EFFECTS OF *Moringa oleifera* LEAF MEAL DIETS ON GROWTH PERFORMANCE, HAEMATOLOGY AND HISTOPATHOLOGY OF KEY BODY ORGANS IN GROWING PIGS

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DECLARATION

This thesis is my original work and has not been presented for a degree in any other University.

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DEDICATION

This work is dedicated to my wife Lucy Sachngor Serem and son Brayden Kiptoo Serem for the support they offered me throughout my research period. It is dedicated to my father Mr. Hosea Rono and Mother Mrs. Ednah Rono for their guidance and financial support throughout my education. Lastly, I would like to dedicate it to My Uncle Mr. Alex Koech for his mentorship and guidance especially during my early years of education.

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LIST OF ABBREVIATIONS

ADF	Voluntary daily intakes
ADG	Average daily weight gains
CF	Crude fibre
CP	Crude protein
DE	Digestible energy
DM	Dry matter
EE	Ether extracts
FCE	Feed conversion efficiency
HDL	High density lipoproteins
HGB	Haemoglobin concentration
LDL	Low density lipoproteins
ME	Metabolizable energy
MID	Mid-range absolute counts
MO	*Moringa oleifera*
MOLM	*Moringa oleifera* leaf meal
MRS	De Man, Rogosa and Sharpe agar
NDF	Neutral Detergent Fibre
PCV	Packed cell volume
PLT	Platelets
RBC	Red blood cells
Se	Selenium
T	Treatment diets

VRBA	Violet red bile agar
WBC	White blood cells

ABSTRACT

This study was designed to determine the effects of inclusion, at varying levels of *Moringa oleifera* leaf meal (MOLM) in growing pig diets on voluntary daily feed intakes, growth performance, carcass quality, haematology, plasma lipid indices, gastrointestinal morphology, bacteriology and histopathology of the spleen, liver and kidneys. Twenty four (24) two and a half months (2.5) old pigs were selected and assigned to 4 treatment diets (T) containing 0% (T1), 3% (T2), 6% (T3) and 12% (T4) MOLM concentrations, each with 2 replications of 3 pigs. The voluntary daily feed intakes and weekly pig weights were recorded for 7 weeks after which 2 sets of blood samples were drawn from 2 pigs per replication for haematology and plasma lipid determination. These pigs were later sacrificed and one segment of tissue from the stomach, distal ileum, and proximal colon collected for histopathological analysis and their contents for pH measurements. Similarly, the spleen, liver and kidney samples were collected for histopathological studies. In addition, faecal samples from the rectum were collected for bacteriology. Data on the voluntary daily feed intakes, growth performance, blood characteristics, pH measurements and bacterial colony counts were analysed for descriptive statistics and ANOVA. Results revealed that the voluntary daily feed intakes for the 12% MOLM pigs were significantly higher than for the control, 3% and 6% MOLM groups. The 3% and 6% MOLM had significantly higher FCE compared to 12% MOLM and control groups. Inclusion of MOLM in the diet significantly increased haemoglobin concentration only to a level of 6% though there was a reduction in 12% MOLM. Also observed was higher Mean Cell Volume for the control compared to 6% MOLM pigs. *Moringa oleifera* leaf meal at 3% in the diet also increased the white blood cell counts compared to the control group. Total

cholesterol in 3% MOLM group was significantly lower compared to those from control. *Moringa oleifera* leaf meal in the diet (>3%) led to the enlargement of splenic follicles (white pulp) as well as capsular and parenchyma fibroses. In the liver, increased MOLM in the diet led to loss of lobular architecture with damaged cellular outlines, dilation of sinusoidal spaces, vascular congestion and occasional nuclear changes in hepatocytes leading to hepatocytic necrosis and distortion of the portal triad. In the kidneys, higher levels of MOLM led to glomerulonephritis essentially presenting as glomerular oedema leading to reduced Bowman's space. In the renal tubules, there were protein casts in the tubular lumen. There was a significant difference in gastric pH of the digesta across the treatment groups where controls had lower pH than all the MOLM diets. Bacteriology results, on the other hand, revealed a significant difference in the coliform counts with the controls having the least counts. In the stomach and ileum, higher levels (6 to 12%) of MOLM in the diet led to the submucosal oedema and fat deposition hence increased thickness. In the ileum, high levels of MOLM in the diet led to the hyperplasia of Peyer's patches. These results imply that, MOLM at lower levels (3% in diet) improves haemoglobin concentration important for oxygen circulation, white blood cell counts, indicating improved innate immunity and hypocholesterolemic properties beneficial in the control of cardiovascular diseases, leading to improved overall productivity of the animals. However, higher levels of MOLM in the diet (>3%) lead to toxicity; distortion of gut morphology, spleen, liver and kidney histo-architecture, that, if fed to the pigs for a prolonged duration may result in organ failure, poor performance and death.

Key words: Peyer's patches, Hepatotoxicity, Renal toxicity, Spleen, *Moringa oleifera*, Haematology, Growing pigs

CHAPTER ONE

1. GENERAL INTRODUCTION

1 Background information

Pig production is gaining importance in societies that are currently undergoing a shift from ruminant to non-ruminant livestock production in Kenya (FAO, 2012).This is because pigs require less space and can multiply and grow fast to reach market weights within 4 months hence making optimal use of limited landholdings resulting from the rapidly increasing human population. However, increasing feed costs, especially the protein sources, have limited the expansion and profitability of pig enterprises. Currently, the main protein ingredients in pig's diet are from fishmeal, soybean, cotton seed cake and sunflower (FAO, 2002). Since humans also rely on some of these protein ingredients for food and other industrial uses, there has been an augmented shortage thus escalating their costs hence necessitating the need to search for alternative protein sources that are more affordable and with additional benefits when fed to the animals (Steinfeld *et al.,* 2006).

In most instances, majority of the feed ingredients are adopted by farmers without being cognizant of their influences on the animal's body systems and no considerations are made to ascertain whether they could have toxic effects to the animals (Etim *et al.,* 2014). It is imperative therefore that, non-conventional feed ingredient are evaluated for toxicity both to humans and animals prior to their adoption into the animal feed industry. In majority of studies, the voluntary feed intake and growth performance have been used to evaluate the quality of feed and if positive results are recorded, then the feed ingredient is presumed safe. However,

these quality indicators do not give a clear indication of the events occurring in the internal milieu of the animal, hence further testing of internal parameters is necessary and one such key parameter to check is haematology. If the blood parameters from animals on these diets are within the normal range, then it is concluded that the diet does not interfere with hematopoietic activity (Etim *et al*., 2014). If on the other hand, the hematologic indices are improved, then there are high chances that the overall performance of the animals were optimized (Isaac *et al*., 2013). Also, the toxicological effects of these ingredients may also be determined through evaluation of the histological architecture of the key detoxifying organs such as the liver, kidney and possibly, the spleen.

Moringa oleifera (MO) is among many plants that have been adopted in the recent past as non-conventional protein source in animal diets (Nouman *et al*., 2014). *Moringa oleifera* belongs to the family Moringaceae which originated from Asia and Middle East and was introduced to eastern Africa from India, at the beginning of the 20^{th} century. *Moringa oleifera* plant can survive well under arid conditions and can grow fast to reach between 6 to 7 m height within a year on less than 400 mm annual rainfall (Foidl *et al*., 2001). Nutritionally, it contains high crude protein of up to 30% and could minimize the use of other protein sources such as soybean and fishmeal in animal feeds (Nuhu, 2010). In addition, the plant has antimicrobial properties and has been used both in humans and animals in disease management (Richter *et al*., 2003; Mathur, 2006).

Despite increased use of the plant in animal nutrition and research, there is limited information available on the performance of pigs under *Moringa oleifera* leaf meal (MOLM) diet. Moreover, little is known about its effects on hematologic parameters

as well as plasma lipids and furthermore, the effects of its prolonged intake on the gastrointestinal tract, liver, kidneys and spleen structure have not been conclusively determined (Pfaff *et al*., 2015). In this study therefore, the effects of MOLM inclusion in growing pig diets on growth performance and carcass quality, blood characteristics and plasma lipid profiles, gut pH, faecal bacteria loads and histopathology of the spleen, liver and kidneys were evaluated.

1.2 Problem statement

Increasing feed costs, especially protein sources, have limited the expansion and profitability of the pig enterprises (FAO, 2012). Alternative protein sources that ensure wider choice as well as reduction in costs are therefore of great importance. Despite increased adoption and research on *Moringa oleifera*, limited information is available on the effects of its leaf meal inclusion in growing pig diets on feed intake, growth performance, carcass quality, hematological parameters, plasma lipid profiles, gut morphology, microbiology as well as the histopathological changes in the spleen, liver and kidneys of growing pigs.

1.3 Research questions

The questions addressed in this study are:

1. What are the effects of MOLM diet on voluntary daily feed intakes, growth performance and carcass quality in growing pigs?
2. What are the effects of MOLM diet on haematological parameters and plasma lipid profiles in growing pigs?

3. What are the effects of MOLM diet on gut pH, faecal lactobacilli, coliform counts and histopathology of the stomach, ileum and colon of growing pigs?
4. What are the effects of inclusion of MOLM in growing pig diets on histopathology of the spleen, liver and kidneys?

1.4 Objectives

1.4.1 Broad objective

The overall objective of this study was to determine the effects of inclusion, at varying levels, of MOLM in growing pig diets on growth performance, blood characteristics, carcass quality, faecal bacterial count and histopathology of the gut, spleen, liver and the kidneys.

1.4.2 Specific objectives

1. To determine the effects of MOLM diet on voluntary daily feed intakes, growth performance and carcass quality in growing pigs.
2. To determine the effects of MOLM diet on haematological parameters and plasma lipid profiles in growing pigs.
3. To determine the effects of MOLM diet on gut pH, faecal lactobacilli, coliform counts and histopathology of the stomach, ileum and colon of growing pigs.
4. To determine the effects of inclusion of MOLM in growing pig diets on histopathology of the spleen, liver and kidneys.

1.5 Justification

Increasing human population in Kenya has led to decline in landholdings resulting in an increased shift from ruminant to non-ruminant livestock production (FAO, 2012). Poultry farming has been the main source of non-ruminant protein food. However, there has been increased reliance on pig production for meat in the recent past. Despite increased dependence on the pigs as a source of human protein, increasing feed costs especially the protein sources have limited their expansion and profitability (FAO, 2012). *Moringa oleifera* is one of the dietary protein alternatives adopted in some parts of the world and increased awareness on its benefits has led to increased usage and research; although it is still greatly underutilized due to the limited available information on its leaf nutritional composition, potential benefits and consequences of its inclusion in pig feeds (Sánchez *et al*., 2006; Nouman *et al*., 2014). This study therefore aims to provide information on pig performance under MOLM diets and assess its physiological effects on the animal's systems in order to recommend its usage as one of the protein ingredients in the formulation of pig feeds in Kenya.

CHAPTER TWO

2. LITERATURE REVIEW

2.1 *Moringa oleifera:* Background information

2.1.1 Botanical and agronomic aspects

Moringa oleifera (MO) is a plant in the family Moringaceae that originated from India, Pakistan, Bangladesh and Afghanistan and has spread to both the tropics and subtropical regions of the world (Fahey, 2005). It was initially utilized by the ancient Greeks and Romans and now has spread to other regions of the world Duke (2001). It was introduced in Kenya by Indians during the construction of Kenya Uganda Railway and where it is currently grown in Kenyan Rangelands such as in the lower Eastern, Baringo and the Coast region (Maundu and Tegnas, 2005). Moringa is a perennial shrub that grows well in a wide variety of soils mostly in the sandy loams (Figure 2.1) and slightly alkaline clay soils due to their good drainage (Abdul, 2007). It thrives best at altitudes of 0 to1800 m above sea level and annual rainfall of 500 to1500 mm (Sanchez *et al*., 2006; Nouman *et al*., 2014). Therefore, it is suitable for hot, humid, dry tropical and subtropical areas performing better under marginal conditions with ample nutritional quality (Nouman *et al*., 2014).

Moringa oleifera has high biomass yields of between 4.2 and 8.3 metric tons/ha and, together with leaf nutritional composition, are greatly influenced by seasons, planting densities, soil factors such as fertilizer use as well as irrigation and harvesting frequencies (Sanchez *et al*., 2006; Radovich, 2011). Just like fodder shrubs, MO establishment requires time to establish strong roots before the first defoliation is done and this period is approximately one year when MO is at least 1-1.5 m high (Sanchez

et al., 2006). Makker and Becker (1997) documented that appropriate spacing for optimal performance of MO range from 1 m by 1 m (10,000 plants per ha) to 2.50 cm by 2.50 cm (16,000,000 plants per ha). The *Moringa oleifera* total yield of fresh matter, total yield of DM, growth rate and height during the first and second year increase significantly also as the cutting interval is increased from 45 to 75 days (Sánchez *et al*., 2006). Furthermore, the same authors established that during the first year of growing Moringa, DM, NDF and ash contents were highest and *in vitro* digestibility were lowest in the longest harvesting frequency, while crude protein (CP) and acid detergent fibre (ADF) contents were not affected by harvesting frequency and recommended that, for intensive biomass production MO should be planted densely, 50 to 75 plants per square meter, and harvested every 75 days.

2.1.2 *Moringa oleifera* leaf nutritional composition and uses

There are a number of studies that have been conducted on the nutritional compositions of MO plant in different geographical locations. *Moringa oleifera* leaves contain between 19.3% - 30% CP in DM, 24.65% DM, 2.23% EE, 19.25% CF, 7.13% ash, 41.98% NFE, 0.33% P and 8.64% Ca (Aregheore, 2002; Nuhu, 2010; Gakuya *et al*., 2014). Due to the rich nutritional composition, the plant found use as human and animal feeds and for medicinal purposes (Richter *et al*., 2003; Sánchez *et al*., 2006).

Other nutritional studies on MO leaves have recorded results as shown in Table 2.1.

Figure 2.1 *Moringa oleifera* plant (block arrow) at the University of Nairobi, Kibwezi field station.

Table 2.1 Nutritional composition (Dry matter basis) of *Moringa oleifera* leaves from different parts of the world

Author	Country	Crude protein (%)	Crude fibre (%)	Lipids (%)	Ash (%)	Nitrogen free extracts (%)	Calcium (mg/100g)
Liaqat *et al*., 2016	Pakistan	29.0	9.3	1.7	--	--	--
Valdez-Solana *et al*., 2015	Mexico	11.1	8.1	10.2	10.7	54.6	2516.5
Asante *et al*., 2014	Ghana	25.7	--	--	--	--	1880.3
Asaolu *et al*., 2012	Nigeria	26.7	11.0	8.1	--	39.54	--
Gupta *et al*., 1989	--	26.4	--	6.5	12	--	--
Al-Kahtani and Abou-Arabi, 2015	Bangladesh	29.0	19.1	5.2	--	--	2100.0

Moringa oleifera leaves also contain relatively high levels of soluble carbohydrates (10%) and β-carotenes (2.33 x 10^2 μg/L) (Mustapha and Babura, 2009). Its leaves are also rich in tocopherols (γ and α), phenolic compounds, vitamin C, essential sulphur amino acids (methionine and cysteine), unsaturated fatty acids, especially oleic acid and minerals (Makkar and Becker, 1997; Ferreira *et al*., 2008; Mustapha and Babura, 2009). Furthermore, these leaves also have a high total antioxidant capacity (260 mg/ 100 g), total polyphenols (260 mg/100 g), quercetin (100 mg/100 g), kaempferol (34 mg/100 g) and β-carotene (34 mg/100 g) (Lako *et al*., 2007). *Moringa oleifera* seeds contain higher amounts of relatively stable oleic acids followed by palmitic acid and behenic acids (Sánchez-Machado *et al*., 2015). Therefore, apart from being an important nutritional agent, *M. oleifera* possess a wide range of additional biological activities including antioxidant, tissue protective (liver, kidneys, heart, testes, and lungs), analgesic, antiulcer, antihypertensive, radioprotective, and immunomodulatory actions (Pal *et al*., 1995; Stohs and Hartman, 2015; Okumu *et al*., 2017).

2.1.3 Antimicrobial activity of *Moringa oleifera* leaf meal

Presence of alkaloids, flavonoids (mainly quercetin and kaempferol), saponins and tannins in all extracts have been linked to various physiological actions in the humans and animals body (Abdulkadir *et al*., 2015). Flavonoids have a hydroxyl group that confers antioxidant activity on MO hence its use as a therapeutic agent (Jang *et al*., 1995). As a result, MO has been used over the years as a traditional remedy for some disease conditions. This is because of its rich phytochemicals that contain effective antibacterial, antimycotic, antiviral and potential anticancer activity. For instance, antiviral activity of MO leaf extracts against Foot and Mouth Disease has been

documented (Younus *et al*., 2015). The MO leaf extracts' antimicrobial properties have also been recorded on both the gram positive and gram negative bacteria such as *Staphylococcus aureus, Escherichia coli* and the *samonella* species (Van Weyenberg and Jacobs, 2013; Abdulkadir *et al*., 2015). Antifungal properties of MO leaves have also been observed (Donli and Dauda, 2003). The modes of extraction of the active ingredients have been found to play a role in the degree of effectiveness of MO extracts against various pathogens. For instance, the antibacterial activity of the methanolic extract of MO had broader antimicrobial action compared to the aqueous extracts (Donli and Dauda, 2003). *Moringa oleifera* also has been known to exhibit both anti-inflammatory and anticancer properties (Cárceres *et al*., 1993; Bharali *et al*., 2003). Dried fruit powder of *Moringa oleifera* also has ameliorative potential against industrial fluorosis in cattle because altered haemato-biochemical parameters were restored after the supplementation of *Moringa oleifera* fruit powder in fluorotic cows (Jena *et al*., 2016).

2.1.4 Anti-nutritive factors in *Moringa oleifera* leaves

Moringa leaves contain relatively low amounts of polyphenols such as tannins (1.4%) and total phenols (2.7 %) that are less harmful if consumed at lower amounts by animals (Makkar and Becker, 1997). These factors, however, when consumed in high amounts may negatively affect the ability of an animal to utilize dietary nutrients and consequently their health. *Moringa oleifera* leaf meal also contain 5.6 % saccharides raffinose and stachyose which produce flatulence in monogastrics, if consumed at higher levels (Gupta *et al*., 1989). Also present are nitrates (0.5 Mmol/100 g), oxalates (4.1 %), saponins (1.2%) and phytates (3.1%). Phytate concentrations in the leaves have been shown to decrease the bioavailability of minerals in monogastrics (Reddy

et al., 1982). Saponins from some plants have an adverse effect on the growth of animals but those present in moringa leaves are safe and don't show haemolytic activity, and thus, humans can consume them without apparent adverse effects (Makkar and Becker, 1997).

2.1.5 Other uses of *Moringa oleifera*

Moringa oleifera seeds contain naturally occurring proteins that are more effective coagulants than alum (Ndabigengesere *et al*., 1995). Furthermore, MO seed extracts significantly improved water quality particularly with regard to number of cryptosporidium oocysts present and water turbidity (Petersen *et al*., 2016). This implies therefore that, the relative lack of toxic compounds in the seed and its ability to clarify and purify muddy water, makes it a suitable alternative to obtaining clean drinking water where it is not available (Ndabigengesere *et al*., 1995; Anwar *et al*., 2007; Ferreira *et al*., 2008). The oxidative stability of the MO seed oil is high making it fit for the manufacture of cosmetics (Sánchez-Machado *et al*., 2015). The pods, on the other hand, can be used as litter in poultry and other housed livestock, hence, ensuring a sustainable utilization of the natural resources and environmental conservation.

2.2 *Moringa oleifera* as a non-ruminant feed

Moringa oleifera has a great potential as a livestock feed (Richter *et al*., 2003). The relatively low amounts of anti-nutritive factors, high protein, lipid and sulphur containing amino acid contents make it an excellent source of protein for the non-ruminants (Richter *et al*., 2003; Ferreira *et al*., 2008). Its leaves also contain all essential amino acids, including Sulphur-containing amino acids and, in fact, they

occur in amounts higher than the recommended body requirements thus making it a good source of protein for monogastrics. In Ghana, improved weight gains were reported in rabbits fed on MOLM and consequently, it was recommended for use either as partial or total replacement for soybean meal without any adverse effects on the productive performance and blood indices of weaned rabbits (Nuhu, 2010). In broiler chicken, it was established that MOLM can replace soybean to a level of 25% of the total diet without affecting negatively the growth performance as well as both the feed intakes and feed conversion efficiency (Gadzirayi *et al*., 2012). In pigs, MOLM in the diet up to a level of 10% was used to replace the commercial pig pre-starter feed without affecting the pig's daily feed intake, feed conversion efficiency and growth rates (Acda *et al*., 2010). In finishing pigs, inclusion of 2.5% and 5% of MOLM in finisher pig feed had no detrimental effects on feed conversion efficiency (Mukumbo *et al*., 2014).

Despite the increasing number of research on MO as a potential feed, there is no consensus on the optimal levels of its inclusion on the animal diets, hence, presenting the need for further studies (Nouman *et al*., 2014). Furthermore, the effects of MOLM in the diet on the feed intake, growth performance and carcass quality of pigs under its diet have not been conclusively documented.

2.3 Pig nutritional requirements

Growth in any animal is dictated by the genetic composition but nutrition can enhance its development (Udofia *et al*., 2007). Pigs are non-ruminants and require a number of essential nutrients to meet their needs for maintenance, growth, reproduction, lactation, and other functions. The National Research Council (NRC) provides

estimates of the amounts of these nutrients for various classes of swine under standard conditions (Table 2.2).

Table 2.2 Dietary nutrient requirements of growing pigs allowed *ad lib* feed (90% dry matter)

	Body Weight (kg)					
	3–5	**5-10**	**10–20**	**20–50**	**50–80**	**80–120**
Digestible energy (kcal/kg)	3,400	3,400	3,400	3,400	3,400	3,400
Metabolizable energy (kcal/kg)	3,265	3,265	3,265	3,265	3,265	3,265
Estimated Digestible energy intake (kcal/day)	855	1,690	3,400	6,305	8,760	10,450
Estimated Metabolizable energy intake (kcal/day)	820	1,620	3,265	6,050	8,410	10,030
Estimated feed intake (g/day)	250	500	1,000	1,855	2,575	3,075
Crude protein (%)	26	23.7	20.9	18	15.5	13.2

Adapted from NRC (2012).

2.4 Pig nutrition and performance

Pigs are non-ruminants, hence, weaning time is a crucial period in the management of piglets and the risk of developing post-weaning diarrhoea (PWD) in piglets is high (Vondruskova, *et al*., 2010). Also, weaned piglets still have their immune and digestive systems in development, insufficient production of specific enzymes for digestion of plant ingredients and high demand for nutrients (Hedemann *et al*., 2006). Weaning stress causes physiological and metabolic responses which may also alter blood parameters, thus weakening their immune system. Furthermore, some nutrients and substances present in some ingredients may affect these metabolic responses or stimulate inflammatory response due to the presence of anti-nutritional or allergenic factors (Pascoal *et al*., 2012). Still, other elements may have probiotic function and improve the health status of piglets. The maintenance of intestinal health is therefore an important factor to minimize or prevent poor performance, morbidity and mortality of piglets (Pascoal *et al*., 2012).The dietary ingredients should therefore be carefully selected to prevent disturbances in the digestive tract. MO has been used as protein sources in non-ruminants and though positive responses when included in the diet have been reported, undesirable performance have also been reported with the high MO concentrations in the diet (Table 2.3).

Table 2.3 Studies on the effects of *Moringa oleifera* diets in non-ruminants from different parts of the world

Author	Study site	Animals	MOLM Levels of inclusion (%) in the diet	Results
Ruckli and Bee, 2016	Switzerland	Finishing pigs	15.5%	Depressed feed intakes and lower weight gains at higher MOLM.
Raphael *et al*., 2016	Cameroon	Chicken	5%, 10%	Improved egg laying percentages.
Kaijage *et al*., 2015	Tanzania	Chicken	11.1%	Improved egg laying percentages.
Gakuya *et al*., 2014	Kenya	Broilers	7.5%, 30%	Depressed feed intakes, feed digestibility and growth rates at >7.5% MOLM.
Gadzirayi *et al*., 2012	Zambia	Broilers	25%	No difference in growth and FCR of MOLM diets with those of controls.

Key: *Moringa oleifera oleifera* leaf meal

2.5 Effects of *Moringa oleifera* leaf meal diets on haematology and plasma lipid levels in animals

Haematological parameters can give a good indication of dietary influences on hematologic and immune systems of farm animals and those with improved blood composition are likely to show good performance (Isaac *et al*., 2013). Increase in Packed cell volume (PCV) together with red blood cells (RBC) is indicative of more efficient red blood cell forming processes in the experimental animals and implies that diets positively influenced blood forming processes (Togun and Oseni, 2003). On the other hand, when white blood cells fall within the normal range, it indicates that the feeding pattern did not affect negatively the immune system (Ameen *et al*., 2007). Increase in neutrophils: lymphocytes ratio is also an indicator of stress Minka and Ayo (2007), whose source could be nutritional (Etim *et al*., 2014).

Plasma lipid profiles such as triglycerides, total cholesterol, high density lipoproteins (HDL), low density lipoproteins (LDL), LDL: HDL ratio and total cholesterol: HDL ratios are important profiles which are mainly influenced by dietary factors (Kris-Etherton *et al*., 2002). Previous studies indicated that a combination of dietary fat and MO leaves as a source of antioxidant reduced plasma cholesterol and lipid peroxidation in rats (Ghasi *et al*., 2000; Mehta *et al*., 2003; Neveda *et al*., 2012). In rabbits, MO and lovastatin were found to lower the serum cholesterol, phospholipids, triglyceride, VLDL, LDL, cholesterol to phospholipids ratio and atherogenic index (Mehta *et al*., 2003). Although plasma lipid profiles in pigs are important both to pig

health and pig meat consumers, little information is available on the effects of MOLM pig diet on their lipid profiles.

2.6 Effects of *Moringa oleifera* and pig nutrition on pork quality

Pork quality is an important attribute in pig production modified by nutritional factors (Xu *et al*., 2010). For instance, it has been shown that feeding pigs on vitamin E and Selenium (Se) rich diet increases their levels in muscles thereby reducing lipid oxidation and thus enhanced pork quality (Ellis and McKeith, 1999). Similarly, feeding of high levels of vitamin D_3 in the final 10 days prior to slaughter improves the pork colour and reduces drip loss. Inclusion of MOLM in pig finishing diet has been shown to significantly improve pork shelf life by prolonging colour and odour acceptability during 10 days of refrigerated storage and reduces muscle fat content of pork thereby improving its quality (Mukumbo *et al*., 2014). *Moringa oleifera* is rich in Vitamins A and C and it is this attribute that is believed to improve the carcass quality by improving meat colour in broiler chicken (Gakuya *et al*., 2014). Dietary supplementation of MOLM had positive effects on proximate composition and shelf-life quality indicators of broiler breast meat (Nkukwana *et al*., 2016). Despite the numerous studies, there is still limited information appertaining to the effects of MOLM inclusion in the diet of growing and finishing pigs.

2.7 Effects of *Moringa oleifera* leaf meal on gut morphology and microbial changes

Gut health is an important feature in animal production because it determines the general animal health and level of utilization of dietary nutrients (Lalles *et al*., 2007). There are a number of gut health indicators in animals. First, gut morphology as well

as villi heights which determine the animal's capacity for nutrient absorption (Pluske *et al*., 1997). Secondly, gut anaerobic commensal bacteria such as Lactobacilli and protozoa which include the anaerobic lactic and butyric acid producing bacteria that utilize carbohydrates in the gut forming organic acids. These organic acids lower the gut pH making it difficult for the pathogenic organisms and coliforms to survive under the acidic conditions (Hammes and Vogel, 1995; Williams *et al*., 2001). Moreover, lactobacilli have shown some probiotic features and have consequently been used to promote nutrient utilization as well as boosting the animal's immune system (De Angelis et al., 2006). Animals fed on *Moringa oleifera* diet have shown improved feed utilization although its effects on the gut microbiology and morphology have not been conclusively documented.

2.8 Effects of *Moringa oleifera* on the histopathology of spleen, liver and kidneys

Histopathology is used to assess the physiological performance of animals especially those under test diets. The main organs involved in detoxification are the liver and the kidneys and their tissue structures may be affected in due process. Spleen, on the other hand, is involved in hematopoietic and immune functions and would also give an indication on how the diet affects the two important body functions. Moreover, spleen is the site of both direct and indirect toxicity and many systemic or generalized diseases have splenic involvement (Suttie, 2006). *Moringa oleifera* leaf extract treatment did not affect negatively the rats' spleen tissue structures and, in fact, at lower levels, it exerted structural improvements on the tissues translating into improved physiological functioning of the tissues (Owolabi and Ogunnaike, 2014). Later, it was established that aqueous extract of *Moringa oleifera* seed, if consumed

at higher quantities (>800mg/kg), negatively affects the normal histological appearance of the spleen causing mild and moderate expansion of white pulp which leads to decrease in white blood cells counts (WBC) and platelet concentrations in rats (Dike and Luteino, 2015).

At moderate doses, MO leaf extract ingestion has been found safe for the renal tissues even in cases of prolonged administration (Paliwal *et al*., 2011; Awodele *et al*., 2012; Ezejindu et al., 2014). Other studies have however established that moringa leaf consumption at higher doses or chronic use could predispose animals to hepatic and kidney damages and may result in renal failure (Oyagbemi *et al*., 2013; Owolabi and Ogunnaike, 2014). Furthermore, *Moringa oleifera* seed though containing natural antioxidant did not confer hepatoprotective effects on the liver architecture at higher doses (600mg/kg) on paracetamol induced hepatotoxixity in rats (Inyang *et al*., 2015). Despite the increased use of MO plant in animal feeds and research, there is no consensus on the optimal levels of inclusion in animal diets and, even so, its effects on the spleen, liver and kidney morphological structures have not been conclusively documented.

CHAPTER THREE

GROWTH PERFORMANCE AND CARCASS QUALITY OF GROWING PIGS FED ON *Moringa oleifera* LEAF MEAL DIETS

Abstract

To determine the effects of inclusion of different levels of *Moringa oleifera* leaf meal (MOLM) in growing pig diets on pig's daily feed intakes (DFI), growth performance, feed conversion efficiency (FCE) and carcass quality, 24, 2.5 month old pigs were selected and assigned to 4 treatment diets (T) containing: 0% (T1), 3% (T2) 6% (T3) and 12% (T4) MOLM concentrations, with 2 replications of 3 pigs each. The DFI, weekly pig weights were taken for 7 weeks after which pigs were slaughtered and the size of the loin eye area, tenth-rib back fat thicknesses measured and the dressing percentage calculated. Data from the experiment were analysed for descriptive statistics and ANOVA. The MOLM utilized in this study had a high crude protein level and low crude fibre compared to the conventional leguminous forages. The DFI for the 12% *Moringa oleifera* leaf meal pigs were significantly higher than control, 3% and 6% *Moringa oleifera* leaf meal. The 3% and 6% *Moringa oleifera* leaf meal had significantly higher FCE compared to T4 and T1. The carcass dressing percentage, carcass lengths and the loin eye area were within the recommended ranges and did not vary significantly with diets (P >0.05). In conclusion, MOLM grown in Kenya have higher levels of crude protein and could be included in pig diet formulations up to a level of 3% without affecting negatively pigs feed intakes, weight gains and feed conversion efficiency and pig carcass quality.

Key words: Backfat, Daily feed intakes, Feed efficiency, Dressing Percentage

3.1 Introduction

Growth performance, feed intakes, feed conversion efficiency and carcass quality are fundamental to pig production as they contribute to the overall profitability of pig production (Niemi *et al.,* 2010; Bilbrey, 2012). Feed conversion efficiency gives an indication of the effectiveness of a production system while the growth rates determine time to market and marketability of pork and other products (Anderson *et al*., 1997). These parameters are dependent on genetic factors, housing and more importantly, the feed. In most instances, feed becomes the most important expense contributing up to 70% of the total recurrent costs in a pig enterprise. In this case, the most limiting nutrients are proteins (Niemi *et al.*, 2010).

In pig nutrition, fish meal, cotton seedcake, sunflower and soya bean meal are the main protein sources in the diet (FAO, 2002). However, their scarcity due to competing usage has led to the adoption of a wide variety of ingredients to substitute the above protein sources (FAO, 2002). *Moringa oleifera* leaves rich in protein has led to its increased adoption as one of the non-ruminant protein source and has shown promising results (Gakuya *et al.,* 2014, Raphael *et al.,* 2016). These include enhanced weight gains in rabbits, chicken and pigs fed on diets containing MO (Acda *et al.*, 2010; Gakuya *et al.*, 2014; Mukumbo *et al.,* 2014). In pigs, Acda *et al*. (2010) reported that MOLM in the diet can be used up to a level of 10% to replace the commercial pig pre-starter feed without affecting piglets' body weight, feed intake, and feed conversion ratio. However, Mukumbo *et al.* (2014) later documented that inclusion of MOLM above 5% in finisher pig feed had a negative effect on their feed

conversion efficiency. MO has also been shown to enhance meat colour and reduce carcass fat, hence, improved quality (Gakuya *et al.,* 2014; Mukumbo *et al.,* 2014). Despite the increasing number of research on MOLM as a potential pig feed, there is no consensus on the optimal levels of its inclusion in pig diets and information on its effects on pork quality is still limited (Nouman *et al.,* 2014; Mukumbo *et al.,* 2014).

This study was therefore designed to determine feed intake, weight gain, feed conversion efficiency and carcass quality of growing pigs fed on diets compounded with different levels of MOLM.

3.2 Materials and Methods

3.2.1 Study site

This study was conducted at the University of Nairobi, College of Agriculture and Veterinary Sciences, Nairobi County. The area receives an average of 869mm annual rainfall with average daily temperature of 19^{o}C.

3.2.2 Experimental animals and study design

This study was conducted in accordance with the University of Nairobi Faculty of Veterinary Medicine Biosafety, Research Animal Use and Ethics guidelines. Twenty four (24) large white growing pigs (2.5 months old) were selected and assigned to four dietary treatments (T) containing: 0% (T1), 3% (T2), 6% (T3), and 12% (T4) MOLM, each with 2 replicates of 3 pigs.

3.2.3 Formulation of growing pig diets for the experiment

Growing pig diets were formulated using the NRC (2012), guidelines using maize meal, wheat pollard and vegetable oil as energy sources while MOLM, cotton seed

cake, sun flower cake, fish meal and soybean meal as protein sources. Vitamin mineral premix, dicalcium phosphate and limestone were also included as vitamin and mineral sources (Table 3.1). The pig feed raw materials were sourced from a reliable local feed manufacturer and preliminary laboratory tests done to confirm their proximate nutritional composition. Fresh *Moringa oleifera* leaves were obtained from the University of Nairobi field station, Kibwezi which is a rangeland and receives an annual rainfall less than 500mm and average temperature of 30^{o} C. All the mixed feeds and the raw materials were stored in a dry, well ventilated, slatted raised floor system, storage facility within the pig experimental premises.

Table 3.1 Feed ingredients and composition of the growing pig diets

Feed ingredients	Treatment diets (groups)			
	0% MOLM	**3% MOLM**	**6% MOLM**	**12% MOLM**
Maize	40.0	40.0	40.0	40.0
Moringa oleifera	0.0	3.0	6.0	12.0
Wheat pollard	30.0	26.0	23.0	16.8
Vegetable oil	1.5	2.4	3.3	3.5
Cotton seed meal	3.9	3.5	3.1	2.8
Sunflower meal	1.4	1.3	1.2	1.3
Fish meal	10.0	10.0	10.0	10.0
Soybean meal	10.0	10.0	10.0	10.0
Dicalcium phosphate	0.0	0.0	0.0	3.3
Limestone	3.4	3.1	2.7	0.0
Vitamin mineral premix	0.3	0.3	0.3	0.3
Total (%)	**100.0**	**100.0**	**100.0**	**100.0**
Diet cost/kg	**29.34**	**30.92**	**32.69**	**38.36**

Key: MOLM = *Moringa oleifera* leaf meal.

The calculated amino acids, composition, minerals and proximate tests for the different treatment diets are shown in Table 3.2.

Table 3.2 Calculated amino acids, minerals, crude protein, crude fibre and energy levels of the experimental diets

	Treatment diets			
Nutrient	**0% MOLM**	**3% MOLM**	**6% MOLM**	**12% MOLM**
Lysine	0.9	1.0	1.1	1.3
Threonine	0.6	0.7	0.8	1
Methionine	0.3	0.4	0.4	0.4
Methionine + Cysteine	0.6	0.5	0.5	0.5
Tryptophan	0.2	0.3	0.3	0.4
Isoleucine	0.7	0.8	0.8	1.2
Leucine	1.3	1.5	1.7	1.1
Valine	0.8	0.9	1	1.3
Calcium	0.9	1.9	1.9	1.6
Phosphorus	0.7	0.7	0.7	0.6
Crude Protein, %	19.6	19.6	19.7	20.2
Crude fibre,%	4.7	4.6	4.5	4.5
ME (Kcal/kg)	2.8	2.8	2.8	2.8

Key: MOLM= *Moringa oleifera* leaf meal, ME= Metabolizable energy

3.2.4 Proximate analysis of the *Moringa oleifera* leaf meal and feed used in the study

Fresh MO leaves were harvested from the Kibwezi field station, air dried and packed into plastic sample bags for proximate analysis at the University of Nairobi Animal Nutrition Laboratory as per the procedure documented by Hart and Fisher (1971). *Moringa oleifera* leaf meal dry matter (DM) level was determined by oven drying samples at 105^{O} C for 6 hours and ash determination done by burning the sample at 550^{O} C for 8 hours. Total N were determined by the Kjeldahl procedure and CP calculated as (N X 6.25).

3.2.5 Housing and management

Pigs were housed in groups of three, each group with a space of $12m^2$ concrete floor system. These housing were well lit, well ventilated and no environmental modifications such as ambient temperature regulations were used. Concrete floor feed and water troughs were also used with a high level of hygiene standards being maintained. Pig houses were cleaned twice daily; in the morning and evening together with feed troughs to ensure fresh feed was available to the pigs on each day.

3.2.6 General body examination and weighing of the animals

At the start of the experiment, each pig was examined and weighed as recommended by Whittington *et al*. (2003), followed by weekly weighing for a total of 7 weeks. Furthermore, each pig was monitored closely throughout the experiment period so that in the unlikely event of injuries and disease, there was a timely response in terms of treatment and management of the health conditions.

3.2.7 Feeding management and calculation of feed conversion efficiency

Feed were weighed each morning and fed in 3 portions to minimize wastage. At the end of the day, feed left in the troughs were weighed for the calculation of average daily feed intake as follows:

Feed intake= Feed provided in the trough in the morning (kg) - Feed left in the troughs at the end of the day (kg).

Feed conversion efficiency was calculated as follows:

$$\text{Feed conversion efficiency (\%)} = \frac{\text{Average daily gains (kg)}}{\text{Average daily feed intakes (kg)}} \times 100\%$$

Water was availed to the pigs *ad libitum*.

3.2.8 Carcass quality evaluation

After blood sample collection, pigs were humanely stunned and slaughtered as recommended (Ray, 2004). After manual evisceration, the size of the loin eye area and tenth-rib back fat thicknesses were measured and the dressing percentage calculated as the ratio between carcass weight after evisceration and the live weight before slaughter following the guidelines by Ray (2004).

Loin eye area was measured by making a transverse section at the loin area and a transparency with $1cm^2$ transparency and the squares counted to ascertain on the surface area.

$$\text{Dressing percentage (\%)} = \frac{\text{Pig carcass weight after evisceration (kg)}}{\text{Pig live weight before slaughter (kg)}} \times 100\%$$

3.2.9 Data management and analysis

Data on pigs' voluntary daily feed intake, weekly weight measurements, haematological and lipid profiles, live weight at slaughter, hot carcass weight, carcass length (from the poll to the tail where it joins the body on the dorsal midline), back fat thickness at tenth rib and loin eye area were stored in the Ms office excel. Data analysis were performed with the statistical pack SAS v9.0 SAS Institute Inc (2002) for the generation of means and frequencies, and to establish whether there were statistical differences between the treatment groups through the Analysis of Variance.

3.3 Results

3.3.1 Nutritional composition of *Moringa oleifera* leaf meal used in this experiment

Moringa oleifera leaf meal used in formulating the pig diets had 91.89% Dry Matter (DM), 27.37% Crude Protein (CP), 8.9% Crude Fibre (CF), 46.01% Nitrogen Free Extractives (NFE), 5.73% Ether Extract (EE) and 11.91% Ash (Table 3.3).

Table 3.3 Proximate composition of *Moringa oleifera* leaf meal used in the treatment diets

Proximate fraction	Values in percentages
Dry matter	91.89
Ash	12.10
Ether extract	5.73
Crude protein	27.37
Crude fibre	8.90
Nitrogen free extractives	46.01

3.3.2 Feed intake, weight gain and feed conversion efficiency of pigs under *Moringa oleifera* leaf meal treatment diets

For the entire experiment period, no signs of physical toxicities were noted in pigs. Feed trial results indicated that T4 consumed significantly higher (p<0.05) amounts of feed than the T2 and T3 but was not statistically different from the controls (T1) (Table 3.4). The T2 and T3 had significantly (P<0.05) higher daily gains compared to T1 and T4. Furthermore, Feed Conversion Efficiency (FCE) was higher in 3% and 6% MOLM compared to 0% and 12% MOLM treatment groups.

Table 3.4 Mean (± standard error of the mean) voluntary daily feed intake, weight gains and feed conversion efficiency of pigs fed on MOLM diets (n= 24)

	Dietary treatments (#MOLM inclusion levels, %)			
Variable	**0% MOLM**	**3% MOLM**	**6% MOLM**	**12% MOLM**
Starting weight (kg)	26.35 ± 0.10 [a]	25.95 ± 0.11 [a]	26.10 ± 0.11 [a]	26.31 ± 0.10 [a]
Final weight (kg)	66.46 ± 0.54 [a]	65.24 ± 0.55 [a]	66.55 ± 0.57 [a]	65.16 ± 0.53 [a]
Daily feed intake (kg/day)	2.90 ± 0.09[a]	2.61 ± 0.09[b]	2.54 ± 0.09[b]	3.153 ± 0.09[c]
Daily gains (kg/day)	0.807 ± 0.04[a]	0.836 ± 0.05[b]	0.810 ± 0.05[a]	0.810 ± 0.05[a]
Feed conversion efficiency (%)	28.05 ± 0.49[c]	31.57 ± 0.48[a]	31.23 ± 0.48[a]	30.31 ± 0.48[b]

Key: n= sample size, #MOLM=*Moringa oleifera* leaf meal. The treatment means denoted by the same superscripts ([a, b and c]) in the same row did not have significant differences at $P < 0.05$.

3.3.3 Effects of *Moringa oleifera* leaf meal diet on pig carcass characteristics

The carcass dressing percentage, carcass lengths and the loin eye area were within the normal ranges (Ray, 2004) and did not vary significantly with diets (P >0.05). Furthermore, backfat thickness on the other hand did not vary significantly with diet although there was an indication of its reduction with increased MOLM in the diet (Table 3.5).

Table 3.5 Effects of *Moringa oleifera* leaf meal diets on pig average dressing percentage, carcass length, backfat thickness and loin eye area (± standard error of the mean) (n=16)

	Treatment groups			
Carcass parameters	**0% MOLM**	**3%MOLM**	**6%MOLM**	**12% MOLM**
Dressing percentage, %	73.82±0.63	73.79±0.75	73.778±0.62	72.93± 0.77
Carcass length, cm	82.04±2.13	84.24± 2.63	84.19±2.46	84.51±3.05
Backfat thickness, cm	2.00±1.00	1.67± 0.57	1.33±0.57	1.67±0.57
Loin eye area, cm^2	25.67±3.78	25.33±1.15	29.00±3.00	26.00±5.29

Key: n= sample size, MOLM= *Moringa oleifera* leaf meal.

Feeding costs analysis for the different treatment groups were carried out to ascertain on the differences in the costs of utilizing MOLM in the feed. Considering the pig starting weights to be approximately 26kg for all treatment groups and the end weights of 66kg, the total weight gains in the experiment period was calculated to be

40kg. The analysis revealed that the 3% MOLM group could save the farmer Ksh 237.80 per 100 kg feed when compared to control Table 3.6.

Table 3.6 Feeding cost analysis of the different inclusion levels of *Moringa oleifera* leaf meal in the diet (n=24)

Parameters	0% MOLM	3% MOLM	6% MOLM	12% MOLM
Feed Conversion Ratio (%)	3.56	3.17	3.20	3.30
Total feed to 40kg Pig (Kg)	142.57	126.69	128.08	131.95
Total feed costs to market (KES)	4163.15	3925.36	4195.72	5057.21
Savings compared to control (KES)	0.00	237.80	-32.57	-894.06

Key: n= sample size, KES=Kenya shillings, MOLM= *Moringa oleifera* leaf meal.

3.4 Discussion

The crude protein levels of MOLM in this study were close to 27.51% as previously reported by Oduro *et al.* (2008), 29.55% by Nuhu (2010) in Ghana and 29% by Liaqat *et al.* (2016) but higher than 23.3% recorded by Gakuya *et al.* (2014) in Kenya. This could be attributed to differences in ecological zones and the physiological stages of harvesting where younger fresh materials could have higher protein levels, NFE and lower crude fibre (Samkol *et al.,* 2005; Gakuya *et al.,* 2014). The relatively lower levels of crude fibre imply that MOLM could be a potential feed for the non-ruminants. This is because non ruminants lack the enzymes responsible for the breakdown of complex cellulose into the simple carbohydrate forms that can easily be utilized by the animals. Furthermore, MOLM has been found to have a high NFE implying it can easily be utilized by the pigs.

The MOLM diets in all treatment groups were well tolerated by the pigs; this was also reported by Gakuya *et al.* (2014) in chicken and Nuhu (2010) in rabbits. This can be

confirmed by the earlier studies that stated that MOLM diets had low levels of toxins such as tannins and flavonoids that could have adverse effects especially when consumed at high levels and over a prolonged period of time (Gupta *et al.,* 1989). There are a number of factors that influence the average daily feed intake in pigs. One is driven by the energy levels in the diet, whereby, feed with low energy levels will positively influence the feed intake rates as the pigs attempt to replenish the body energy needs. Secondly, high fibre levels in the diet may increase the rate of passage of feed in the gut hence the need for higher feed consumption (Afuang *et al.,* 2003). Therefore, the higher pig feed intakes recorded in the control and 12% MOLM groups compared to those from 3% and 6% MOLM groups in this study could be attributed to the availability of the nutrients in the diets. The highest average daily gains recorded in T2 (3% MOLM) were close to those of Mukumbo *et al.* (2014) and Oduro-Owusu *et al.* (2015) who reported highest pig weight gains at 5% MOLM as well as highest feed conversion efficiency which could be attributed to the high protein content observed and the higher digestibility noted due to low fibre content amongst the diets. Furthermore, the calculated amino acid compositions for the different diets also justify the higher growth rates observed in the MO diets. This is because amino acids form the building blocks for the various proteins hence, rapid growth rates are likely to be observed under MOLM diets. However average daily gains in our study was different from those of Acda *et al.* (2010) who tried MOLM on piglets and stated that MOLM up to 10% could substitute commercial pig pre-starter diets.

The carcass dressing percentages, back fat thickness, loin eye area and carcass lengths did not differ significantly in all treatments, implying that MOLM diets did not have

any negative effect on the carcass quality; a finding similar to that of Mukumbo *et al.* (2014) and Oduro-Owusu *et al.* (2015) who stated that the different levels of MOLM in the diet did not affect negatively the carcass dressing percentage, back fat thickness, carcass length and loin eye area in finishing pigs.

The analysis of pig production costs in this study show that MOLM at low levels (3%) could be more profitable to include in pig's diets. This can be explained by the higher feed conversion efficiency recorded in the same group. However, it implies that further increase in MOLM in the diet beyond 3% is more expensive because of the higher costs of Moringa leaves.

In conclusion, MOLM grown in Kenya have high levels of crude protein and low fibre content that can allow inclusion in growing pig's diet formulations up to a level of 6% without affecting negatively pigs feed intake, weight gain and feed conversion efficiency and carcass quality. However in terms of feeding costs, it is more economical to include it in the diet up to a level of 3% after which the costs of making the feeds will begin to increase due to the higher cost of MOLM.

CHAPTER FOUR

EFFECTS OF DIETARY *Moringa oleifera* LEAF MEAL ON HAEMATOLOGY AND PLASMA LIPID PROFILES IN GROWING PIGS

Abstract

This study was designed to determine the effects of inclusion of different levels of *Moringa oleifera* leaf meal (MOLM) in growing pig diets on pig's haematology and plasma lipid indices. Twenty four pigs two and half month old were selected and assigned to 4 treatment diets (T) containing: 0% (T1), 3% (T2) 6% (T3) and 12% (T4) MOLM concentrations, with 2 replications of 3 pigs each. The daily feed intakes and weekly pig weights were recorded for 7 weeks after which 2 sets of blood samples were drawn from 2 pigs per replication for haematology and plasma lipid determination. Data from the experiment were analysed for descriptive statistics and ANOVA. Inclusion of *Moringa oleifera* leaf meal in diet significantly increased haemoglobin concentration only to a level of 6% after which there was a reduction at 12% MOLM. Also observed was higher Mean Cell Volume for the control compared to 6% MOLM. *Moringa oleifera* leaf meal in the diet also improved the White Blood Cell Counts (3% MOLM) compared to those in control group. Total cholesterol in 3% MOLM was significantly reduced compared to control group. This implied that MOLM at lower levels (3%) improved haemoglobin concentration and white blood cell counts and exhibited hypocholesterolaemic effects, hence improving the overall productivity of the animals.

Key words: Haematology, Red blood cells, Total cholesterol, Total cell counts.

4.1 Introduction

Moringa oleifera leaf meal has been shown to have numerous haematological, plasma lipid actions as well as immune-modulatory features (Gupta *et al.,* 1989). This has been attributed to the wide variety of polyphenols and phenolic acids as well as flavonoids, glucosinolates, and alkaloids (Stohs and Hartman, 2015). This is fundamental to animal production because improved red blood cell and haemoglobin indices is a sign of improved oxygen circulation and thus improved productivity of the animal (Olugbemi *et al.,* 2010). On the other hand, improved white blood cell parameters are important since it gives an indication on the performance of the immune system and the ability of the animal to counter infections and in this case may minimize use of antimicrobials (Gupta *et al.*, 2012). Hypocholesteraemic features of MOLM have also been reported and attributed to β-sistosterol present in MO that enhances conversion of cholesterol to forms that can be excreted in bile.

A number of studies have been conducted on the hematopoietic ability of MOLM and diverse results have been recorded. Despite increasing use of the plant in animal nutrition and research on the systemic effects, varying information is available on the effects of its inclusion at varying levels in diets on haematological and lipid profiles in growing pigs hence the need for the study. This study therefore was designed to determine the effects of inclusion of MOLM at different levels in pig's diet on haematological parameters and plasma lipid profiles in growing pigs.

4.2 Materials and Methods

4.2.1 Study site

See sub section 3.2.1

4.2.2 Animals and experimental design

See sub section 3.2.2

4.2.3 Treatment diets

See sub section 3.2.3

4.2.4 Blood sample collection, processing and laboratory analysis

At the end of the experiment, pigs were starved for 12 hours with provision of drinking water only. Four pigs from each treatment were randomly selected and 2 sets of blood (5ml each) drawn from caudal jugular vein using 9ml vacutainers; one with EDTA and the second with serum clot activator. Pigs were restrained to stand square on all four legs by a snout rope, head slightly forward and slightly raised and the operator standing parallel with the pig facing forward and working to the left. Red Blood Cells, Total White Blood Cell Counts, granulocyte, lymphocyte, mid-range absolute count (MID) differential counts were determined in the laboratory using an automated haematology analyser machine. Blood for plasma lipid profiling were centrifuged for 15 min at 3,000 revolutions per minute (rpm) (Li and Kim, 2013). Plasma triglycerides, total cholesterol, HDL and LDL were analysed using the standard plasma lipid profiling test kits.

4.2.5 Data management and analysis

Data on pigs' haematological and lipid profile results, were stored in MS office excel after which means of red blood cells, white blood cells and platelets as well as haemoglobin concentration and analysis of variance were performed using statistical pack SAS v9.0 (SAS Institute Inc, 2002).

4.3 Results

4.3.1 Effects of *Moringa oleifera* leaf meal diet on pig blood parameters

All the blood parameters measured in this study (haematological and plasma lipid profiles) were within the normal ranges for the 0%, 3%, 6% and 12% MOLM (Friendship *et al*., 1984). However, there were variations in the hematologic and plasma lipid profile levels with variations in MOLM levels in the diets.

4.3.2 Effects of *Moringa oleifera* leaf meal on the red blood cell parameters of growing pigs

From the study, the haemoglobin concentration was significantly higher (P <0.05) in 6% MOLM compared to control, 3% and 12% MOLM (Table 4.1). However, Mean cell volume (MCV) was significantly higher (P <0.05) in control group compared to those of 3% and 6% MOLM. Red blood cell distribution width standard deviation (RDWs) were significantly higher (P<0.05) in 12% MOLM and control groups compared to 3% and 6% MOLM.

Table 4.1 Mean (± standard error of the mean) red blood cell parameters from pigs fed on varying levels of *Moringa oleifera* leaf meal in the diet (n=16)

	Treatment groups				
Parameters	**0% MOLM**	**3% MOLM**	**6% MOLM**	**12% MOLM**	**Reference (Friendship *et al*., 1984)**
Red blood cells ($x10^6/mm^3$)	7.5±0.7[a]	8.0± 1.0 [a]	8.7±0.6 [b]	7.1± 0.2 [a]	5.0-8.0
Haemoglobin concentration (g/dL)	13.5± 0.7 [a]	13.7± 0.6 [a]	14.7±0.6 [b]	12.3± 0.6 [c]	10.0-16.0
Haematocrit concentration (%)	45.0 ± 1.4[a]	44.3±2.1[a]	45.0±1.0 [a]	43.4±1.6 [a]	32.0-50.0
Mean Cell Volume (fL)	60.0± 0.0[b]	56.7±1.5[b]	52.3±0.6[a]	58.0±2.6[b]	53.0-79.0
Mean Corpuscular Haemoglobin (pg)	18.0±0.0[a]	17.3±1.2 [a]	17.3±0.6 [a]	17±1.0 [a]	17.0-21.0
Mean Corpuscular Haemoglobin Concentration (g/dL)	30.5± 0.7 [a]	31.0±1.0 [a]	32.3±0.6 [a]	30.3±4.4 [a]	30.0-34.0
Red Cell Distribution Width (Counts) (%)	22.0±0.0 [a]	23.0± 1.0 [a]	23.3±0.6 [a]	22.4±0.6 [a]	17.0-29.0
Distribution Width standard deviation (fL)	48.5±0.7 [a]	47.3±1.2 [a]	44.7±1.2 [a]	49±1.0 [a]	38.0-55.0

Key: [#]MOLM=*Moringa oleifera* leaf meal. The treatment means denoted by the same superscripts ([a, b and c]) in the same row did not have significant differences at $P < 0.05$.

4.3.3 Effects of *Moringa oleifera* leaf meal diet on the white blood cell parameters of growing pigs

The 3% MOLM group had a higher (P =0.05) concentration of white blood cells compared to control, 12% and 6% MOLM treatment groups. There was also an increase in lymphocytic concentration with increase in MOLM in diet as shown in Table 4.2. Granulocyte cell concentration was, on the other hand, lower (P <0.05) in the MOLM treatment groups compared to the control (T1) groups. Differential cell counts showed that granulocyte proportions declined with increased MOLM in the diet, but started to rise again with increased MOLM in T4 (12% MOLM). The mid-range absolute counts (MID) cell proportions further increased (P <0.05) with increase in MOLM in the diet and similarly for the lymphocytes (LMP) (Figure 4.1).

4.3.4 Effects of *Moringa oleifera* leaf meal on platelet parameters of growing pigs

Platelet counts, platelet distribution width (counts) and platelet distribution width (standard deviation) were significantly lower (*P* <0.05) among pigs on MOLM diets compared to controls (T1). The T2 group had the highest platelet concentration while the controls had the least number of cells per mL of blood (Table 4.3).

Table 4.2 Mean white blood cell parameters of growing pigs fed on the different levels of *Moringa oleifera* leaf meal in diet (± standard error of the mean) (n=16)

White blood cell indices	Dietary treatments (#MOLM inclusion levels, %)				
	0% MOLM	3% MOLM	6% MOLM	12% MOLM	Normal Reference (Friendship *et al*., 1984)
White blood cell counts (10^9/L)	14.50^{a} ±0.20	16.62^{b} ±0.23	15.63^{a} ± 0.23	14.87^{a} ± 0.42	6.00-21.70
Lymphocyte concentration (10^9/L)	7.50^{a} ± 0.20	8.70^{b} ± 0.23	9.30^{b} ± 0.25	8.70^{b} ± 0.23	3.80-16.50
Mid – range absolute counts (10^9/L)	1.00^{a} ± 0.17	1.00^{a} ± 0.17	1.00^{a} ± 0.17	1.30^{a} ± 0.25	0.10-5.00
Granulocyte concentration (10^9/L)	6.50^{b} ± 0.20	7.00^{b} ± 0.23	5.00^{a} ± 0.23	5.30^{a} ± 0.25	5.00-13.90
Lymphocytes (%)	50.00^{a} ±1.42	52.09^{a} ±1.70	60.70^{b} ± 2.1	56.80^{c} ± 1.30	39.00-62.00
Granulocytes (%)	43.50^{a} ± 1.40	41.90^{a} ±0.25	32.60^{b} ± 0.42	34.64^{b} ± 0.65	28.00-50.00
Mid – range absolute proportion (%)	6.50^{a} ± 0.54	6.00^{a} ± 0.65	6.53^{a} ± 0.23	8.40^{b} ± 0.33	4.5.00-13.00

Key: #MOLM=*Moringa oleifera* leaf meal. The treatment means denoted by the same superscripts ($^{a, b \text{ and } c}$) in the same row did not have significant differences at $P < 0.05$.

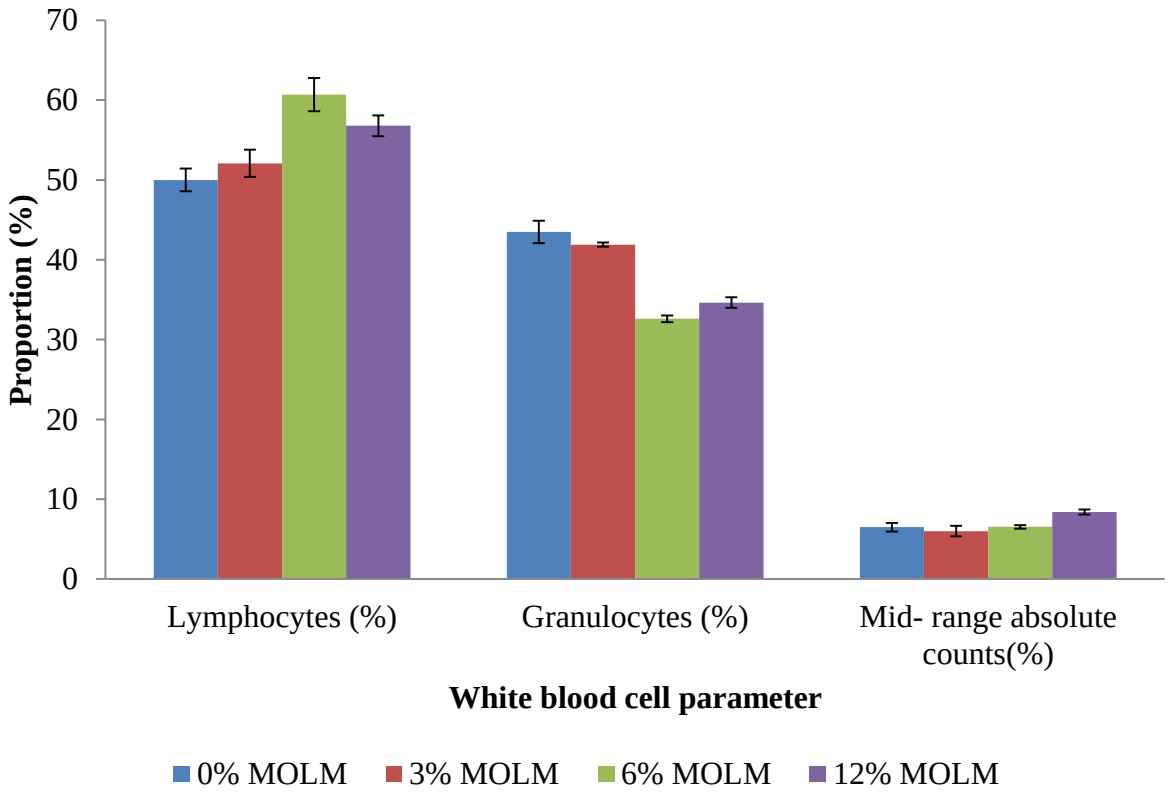

Figure 4.1 Mean (± Standard error) white blood cell proportions (%) in pigs under different *Moringa oleifera* leaf meal diets

Key: MOLM = *Moringa oleifera* leaf meal

Table 4.3 Mean platelet parameters of the *Moringa oleifera* leaf meal treatment groups (n=16)

	Treatment groups				
Variable	**0%MOLM**	**3%MOLM**	**6%MOLM**	**12%MOLM**	**P value**
Platelet counts ($x10^9$/l)	200.0± 29.7	343.0± 42.5	312.0± 44.2	312.3± 2.5	0.02
Mean platelet volumes (fl)	11.5±0.7	10.3± 0.6	10.0±0.0	12.1± 0.1	0.51
Platelet distribution width counts (%)	42.0± 0.0	42.0±0.0	40.0±0.0	42.7±1.2	0.01
Platelet distribution with standard deviation (fL)	20.0±0.0	19.3±0.6	15.7± 0.6	21.6±1.4	0.00

Key: MOLM =*Moringa oleifera* leaf meal

4.3.5 Effects of *Moringa oleifera* leaf meal on plasma lipids in growing pigs

Total cholesterol reduced significantly (P <0.05) with increase in MOLM in the diet, but again increased marginally at the highest level of dietary MOLM supplementation (Figure 4.2). Control also had the highest level of LDL (2.89mg/ml) compared to 3%MOLM (2.27mg/ml), 6% (2.26mg/ml) and 12 %MOLM (2.5mg/ml). TGS and HDL however did not vary significantly with diet. However, all plasma lipids were within the normal ranges (Friendship *et al.*, 1984)

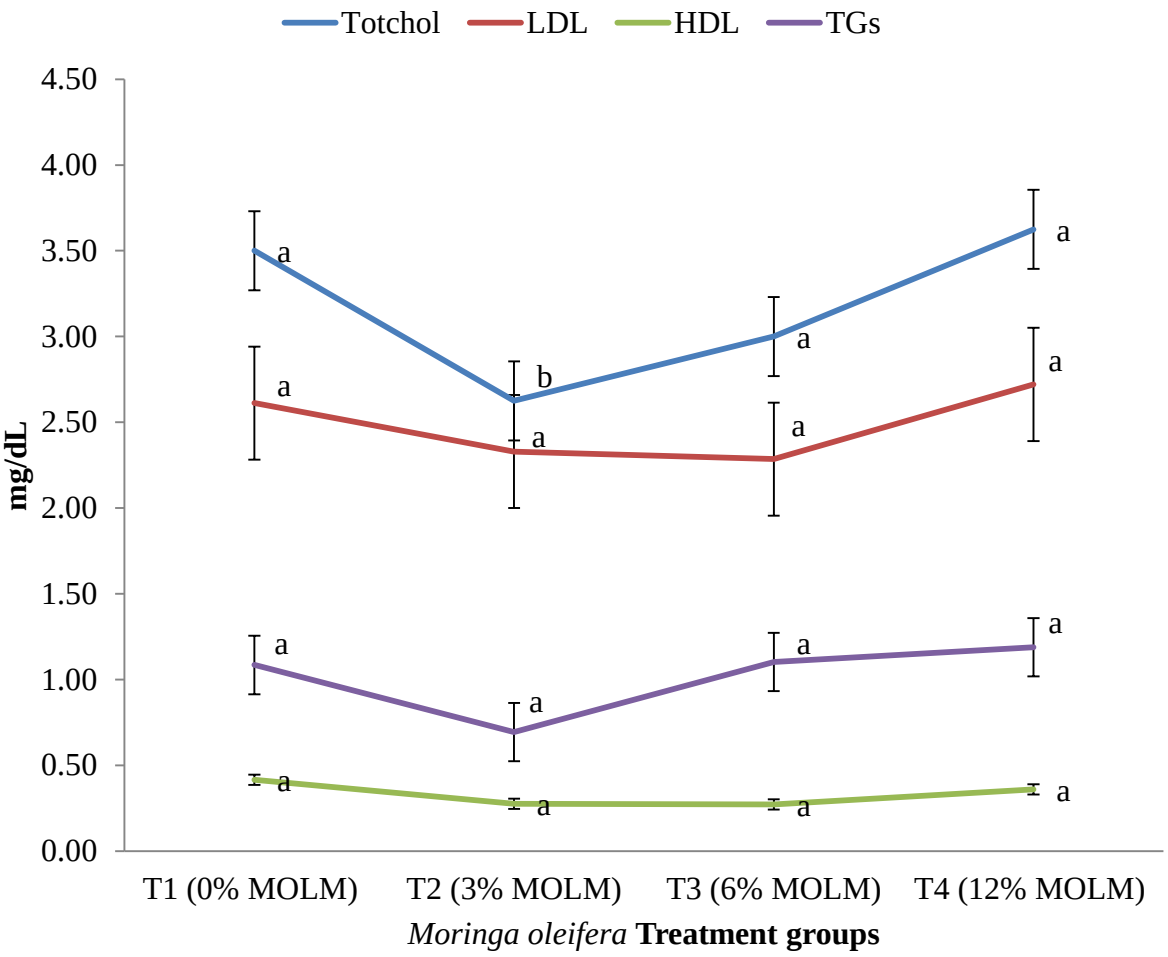

Figure 4.2 Mean (± Standard deviation) plasma lipid profiles of growing pigs fed on different levels of *Moringa oleifera* leaf meal diets (n=16)

Key: Totchol= Total cholesterol, LDL= Low density lipoproteins, TGs= Total triglycerides, HDL=High density lipoproteins, MOLM= *Moringa oleifera* leaf meal, the treatment means denoted by the same superscripts ($^{a, b \text{ and } c}$) in the same series did not have significant differences at $P < 0.05$.

4.4 Discussion

Diet has been found to influence hematological parameters (Etim *et al.,* 2014). *Moringa oleifera* leaf meal up to 6% in diet improved the red blood cell counts and hemoglobin (Hb) concentration in blood after which the levels declined significantly at 12 %MOLM. These results were similar to those of El Tazi and Tibin (2014) who recorded higher levels of Hb in broiler chickens fed on MOLM diets. This has been attributed to higher levels of protein and minerals mostly iron in the MO plant which is responsible for the formation of haemoglobin (El Tazi and Tibin, 2014). The higher the haemoglobin concentration the better the oxygen circulation in the body, hence, better performance of the animal (Olugbemi *et al.,* 2010). At higher levels (>6% MOLM) however, haemoglobin concentration declined and could possibly be due to the potential toxicities by high levels of flavanoids and tannins in the plant leaves (El Tazi and Tibin, 2014). Increased MOLM (12% MOLM) led to increased MCV implying that there might have been increased release of immature RBC or increased iron or folic acid levels that enhanced red blood cell formation (Fahey, 2005). Higher levels of MCV could further imply existence of chronic liver diseases hence inefficiency of liver detoxification. This also could be as a result of increased levels of flavonoids which might have led to impairment of liver function at the highest MOLM in the diet (Fahey, 2005). This therefore implies that MOLM should be used

in moderation since high levels (>3%) in the diet may lead to toxicity and reduced efficiency in oxygen transportation in the body, hence, reduced performance.

The group on 3%MOLM had the highest white blood cell counts followed by 6% and 12% MOLM. The control group however had the least WBC counts. These results were similar to those of Gupta *et al.* (2012) which implied that higher vitamin and protein concentrations in MOLM may have led to improved immune system in animals; indicated by higher body defence cell levels. This is important since the treatment groups would be able to fight diseases compared to controls, hence, minimizing drug usage and thereby reducing the cost of production and, subsequently, the safety of pork (Pascoal *et al.*, 2012). Mid-range absolute cell counts (MID) increased with increased MOLM in the diet, meaning that the white blood cell precursors had increased therefore enabling the animal to readily counter any infections that may arise. These findings support those of Gaikwad *et al.* (2011) and Stohs and Hartman (2015) who documented that MO stimulate both cellular and humoral immune systems. This immunomodulatory potential of *M. oleifera* leaves could be attributed to the presence of flavonoids, polyphenols and terpenoids which may modulate one of the above mentioned immune-mechanisms. Granulocytes in most instances are responsible for the immune defence against bacterial infections. In this case, MOLM antimicrobial properties may have led to suppression of the pathogenic microbes hence resulting in declined granulocyte levels, with higher levels of *M. oleifera* in the diet.

Fahey (2005) and Ghebreselassie *et al.* (2011) reported that, MOLM exerts hypocholesterolemic effects when taken in the diet. This has also been supported by

this study. Increased MOLM reduced cholesterol levels significantly, perhaps by lowering the serum concentrations of LDL by β-sitosterol; the bioactive phytoconstituents isolated from *Moringa oleifera* (Ghasi *et al.,* 2000). However, pigs on the highest concentration of MOLM showed increased cholesterol levels therefore necessitating further studies to establish reasons for the increased cholesterol levels. This study therefore concludes that low levels of MOLM in the pig's diet could enhance haemoglobin and WBC formation which, could increase efficiency in oxygen circulation in the body and boost animal's immunity and enhance better performance. However, higher levels beyond 6% could interfere with the normal haematological parameters and subsequently affect negatively the pig's performance. *Moringa oleifera* leaf meal also has hypocholesterolaemic effects, hence, could reduce both the total cholesterol and LDL levels thereby minimizing chances of cardiovascular diseases associated with higher levels of cholesterol. Further studies however, ought to focus on the actual immune response in relation to specific infectious agents in pigs.

CHAPTER FIVE

EFFECTS OF *Moringa oleifera* LEAF MEAL DIETS ON GUT pH, MORPHOLOGY AND MICROBIAL CHANGES IN GROWING PIGS

Abstract

This study was designed to determine the effects of inclusion at different levels of *Moringa oleifera* leaf meal on gut pH, lactobacilli, coliform counts and histopathology of the stomach, ileum and the colon. A total of 24 pigs were selected and assigned to four treatment diets (T) containing 0% MOLM (T1) (control), 3% MOLM (T2), 6% MOLM (T3) and 12% MOLM (T4). After 7 weeks of the experiment, pigs were slaughtered and one segment of tissue from the stomach, distal ileum, and proximal colon were collected for histopathological examination and their contents for pH measurements. Faecal sample from the rectum of these animals were also collected for bacteriology. Data on the pH measurements, lactobacilli and coliform counts were stored in Microsoft Office excel and later analysed for descriptive statistics and analysis of variance. There was a significant difference (P <0.05) in gastric pH of the digesta across the treatment groups where controls had significantly lower pH than 3%, 6% and 12% MOLM diets. Bacteriology results, on the other hand, revealed a significant difference (P <0.05) in coliform counts with the controls having the least counts compared to the treatment diets. Lactobacilli counts were not affected by the diet. Higher levels of MOLM (6 to 12%) in the diet led to the stomach and ileum submucosal oedema, fat infiltration and increased thickness. Observed also were reactions indicated by the deposition of lymphatic nodules, implying an inflammatory reaction with increasing levels of MOLM in the diet. It was

concluded therefore that, high levels of MOLM >3% in the diet affected the gut morphology and may have exerted a negative effect on nutrient utilization and performance of the pigs.

Key words: *Moringa oleifera*, Growing pigs, Lactobacilli, Coliforms, Peyer's patches

5.1 Introduction

Diet has a great influence on the diversity of gut microbial population composition, gut pH and morphology. This therefore determines the capacity for digestion, absorption and utilization of the dietary nutrients hence the overall performance of the animal (Kogut and Arsenault, 2016).

Gastric pH measurements are important when undertaking studies on gut microbiology and morphology. Beasley *et al.* (2015) established that gastric pH can vary between and within animal species depending on the diet fed. The same authors recorded that, those animals feeding on the higher diets in the food chain require lower pH because their stomachs regulate the microbes entering into the animal's body system through the gut. A study on the survival of bacteria under different pH levels revealed that at pH of less than 3.5, the bacterial activity are diminished while killing of the bacteria occurred at pH less than 2.5 (Zhu *et al.,* 2006). Increased MO in broiler chicken diet led to increased pH in the proventriculus and caecal pH (Nkukwana *et al.,* 2015). On morphology, duodenal villous lengths were longest at low levels of MO diet but shortest in the highest MO diet concentrations. The highest MO diet birds had the widest jejunal villi while the least MO diet birds had the shortest. Increased MO in the diet led to a significant increase in the number of enterocytic goblet cells and decreased villi heights (Hlophe and Moyo, 2014).

Animals fed on *Moringa oleifera* diet have shown improved feed utilization even though the effects on gut microbial composition and morphology have not been conclusively documented (Pfaff *et al.,* 2015). The main objective of this study therefore was to determine the effects of MOLM diet on gut morphology, gut microbial composition and pH in growing pigs.

5.2 Materials and Methods

5.2.1 Study site

See sub section 3.2.1

5.2.3 Animals and experimental design

See section 3.2.2

5.2.4 Treatment diets

See sub section 3.2.3

5.2.5 Animals housing design

See sub section 3.2.5.

5.2.6 Gut morphology and contents sampling assessment

After the slaughter of the pigs, one segment of tissue (3 cm) from the distal ileum (50 cm cranial to the ileocecal valve) and one segment from the proximal colon (20 cm from the cecum) were collected for histopathological analysis (De Angelis *et al.,* 2006). The gut sections were prepared for histological sections using the procedure used by Yamsakul *et al.* (2013).

Small pieces of each organ were fixed by immersion in phosphate buffered formalin (10%) and routinely processed (ethanol dehydration) to serial paraffin sections (5 µm

thick) which were then stained with haematoxylin and eosin. The stained sections were examined and photographed using a Leica ® DM 500 light microscope. Photographs of the tissue sections from the treatment groups were analysed for any changes by comparing to the control group. Tissue analysis was done using qualitative methods with emphasis on histo-morphology and general histo-architecture.

The gut contents were also sampled for pH measurements (De Angelis *et al.,* 2006). Fecal sample (50g) from the rectum of each animal were collected using sterile wooden tongue depressor into sterile centrifuge tube for bacteriology (De Angelis *et al.,* 2006). After collection, faeces were mixed with Amies Transport medium and later plated on MRS agar (Oxoid Ltd.) for total count of lactobacilli in the laboratory. Another sample was plated in the VRBA agar (Oxoid Ltd.) for total coliform counts.

5.2.7 Data management and analysis

Data on pigs' gut pH, lactobacilli and coliform colony counts were stored in the Ms Office excel after which they were exported to statistical pack SAS v9.0 SAS Institute Inc (2002) for descriptive statistics and analysis of variance.

5.3 Results

5.3.1 Effects of *Moringa oleifera* leaf meal diet on the gastro intestinal pH

From the results, there were significant differences (P <0.05) in gastric pH across the treatment groups. The control (T1) groups exhibited significantly lower pH (P <0.05) compared to the groups fed MOLM diets which showed consistently higher pH (Table 5.1). However, the duodenal, ileum, caecum and colon pH did not vary significantly (P >0.05).

Table 5.1 Mean (± Standard deviation) gastrointestinal pH of pigs under different levels of *Moringa oleifera* leaf meal diets (n=12)

	***Moringa oleifera* treatment diets**				
Gut	**0% MOLM**	**3% MOLM**	**6% MOLM**	**12% MOLM**	**P Value**
Gastric	3.54±0.05	5.44± 0.24	4.49±0.76	5.22±0.78	0.039
Duodenum	6.54± 0.65	5.92± 1.01	6.50± 0.71	6.66± 0.57	0.675
Ileum	5.14±0.19	6.16±0.76	7±1.41	7.3±0.61	0.100
Caecum	6.2±0.28	5.74±0.45	6 ±0.01	6±0.00	0.417
Colon	5.5 ± 0.70	6.14±0.79	6 ±0.01	6.67± 0.58	0.331

Key: n= Sample size, MOLM= *Moringa oleifera* leaf meal

5.3.2 Effects of diet on lactobacilli and coliform counts

Bacteriology results, on the other hand, revealed a significant difference (P <0.05) in the faecal coliform counts with the T1 (controls) having the least counts compared to the *Moringa oleifera* treatment diets (Table 5.2). Lactobacilli counts, however, remained constant among the treatment groups.

Table 5.2 Mean (± Standard deviation) faecal lactobacilli and coliform counts from *Moringa oleifera* leaf meal pig treatment diets (n=16)

	Moringa oleifera **treatment diets**				
	0% MOLM	**3% MOLM**	**6% MOLM**	**12% MOLM**	**P Value**
Coliforms (10^4 CFU/ml)	2.0± 1.4	18.5±10.5	65.3±8.3	77 ± 79.3	P<0.001
Lactobacillus (10^7 CFU/ml)	300.0±0.0	259.3± 70.4	300.0±0.0	300.0±0.0	P>0.05

Key: CFU= colony forming units, MOLM= *Moringa oleifera* leaf meal

5.3.3 Histopathology of the stomach of pigs fed on *Moringa oleifera* leaf meal diets

In the control diets, all the histological layers of the stomach (the tunica mucosa, submucosa, muscularis interna, and the muscularis externa) appeared normal and evenly distributed (Figure 5.1a). In the T2 group, the tunica mucosa was reduced in thickness, the tunica submucosa enlarged and filled with both fat and fibrous connective tissue. The intermuscular spaces in the tunica mascularis were also enlarged and filled with oedema (Figure 5.1b). In T3, the mucosa appeared degenerated, tunica submucosa was more oedematous and the tunica mascularis appeared degenerated (Figure 5.1c). The mucosa and mucosal glands of T4 appeared degenerated and the sub mucosa also was prominent and filled with fatty tissue and oedema of the submucosa (Figure 5.1d)

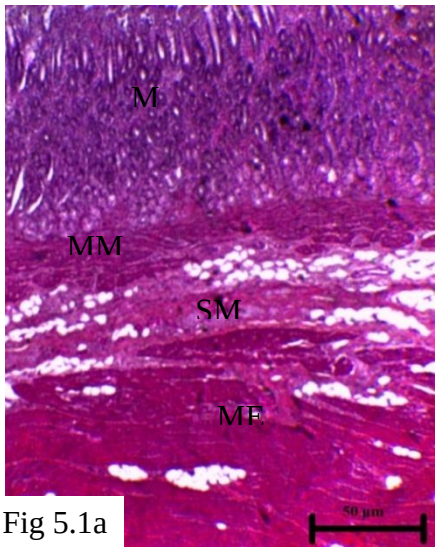

Fig 5.1a

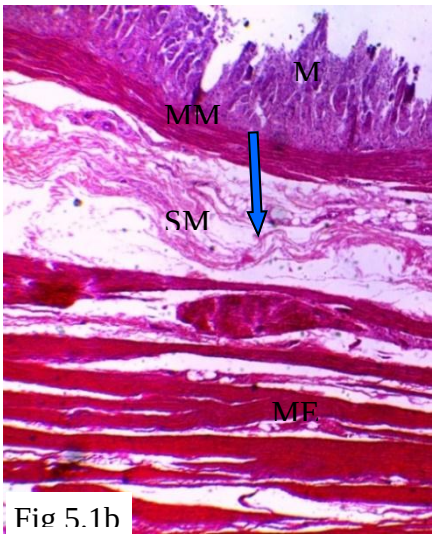

Fig 5.1b

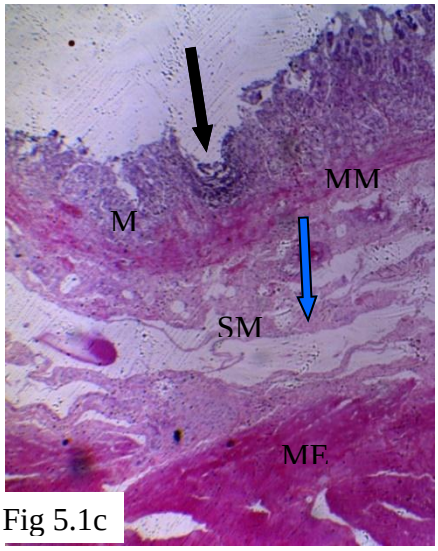

Fig 5.1c

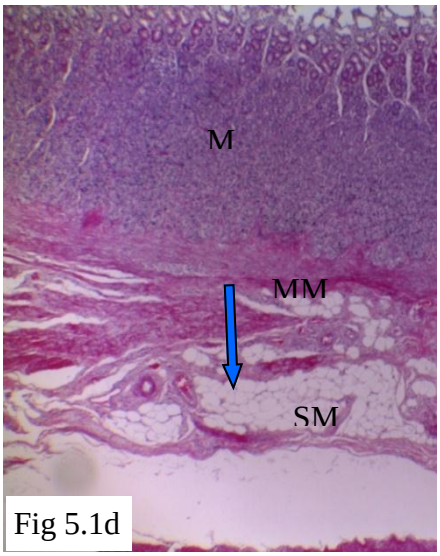

Fig 5.1d

Figure 5.1: Cross sections of stomachs from pigs fed on different levels of *Moringa oleifera* leaf meal diets.

Figure 5.1a: A cross-section of the stomach of pigs in the control group. The stomach is normal with all the layers evenly distributed. Mucosa (M), sub-muscularis (SM), submucosa (SM) and muscularis externa (ME).

Figure 5.1b: Histological sections of the stomach of pigs in T2 group. The mucosa (M) seems to be reduced in thickness and submucosa (SM) enlarged and filled with both fat and fibrous connective tissue. The intermascular spaces in muscularis mucosa (MM) are also enlarged and oedematous (blue arrow). Muscularis externa (ME)

Figure 5.1c: A histological section of stomach from pigs in T3. The mucosa (M) is considerably reduced and degenerated (Black lock arrow), the muscularis mucosa (MM) degenerated and the submucosa conspicuously oedematous (blue arrow)

Figure 5.1d: A cross section of the stomach of pigs in T4. There is degeneration of the mucosa (M) and the mucosal glands. The sub mucosa (SM) appears oedematous and filled with fatty tissue. Muscularis mucosa (MM), and muscularis externa (ME).

5.3.4 Histopathology of ileum from pigs fed on *Moringa oleifera* leaf meal

Ileum from the controls (T1) had normal histoarchitecture (Figure 5.2a). The submucosa, muscularis mucosa and muscularis externa layers appeared normal. Similarly, ileal sections from pigs from T2 group appeared normal although the sub mucosa was infiltrated by fatty tissue and enlarged (Figure 5.2b). Lymph nodules were also observed in the mucosa. In T3, the submucosa was enlarged and lymph nodule (PP) increased in number and are more conspicuous (Figure 5.2c). In T4 group, the submucosa was enlarged and lost its normal outline. Lymph nodules (PP) were more diffuse and muscularis mucosa became less distinctive (Figure 5.2d).

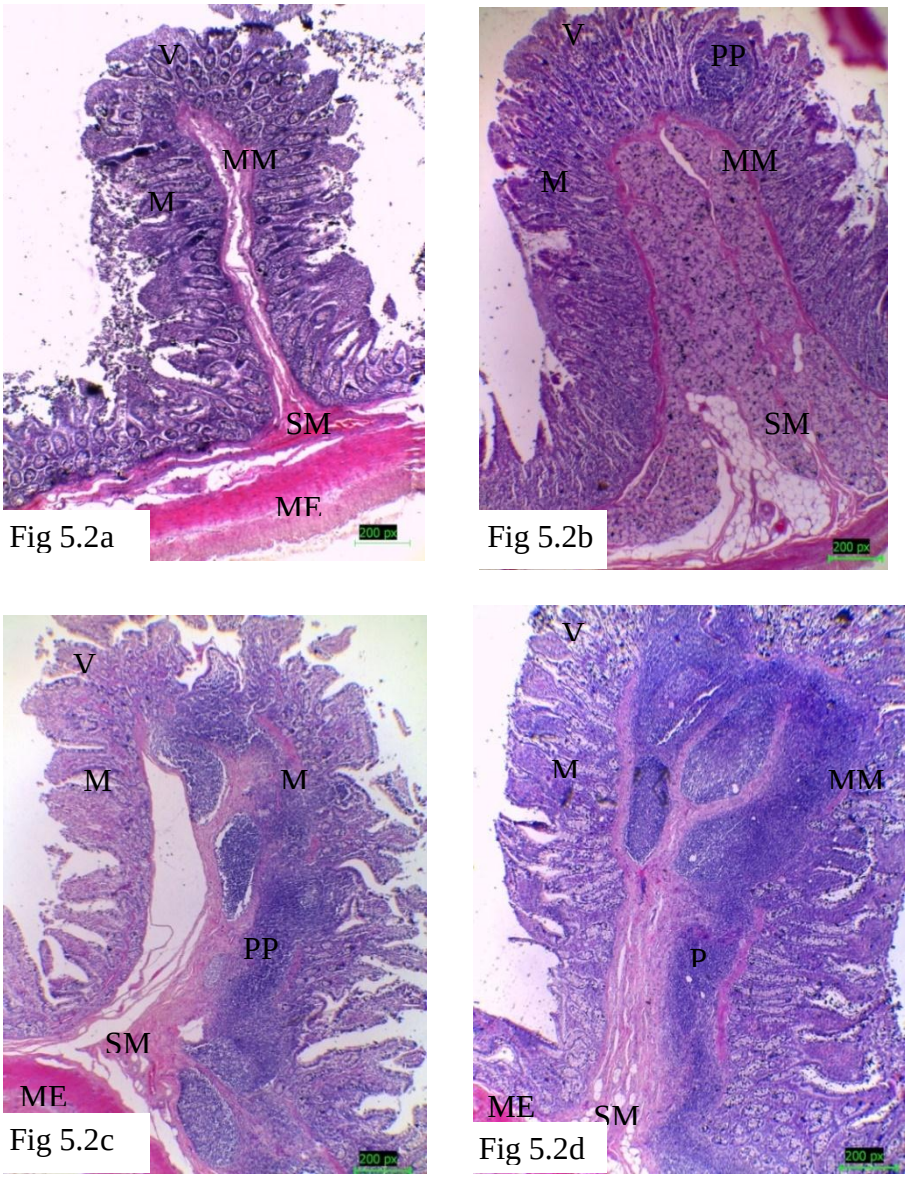

Figure 5.2: Cross sections of the ileum from pigs fed on varying levels of *Moringa oleifera* leaf meal.

Figure 5.2a: A histological section of ileum from control group of pigs (T1) showing normal histoarchitecture. Mucosa (M), muscularis mucosa (MM), submucosa (SM), muscularis externa (ME) and villi (V).

Figure 5.2b: A section of ileum from pigs in T2 group showing generally normal structure except enlarged submucosa infiltrated with fatty tissue. Mucosa (M), muscularis mucosa (MM), villi (V) and Peyer's patches (PP).

Figure 5.2c: An ileal section of pigs' in T3 showing enlarged submucosa (SM) and proliferation of Peyer's patches. Mucosa (M), muscularis mucosa (MM), muscularis externa (ME) and villi (V).

Figure 5.2d: An ileal section of T4 pigs showing further enlargement and fatty infiltration of submucosa (SM). Peyer's patches (PP) are also enlarged and prominent dominating the entire muscularis mucosa (MM) and part of the sub mucosa. Mucosa (M), villi (V) and muscularis externa (ME).

5.3.5 Histopathology of the colon from pigs fed on *Moringa oleifera* leaf meal diets

Colon section from the controls (T1), had normal histoarchitecture with the mucosal glands and submucosa clearly visible (Figure 5.3a). In the T2 group, just like the controls, the structure appeared normal with all the layers of the colon wall; mucosa, submucosa, muscularis, submucosa and the mucosal glands distinguishable (Figure 5.3b). In T3 group submucosa was slightly enlarged with fat deposits, and the mucosal glands decreased in numbers but have become more conspicuous (Figure 5.3c). Lastly, in T4, colon sections from the submucosa were slightly reduced in size, the mucosa appeared degenerated and the mucosal glands appeared reduced and their outlines nearly lost (Figure 5.3d).

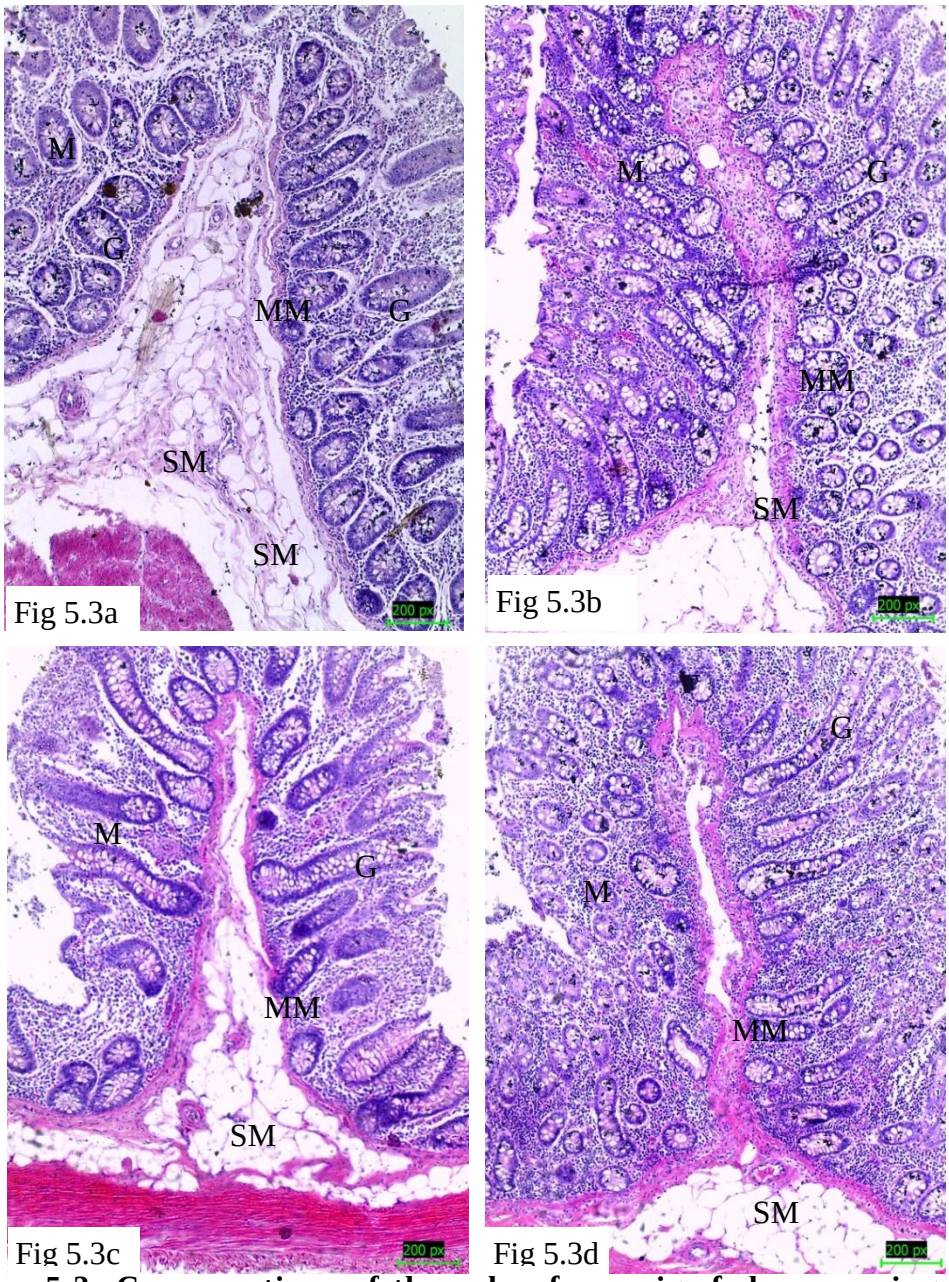

Figure 5.3: Cross sections of the colon from pigs fed on varying levels of *Moringa oleifera* leaf meal.

Figure 5.3a: Colon section from control pigs (T1) showing normal histological structure with conspicuously discernible mucosa (M) and submucosa (SM), muscularis mucosa (MM), mucosal glands (G).

Figure 5.3b: A section through the colon of T2 pigs showing normal histological structure. Mucosa (M), muscularis Mucosa (MM), and sub mucosa (SM), Submucosa, mucosal glands (G).

Figure 5.3c: A cross section of colon in T3 pigs showing slightly enlarged submucosa (SM) with fatty infiltration and reduced number of mucosal glands (G) though more conspicuous. Mucosa (M), muscularis mucosa (MM).

Figure 5.3d: A cross section of colon in T4 pigs displaying degenerated mucosa (M) and slight atrophy of the submucosa (SM). The mucosal glands (G) also appear fewer in number and degenerated. Muscularis mucosa (MM).

5.4 Discussion

The normal gastric pH in pigs is 4.4, small intestines 6.1 - 6.7, caecum 6.0 - 6.4 and colon 6.1-6.6 (Merchant *et al*., 2011). In this study, the pH of various gut sections varied across the treatments. This supports the findings by Beasley *et al.* (2015) who reported that gastric pH may vary within the species depending on the diet in which an animal is feeding on. From this study, gastric pH from the controls were slightly lower than 4.4. However, increased MOLM in the diet increased the gastrointestinal tract pH. These results are similar to those recorded by Nkukwana *et al.* (2015) in chicken where increased MOLM in the diet led to increased pH of the proventriculus as well as caecum. This is attributable to increased coarseness of the diet with higher levels of MOLM leading to higher viscosity. Furthermore, increased MOLM in the diet led to degeneration of the stomach mucosa which may have led to ineffective gastric juice secretion hence raising the pH of the stomach contents. The rich mineral content, especially calcium in moringa may have acted as a buffer, thus explaining the higher pH of the pig ingesta, with the increasing levels of MOLM in the diet. The duodenal, ileal, caecal and colon pH did not vary significantly with changes in MOLM levels in the diet.

Diet did not have a significant effect on the lactobacilli counts but the total faecal coliform counts increased with increasing MOLM in the diet. This implies therefore that higher levels of MOLM may have led to a higher pH which may have favoured the growth of coliforms. This supports the findings by Hammes and Vogel (1995) who reported that lower gut pH make the environment difficult for the coliforms to thrive.

High levels of *Moringa oleifera* (T3 and T4) negatively affected gut morphology. With increase in MOLM in the diet, there seemed to be increased goblet cells and inflammatory cell infiltration in the stomach. The submucosa seemed oedematous and in some instances, fat deposition was observed. The muscularis mucosa at higher levels of MOLM appeared degenerated and reduced in size. These results are similar to those of Hlophe and Moyo (2014) who reported a significant increase in the number of goblet cells and reduced villi heights in the small intestines with higher levels of MOLM in the diets in poultry. However, at lower levels, MOLM appeared to have enhanced the digestive tract of chicken and, for instance, Nkukwana *et al.* (2015) established that duodenal and ileal surface area for absorption was larger in the lower levels of *Moringa oleifera* in the diet and least in the controls and highest concentrations of MOLM. However, Madalla *et al.* (2014) recorded no obvious intestinal and liver pathological changes which could be linked to low levels of *Moringa oleifera* dietary treatment in fish. Submucosal oedema observed in the upper GIT such as the stomach and ileum has been attributed to inflammatory reactions in the gut and maybe of dietary origin. This has also been observed as a reaction to some drug administration in rodents.

Lymphatic nodules (Peyer's patches) are an important feature of the ileum. They are found in the lamina propria and the submucosa of the ileum. They are the immune organs of the intestines due to their ability to counter luminal antigens and bacteria (Jung *et al.,* 2010). Furthermore, they are responsible for tolerance to both foreign antigens as well as commensal microbiota in the gut (Mescher, 2009). The increase in numbers or the hyperplasia of Peyer's patches has been associated with gastrointestinal mediated allergy (Van Weyenberg and Jacobs, 2013). Furthermore,

this increase in the lymphoid tissue could imply enhanced immune mucosal response to gut antigen stimulation or as a result of infection (Krauss *et al.,* 2010). At lower levels, herbal remedies due to their carbohydrate and polysaccharide combinations with lignin have been reported to modulate enteric immune functions by enhancing production of immunoglobulin A and may also stimulate systemic defence reactions. However, at higher levels, hyperplasia of the enteric lymphoid tissue may arise from proteinaceous phytochemicals that are toxic to the animal hence eliciting immune reactions presenting in the Peyer's patches reaction.

In conclusion, MOLM in the diet may be used at lower levels since higher levels (> 3% in the diet) affects the gastrointestinal pH negatively and may enhance the multiplication of coliforms, some of which may be pathogenic. Furthermore, this may extend to influence negatively the gastrointestinal morphology, hence, affecting the nutrient utilization and decreased animal productivity.

CHAPTER SIX

HISTOPATHOLOGICAL EFFECTS OF *Moringa oleifera* LEAF MEAL DIETS ON THE SPLEEN, LIVER AND KIDNEY OF GROWING PIGS

Abstract

The rich nutritional value of *Moringa oleifera* has been used over the years both as food and medicine by man and animals and the main plant parts utilized are the leaves and seeds. Despite increased usage, little information is available on its application as a nutraceutical agent in pigs especially in regard to its toxicity. The objective of this study was to determine the histopathological consequences of prolonged inclusion at varying levels of *Moringa oleifera* leaf meal (MOLM) diets on the spleen, liver and kidneys of growing pigs. Twenty four growing pigs were selected and assigned to four treatment diets (T) containing; 0% (T1), 3% (T2), 6% (T3) and 12% (T4) MOLM concentrations. Each treatment had two replicates of 3 pigs and the experimental period lasted 7 weeks after which four pigs from each treatment were selected, sacrificed and the spleen, liver and kidney samples collected for histopathological analysis. Grossly, the spleen, liver and the kidney weights across the treatment groups did not vary significantly. The histological results however revealed that increased MOLM in the diet (>3%) led to red pulp atrophy, enlargement of splenic follicles (white pulp) as well as capsular and parenchymal fibroses. In the liver, increased MOLM in the diet led to loss of lobular architecture with damaged cellular outlines, dilation of sinusoidal spaces, vascular congestion and occasional nuclear changes in hepatocytes leading to hepatocytic necrosis and distortion of the portal triad. In the kidneys, higher levels of MOLM led to glomerulonephritis essentially presenting as

glomerular edema leading to reduced Bowman's space. In the renal tubules, there were protein casts in the tubular lumen. Prolonged inclusion of MOLM (>3%) in the diet negatively affected the histoarchitecture of the spleen, liver and the kidneys and may, in extreme circumstances, result in reduced animal performance as a result, inefficiency in red blood cell formation and detoxification by the liver and the kidneys.

Key words: Histopathology, Nephrotoxicity, *Moringa oleifera*, Hepatotoxicity, Spleen, Growing pigs

6.1 Introduction

The rich nutritional composition of *Moringa oleifera* (MO) has resulted in its adoption for numerous purposes such as human food, animal feeds and medicinal purposes (Richter *et al.*, 2003; Sánchez *et al.*, 2006; Popoola and Obembe, 2013). Despite recorded higher growth rates in MO based diets in majority of the animal nutrition studies, depressed feed intakes and reduced growth rate associated with higher levels of MO in the diet have similarly been documented (Gakuya *et al.*, 2014). This implies therefore that MO have phytochemicals such as flavonoids and tannins that influence either positively or negatively the physiological functioning of the body systems through modes of action that have not been conclusively established.

Histopathology can be used to assess the toxicological effects of feed ingredients used in formulations of animal feeds, with the key organs of interest being the liver and kidneys due to their roles in detoxification. Liver functions include; protein and lipid synthesis, Liver metabolises toxins ingested or generated in the body into less harmful molecules that can be excreted in urine (Tang *et al.*, 2013). Spleen is also important

since it is involved in haematopoietic and immune functions and would also give an indication on how the diet affects these two important body functions. Moreover, spleen is the site of both direct and indirect detoxification and many systemic or generalized diseases have splenic involvement (Suttie, 2006).

In rats, *Moringa oleifera* leaf extracts exerted structural improvement on the kidney tissues translating into improved physiological functioning (Owolabi and Ogunnaike, 2014). Later, it was established that aqueous extract of *Moringa oleifera* seed, if consumed at higher quantities (greater than 800mg/kg), negatively affect the normal histological appearance of the spleen causing mild and moderate expansion of white pulp leading to a decrease in WBC count and platelet concentration in rats (Dike and Luteino, 2015).

At moderate doses, MO leaf extract ingestion has been found safe for the renal tissues and has shown nephron-protective effects in cases of drug administration (Paliwal *et al.,* 2011; Awodele *et al.,* 2012; Ouédraogo *et al.,* 2013; Ezejindu *et al.,* 2014, Okumu *et al.,* 2017). However, other studies have established that moringa leaf consumption at higher doses or chronic use could predispose animals to hepatic and kidney damage and may result in renal failure (Oyagbemi *et al.,* 2013; Owolabi and Ogunnaike, 2014).

In spite of the increased use of MO plant in animal feeds and research, there is no consensus on the optimal levels of inclusion in animal diets and, even so, their effects on the spleen, liver and kidney morphology remain conflicting. This study therefore was designed to determine the effects of prolonged inclusion of different levels of MO plant leaves in the pig's diet on the spleen, liver and kidney structures in growing pigs.

6.2 Materials and Methods

6.2.1 Study site

See sub section 3.2.1

6.2.2 Animals and experimental design and treatment diets

See sub section 3.2.2

6.2.3 Treatment diets

See sub section 3.2.3

6.2.4 Animals housing design

See sub section 3.2.5

6.2.5 Sample collection for histopathological analysis

After slaughtering of the pigs, abdominal cavity were opened and after manual evisceration, visceral organs from four randomly selected pigs per treatment (spleen, liver and kidneys) were weighed after which approximately 50 grams from each organ picked for histopathological analysis. Small pieces of each organ were fixed by immersion in phosphate buffered formalin (10%) and routinely processed (ethanol dehydration) to serial paraffin sections (5 µm thick) which were then stained with haematoxylin and eosin. The stained sections were examined and photographed using a Leica ® DM 500 light microscope. Photographs of the tissue sections from the treatment groups were analyzed for any changes by comparing to the control group. Tissue analysis was done using qualitative methods with emphasis on the histomorphology and general histoarchitecture.

6.2.6 Data management and analysis

Data on the spleen, liver and kidney of pigs were stored in the Ms office excel and subsequently exported to statistical pack SAS v9.0 SAS Institute Inc (2002), for the generation of means and frequencies, and to establish whether there were statistical differences between the treatment groups through the Analysis of Variance.

6.3 Results

6.3.1 General body condition of the pigs

The general body conditions of the pigs across the treatments were good and no animal mortality was observed during this study. The starting pig weights averaged 26kg while the final weights were 67.3kg (T1), 66.3kg (T2), 65.1kg (T3) and 64.9 (T4). With advancement in the experimental time, there appeared to be a reduction in the rates of weight gains in pigs with higher levels of *Moringa oleifera* in the diet. However, final weights for the different treatment groups did not differ significantly ($P>0.05$).

6.3.2 Effects of *Moringa oleifera* leaf meal diets on organ weights

The liver, spleen and kidney weights for the T1, T2, T3 and T4 did not differ significantly ($P>0.05$) (Table 6.1).

Table 6.1 Average (± Standard deviation) liver, spleen and kidney weights from pigs fed on different levels of *Moringa oleifera* leaf meal diets

	Dietary treatments (#MOLM inclusion levels, %)				
Organ	**0% MOLM**	**3% MOLM**	**6% MOLM**	**12% MOLM**	**P Value**
Liver (g)	1294.2±212.3	1589.0±127.9	1627.5±101.1	1653.8±98.7	0.52
Spleen (g)	107.0±25.1	164.7±17.0	133.3±16.7	108.5±19.1	0.31
Kidney (g)	165.2±51.7	229.1±41.9	187.7±42.2	299.3±42.2	0.28

#MOLM= *Moringa oleifera* leaf meal, g= Grams.

6.3.3 Histopathological changes in the pigs' spleen attributable to *Moringa oleifera* leaf meal

Spleen from control (T1) and 3% MOLM experimental groups had normal histoarchitecture where the white pulp contained splenic lymphoid follicles embedded within the red pulp and trabecular extensions of the capsule extended into the tissues (Figures 6.1 a and b). The follicles appeared as nodular aggregations of lymphocytes surrounded by the red pulp (Figure 6.1 c and d). The splenic parenchyma was enveloped by an extensive capsule (Figures 6.1 e and f). Spleen from T3 group, on the other hand, showed red pulp atrophy characterized by reduced red blood cell cellularity (Figure 6.2a). This atrophy was further aggravated in T4 (Figure 6.2b), leaving prominent white pulp in both cases. Red pulp atrophy was accompanied by white pulp fibrosis in T3 (Figure 6.2c) culminating into mild focal hyperplasia in T4 (figure 6.2d). The degeneration of parenchyma was further accompanied by fibroses (Figures 6.2 e and f).

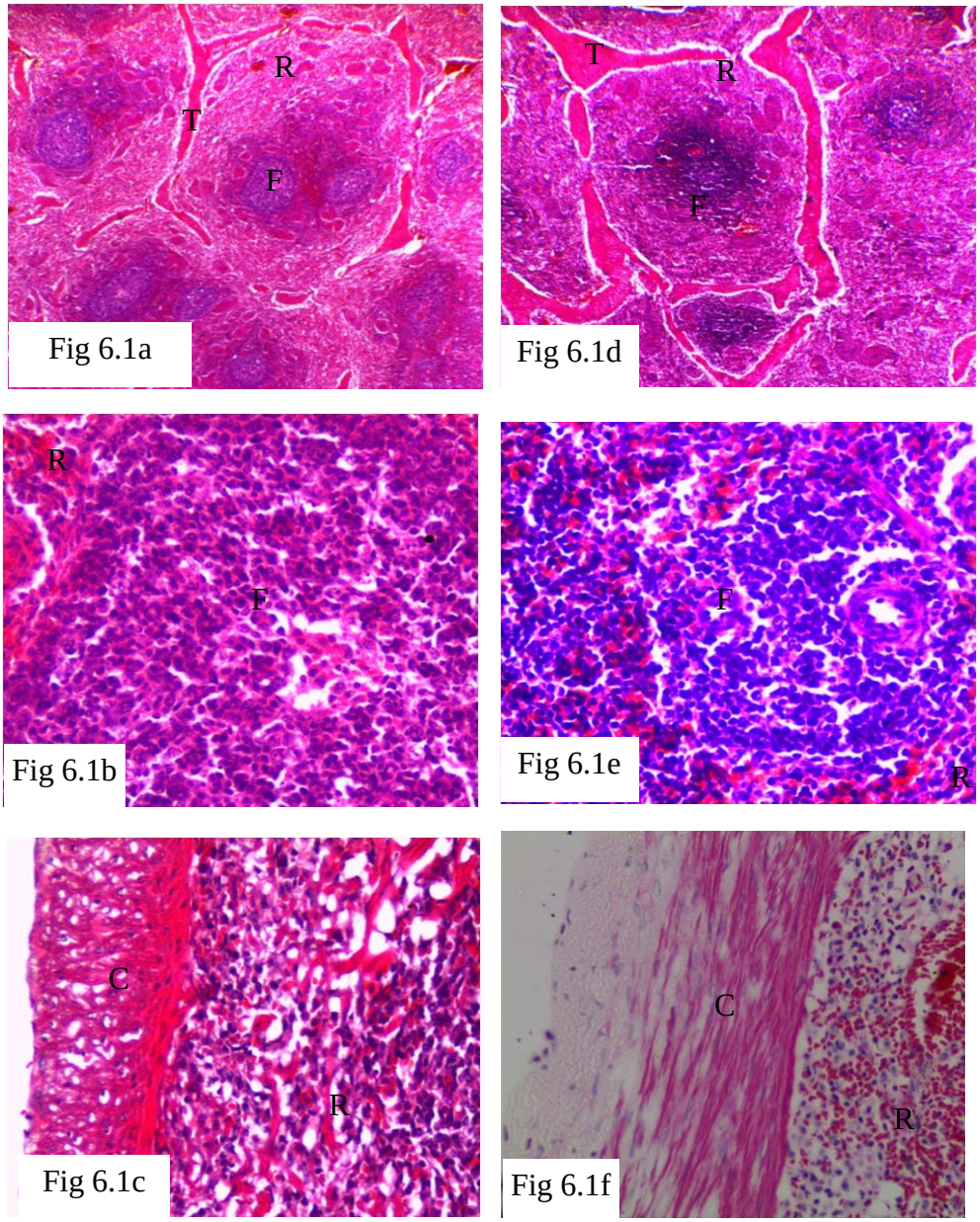

Figure 6.1: Sections of the normal spleen parenchyma in the control (a-c) and 3% (d-f) *Moringa oleifera* leaf meal experimental groups.

Control, Figures 6.1 (a, b and c) and 3% *Moringa oleifera* leaf meal group (Fig 6.1 d, e and f) showing in both cases the normal red pulp (R), white pulp (F) and trabecular (T) extensions of the capsule into the tissue (figures 6.1 a and d). The white pulp (F) appears as nodular aggregations of lymphocytes surrounded by red pulp (R) (Figures 6.1 b and e). Underneath the capsule (C) lies normal red pulp (R) tissue (Figures 6.1 c and f).

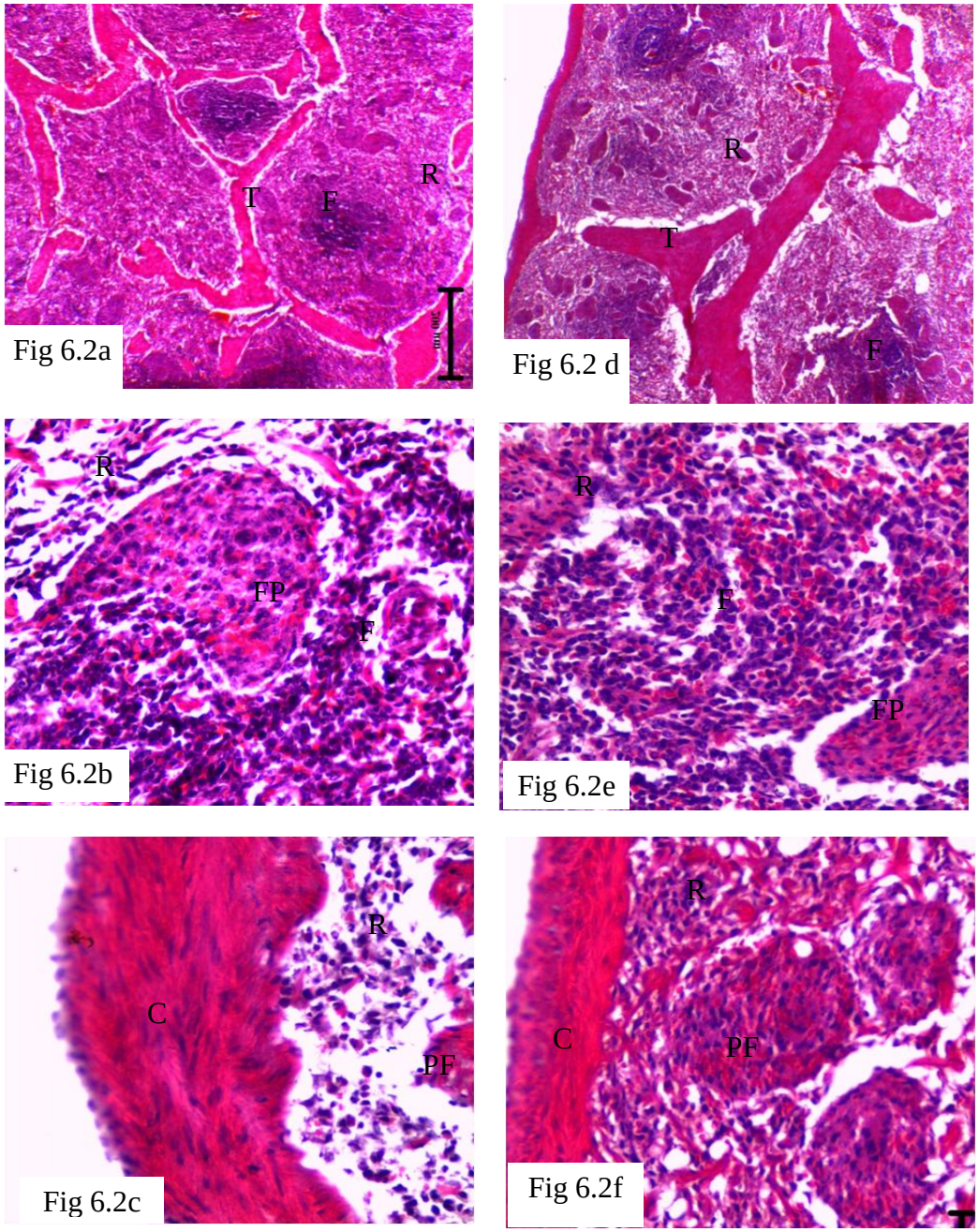

Figure 6.2: Sections through splenic parenchyma in 6% (a-c) and 12% (d-f) *Moringa oleifera* leaf meal treatment groups exhibiting various degrees of structural alterations.

In 6% *Moringa oleifera* leaf meal, the red pulp (R) demonstrated red pulp atrophy characterized by reduced cellularity (Figure 6.2a). This was further aggravated in 12% *Moringa oleifera* leaf meal (Figure d) and in both cases; this atrophy left prominent white pulp (F). Trabeculae (T) extended into parenchyma from the capsule and appear expanded. Accompanying the red pulp atrophy were the white pulp (F) atrophy in T3 (Figure 6.2b) culminating in white pulp hyperplasia in T4 (Figure 6.2e). In both cases, capsular and (C) and parenchyma fibrosis (PF) were common features (Figure 6.2c and f).

6.3.4 Histopathological effects of *Moringa oleifera* leaf meal diets on the growing pigs liver

The liver in controls (T1) showed normal lobular architecture conspicuously outlined by the surrounding interlobular septae (Figure 6.3a). In T2, the liver also appeared normal with normal lobular architecture and cellular outline (Figures 6.3 c and d) but the sinusoids showed mild dilatation around the central veins. Vascular congestion was also evident at the hepatic triads (Figures 6.3e, f). In the T3 and T4 groups, the liver maintained normal lobular outline as in T2 but with apparent loss of lobular architecture (Figures 6.4 a, b) characterized by hepatic degeneration presented as damaged cellular outlines, nuclear pyknosis in hepatocytes and leucocytic infiltration (Figures 6.4 c, d). In addition, there was sinusoidal congestion. These changes were comparatively more amplified in T4 group leading to hepatic necrosis. In both cases, there was vascular congestion around central veins (Figure 6.4 e, f).

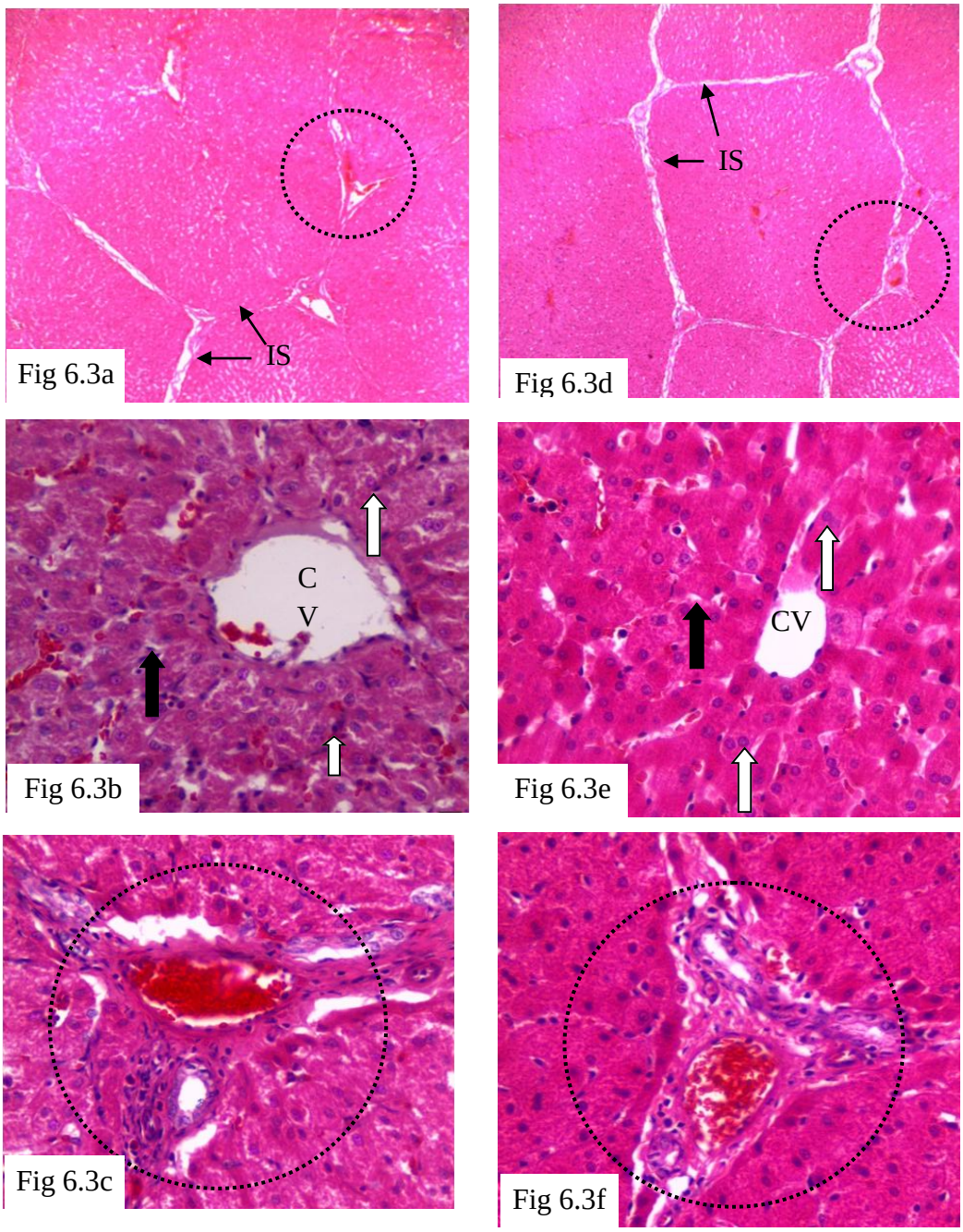

Figure 6.3: Sections through liver parenchyma of control (a-c) and 3% (d-f) *Moringa oleifera* leaf meal treated groups.

In control, and 3% *Moringa oleifera* leaf meal the lobules show normal architecture and are conspicuously demarcated by the interlobular septae (IS) (Figures 6.3 a, d). Hepatic triads are clearly demonstrated (hatched circle). Hepatocytes are normal with normal cellular outlines (white block arrows) but the sinusoids appear mildly dilated (black block arrows) around the central veins (CV) (Figures 6.3 b, e). In both cases, vascular congestion was evident hatched circle (figures 6.3 c, f).

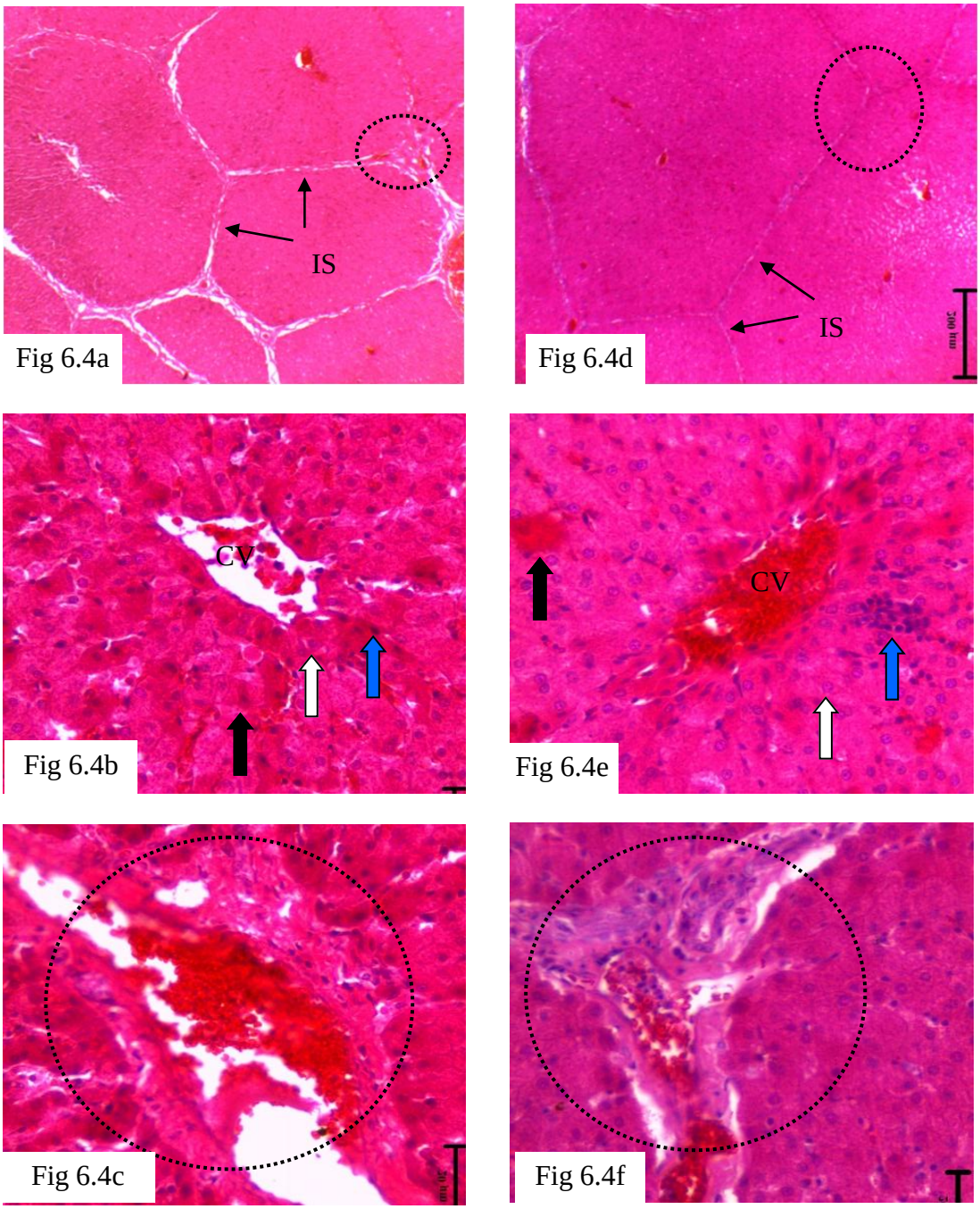

Figure 6.4: Sections through the liver parenchyma of pigs in groups 6% (a-c) and 12% (d-f) *Moringa oleifera* leaf meal.

The liver lobules in T3 and T4 showed normal outline but with apparent loss of lobular architecture (Figure 6.4 a, d). There was apparent hepatocytic degeneration characterized by nuclear pyknosis (white block arrow) and leucocytic infiltration (blue block arrow) (Figures 6.4 b, e). These changes were more aggravated in T4 group compared to T3. Accompanying these changes was sinusoidal congestion (black block arrow). Vascular congestion around the hepatic triad (hatched circles) was also evident (Figures 6.4 c, f). IS=interlobular septae, CV=central vein.

6.3.5 Kidney histopathological changes occurring as a result of *Moringa oleifera* leaf meal diets

The kidneys of controls (T1) and T2 groups displayed normal architecture (Fig 6.5 a-f). The glomerulus appeared normal, and clearly delineated by the Bowman's capsule as well as the parietal squamous cell epithelium and circumscribed by a normal Bowman's space. The glomerular tuft appeared lobulated. In T3, the kidney showed extra capillary proliferative glomerulonephritis presented in the form of edema and congestion of the glomerulus with mononuclear infiltration (Fig 6.6a-c). Kidneys of T4 group showed extra capillary necrotizing glomerulonephritis (Fig 6.6d-f). There were sharply demarcated sub-capsular necrotic areas with inflammatory cells surrounded by oedema and severe glomerular degeneration. Renal tubules had cystic dilatations and the tubular lumina were completely obliterated and filled with fluid. Protein casts were observed in the lumina of tubules and the tubules seemed to be surrounded by cell debris (Figure 6.6 c, f).

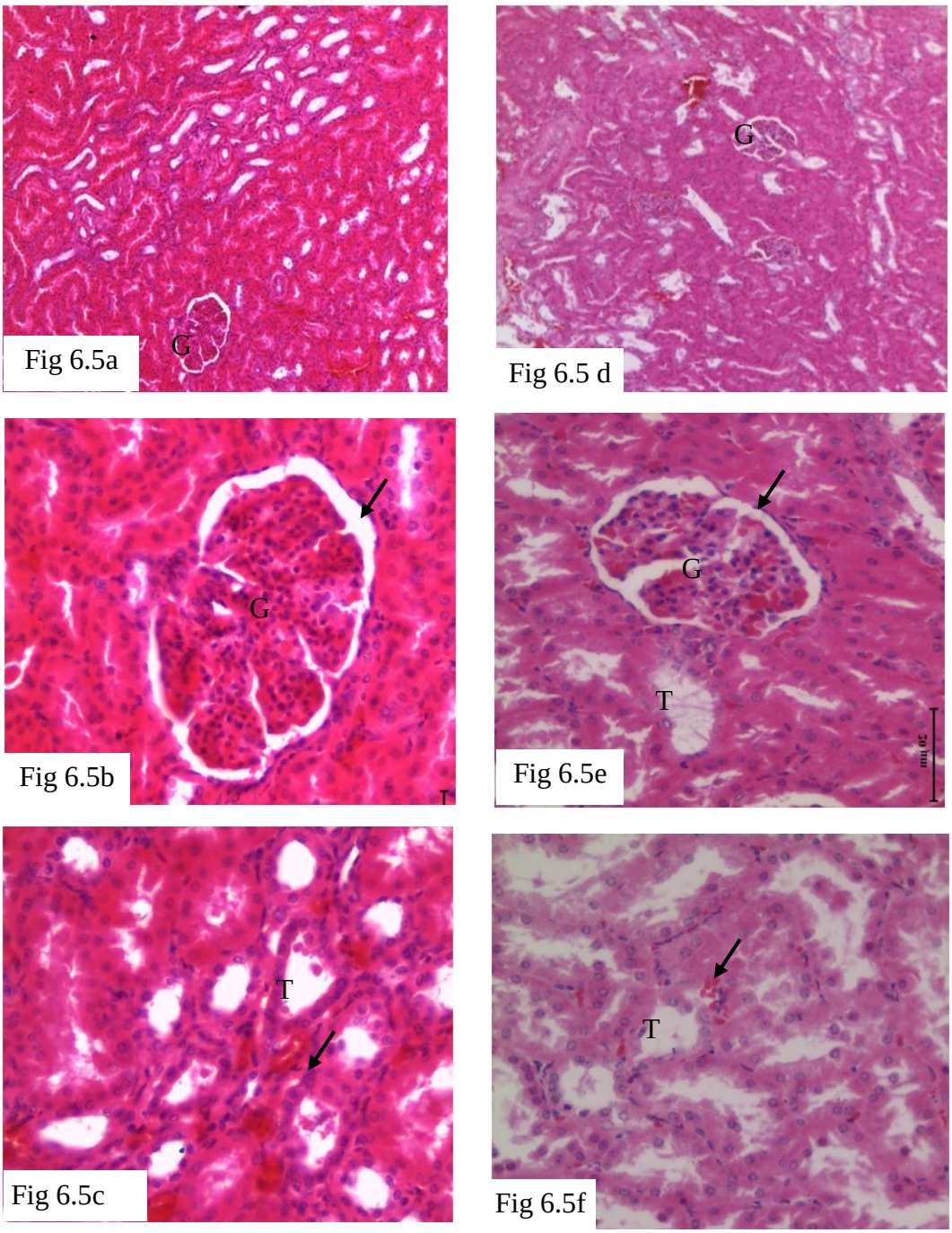

Figure 6.5: Sections through kidney parenchyma of the control (a-c) and 3% (d-f) *Moringa oleifera* leaf meal pigs respectively.

Figures 6.5a (control) and 6.5d (3% *Moringa oleifera* leaf meal) show normal architecture with glomeruli (G) delineated by Bowman's capsule circumscribing the normal Bowman's space (BS) (Figures Fig 6.5b and Fig 6.5e). The tubules (T) appeared normal (Figures 6.5c and 6.5f). G=Glomerulus, BS=Bowman's space.

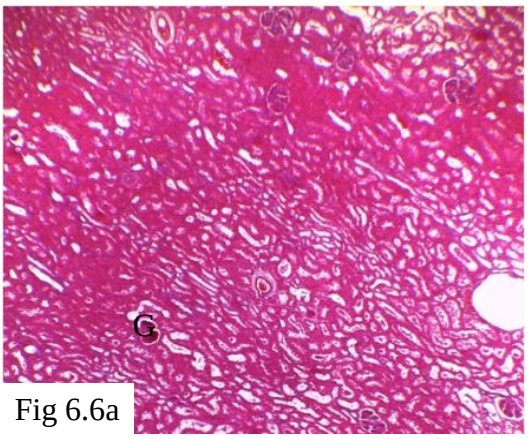

Fig 6.6a

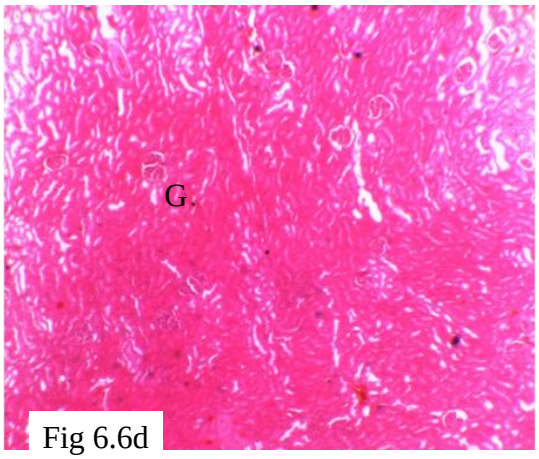

Fig 6.6d

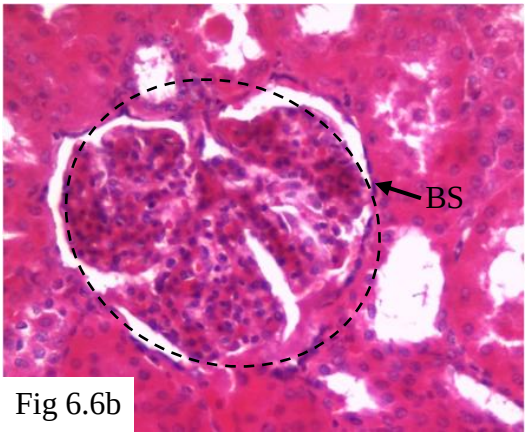

Fig 6.6b

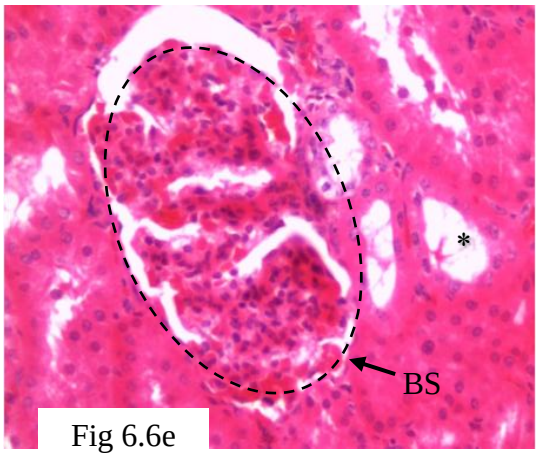

Fig 6.6e

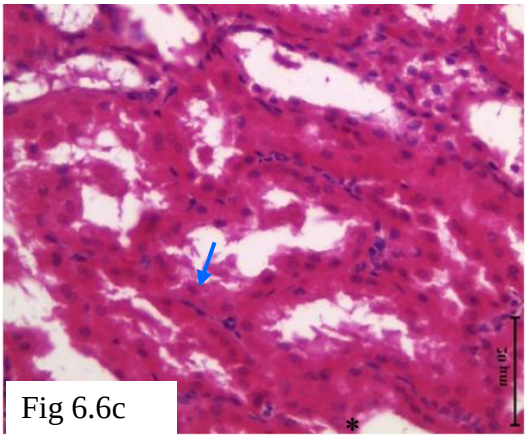

Fig 6.6c

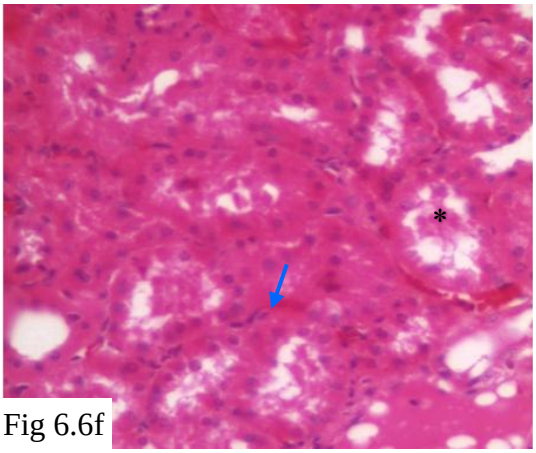

Fig 6.6f

Figure 6.6: Sections through the kidneys of 3% (a-c) and 12% (d-f) *Moringa oleifera* leaf meal.

Figure 6.6b and 6.6d show extra capillary proliferative glomerulonephritis (hatched circle) presented as oedema and congestion of glomeruli (G) with mononuclear infiltration (Figure 6.6b). In T4, there is progression of the inflammatory reaction to the extra capillary necrotizing glomerulonephritis (hatched circle) appearing as sharply demarcated sub capsular necrosis, edema and severe hydropic glomerular degeneration (Figure 6.6e). Tubular degeneration accompanied by cystic dilation (T) proteinaceous material filled lumen (asterisk) and perivascular edema and congestion (blue arrow) are evident (Figures 6.6c and 6.6f). G=glomerulus, BS=Bowmans space.

6.4 Discussion

Throughout the experiment period, no mortalities were recorded in the pigs and the overall pig growth rates did not vary significantly. Spleen contains hematopoietic and lymphoid elements, hence it is a primary site for extramedullary haematopoiesis and also removes degenerate, aged red blood cells as well as particulate materials and circulating bacteria from the blood circulation (Suttie, 2006). Results from this study show that MOLM in higher concentrations (>6%) in diet resulted in alterations in splenic histoarchitecture characterized by parenchyma and capsular fibrosis, reduced cellularity, white pulp hyperplasia and degenerated red pulp. Parenchyma fibrosis have also been reported in previous studies and mainly occurs in association with inflammatory, toxic, or neoplastic lesions of the spleen (Suttie, 2006). In this case parenchyma fibroses may have resulted from toxicity arising from the phytochemicals (tannins and flavonoids) present in MO leaves. Toxicity from plants has been associated with phytochemicals stored in plant organs (El Hilaly *et al.,* 2004). It is therefore plausible to suggest that higher concentrations of MOLM in the diet led to accumulation of tannins, flavonoids and other phenolic compounds in the animal's body systems and may have resulted in the inflammatory reactions in the spleen. This is supported by the findings of Dike and Luteino (2015) who reported that, sections of the rat's spleen treated with 800mg/kg and 1600mg/kg aqueous extract of *Moringa oleifera*, showed mild and moderate expansion of white pulp. Consequently, it is logical to suggest therefore that MOLM in the pig diets should be included at lower

levels (<6%) to avoid negative effects on the spleen which could ultimately impair its performance.

The liver is an accessory organ of the digestive system and plays a key role in blood detoxification. This means therefore that if the diet had some toxic elements, the first organ to be affected is the liver. In controls (T1) and the lower levels of MOLM (T2) the normal histological structures of the liver were retained. At higher concentrations of MOLM in the diet (T3 and T4), it appeared MOLM had some mild toxic effects since the normal hepatic outline appeared lost and central veins appeared congested. Hepatic necrosis was also noted as a sign of hepatitis. Hepatic reactions at increased levels of MOLM (>3%) may have been due to the accumulation of tannins, flavonoids and other phenolic compounds in the liver. These results confirm previous reports which suggested low levels of MO (2%) had no negative effects in rat liver Owolabi and Ogunnaike (2014), but chronic use could predispose animals to hepatic and kidney damage Oyagbemi *et al.* (2013). Indeed, this latter suggestion seems to have vindicated the result of high level use of MOLM in this study. Tannins, flavonoids and phenolic compounds are known to have serious effects on the liver structure and function.

Kidneys play an important role in excretion mainly through ultra-filtration and if affected negatively by any dietary compound, the detoxification process may be compromised and subsequently, the normal functioning of the body may as well be affected (Paliwal *et al.*, 2011). In this study, higher levels of MOLM in the diet negatively affected the glomeruli, renal tubules and renal cells. These results seems to contradict those of Ezejindu *et al.* (2014) where it was reported that *Moringa oleifera*

extracts did not have a negative effect on the renal histo-architecture of Winster rats. This may be attributed to the lower dosages and short duration of administration of *Moringa oleifera* extracts in their study as opposed to this study where the duration was relatively longer. Oedema noted in the glomeruli may have resulted from the obstruction of ultra-filtration process caused by the phenolic compounds found in MOLM. Furthermore, these compounds in MOLM diets may also have had a negative effect on the renal cells hence the observed marked nephritis in the highest concentration of MOLM (12%) in the diet.

In conclusion, MOLM can be included in the diet of pigs up to a level of 3% after which it will exert toxicological effects on the spleen, liver and the kidneys, and under a prolonged inclusion in the diet may cause organ failure and consequently may result in death of the animal. However, further studies need to be conducted to ascertain the exact nature of phytochemicals in the MO leaves associated with the histopathological changes observed in these organs.

CHAPTER SEVEN

GENERAL DISCUSSION, CONCLUSIONS AND RECOMENDATIONS

7.1 General Discussion

Moringa oleifera leaves harvested from Kibwezi field station were fresh (at a younger stage) translating to the relatively high crude protein levels (27.3%) and low crude fiber levels (8.9%). These imply that, it can be included in the non-ruminant feeds without causing digestive exertion. However, there are some variations in the nutritional compositions recorded in various parts of the world attributable to differences in climatic zones and physiological stages of harvesting (Samkol *et al.,* 2005; Gakuya *et al.,* 2014). No mortalities were observed in this study thus MOLM can be tolerated by pigs to a high level of 12% in the diet. This support the findings that MOLM has relatively low levels of saponins, tannins and phenols that are toxic to animals, hence high levels are well tolerated in the diet (Gupta *et al.,* 1989). Such tolerance has been recorded in rabbits and chickens (Nuhu, 2010; Gakuya et al., 2014). In this study though, the experiment ran for seven weeks and it is however postulated that if the study had continued for a further prolonged period, at levels greater than 3% in the diet might have resulted in mortalities due to its toxicological effects recorded on various tissues and organs in the body. High protein content in MOLM and high digestible proteins in diet have been shown to improve nutrient digestibility and utilization, hence, enhancing growth performance in animals (Gakuya *et al.,* 2014; Mukumbo *et al.,* 2014). This was also observed in this study where pigs at 3% MOLM had the highest daily weight gains as well as feed conversion efficiency. However, at high levels of MO (12%) in the diet, there were

lower growth rates and feed conversion efficiency, a situation similar to that observed by Liaqat *et al.* (2016). Apart from better growth performance at lower levels of MO, MOLM in the diet appeared to have had additional positive effects in pigs. For instance, MOLM at 3% and 6% in the diet enhanced red blood cell parameters especially haemoglobin concentrations and red blood cell counts. This may have boosted the oxygen circulation in the body therefore enhancing the nutrient utilization by the animals and thus improved overall growth performance. *Moringa oleifera* leaf meal at concentrations of 3% and 6% in the diet also led to increased total cell counts, an indication of enhanced cellular immune response therefore increasing the chances body defence against diseases, thus enhancing the feed conversion efficiency and overall growth performance. High cholesterol levels in an animal's diet have been associated with slower growth rates. In this study, increased MOLM in the diet reduced significantly total blood cholesterol in pigs, even though pigs taking 12% of MOLM had increased cholesterol levels, a feature that may need further investigation. Reduced total cholesterol observed in this study therefore, may have enhanced nutrient utilization and growth in pigs. The hypocholesterolaemic effects exerted by MO are due to lowered concentrations of LDL by B-sitosterol, the bioactive phyto constituents of *Moringa oleifera*, with similar structure to cholesterol that inhibit cholesterol synthesis in the liver through negative feedback (Ghasi *et al.,* 2000; Ghebreselassie *et al.,* 2011). This is an attribute that may be of great use in humans, as MO tends to reduce cholesterol thus protecting against cardiovascular diseases such as hypertension, stroke and heart attack. *Moringa oleifera* leaf meal is rich in vitamins, protein and minerals such as iron required for the formation of haemoglobin and this leads to improved growth performance (Olugbemi *et al.,* 2010; El Tazi and

Tibin, 2014). In this study, MOLM level of 6% improved haemoglobin concentration in blood after which the levels declined significantly. The decline in haemoglobin and red blood cell levels at higher levels of MOLM, can be attributed to toxicities associated with high levels of flavanoids and tannins in plant leaves (El Tazi and Tibin, 2014). A high mean cell volume recorded at 12% MOLM is undesirable and implies increased release of immature RBCs or increased iron or folic acid levels or it may be due to impairment of liver functions caused by high flavonoid levels present in MOLM (Fahey, 2005). It implies therefor that MOLM should be used in moderation as high levels (>3%) may negatively affect these red blood cells indices.

High vitamin and protein concentrations in the diet led to improved immune system in animals, as indicated by high body defence cell levels (Gupta *et al.,* 2012). This is important since the treatment groups would be able to fight diseases compared to the controls, hence, may minimize drug usage reducing costs of production and subsequently improve safety of the pork. *Moringa oleifera* methanol extract stimulate both cellular and humoral immune systems attributable to flavonoids, polyphenols and terpenoids (Gaikwad *et al.,* 2011). In this study, the 3% MOLM diet groups had the highest white blood cell counts while 0% MOLM group (controls) had the least counts. Mid - range absolute cell concentrations also increased with increased MOLM in the diet indicating enhanced immunity. This has previously been reported in chickens where inclusion of moringa leaf meal in broiler diet as a vegetable protein source appeared to have enhanced immune response to Newcastle disease and infectious bursal disease vaccination without any change in weight gain, body organ weight, and blood haematology (Liaqat *et al.,* 2016).

In this study, MOLM in the diet did not have an effect on the carcass quality. The measured parameters such as carcass dressing percentage, back fat thickness, loin eye area and carcass lengths did not vary significantly with variations in MOLM in diets. This observation is similar to that recorded in chicken and pigs, and in fact, in some instances MOLM enhanced the colour and prolonged the refrigeration period of the meat (Gakuya *et al.*, 2014; Mukumbo *et al.,* 2014; Oduro-Owusu *et al.,* 2015).

he normal gastric pH in pigs is 4.4, small intestines 6.1-6.7, caecum 6.0-6.4 and colon 6.1-6.6 (Merchant *et al.*, 2011). In this study, the pH of various gut sections varied across treatments. Gastrointestinal pH can vary within an animal species depending on the diet (Beasley *et al.*, 2015). In this study, gastric pH from the controls was slightly lower than the normal. Increased MOLM in the diet, on the other hand increased significantly ($P<0.05$) the gastrointestinal pH. In chicken, increased MOLM in diet led to increased pH of the proventriculus attributed mainly to increased coarseness of the diet leading to higher viscosity, hence, higher pH (Nkukwana *et al.*, 2015). Furthermore, increased MOLM in the diet led to increased number of goblet cells which may have led to increased mucous secretion in the stomach thereby increasing the pH.

Diet did not have a significant effect on the lactobacilli counts. However, total fecal coliform counts increased with increasing MOLM in diet. This implies therefore that high levels of MOLM led to a higher pH which may have favoured the growth of the coliforms (Hammes and Vogel, 1995). High levels of coliforms in the gut may be undesirable in animal production owing to the fact that some, especially the *Escherichia coli* and Salmonella, may be highly pathogenic. They also increase the costs of production and also make the animal products unsafe for human consumption

due to possible transmission to humans. This then imply that high levels of Moringa in the diet may cause gut microbial disturbance and may also result in higher disease incidences as well as reduced growth rate of the pigs. It is important therefore that any inclusion of MO in the diet is done in moderation (that is at levels <3%).

A significant increase in the number of goblet cells and reduced villi heights in poultry fed with high levels of MOLM have previously been reported (Hlophe and Moyo, 2014). However, at lower levels, MOLM appeared to have enhanced the digestive tract of chicken (Nkukwana *et al.*, 2015). In this study, the stomach submucosa seemed oedematous and, in some instances, fat deposition was observed. The muscularis mucosa at higher levels of MOLM appeared degenerated and reduced in size and may have been one of the causes of poor performance in pigs on high levels of Moringa. In the ileum, also observed was the pronounced hyperplasia of the Peyer's patches with increased levels or MOLM in the diet. Peyer's patches are immune organs of the gut and their prominence is influenced by dietary features; either presence of allergens or if the dietary features have an immunomodulatory effect. In this study, the overall pig growth performance may have been a reflection of the changes observed as a result of the different levels of *Moringa oleifera* in the diet. The immunomodulatory effects of moringa may have been as a result of improved immune system at the gut level, also reflected in the spleen, evidenced by the enlargement of the white pulp and subsequently the high levels of the white blood cell counts observed in the haematological results. This therefore means that if proper levels of Moringa are included in the diet, it may reduce the need to use the antibiotics or other synthetic chemical immune boosters to protect the animals against diseases.

Spleen is a hematopoietic and lymphoid organ and is a primary site of extramedullary hematopoiesis (Suttie, 2006). Results from this study show that MOLM in high concentrations affected negatively the normal spleen architecture including red pulp atrophy, white pulp hyperplasia parenchymal and capsular fibroses. Toxicity from plants arises mainly from phytochemicals stored in plant organs (El Hilaly *et al.*, 2004). *Moringa oleifera* leaf meal contains tannins, flavonoids and other phenolic compounds at lower concentrations (Gupta *et al.*, 1989). In this study, MOLM was included in pig's diet for a prolonged period and may explain the negative effects in the pig organs and subsequently the overall growth performance due to the accumulation of these toxins in the body.

The observed parenchymal and capsular fibroses observed here are similar to those associated with inflammatory, toxic or neoplastic lesions of the spleen. Sections of the rat's spleen treated with 800mg/kg and 1600mg/kg aqueous extract of *Moringa oleifera* have also shown mild and moderate expansion of white pulp (Dike and Luteino, 2015). These changes in the splenic morphology may have also affected haematological parameters such as the white blood cells as well as the red blood cells levels. The expansion of the white pulp indicates increased white blood cell flow while red pulp atrophy imply interference with red blood cell formation therefore low red cell levels as well as low haemoglobin concentrations and therefore reduced growth at higher levels of MO in the diet.

Flavonoids present in MOLM have been shown to have hepatoprotective features and when fed at lower levels to rats, it did not affect negatively liver histological appearance (Dike and Luteino, 2015). However, chronic use could lead to accumulation of tannins, flavonoids and other phenolic compounds and may

predispose animals to hepatic and kidney damages (Oyagbemi *et al*., 2013). In this study, MOLM at high concentrations (>6%MOLM) in the diet, affected negatively the hepatic histological appearance, central veins were congested and invasion by lymphoid cells. Hepatic necrosis was also noted as a sign of hepatitis. At lower levels of Moringa (3%), there were increased levels or red blood cells in the central veins which could imply increase in erythropoiesis in the liver. However at higher levels, it may have led to reactions that led to the increased white blood cells that could be spotted in the liver parenchyma.

In this study, higher levels of MOLM (>3%) in the diet negatively affected the glomeruli, renal tubules and the renal cells, hence, may have contributed to lower performance of the pigs. These results were different from those recorded by Ezejindu *et al.* (2014) who documented that *Moringa oleifera* extracts exerted nephro-protective effect on the kidneys of Winster rats at 5% levels of administration. This difference may be attributed to the lower dosages and short duration of administration of *Moringa oleifera* extracts in their study unlike in this study where the duration was relatively longer. Oedema noted in the glomeruli may have been as a result of obstruction of ultrafiltration process caused by the phenolic compounds found in MOLM. Furthermore, these compounds in the MOLM diets may also have had a negative effect on the renal cells, hence, the observed marked nephrosis in the 12% concentration of MOLM in the diet. This means therefore that if MOLM at high concentration was used in the diet for a prolonged duration, may result in renal failure.

7.2 Conclusions

1. *Moringa oleifera* leaf grown in Kenyan rangelands have higher levels of crude protein and could be included in pig diet formulations up to a level of 6% without affecting negatively pig's voluntary feed intake, weight gain and feed conversion efficiency. However, MOLM used in pig diets up to a level of 12% does not have negative effects on the pigs' carcass quality.
2. *Moringa oleifera* leaf meal at levels <6% in pig's diet enhances haematological parameters mainly, haemoglobin and white blood cell formation which could increase efficiency of oxygen circulation in the body and also boost animal's immunity. However, higher levels beyond 6% interfered with the normal haematological parameters.
3. *Moringa oleifera* leaf meal at low levels (3%) in diet has hypocholesterolaemic effects due reduced low density lipoproteins therefore enhancing growth performance. This is also important to humans since this could lower the risks of cardiovascular diseases therefore enhancing human health.
4. *Moringa oleifera* leaf meal greater than 3% in the diet affected negatively the normal histoarchitecture of the spleen, liver and Kidneys.
5. Higher levels of MOLM in the diet (>3%) negatively affected the gastrointestinal pH and may enhance multiplication of coliforms and is therefore undesirable. Furthermore, this extended to influence negatively the gastrointestinal morphology, hence, affecting the nutrient utilization leading to decreased animal productivity.

7.3 Recommendations

1. Further molecular studies are needed to determine the actual causes of improved feed conversion efficiency at lower levels of MOLM and relative reduction of pig growth and FCE at higher levels (>6% in the diet) of MOLM.
2. Future studies ought to determine the effectiveness of the immune response exerted by the plant on pigs in relation to specific infectious agents.
3. There is need for further exploration of the hypocholesterolaemic effects of *Moringa oleifera* in humans to explore the potential of MOLM in control of cardiovascular diseases especially in older persons.
4. This study recommends that further studies be conducted to ascertain the exact phytochemicals in the MO leaves associated with the histopathological changes observed in the liver, kidney, spleen, and gastrointestinal tract.
5. Further studies ought to be conducted to determine the role of MOLM in changes in gut microbial composition specifically in relation to lactobacillus and coliforms.
6. More studies should be conducted to determine safe doses of *Moringa oleifera* in humans to ensure safe utilization both in human diets.
7. There is also need to determine ways of eliminating toxic effects in MOLM especially in regard to the parameters tested in this study.

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APPENDICES

Appendix 1 Average starting weights (kg) for pigs under different levels of MOLM diets

Treatment	Mean	Std. Error	95% Confidence Interval	
			Lower Bound	Upper Bound
1	26.353[a]	0.224	25.88	26.825
2	25.959[a]	0.211	25.515	26.404
3	26.101[a]	0.217	25.642	26.559
4	26.313[a]	0.202	25.887	26.739

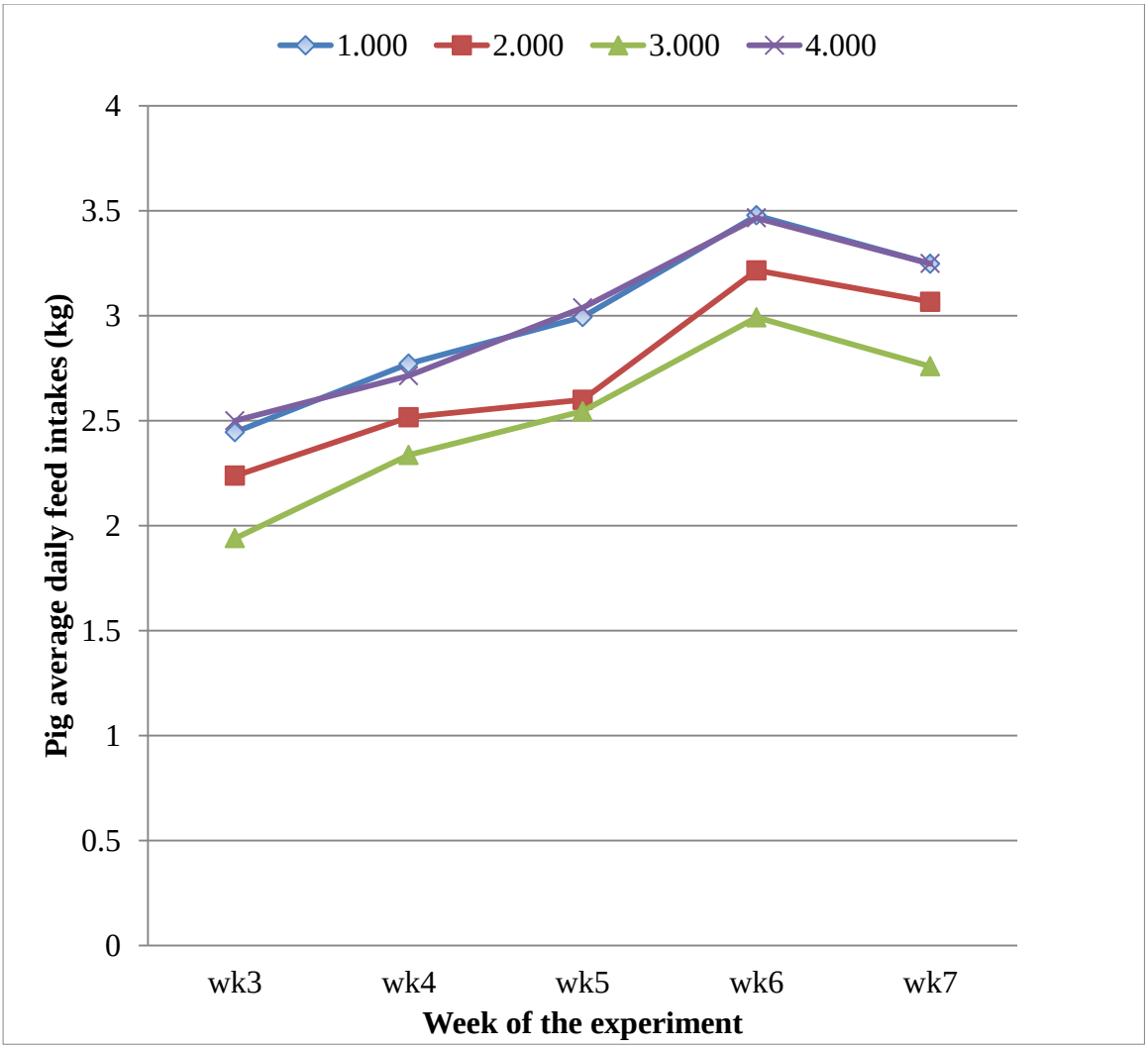
a. Covariates appearing in the model are evaluated at the following values:

Weight at weaning = 24.41

Appendix 2 Average end weights (kg) for pigs under different levels of MOLM diets

			95% Confidence Interval	
Treatment	Mean	Std. Error	Lower Bound	Upper Bound
1.000	66.466[a]	1.138	64.064	68.868
2.000	65.247[a]	1.071	62.988	67.507
3.000	66.556[a]	1.104	64.228	68.885
4.000	65.165[a]	1.025	63.001	67.328

a. Covariates appearing in the model are evaluated at the following values: Weight at weaning = 24.41



Appendix 3 Pig average daily feed intakes (kg) during the experiment period.

Appendix 4 Feeding costs analysis of the *Moringa oleifera* pig diets

Treatment (T)	MZ	MO	WP	VO	CSC	SF	FM	SB	DP	LM	VP	CC	TTC
Cost/kg	23.0	70.0	19.0	50.0	41.0	28.0	45.0	65.0	83.0	3.9	189.0	522.0	
T1 (KES)	920.0	0.0	562.0	74.5	159.5	38.4	450.0	650.0	0.0	13.3	47.3	5.2	2920.1
T2 (KES)	920.0	210.0	502.6	121.0	144.7	35.6	450.0	650.0	0.0	12.0	47.3	5.2	3098.3
T3 (KES)	920.0	420.0	445.2	166.5	128.7	32.5	450.0	650.0	0.0	10.5	47.3	5.2	3275.8
T4 (KES)	920.0	840.0	320.9	174.0	114.4	35.3	450.0	650.0	275.6	0.0	47.3	5.2	3832.6

RM= Ration, MZ= Maize, MO=*Moringa oleifera* leaf meal, WP=Wheat pollard, VO=Vegetable oil, CSC= Cotton Seed Cake, SF=Sunflower, FM=Fish meal, SB= Soybean, DP=Di calcium Phosphate, LM=Limestone, VP=Vitamin Mineral Premix, CC=coccidiostat, TTC= Total cost for a treatment diet