V., Jégo, Y. and Beaumont, C., 2008. Chicken meat quality: genetic variability and relationship with growth and muscle characteristics. *BMC Genetics*, *9*(1), p.53.

Makkar, H.P.S., 2003. Effects and fate of tannins in ruminant animals, adaptation to tannins, and strategies to overcome detrimental effects of feeding tannin-rich feeds. *Small Ruminant Research*, *49*(3), pp.241-256.

Mapiye, C., Chimonyo, M., Dzama, K., Strydom, P.E., Muchenje, V. and Marufu, M.C., 2009. Nutritional status, growth performance and carcass characteristics of Nguni steers supplemented with Acacia karroo leaf-meal. *Livestock Science*, *126*(1-3), pp.206-214.

Mapiye, C., Chimonyo, M., Marufu, M.C. and Dzama, K., 2011. Utility of Acacia karroo for beef production in Southern African smallholder farming systems: A review. *Animal Feed Science and Technology*, *164*(3), pp.135-146.

Mateos, G.G., Jiménez-Moreno, E., Serrano, M.P. and Lázaro, R.P., 2012. Poultry response to high levels of dietary fiber sources varying in physical and chemical characteristics. *The Journal of Applied Poultry Research*, *21*(1), pp.156-174.

Melesse, A., Bulang, M. and Kluth, H., 2009. Evaluating the nutritive values and in vitro degradability characteristics of leaves, seeds and seedpods from Moringa stenopetala. *Journal of the Science of Food and Agriculture*, *89*(2), pp.281-287.

Melesse, A., Getye, Y., Berihun, K. and Banerjee, S., 2013. Effect of feeding graded levels of Moringa stenopetala leaf meal on growth performance, carcass traits and some serum biochemical parameters of Koekoek chickens. *Livestock Science*, *157*(2), pp.498-505.

Mokoboki, H.K., Ndlovu, L.R., Ng'ambi, J.W., Malatje, M.M. and Nikolova, R.V., 2005. Nutritive value of Acacia tree foliages growing in the Limpopo Province of South Africa. *South African Journal of Animal Science*, *35*(4), pp.221-228.

Mountzouris, K.C., Tsirtsikos, P., Kalamara, E., Nitsch, S., Schatzmayr, G. and Fegeros, K., 2007. Evaluation of the efficacy of a probiotic containing Lactobacillus, Bifidobacterium, Enterococcus, and Pediococcus strains in promoting broiler performance and modulating cecal microflora composition and metabolic activities. *Poultry Science*, *86*(2), pp.309-317.

Mtui, D.J., Lekule, F.P., Shem, M.N., Rubanza, C.D., Ichinohe, T., Hayashida, M. and Fujihara, T., 2006. Seasonal influence on mineral content of forages used by smallholder dairy farmers in lowlands of Mvomero District, Morogoro, Tanzania. *Journal of Food Agriculture and Environment 4*(2), p.216.

Ncube, S., Halimani, T.E., Mwale, M. and Saidi, P.T., 2017. Effect of Acacia angustissima Leaf Meal on the Physiology of Broiler Intestines. *Journal of Agricultural Science*, *9*(2), p.53.

Ndou, S.P., Khanyile, M. and Chimonyo, M., 2015. Growth performance and nutrition-related serum metabolites in growing pigs fed on Acacia Tortilis leaf meal. *Livestock Science*, *182*, pp.22-27.

Ng'ambi, J.W., Nakalebe, P.M., Norris, D., Malatje, M.S. and Mbajiorgu, C.A., 2009. Effects of dietary energy level and tanniferous Acacia karroo leaf meal level of supplementation at finisher stage on performance and carcass characteristics of Ross 308 broiler chickens in South Africa. *International Journal of Poultry Science*, *8*(1), pp.40-46.

Nkukwana, T.T., 2012. *The effect of Moringa oleifera leaf meal on growth performance, gut integrity, bone strength, quality and oxidative stability of meat from broiler chickens.* PhD Thesis, University of Fort Hare, Alice, South Africa.

Nkukwana, T.T., Muchenje, V., Pieterse, E., Masika, P.J., Mabusela, T.P., Hoffman, L.C. and Dzama, K., 2014. Effect of Moringa oleifera leaf meal on growth performance, apparent digestibility, digestive organ size and carcass yield in broiler chickens. *Livestock Science*, *161*, pp.139-146.

Nkukwana, T.T., Muchenje, V., Masika, P.J. and Mushonga, B., 2015. Intestinal morphology, digestive organ size and digesta pH of broiler chickens fed diets supplemented with or without Moringa oleifera leaf meal. *South African Journal of Animal Science*, *45*(4), pp.362-370.

Nwoche, G.N., Ndubuisi, E.C. and Iheukwumere, F.C., 2006. Performance of finisher broilers and cost implication of feeding palm oil as energy supplement. *Nigeria Agricultural Journal*, *37*(1), pp.44-49.

Nworgu, F.C. and Fasogbon, F.O., 2007. Centrosema (Centrosema pubescens) leaf meal as protein supplement for pullet chicks and growing pullets. *International Journal of Poultry Science*, *6*(4), pp.255-260.

Nworgu, F.C. and Egbunike, G.N., 2013. Nutritional Potential of Centrosema pubescens Mimosa invisa and Pueraria phaseoloides Leaf Meals on Growth Performance Responses of Broiler Chickens. *American Journal of Experimental Agriculture*, *3*(3), p.506.

Nyamukanza, C.C. and Scogings, P.F., 2008. Sprout selection and performance of goats fed Acacia karroo coppices in the False Thornveld of the Eastern Cape, South Africa. *South African Journal of Animal Science*, *38*(2), pp.83-90.

Onyimonyi, A.E. and Onu, E., 2009. An assessment of pawpaw leaf meal as protein ingredient for finishing broiler. *International Journal of Poultry Science*, *8*(10), pp.995-998.

Onyimonyi, A.E., Olabode, A. and Okeke, G.C., 2009. Performance and economic characteristics of broilers fed varying dietary levels of neem leaf meal (Azadirachta indica). *International Journal of Poultry Science* *8*(3), pp.256-259.

Rubanza, C.D.K., Shem, M.N., Otsyina, R., Bakengesa, S.S., Ichinohe, T. and Fujihara, T., 2005. Polyphenolics and tannins effect on in vitro digestibility of selected Acacia species leaves. *Animal Feed Science and Technology*, *119*(1-2), pp.129-142.

Simol, C.F., Tuen, A.A., Khan, H.H.A., Chubo, J.K., King, P.J.H. and Ong, K.H., 2012. Performance of chicken broilers fed with diets substituted with mulberry leaf powder. *African Journal of Biotechnology*, *11*(94), pp.16106-16111.

Svihus, B., 2014. Function of the digestive system. *Journal of Applied Poultry Research*, *23*(2), pp.306-314.

Viveros, A., Chamorro, S., Pizarro, M., Arija, I., Centeno, C. and Brenes, A., 2011. Effects of dietary polyphenol-rich grape products on intestinal microflora and gut morphology in broiler chicks. *Poultry Science*, *90*(3), pp.566-578.

Wenk, C., 2001. The role of dietary fibre in the digestive physiology of the pig. *Animal Feed Science and Technology*, *90*(1-2), pp.21-33.

Zanu, H.K., Mustapha, M. and Addo Nartey, M., 2012. Response of broiler chickens to diets containing varying levels of Leucaena (Leucaena leucocephala) leaf meal. *Journal of Animal and Feed Research*, *2*(2), pp.108-112.

CHAPTER 3: Response in internal organ weights to *Vachellia tortilis* inclusion in broilers

Abstract

The objective of the current study was to investigate the response in carcass yield and organ weights to *Vachellia tortilis* inclusion in broilers. Five-hundred unsexed cobb 500-day-old broiler chicks were fed on conventional starter mash for 14 days. The chicks were randomly allocated to six dietary treatments. These diets contained 0, 30, 60, 90, 120 and 150 g/kg DM inclusion levels of *Vachellia tortilis* leaf meal. Each diet was offered *ad libitum* to 10 birds per pen for 17 days before they were slaughtered. There was a linear decrease in both slaughter weight (SW) and dressed carcass weight (DCW) ($P<0.05$) as *V. tortilis* leaf meal increased. The SW and DCW decreased by 0.77 and 0.94 g for each g/kg increase in *V. tortilis* inclusion. The scaled weights of the thighs (TW) and drumstick (DW) showed no relationship with levels of leaf meal. The weight of gizzard, intestines and stomach, however, increased linearly ($P<0.05$) as levels of leaf meal increased. Relative gizzard, intestine and stomach weights increased by 0.0028, 0.0059 and 0.0008 g for each g/kg increase in *Vachellia tortilis* ($P<0.05$) inclusion. There was a linear increase in relative heart weight ($P<0.05$) and kidney weight ($P<0.05$) with increasing levels of *V. tortilis* leaf meal in the diet. The relative heart and kidney weight increased by 0.0009, 0.0006 g as *V. tortilis* increased ($P<0.05$). The relative weight of the liver was, however, not related to *V. tortilis* inclusion. It was concluded that carcass yield and organ weights responded differently to increase in *Vachellia tortilis* inclusion.

Keywords: Dressed carcass weight, Gizzard, Heart, Kidney, Intestines, Slaughter weight.

3.1 Introduction

Chicken meat provides high quality protein and high concentration of polyunsaturated fatty acids (Wapi *et al.*, 2013). In Southern Africa, broilers contribute about 70 % of the animal protein for human consumption (Department of Agriculture, Forestry and Fisheries (DAFF, 2013). Poultry provide animal proteins required by humans to meet their protein requirements. Maize and soya-beans contribute over 93% of the poultry diets. These grains are also consumed by humans. Maize, for example, is a staple crop in southern Africa. Soya beans are used for oil extraction and marketed as whole grain. Therefore, consumption of maize and soya beans poses a serious competition between poultry and humans (Halimani *et al.*, 2005). These challenges of feed shortages increase the cost of poultry feeds. There is, therefore, the need to identify and characterize non-conventional feed resources as alternatives to conventional feed ingredients. Inclusion of leaf meals in broiler diets not only reduces the proportion of conventional feed ingredients but may also have beneficial effects on the quality of meat produced. *Vachellia* leaves, for example, have been identified to be important due to their relatively high crude protein content, favorable minerals and vitamins. The protein content of *Vachellia tortilis* leaves range from 160 to 210 g/kg, depending on season, soil fertility and stage of flowering (Khanyile *et al.*, 2014). Assessment of the nutritional value and potential of leaf meal can be useful as an alternative feed for broilers.

Among other challenges, *Vachellia* leaf meal consumption is likely to be limited by the presence of polyphenolic compounds, thorns and high fibre content (Khanyile *et al.*, 2014). Thorns can easily be eliminated mechanically through sieving. These compounds damage the internal mucosa, which consequently, impairs nutrient absorption in the gut (Jansmanet *et al.*, 1993). Polyphenolic compounds, especially proanthocyanidins, form insoluble complexes with divalent ions, reducing their bioavailability in the bird (Stukelj *et al.*, 2010).

Furthermore, marketing of broilers has shifted from whole chicken to portions. When assessing the potential of leaf meals in broiler production, it is, therefore, important to consider carcass and breast muscle yield (Le Bihan-Duval *et al.*, 1999; Rostagno *et al.*, 2007). Determining the response in organ weights is also important, since organ weights reflect the extent of metabolism and presence of anti-nutritional factors in the diet. For example, the liver is one of the sensitive visceral organs that respond to toxic substances and protein deficiency. The anatomy and weight of internal organs may therefore, change in response to dietary tannins and fibre content (Agyekum *et al.*, 2012). There is, however, little, if any, information on the response of carcass yield and organ weights to *Vachellia tortilis* inclusion in broilers.

Given that leaf meals may depress feed intake and growth performance, it is essential to conduct dose-response trials for farmers to predict performance at any given inclusion level when feeds are mixed on-farm. It is crucial that inclusion levels of the leaf meals do not constrain feed intake. The objective of the study was, therefore, to determine the response in carcass yield and organ weights of broilers fed on *Vachellia tortilis*. It was hypothesized that carcass weight and organ weight would change with inclusion levels of *V. tortilis*.

3.2 Materials and methods

3.2.1 Description of study site

The study was conducted at Ukulinga Research Farm, University of KwaZulu-Natal (UKZN), Pietermaritzburg, South Africa, sited in a subtropical hinterland, located at $30^{0}24$'S, $29^{0}24$'E and is approximately 700 m above sea level. The climate is characterized by an annual rainfall of 735 mm, which falls regularly in the rainy season between October and April. Mean annual maximum and minimum temperatures are 25.7 and 8.9°C, respectively, light to mild frost occurs in the cool dry season.

3.2.2 Leaf meal collection

Vachellia tortilis leaves were hand harvested at an advanced stage of maturity at Makhathini Research Station, Jozini, South Africa. The leaf meal was harvested between April and May 2013 after the rainy season at advance stage of maturity. The leaves were dried under shade for three days and passed through a 2 mm sieve to get rid of thorns, pods and twigs. Dried leaf meals were bagged in a form of powder and stored in a well ventilated dry room.

3.2.3 Experimental diets and design

Six experimental diets were formulated using a Winfeed® diet formulation software programme. The experimental diet had the inclusion levels as follows: 0, 30, 60, 90, 120 and 150 g/kg of *Vachellia tortilis*. All experimental diets were supplemented with vitamins and minerals to meet the National Research Council (NRC, 2012) recommended specifications for broilers. The trial ran for 31 days.

Ten birds were allocated randomly in each pen, with six pens for each inclusion level. The 10 birds per pen constituted an experimental unit. Table 3.1 shows the ingredient composition of broiler finisher diet used in the experiment.

3.2.4 Chemical composition of feeds

The feed had inclusion levels of 0, 30, 60, 90, 120 and 150 g/kg of *Vachellia tortilis*. The experimental feed was formulated using Winfeed formulation programme to contain similar amounts of energy and protein. The experimental diets were supplemented with vitamins and minerals to meet the National Research Council (NRC, 2012) recommended specifications for broilers. The raw materials were mixed as specified in Table 3.1.

Moisture content was analysed by weighing crucibles and feed before and after being placed into an oven which will be 95°C for 72 hours. Feed sample was approximately 5 grams. Dry matter was calculated from moisture content. Moisture content was calculated as described by the Association of Official Analytical Chemists (AOAC Official Methods 934.01). Nitrogen was analysed using the LECO TruSpec Nitrogen Analyzer. The nitrogen was multiplied by a factor of 6.25, as described by the AOAC Official Methods 990.03. Ash analysis, crucibles and feed was weighed and placed into a furnace overnight at 550°C. Ash was then calculated and organic matter from ash as described by the AOAC Official Methods 942.05. Crude fat was analysed using Soxhlet extraction according to the AOAC official methods (2003). Table 3.2 shows the chemical composition of the experimental diets, including that of *V. tortilis* leaf meal. Metabolizable energy was determined as described by the following equation.

NFE = 100- moisture- Crude protein- Crude fibre- Ash%; and

ME (Kcal/kg) = 10 (3-5x Crude protein) + (8.5x Crude fibre) + (3.5x NFE)

Table 3.1: Ingredient composition (g/kg) of broiler finisher diets containing *Vachellia tortilis* leaf meal

Ingredient	*Vachellia tortilis* inclusion level (g/kg)					
	0	30	60	90	120	150
White maize	545.0	519.0	493.0	469.0	445.0	421.0
Soybeans 46 %	392.0	388.0	383.0	376.0.	370.0	363.0
Vachellia tortilis	0	30.0	60.0	90.0	120.0	150.0
Oil-sunflower	45.0	46.1	47.0	47.9	48.8	49.8
Limestone	1.4	1.4	1.3	1.2	1.2	1.2
Monocalcium	1.5	1.4	1.4	1.3	1.3	1.2
Salt	5.0	5.0	5.0	5.0	5.0	5.0
Vitamin-mineral premix	5.0	5.0	5.0	5.0	5.0	5.0
Amino acids	3.2	4.6	4.4	4.3	4.1	3.8

Table 3.2: Chemical composition of the experimental diets and *Vachellia tortilis*

Component	*Vachellia tortilis* inclusion level (g/kg DM)						*Vachellia tortilis*
	0	30	60	90	120	150	
Dry matter	993	987	988	973	974	963	944
ME (MJ/kg)	140	14	14.1	14.2	14.1	14.3	-
Ash	41	39	42	43	46	47	650
Crude protein	194	203	204	194	221	201	218
Ether extracts	68.9	67.9	75.1	79.5	75.7	85.9	40.1
Acid detergent fibre	20.1	29.2	28.4	32.6	41.1	42	298
Neutral detergent fibre	80.9	106.7	100.4	115	129.5	129.3	494
Proanthocyanidins	0.1	0.6	1.7	2.2	5.9	9.1	51.5

3.2.5 Management of birds

The care and use of broilers were done according to the ethical guidelines stated by the certification of authorization to experiment on living animals given by the University of KwaZulu-Natal Animal Ethics Committee protocol (Reference Number: AREC/014/017).

Prior to arrival, these broilers were vaccinated, and the house was disinfected and cleaned. All chicks were fed on commercial starter feed for 14 days, after which they were allocated to the experimental diets. Unsexed Cobb 500-day-old broiler chicken with average body weight of 44 g were allocated to a pen containing fresh wood shavings and infrared lighting for 14 days. Each pen had two water drinkers and one plastic feeder. Drinking water was also provided *ad libitum* through water founts fitted on the opposite side to the feeder. The brooder facility had a temperature of 31°C during the first two weeks, then the temperature was reduced per week until 21°C was reached. The temperature decreased from 31 to 21°C at intervals up to 31 days. Chick behavior was used as an indicator of correct temperature. An appropriate temperature was indicated by movement and behavior of chicks. A foot bath was placed by the entrance for biosecurity.

3.2.6 Measurements of growth

All birds were weighed on their arrival and initial body weights during acclimatization period was recorded. Body weight and feed intake were recorded independently per bird in each pen after every seven days. Weekly feed intake (WFI) for birds was determined as the difference between the weight of the feed offered in the beginning and feed left in troughs at the end of each week. The average daily feed intake (ADFI) was calculated by dividing the weekly measurements by 7. The birds were weighed per pen independently. Weighing was done every week on Sunday from 0730h to 0830 h throughout the trial. The weighing of the birds was done using the electric balanced scale, and the following variables were calculated.

$$ADFI = \frac{Feed\ \ intake}{number\ of\ days\ fed(days)}$$

$$ADG = \frac{Body\ weight\ gain}{number\ of\ days(7days)}$$

$$\text{FCR} = \frac{Average\ daily\ feed\ intake}{Average\ daily\ body\ weight\ gain}$$

3.2.7 Measurements of carcass yield

On day 31, these birds were starved for 12 hours prior to slaughter at 0800h the next day. Slaughter weight (SW) was taken as the body weight of bird after overnight fasting. All these birds were slaughtered and exsanguinated at Ukulinga Research Farm. The birds were then dipped in hot water and de-feathered. heads, legs and internal organs were removed. Dressed carcass weight (DCW) was recorded. The SW and dressing percentage (DP) for each bird was recorded. The DP was estimated by dividing the DCW by the SW and expressed as a percentage. The weight of the breast, drumstick and thigh were recorded using a digital scale. The scaled portion were expressed as percentage by dividing the portion weights by slaughter weight.

3.2.8 Weights of internal organs

The giblets (liver, gizzards and heart), the empty weight of small intestines and large intestines, including the caeca and stomach, were recorded. Gizzards were opened and weighed after removal of its contents. The scaled organs were determined by dividing the weights of the organs by the SW and expressed as a percentage. In the small intestines of each bird, jejunum segments were measured from the midway between the point of entry of the bile ducts and Meckel's diverticulum.

3.2.9 Statistical analyses

Relationships between *Vachellia tortilis* leaf meal inclusion and performance characteristics, carcass yield and organ weights were determined with the response surface regression (RSREG) procedure of (SAS, 2009).

The model used was: $Y = \beta 0 + \beta 1\ D + \beta 2\ D^2 + E$

Where: Y is the response variables (Carcass yield and organ weight)

β0, β1, β2 regression coefficients

D is the inclusion level of *Vachellia tortilis* leaf meal

E is the residual error

3.3 Results

3.3.1 Influence of leaf meal inclusion on carcass yield

The influence of V. tortilis inclusion on broiler performance is summarized in Table 3.3. The least square means for carcass characteristics of broilers fed on increment levels of *V. tortilis* are shown in Figure 3.1. *Vachellia tortilis* leaf meal inclusion was not related to DP. SW (P<0.001) and DCW (P<0.05) showed a relationship with increasing levels of *V. tortilis*. There was no relationship in relative breast weight, TW and DW (P>0.05) to leaf meal inclusion. There was a linear decrease in SW (P<0.001) and dressed carcass weight (P< 0.05) with increasing levels of leaf meal. The R^2 values for DCW and SW were 0.96 and 0.83, respectively. For DP, there was no relationship (P>0.05) with *V. tortilis* inclusion.

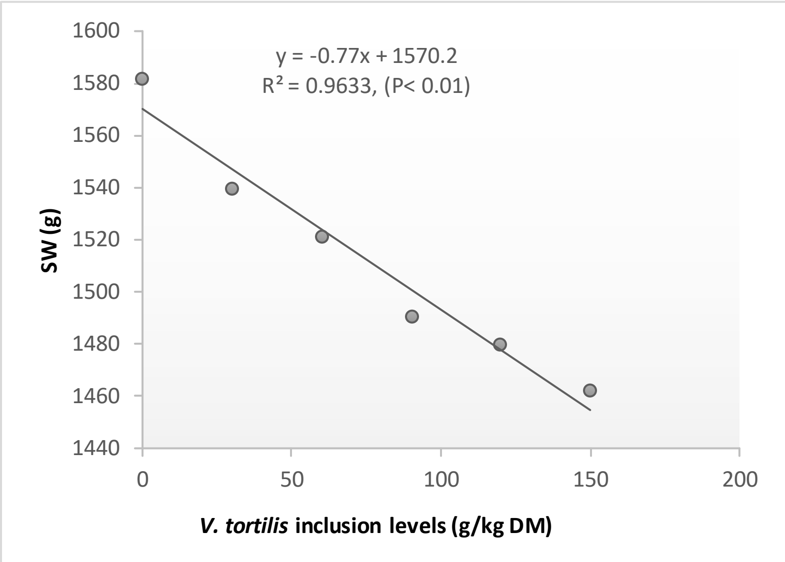
3.3.2 Response in organ weights to leaf meal inclusion

Responses of relative organ weight to incremental levels of *V. tortilis* leaf meals in diets are given in Table 3.4. The relative weight of the liver showed no relationship with increasing levels of *V. tortilis* leaf meal-based diet. The relative organ weight, including intestines, gizzard, heart, kidney and proventriculus were related to increasing inclusion levels of *V. tortilis* leaf meal in the diet. There was a linear increase in intestinal weight (P<0.01), proventriculus weight (P<0.001) and gizzard weight (P<0.001) as the inclusion level of *V. tortilis* increase. The heart weight linearly increases (P<0.05) with increasing leaf meal levels with the R^2 value was 0.96. Kidney weight also showed a linear increase (P<0.05). The relative weight of the liver was, however, not related to *Vachellia tortilis i*nclusion (P>0.05).

Table 3.3: Relationship between *Vachellia tortilis* leaf meal inclusion level and broiler performance

	Inclusion level of *Vachellia tortilis* leaf meal (g/kg DM)						SEM	R^2	Equations	Significance
Parameter	0	30	60	90	120	150				
ADFI	0.094	0.099	0.099	0.091	0.091	0.077	0.009	0.59	y=-0.004x+0.09	***
ADG	0.060	0.063	0.058	0.055	0.047	0.039	0.003	0.85	y=-0.002x+0.06	***
FCR	1.577	1.559	1.673	1.618	1.972	1.98	0.211	0.77	y=-0.057x+1.61	**

ADFI: average daily feed intake (kg DM/d); ADG: average daily gain (kg BW/d); FCR: feed conversion ratio (kg/kg); SEM: standard error of mean; (***P<0.001; **P<0.01.



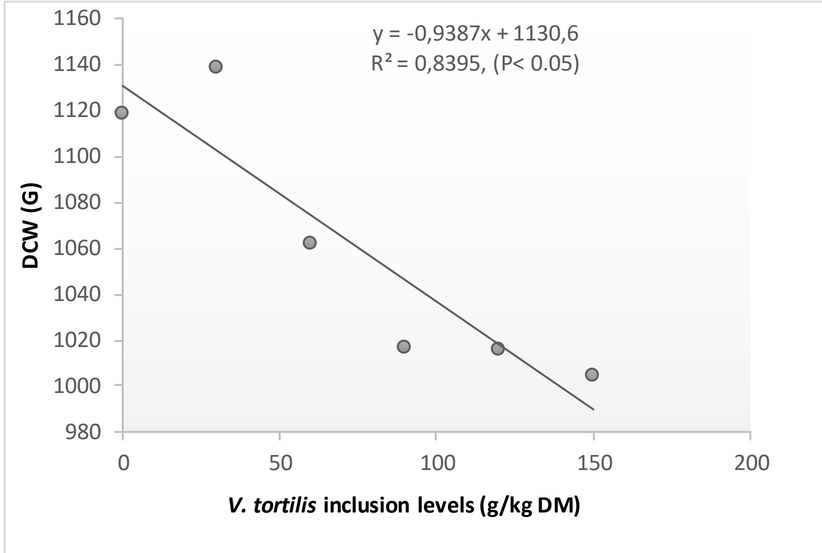


Figure 3.1: Response in slaughter weight (SW) and dressed carcass weight (DCW) to *Vachellia tortilis* in broilers.

Table 3.4: Effect of inclusion level of *Vachellia tortilis* leaf meal on the relative organ weight and length (% SW) in broilers

	Inclusion levels						Regression			
	0	**30**	**60**	**90**	**120**	**150**	**SEM**	**Quadratic**	**Linear**	**Significance**
Organ										
Gizzard	2.1	2.3	2.4	2.5	2.5	2.6	0.07	NS	0.003	**
Heart	0.39	0.42	0.44	0.47	0.49	0.54	0.03	NS	0.001	**
Kidney	0.13	0.16	0.18	0.2	0.21	0.23	0.01	NS	0.03	**
Liver	1.8	1.8	1.7	1.7	1.7	1.7	0.06	NS	0.04	NS
Proventriculus	0.38	0.41	0.44	0.47	0.48	0.5	0.01	NS	0.001	**
Small intestines	3.5	3.8	4.4	4.8	5	5.4	0.22	NS	0.001	**
Large intestines	0.1	0.2	0.3	0.3	0.4	0.3	0.01	NS	0.002	**
Caecum	5.2	6.3	7.2	7.7	8.3	8.6	0.5	NS	0.02	*
Length										
Duodenum	1.7	1.8	1.9	2	2.01	2.1	0.07	NS	0.002	NS
Jejunum	4.9	5.1	5.2	5.3	5.4	5.6	0.17	NS	0.004	NS
Ileum	4.6	4.9	5.1	5.2	5.3	5.4	0.18	NS	0.005	NS

SW: slaughter weight; SEM: standard error of mean; (*** $P < 0.001$; ** $P < 0.01$; *$P < 0.05$); NS: not significant $P > 0.05$

3.4 Discussion

In the current study, all birds were clinically healthy throughout the trial. The increase of human population has stimulated increased cost of grain such as soybeans and maize as ingredient in formulating animal diets (FAO, 2011). Nutrition is one of the main factors affecting carcass yield. The *Vachellia tortlis* leaf meal has high crude protein content and has been used in formulating diets to meet nutrient requirement for broilers (NRC, 2012). The recommended crude protein requirement in the diet for broilers is between 200 and 220 g/kg for potential development, growth and maintenance. *Vachellia tortilis* leaves are used due to their high crude protein range from 160 to 210 g/kg. In broiler production, leaf meal can be a good source of crude protein, amino acids, energy and favorable minerals and vitamins (Woyengo *et al.*, 2014). Therefore, better consumption of leaf meal is important for sustainable broiler production system.

Dressing percentage is the major tool used to assess carcass yield. Increasing *V. tortilis* inclusion is expected to produce a linear relationship with carcass yield of broiler. Given that leaf meal is associated with decreasing feed intake and growth performance, it is important to investigate carcass yield and internal organ weights of broilers fed *Vachellia tortilis* leaf meal for broiler farmer to predict performance at any inclusion level when feed is mixed on-farms. Determining the relationship of DCW with inclusion levels of *V. tortilis* leaf meal will improve our knowledge onit utilization, since leaf meals supply high crude protein and energy, given that these leaf meals suppress feed intake and growth performance.

The linear relationship between *Vachellia tortilis* inclusion and feed intake is because the fibre in broiler diets is not digestible, and thus contributes to gut fill, thereby reducing feed intake. Esonu *et al.* (2003), on the other hand, showed that high fibre content of leaf meals in the diet is related to the energy dilution effect and resulting to increase in feed intake. The indigestible residues from fibre can decrease feed intake by inducing bulkiness in the stomach causing decreased feed digestibility and impacting the intestinal tract and leading to decreased broiler growth performance (Agbede and Aletor, 2003; Gadzirayi *et al*., 2012). The decrease in feed intake indicates that *Vachellia* leaf meal could have a negative influence due to polyphenolic compounds presence in leaf meals. The decrease in feed intake with *Vachellia tortilis* leaf meal inclusion suggest that birds decrease the consumption of nutrients. Polyphenolic compounds are most abundant and known to have several negative effects in the broilers' performance including, low feed intake, depressed digestibility, decrease consumption of absorbed nutrients and poor performance (Schiavone *et al*., 2008).

The linear decrease between *V. tortilis* inclusion and slaughter weight suggest that a leaf meal influenced carcass yield parameters. Slaughter weight decreased by 0.77 g for each g/kg increase in *V. tortilis* inclusion. The R^2 value for SW of 0.96 indicates the strength of the linear relationship between SW and incremental inclusion levels *V. tortilis*. The observation that body weight decreased with increased inclusion levels of *V. tortilis* agrees with Tsega and Tamir (2009), who reported that increasing levels of dried leaves of sweet potatoes (*Ipomoea batatas*) decrease body weight gain of broilers. Al Mamary *et al*. (2001) observed that a low concentration of tannins in the diet should not decrease feed intake of monogastric animals while higher concentrations of tannins negatively influence growth performance.

D'Mello (1992) reported that birds fed incremental levels of *Leucaena leucocepha* leaf meal showed depressed growth. The influence of polyphenolic compounds varies on the type, levels and the nutrients contained in the diet. Olugbemi *et al*. (2010) reported decreases in final body weight and weight gain as level of *Moringa oleifera* leaf meal increase in diets fed to broilers.

The influence of nutrients imbalance and poor metabolism on monogastrics a fed high levels of leaf meal ingredients have been reported earlier. The lower SW for birds at the inclusion level of leaf meal increased could also be explained by a decrease in energy intake due to higher fibre intake (D'Mello, 1995).

Incremental level of fibre content in the diet increased feed volume and this restricted efficient utilization of nutrient by birds (Gous *et al*., 1990). The decrease in body weight might be due to different dietary inclusion level of leaf meal in ration. The decrease in body weight may be due to imbalanced of nutrients and improper metabolism which altered the gut functioning as the levels of leaf meal increase (Onyimonyi *et al*., 2009). Onyimonyi *et al*. (2009) reported that the decrease in growth performance at higher levels of the leaf meal inclusion could be caused by nutrient imbalances and improper metabolism that distorted the harmonious gut environment.

There was a linear decrease in dressed carcass weight as *V. tortilis* inclusion level increased in the diet. Dressed carcass weight decreases on average by 0.94 g as the inclusion level of *V. totilis* increased by 1 g/kg. The R^2 value was 0.84, which indicated the strength of the linear relationship between DCW and inclusion level of *V. tortilis*. The linear decrease in DCW with increasing inclusion level of *Vachellia tortilis* in the diet agrees with Ncube *et al*. (2018), who also reported a decrease in carcass weight of broilers fed increasing inclusion of *Vachellia angustissima* leaf meal. The linear decrease between *V. tortilis* inclusion level and DCW suggest that broilers were not absorbing sufficient nutrient required for muscle deposition for the increased carcass yield. The decrease in DCW shows that increased in *V. tortilis* inclusion increases the ability of polyphenolic compounds to bind protein. Polyphenolic compounds in leaf meals are known to decrease feed intake, digestibility and absorption of nutrients (Ndou *et al.,* 2015), thereby induce depression in growth of broilers.

Nworgu *et al.* (2007) reported that proteins are negatively influenced by binding of polyphenolic compounds to protein, which reflects nutritional insufficient of the dietary protein available for broilers to use in muscle deposition. This resulted in insufficient consumption of digestible proteins and metabolizable energy that were required for growth (Onyimonyi *et al.*, 2009). This agrees with Smith *et al.* (2001) who observed suppression in growth performance for rats with increasing inclusion levels for *V. angustissima* leaf meal. Protein content, availability and efficiency of utilisation determines muscle development. Depressed carcass deposition result from low feed, energy and protein intake in *Vachellia* species can be as a result of proanthocyanidins (Mapiye *et al.*, 2009). Proanthocyanidins concentration, nature and composition in diet subsequently determines the interaction and efficiency of utilisation in nutrients primarily proteins. There was no relationship between *V. tortilis* inclusion dressing percentage, which agrees with Ncube *et al.* (2018), who reported that *A. angustissima* inclusion had no effect on dressing percentage.

Hetland and Svihus (2001) reported that inclusion level of leaf meal has the influence of stimulating gizzard activity and increasing the volume of the gizzard when added into the diet. It is essential to determine the relationship of gizzard with inclusion level of *V. tortilis*, since *Vachellia* is known to have high crude protein and contains polyphenolic compound such as tannin, which in turn, suppresses broiler performance. There was a linear increase in relative weights of the gizzard with increasing dietary level of *V. tortilis* leaf meal. From the equation $y= 0.003x + 2.17$, the relative gizzard weight increased by 0.003g for each g/kg increase in *V. tortilis* inclusion. The R^2 value of 0.91, indicating that there was a strong linear relationship of relative gizzard weight with increasing *V. tortilis* inclusion. The increase in relative gizzard weights with increasing of *Vachellia* could be associated with fibre, which stimulates the increase in muscular activities. The gizzard breaks down ingested feed by muscular action and higher dietary leaf meal would prone high thickening of the muscle (Onibi *et al.,* 2008). This might be due to increased levels of fibre, which would stimulate the activity of the organ thus resulting to increased volume of the gizzard (Hetland and Syihus, 2001). A variety of plant inclusions has been researched with most influencing the digestibility of nutrients and the degree of lignification, in which this will impact the productivity due to rate of passage in the gastrointestinal tract; therefore, broiler require a minimal fibre content in the diet to keep in good condition the activity of gizzard and gastrointestinal tract functionality.

The linear relationship between *V. tortilis* level and relative heart weight was such that the relative heart weight increased by 0.0009 g for each gram increase in *V. tortilis*. The results of such increase indicated that leaf meal influenced the heart weight gain. This can be explained by an attempt to supply oxygen and an increase in blood circulation (Khanyile, 2013). Moreover, this might be due to an increase in other internal organs such as gizzard and intestines. The increase in gizzard and intestinal weight can also increase the metabolic activities such as digestion, which led to increased demand for oxygen and blood circulation, and thus contributes to increase in heart weights. The results agree with Agbede and Aletor, (2003), who reported an increase in relative heart weight with increasing levels of *Glyricidia sepium* leaf meal. Ayssiwede *et al.* (2011) reported the increase in heart weight to broilers fed on incremental level of *Leucaena leucocephala* leaf meal. Ekenyem and Medubuike, (2006) on the other hand, reported a decrease in heart weight with graded level of *Lpomoea asarifolia* leaf meal.

The catabolism of nitrogenous compounds should increase the concentration of wastes in blood, thereby increasing the work load of the kidneys, causing them to increase in size, due to consumption of protein rich leaf meal-based diets (Khanyile, 2013). The relative kidney weight increased linearly as *Vachellia tortilis* level increased. The leaf meal inclusion had no effect on the relative weight of the liver. The linear relationship was observed between *V. tortilis* inclusion levels and relative kidney weight. The kidney weight increased by 0.0006 g for each gram increase in leaf meal. These results suggest that broiler gain kidney weight to achieve increasing work load for excretion of excess protein from leaf meal that is not required.

There was a linear increase in relative proventriculus weight as *V. tortilis* level increased. Increasing inclusion level of this leaf meal resulted to an increase in proventriculus weight of 0.0008 g for each increase of *Vachellia tortilis* level. The linear relationship between proventriculus and *V. tortilis* inclusion could be explained by the additional bulk and greater volume of digesta staying in the gastrointestinal tract during enzyme digestion (Uchegbu *et al.*, 2004). Thorburn and Wilcox (1985) reported that structural carbohydrate in monogastric diets specifically had a mechanical influence on the intestinal wall and caused the gastrointestinal tract to increase and thicken.

Three distinct segments including the duodenum, jejunum and ileum, are site where about 90 % of digestion and absorption of nutrients and minerals occurs; the other 10 % taking place in the stomach and large intestines. Therefore, it is useful to understand the organ to predict performance at any given inclusion level when feed is mixed on-farm, since it reflects the extent of metabolism. There was a linear increase in relative weight of intestines in respond to increasing of *Vachellia tortilis* leaf meal inclusion in the diet. This could be explained by adaptation to assist in the increased feed volumes related with the incremental levels of the leaf meal in the diet. Zhang *et al.* (2012) reported that the presence of leaf meal influenced increased the capacity and volume of intestines. The increase in intestinal size could be the results of thickening of the digestive tract with incremental level of leaf meal in the diet, which could be reflection of extreme workload by the intestines in pushing digesta along the digestive tract as the dietary fibre increase in the diet (Ncube *et al.*, 2017). The increase of the gastrointestinal tract indicates stronger movement during digesta transportation and be explained by highly activated digestion and absorption (Nkukwana *et al.*, 2015). Svihus (2014) suggested morphological changes in the duodenum and colon implied intensified digestive and absorption capacity of the intestines.

The weight of the large intestines and caecum relative to body weight of broilers were associated with increase inclusion of *V. tortilis*. The weight of large the intestine and caecum, however, increased linearly as levels of leaf meal increased. Relative weight of large intestines and caecum increased by 0.0015 and 0.022 g for each g/kg increase in *V tortilis* inclusion. The observed increase in relative weight of large intestines with inclusion level of *Vachellia* might be attributed to the prolonged presence of fibre in the gut. The increase caecum of the broilers would increase the digestive capacity with respect to dietary leaf meal. In addition, the result of such increase of caecum weight indicated that birds contain higher concentrations of bacteria and therefore play an essential role in the microbial degradation of some carbohydrates (Jorgensen *et al.*, 2010).

The presence of leaf meal in the diet of broiler was found to increase the length and weight of the intestinal segments and cause physical adaptation. There was a linear increase in length of intestinal segments with inclusion level of *V. tortilis*. The length of duodenum, jejunum and ileum increased by 0.0026, 0.0043 and 0.005 cm for each g/kg in *V. tortilis* inclusion. Borin *et* *al*., (2006) revealed that leaf meal influences the development of the intestinal segments, increase in capacity and volume. Nkukwana *et al*. (2015) stated that result of an increase in duodenal length could also be triggered by the dietary leaf meal associated with high fibre content.

The increase in duodenum, jejunum and ileum length is in agreement with previous studies (Wu *et al*., 2004), reported that plant material inclusion in the diet increased the lengths of the intestinal sections. The increase in intestinal length might be attributed to the presence of fibre for a long time in the gut. According to Forbes and Covasa (1995), bird requires more time to adapt to first access of dietary fibre. Thus, with the inclusion of *Vachellia tortilis* leaf meal inclusion in the diet of broiler, the intestinal morphology of the jejunum was more favorable for the birds and contribute to improve digestion and absorption of nutrients.

3.5 Conclusions

Vachellia tortilis leaf meal could decrease the proportion of other feed in broiler diets. The concentration of polyphenolic compounds and fibrous properties could constrain the optimal exploration of *Vachellia* leaves in the diets. The slaughter weight and dressed carcass weight showed a linear decrease with increasing inclusion levels of *Vachellia tortilis* leaf meal, whereas increasing inclusion levels of *V. tortilis* leaf meal increase internal organ weights linearly. However, it is necessary to investigate the effect of *V. tortilis* leaf meal inclusion on villi height, apparent villus surface area, villus height and depth ratio, crypt depth, epithelial thickness, submucosa and muscularis of broilers without constraining gut health of broilers. Since digestion and absorption of nutrients depends on the morphology and anatomy of intestines, it is crucial to assess the response of the gut morphology to leaf meal inclusion.

3.6 References

Agbede, J.O. and Aletor, V.A., 2003. Evaluation of fish meal replaced with leaf protein concentrate from Glyricidia in diets for broiler-chicks: Effect on performance, muscle growth, haematology and serum metabolites. *International Journal of Poultry Science*, *2*(4), pp.242-250.

Agyekum, A. K., Slominski, B. A. and Nyachoti C. M., 2012. Organ weight, intestinal morphology, and fasting whole-body oxygen consumption in growing pigs fed diets containing distillers dried grains with solubles alone or in in combination with a multienzyme supplement. *Journal of Animal Science* 90(11), 3032-3040.

Al-Mamary, M., Molham, A.H., Abdulwali, A.A. and Al-Obeidi, A., 2001. In vivo effects of dietary sorghum tannins on rabbit digestive enzymes and mineral absorption. *Nutrition Research*, 21(10), pp.1393-1401.

Ayssiwede, S.B., Chrysostome, C.A.A.M., Zanmenou, J.C., Dieng, A., Houinato, M.R., Dahouda, M., Akpo, Y., Hornick, J.L. and Missohou, A., 2011. Growth performance, carcass and organs characteristics and economics results of growing indigenous Senegal chickens fed diets containing various levels of Leuceana leucocephala (Lam.) leaf meal. *International Journal of Poultry Science*, 10 (3), pp.734-749.

Borin, K., Lindberg, J.E. and Ogle, R.B., 2006. Digestibility and digestive organ development in indigenous and improved chickens and ducks fed diets with increasing inclusion levels of cassava leaf meal. *Journal of Animal Physiology and Animal Nutrition*, *90*(5-6), pp.230-237.

D'Mello, J.P.F., 1992. Chemical constraints to the use of tropical legumes in animal nutrition. *Animal Feed Science and Technology*, *38*(2-3), pp.237-261.

D'Mello JPF (1995) Leguminous leaf meals in non-ruminant nutrition. In: D'Mello JPF and Devendra C (eds) Tropical Legumes in Animal Nutrition, 6(2), pp 247-282.

Ekenyem, B.U. and Madubuike, F.N., 2006. An assessment of Ipomoea asarifolia leaf meal as feed ingredient in broiler chick production. *Pakistan Journal of Nutrition*, *5*(1), pp.46-50.

Esonu, B.O., Iheukwumere, F.C., Iwuji, T.C., Akanu, N. and Nwugo, O.H., 2003. Evaluation of Microdesmis puberula leaf meal as feed ingredient in broiler starter diets. *Nigerian Journal of Animal Production*, *30*(1), pp.3-8.

Forbes, J.M. and Covasa, M., 1995. Application of diet selection by poultry with particular reference to whole cereals. *World's Poultry Science Journal*, *51*(2), pp.149-165.

Gadzirayi, C.T., Masamha, B., Mupangwa, J.F. and Washaya, S., 2012. Performance of broiler chickens fed on mature Moringa oleifera leaf meal as a protein supplement to soyabean meal. *International Journal of Poultry Science*, *11*(1), pp.5-10.

Halimani, T.E., Ndlovu, L.R., Dzama, K., Chimonyo, M. and Miller, B.G., 2005. Metabolic response of pigs supplemented with incremental levels of leguminous Acacia karroo, Acacia nilotica and Colophospermum mopane leaf meals. *Animal Science*, *81*(1), pp.39-45.

Hetland, H. and Svihus, B., 2001. Effect of oat hulls on performance, gut capacity and feed passage time in broiler chickens. *British Poultry Science*, *42*(3), pp.354-361.

Iheukwumere, F.C., Ndubuisi, E.C., Mazi, E.A. and Onyekwere, M.U., 2008. Performance, nutrient utilization and organ characteristics of broilers fed cassava leaf meal (Manihot esculenta Crantz). *Pakistan Journal of Nutrition*, *7*(13), pp.13-16.

Jansman, A.J.M., 1993. Tannins in feedstuffs for simple-stomached animals. *Nutrition Research Reviews*, *6*(1), pp.209-236.

Jørgensen, H., Theil, P.K. and Knudsen, K.E.B., 2010. Satiating properties of diets rich in dietary fibre fed to sows as evaluated by physico-chemical properties, gastric emptying rate and physical activity. *Livestock Science*, *134*(1-3), pp.37-40.

Khanyile M., 2013. Nutritive value of Acacia species and response of pigs fed on incremental levels of *Acacia tortilis* leaf meal-based diets. MSc Thesis, University of KwaZulu-Natal, Pietermaritzburg, South Africa.

Khanyile, M., Ndou, S.P. and Chimonyo, M., 2014. Influence of Acacia tortilis leaf meal-based diets on growth performance of pigs. *Livestock Science*, *167(5)*, pp.211-218.

Khanyile, M., Ndou, S.P. and Chimonyo, M., 2017. Influence of Acacia tortilis leaf meal-based diet on serum biochemistry, carcass characteristics and internal organs of finishing pigs. *Animal Production Science*, *57*(4), pp.675-682.

Mapiye, C., Chimonyo, M., Dzama, K., Strydom, P.E., Muchenje, V. and Marufu, M.C., 2009. Nutritional status, growth performance and carcass characteristics of Nguni steers supplemented with Acacia karroo leaf-meal. *Livestock Science*, *126*(1), pp.206-214.

Ncube, S., Halimani, T.E., Mwale, M. and Saidi, P.T., 2017. Effect of Acacia angustissima leaf meal on the physiology of broiler intestines. *Journal of Agricultural Science*, *9*(2), p.53.

Nkukwana, T.T., Muchenje, V., Masika, P.J. and Mushonga, B., 2015. Intestinal morphology, digestive organ size and digesta pH of broiler chickens fed diets supplemented with or without Moringa oleifera leaf meal. *South African Journal of Animal Science*, *45*(4), pp.362-370.

Nworgu, F.C. and Fasogbon, F.O., 2007. Centrosema (Centrosema pubescens) leaf meal as protein supplement for pullet chicks and growing pullets. *International Journal of Poultry Science*, *6*(4), pp.255-260.

Ncube, S., Saidi, P.T., Tivapasi, M.T., Imbayarwo-Chikosi, V.E. and Halimani, T.E., 2018. Serum lipid, enzyme, and hematological responses of broilers fed Acacia angustissima leaf meal-based diets. *Tropical animal health and production*, *50*(3), pp.665-670.

Olugbemi, T.S., Mutayoba, S.K. and Lekule, F.P., 2010. Effect of Moringa (Moringa oleifera) inclusion in cassava-based diets fed to broiler chickens. *International Journal of Poultry Science*, *9*(4), pp.363-367.

Onibi, G.E., Folorunso, O.R. and Elumelu, C., 2008. Assessment of partial equi-protein replacement of soyabean meal with cassava and leucaena leaf meals in the diets of broiler chicken finishers. *International Journal of Poultry Science*, *7*(4), pp.408-413.

Onyimonyi, A.E. and Onu, E., 2009. An assessment of pawpaw leaf meal as protein ingredient for finishing broiler. *International Journal of Poultry Science*, *8*(10), pp.995-998.

Smith, A.H., Odenyo, A.A., Osuji, P.O., Wallig, M.A., Kandil, F.E., Seigler, D.S. and Mackie, R.I., 2001. Evaluation of toxicity of Acacia angustissima in a rat bioassay. *Animal feed science and technology*, *91*(1-2), pp.41-57.

Schiavone, A., Guo, K., Tassone, S., Gasco, L., Hernandez, E., Denti, R. and Zoccarato, I., 2008. Effects of a natural extract of chestnut wood on digestibility, performance traits, and nitrogen balance of broiler chicks. *Poultry Science*, *87*(3), pp.521-527.

Tsega, W. and Tamir, B., 2009. The effect of increasing levels of dried leaves of sweet potato (Ipomoea batatas) on dry matter intake and body weight gain performance of broiler finisher chickens. *Livestock Research for Rural Development*, *21(10)*, p.12.

Thorburn, C.C. and Wilcox, J.S., 1985. Cited in Faniyi, G. F., Ologhobo, AD, Adeniran, GA and Alaka, OO (1988). Replacement value of biodegraded cowpea and sorghum seedhulls for brewers dried grain in broiler diets. In *Proc. 3 annual conf. Animal Science Association of Nigeria Sept*, 22 (6), pp. 84-87.

Tsega, W. and Tamir, B., 2009. The effect of increasing levels of dried leaves of sweet potato (Ipomoea batatas) on dry matter intake and body weight gain performance of broiler finisher chickens. *Livestock Research for Rural Development*, 208(*21)*, pp.12.

Uchegbu, M.C., Okoli, I.C., Anyanwu, C.E., Etuk, E.B., Esonu, B.O. and Udedibie, A.B.I., 2004. Performance, carcass and organ characteristics of finisher broilers fed graded levels of raw Napoleona imperialis seed meal. *Livestock Research for Rural Development*, *16*(6), pp.24-30.

Wapi, C., Nkukwana, T.T., Hoffman, L.C., Dzama, K., Pieterse, E., Mabusela, T. and Muchenje, V., 2013. Physico-chemical shelf-life indicators of meat from broilers given Moringa oleifera leaf meal. *South African Journal of Animal Science*, *43(1)*, pp.43-47.

Woyengo, T.A., Beltranena, E. and Zijlstra, R.T., 2014. Non-ruminant nutrition symposium: Controlling feed cost by including alternative ingredients into pig diets: A review. *Journal of Animal Science*, *92*(4), pp.1293-1305.

Wu, Y.B., Ravindran, V., Thomas, D.G., Birtles, M.J. and Hendriks, W.H., 2004. Influence of phytase and xylanase, individually or in combination, on performance, apparent metabolisable energy, digestive tract measurements and gut morphology in broilers fed wheat-based diets containing adequate level of phosphorus. *British Poultry Science*, *45*(1), pp.76-84.

Zhang, X., Cao, F., Sun, Z., Yu, W., Zhao, L., Wang, G. and Wang, T., 2012. Effect of feeding Aspergillus niger-fermented Ginkgo biloba-leaves on growth, small intestinal structure and function of broiler chicks. *Livestock Science*, *147*(1-3), pp.170-180.

CHAPTER 4: Response in gut morphology to *Vachellia tortilis* leaf meal inclusion in broiler chickens

Abstract

The objective of the current study was to investigate the response in gut morphology to *Vachellia tortilis* inclusion in broiler chickens. Five-hundred-day-old broiler chicks were fed on conventional starter mash for 14 days. Unsexed Cobb 500 chicks with average body weight of 44g were randomly allocated to six dietary treatments. These treatments contained 0, 30, 60, 90, 120 and 150 g/kg DM inclusion levels of *Vachellia tortilis* leaf meal. Each diet was offered *ad libitum* to 10 birds per pen for 17 days before they were slaughtered. The histological measurements of mucosa of the jejunum including villus height, crypt depth, epithelial, submucosa and muscularis thickness were measured. The apparent villus surface area and villus to crypt ratio were calculated. There was a linear increase in VH, AVSA and villus height: depth ratio (*P<0.05*) with increasing inclusion level of *V. tortilis* leaf meal. The VH, AVSA and CD/VH ratio increased by 0.12, 1.08, and 1.96 g for each g/kg increase in *V. tortilis* inclusion. There was no relationship between *V. tortilis* leaf meal inclusion and CD (*P>0.05*). The thickness of submucosa, mascularis and epithelial, however, increased linearly (P<0.05) as levels of the leaf meal increased. Submucosa, mascularis and epithelial thickness increased by 8.73, 1.15, and 0.38 g for each g/kg increased in *Vachellia tortilis* inclusion. Increasing inclusion level of *V. tortilis* leaf meal was associated with increased villus height, apparent villus surface area, villus height and depth ratio, thickness of submucosa, muscularis and epithelial. Increases in morphology measurements suggest that leaf meal is effective in improving gut digestion and absorption of broilers.

Keywords: Jejunum, leaf meal, mucosal lining, small intestines, tannins, villi

4.1 Introduction

Poultry production plays a major role in the economy of developing countries (Ncube, 2017). Broilers are an important source of protein for urban consumers (Boer *et al.*, 2001). Most broiler industries are intensively managed; therefore, it is important to supply a quality feed to optimize performance. Feed accounts for over 75 % of variable cost in production systems (Agbede and Aletor, 2003). Grains such as maize and soybean poses competition between humans and the poultry industry. The shortage of supply of these grains increases the cost of poultry feeds. Therefore, consumption of the leguminous leaf meals could be a potential protein source to substitute the expensive grains. Leaf meals have a great potential because of their high crude protein content (Dube *et al.*, 2001; Halimani *et al.*, 2005; Ng'ambi *et al.*, 2009) and may improve the quality of the chicken meat.

The utilization of *Vachellia tortilis* leaf meal in diets of broilers is constrained by the presence of polyphenolic compounds, high fibre content and presence of thorns (Halimani *et al.*, 2005; Martens *et al.*, 2013). High concentrations of polyphenolic compounds damage the internal mucosal membranes and compromising nutrient absorption (Ncube *et al.*, 2017), and, consequently, growth performance.

The gastrointestinal tract plays a role in digestion, absorption and protection of these birds from infection. The intestinal mucosa is the first tissue to experience dietary ingredients and contaminants (Dibner *et al.*, 1996). The structural change of the mucosa has important implications for broiler performance. Such changes in mucosal architecture are associated with villi height (VH) and crypt depth (CD) and is influenced by the composition of diet and feeding level. Ncube *et al.* (2017), for example, conducted a study based on the effect of *Acacia angustissima* on the gut of broilers and found that leaf meal inclusion resulted in an increased intestinal wall thickness, epithelial thickness and villi height of the duodenum. Impairment to the gut mucosa raises the birds' maintenance requirement markedly, reducing nutrients required for growth (Dibner *et al.*, 1996). Gut morphology of broilers fed increasing levels of *V. tortils* leaf meal gives insight about the digestion and absorption of nutrients, internal and gut health state of broilers. The effect of *V. tortils* leaf meal inclusion on the gut morphology of broilers has not been established.

Given that high levels of leaf meal inclusion may suppress growth performance, it is important to conduct dose-trials for farmers to predict changes in gut morphology in broilers to increasing levels of *Vachellia tortilis* inclusion. The objective of the current study was, therefore, to determine the response in gut morphology of broilers fed on *Vachellia tortilis*. It was hypothesized that gut morphology increases with *V. tortilis* inclusion levels.

4.2 Materials and methods

4.2.1 Study site

The study site has been described in detail in section 3.2.1.

4.2.2 Leaf meal collection

Collection of *Vachellia tortilis* leaves has also been described in section 3.2.2.

4.2.3 Experimental diets and design

Experimental diets and design were described in section 3.2.3.

4.2.4 Chemical composition of feeds

Chemical composition of the feed is described in section 3.2.4.

4.2.5 Management of birds

Management of birds are given in section 3.2.5.

4.2.6 Intestinal histomorphometry

From small intestines of each bird, samples of jejunum segments were used to measure 3.0 cm at its midpoint pieces and fixed immediately in 10 % neutral formalin prior to preparation and analysis. The intestinal samples were sectioned and stained with haematoxylin-eosin stain. These sections were observed at 100 x magnification using a light microscope. Morphometric variables were measured, including villus height (VH), crypt depth (CD) and villus width (VW). The AVSA was calculated from the villus height and villus width and villus height to crypt dept ratio was also calculated. The epithelial thickness and mucosal layer thickness were also measured. Villi was selected from each section (2 villi per section) that demonstrated the longitudinal view of villus from base to tip. The villus height was measured from the tip to the bottom excluding crypt, while villus width was basal and apical parts. The crypt was also measured from the tip of the crypt to the point where it meets the muscularis mucosa (Velayudhan *et al.*, 2008). The average villus height from two birds (16 villi from eight different sections in each segment per bird) was expressed as a mean villus height for one group. The value of villus area was calculated from the villus height, basal and apical width according to the method described by Iji *et al.* (2001) the average villus from two birds (16 calculation of villus area from 8 different sections in a segment per bird).

4.2.4 Statistical analyses

Relationships between *Vachellia tortilis* leaf meal inclusion and intestinal histomorphometry (VH, AVSA, CD, villus to crypt ratio, villus apical width, intestinal wall thickness epithelial thickness and mucosal layer thickness) were determined with the response surface regression (RSREG) procedure of (SAS, 2009). The model used was: $Y = \beta_0 + \beta_1 D + \beta_2 D^2 + E$, where: Y is the response variables (VH, AVSA, CD, villus to crypt ratio and mucosal layer thickness)

β_0, β_1, β_2 regression coefficients;

D is the inclusion level of *Vachellia tortilis* leaf meal; and

E is the residual error.

4.3 Results

4.3.1 Intestinal histomorphotry

Table 4.1 shows the relationships among histological measurements of the mucosa of the intestines of broilers fed on diets of varying inclusion level of *Vachellia tortilis*. Histological measurements of the jejunum had a linear relationship (P<0.05) with *Vachellia tortilis* inclusion level. Villi height increased with inclusion levels of *V. tortilis* in the jejunum segment of the small intestines (P< 0.05). The same trend was also observed for apparent villus surface area (AVSA), which increase with inclusion level of *Vachellia tortilis* leaf meal (P< 0.05). Crypt had no relationship (P>0.05) with inclusion levels of *V. tortilis*. The thickness measurement of the mucosa of the jejunum had a linear relationship with *V. tortilis* inclusion (P<0.05). Epithelial thickness, submucosa and muscularis increased with inclusion levels of *V. tortilis* in the jejunum segment.

Table 4.1: Histological measurements (µm/ *slaughter weight* g) of mucosa of the jejunum in broiler fed diets with incremental *Vachellia tortilis* leaf meal

	Inclusion levels						SEM	Regression coefficient		Significance
Parameters	**0**	**30**	**60**	**90**	**120**	**150**		**Quadratic**	**Linear**	
Villi height	30.2	44.1	42.2	52.5	42.3	56.3	2.73	NS	6.79 ± 3.89	**
AVSA	113	125	157	160	248	266	17.55	NS	9.79 ± 15.72	NS
VH/ CD	6.5	7.29	6.04	9.06	9.21	17.29	3.14	NS	1.96 ± 2.34	NS
Crypt depth	4.84	7.82	7.79	7.41	5.85	6.01	1.62	NS	1.79 ± 1.22	NS
Epithelial thickness	1.24	1.76	1.99	2.11	2.47	2.63	0.27	NS	0.38 ± 0.19	NS
Submucosa	3.61	3.41	3.74	4.03	4.42	4.73	0.24	NS	0.005 ± 0.15	*
Muscularis	7.29	8.1	9.54	9.78	10.37	10.88	0.78	NS	1.15 ± 0.55	NS

AVSA: apparent villi surface area; VH/ CD: villus height to crypt depth ratio; NS: not significant ($P>0.05$); * $P<0.05$; ** $P<0.01$; SEM: standard error mean

Table 4.2: Relationship between inclusion level of *Vachellia tortilis* leaf meal and histological measurements

Parameter	R^2 value	Equation	P value
Villus height	0.64	y= 6.79x + 32.96	**
AVSA	0.90	y= 9.79x + 111.77	***
VH/CD	0.39	y= 1.96x + 7.26	*
Submucosa	0.81	y= 0.15x + 3.51	***
Muscularis	0.76	y= 1.15x + 7.25	**
Epithelial thickness	0.72	y= 0.38x + 1.29	***

AVSA: apparent villus surface area; VH/VCD: villus height to crypt depth ratio

* P<0.05; ** P<0.01; *** P<0.001; SEM: standard error mean

4.4. Discussion

The gastrointestinal mucosa is the microscopic structure and the first tissue that comes in contact with dietary nutrients. Optimal gut health refers to a scenario where the gut is well functioning to ensure effective digestion and absorption, non-existence of gastrointestinal tract illnesses, effective immune status and state of wellbeing of the host animal. Determining gut morphology is important, since the gut reflects the extent of digestion and absorption of nutrients. Several studies have reported on changes in intestinal morphology with inclusion of leaf meals in monogastric animals (e.g. Viveros *et al.*, 2011). Components of these diet and feeding level have a huge influence on the mucosal architecture of the gut. The architecture refers to villi height, apparent villi surface area (AVSA), crypt dept and the mucosa thickness (Jamroz *et al.*, 1992). A healthy intestinal mucosa in broiler industry is of great importance to attain target growth performance and feed efficiency. When determining optimum inclusion levels for broilers, the associated changes in intestinal morphology should be considered.

Villi height (VH) and apparent villus surface area (AVSA) was related to an increased inclusion levels of *V. tortilis* increase in the diet. The VH and AVSA increased by 6.79 and 1.08 g for each g/kg increased in *Vachellia tortilis* inclusion. The R^2 value for VH and AVSA were 0.64 and 0.90, respectively, indicating a strong linear relationship between VH, AVSA and incremental inclusion levels of *V. tortilis*. The linear increase of VH and AVSA suggest an intense digestion of leaf meal associated with increase fibre in the diet as *V. tortilis* inclusion level increase. The findings in the current study agrees with those of Nkukwana *et al.* (2015), who reported effect of Moringa leaf meal inclusion on villi height in the jejunum. Increases in VH and AVSA are an adaptation mechanism to improve the digestion and absorption of nutrients from the gut (Bakare and Chimonyo, 2017). Villi in the jejunum is a protective barrier to toxic substances such as polyphenolic compounds that could be ingested by birds through feeding. Gastrointestinal mucosa is the tissue that can adapt to the nutritional environment to improve digestion and absorption capacity of nutrients (Mitchell and Moreto, 2006). The increase in villi height could be stimulated by digestive and absorptive capacity related with bulk of leaf meals in the diet. The observed change in villi height showed that inclusion level of *V. tortilis* leaf meal in the diets had an influence on the gut.

Changes in the gastrointestinal mucosa with leaf meal inclusion could be a physiological adaptation of broilers to changes in diets. Such adaptations to changes in the digestion and absorption of nutrients are reflected by the increase in size of gastrointestinal components. Bedford (2006) concluded that such changes highlight the suboptimal digestion that birds are exposed to. These changes of gastrointestinal tract might contribute to increased VH and AVSA as leaf meal content is increased. At high leaf meal inclusion levels, the proportion and amount of dietary fibre is also high. High fibre in the diet might stimulate a natural increase in digestion and absorption of nutrients by increasing VH and AVSA. Increases in the VH and AVSA, therefore, suggests that nutrients are becoming more limiting. High inclusion of fibre in the diets reduces nutrient density. Hence, VH and AVSA increase absorptive functions to absorb as much nutrients as is possible before they are excreted through faeces. The intention of the bird is to meet its requirements for essential nutrients.

Nkukwana *et al.* (2014) reported the enhanced growth performance in birds that were fed MOLM is associated to the improved intestinal morphology and the villus surface area. Such findings were supported by Yu *et al.* (2015) who reported that gastrointestinal tract health in broilers to contribute to their overall health and performance. Leaf meals, therefore, have the impact on morphological changes in gastrointestinal tissues, which gives further information on the possible benefits to the digestive tract (Jamroz *et al.,* 2006). The jejunum is mainly responsible for digestion and absorption of all the major nutrients. Hence, a consequence of increased VH may be attributed to greater need for digestion capacity (Svihus, 2014). Khalaji *et al.* (2011) reported that the jejunal villus height increased as the inclusion levels of *Artemisia* leaf meal increased, further suggesting that the gastrointestinal tract undergoes morphological changes, which may result in increases intestinal length and villus height.

In the current study, increases of villus height to crypt depth ratio in the jejunum was observed with increasing inclusion levels of *Vachellia tortilis*. Thus, changes in the villus height to crypt depth ratio in the intestinal morphology may impact nutrient metabolism and broiler performance. This may be the reason for the largest jejunal surface area that was observed at high inclusion levels of the leaf meal. The regeneration of tissue turnover increases nutrient requirements for maintenance and therefore, decreases efficiency with which dietary nutrients are converted to body tissues and growth. The requirement of protein and energy for gut maintenance is higher compared to other organs (Xu *et al.*, 2003). A fast growing broiler generates about 12 % of the newly synthesized digestive tract. Wu *et al.* (2004) reported that the villus height to crypt depth ratio, villus height and crypt depth reflect intestinal health. However, longer villi lead to thicker epithelial thickness and consequently absorption ability will be improved. The current study showed a linear response of villus height to crypt depth ratio with increasing levels of *V. tortilis* leaf meal. The villus height to crypt depth ratio increased with a decreasing rate by 1.96 g for each g/kg increase in *V. tortilis* inclusion with R^2 value of 0.39. Abbasi *et al.* (2014) reported that an increase in villus height to crypt dept ratio in the intestinal morphology is associated with an improvement in digestion and absorption of nutrients.

There was a linear increase in submucosa measurement with increasing level of *V. tortilis* leaf meal from the equation y= 0.15x +3.51, the submucosa increased by 0.15 g for each g/kg increase in *V. tortilis* inclusion. The R^2 value 0.81, indicating that there was a stronger linear relationship of submucosa with increasing *V. tortilis* inclusion. The thickening of the muscle layer in the jejunum segment is explained by stronger movements of muscles during digestion (Xu *et al.*, 2003). The thickening of submucosa also suggests that digestion and absorption function is highly activated (Al-Tememy *et al.*, 2011). As a result, the physiological adaptations to match digestion and absorption may possibly increase the cost to the broilers in terms of energy demand. As a result, growth and carcass yield may be negatively affected. Nutrients are prioritized for maintenance of the birds' current biological state. After maintenance requirements are met, then any additional nutrients, be directed towards muscle deposition for meat production (Xu *et al.*, 2003). That could explain that observation that although an increase in inclusion of *Vachellia tortilis* leaf meal was associated with an increase in submucosa thickness to improve the digestion and absorptive capacities of the intestine, carcass yield was negatively affected. Greater demand for nutrients is required during the growing phase of birds. Decreasing weight of broilers as the level of *V. tortilis* leaf meal increase could be associated with increase in the maintenance requirements of the gastrointestinal tract.

A linear increase in muscularis with increasing inclusion level of *V. tortilis* was observed. The muscularis increased by 1.15 g for each g/kg increase in *V. tortilis*. The R^2 value of 0.76, suggesting a stronger relationship between muscularis and increasing level of *V. tortilis*. Changes were found in the thickness of the muscularis wall, which might be attributed to structural fibre such as neutral detergent fibre (NDF) and acid detergent fibre (ADF), that may have a mechanical effect on the intestinal wall on young bird fed diet with high inclusion of *V. tortilis*. The results agree with previous studies (Jamroz *et al.*, 2006), who reported an increase in thickness of mucosa as leaf meal content is increased. The mucosal state provides a barrier for pathogenic micro-organisms. The lymphoid present in gut tissue participate in the response of the intestinal wall on ingredients of the diet. Jorgense *et al.* (1996) reported that high intake of plant material is related to a substantial expansion of the gastrointestinal tract, and Jamroz *et al.* (2001) also reported that dietary fibre might be expected to have an effect on the peristaltic activity and thus the intestinal mucosa. Intestinal wall increase was associated with *Vachellia tortilis* increasing levels thus increase the digestion of bulk leaf meal in the diets

There was a linear increase in epithelium thickness with increasing level of *V. tortilis*. The thickness of the epithelium increased by 0.37g for each g/kg increase in *V. tortilis*. The increase in epithelial thickness with increasing inclusion level of *V. tortilis* leaf meal could be attributed to absorption of nutrients, secretion of electrolytes and fluids, replenishment of impaired cells or those lost to normal attribution (Yu *et al.*, 2001). This represent that epithelium have been triggered possibly due to presence of leaf meal in the diet. Epithelial thickening in the study may suggest an enhanced rate of absorption.

4.5 Conclusions

Vachellia tortilis inclusion level had the positive relationship on the intestinal morphology including villus height, apparent villus surface area (AVSA), villus height to crypt ratio, epithelial thickness and mucosa thickness. The linear increase of these morphological measurements suggests that of *V. tortilis* is effective in improving the gut health, digestion and absorption of broilers. This denotes that gut of broilers can tolerate *V. tortilis* leaf meal increase in the diet, whereas increasing leaf meal in the diets decreased carcass yield of broiler.

4.6 References

Abbasi, M.A., Mahdavi, A.H., Samie, A.H. and Jahanian, R., 2014. Effects of different levels of dietary crude protein and threonine on performance, humoral immune responses and intestinal morphology of broiler chicks. *Revista Brasileira de Ciência Avícola*, *16*(1), pp.35-44.

Agbede, J.O. and Aletor, V.A., 2003. Evaluation of fish meal replaced with leaf protein concentrate from Glyricidia in diets for broiler-chicks: Effect on performance, muscle growth, haematology and serum metabolites. *International of poultry science*, 21(4), pp.242-250.

Al-Tememy, H.S., Al-Jaff, F.K., Al-Mashhadani, E.H. and Hamodi, S.J., 2011. Histological effect of inclusion different levels of coriander oil in broiler diet on small intestine. *Diyala Agricultural Sciences Journal*, *3*(2), pp.1-11.

Amerah, A.M., Ravindran, V. and Lentle, R.G., 2009. Influence of insoluble fibre and whole wheat inclusion on the performance, digestive tract development and ileal microbiota profile of broiler chickens. *British poultry science*, *50*(3), pp.366-375.

Bedford, M.R., 2006. Effect of non-starch polysaccharidases on avian gastrointestinal function. *Avian gut function in health and disease. Oxon: Wallingford*, 7(3), pp.159-170.

Borin, K., Lindberg, J.E. and Ogle, R.B., 2006. Digestibility and digestive organ development in indigenous and improved chickens and ducks fed diets with increasing inclusion levels of cassava leaf meal. *Journal of Animal Physiology and Animal Nutrition*, *90*(5-6), pp.230-237.

Dibner, J.J., Kitchell, M.L., Atwell, C.A. and Ivey, F.J., 1996. The effect of dietary ingredients and age on the microscopic structure of the gastrointestinal tract in poultry. *Journal of Applied Poultry Research*, *5*(1), pp.70-77.

Dube, J.S., Reed, J.D. and Ndlovu, L.R., 2001. Proanthocyanidins and other phenolics in Acacia leaves of Southern Africa. *Animal Feed Science and Technology*, *91*(1), pp.59-67.

Fasuyi, A.O. and Nonyerem, A.D., 2007. Biochemical, nutritional and haematological implications of Telfairia occidentalis leaf meal as protein supplement in broiler starter diets. *African Journal of Biotechnology*, 6: 1055-1063.

Halimani, T.E., Ndlovu, L.R., Dzama, K., Chimonyo, M. and Miller, B.G., 2005. Metabolic response of pigs supplemented with incremental levels of leguminous Acacia karroo, Acacia nilotica and Colophospermum mopane leaf meals. *Animal Science*, *81*(1), pp.39-45.

Jamroz, D., Jakobsen, K., Orda, J., Skorupinska, J. and Wiliczkiewicz, A., 2001. Development of the gastrointestinal tract and digestibility of dietary fibre and amino acids in young chickens, ducks and geese fed diets with high amounts of barley. *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology*, *130*(4), pp.643-652.

Jamroz, D., Wertelecki, T., Houszka, M. and Kamel, C., 2006. Influence of diet type on the inclusion of plant origin active substances on morphological and histochemical characteristics of the stomach and jejunum walls in chicken. *Journal of Animal Physiology and Animal Nutrition*, *90*(5-6), pp.255-268.

JøRgensen, H., Zhao, X.Q., Knudsen, K.E.B. and Eggum, B.O., 1996. The influence of dietary fibre source and level on the development of the gastrointestinal tract, digestibility and energy metabolism in broiler chickens. *British Journal of Nutrition*, *75*(3), pp.379-395.

Khalaji, S., Zaghari, M., Hatami, K.H., Hedari-Dastjerdi, S., Lotfi, L. and Nazarian, H., 2011. Black cumin seeds, Artemisia leaves (Artemisia sieberi), and Camellia L. plant extract as phytogenic products in broiler diets and their effects on performance, blood constituents, immunity, and cecal microbial population. *Poultry Science*, *90*(11), pp.2500-2510.

Khanyile, M., Ndou, S.P. and Chimonyo, M., 2017. Influence of Acacia tortilis leaf meal-based diet on serum biochemistry, carcass characteristics and internal organs of finishing pigs. *Animal Production Science, 57*(4), pp.675-682.

Makkar, H.P.S., 2003. Effects and fate of tannins in ruminat animals, adaptation to tannins, and strategies to overcome detrimental effects of feeding tannin-rich feeds. *Small Ruminant Research*. 49: 241-256.

Mateos, G. G., R. Lázaro, and M. I. Gracia., 2002. The feasibility of using nutritional modifications to replace drugs in poultry feeds. *Journal of Applied Poultry Research* 11: 437-452.

Mlozi, M.R.S., Kakengi, A.V.M., Minga, U.M., Mtambo, A.M. and Olsen, J.E., 2003. Marketing of free-range local chickens in Morogoro and Kilosa urban markets, Tanzania. *Livestock Research for Rural Development*, *15*(2), p.2003.

Ncube, S., Halimani, T.E., Mwale, M. and Saidi, P.T., 2017. Effect of Acacia angustissima leaf meal on the physiology of broiler intestines. *Journal of Agricultural Science*, *9*(2), p.53.

Ndou, S.P., Khanyile, M. and Chimonyo, M., 2015. Growth performance and nutrition-related serum metabolites in growing pigs fed on *Acacia tortilis* leaf meal. *Livestock Science,* 182: 22-27.

Ng'ambi, J.W., Nakalebe, P.M., Norris, D., Malatje, M.S. and Mbajiorgu, C.A., 2009. Effects of dietary energy level and tanniferous Acacia karroo leaf meal level of supplementation at finisher stage on performance and carcass characteristics of Ross 308 broiler chickens in South Africa. *International Journal of Poultry Science*, *8*(1), pp.40-46.

Nkukwana, T.T., Muchenje, V., Masika, P.J. and Mushonga, B., 2015. Intestinal morphology, digestive organ size and digesta pH of broiler chickens fed diets supplemented with or without Moringa oleifera leaf meal. *South African Journal of Animal Science*, *45*(4), pp.362-370.

Nkukwana, T.T., Muchenje, V., Pieterse, E., Masika, P.J., Mabusela, T.P., Hoffman, L.C. and Dzama, K., 2014. Effect of Moringa oleifera leaf meal on growth performance, apparent digestibility, digestive organ size and carcass yield in broiler chickens. *Livestock Science*, *161(3)*, pp.139-146.

Olugbemi, T.S., Mutayoba, S.K. and Lekule, F.P., 2010. Effect of Moringa (Moringa oleifera) inclusion in cassava-based diets fed to broiler chickens. *International Journal of Poultry Science*, *9*(4), pp.363-367.

Reed, J.D., McDowell, R.T., Van Soest, P.J., Horvath, P.R., 1982. Condensed tannins: a factor limiting the use of cassava forage. J. Sci. Food Agric. 33, 213–220.

Svihus, B., 2014. Starch digestion capacity of poultry. *Poultry science*, *93*(9), pp.2394-2399.

Thiex, N., 2008. Evaluation of analytical methods for the determination of moisture, crude protein, crude fat, and crude fiber in distillers dried grains with solubles. *Journal of AOAC International*, *92*(1), pp.61-73.

Velayudhan, B.T., Daniels, K.M., Horrell, D.P., Hill, S.R., McGilliard, M.L., Corl, B.A., Jiang, H. and Akers, R.M., 2008. Developmental histology, segmental expression, and nutritional regulation of somatotropic axis genes in small intestine of preweaned dairy heifers. *Journal of dairy science*, *91*(9), pp.3343-3352.

Wu, Y.B., Ravindran, V., Thomas, D.G., Birtles, M.J. and Hendriks, W.H., 2004. Influence of phytase and xylanase, individually or in combination, on performance, apparent metabolisable energy, digestive tract measurements and gut morphology in broilers fed wheat-based diets containing adequate level of phosphorus. *British Poultry Science*, *45*(1), pp.76-84.

Xu, Z.R., Hu, C.H., Xia, M.S., Zhan, X.A. and Wang, M.Q., 2003. Effects of dietary fructooligosaccharide on digestive enzyme activities, intestinal microflora and morphology of male broilers. *Poultry Science, 82*(6), pp.1030-1036.

Yu, W., Zhang, X., Hussain, A., Zhao, L., Wang, T. and Cao, F., 2015. Intestinal absorption function of broiler chicks supplemented with Ginkgo leaves fermented with Bacillus species. *Pakistan Journal of Zoology*, *47*(2), pp.76

CHAPTER 5: General Discussion, Conclusions and Recommendations

5.1 General discussion

The main hypothesis tested was that the inclusion level of *V. tortilis* leaf meal in the diet decreases SW, DCW and that the incremental level of *V. tortilis* leaf meal has no effect on DP, but increases the relative weight gizzard, heart, kidney, proventriculus, intestines, ceacum and the length of intestinal segment. The histomorphology of the jejunum was determined to test hypothesis that there was an increase of VH, AVSA, VH:CD, epithelial and submucosa and mascularis with the increasing level of *Vachellia tortilis* leaf meal, but the incremental level of leaf meal has no effect on CD. The relationship between inclusion level of *V. tortilis* leaf meal carcass yield, organ weight and gut morphology measurement of broilers improve our knowledge on utilization of leaf meal diets and stimulate the development of technologies to reduce the negative impact on the leaf meal in birds.

In chapter 3, the dose response trial using regression was conducted to test hypothesis that increasing levels of *Vachellia tortilis* leaf meal in SW, DCW and DP. The utilization of *V. tortilis* leaves by broilers is constrained by the presence of polyphenolic compounds that results in low performance of broilers with incremental level of leaf meal. *Vachellia tortilis* leaf meal inclusion levels are expected to produce a negative relationship with carcass yield of broilers because increment of leaf meal also increases the level of polyphenolic compounds. The incremental levels of *V tortilis* leaf meal decreased SW and DCW by 0.77 and 0.94, respectively. There was a decrease of feed intake with incremental level of leaf meal, this could be due to astringency effect of fibre present in leaf meal. In poultry, the bulk of the fibre is not digestible, and thus contributes to gut fill, thereby reducing feed intake. Reduced DCW was observed when *V. tortilis* leaf meal levels increase in the diet.

The increasing inclusion levels of *V. tortilis* leaf meal produced a linear relationship in the organ weights measurement. The increase in scaled weights for the internal organs with increasing levels for the leaf meal suggest that broilers had developed organs, therby adapted to leaf meal inclusion in the diets. The increase in gizzard observed with increasing levels for the leaf meal can be explained by compaction and bulking effect of the fibre that resulted to the increase in sizes as the mechanism for adaptation. The heart weight increase was attributed to increase blood circulation and oxygen related with lean carcass that was evidenced by a decrease in carcass yield traits with increasing levels of leaf meal.

The histomorphology of small intestines were determined in chapter 4 to test the hypothesis that incremental levels of *V. tortilis* leaf meal has positive effect on the VH, AVSA, CD ratio, but has no effect on CD. The use of different inclusion levels of *V. tortilis* leaf meal was expected to provide a positive relationship in gut morphology of broilers. In relation to histological measurement of mucosa of the jejunum of broilers fed diets on *Vachellia tortilis* inclusion, VH, AVSA, and epithelial thickness of broiler fed on *V. tortilis* was expected to differ with the inclusion levels of leaf meal. The VH and AVSA establish the digestion and absorptive function for nutritional requirements to be met. An increase in VH and AVSA is considered as an adaptive mechanism to improve the absorption of nutrients from the gut. The CD relative to SW of broiler was not affected by the *V. tortilis* leaf meal in the diets.

Increasing levels of *V. tortilis* leaf meal produced a positive linear relationship in the intestinal epithelial and mucosal layer thickness of broilers. The epithelial, submucosa and muscularis thickness measurement in the jejunum of broilers on *V tortilis* diets were expected to differ with inclusion level of the leaf meal. The observed relationship between inclusion of *V. tortilis* leaf meal and mucosal thickness measurement, suggest that increasing level of *Vachellia* leaves in broilers 'diets increased digestive and absorptive capacity of the intestines. The increase in intestinal volume was therefore a physiological adaptation to establish the function for increased feed volume related with leaf meal-based formulations.

5.2 Conclusions

Vachellia tortilis could reduce the proportion of expensive protein source ingredients in broiler diets. *V. tortilis* leaf meal have great potential because of their high crude protein content. The concentration of proanthocynidins and fibrous properties could constrain the optimal performance and exploration of *Vachellia* for feeding broilers. Incorporation of *V. tortilis* leaf meal in broilers' diets increases histological measurements and organ weights with inclusion level, whereas, inevitably suppress carcass yield parameters. The extent with which broiler utilize leaf meal-based diet will improve with continuous investigation of *V. tortilis* in diet. The leaf meal may improve the quality of the chicken meat.

5.3 Recommendations and further research

The utilization of *V. tortilis* leaf meal in broiler production obligates farmers to be advocate on the harvesting and processing of leaves before use in feed formulation. It can be recommended that farmers should use *V. tortilis* leaf meal to minimize the competition between human and livestock for protein sources. In addition, leaf meal can decrease the proportion of other expensive grains in the diets. Future research is required to be conducted to:

1. Determine fat deposition and lipogenic enzyme activities of broilers fed on increasing level of *Vachellia tortilis*;
2. Determine nutrient digestibility of broilers fed on inclusion level of *V. tortilis* leaf meal;
3. Response in growth performance and carcass yield in broilers fed on *V. tortilis* treated with polyethylene glycol; and

4. Investigate the haematological parameters of broilers fed increasing levels of *V. tortilis* leaf meal.

Appendix 1: Ethical Approval of Research Project on Animals

UNIVERSITY OF KWAZULU-NATAL
INYUVESI YAKWAZULU-NATALI

26 May 2016

Professor Michael Chimonyo (28007)
School of Agricultural, Earth & Environmental Sciences
Pietermaritzburg Campus

Dear Professor Chimonyo,

Protocol reference number: AREC/014/017
Project title: Response in growth performance, nematode counts and meat quality of broilers fed on incremental levels of *Vachellia tortilis*

Full Approval – Research Application

With regards to your revised application received on 21 May 2017. The documents submitted have been accepted by the Animal Research Ethics Committee and **FULL APPROVAL** for the protocol has been granted.

Any alteration/s to the approved research protocol, i.e Title of Project, Location of the Study, Research Approach and Methods must be reviewed and approved through the amendment/modification prior to its implementation. In case you have further queries, please quote the above reference number.

Please note: Research data should be securely stored in the discipline/department for a period of 5 years.

The ethical clearance certificate is only valid for a period of one year from the date of issue. Renewal for the study must be applied for before 26 May 2018.

Attached to the Approval letter is a template of the Progress Report that is required at the end of the study, or when applying for Renewal (whichever comes first). An Adverse Event Reporting form has also been attached in the event of any unanticipated event involving the animals' health / wellbeing.

I take this opportunity of wishing you everything of the best with your study.

Yours faithfully

....

Prof S Islam, PhD
Chair: Animal Research Ethics Committee

/ms

Cc Academic Leader Research: Professor O Mutanga
Cc Registrar: Mr Simon Mokoena
Cc NSPCA: Ms Stephanie Keulder
Cc Ukulinga Research Farm

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