Effects of dietary inclusion of *Moringa oleifera* leaf meal on growth performance, physico-chemical attributes, oxidative stability and sensory quality of pork

By

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Declaration

I, **Xola Pauline Nduku**, declare that this dissertation is my original work and has not been submitted to any University. This work was conducted under the supervision of Prof. V. Muchenje and Dr. T. T. Nkukwana. All assistance towards the production of this work and all the references contained herein have been fully acknowledged.

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Abstract

The objective of the study was to determine growth performance, physico-chemical attributes, oxidative stability and sensory quality of pork from pigs fed one of three dietary treatments, eac containing 0%, 2.5% or 5% (T1, T2 and T3, respectively) *Moringa oleifera* leaf meal (MOLM). Dietary treatments were formulated to be isonitrogenous and isoenergetic for weaner (6 – 8 weeks) and grower (9 – 13 weeks) phases. Twelve Large White (LW) and 12 Kolbroek (KB) male pigs at 6 weeks of age, initially weighing an average 10 kg, were randomly allocated to one of the dietary treatments, each with four replicates, in individual pens. Feed and water were offered *ad libitum.* The *Longissimus thoracis et lumborum* (LTL) muscle of each carcass (n = 24) was sampled for meat quality and fatty acid analyses. Breed differences in measured parameters were observed. In pigs receiving 5% MOLM, the LW had significantly the highest ADFI (P<0.05) than KB pigs. In pigs receiving 2.5% and 5% MOLM, backfat thickness was significantly highest (*P*<0.05) compared to those receiving 0% MOML. Dietary inclusion of MOLM had no significant effects (*P*>0.05) on the physico-chemical quality of pork from LW pigs; although it significantly (*P*<0.05) increased a* and reduced WBSF values in pork from KB pigs. Pork from LW pigs receiving 5% MOLM had significantly increased (*P*<0.05) *n*-3 content of the subcutaneous tissue of LW pigs compared to KB, and also when compread to other treatment groups. In both breeds, the composition of PUFA: SFA and *n*-6: *n*-3 in the subcutaneous adipose tissue was significantly (*P*<0.05) lower in T2 and T3 than in T1. The *n*-3 levels for pork muscle from LW pigs receiving 2.5% and 5% MOLM, and from KB pigs receiving 5% MOLM, were significantly (*P*<0.05) lower. Inclusions of MOLM significantly (*P*<0.05) reduced the *n*-6: *n*-3 fatty acids in pork from both LW pigs and KB pigs. Consumer scores on sensory attributes (aroma intensity, initial impression of juiciness, first bite, sustained impression of juiciness, muscle fibre and overall tenderness and overall flavor intensity) significantly (*P*<0.05) increased as the level of

MOLM inclusion in the diet increased; and higher scores were observed in fried meat than in boiled for most sensory attributes. It may be concluded that inclusion of MOLM in pig diets up to 5% improved the ADFI, a*, tenderness of pork without adversely affecting the FCR and other physico-chemical quality attributes; and resulted in desired increase in levels of *n*-3 and reduced the *n*-6: *n*-3 fatty acid ratio.

Keywords: Fatty acids, lipid oxidation, *Moringa oleifera*, pork quality, sensory evaluation.

List of abbreviations

a*	Redness
ADFI	Average daily feed intake
ADG	Average daily gain
AI	Atherogenicity index
b*	Yellowness
BFT	Backfat thickness
BWG	Body weight gain
C	Chroma value
CL %	Cooking loss percentage
DFI	Daily feed intake
DI	Desaturase index
FA	Fatty acid/s
FAME	Fatty Acid Methyl Ester/s
FCE	Feed conversion ratio
h^{o}	Hue angle
IMF	Intramuscular fat
KB	Kolbroek
L*	Lightness
LTL	*Longissimus thoracic et. Lumborum*
LW	Large White
MOLM	*Moringa oleifera* leaf meal
MUFA	Monounsaturated fatty acid
n-3	Omega-3 fatty acid/s
n-6	Omega-6 fatty acid/s
P	Significance level
%	Percentage
PUFA	Polyunsaturated fatty acid/s
SAS	Statistical Analysis System
SCF	Subcutaneous fat
SFA	Saturated fatty acid
TBARS	Thiobarbituric acid reactive substances
TL %	Thawing loss percentage
WBSF	Warner Bratzler Shear Force

Table of contents

List of tables

List of figures

List of appendices

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Chapter 1

Introduction

1.1 Background

Pigs have diverse ability to forage from a variety of feedstuffs, but the nature of the diet affects their growth performance and the overall pork quality (Edwards, 2003). Pig growth results from a multitude of biological processes, and the genotype of the animal determines the maximum level at which these processes occur (Frank *et al.*, 1998). This suggests possible pig breed differences in growth characteristics, and hence, pork quality. In addition to genetics, interactions between various other factors such as environment and nutrition have been shown to influence pig growth performance (Hossner, 2005; Lynch *et al.*, 2006). Pig characteristics that are positive for profitability are high growth rate, feed conversion ratio and low carcass fatness (McPhee and MacBeth, 2000).

A pig's genetic potential is determined by its ability to grow maximum lean meat percentage (LMP), which is assumed to be associated with average daily weight gain (ADG), in particular low ADG and vice versa (Stege *et al.*, 2011). Lean meat accretion is equivalent to its protein deposition, and is driven by the intake of energy, but limited by the animal's genetic potential (Lynch *et al.*, 2006). Generally, the type of nutrition provided to the animal will influence its genetic potential to grow (Wen-qian and Fu-chang, 2010), which implies that a pig that over-consumes energy more than it requires for maximum lean growth will direct excess feed towards fat deposition. As such, the fatty acid composition of meat is greatly influenced by the FA composition of the feed consumed (Wood *et al.*, 2003).

According to Hernandez *et al.* (1998), consumers have a negative view of pork. They consider it to contain excess fat. Increased concerns among consumers on meat safety and quality has led to demands for lean muscle with less fat (Moelich *et al*., 2003), having no undesirable effects on human health (Blanchard, 1995; Andersen *et al*., 2005; Muchenje *et al*., 2009). Meat quality is considered in terms of sensory properties, nutritive value, food safety aspects and technological factors (Hoffman, 1994; Wood *et al.*, 2003; Hugo and Roodt, 2007).

The FA composition has a major impact on consumer's perception on the health status of the meat (Wood *et al.*, 2008). Pork contains more polyunsaturated fatty acids (PUFAs) (Ovaskainen *et al.*, 2001). Nonetheless, the high PUFA content of pork makes it more susceptible to oxidation (Hugo and Roodt, 2007; Warriss, 2010). Lipid oxidation is the most common process of meat quality deterioration, and is responsible for the formation of toxic compounds in the meat. This results in the development of rancidity, thereby negatively affecting sensory characteristics (colour, texture and flavour) and nutritional quality of the meat (Min and Ahn, 2005; Qwele *et al.*, 2013b). The development of rancidity in meat by lipid oxidation begins at the time of slaughter and continues during storage. The inclusion of antioxidants in pig diets may counteract the process of lipid oxidation (Kumaran and Karunakaran, 2007; Lee *et al.*, 2010). Over the years, synthetic antioxidants have been found to be more effective and less expensive, but they are known to cause cancer and cardiovascular diseases (Borek, 2006). Recently, natural antioxidants such as tocopherol, ascorbic acid and beta carotene, which occur naturally in certain plants such as *Moringa oleifera* Lam. leaves have received extensive research attention as alternatives to synthetic antioxidants (Kang *et al.*, 2012; Mudronov *et al.*, 2012).

Moringa oleifera Lam. (*M. oleifera*) of the family Moringaceae, is a highly valued nutritive plant, distributed in various countries of the tropic and subtropic regions (Anwar *et al.*, 2007). This plant has had enormous attention as a significant food product and natural nutrition of the tropics (Anwar *et al.*, 2007). *M. oleifera* is reported to be of high nutritional value because of its vast nutritional significance, with high levels of protein, vitamins, amino acids, minerals and FAs, with remarkably high levels of α-linoleic acid (Reyez-Sànchez *et al.*, 2006; Moyo *et al.*, 2011). Studies have shown *M. oleifera* leaves to contain high amount of nutritional description and antibiotic properties, and hence, a potential valuable resource for improving the quality and oxidative stability of meat (Reyez-Sànchez *et al.*, 2006; Anwar *et al.*, 2007; Moyo *et al.*, 2011). *M. oleifera* leaves have been reported to be a natural source of antioxidant compounds such as ascorbic acid, flavonoids, phenolics and carotenoids (Siddhurju and Becker, 2003; Qwele *et al.*, 2013a, 2013b; Nkukwana *et al.*, 2014). Natural products such as *M. oleifera* are preferred by consumers because they are perceived as non-harmful and have numerous health benefits (Tanabe *et al*., 2002; Rasooli, 2007). The current study aimed at determining the effect of including different levels of *Moringa oleifera* leaf meal (MOLM) on growth performance, physico-chemical attributes, oxidative stability and sensory quality of pork.

1.2 Problem Statement

The composition of fat in pork is a problem, as consumers demand lean and healthy pork. Pork has a high *n*-6: *n*-3 ratio, which is associated with cancer, hypertension and cardiovascular diseases (Wood *et al.*, 2003). Unfavourable levels of PUFAs in pork negativley affect the oxidative stability and shelf life of the meat, resulting in lipid oxidation (Nilzen *et al.*, 2001). The development of lipid oxidation results in pork quality deterioration and strongly reduces the consumer's acceptability of the product. Nutritionists recommend pig diets to be balanced between *n*-3 PUFA and *n*-6 PUFA (Wood *et al*., 2003) to produce good quality pork with no human health implications. While diet formulation is frequently emphasized by nutritionists, the composition, mixing and form of the complete diet can have important implications for pig growth performance and pork quality.

1.3 Justification

The use of natural antioxidants to stabilize meat is increasing and considered to be safer than synthetic antioxidants (Van Lunen, 2003). Natural plant sources with high nutritive quality and antioxidant properties, such as *Moringa oleifera*, have a potential to be used in this regard (Reyez-Sànchez *et al.*, 2006). Qwele *et al*. (2013b) and Moyo *et al.* (2012) showed the effects of feeding *Moringa oleifera* leaf meal (MOLM) on quality of meat from goats. Furthermore, studies on effects of MOLM in growth performance and meat quality characteristics in broiler chickens have been conducted (Wapi *et al.*, 2013; Nkukwana *et al.*, 2014). Mukumbo *et al.* (2014) studied the inclusion of MOLM in finisher pig diets; however, evidence of any reliable effect of feeding MOLM to two different pig breeds at an early stage of production is still scarce. Findings from the current study will provide pig producers and the meat industry with potential strategies that they can use to alter pig diets for production of desirable pork with good quality and increased oxidative stability.

1.4 Objectives

The main objective of the study was to determine the effect of including different levels of *Moringa oleifera* leaf meal (MOLM) on growth performance, physico-chemical attributes, oxidative stability and sensory quality of pork from two pig breeds.

Specific objectives

To determine the effect of dietary inclusion of *Moringa oleifera* leaf meal on:

- Growth performance and physico-chemical characteristics of pork;
- Fatty acids composition, health lipid indices and thiobarbituric acid reactive substances of pork;
- Consumer sensory scores on pork prepared using two cooking methods.

1.5 Hypotheses

The null hypotheses tested are:

- Dietary inclusion of MOLM has no effect on growth performance ratio and physico-chemical quality of pork;
- Dietary inclusion of MOLM has no effect on the fatty acids composition, health lipid indices and thiobarbituric acid reactive substances of pork;
- Dietary inclusion of MOLM has no effect on consumer sensory scores on pork prepared using two cooking methods, frying and boiling, respectively.

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Chapter 2

Literature Review

2.1 Introduction

Pork quality covers characteristic properties that influence its suitability for further processing, storage, and retail display until it is consumed. The main qualities of interest include the meat fat content, fat composition and oxidative stability (Jung *et al.*, 2010; Sirri *et al.*, 2011). Consumers consider pork meat to contain an excess of fat, saturated fatty acids (SFAs) and cholesterol (Hernández *et al.*, 1998). Meat fat content and fatty acid (FA) composition is largely correlated to its healthiness and nutritional quality (Fisher *et al.*, 2000). The meat industry has been effective in reducing the fat content and is continually working towards modifying the FA profile of meat in accordance with the demands by health conscious consumers for lean pork (Higgs, 2000; Warriss, 2000; Van Schalkwyk and Hoffman, 2010).

2.2 Lean pork growth

Growth efficiency has important implications for animal production because of its influence on the value of animal and product being produced (Oke *et al.*, 2006). Pig growth results from a multitude of biological processes and the genotype of an animal determines the maximum level at which these processes can occur (Frank *et al.*, 1998). Interactions between pig genotype, nutrition and environmental factors have been reported to determine the degree to which genetic potential is expressed, and influence the lean growth rate (Wondra *et al.*, 1995; Frank *et al.*, 1998; Lynch *et al.*, 2006). High growth rates, feed conversion ratio and low carcass fatness are pig characteristics that are desirable for profit optimasation (Alfonso *et al.*, 2005).

According to Choi *et al.* (2013), high growth rates and body weight gain are efficient indicators in pig selection, since they are associated with a lower maintenance requirement. Nowadays, pig producers have economic incentives based on consumer demands and carcass value marketing programs to produce lean pork as efficiently as possible (Lynch *et al.*, 2006). A pig's ability to grow lean meat determines its genetic potential, and high lean meat percentage (LMP) is assumed to be associated with low average daily weight gain (ADG) (Stege *et al.*, 2011). Lynch *et al.* (2006) reported lean meat deposition to be equivalent to its protein deposition, and driven by the consumption of feed energy, and is limited by the animal's genetic potential. This implies that any pig that consumes more energy than is required for maximum lean growth will direct excess feed towards fat growth. According to Dube *et al.* (2011), valuable pigs are those which for each unit of food energy, retain most by favouring conversion of metabolizable energy to lean than conversion to fat tissue. Therefore, pork processors have the objective of marketing lean pork products that are uniform in weight and composition (King, 1999). This offers the opportunity to increase genotype variation for which leaner and more efficient pigs can be selected.

2.3 Description of Large White and Kolbroek pig breeds

2.3.1 Large White pigs

As the name suggest, Large White (LW) pigs are large-framed, having white, pinkish skin colour, which is free from black hair. They are also characterised by moderately long and slightly dished face, and the ears are pricked. The LW pig breed is the most popular exotic breed in South Africa (Agricultural Research Council, 2013) due to their fast growth rate potential, and excellent feed conversion efficiency (Mushandu *et al.*, 2005). In addition, Large White pigs have been valued for producing good quality pork and bacon products. This

breed can be used for commercial production, communal pig production and is commonly used in crossbreeding programs (Taylor and Roese, 2005). Pig breeding programs produce for market that meets consumer's requirements of low amounts of fat and high levels of lean meat content. In general, pork quality of the imported pig genotypes like the LW, is superior when compared with local genotypes. The LW pigs have been reported to possess superior pork quality measures such as colour, water-holding capacity, pH, and drip loss (Kanengoni *et al.,* 2004); and these are characterised by greater content of intramuscular fat compared to slower growing pigs, thus better tenderness and flavour (Zak *et al.*, 2009).

2.3.2 Kolbroek pigs

Kolbroek (KB) pigs are small in size, have long nose and have many variations of coat colour, with black and brown being the most. (Halimani *et al.,* 2010). Kolbroek pigs have a large head, the hams are fairly well developed with study legs and strong feet (Holness, 1991), with very short, pricked ears and a squashed face (Crafter and Morton, 2010). Kolbroek pigs are early maturing, but grow slower than the exotic breeds (Madzimure, 2011). These pigs are tolerant to warm climates, and are often found near rural villages where they are reared at free range or backyard. In communal productions systems of South Africa, KB pigs are owned as local genotypes by the community (Halimani *et al.*, 2010). In these areas, KB pigs have a potential to increase food security, reduce poverty and improve the livelihood of resource limited farmers (Madzimure, 2011). In addition, they are viewed as a suitable breed for resource limited farmers because of their tolerance to various diseases and adaptability to adverse conditions. These pigs survive under conditions with inefficient breeding management, insufficient veterinary care, inadequate feeds and feeding management (Chimonyo *et al.*, 2005). Halimani *et al.* (2010) reported that all local pigs, the most popular being Kolbroek, Mukota and the Windsnyer pig are essentially one genotype based on molecular genetic characterisation. Local genotype pigs can survive on cheaper

high-fibre diets and can convert feed with a low nutrient content very efficiently (Ndindana *et al.*, 2002). Kolbroek pigs produce tasty pork, and have an excellent foraging ability (Chimonyo *et al.*, 2005). These are traits of economic importance that make them favourable with communal farmers, and determine the potential profit for the farmers. As compared to imported breeds, local pigs such as the KB fail to meet or achieve good grades for pork market demands for slaughter masses as part of grading scheme (Kanengoni *et al*., 2004). Thus, these pigs tend not to be considered for pork quality because of their short carcasses, which cannot be simply prepared into specialised pork portions (Chimonyo *et al*., 2010).

2.4 Meat quality

A quality characteristic is related to a meat product's excellence, and can be evaluated without consumption (Poulsen *et al.*, 1996; Grebitus and Bruhn, 2008). The major criteria used in measuring meat quality are colour, ultimate pH, tenderness and marbling (Muchenje *et al*., 2008a). Studies on pork quality reported that fresh pork is classified into three quality categories according to measurements of colour, firmness and drip loss, where, pale, soft, exudative (PSE); reddish-pink, firm, non-exudative (RFN; normal pork) and dark, firm, dry (DFD) can be measured (Faucitano *et al.*, 2010). Pale, soft, exudative meat is associated with very low meat ultimate pH (pH < 5.4) that declines during in a few minutes after slaughter (Barbut *et al.*, 2008). This is not desirable for meat quality, hence, the objective of the meat industry is to reduce PSE meat and improve the colour, tenderness, juiciness and flavour of meat to meet consumer demands. Other aspects of meat quality include attributes such as juiciness, flavour and aroma. Consumers' initial and continued interest and demands in meat are mainly affected by these quality measures (Muchenje *et al*., 2009a).

2.4.1 Meat pH

The acidity or alkalinity of meat measured 24 hours after slaughter is called ultimate pH (pH_u). The meat pH values vary depending on pre-slaughter conditions and animal stress, which reduce post mortem muscle glycolises (Chulayo and Muchenje, 2013). Other pre-slaughter stress conditions that occur during transportation (injury due to overcrowding, and temperature fluctuations) result in increased glycogen depletion, leading to high pH values (Muchenje *et al.*, 2009b; Qwele, 2012; Chulayo and Muchenje, 2013). In addition, nutritional stress results in dehydration which exarcebates electrolyte imbalance and glycogen depletion thus increasing the pH of meat (Qwele, 2012). According to Stanišić *et al.* (2012), post mortem rise in pH is due to progressive alkalization caused by the release of amino acids and peptides during protein breakdown. Antioxidants are used to lower pH of meat during lipid peroxidation. The antioxidants donate electrons and became reduced themselves thus facilitating in maintaining low pH values (Velasco and Williams, 2011; Li *et al.*, 2013). The normal pH decline in muscles is from approximately 7.0 - 7.2 reduced to near pH 5.5 - 5.7 within 24 hours (Lammers *et al.*, 2007). A very low ultimate pH (< 5.4) will result in a very pale and soft (PSE) muscle colour (Drummond and Sun, 2005). If the pH does not decline much post mortem, the meat will be dark with dry surface. The darkening of colour becomes visible when the muscle pH exceeds 5.7 (Murphy and Marks, 2000). This implies that the level that muscle pH changes post mortem is variable and have a great impact on the overall colour of meat and meat products Muchenje *et al.* (2009b, 2009c).

2.4.2 Meat colour

Meat colour is mainly affected by muscle myoglobin content, the chemical state and reactions of the myoglobin and the muscle pH (Qwele, 2012; Muchenje *et al.*, 2008a; Muchenje *et al.*, 2009a). Three myoglobin derivatives namely deoxymyoglobin (reduced myoglobin),

oxymyoglobin (oxygenated myoglobin), and metmyoglobin (oxidised myoglobin) determine the meat colour (Rosenvold and Anderson, 2005). Deoxymyoglobin is a purple pigment of deep muscle obtained from meat under vacuum, oxymyoglobin is bright cherry red and is considered to signify fresh meat by the consumer. For pork, a dark pink colour is preferable, with a colour scale ranging from one to six (1= pale pinkish gray; 2= grayish pink; 3= reddish pink; 4= dark, reddish pink; 5= purplish pink; 6= dark purplish red) (Allen *et al*., 1997). Measurement of meat colour can be done using several instruments such as a Colour guide and visual appraisal following a given scale. Colour coordinates used when the Commission International De I' Eclairage colour system is employed are lightness (*L**), redness (*a**) and yellowness (*b**) (Commision International De I' Eclairage, 1976). Colour *L**, *a**, and *b** ranges are; for *L** 33.2- 41, *a** 11.1- 23.6 and *b** 6.1- 11.3 (Muchenje *et al*., 2009a). Meat colour is important because consumers associate it with the products freshness, and it is a significant factor affecting consumer acceptance, buying decisions and satisfaction of meat products (Muchenje *et al*., 2009a; Stanišić *et al*., 2012).

2.4.3 Meat tenderness

Meat tenderness refers to the ability of meat to resist fragmentation when being chewed. It includes initial bite by the teeth, the breakdown of meat into fragments and the amount of residue remaining after chewing (Lawrie, 1998). Pre-slaughter handling, freezing, thawing and aging have a significant effect on tenderness (Gregory, 2007). The rate and extent of the chemical and physical changes in conversion of muscle to meat also has an impact on the tenderness of meat (Sentandreu *et al*., 2002). As meat ages, high meat pH influences tenderization because it improves muscle catabolism through elevated calpain or reduced calpastatin activities. The toughness of meat may also be due to its lower µ- and m-calpain activities (Claeys *et al*., 2001) and associated muscle enzymatic activities (Muchenje *et al*.,

2009a). Some reports indicate positive associations between the tenderness of meat and the level of intramuscular fat content, because it is generally perceived to be tender, juicy and with good flavour (Cannata *et al.*, 2010). However, there has been emphasis on a high lean-to-fat ratio in meat, due to consumer demands for healthier products (Higgs, 2000; Williams, 2007). Tenderness is the most important characteristic that regulates meat palatability and acceptability by consumers (Cain *et al.*, 2003; Maltin *et al.*, 2003). Meat tenderness can be measured by instrumental means, using the Warner Bratzler shear device, or using consumer sensory analysis (Tornberg, 1996).

2.4.4 Consumer sensory evaluation of meat

Consumer perceptions on meat are influenced by various attributes such as gender, culture and religion within various ethical groups (Xazela *et al.*, 2011; Ngambu *et al.*, 2011). Irrespective of the differences in consumers' backgrounds and perceptions, good quality meat is highly preferred by consumers (Muchenje *et al.*, 2008b; Tshabalala *et al.*, 2003; Xazela *et al.*, 2011). Muchenje *et al.* (2008a, 2008b) stated that consumers consider sensory characteristics to determine the acceptance of meat products. Consumer meat sensory characteristics are used to determine meat quality, using meat palatability factors such tenderness, juiciness, colour and flavour (Tshabalala *et al.*, 2003; Muchenje *et al.*, 2008b). In a study by Bredahl and Poulsen (2002), consumers defined high quality pork in terms of good taste, tenderness, freshness, leanness and healthiness.

Consumer's decisions on the quality of meat have been reported to be affected also by the cooking method of meat (Nour *et al.*, 1994). Ngambu (2011) reported significant effect of thermal preparation (boiling and roasting) on meat sensory characteristic scores. According to Gheisan and Ranjbar (2012), consumers have become more concerned about the safety of the synthetic food preservatives used to store meat and they demand additive-free or natural

products (Ahn *et al.*, 2002). Natural antioxidants from plant extracts are considered to be safer than synthetic antioxidants, and increasingly used to enhance growth and stabilize meat (Jung *et al.*, 2010; Qwele, 2012). It has also been reported that these natural antioxidants have greater application potential for consumer's acceptability, palatability, stability and shelf-life of meat products (Jung *et al.*, 2010; Moyo *et al.*, 2011). *Moringa oleifera* Lam. (*M. oleifera*) tree is a major source of natural antioxidants and is reported to be of high nutritional value. All parts of the *M. oleifera* tree are palatable; however, the leaves and seeds are mainly used for animal feed (Fahey, 2005; Moyo *et al.*, 2011).

2.5 *Moringa oleifera* Lam. (*M. oleifera*)

Moringa oleifera Lam. (*M. oleifera*), commonly referred to as the drumstick tree, is a multipurpose plant of Moringaceae family, widely distributed in the tropic and subtropic regions worldwide (Makkar and Becker, 1997; Mbikay, 2012). It has been highly valued due to its significant use for human food production, medicinal purposes and in animal diets (Khalafalla *et al.*, 2010). In India, Philippines, and tropical Africa, almost every part of *M. oleifera* has been considered to be a functional food commodity, where edible fruits, leaves, flowers, roots, and seed oil are cooked and consumed as vegetable (Anwar and Bhanger, 2003; Ghazali and Mohammed, 2009). In addition to human food production, several medicinal properties have been attributed to the various parts of this highly valued tree. The roots, stem, leaves, flowers and seeds have been used in South Asia for treating bacterial infection, fungal infection, inflammation, infectious diseases along with cardiovascular, gastro-intestinal, and haematological disorders (Morimitsu *et al.*, 2000; Siddhuraju and Becker, 2003; Rahman *et al*., 2009). In Uganda, *M. oleifera* is extensively grown and it is used in public hospitals as theraphy agaist human immunodefieciency virus (HIV) (Kasolo *et*

al., 2010). Table 2.1 summarises some common medicinal uses of various parts of *M. oleifera* as reported by Anwar *et al*. (2007), citing various authors.

2.5.1 Nutritional description of Moringa oleifera Lam. leaves

M. oleifera leaves have immense nutritional value, with prophylactic and antioxidant properties and can be used to improve animal growth performance, as well as the quality and nutritional composition of the meat (Fahey, 2005; Anwar *et al.*, 2007; Nkukwana *et al.*, 2014). Studies have shown *M. oleifera* to contain high levels of some essential amino acids, crude protein (30.29% Dry leaf nutritive content), minerals, vitamins and fatty acid properties (Moyo *et al.*, 2011). The reported essential amino acids contained include threonine, tyrosine, methionine, phenylalanine, isoleucine, leucine, histadine, lysine and tryptophan and alanine (Foidl *et al.*, 2001; Sanchez-Machado *et al.*, 2009; Moyo *et al.*, 2011). In addition, *M. oleifera* leaves have been reported to contain some polyunsaturated fatty acids (PUFAs), namely α-linolenic, linoleic and g-linolenic (Sanchez- Machado *et al.*, 2009, Moyo *et al.*, 2011).

Table 2.1 Medicinal uses of various parts of *Moringa oleifera* Lam.

Plant part	Medicinal uses
Root	Antifertility, antilithic, anti-inflammatory, stimulant in paralytic afflictions, act as a cardiac/circulatory tonic, used as a laxative, articular pains and constipation
Stem	Rubefacient, prevent enlargement of the spleen and formation of tuberculous glands of the neck, to destroy tumors and to heal ulcers, tooth cavity as a pain killer and has anti tubercular activity
Leaves	Purgative, applied as poultice to sores, rubbed on the temples for headaches, used for piles, fevers, sore throat, bronchitis, eye and ear infections, scurvy and catarrh; leaf juice is assumed to control glucose levels, applied to reduce glandular swelling
Flower	High medicinal value as a stimulant, aphrodisiac, abortifacient, cholagogue; used to cure inflammations, muscle diseases, hysteria, tumors, and enlargement of the spleen
Seed	Seed extract exerts its protective effect by decreasing liver lipid peroxides, antihypertensive compounds thiocarbamate and isothiocyanate glycosids have been isolated from the acetate phase of the ethanolic extract of *M. oleifera* pods

(Adopted from Anwar *at al.* (2007)

2.5.2 Antioxidants found in Moringa oleifera Lam. leaves

Antioxidants are substances that reduce the destruction of cellular components by free radicals, which may cause lipid oxidation in meat and meat products (Young and Woodside, 2001). *M. oleifera* leaves can be used as antioxidants to scavenge free radicals because they contain high amounts of phytochemicals and can tolerate antioxidant activity (Qwele *et al.*, 2011). Among phytochemicals, *M. oleifera* leaves contain high levels of antioxidants such as α-tocopherol ascorbic acid, beta carotene, flavonoids and phenols, which have been demonstrated to have significant antioxidant activity (Sreelatha and Padma, 2009; Verma *et al.,* 2009; Qwele *et al.,* 2013). The antioxidants, found in *M. oleifera* leaves, diminish free radicals by donating an electron and stabilizing the compound. Thus, *M. oleifera* leave have shown to have a defensive role against oxidative damage and can be used as an antioxidant to prevent meat oxidative damage. Table 2.2 summarizes the different antioxidants found in *M. oleifera* leaves, in combination as reported in literature.

Table 2.2 Reported antioxidants found in *Moringa oleifera* Lam. leaves

Antioxidants	Literature
Vitamin A, Vitamin C, Vitamin E, Vitamin K, Vitamin B (Choline), Vitamin B1 (Thiamin), Vitamin B2 (Riboflavin), Vitamin B3 (Niacin), Vitamin B6, Alanine, Alpha-Carotene, Arginine, Beta-Carotene, Beta-sitosterol, Caffeoylquinic Acid, Campesterol, Carotenoids, Chlorophyll, Delta-5-Avenasterol, Delta-7-Avenasterol, Glutathione, Histidine, Indole Acetic Acid, Indoleacetonitrile, Kaempferal, Leucine, Lutein, Methionine, Myristic-Acid, Palmitic-Acid, Proline, Quercetin, Rutin, Selenium, Threonine, Tryptophan, Xanthins, Xanthophyll, Zeatin, Zeaxanthin, Chromium, Zinc.	(Group, 2009; Qwele, 2012, Nkukwana, 2012).

2.5.3 Potential of Moringa oleifera Lam. leaves in improving meat quality

Animals need dietary nutrients and antioxidants to combat body infections and protect the muscle against oxidative attach (Buckley *et al.*, 1995; Min *et al.*, 2008; Moyo *et al.*, 2011). Animal nutrition plays a major role in animals' ability to produce good quality meat. Due to the nutritional characterisation, representing a good source of protein and amino acids, vitamins, β -carotene and various phenolics (Moyo *et al*., 2011), *M. oleifera* leaves have potential to be a valuable resource for animal diets (Makkar and Becker, 1996; Reyez-Sànchez *et al.*, 2006). This plants contain tannins and essential with antioxidant properties which pass antioxidant compounds to meat (Lahucky *et al.*, 2010). The antioxidative properties fed to animals may also have an effect on the fatty acid composition and oxidative stability of the meat produced (Koreleski and Swiatkiewickz, 2006). Plant products are also known to be rich sources of PUFAs of the *n*-3 and *n*-6 family (Geay *et al.*, 2001). The addition of natural sources of antioxidants to animal diets, can enhance animal growth and improve the oxidative stability, sensory quality, shelf life and the acceptability of meat (Coetzee and Hoffman, 2001). Antioxidants in feed guarantees high deposition of α-tocopherol in meat and protects it against lipid oxidation (Kemin, 2009; Qwele, 2012; Moyo *et al.*, 2012).

2.6 Lipid oxidation

The biochemical changes that go with pre-slaughter metabolism and postmortem aging in the conversion of muscle to meat give rise to conditions that destroys the balance between prooxadative and antioxidative factors, resulting in initiation and propagation of lipid oxidation (Buckley *et al.*, 1995; Min *et al.*, 2008). Lipid oxidation involves an imbalance between the antioxidant defence mechanism and the reactive oxygen species, leading to oxidative stress (Moyo *et al*., 2012). Free radicals attract electrons from the lipids in cell membranes which results in the development of rancidity in meat by lipid oxidation.

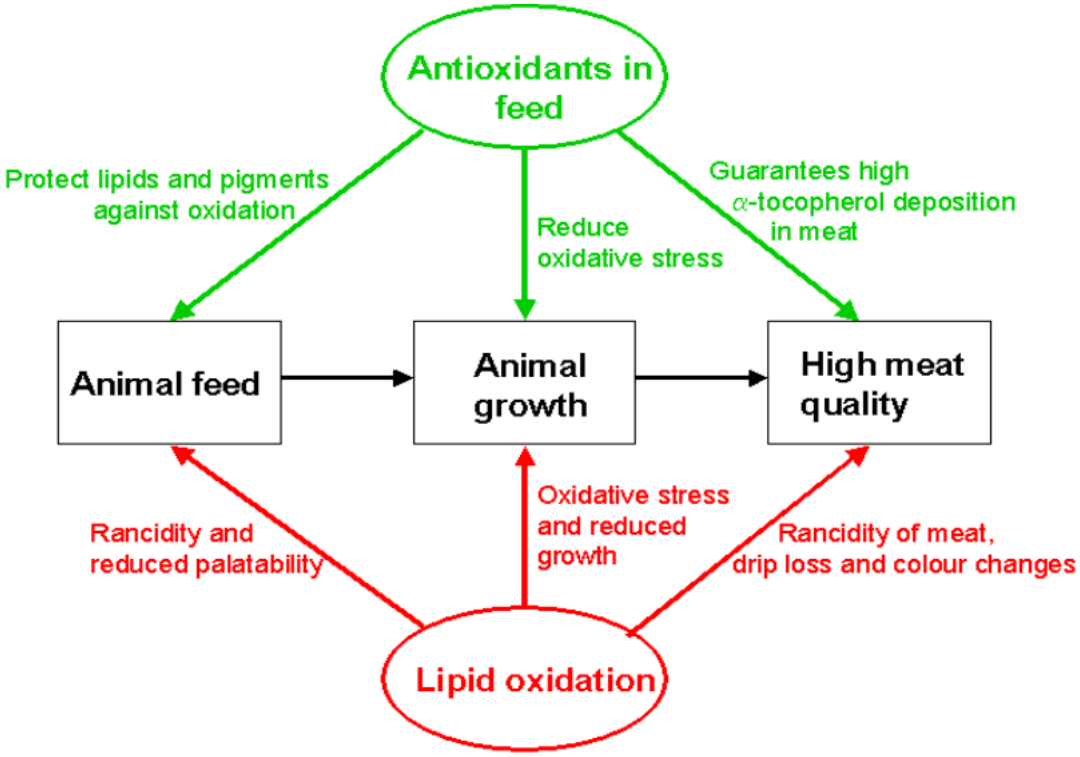

Figure 2.1 Antioxidants in feed reduce meat oxidative stress (Source: Kemin, 2009)

The meat becomes rancid and negatively affects the colour, flavour and nutritional quality of meat (Fasseas *et al*., 2008). Lipid oxidation begins at the time of slaughter and continues during storage (Wood *et al.*, 2003; Jensen *et al.*, 2008). Lipids are protected against oxidative attack by naturally occurring antioxidants, such as α-tocopherol. The rate and extent of lipid oxidation in meat depends on the α-tocopherol concentration in the tissue (Buckley and Morrissey, 1992). Dietary supplementation of the animals' diet with α-tocopherol increases stabilization of polyunsaturated fatty acids (PUFAs) and cholesterol in muscle against oxidative deterioration (Monahan *et al.*, 1990; Jensen, 1995). Oxidative deterioration most often affects PUFAs, because they contain multiple double bonds in between which lies methylene -CH2- groups that possess especially reactive hydrogens. Amongst meat products, pork is considered to be more prone to the development of oxidative rancidity compared to other meat, due to the higher content of PUFAs it contains (Kemin, 2009).

2.7 Fatty acid composition

The quality of meat is largely associated to its fat content and its fatty acid composition (Muchenje *et al*., 2009c). Fatty acid composition determines the oiliness of adipose tissue and the oxidative stability of muscle and, therefore, affects flavour and colour of the meat (Wood *et al.* 2008). Fatty acids are either saturated, monounsaturated and polyunsaturated (Qwele, 2012). Saturated fatty acids (SFAs) found in meat are reported to be responsible for various cardiovascular diseases and hence, they are less desirable (Wood and Enser, 1997). Dietary recommendations encourage the consumption of less saturated fatty acids and have led to an increase in the demand for meat containing higher levels of unsaturated fatty acids (Jensen, 1998). The ability of unsaturated fatty acids, particularly those with more than two double bonds, to rapidly oxidize, is significant in regulating rancidity, colour deterioration and shelf life of meat (Wood *et al*., 2003).

Polyunsaturated fatty acids (PUFAs) are essential fatty acids which are considered as desirable dietary fats, with lower risks of disease implication and are transferred to meat through diet (Whetsell *et al.*, 2003). However, Wood *et al.* (2003) stated that meat should have a favourable balance between PUFA and SFA (PUFA: SFA) and a satisfying omega 3 and omega 6 (*n*-3: *n*-6 PUFA) ratio. For this reason, the PUFA: SFA and *n*-6: *n*-3 PUFA ratios are some of the most significant parameters to evaluate the nutritional value and freshness of meat (Mapiye *et al*., 2011). Nowadays, consumers demand safe and fresh meat with increased nutritional value (Bou *et al.*, 2009). In that respect, the chemical composition of muscle tissue becomes an important component of meat quality.

2.8 Summary of review

Due to the immense nutritional value, indicating a good source of prophylactic and antioxidant properties, *M. oleifera* leaves have potential to be a valuable resource for animal diets. The addition of natural sources of antioxidants to animal diets, can enhance animal growth and improve the oxidative stability, sensory quality, shelf life and the acceptability of meat (Coetzee and Hoffman, 2001). *M. oleifera* leaves contain high levels of antioxidants such as α-tocopherol ascorbic acid, beta carotene, flavonoids and phenols, which have been demonstrated to scavenge free radicals and protect meat against lipid oxidation. The use of *M. oleifera* leaves in the feed may be beneficial in improving the nutritional quality and oxidative stability of meat.

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Chapter 3

Effects of dietary inclusion of *Moringa oleifera* leaf meal (MOLM) on growth performance and physico-chemical quality of pork

Abstract

The effect of different levels of *Moringa oleifera* leaf meal (MOLM) on growth parameters, carcass and meat quality were determined for a total of 24 male pigs, i.e., 12 Large White (LW) and 12 Kolbroek (KB) aged six weeks and weighing an average of 10 kg. The pigs were individually housed and randomly subjected to three dietary treatments formulated to contain 0% (T1), 2.5% (T2) and 5% (T3) MOLM, at four pigs per treatment per breed, for eight weeks. The dietary treatments were formulated to be isonitrogenous and isoenergetic for weaner (6 – 8 weeks) and grower (9 – 13 weeks) phases. The body weight gain (BWG) of the pigs were measured on a weekly basis. The changes in average daily gain (ADG) and daily feed intake (ADFI) were measured; and feed conversion ratio (FCR) was calculated accordingly. After the feeding trial of eight weeks, the pigs were slaughtered and back fat thickness, pH at 45 minutes (pH_{45}) and pH at 24 hours (pH_u) *post mortem* were measured. Meat samples were taken from the *Muscularis Longissimus thoracic et. lumborum* (LTL) and were analysed for colour (L*, a*, b*), Chroma value (C), and Hue angle (h°). The LTL muscle samples were also used to measure thawing loss (TL %), cooking loss (CL %) and Warner Bratzler Shear Force (WBSF). Diet had no significant (P>0.05) effect on ADFI, ADG and FCR in both breeds. Breed and treatment interactions in ADFI were observed in pigs fed 5% MOLM, where LW pigs had the highest ADFI as compared to KB pigs (P<0.05). In KB pigs, those that were fed diets with 2.5% and 5% MOLM, respectively, had the highest BFT (P<0.05) than in the pigs that received 0% MOLM 21.5 mm vs. 11.7 mm). The various inclusion levels of MOLM in pig diet had no significant effects (P>0.05) on pH_u,

L*, a*, b*, C, h^{o}, TL %, and WBSF values. In KB pigs however, dietary treatments resulted in significant (P<0.05) differences in a*, h^{o}, TL %, CL % and WBSF values. Pork from pigs receiving 0% MOLM (4.3) had the lowest a* value, while pigs receiving 2.5% MOLM (5.7) had the highest. Pork from LW pigs had the lowest (P<0.05) WBSF values than from KB in pigs that received 0% and 2.5 % MOLM (P<0.05). In KB pigs, the WBSF value of pork was the lowest in T3. Therefore, MOLM inclusion levels in pig diets can improve the ADFI (P<0.05), a* and tenderness of pork without adversely affecting the FCR and other physico-chemical quality attributes.

Keywords: Feed conversion ratio, lean pork, pork colour, pig diet.

3.1 Introduction

Breed and diet have important effects on carcass and meat quality (Wood *et al.*, 2004; Hoffman *et al*., 2005); and there has been a great interest in the variation between breeds in pork quality (Ramakrishnan, 2013). The available reports show a significantly lower growth rate and a higher production cost in indigenous stock when compared to exotic pigs (Sasendran and Rajagopalan, 1982; Ramakrishnan, 2013). Wood *et al.* (2004) showed that exotic breeds (Large White and Duroc) grew faster and had less fat carcasses and high pork quality than indigenous breeds (Berkshire). Improvements in carcass and physico-chemical attributes of meat such as colour and pH, colour and tenderness may be achieved by means of adequate animal diet nutrition (Pettigrew and Esnaola, 2001).

There is considerable evidence indicating that manipulating the nutrient composition of pig diets may offset the negative effects on pork quality. Thus, products with antioxidants from plants, such as *Moringa oleifera* Lam, have been reported to be safer than synthetic antioxidants and are increasingly being used to enhance growth performance and improve meat quality (Moyo *et al*., 2011; Kang *et al*., 2012; Mudronov *et al.*, 2012). *Moringa oleifera* Lam. (*M. oleifera*) leaves have been reported to be an enormous potential source of crude protein, amino acids, minerals and they have high levels of prophylactic and antioxidant properties (Reyes-Sanchez *et al.*, 2006; Moyo *et al.*, 2011; Qwele *et al.*, 2013; Nkukwana *et al.*, 2014). Evidence of any effect of feeding of *M. oleifera* leaves on growth and pork quality of different pig breeds is lacking. Similarly, the effects is still a matter of speculation. The objective of the current study was to assess the use of *Moringa oleifera* leaf meal (MOLM) in formulated pig feeds and its effect on growth performance and quality of pork from Large White and Kolbroek pigs.

3.2 Materials and Methods

3.2.1 Study site description

The study was conducted at Fort Cox College of Agriculture and Forestry Farm near Keiskamahoek, situated in the Eastern Cape province of South Africa. This area is located in the False Thornveld and is characterized by mean annual rainfall of 480 mm and mean annual temperature of 18.7°C (Acocks, 1988). The site lies along latitude 27°01'E and latitude 32°46'S at an altitude of 450-500 m above sea level and receives 550 mm of rainfall 550 mm mainly during summer months (November-April), but also some winter rainfalls can be received.

3.2.2 Animal management and experimental design

Ethical considerations were made in this study to conform to the national and international standards governing the usage of animals. Permission to use animals was obtained from the Ethical Clearance Committee of the University of Fort Hare (Certificate Reference Number: MUC011 SNDU01). Twenty four male pigs (12 Large White, 12 Kolbroek) at six weeks of age, weighing an average of 10 kg were obtained from a small scale commercial piggery in Fort Cox College of Agriculture and Forestry. All the pigs wer dewormed agaist internal parasites and were housed individually housed in 3 x 2 m concrete floored and zinc-roofed pens with 1.5 m high walls, and each pen was equipped with a 1 x 0.3 x 0.3 m concrete feeding trough for feed and nipple water. Each pig served as an experimental unit. The pigs were randomly assigned to one of three maize-soyabean meal basal feeds in mash form that were formulated, and consisted of the control (no MOLM); treatments 1 (T1) and treatment 2 (T2) which contained 2.5% and 5%, respectively. All the dietary treatments were formulated for weaner (6 -8 weeks) and grower (9-13 weeks) phases (National Research Council, 1998). The ingredient and chemical composition of the dietary treatments for both the weaner and the grower phase are shown in Table 3.1.

Table 3.1 Ingredient composition of dietary treatments, on fed basis

Feeding phase	**Weaner**			**Grower**		
Dietary treatments	T1	T2	T3	T1	T2	T3
MOLM Inclusion level	0% MOLM	2.5% MOLM	5% MOLM	0% MOLM	2.5% MOLM	5% MOLM
Feed ingredients (g/kg)						
Yellow Maize	704.01	703.88	683.09	612.49	673.07	648.07
Moringa	**0.00**	**25.00**	**50.00**	**0.00**	**25.00**	**50.00**
Wheat Middlings	20.00	0.00	0.00	167.82	100.00	100.00
Soya Hi Protein	224.09	223.27	219.32	181.40	155.87	155.87
Soya Oil	5.00	5.00	5.00	5.00	5.00	5.00
Limestone Powder	13.72	13.41	13.47	14.24	16.87	16.87
Monocalcium Phosphate	7.10	6.93	6.69	3.87	5.52	5.52
Fine Salt	6.19	3.63	6.00	4.74	7.15	7.15
Methionine	2.71	2..56	2.53	1.21	1.24	1.24
Typtophan	0.65	0.65	0.63	0.30	0.46	0.46
Threonine	1.76	1.70	1.71	0.61	0.72	0.72
Biolysin	8.00	8.00	8.00	4.25	5.53	5.53
Chlorine Chloride Powder	0.58	0.58	0.58	0.58	0.58	0.58
Vitamin/Mineral Premix	0.00	3.00	3.00	3.00	3.00	3.00
Formulated nutrient specification						
Net Energy (MJ/Kg)	10.07	10.07	10.07	9.65	9.65	9.65
CP (%)	26	26	26	22	22	22
Available lysine (%)	1.14	1.14	1.14	0.89	0.89	0.89
Calcium (%)	0.67	0.67	0.67	0.62	0.62	0.62
Total Phosphorus (%)	0.42	0.42	0.42	0.49	0.49	0.49
Fat (%)	3.37	3.37	3.37	2.91	2.91	2.91

*Supplied the following per kg of feed: 3300 IU of vitamin A, 330 IU of vitamin D3, 19.8 IU of vitamin E, 1.32 of vitamin K, 0.05 mg of biotin, 0.14 mg of folic acid, 4.4 mg of niacin, 11 mg of pantothenic acid, 0.0132 mg of vitamin B12, 12.4 mg of zinc, 25 mg of iron, 5.3 mg of manganese, 2.6 mg of copper, 0.05 mg of selenium. MOLM (*Moringa oleifera* leaf meal); T1 (control, 0% MOLM)); T2 and T3 contains levels of MOLM at 2.5% and 5%, respectively.

The pigs were put into 3 treatments groups, at the rate of 4 pigs per treatment per each of the two breeds. Ear tags were used to identify the animals. The experimental pigs were weighed before the beginning of the trial and were allowed seven days feed adaptation. They were then fed the experimental diets for a period of seven weeks. From week 1 – 3, the pigs were fed formulated pig weaner feed, and from week 4 – 7 they were fed formulated pig grower feed. Proximate analysis for moisture, crude protein, ash and ether extract; and mineral composition (Table 3.2) was performed on all experimental diets and on MOLM samples according to methods of the Association of Official Analytical Chemists (AOAC, 2003). Pigs had *ad libitum* access to feed and water and feeding was done twice daily, i.e., at 0800 hours and at 1500 hours, with half/half rations, respectively. The pigs were washed two times each day and each pen was cleaned on a daily basis, using a hard broom and running water to maintain a clean environment.

3.2.3 Production efficiency parameters and slaughter procedure

The pigs were weighed on a weekly basis to measure body weight gain (BWG). Average daily gain (ADG), daily feed intake (DFI), and feed conversion ratio (FCR) of the pigs were recorded at the beginning of each week. Weekly body weight gain for each pig was measured as:

BWG = week 2 body weight – week 1 body weight

The average daily gain for each pig was measured as:

ADG = (week 2 body weight – week 1 body weight) number of days between weighing

The daily feed intake for each pig was measured on a daily basis as:

DFI = amount of feed offered − amount of feed refusals

Table 3.2 Nutrient composition of the experimental diets and *Moringa oleifera* leaf meal (MOLM), on dry matter basis

ITEM	Feeding Phase						MOLM
	Weaner			Grower			
	Dietary Treatments						
	T1	T2	T3	T1	T2	T3	
Proximate composition							
Crude protein (%)	16.82	18.58	17.77	14.59	17.10	15.58	22.78
Crude fibre (%)	4.17	3.76	3.89	5.23	4.27	5.06	10.28
Ash (%)	5.36	5.19	5.11	4.44	5.86	5.27	11.98
Crude fat (%)	2.12	1.60	1.86	2.40	2.47	1.93	6.59
Mineral composition							
Calcium (%)	0.91	0.85	0.75	0.87	1.09	0.74	0.49
Copper (ppm)	19.95	17.22	19.46	19.70	29.33	21.48	2.90
Iron (ppm)	162.9	155.70	172.80	166.80	189.10	165.00	416.80
Potassium (%)	0.79̂	0.82	0.800	0.80	0.80	0.79	1.39
Magnesium (%)	0.18	0.19	0.19	0.21	0.21	0.21	0.48
Manganese (ppm)	30.19	25.64	26.50	33.30	36.60	35.02	31.97
Sodium (%)	0.28	0.23	0.23	0.18	0.18	0.17	0.03
Phosphorus (%)	0.58	0.56	0.54	0.54	0.61	0.46	0.28
Sulphur (%)	0.23	0.25	0.27	0.24	0.25	0.25	0.75
Zinc (ppm)	45	59	49	40	57	40	9

MOLM (*Moringa oleifera* leaf meal); T1 (control, 0% MOLM); T2 and T3 contains levels of MOLM at 2.5% and 5%, respectively.

Subsequently, the FCR was determined as a measure of the amount of feed required to attain one unit of weight gain. That is:

$$FCR = DFI / ADG$$

At the end of the eight week feeding trial, the pigs were prepared for slaughter. The pigs were weighed in the morning just before the 0800 hours feeding. The pigs were loaded and transported to and slaughtered at a small commercial abattoir in Adelaide, which is situated approximately 90 km away from piggery. The pigs were loaded and transported in the evening at 1700 hours. The pigs were held in lairage at the abattoir to be slaughtered the next morning at 0500 hours. No feeding was done during this time, i.e. the pigs were fasted for 12 hours prior to slaughter. Pre-slaughter weights were measured. This was done under conditions of minimal stress. An electric stunner was used to render the pigs unconscious prior slaughter, before the jugular vein was cut. Electric stunning was done by placing the electrodes tongs behind each ear in the pig for 3 – 4 seconds, releasing 340 V of electricity through the pig's head. After cutting the jugular vein, each pig was left to bleed for a period of 5 minutes and the hoisted by a chain around its right ankle into a scalding tank of hot water at 65° C for 3 seconds in order to loosen the hair for easy removal. After scalding, the hair was scraped off each carcass using sharp knives. Each carcass was singed using a gas flame after scraping in order to remove any remaining hair and to sterilise the carcass. Each carcass was then eviscerated and the offals were inspected for any signs of illness or infections.

3.2.4 Carcass and meat quality measurements

Back fat thickness was measured between the 2nd and 3rd last ribs, 45 mm from the dorsal midline using a calliper. pH and temperature measurements were taken 45 minutes (pH_{45}) *post mortem* in the right side of the each hanging carcass between the second and third last rib in the *Longissimus thoracic et. lumborum* (LTL) muscle using a portable pH meter

equipped with an electrode probe and thermometer (Crison pH 25, Crison Instruments, S. A., Alella, Spain). After every four readings pH meter was calibrated with pH 4.01, 7.00 and 9.21 CRISON available standard solutions. Carcasses were then moved to the cold room and chilled at -2°C. The ultimate pH (pHu) was taken 24 hours *post mortem* in the right side of the LTL muscle on each hanging carcass in the cold room and cold carcass weights were measured. Loin chops from the LTL muscle were cut from the right of each carcass. Fresh meat samples were taken, vacuum sealed (Gastrovac Pro, Henkovac, Netherlands) and stored at -20 °C until the time of measurements. These were used to measure the colour co-ordinates, thawing and cooking losses and Warner Braztler Shear Force (WBSF) of meat.

3.2.5 Meat colour measurement

Measurements were made 24 hours *post mortem* on the exposed cut surface of the LTL muscle using Minolta colour guide 45/0 BYK-Gardener GmbH with a 20 mm diameter measurement area, illuminant D65- day light and 10° standard observer on the same fillets. The CIE colour co-ordinates for L* = Lightness (0 = dark, 100 = light); a* = Redness (red-green spectrum); and b* = Yellowness (yellow-blue spectrum) (Commission International de I'Elclairage, 1976) were measured. Before each measurement, the colour guide was first calibrated using black and white standards. Three readings were taken on the surface of each sample by rotating the colour guide by 90° between each measurement, in order to obtain a representative average value of the colour. The values for a* and b* were then used to calculate colour saturation (chroma value) and hue angle according to the following equations:

$$\text{Chroma value} = \sqrt{(a^{*2} + b^{*2})}$$

$$\text{Hue angle } (^{o}) = \tan^{-1} (b^{*}/a^{*})$$

3.2.6 Thawing and cooking loss

The frozen meat sample from each carcass was weighed (weight form freezer) and left to thaw at room temperature for 12 hours after which each sample was weighed again (weight after melting). The thawing loss percentage (TL %) was then calculated as:

TL % = [(weight from freezer – weight after melting) / (weight from freezer)] × 100%

The same meat samples were then individually sealed in a water tight PVC-plastic bag and boiled in a water bath at 85°C for 45 minutes. Thereafter, the samples were cooled and reweighed. The recorded weight differences were expressed as cooking loss, and the percentage (CL %) was calculated using the following formulae:

CL % = [(weight before cooking – weight after cooking) / (weight before cooking)] × 100%

3.2.7 Tenderness (Warner Bratzler Shear Force)

After determining the cooking loss, the connective tissue was removed from the cooked sample pieces and three sub samples measuring 3 x 1.5 x 1.5 cm each were removed from each cooked muscle and each sub sample was sheared in a direction perpendicular to that of the fibre using an Instron Universal Testing Machine equipped with a Warner Bratzler Shear Force apparatus (cross head speed at 400 mm/min, one shear in the centre of each core). The Warner Bratzler Shear Force values are measured in Newtons (N).

3.2.8 Statistical analysis and model

Data on growth performance, carcass traits and meat quality were analysed using PROC GLM procedures of SAS (2003) and pair wise comparisons of LSMeans were done. The least significant difference (LSD) method was used to separate the means.

The statistical model used was:

$$Y_{ijk} = \mu + \alpha_i + \beta_j + \alpha\beta_{ij} + \varepsilon_{ijkl}$$

Where Y_{ijk} = response variables (growth performance, physico-chemical meat quality)

μ = overall constant mean

α_i = effect of diet (Control, 2.5% MOLM, 5% MOLM)

β_j = breed effect (LW, KB)

$\alpha\beta_{ij}$= diet by breed effect

ε_{ijkl} = random error

3.3 Results and Discussion

3.3.1 Growth efficiency parameters

Results for the weekly pig live weights are presented in Figure 3.1. There were no significant differences (P>0.05) in live weight rates for Large White (LW) and Kolbroek (KB) pigs in all the dietary treatments over a period of seven weeks. Table 3.3 presents the effects of different levels of *Moringa oleifera* leaf meal (MOLM) on growth efficiency parameters of LW and KB pigs. The inclusion of MOLM in the pigs' diet did not significantly (P>0.05) affect the growth parameters (ADFI, ADG and FCR) in the two pig breeds, across the three treatment groups. Furthermore, the results of the current study showed that the slaughter weights of the pigs were not affected (P>0.05) by either diets or breeds. The inclusion of MOLM in the feed had no significant (P>0.05) effect on average slaughter weights between KB and LW pigs across all treatment groups (T1 = 0% MOLM; T2 = 2.5% MOLM; T3 = 5% MOLM). This is in line with Qwele *et al.* (2013) who reported effect of dietary mixture of *M. oleifera* in broiler slaughter weight to be of little significance. The growth performance of experimental pigs observed during the experimental period did not seem to have been affected by inclusion of MOLM in diets. In terms of daily weight gains, Nuhu (2010) showed that daily weight gain increased with increasing levels of MOLM, and animals fed 0% MOML performed poorer (P<0.05) than those fed the MOLM inclusive diets. There are however a few studies reporting the effect of including *M. oleifera* leaf meal on pig growth performance.

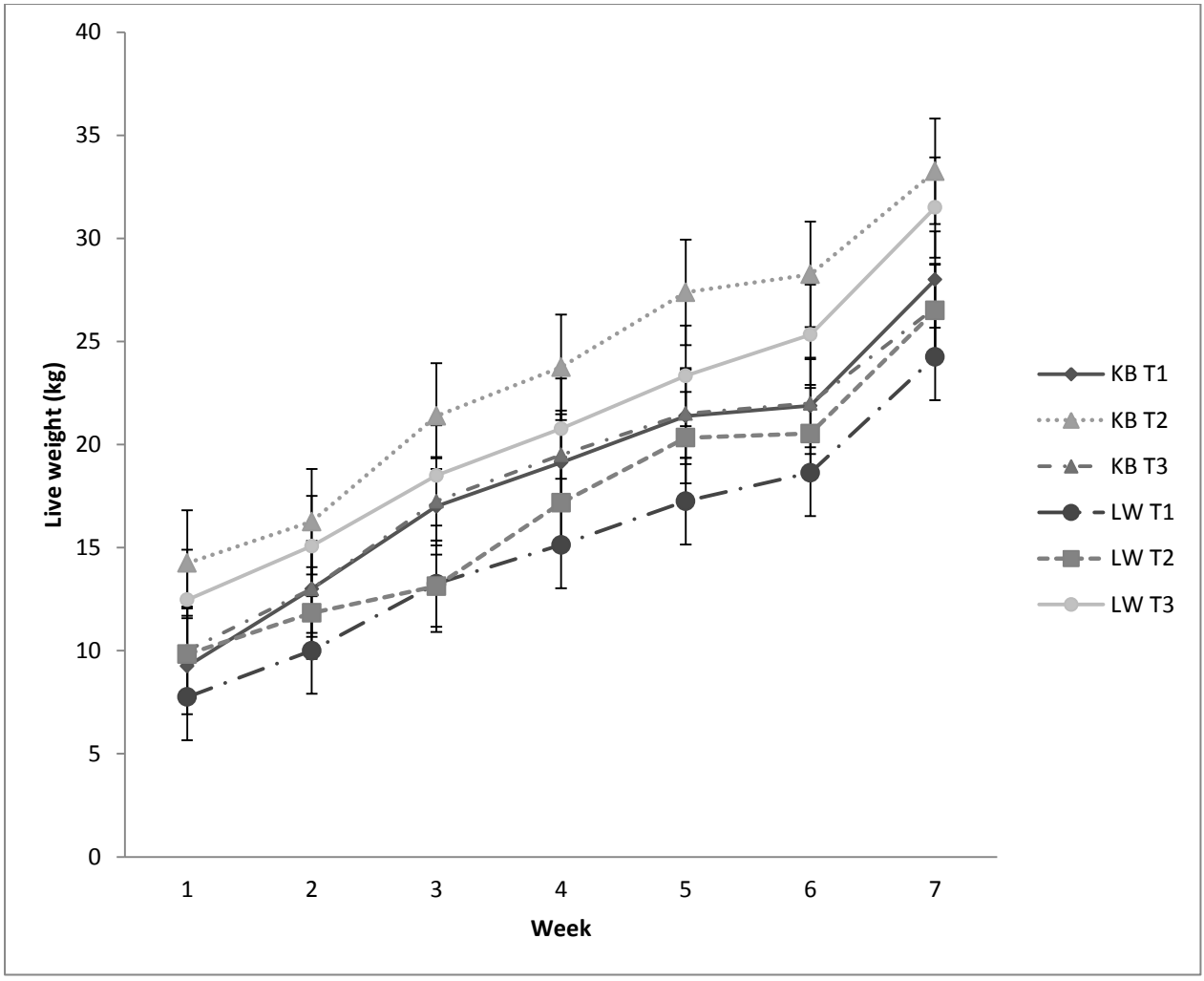

Figure 3.1 Weekly live weights for Kolbroek and Large White pigs receiving different levels of *Moringa oleifera* leaf meal

KB T1 (Kolbroek pigs fed 0% MOLM), KB T2 (Kolbroek pigs fed 2.5% MOLM), KB T3 (Kolbroek pigs fed 5% MOLM), LW T1 (Large White pigs fed 0% MOLM), LW T2 (Large White pigs fed 2.5% MOLM), LW T3 (Large White pigs fed 5% MOLM).

Table 3.3 Effects of feeding graded levels of *Moringa oleifera* leaf meal on growth efficiency parameters of Large White and Kolbroek pigs

Growth efficiency parameter	Dietary treatments		
	T1 (0% MOLM)	**T2 (2.5% MOLM)**	**T3 (5% MOLM)**
Average daily feed intake (kg/day)			
Large White	1.37±0.169	1.54±0.169	1.83^{B}±0.169
Kolbroek	0.99±0.169	1.17±0.196	1.27^{A}±0.196
Average daily gain (kg/day)			
Large White	0.46±0.055	0.50±0.063	0.50±0.063
Kolbroek	0.56±0.055	0.52±0.055	0.51±0.055
Feed conversion ratio			
Large White	3.12±0.738	2.27±0.853	4.25±0.853
Kolbroek	3.40±0.738	3.87±0.37	4.33±0.738
Average slaughter weight (kg)			
Large White	22.3±4.22	26.5±4.88	28.2±4.88
Kolbroek	27.3±4.22	33.3±4.22	25.1±4.22

^{AB}Means in the same column per trait with different superscripts are significantly different (P<0.05); *Moringa oleifera* leaf meal (MOLM).

Mukumbo *et al.* (2014) noted significantly higher (P<0.05) ADFI in pigs that were fed 7.5% MOLM than the pigs that received 0%, 2.5% and 5% MOLM. Similar to the current study, the ADFI of pigs fed 2.5% and 5% MOLM did not significantly differ from the ADFI of pigs fed 0% MOLM. This suggest that at lower inclusion levels of 2.5% and 5%, MOLM does not affect (P>0.05) feed intake, but at 7.5% inclusion and above, significant (P<0.05) increase in the ADFI may be observed. This may be due to the increased fibre content in the diet and the compensatory behaviour of the pigs in accessing adequate nutrients in higher MOLM inclusion levels. In the current study, the highest inclusion level of MOLM was 5%.

The current study showed significant breed differences on feed intake in pigs that received 5% MOLM. The LW pigs had significantly higher (P<0.05) feed intake as compared to Kolbroek pigs. Exotic pigs such as the LW are good converters of feed and thus have a superior feed efficiency compared to indigenous pigs (Ramakrishnan, 2013). Alfonso *et al.* (2005), reported differences in the conversion ratio of Basque and Large White pigs, where Basque pigs had a higher and thus poor feed conversion ratio than Large White pigs. However, there were no significant differences in FCR between LW and KB pigs in the current study.

3.3.2 Carcass quality

Table 3.4 presents the effects of various levels of MOLM on carcass traits in LW and KB pigs. Treatments effect in the dressing percentage was not significant (P>0.05) between the breeds. These results are in line with those of Okubanjo (1998), who showed no significant differences in the dressing percentage of Nigerian indigenous pigs and Duroc, Landrace and Large White pig breeds.

Table 3.4 Effects of feeding graded levels of *Moringa oleifera* leaf meal on carcass characteristics of Kolbroek and Large White pigs

Carcass traits	Dietary treatment		
	T1 (0% MOLM)	**T2 (2.5% MOLM)**	**T3 (5% MOLM)**
Dressing percentage (%)			
Kolbroek	60.7^{a}±4.01	68.9^{ab}±4.01	74.2^{b}±4.01
Large White	61.3±4.01	61.8±4.63	63.1±4.63
BFT (mm)			
Kolbroek	14.1^{a}±2.3	21.5^{Bb}±2.3	21.9^{Bb}±2.3
Large White	9.3±2.3	11.7^{A}±2.7	11.5^{A}±2.7
pH_{45}			
Kolbroek	6.3^{A}±0.11	6.5±0.11	6.4±0.11
Large White	6.9^{Bb}±0.11	6.4^{a}±0.12	6.2^{a}±0.12

^{AB}Means in the same column per carcass traits with different superscripts are significantly different (P<0.05); ^{ab}Means in the same row with different superscripts are significantly different (P<0.05); *Moringa oleifera* leaf meal (MOLM); back fat thickness (BFT); pH measured 45 minutes after slaughter (pH_{45}).

In terms of dietary treatment, the inclusion of MOLM in the feed did not significantly (P>0.05) affect the dressing percentage in LW pigs, across treatments. However, significant (P<0.05) dressing percentage differences were observed in KB pigs. The inclusion of MOLM at 2.5% and 5% resulted in significantly higher (P<0.05) dressing percentages (68.9% and 74.2%, respectively) as compared to 0% MOLM inclusion level (60.7%). This is in line with Safa and Tazi (2014) who found significantly higher dressing percentage in broilers supplemented with 3%, 5% and 7% MOLM as compared to the control group (0% MOLM). There are scarce reports on effects of MOLM on pig dressing percentage.

Breed differences in back fat thickness (BFT) were observed in pigs that received 2.5% and 5% MOLM. Kolbroek pigs showed highest (P<0.05) back fat thickness (21.5 mm) as compared to LW pigs (11.7 mm). Kolbroek pigs could have had higher backfat than Large White because Large White is a popular commercial breed, and a lot of genetic selection for low backfat thickness has been carried out for this breed over the years. This implies that the inclusion of MOLM in pig diets promoted the possible differences between breeds. Measuring the effect of dietary treatments in each breed showed an increase (P<0.05) of BFT in KB pigs after the inclusion of 2.5% and 5% MOLM in the feed. Similar studies on breed differences report that Meishan indigenous pigs have lower lean meat percentages and higher subcutaneous fat than Landrace and LW pigs (Gispert *et al.*, 2007). In addition, Wood *et al.* (2004) showed that exotic breeds (LW and Duroc) have less fat carcasses than local pig breeds (Berkshire). Contrary to these studies, Okeudo *et al.* (2007) showed that LW pigs have significantly higher BFT than the

Nigerian indigenous pigs. Low back fat thickness in indigenous pigs could be attributed to the ability of LW pigs to utilize high protein and energy diets (viz. MOLM) without becoming fat. Due to high back fat thickness in indigenous breeds, pork from these pigs is

poorly accepted by consumers and for commercial use, as consumers are increasingly demanding lean pork. Significant breed differences were observed (P<0.05) in pH_{45} in only the pigs that received 0% MOLM. The KB pigs on 0% MOLM had the lowest (P<0.05) pH_{45} (6.3) value compared to LW pigs (6.9). No significant treatment effects were observed among KB pigs, however, 2.5% and 5% MOLM inclusions resulted in significantly lower pH_{45} in LW pigs (P<0.05).

3.3.3 Physico-chemical meat quality

The effect of dietary inclusion of different levels of MOLM in pig feed on physico-chemical pork quality characteristics are shown in Table 3.5. The current study showed no significant (P>0.05) breed differences in the pH_u, L*, a* b*, C and h^o value measured across all treatments. The muscle ultimate pH values for both breeds in all dietary treatment groups fell within the ultimate range of 5.5 – 5.9 as reported by Gajana *et al.* (2013) and Mukumbo *et al.* (2014). Higher meat pH results in lower L* values, suggesting that high meat pH results in darker meat than normal meat pH (Zhang *et al*., 2005).

The current study did not show any variability in the pH_u values of pork across the different levels of MOLM in the diet. The reason for no variation could be that the pigs were all handled the same way, fed with similar levels of nutrients and were exposed to limited stress during transportation, travelling time, time spent in the lairages and during slaughter. Meat quality attributes for pork from LW pigs were not significantly influenced by dietary treatment. The various inclusion levels of MOLM in the diet had no significant effect (P>0.05) in muscle pH_u, colour characteristics, TL %, and WBSF values. In broilers, Qwele *et al.* (2013) reported that the dietary supplementation of *Moringa oleifera* formulated diets does not result in any differences in the physico-chemical characteristics of broiler meat.

Table 3.5 Effect of feeding graded levels of *Moringa oleifera* leaf meal on meat quality characteristics of Kolbroek and Large White pigs

Meat quality traits	Dietary treatment		
	T1 (0% MOLM)	T2 (2.5% MOLM)	T3 (5% MOLM)
pH_u			
Kolbroek	5.6±0.08	5.6±0.08	5.8±0.08
Large White	5.7±0.08	5.6±0.09	5.6±0.09
Lightness (L*)			
Kolbroek	51.3±2.26	45.2±2.26	46.3±2.26
Large white	54.2±2.26	49.7±2.60	51.3±2.60
Redness (a*)			
Kolbroek	4.3^{a}±0.77	7.2^{b}±0.77	6.7^{b}±0.77
Large White	4.5±0.77	5.7±0.89	5.2±0.89
Yellowness (b*)			
Kolbroek	7.8±0.75	7.8±0.75	7.8±0.75
Large White	7.1±0.75	8.3±0.87	8.2±0.87
Chroma (C)			
Kolbroek	9.0±0.90	10.7±0.90	10.3±0.90
Large White	8.5±0.90	10.7±1.04	9.7±1.04
Hue angle (h^{o})			
Kolbroek	61.0^{b}±3.37	48.0^{a}±3.37	49.2^{a}±3.37
Large White	56.7±3.37	51.5±3.89	58.1±3.89
Thawing loss (%)			
Kolbroek	3.5^{b}±0.54	1.4^{a}±0.54	0.8^{Aa}±0.53
Large White	3.4±0.54	3.0±0.62	2.7^{B}±0.62
Cooking loss (%)			
Kolbroek	15.4±4.98	14.4±4.98	15.5^{A}±4.98
Large White	21.3^{a}±4.98	22.6^{a}±5.75	27.8^{Bb}±5.75
WBSF (N)			
Kolbroek	22.7^{Ab}±1.68	20.7^{Ab}±1.68	12.1^{a}±1.68
Large White	16.3^{B}±1.68	14.5^{B}±1.94	12.8±1.94

^{AB}Means in the same column per meat quality traits with different superscripts are significantly different (P<0.05); ^{ab}Means in the same row with different superscripts are significantly different (P<0.05); *Moringa oleifera* leaf meal (MOLM); pH measures 24 hours after slaughter (pH_u); Warner Bratzler Shear Force (WBSF).

In KB pigs, dietary treatments resulted in significant (P<0.05) differences in redness, h^o (hue angle) and WBSF values. The redness of pork from pigs receiving 0% MOLM was significantly (P<0.05) lowest than pork from pigs receiving 2.5% and 5% MOLM. Similarly, Moyo *et al.* (2013) reported that chevon from the MOLM diet had significantly higher redness (a*) values than those in other diets. The higher redness values in pork from pigs that were fed 2.5% and 5% MOLM could be attributed to the high levels of dietary iron in the two dietary treatments. Iron is contained in the oxymyoglobin which results to red colour in meat. The iron content is indicated by meat pigments (Carpenter and Clark, 1995), and the paleness of meat could be due to its low concentration of muscle pigment (Kadim *et al*., 2003). Redness in meat is preferable because it is more attractive and acceptable by consumers. Meat colour is commonly associated with factors such as breed (Muchenje *et al.*, 2008a, 2008b, 2009a; Ekiz *et al.*, 2010). High redness in KB pigs may also be attributed to the dark black skin colour of the pigs, resulting in darker meat as compared with pork from light skinned pigs. Consequently, pork from KB pigs that received 0% MOLM had a significantly (P<0.05) higher h^o value (61.0) as compared to pork from KB pigs receiving 2.5% and 5% MOLM, which were 48.0 and 49.2 respectively. A higher hue angle is related to lower redness of meat. Hence, the muscle of KB pigs can be characterised as less bright, darker and redder than the muscle of LW pigs.

The WBFS values in T1 and T2 were significantly higher (P<0.05) from T3. This implies that the highest level of MOLM (5%) inclusion in pig feed resulted in decreased WBSF values, thus more tender pork. The tenderness in 5% MOLM meat could be due to a higher amount of intramuscular fat and BFT in KB pigs. Breed differences (P<0.05) in WBSF values of pork were observed in T1 and T2 levels. However, no significant differences were in 5% MOLM (T3) level group. Pork from LW pigs had significantly (P<0.05) lower WBSF value (16.3) in the control group (0% MOLM), and also in T2 (14.5). Pork from KB in T1

and T2 had significantly highest WBSF values of 22.7 and 20.7 for respective treatment groups. These results are in line with Wood *et al.* (2004), where tenderness was affected by breed, and was higher in modern Large White pigs than in indigenous Tamworth pigs. The differences in WBSF values between the two breeds could be attributed to their different types of muscles and reaction to pre-slaughter stress. Pre-slaughter stress is said to be one of the most important influences on meat tenderness (Muchenje *et al.*, 2009b), and it has been shown to have a negative effect on the overall meat quality (King *et al.*, 2006; Gajana *et al.*, 2013). Animals which were more excitable are more expected to produce meat with higher shear force values, which reduces the acceptability of the meat (Voisinet *et al.*, 1997). In addition, animals subjected to pre-slaughter stress tend to have tougher and darker meat due to the depletion of glycogen in the muscle (Muchenje *et al.*, 2009a). From the results of the current study, the higher WBSF values in KB pigs may be attributed to their rapid reaction to pre-slaughter stress processes such as teeth clipping, ear-tagging, vaccination and tail docking. Significant breed differences ($P<0.05$) were observed in the thawing loss percentage (TL %) and cooking loss percentage (CL %). For TL % and CL %, breed differences were seen ($P<0.05$) in pork from pigs that received 5% MOLM. Pork from KB pigs showed the lowest ($P<0.05$) TL % (0.8) and CL % (15.5) than LW pigs (2.7 and 27.8, respectively).

3.4 Conclusion

From this study it can be concluded that the growth efficiency parameters of the pigs did not seem to have been affected by inclusion of MOLM in diets. Inclusion of MOLM at the levels used in this study had no significant effects on physico-chemical attributes, especially in LW pigs, but, in KB pigs however, inclusions of MOLM resulted in increased a* without causing DFD (dark, firm, dry) pork, and reduced the WBSF value. Therefore MOLM can be included in pig diets at levels of 5% and may improve the a* and tenderness of pork without adversely affecting the growth performance and other physico-chemical quality attributes. The physico-chemical quality of meat is also influenced by the amount of fatty acids in diet. Unfavourably high levels of polyunsaturated fatty acids in pork adversely affect the oxidative stability and shelf life of the meat, resulting in lipid oxidation.

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Chapter 4

The effect of *Moringa oleifera* leaf meal (MOLM) on fatty acid composition, health lipid indices and oxidative stability of pork

Abstract

The effect of inclusion of MOLM as a feed additive on fatty acid (FA) composition, the health lipid indices of atherogenicity (AI) and desaturase (DI) and Thiobarbituric acid reactive substances (TBARS) of subcutaneous fat (SCF) tissue and *Longissimus thoracis et lumborum* (LTL) muscle samples was determined. Twelve Large White (LW) and 12 Kolbroek (KB) male pigs at 6 weeks of age, were individually housed and randomly subjected to three dietary treatments formulated to contain 0% (T1), 2.5% (T2) and 5% (T3) MOLM, at four pigs per treatment per breed, for eight weeks. were randomly allocated to one of the dietary treatments, either containing 0%, 2.5% or 5% (T1, T2 and T3, respectively) *Moringa oleifera* leaf meal (MOLM), each with four replicates. Dietary treatments were formulated to be isonitrogenous and isoenergetic for starter (6 – 8 weeks) and grower (9 – 13 weeks) phases. Pigs were slaughtered after eight weeks of feeding trial. Fatty acid analysis was done on subcutaneous adipose tissue and *Longissimus thoracis et lumborum* (LTL) pork samples taken from the carcass of each of the pigs (n = 24). Health lipid indices (AI and DI) were calculated and oxidative stability was evaluated by TBARS. Lower (P<0.05) SCF% and higher moisture% were observed in LW pigs compared to KB pigs. Likewise, there were significant (P<0.05) breed differences observed in total PUFA and *n*-6 content of the subcutaneous tissue, with LW pigs having higher amounts in all the treatment groups. The inclusion of MOLM at levels of 2.5% and 5% resulted in significantly (P<0.05) lower *n*-6 levels in the subcutaneous tissue. In T3, the *n*-3 content was significantly (P<0.05) higher in LW pigs than KB, and when compared to T1 and T2. Inclusion levels of MOLM at 5%

resulted in favourably higher *n*-3 amounts in LW pigs. The PUFA: SFA and *n*-6: *n*-3 fatty acids in the subcutaneous adipose tissue, was significantly (P<0.05) lower in T2 and T3 than in T1. For muscle samples, there were no significant differences (*P*>0.05) as a result of any dietary treatment in intramuscular fat (IMF %), fat free dry matter (FFDM%) and moisture content in both breeds. The LW pigs had a significantly (*P*<0.05) higher content of PUFA: SFA than KB pigs in T3. The inclusion levels of MOLM at 2.5% and 5% levels in LW pigs, and 5% in KB pigs, resulted in lower (*P*<0.05) *n*-3 in pork muscle. The *n*-6: *n*-3 ratio of pork from LW and KB pigs was significantly (*P*<0.0) reduced by the inclusion of MOLM in their diets. There were no significant (*P*>0.05) breed or dietary treatment effects for the health lipid indices of atherogenicity (AI) and desaturase (DI). The current study showed no difference (*P*>0.05) caused by the inclusion of MOLM in pig diets on TBARS of pork muscle, in both breeds. Therefore, *Moringa oleifera* leaf meal at 5% inclusion level results in desired increase in levels of *n*-3 fatty acids and reduced *n*-6: *n*-3 fatty acid ratio and the may be a proficient source of natural antioxidants for pork.

Keywords: *Moringa oleifera*, Omega-3 fatty acids, omega-6/omega-3 fatty acids, TBARS.

4.1 Introduction

In pigs, the FA composition of pig muscle and adipose tissue can be changed through altering the FA composition of the dietary fat (Kouba *et al.*, 2003; Nurnberg *et al.*, 2005). Fatty acid composition of the fat tissue in pigs is affected by environmental factors, such as diet, and by genetic factors, such as sex, breed and halothane genotype (De-Smet *et al.*, 2004). However, there is limited research on the effects of genetic factors, especially breed on fat acid composition (Piedrafita *et al.*, 2001; Wood *et al.*, 2004; LoFiego *et al.*, 2005). Numerous studies have reported the effects of modified dietary treatments on FA composition of pig muscle and adipose tissue (Gatlin *et al.*, 2002, Kouba *et al.*, 2003; Nurnberg *et al.*, 2005). The FA composition of muscle and fat tissues is manipulated in order to produce meat with desirable nutritional and technological qualities (Wood *et al.*, 2003). Lipid oxidation is a major cause of meat quality deterioration, resulting in rancidity and the formation of undesirable colour, flavours, nutritional value; and therefore, consumer acceptability (Bou *et al.*, 2004; Min and Ahn, 2005; Giannenas *et al.*, 2009). This process of detelioration may be delayed by the addition of antioxidants in the animal feed (Qwele *et al.*, 2013; Nkukwana *et al.*, 2014).

A search for natural antioxidants, especially from plants, has increased in recent years (Meyer *et al.*, 2002; Moyo *et al.*, 2012; Qwele *et al.*, 2013; Nkukwana *et al.*, 2014). Plant products are also known to be rich sources of PUFAs of the *n*-3 and *n*-6 family (Geay *et al.*, 2001). These plants contain tannins and essential with antioxidant properties which pass antioxidant compounds to meat (Lahucky *et al.*, 2010). The antioxidative properties fed to animals may also have an effect on the fatty acid composition and oxidative stability of the meat produced (Koreleski and Swiatkiewickz, 2006). One such plant with a potential to be used as an antioxidant and could have influence on the fatty acid composition in pig tissue is *Moringa*

oleifera (*M. oleifera*). The *M. oleifera* plant can be used as a natural antioxidant due to the presence of phytochemicals such as, carotenoids, vitamins, minerals, amino acids, flavonoids and phenolics present in the leaves (Verma *et al*., 2009). Furthermore, Moyo *et al.* (2011) reported *M. oleifera* leaves to contain favourably high levels of *n-3* fatty acids.

Studies have reported dietary supplementation with *M. oleifera* leaf meal (MOLM) to result in improved fatty acid profiles in breast meat from broiler chickens (Nkukwana *et al.*, 2014) and significantly lower *n*-6: *n*-3 ratios in goat meat (Qwele *et al.*, 2013). There is, however, scarce information on the dietary use of MOLM in on the fatty acid composition in pork from different pig breeds fed in the early phase of production. The present study, therefore evaluated the potential of MOLM as a feed additive on the fatty acid profile and oxidative stability of pork meat from Large White and Kolbroek pigs.

4.2 Materials and Methods

4.2.1 Study site description

The feeding trial was conducted at a small scale commercial piggery at Fort Cox College of Agriculture and Forestry. A detailed study site description was given in Chapter 3, Section 3.2.1.

4.2.2 Animal management and experimental design

Ethical principles were considered in this study to conform to the national and international standards governing the usage of animals. Permission to use animals was obtained from the Ethical Clearance Committee of the University of Fort Hare (Certificate Reference Number: MUC011 SNDU01). Twenty four pigs (12 LW and 12 KB) male pigs, weighing on average 10 kg, were randomly divided into three groups of four for each breed. The groups were then randomly assigned to each of three dietary treatments, that consisted of a control diet (0% MOLM), 2.5% MOLM and 5% MOLM. The management of pigs and experimental design are fully described in Section 3.2.2. The ingredient composition of dietary treatments and the nutrient composition of the experimental diets and MOLM are presented in Tables 3.1 and 3.2, respectively. Table 4.1 presents the fatty acid composition of the experimental dietary treatments and MOLM.

4.2.3 Meat sample preparation

Upon the end of the eight week feeding trial, the pigs were transported to and slaughtered at a small commercial abattoir as described in Section 3.2.3. Meat samples from the *Longissimus thoracic et. lumborum* LTL muscle were collected 24 hours post mortem from the right side of each carcass, cut into 25 mm thick loin chops, vacuum sealed (Gastrovac Pro, Henkovac, Netherlands) and stored at -20°C until the time of analysis. Meat sample collection and preparation are described in Section 3.2.4.

Table 4.1 Fatty acid composition of experimental dietary treatments and *Moringa oleifera* leaf meal

Fatty acids	**Starter**			**Grower**			**MOLM**
Dietary treatment	**T1**	**T2**	**T3**	**T1**	**T2**	**T3**	
C14:0	0.04	0.08	0.08	0.04	0.08	0.10	2.42
C16:0	10.99	10.37	10.51	10.61	10.87	11.18	21.07
C16:1c9	0.07	0.07	0.07	0.07	0.07	0.07	0.21
C17:0	0.06	0.05	0.05	3.05	0.06	0.06	0.30
C18:0	2.70	2.78	2.58	2.78	2.83	2.51	3.46
C18:1c7	0.04	1.31	1.32	0.04	0.06	0.07	0.84
C18:1c9	32.32	33.09	32.83	31.34	32.00	31.08	5.00
C18:2c9,12 (n-6)	50.46	48.63	48.19	52.55	50.17	49.70	12.47
C20:0	0.43	0.42	0.43	0.39	0.43	0.42	1.04
C20:1c11	0.19	0.16	0.14	0.23	0.21	0.9	0.35
C18:3c9,12,15 (n-3)	2.25	2.53	3.23	1.36	2.65	3.37	48.71
C22:0	0.22	0.22	0.25	0.25	0.23	0.25	1.50
C23:0	0.02	ND	0.02	ND	0.02	0.02	0.14
C24:0	0.17	0.20	0.23	0.18	0.22	0.23	2.24
C24:1c15	0.09	0.10	0.09	0.12	0.12	0.13	ND
C22:5c7,10,13,16,19 (n-3)	ND	ND	0.02	ND	0.04	0.04	0.55
Total SFA	14.61	14.13	14.12	14.30	14.72	14.76	32.39
Total MUFA	32.68	34.72	34.44	31.80	32.42	32.13	5.90
Total PUFA	52.71	51.16	51.44	53.91	52.86	53.11	61.72
Total *n*-6	50.46	48.63	48.19	52.55	50.18	49.71	12.47
Total *n*-3	2.25	2.53	3.25	1.36	2.69	3.40	49.25
PUFA: SFA	3.61	3.62	3.65	3.78	3.59	3.60	1.91
PUFA: MUFA	1.61	1.47	1.49	1.70	1.63	1.66	10.47
n-6: *n*-3	22.46	19.25	14.82	38.62	18.70	14.62	0.25

MOLM – *Moringa oleifera* leaf meal; ND – not detected T1 (0% MOLM); T2 (2.5% MOLM); T3 (5% MOLM); SFA – saturated fatty acids; MUFA – monounsaturated fatty acids; PUFA – polyunsaturated fatty acids; *n*-6 – omega 6 fatty acids; *n*-3 – omega 3 fatty acids; PUFA: SFA – polyunsaturated fatty acids/saturated fatty acids; *n*-6: *n*-3 – omega 6 fatty acids/omega 3 fatty acids.

4.2.4 Fatty acid profile determination of feed materials and pork samples

The total lipid from samples of the formulated feed treatments was extracted with a Soxhlet extraction according to AOAC (2003) procedures for determination of fats. Total lipid from muscle samples were quantitatively extracted, according to the method of Folch *et al.* (1957), using chloroform and methanol in a ratio of 2:1. An antioxidant, butylated hydroxytoluene was added at a concentration of 0.001 % to the chloroform: methanol mixture.

A rotary evaporator was used to dry the fat extracts under vacuum and the extracts were dried overnight in a vacuum oven at 50°C, using phosphorus pentoxide as a moisture absorbent. Total extractable fat was determined gravimetrically from the extracted fat and expressed as percent fat (w/w) per 100 g tissue. The extracted fat from feed, subcutaneous fat and muscle was stored in a polytop (glass vial, with push-in top) under a blanket of nitrogen and frozen at –20°C pending fatty acid analyses.

A lipid aliquot (20 mg) of feed, subcutaneous and muscle lipid was transferred into a Teflon-lined screw-top test tube by means of a disposable glass pasteur pipette. Fatty acids were transesterified to form methyl esters using 0.5 N NaOH in methanol and 14 % boron trifluoride in methanol (Park and Goins, 1994). FAMEs from subcutaneous fat, feed and muscle were quantified using a Varian 430 flame ionization GC, with a fused silica capillary column, Chrompack CPSIL 88 (100 m length, 0.25 mm ID, 0.2 μm film thicknesses). Analysis was performed using an initial isothermic period (40°C for 2 minutes). Thereafter, temperature was increased at a rate of 4°C/minute to 230°C. Finally an isothermic period of 230°C for 10 minutes followed. FAMEs n-hexane (1μl) were injected into the column using a Varian CP 8400 Autosampler. The injection port and detector were both maintained at 250°C. Hydrogen, at 45 psi, functioned as the carrier gas, while nitrogen was employed as the makeup gas. Galaxy Chromatography Software recorded the chromatograms.

Fatty acid methyl ester samples were identified by comparing the retention times of FAME peaks from samples with those of standards obtained from Supelco (Supelco 37 Component Fame Mix 47885-U, Sigma-Aldrich Aston Manor, Pretoria, South Africa). All other reagents and solvents were of analytical grade and obtained from Merck Chemicals (Pty Ltd, Halfway House, Johannesburg, South Africa). Fatty acids were expressed as the proportion of each individual fatty acid to the total of all fatty acids present in the sample. The following fatty acid combinations were calculated: omega-3 (*n*-3) fatty acids, omega-6 (*n*-6) fatty acids, total saturated fatty acids (SFA), total monounsaturated fatty acids (MUFA), polyunsaturated fatty acids (PUFA), PUFA:SFA ratio and *n*-6: *n*-3 ratio.

4.2.5 Lipid health indices

The fatty acid composition (i.e total saturated fatty acids (SFA), total monounsaturated fatty acids (MUFA), total polyunsaturated fatty acids (PUFA), total omega-6 fatty acids, total omega-3 fatty acids, PUFA/SFA and omega-6/omega-3 ratio) data were used to calculate the atherogenicity and desaturase index. Atherogenicity indices were calculated as the content ratio of SFA/unsaturated FA using the following formula proposed by Ulbricht and Southgate (1991):

$$\text{Atherogenicity index (AI)} = [C12:0 + 4(C14:0) + (C16:0)] / \sum (\text{MUFA} + \text{PUFA})$$

The Δ^9 desaturase indices were used as indicators of the Δ^9 desaturase activity using fatty acids that are substrates and products for Δ^9 desaturase and calculated using the following model proposed by Lock and Garnsworthy (2003):

$$\text{Desaturase index (DI)} = C14:1/C14:0$$

4.2.6 Oxidative stability of feed materials and of fresh pork samples

A 5 g sample of lean meat (removed from the middle of each cut) was used for TBARS analysis using the aqueous acid extraction method of Raharjo *et al.* (1992) to determine lipid oxidation. A 2 g sample of back fat (BF) (inner + outer layer) was also removed for the lipid extraction, using the Folch *et al.* (1957) method. Thiobarbituric acid-reacting substances (TBARS) were expressed as micrograms of malonaldehyde (MDA) per gram of meat.

4.2.7 Statistical analysis

Data on fatty acid composition, health lipid indices and TBAR values were analysed using General Linear Model (GLM) procedures of SAS (2003). Interactions were significant at (P<0.05). The statistical model used was:

$$Y_{ijk} = \mu + \alpha_i + \beta_j + (\alpha\beta)_{ij} + E_{ijkl}$$

Where Y_{ijk} = dependent variables (fatty acid and health index variables, oxidative stability)

μ = Overall mean

α_i = i^{th} effect of breed (Large White, Kolbroek)

β_j = j^{th} effect of dietary treatment (0% MOLM, 2.5% MOLM, 5% MOLM)

$(\alpha\beta)_{ij}$ = breed and treatment interactions

E_{ijkl} = random error

The PDIFF option of SAS (2003) was used to compare the means.

4.3 Results and discussion

4.3.1 Fatty acid composition of the subcutaneous adipose tissue

The effect of pig breed and dietary treatment on the total percentages of subcutaneous fat (SCF %), fat-free dry matter (FFDM %) and moisture of subcutaneous adipose tissue is shown in Table 4.2. Breed differences in SCF% and moisture % were observed ($P<0.05$) in T1 and T3 treatment groups, however, no difference ($P>0.05$) were observed in FFDM% across all three treatment groups. The LW pigs had lower SCF% ($P<0.05$) and higher moisture% ($P<0.05$) as compared to KB pigs. In a study by Wood *et al.* (2004), two indigenous breeds (Berkshire and Tamworth), had higher fat content than the two exotic breed (Duroc and Large White). In the current study, there were no breed difference ($P>0.05$) due to dietary treatment in SCF%, FFDM% and moisture % across all treatments. This concurs with Mukumbo *et al.* (2014) who found no significant effect caused by the inclusion of MOLM in pig feed, on pork SCF %, FFDM % and moisture % in pork.

The fatty acid profiles of subcutaneous adipose tissue of the two breeds from the three dietary treatments are presented in Tables 4.3. The current study showed breed differences in C18:0 in pigs in T3, but no significant ($P>0.05$) differences were observed in pigs in T1 and T2. The level of C18:0 was significantly ($P<0.05$) higher in LW than in KB pigs. There were however no significant ($P>0.05$) differences as due to dietary MOLM inclusion in LW pigs. However, in KB pigs, 5% MOLM inclusion resulted in significantly ($P<0.05$) higher C18:0. There were significant breed differences in the content of C18:2c9,12 (n-6) across all treatment groups, where significantly ($P<0.05$) higher amounts were in LW pigs as compared to KB.

Table 4.2 Proximate composition of the subcutaneous adipose tissue as affected by breed and dietary treatment

Nutrient composition	Dietary Treatments		
	T1 (n=8)	T2 (n=8)	T3 (n=8)
Subcutaneous fat %			
Large White	60.57^{A}±2.579	63.09±2.978	56.62^{A}±2.978
Kolbroek	70.70^{B}±2.579	69.98±2.579	70.35^{B}±2.579
Fat free dry matter %			
Large White	10.42±0.917	10.74±1.059	11.94±1.059
Kolbroek	8.86±0.917	10.22±0.917	9.73±0.917
Moisture %			
Large White	29.02^{B}±2.217	26.17±2.560	31.44^{B}±2.560
Kolbroek	20.44^{A}±2.217	19.80±20217	19.92^{A}±2.217

^{AB}Means in the same column with different superscripts are significantly different (P<0.05); MOLM – *Moringa oleifera* leaf meal; T1 (0% MOLM); T2 (2.5% MOLM); T3 (5% MOLM).

Table 4.3 Total % fatty acid composition of subcutaneous adipose tissue as affected by breed and dietary treatment

Fatty acids (%)	Breed	Dietary Treaments		
		T1 (n =8)	T2 (n = 8)	T3 (n = 8)
C14:0	Large White	1.37±0.068	1.30±0.079	1.29^{A}±0.079
	Kolbroek	1.17^{ab}±0.068	1.27^{b}±0.068	1.01^{Ba}±0.068
C15:0	Large White	0.11±0.016	0.10^{B}±0.019	0.14^{B}±0.019
	Kolbroek	0.08±0.019	0.05^{A}±0.016	0.08^{A}±0.019
C16:0	Large White	24.95±0.537	25.06±0.620	24.52±0.620
	Kolbroek	23.43±0.537	26.12±0.537	24.77±0.537
C16:1c9	Large White	2.57±0.278	2.30±0.321	2.94^{A}±0.321
	Kolbroek	2.44±0.278	1.95±0.278	1.66^{B}±0.278
C17:0	Large White	0.67±0.147	0.75±0.147	1.00±0.147
	Kolbroek	0.62±0.128	0.51±0.128	0.64±0.128
C18:0	Large White	11.43±0.960	13.61±1.109	11.89^{B}±1.109
	Kolbroek	12.09^{a}±0.960	14.66^{ab}±0.960	15.60^{Ab}±0.960
C18:1c7	Large White	3.65^{a}±0.249	4.12^{ab}±0.288	4.70^{Bb}±0.288
	Kolbroek	4.08±0.249	3.87±0.249	3.60^{A}±0.249
C18:1t9	Large White	0.34^{B}±0.058	0.13±0.082	0.20±0.067
	Kolbroek	0.04^{A}±0.058	0.02±0.058	0.03±0.058
C18:1c9	Large White	35.73^{A}±0.815	36.51^{A}±0.941	36.78^{A}±0.941
	Kolbroek	40.07^{B}±0.815	39.76^{B}±0.815	40.36^{B}±0.815
C18:2c9,12 (n-6)	Large White	16.94^{Bb}±0.926	13.60^{Ba}±1.070	13.89^{Ba}±1.070
	Kolbroek	13.24^{Ab}±0.926	9.64^{Aa}±0.926	9.86^{Aa}±0.926
C20:0	Large White	0.16^{A}±0.018	0.19±0.021	0.14^{A}±0.021
	Kolbroek	0.21^{Ba}±0.018	0.23±0.018	0.26^{B}±0.018
C20:1c11	Large White	0.47±0.086	0.53±0.099	0.47^{A}±0.099
	Kolbroek	0.65±0.086	0.72±0.086	0.76^{B}±0.086
C18:3c9,12,15 (n-3)	Large White	0.73^{a}±0.066	0.84^{a}±0.076	1.09^{Bb}±0.076
	Kolbroek	0.70±0.066	0.72±0.076	0.70^{A}±0.076
C20:2c11,14 (n-6)	Large White	0.46±0.047	0.45±0.047	0.41±0.054
	Kolbroek	0.52±0.047	0.45±0.047	0.05±0.047
C20:3c8,11,14 (n-6)	Large White	0.12^{B}±0.009	0.09^{B}±0.010	0.10^{B}±0.010
	Kolbroek	0.07^{A}±0.009	0.05^{A}±0.013	0.05^{A}±0.009
C20:3c11,14,17 (n-3)	Large White	0.05±0.016	0.08±0.016	0.09±0.016
	Kolbroek	0.06±0.014	0.10±0.016	0.11±0.014
C20:4c5,8,11,14 (n-6)	Large White	0.37^{B}±0.026	0.28^{B}±0.031	0.31^{B}±0.031
	Kolbroek	0.20^{Ab}±0.026	0.09^{Aa}±0.026	0.16^{Aab}±0.026

^{AB}Means in the same column with different superscripts are significantly different (P<0.05); ^{ab}Means in the same row with different superscripts are significantly different (P<0.05); MOLM – *Moringa oleifera* leaf meal; T1 (0% MOLM); T2 (2.5% MOLM); T3 (5% MOLM).

In both breeds, the 2.5% and 5% MOLM inclusion resulted in significantly (P<0.05) lower amounts of C18:2c9,12 (n-6) as compared to 0% MOLM level. The inclusion level of MOLM at 5% resulted in breed and dietary treatment effects (P<0.05) in C18:3c9,12,15 (n-3). The LW pigs had significantly (P<0.05) higher C18:3c9,12,15 (n-3) than KB pigs, and when compared with other treatment groups (T1 and T2). As it is naturally expected, the LW pigs were prominent in having higher amounts of FAs than KB pigs across total FA profiles, and breed differences occurred in treatment groups where MOLM was included.

The effect of pig breed and dietary treatment on the saturated fatty acids (SFA), mono-unsaturated fatty acids (MUFA), polyunsaturated fatty acids (PUFA), omega-6 (*n*-6) fatty acids, omega-3 (*n*-3) fatty acids, and PUFA: SFA, PUFA: MUFA, *n*-6: *n*-3 ratios in the subcutaneous fat of pork is shown in Table 4.4. Both breed and treatment had no significant effect on the SFA content of fat (P>0.05). However, there were significant (P<0.05) breed differences observed in total PUFA and *n*-6 content, with LW pigs having a higher amounts in all the treatment groups. From the current study, LW pigs which had thinner backfat thickness had significantly (P<0.05) higher amounts of PUFA than KB pigs, across all dietary treatments. In both breeds, the inclusion levels of MOLM at 2.5% and 5% levels resulted in significantly (P<0.05) lower *n*-6 levels in the subcutaneous tissue. In T3, the *n*-3 content was significantly (P<0.05) higher in LW pigs than KB, and when compared to T1 and T2. Inclusion levels of MOLM at 5% resulted in favourably higher (P<0.05) *n*-3 amounts in LW pigs. Additionally, the PUFA: SFA and *n*-6: *n*-3 fatty acids ratio in the subcutaneous adipose tissue, was significantly lower (P<0.05) in T2 and T3 than in T1.

Table 4.4 Total % and fatty acid ratios of subcutaneous adipose tissue as affected by breed and dietary treatment

Fatty acids (%)	Breed	Dietary Treatments		
		T1 (n = 8)	T2 (n = 8)	T3 (n = 8)
Total SFA	Large White	38.57±1.24	41.08±1.43	39.03±1.43
	Kolbroek	37.63±1.24	42.87±1.24	42.35±1.24
Total MUFA	Large White	42.78^{A}±1.037	43.55^{A}±1.198	45.09^{A}±1.198
	Kolbroek	47.57^{B}±1.037	46.31^{A}±1.037	46.41^{A}±1.037
Total PUFA	Large White	18.67^{B}±1.035	15.37^{B}±1.194	15.88^{B}±1.195
	Kolbroek	14.80^{Ab}±1.035	10.81^{Aa}±1.035	11.25^{Aa}±1.035
Total *n*-6	Large White	17.89^{Bb}±0.956	14.43^{Ba}±1.104	14.71^{Ba}±1.104
	Kolbroek	14.04^{Ab}±0.956	10.20^{Aa}±0.956	10.61^{Aa}±0.956
Total *n*-3	Large White	0.79^{a}±0.107	0.94^{a}±0.123	1.18^{Bb}±0.123
	Kolbroek	0.76±0.107	0.82±0.123	0.64^{A}±0.107
PUFA: SFA	Large White	0.49^{b}±0.031	0.38^{Ba}±0.036	0.41^{Bab}±0.036
	Kolbroek	0.39^{b}±0.031	0.26^{Aa}±0.031	0.27^{Aa}±0.031
PUFA: MUFA	Large White	0.44^{Bb}±0.026	0.35^{Ba}±0.030	0.35^{Ba}±0.030
	Kolbroek	0.31^{A}±0.026	0.24^{A}±0.026	0.24^{A}±0.026
n-6: *n*-3	Large White	22.87^{b}±1.448	15.58^{a}±1.672	12.48^{a}±1.672
	Kolbroek	19.44^{b}±1.448	13.49^{a}±1.672	14.58^{a}±1.448

^{AB}Means in the same with different superscripts are significantly different (P<0.05); ^{ab}Means in the same row with different superscripts are significantly different (P<0.05); MOLM – *Moringa oleifera* leaf meal; T1 (0% MOLM); T2 (2.5% MOLM); T3 (5% MOLM); SFA – saturated fatty acids; MUFA – monounsaturated fatty acids; PUFA – polyunsaturated fatty acids; *n*-6 – omega 6 fatty acids; *n*-3 – omega 3 fatty acids; PUFA: SFA – polyunsaturated fatty acids and saturated fatty acids ratio; PUFA: MUFA – polyunsaturated fatty acids and monounsaturated fatty acids ratio; *n*-6: *n*-3 – omega 6 fatty acids and omega 3 fatty acids ratio.

The KB pigs had significantly (P<0.05) lower ratio of PUFA:SFA than LW pigs in T2 and T3, but no significant (P>0.05) breed differences were observed in the *n*-6:*n*-3 across all treatment groups. The current study aimed at feeding MOLM to try and improve the *n*-3 and reduce the *n*-6:*n*3 content of pork because higher level of *n*-3 FAs increases the nutritional value of pork and is considered to be good for health. In a study by Mukumbo *et al.* (2014), the fatty acid profiles of all subcutaneous pork fat samples analysed were similar and there was no significant differences (P>0.05) in the fatty acid composition across all samples from the different dietary levels of MOLM (2.5%, 5% and 7.5%).

4.3.2 Fatty acid composition of the Longissimus thoracic et. lumborum muscle

The effect of pig breed and dietary treatment on the total percentages of intramuscular fat (IMF %), fat-free dry matter (FFDM %) and moisture % of pork muscle is presented in Table 4.5. There were no significant breed differences (P>0.05) in IMF% and moisture % of the pigs across all dietary treatments. Breed differences were observed in the FFDM% of pigs in T1, where LW pigs had significantly (P<0.05) lower FFDM% than KB pigs. At 2.5% and 5% MOLM inclusion levels, no significant (P>0.05) breed differences were observed in FFDM %.

The fatty acid composition of pork muscle samples from the three dietary treatments is presented in Tables 4.6. More recently, nutritionists have focussed on the type of PUFA and the balance in the diet between *n*-3 PUFA formed from α-linolenic acid (18:3) and *n*-6 PUFA formed from linoleic acid (18:2) (Williams, 2000).

Table 4.5 Proximate composition of the *Longissimus thoracic et. lumborum* muscle as affected by breed and dietary treatment

Nutrient composition	Dietary Treatments		
	T1 (n=8)	T2 (n=8)	T3 (n=8)
Intramuscular fat %			
Large White	2.42±0.464	2.76±0.536	1.67±0.536
Kolbroek	2.75±0.464	3.48±0.464	2.36±0.464
Fat free dry matter %			
Large White	18.95^{Aa}±0.605	21.34^{Ab}±0.699	22.35^{Ab}±0.699
Kolbroek	20.86^{Ba}±0.605	22.45^{Aa}±0.605	22.13^{Aa}±0.605
Moisture %			
Large White	77.68±0.872	75.90±1.007	76.00±1.007
Kolbroek	75.59±0.872	73.49±0.872	75.51±0.872

^{AB}Means in the same column with different superscripts are significantly different (P<0.05); ^{ab}Means in the same row with different superscripts are significantly different (P<0.05); MOLM – *Moringa oleifera* leaf meal; T1 (0% MOLM); T2 (2.5% MOLM); T3 (5% MOLM); SFA – saturated fatty acids.

Table 4.6 Total % fatty acid composition of *Longissimus thoracic et. lumborum* muscle as affected by breed and dietary treatment

Fatty acids (%)	Breed	Dietary Treatments		
		T1 (n = 8)	T2 (n = 8)	T3 (n = 8)
C14:0	Large White	1.08±0.961	1.01±0.111	0.85±0.111
	Kolbroek	1.19±0.961	1.15±0.961	0.93±0.961
C15:0	Large White	0.12^{ab}±0.014	0.09^{Ba}±0.014	0.14^{b}±0.014
	Kolbroek	0.08^{b}±0.014	0.04^{Aa}±0.014	0.11^{b}±0.014
C16:0	Large White	25.18±0.644	24.81±0.744	23.95±0.744
	Kolbroek	23.95^{a}±0.644	25.92^{b}±0.644	24.46^{ab}±0.644
C16:1c9	Large White	2.50±0.387	2.39±0.447	2.26±0.447
	Kolbroek	2.47±0.387	2.94±0.387	2.35±0.387
C17:0	Large White	0.54±0.075	0.59±0.087	0.71±0.087
	Kolbroek	0.45±0.075	0.37±0.075	0.58±0.075
C17:1c10	Large White	0.30^{Aa}±0.113	0.36^{a}±0.130	0.79^{b}±0.113
	Kolbroek	0.91^{Bb}±0.130	0.21^{a}±0.113	0.50^{a}±0.113
C18:0	Large White	13.12±0.657	13.80±0.759	12.91±0.930
	Kolbroek	12.93±0.657	13.54±0.657	12.97±0.657
C18:1c7	Large White	4.34±0.289	2.83±0.333	5.18±0.333
	Kolbroek	4.52±0.289	4.92±0.289	4.73±0.289
C18:1t9	Large White	0.05^{b}±0.010	0.01^{a}±0.011	0.06^{b}±0.014
	Kolbroek	0.03±0.014	0.02±0.010	0.03±0.010
C18:1c9	Large White	34.06±1.318	35.88±1.522	33.59±1.522
	Kolbroek	37.91±1.318	38.71±1.318	36.68±1.318
C18:2c9,12 (n-6)	Large White	15.33^{Bb}±1.048	11.62^{a}±1.210	13.84^{ab}±1.210
	Kolbroek	12.12^{A}±1.048	8.99±1.048	11.53±1.048
C20:0	Large White	0.14±0.018	0.14±0.020	0.11±0.020
	Kolbroek	0.18±0.020	0.16±0.017	0.15±0.018
C18:3c6,9,12 (n-3)	Large White	0.10±0.012	0.09^{B}±0.017	0.10±0.014
	Kolbroek	0.06±0.024	0.04^{A}±0.012	0.07±0.012
C20:1c11	Large White	0.48±0.064	0.51±0.074	0.44^{A}±0.074
	Kolbroek	0.61±0.064	0.63±0.064	0.66^{B}±0.064
C18:3c9,12,15 (n-3)	Large White	0.46^{a}±0.051	0.53^{a}±0.059	0.72^{Ab}±0.059
	Kolbroek	0.45^{a}±0.059	0.42±0.051	3.50^{B}±0.051
C20:2c11,14 (n-6)	Large White	0.38±0.027	0.33±0.031	033±0.031
	Kolbroek	0.04±0.031	0.28±0.027	0.30±0.027
C22:0	Large White	0.08±0.022	0.11±0.031	0.14±0.025
	Kolbroek	0.05±0.031	0.05±0.031	0.08±0.025
C20:3c8,11,14 (n-6)	Large White	0.29±0.059	0.29±0.029	0.37±0.068
	Kolbroek	0.19±0.068	0.19±0.059	0.28±0.059

C20:3c11,14,17 (n-3)	Large White	0.03±0.012	0.04±0.012	0.03±0.010
	Kolbroek	0.07±0.017	0.05±0.008	0.05±0.010
C20:4c5,8,11,14 (n-6)	Large White	3.09±0.705	2.62±0.815	3.78±0.815
	Kolbroek	1.63±0.705	1.63±0.705	2.69±0.705
C20:5c5,8,11,14,17 (n-3)	Large White	0.07^{a}±0.028	0.15^{Ba}±0.028	0.16^{b}±0.028
	Kolbroek	0.07^{a}±0.048	0.05^{A}±0.034	0.09±0.024
C22:5c7,10,13,16,19 (n-3)	Large White	0.16^{a}±0.051	0.27^{a}±0.070	0.41^{b}±0.058
	Kolbroek	0.14^{a}±0.058	0.16^{a}±0.070	0.33^{b}±0.051

^{AB}Means in the same column with different superscripts are significantly different (P<0.05);
^{ab}Means in the same row with different superscripts are significantly different (P<0.05);
MOLM – *Moringa oleifera* leaf meal; T1 (0% MOLM); T2 (2.5% MOLM); T3 (5% MOLM).

Information in the current study is restricted to the major fatty acids in intramuscular fat in relation to human health considerations. Pig breed and dietary treatment had no significant (P>0.05) effect in C18:0 across all treatment groups. In T1, significant (P<0.05) breed differences were shown in the C18:2c9,12 (n-6), where LW pigs had higher amounts than KB pigs an when compared with T2 and T3.

The effects of pig breed and dietary treatment on the composition of saturated fatty acids (SFA), mono-unsaturated fatty acids (MUFA), polyunsaturated fatty acids (PUFA), omega-6 (*n*-6) fatty acids, omega-3 (*n*-3) fatty acids, and PUFA: SFA, PUFA: MUFA and *n*-6:*n*-3 ratios in pork muscle are shown in Table 4.7. There were no significant (P>0.05) breed differences in the SFA across treatment groups. Treatment effects were observed in LW pigs, where inclusion of MOLM at 5% resulted in significantly (P<0.05) lower SFA level. Lower levels of saturated fatty acids are more desirable in meat. The values for the other fatty acids did not differ significantly (P>0.05) in both breeds, across the dietary treatments. There was however a general trend of increasing amounts of PUFAs and *n*-6 FAs as the MOLM inclusion level was increased. Although pork muscle samples from pigs fed on MOLM had slightly higher levels of PUFAs and *n*-6, they were not significantly (P>0.05) different from the 0% MOLM samples. There were breed differences observed in PUFA: SFA fatty acid ratio in pork muscle in T3 MOLM inclusion levels, where LW pigs had a significantly (P<0.05) higher content (0.60) than KB pigs (0.41). A high PUFA: SFA ratio of 0.4 or above is recommended in the human diet (Teye *et al.*, 2006).

Table 4.7 Total % and fatty acid ratios of *Longissimus thoracic et. lumborum* muscle as affected by breed and dietary treatment

Fatty acids (%)	Breed	Dietary Treatments		
		T1 (n = 8)	T2 (n = 8)	T3 (n = 8)
Total SFA	Large White	40.3^{b}±1.680	40.51^{b}±1.94	34.01^{a}±1.941
	Kolbroek	40.09±1.680	41.64±1.680	39.23±1.680
Total MUFA	Large White	40.78±2.379	43.98±2.747	46.22±2.747
	Kolbroek	45.93±2.379	47.43±2.379	44.94±2.379
Total PUFA	Large White	18.96±1.862	15.51±2.150	19.77±2.150
	Kolbroek	13.98±1.862	12.96±1.862	15.83±1.862
Total *n*-6	Large White	18.18±1.873	14.55±2.162	18.31±2.162
	Kolbroek	13.49±1.873	10.72±1.873	14.80±1.873
Total *n*-3	Large White	0.78^{a}±0.106	0.96^{Ba}±0.123	1.46^{Bb}±0.123
	Kolbroek	0.65^{a}±0.123	0.60^{Aa}±0.106	1.03^{Ab}±0.106
PUFA:SFA	Large White	0.47^{ab}±0.056	0.38^{a}±0.064	0.60^{b}±0.064
	Kolbroek	0.35±0.056	0.28±0.056	0.41±0.056
PUFA:MUFA	Large White	0.49±0.065	0.36±0.075	0.44±0.074
	Kolbroek	0.31±0.065	0.24±0.065	0.36±0.065
n-6:*n*-3	Large White	23.37^{b}±1.636	15.10^{a}±1.889	12.61^{a}±1.889
	Kolbroek	20.82^{b}±1.889	18.46^{ab}±1.636	14.25^{a}±1.636

^{AB}Means in the same column with different superscripts are significantly different (P<0.05); ^{ab}Means in the same row with different superscripts are significantly different (P<0.05); *Moringa oleifera* leaf meal; T1 (0% MOLM); T2 (2.5% MOLM); T3 (5% MOLM); SFA – saturated fatty acids; MUFA – monounsaturated fatty acids; PUFA – polyunsaturated fatty acids; *n*-6 – omega 6 fatty acids; *n*-3 – omega 3 fatty acids PUFA: SFA – polyunsaturated fatty acids and saturated fatty acids ratio; PUFA: MUFA – polyunsaturated fatty acids and monounsaturated fatty acids ratio; *n*-6: *n*-3 – omega 6 fatty acids and omega 3 fatty acids ratio.

Meat fatty acid composition is influenced by genetic factors, although to a lower extent than dietary factors (De Smet *et al.*, 2004). There is no available information of fatty acid profiles and their comparison in the Large White and Kolbroek pigs. The inclusion levels of MOLM at 2.5% and 5% levels in LW pigs, and 5% in KB pigs significantly increased ($P<0.05$) *n*-3 in pork muscle. Generally, *M. oleifera* leaves contain plant bioactive compounds which modify the cholesterol metabolism of the body, therefore leading to a product with healthier implications for human consumption (Wallace *et al.*, 2010). The *n*-3 and *n*-6 FAs play an important role in human nutrition and are essential to human health (Simopoulus, 2002), in the regulation of the cardiovascular system and immunological processes (Grashorn, 2007). Hence, the lowest ratio of *n*-6:*n*-3 fatty acids tends to support better cardiovascular health (Simopoulus, 2002). Inclusions of MOLM at 2.5% and/or 5% resulted in significantly ($P<0.05$) lower *n*-6:*n*-3 fatty acids in pork from LW pigs and KB pigs. The recommended value of *n*-6: *n*-3 ratio is 4.0, even though it can be manipulated to be higher than this in some meats (Muchenje *et al.*, 2009; Wood *et al.*, 2003); with dietary inclusions that are likely to influence the long chain *n*-3 fatty acids (Givens *et al.*, 2011). The values obtained from the current study were above 4.0. Pig meat normally has a high 18:2 content, producing an unfavourably high *n*-6:*n*-3 ratio (Enser *et al.*, 2000). Although higher values of the *n*-6: *n*-3 ratio were obtained from the current study, they were reduced by the inclusion of MOLM in the pig diets.

4.3.3 Health lipid indices

The calculated atherogenicity index (AI) and desaturase index (DI) for both pork fat and LTL muscle samples from all three dietary treatments and both breeds are presented in Table 4.8. Neither the effect of breed nor dietary treatment was found to be significant for both indices ($P>0.05$). However, the AI values observed in this study are acceptably low, with highest value of 0.55 in LW pigs, and 0.57 in KB pigs.

Table 4.8 Effect of dietary treatment on atherogenicity and desaturase indexes of subcutaneus fat and intramuscular fat

Index	Breed	Dietary Treatments		
		T1 (n = 8)	T2 (n = 8)	T3 (n = 8)
Subcutaneus fat				
AI	Large White	0.50±0.020	0.51±0.022	0.49±0.023
	Kolbroek	0.45±0.020	0.55±0.020	0.50±0.020
DI	Large White	3.18±0.241	2.68±0.278	3.12±0.278
	Kolbroek	3.38±0.241	2.75±0.241	2.68±0.278
Intramuscular fat				
AI	Large White	0.50±0.045	0.48±0.053	0.39±0.053
	Kolbroek	0.57±0.045	0.53±0.045	0.46±0.045
DI	Large White	2.59±0.219	2.60±0.253	2.50±0.310
	Kolbroek	3.00±0.219	2.87±0.219	2.83±0.219

MOLM (*Moringa oleifera* leaf meal); T1 (0% MOLM); T2 (2.5% MOLM); T3 (5% MOLM); AI (athorogenicity index); DI (desaturase index).

This lack of significant effect on AI is in agreement with findings by Nkukwana *et al.* (2014) who also reported no MOLM effect in AI of meat. Other studies have shown increased SFAs and decreased MUFAs of the subcutaneous fat and attributed this to a reduction in desaturase index (Sun *et al.*, 2004; Cordero *et al.*, 2010). The insignificant results in DI may be explained by the insignificant differences in SFA and MUFA content observed in the subcutaneous fat as shown in Table 4.4. The observed values of DI in the current study were lower than those reported by Bothma (2012).

4.3.4 Thiobarbituric acid reactive substances (TBARS)

Table 4.9 presents the TBARS as a measure of oxidative stability in pork muscle. The means (±SE) for average TBARS (mg MDA/kg meat) of pork muscle as affected by breed and dietary treatment were analysed. The inclusion of MOLM at 2.5% and 5% levels did not result in any significant breed differences ($P>0.05$) in pork. Significant ($P<0.05$) breed differences were observed at 0% MOLM (T1) inclusion level, where pork from LW pigs had significantly ($P<0.05$) higher TBARS values (0.26) than pork from KB pigs (0.15). This suggests that, the KB pigs at this treatment group can resist lipid oxidation or improve in oxidative stability than LW pigs. There are limited studies that report the effect of pig breed on the oxidative stability of pork. Oil extracted from *M. oleifera* leaves was shown to have significantly high oxidative stability, demonstrating the presence of natural antioxidants (Anwar and Bhanger, 2003). The current study showed no significant differences ($P>0.05$) caused by the inclusion of MOLM in pig diets, in TBARS (measures of oxidative stability) of pork in both breeds. However, for all treatments, pork had lower TBARS values ranged between 0.15 to 0.26 in LW pigs, and 0.09 to 0.15 in KB pigs. The TBARS values observed in this study were lower than those reported by Asghar *et al.* (1991).

Table 4.9 Means (±SE) for average TBARS (mg MDA/kg meat) of *Longissimus thoracic et. lumborum* muscle as affected by breed and dietary treatment

Breed	TBARS (mg MDA/kg meat)		
	T1 (n=8)	T2 (n=8)	T3 (n=8)
Large White	0.26^{B}±0.035	0.15±0.041	0.18±0.041
Kolbroek	0.15^{A}±0.035	0.14±0.035	0.09±0.035

^{AB}Means in the same column with different superscripts are significantly different (P<0.05); MOLM – *Moringa oleifera* leaf meal; T1 (0% MOLM); T2 (2.5% MOLM); T3 (5% MOLM); TBARS (thiobarbituric acid reactive substances).

4.4 Conclusion

Results of the current study demonstrated that inclusion of MOLM at additive levels of up to 5% influenced the fatty acid composition in subcutaneous adipose tissue and muscle tissues of pork. The inclusion of 2.5% and 5% MOLM in pig feed resulted in desirably higher levels of *n*-3 fatty acids and lower levels of *n*-6:*n*-3 fatty acids in the subcutaneous adipose tissue and the *Longissimus thoracic et. lumborum* muscle in both breeds. The inclusion of MOLM did not cause significant differences in the muscle TBARS, although it tended to generally lower the TBARS in pork, indicating a potential to reduce lipid oxidation. Therefore, in observing the fatty acid profiles and TBARS in meats from pigs fed MOLM diets in the current study confirm the potential of *Moringa oleifera* leaf meal to improve levels of *n*-3 fatty acids and reduce the *n*-6: *n*-3 fatty acid ratio, as well as a proficient source of natural antioxidants for pork. Fat is a major determinant of meat flavour, tenderness and juiciness, which are used by consumers to evaluate meat quality.

4.5 References

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Chapter 5

The effect of cooking method on consumer sensory scores of pork from Large White and Kolbroek pigs fed with different levels of *Moringa oleifera* leaf meal

Abstract

The effect of cooking method on consumer sensory evaluation of pork quality from pigs that were supplemented with different levels of *Moringa oleifera* leaf meal (MOLM) was studied. Twelve Large White (LW) and 12 Kolbroek (KB) male pigs at 6 weeks of age, were individually housed and randomly subjected to three dietary treatments formulated to contain 0% (T1), 2.5% (T2) and 5% (T3) MOLM, at four pigs per treatment per breed, for eight weeks. Dietary treatments were formulated for weaner (6 – 8 weeks) and grower (9 – 13 weeks) phases. Pigs were slaughtered after eight weeks of feeding trial. Sample cuttings were made from the *Longissimus thoracic et. Lumborum* muscle from the right side of each carcass, and were stored at -20°C until the time of analysis. Meat for tasting was prepared using two thermal conductivities, namely boiling and frying. Consumer sensory evaluation was done with 120 consumers of different ages, gender and tribes. A meat sensory characteristic evaluation form containing a six-point rating scale of meat characteristics was used to give scores to different pork sensory characteristics. The evaluated sensory characteristics were aroma intensity, initial impression of juiciness, first bite, sustained impression of juiciness, muscle fibre and overall tenderness, amount of connective tissue, overall flavour intensity, and off-flavour presence and intensity. Consumer sensory scores on sensory attributes significantly (P<0.05) increased as the level of MOLM inclusion in the diet increased. Significant (P<0.05) breed differences were observed in the sensory scores for OFPI, where KB pigs had higher scores for as compared to LW pigs. In both breeds, the 2.5% and 5% MOLM inclusion resulted in significantly (P<0.05) higher scores for aroma intensity (AI), initial impression of juiciness (IIJ), first bite (FB), sustained impression of

juiciness (SIJ), muscle fibre and overall tenderness (MFT) and overall flavour intensity (OFI) with generally higher scores observed in LW pigs than KB pigs. In most sensory attributes, higher scores were observed in fried meat than in boiled, across the dietary treatments. The inclusion of *Moringa oleifera* leaf meal in pig diets significantly improved the sensory attributes of pork in both breeds.

Keywords: Boiled pork, consumer perceptions, fried pork, pork quality, tasting panel.

5.1 Introduction

Consumer acceptance refers to the willingness and preference of consumers to consume meat based on its appearance, taste and flavour when it is consumed. In determining meat quality and acceptability, consumers consider sensory characteristics (Muchenje *et al.*, 2008; Xazela *et al.*, 2011). Sensory characteristics used to assess pork eating quality also include aroma, tenderness, flavour intensity, level of juiciness and the presence of off-flavours (Tshabalala *et al.*, 2003; Webb *et al.*, 2005; Lawrie and Ledward, 2006). Nevertheless, differences can be observed in meat sensory characteristic due to different factors such as animal's genotype, diet and meat cooking method (Ibañez and Barcina, 2001; Esenbuga *et al.*, 2009). Reports have indicated that the meat eating quality could be changed by different cooking techniques and heat treatments (Xazela *et al.*, 2011), leading to various perceptions of meat (Hoffman and Wiklund, 2006).Various cooking methods that have been shown to affect sensory and nutritional properties of meat include boiling, frying, roasting and grilling, (Dzudie *et al.*, 2000; Xazela *et al.*, 2011). This implies that the cooking method has a significant impact on nutritional value, eating quality and general consumers 'acceptability of pork. There is, however, scarce information of how various meat sensory qualities interact with these various factors.

To determine the consumers' judgement on meat, most studies have used trained taste panels (Tshabalala *et al.*, 2003; Simela *et al.*, 2008; Muchenje *et al.*, 2008; Muchenje *et al.*, 2010). Trained panel evaluations of meat can correspond with that of consumers (Sveinsdóttir *et al.*, 2009). Simela *et al.* (2008) reported trained panel sensory scores on meat to match the results of instrumental evaluations of meat quality. Therefore, although laboratory procedures can be used to determine the physico-chemical quality of meat; consumers are also able to indicate practical data about the acceptability of the meat. Consumers are the end users of meat and

they give reliable information on meat quality. Studies have been conducted on the effects of MOLM on the physico-chemical characteristics of meat from goats, poultry and pigs (Qwele *et al.*, 2013; Wapi *et al.*, 2013; Nkukwana *et al.*, 2014; Mukumbo *et al.*, 2014). In addition, Moyo *et al.* (2013) studied the effect of MOLM on sensory characteristics of goat meat. Of these authors, none have studied the consumer sensory scores for pork from Large White and Kolbroek pigs fed different levels of MOLM. Additionally, there is little research on the influence of cooking method on consumer sensory scores of pork. Therefore, the objective of the current study was to determine the effect of the inclusion of *Moringa oleifera* leaf meal on consumer sensory scores of boiled and fried pork from Large White and Kolbroek pigs.

5.2 Materials and Methods

5.2.1 Study site description

The feeding trial was conducted at a small scale commercial piggery at Fort Cox College of Agriculture and Forestry. A detailed study site description is given in Chapter 3, Section 3.2.1.

5.2.2 Animal management and experimental design

Twenty four pigs (12 LW and 12 KB) male pigs, weighing on average 10 kg, were randomly divided into three groups of four for each breed. The groups were then randomly assigned to each of three dietary treatments, that consisted of a control diet (0% MOLM), 2.5% MOLM and 5% MOLM. The management of pigs and experimental design are fully described in Section 3.2.2. The ingredient composition of dietary treatments and the nutrient composition of the experimental diets and MOLM are presented in Tables 3.1 and 3.2, respectively. Table 4.1 presents the fatty acid composition of the experimental dietary treatments and MOLM.

5.2.3 Meat sample preparation and thermal treatments

After the feeding trial of eight weeks, the pigs were transported to the abattoir and slaughtered as described in Section 3.2.3. The *Longissimus thoracis et lumborum* (LTL) muscle samples were taken 24 hours after slaughter from the right side of each carcass. The entire muscle was cut into 25 mm thick loin chops and samples were vacuum sealed (Gastrovac Pro, Henkovac, Netherlands) and stored at -20°C until the time of consumer sensory evaluation. Before preparation, the samples were thawed over 12 hours at room temperature. Pork loin chops were prepared using two thermal treatments, namely boiling and frying. Meat from each treatment in each breed was boiled or fried alone. The samples were boiled in water at 80°C for 40 minutes. The other samples were prepared in frying pans at,

using cooking oil. Salt was added for taste. Every piece was cut into approximately 2 × 2 × 2 cm samples and stores in warm pans at 50°C until tasting.

5.2.4 Sensory evaluation

The sensory quality of pork was evaluated was by a consumer panel composed of 120 students and staff from the University of Fort Hare. The panel of evaluators were invited using e-mails and they were of different tribes (Xhosa, Shona, Zulu, Other), different age group (≤ 20, 21-25, 26-30, 31-35, ≥ 40) and gender (female, male). Each participant was given scoring sheet to record the sensory scores for each sample. A pork sensory characteristic evaluation form (Appendix 1), containing a six-point rating scale of meat characteristics was used to give scores to different pork sensory characteristics. The evaluated sensory characteristics were: aroma intensity (AI), where score 1 is extremely bland and score 6 is extremely intense; initial impression of juiciness (IIJ), where score 1 is extremely dry and score 6 is extremely juicy; first bite (FB), where score 1 is extremely tough and score 6 is extremely tender; sustained impression of juicinesss (SIJ), where score 1 extremely dry and score 6 is extremely juicy; muscle fibre and overall tenderness (MFT), where score 1 is extremely tough and score 6 is extremely tender; amount of connective tissue (ACT), where score 1 is extremely bland and score 4 is none; overall flavour intensity (OFI), where score 1 is extremely bland and score 6 is extremely intense; and off-flavour presence and intensity (OFPI), where score 1 is none and score 5 is extremely intense. The off-flavour indicators were livery/bloody, cooked vegetable, pasture/grassy, animal like/kraal (manure), metallic, sour, unpleasant and none.

Samples were put in plates and were randomly circulated among the consumer panelist. The panelists were allowed to evaluate twelve samples, corresponding to the two methods of

cooking samples from the three treatments in each of the two breeds. The participants were trained on making inferences and recording the scores for each of the six samples. The panellists evaluated the samples after every 10 minutes so as to limit the crossover of the treatments. The evaluation form was accompanied by confidentiality and informed consent form and the participants had to sign the form, as an agreement to participate freely and without being forced to do so. Ethical principles were considered to conform to the national and international standards governing the nature of this study. Permission to work with people was obtained from the Ethical Clearance Committee of the University of Fort Hare (Certificate Reference Number: MUC011 SNDU01) (Appendix 2).

5.2.5 Statistical analysis

The general linear model procedure of the SAS (2003) was used to analyse the effects of MOLM inclusion in pig diet and cooking method, on their scores on pork sensory attributes. The model is:

$$Y_{ijk} = \mu + \alpha_i + \beta_j + (\alpha\beta)_{ij} + E_{ijkl}$$

Where Y_{ijk} = response variable (aroma intensity, initial impression of juiciness, first bite, sustained impression of juiciness, muscle fibre and overall tenderness, amount of connective tissue, overall flavour intensity and off-flavour presence and intensity),

μ = overall mean common to all observations

α_i = effect of 0%, 2.5% and 5% MOLM inclusion

β_j = effect of cooking method (boiled, fried)

$(\alpha\beta)_{ij}$ = cooking method and MOLM inclusion interaction

E_{ijkl} = random error

5.3 Results and Discussion

5.3.1 Consumer demographic information

The demographic data of consumers used in the study are shown in Table 5.1. Majorities (56.3 %) of the panelist were relatively young, between the ages of 21 to 25 years, and 4.2 % were above the age of 40 years. Many of them were males (57 %) and 43 % were females. Majorities (85.7 %) of the consumer panelist were of the Xhosa tribe and few of them were from the Zulu (6.7 %), Shona (5.9 %) and other tribes (1.7 %).

5.3.2 Consumer sensory scores of pork

Table 5.2 shows the effect of MOLM inclusion on the sensory attributes of pork from LW and KB pigs. The results from the current study showed significant (P<0.05) breed differences only in the IIJ, FB and OFPI. Compared to KB pigs, pork from LW pigs had significantly higher (P<0.05) scores for IIJ and FB and significantly (P<0.05) lower scores in OFPI. There were no significant (P>0.05) breed differences observed in the sensory scores for AI, SIJ, MFT, ACT and OFI. However, in all of the sensory attributes, significant (P<0.05) differences were observed due to dietary treatment. The scores significantly (P<0.05) increased as the level of MOLM inclusion in the diet increased. In both breeds, pork from pigs that had MOLM inclusions in their diet had generally higher sensory scores compared to those that did not receive MOLM in the diet. For OFPI, higher (P<0.05) scores were observed in pork from the 0% MOLM inclusion group, in both breeds. The consumer scores for aroma intensity were significantly (P<0.05) higher at 5% MOLM inclusion in LW pigs, while pork from KB pigs received significantly (P<0.05) higher scores both at 2.5% and 5% MOLM inclusion levels.

Table 5.1 Demographic information of consumers used in the study

Variable	Category	Number	Proportion (%)
Age	≤ 20	12	10.1
	21 – 25	68	56.3
	26 – 30	22	18.5
	31 – 35	10	8.4
	36 – 40	3	2.5
	≥ 40	5	4.2
Tribe	Xhosa	103	85.7
	Zulu	8	6.7
	Shona	7	5.9
	Other	2	1.7
Gender	Male	69	57%
	Female	51	43%

Table 5.2 Effect of *Moringa oleifera* inclusion on consumer sensory scores of pork

Sensory attributes	Dietary treatments		
	T1 (0% MOLM)	T2 (2.5% MOLM)	T3 (5% MOLM)
Aroma intensity			
Large White	4.44^{a}±0.101	4.56^{a}±0.101	4.80^{b}±0.101
Kolbroek	4.30^{a}±0.101	4.54^{b}±0.101	4.87^{c}±0.101
Initial impression of juiciness			
Large White	4.14^{Ba}±0.096	4.40^{Bb}±0.096	4.86^{Bc}±0.096
Kolbroek	3.88^{Aa}±0.096	4.20^{Ab}±0.096	4.48^{Ac}±0.096
First bite			
Large White	4.07^{Ba}±0.093	4.26^{b}±0.093	4.77^{Bc}±0.093
Kolbroek	3.86^{Aa}±0.093	4.20^{b}±0.093	4.44^{Ac}±0.093
Sustained impression of juiciness			
Large White	4.15^{a}±0.087	4.48^{b}±0.087	4.83^{c}±0.087
Kolbroek	4.19^{a}±0.087	4.42^{b}±0.087	4.70^{c}±0.087
Muscle fibre and overall tenderness			
Large White	4.05^{a}±0.093	4.29^{b}±0.093	4.71^{c}±0.093
Kolbroek	3.96^{a}±0.093	4.32^{b}±0.093	4.80^{c}±0.093
Amount of connective tissue			
Large White	3.04^{a}±0.081	3.29^{b}±0.081	3.22^{b}±0.081
Kolbroek	2.94^{a}±0.081	3.15^{b}±0.081	3.12^{b}±0.081
Overall flavour intensity			
Large White	4.03^{a}±0.096	4.48^{b}±0.096	4.72^{c}±0.096
Kolbroek	4.02^{a}±0.096	4.41^{b}±0.096	4.75^{c}±0.096
Off-flavour presence and intensity			
Large White	1.23^{Ab}±0.041	1.07^{Aa}±0.041	1.19^{Ab}±0.041
Kolbroek	1.44^{Bb}±0.041	1.30^{Ba}±0.041	1.32^{Ba}±0.041

^{AB}Means in the same column per carcass traits with different superscripts are significantly different (P<0.05); ^{ab}Means in the same row with different superscripts are significantly different (P<0.05); MOLM (*Moringa oleifera* leaf meal).

In both breeds, sensory scores for IIJ, FB, SIJ, MFT and OF were significantly (P<0.05) higher as the level of MOLM increased in the diet. In both breeds, inclusion levels of MOLM at 2% and 5% resulted in significantly (P<0.05) higher scores. Sensory characteristics of pork such as tenderness, flavour and aroma are critical aspects of pork quality that determine the eating preference of consumers and can influence decisions on consumption of pork (Tshabalala *et al.*, 2003; Muchenje *et al.*, 2008). The sensory attributes of meat are manipulated by variations in diet nutritional and quality composition and feed consumption (Muchenje *et al.*, 2008). Meat juiciness is directly related to the intramuscular fat and moisture content of the meat, which improves its palatability (Webb *et al.*, 2005; Muchenje *et al.*, 2008). High scores for juiciness in the current study could be because of the high amount of fat in pork meat, which allows marbling. In the current study, the positive effect of MOLM dietary inclusion on pork sensory attributes, mainly tenderness, juiciness and flavour is attributed to its nutrient composition of *M. oleifera* leaves. The leaves are reported to be a good source of proteins and minerals which are the dietary requirements for the development of meat sensory attributes (Moyo *et al.*, 2013). *M. oleifera* leaves also have high antibacterial, antihelmintic (Moyo *et al.*, 2011) and antioxidant properties (Siddhuraju and Becker, 2003; Qwele *et al.*, 2013) which improve meat quality and improve the nutritional composition of the meat.

Table 5.3 shows the effect MOLM inclusion and cooking method (boiled and fried meat) on sensory scores of pork form LW and KB pigs. There were significant (P<0.05) breed differences in IIJ and FB, where LW pigs had higher scores than KB in both boiled and fried meat. Once meat has been cooked, Consumers tend to evaluate cooked meat quality on the basis of its flavour, aroma and juiciness (Xazela *et al.*, 2011). In both breeds, the 2.5% and 5% MOLM inclusion resulted in significantly (P<0.05) higher scores for AI, IIJ, FB and SIJ, with generally higher scores observed in fried meat.

Table 5.3 Effect of *Moringa oleifera* inclusion and cooking methods on consumer sensory scores of pork

Sensory attributes	Dietary treatments					
	T1 (0% MOLM)		T2 (2.5% MOLM)		T3 (5% MOLM)	
	Boiled	Fried	Boiled	Fried	Boiled	Fried
Aroma intensity						
Large White	$4.40^{a}\pm0.142$	$4.47^{ab}\pm0.142$	$4.50^{ab}\pm0.142$	$4.63^{ab}\pm0.142$	$4.63^{ab}\pm0.142$	$4.99^{bc}\pm0.142$
Kolbroek	$4.17^{a}\pm0.142$	$4.43^{a}\pm0.142$	$4.49^{b}\pm0.142$	$4.60^{bc}\pm0.142$	$4.80^{c}\pm0.142$	$4.94^{c}\pm0.142$
Initial impression of juiciness						
Large White	$3.89^{a}\pm0.136$	$4.38^{Bb}\pm0.136$	$4.40^{Bb}\pm0.136$	$4.41^{b}\pm0.136$	$4.65^{Bb}\pm0.136$	$5.07^{c}\pm0.136$
Kolbroek	$3.69^{a}\pm0.136$	$4.07^{Ab}\pm0.136$	$3.94^{Aab}\pm0.136$	$4.45^{bc}\pm0.136$	$4.14^{Ab}\pm0.136$	$4.83^{bc}\pm0.136$
First bite						
Large White	$4.12^{a}\pm0.131$	$4.02^{Ba}\pm0.131$	$4.44^{b}\pm0.131$	$4.08^{ab}\pm0.131$	$4.86^{Bc}\pm0.131$	$4.68^{Bbc}\pm0.131$
Kolbroek	$3.75^{ab}\pm0.131$	$3.98^{Aab}\pm0.131$	$4.21^{b}\pm0.131$	$4.20^{b}\pm0.131$	$4.56^{Abc}\pm0.131$	$4.32^{Ab}\pm0.131$
Sustained impression of juiciness						
Large White	$4.05^{a}\pm0.124$	$4.25^{a}\pm0.124$	$4.36^{b}\pm0.124$	$4.59^{bc}\pm0.124$	$4.68^{bc}\pm0.124$	$4.99^{c}\pm0.124$
Kolbroek	$4.19^{a}\pm0.124$	$4.20^{a}\pm0.124$	$4.21^{a}\pm0.124$	$4.63^{b}\pm0.124$	$4.59^{b}\pm0.124$	$4.81^{b}\pm0.124$
Muscle fibre and tenderness						
Large White	$4.07^{a}\pm0.124$	$4.04^{a}\pm0.124$	$4.07^{a}\pm0.124$	$4.52^{b}\pm0.124$	$4.68^{b}\pm0.124$	$4.74^{b}\pm0.124$
Kolbroek	$3.96^{a}\pm0.124$	$3.95^{a}\pm0.124$	$4.17^{ab}\pm0.124$	$4.48^{b}\pm0.124$	$4.62^{bc}\pm0.124$	$4.98^{c}\pm0.124$
Amount of connective tissue						
Large White	$2.92^{a}\pm0.114$	$3.15^{a}\pm0.114$	$3.19^{b}\pm0.114$	$3.39^{Bbc}\pm0.114$	$3.12^{a}\pm0.114$	$3.32^{bc}\pm0.114$
Kolbroek	$2.73^{a}\pm0.114$	$3.15^{b}\pm0.114$	$3.21^{b}\pm0.114$	$3.10^{Ab}\pm0.114$	$3.01^{b}\pm0.114$	$3.23^{b}\pm0.114$
Overall flavour intensity						
Large White	$3.90^{a}\pm0.136$	$4.15^{ab}\pm0.136$	$4.41^{b}\pm0.136$	$4.55^{bc}\pm0.136$	$4.68^{c}\pm0.136$	$4.75^{c}\pm0.136$
Kolbroek	$3.83^{a}\pm0.136$	$4.21^{b}\pm0.136$	$4.30^{bc}\pm0.136$	$4.51^{c}\pm0.136$	$4.62^{cd}\pm0.136$	$4.87^{d}\pm0.136$
Off-flavour presence						
Large White	$1.28^{b}\pm0.058$	$1.18^{Ab}\pm0.058$	$1.11^{Aab}\pm0.058$	$1.03^{a}\pm0.058$	$1.20^{Ab}\pm0.058$	$1.17^{b}\pm0.058$
Kolbroek	$1.54^{bc}\pm0.058$	$1.34^{Bb}\pm0.058$	$1.43^{Bb}\pm0.058$	$1.17^{a}\pm0.058$	$1.42^{Bb}\pm0.058$	$1.22^{a}\pm0.058$

^{AB}Means in the same column per carcass traits with different superscripts are significantly different ($p<0.05$); ^{ab}Means in the same row with different superscripts are significantly different ($P<0.05$); MOLM (*Moringa oleifera* leaf meal).

There were no significant (P>0.05) differences in the MFT in boiled and fried meat in T1, but the inclusion of MOLM in the diet resulted in significantly (P<0.05) higher scores in both breeds. The OFI of meat had higher (P<0.05) scores in T1 and T3 than in T2. Fried meat was given higher (P<0.05) scores by consumers in most sensory attributes and across the dietary treatments. Differences observed on meat sensory characteristics between boiled and fried meat can be associated with consumer experience and familiarity with a particular cooking method of meat (Sveinsdóttir *et al*., 2009; Xazela *et al*., 2011). This may also be attributed to the enhanced flavour as a result of the cooking oil used in preparing fried meat. Boiled meat was prepared using just water, and this may have reduced the flavour intensity of pork.

5.4 Conclusion

The inclusion of *Moringa oleifera* leaf meal in pig diets improved all the sensory attributes of pork in both breeds. Consumer sensory scores significantly increased as the level of MOLM inclusion in the diet increased. The cooking method affected all the pork quality attributes, where fried meat had higher sensory scores than the boiled meat. However, in all of the sensory attributes, significant ($P<0.05$) differences were observed due to dietary treatment.

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Chapter 6

General discussion, conclusions and recommendations

6.1 General discussions

The objective of the study was to determine effect of inclusion of *Moringa oleifera* (MOLM) in Large White and Kolbroek pigs' diet, on their growth performance, physico-chemical attributes, oxidative stability and sensory quality of pork. Twelve Large White (LW) and twelve Kolboek (KB) pigs were fed one of three dietary treatments (isonitrogenous and isoenergetic formulated), containing either 0%, 2.5% or 5% (T1, T2 and T3, respectively) MOLM for a period of eight weeks. The ingredient composition of dietary treatments and the nutrient composition of the experimental diets and MOLM are presented in Tables 3.1 and 3.2, respectively.

In chapter 3, the growth parameters (ADG, ADFI and FCR), carcass and meat quality of pork from LW and KB pigs were determined. Breed differences on ADFI were observed in pigs in T3, where LW pigs had significantly higher (P<0.05) ADFI as compared to KB pigs. The inclusion of MOLM in the pigs' diet did not significantly (P>0.05) affect the growth parameters (ADFI, ADG and FCR) in both breeds, across the three treatment groups. The KB pigs had significantly (P<0.05) higher BFT as compared to LW pigs, and significantly (P<0.05) higher BFT was in T2 and T3 than in T1 group. Similar studies on breed differences reported that Meishan indigenous pigs have lower lean meat percentages and higher subcutaneous fat than Landrace and LW pigs (Gispert *et al.*, 2007). The dietary inclusion of MOLM did not significantly (P>0.05) affect the physico-chemical quality of pork from LW pigs. However, significantly (P<0.05) increased the a* and reduced WBSF values in pork from KB pigs. Similarly, Moyo *et al.* (2013) reported that chevon from the MOLM diet had higher a* values (P<0.05) than those in other diets. In broilers, Qwele *et al.* (2013) reported

that the dietary supplementation of *Moringa oleifera* formulated diets does not result in any differences in the physico-chemical characteristics of broiler meat.

In Chapter 4, the effects of inclusions of MOLM as a feed additive on fatty acid (FA) composition, the health lipid indices of atherogenicity (AI) and desaturase (DI) and Thiobarbituric acid reactive substances (TBARS) of subcutaneous fat (SCF) tissue and *Longissimus thoracis et lumborum* (LTL) muscle samples were determined. The results showed that the dietary group T3, significantly (P<0.05) increased the *n*-3 content of the subcutaneous tissue in LW pigs than KB, and when compared to T1 and T2. The PUFA:SFA and *n*-6:*n*-3 fatty acids in the subcutaneous adipose tissue, was significantly (*P*<0.05) lower in T2 and T3 than in T1. For muscle samples, T2 and T3 in LW pigs, and T3 in KB pigs, resulted in significantly (*P*<0.05) lower *n*-3 in pork muscle. Inclusions of MOLM significantly (P<0.05) reduced the *n*-6:*n*-3 fatty acids in pork from LW pigs and KB pigs. The *n*-3 and *n*-6 FAs play an important role in human nutrition and are essential to human health, hence, the lowest ratio of *n*-6:*n*-3 fatty acids is reported to support better cardiovascular health (Simopoulus, 2002). There were no significant (*P*>0.05) breed or dietary treatment effects on health lipid indices and on the TBARS of pork, in both breeds.

In Chapter 5, the effect of different levels of MOLM and cooking method on consumer sensory evaluation of pork quality was determined. Consumer gave significantly (P<0.05) higher scores on sensory attributes as the level of MOLM inclusion in the diet increased. In both breeds, T2 and T3 resulted in significantly (*P*<0.05) higher scores for aroma intensity, initial impression of juiciness, first bite, sustained impression of juiciness, muscle fibre and overall tenderness and overall flavour intensity; with generally higher scores observed in LW pigs than KB pigs. In most sensory attributes, higher scores were observed in fried meat than in boiled.

Qwele *et al*. (2013) and Moyo *et al.* (2012) reported the effects of feeding MOLM on quality of meat from goats. Furthermore, studies on effects of MOLM in broiler chickens (Nkukwana *et al.*, 2014) were reported in literature. Mukumbo *et al.* (2014) studied the inclusion of MOLM in finisher pig diets. However, evidence of any reliable effect of feeding MOLM to two different pig breeds at an early stage of production is still scarce. Additionally, there are limited studies that report the effect of pig breed on the oxidative stability of pork.

6.2 Conclusions

The inclusions of MOLM in pig diets at levels of 2.5% and/or 5% was shown improve the ADFI, the a* and tenderness of pork without adversely affecting the growth performance and other physico-chemical quality attributes. *Moringa oleifera* leaf meal inclusion resulted in ideal increase in levels of *n*-3 fatty acids and reduced *n*-6:*n*-3 fatty acid ratio. There were no significant effects of MOLM inclusions in the TBARS of pork muscle, however they were generally improved. Hence, MOLM may be a proficient source of natural antioxidants for pork. Increases in consumer sensory scores for pork from pigs that received MOLM, were also observed in the study.

6.3 Recommendations

- Based on the findings from this study, the use of *Moringa oleifera* leaf meal in pig diets to enhance growth and improve pork quality is recommended.
- Consumer panellist need to be well trained on making sensory evaluations on differently cooked meat

The following areas need further research investigations:

- The effect of including *Moringa oleifera* leaf meal in pig diets on the growth performance and physico-chemical pork quality, including fatty acid profiles, using indigenous breeds found in communal areas.
- The effect of higher inclusion levels of *Moringa oleifera* leaf meal on the health lipid indices of pork. The health lipid indices of atherogenicity (AI) and desaturase index (DI); were determined in this study, but no significant differences were observed at 2.5% and 5% MOLM inclusions.
- A more detailed and particular study on the oxidative stability of pork from grower pigs fed MOLM, using higher inclusion levels than used in the current study.

6.4 References

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Appendix 1. Score sheet: The effect of different levels of *Moringa leifera* leaf meal (MOLM) and thermal preparation on consumer sensory analysis of pork from Large White and Kolbroek pigs

CONSUMER ANALYSIS OF PORK

Demographic information

Age: ≤ 20_____, 21-25_____, 26-30_____, 31-35_____, 36-40_____

Tribe: Xhosa_____, Zulu_____, Shona_____, Other (specify) _____

Gender: Male_____, Female_____

Date: _____

Please evaluate the following samples of MEAT (pork) for the designated characteristics

Characteristics	Rating scale	No.	Boiled	Grilled
1. Aroma intensity Take few sniffs as soon as you remove foil. Typical pork meat aroma	**1= Extremely bland** **2= Fairly bland** **3= Slightly bland** **4= Slightly intense** **5= Fairly intense** **6= Extremely intense**	**LW1**		
		LW2		
		LW3		
		KB1		
		KB2		
		KB3		
2. Initial impression of juiciness The amount of fluid exudes on the cut surface when pressed between thumb and finger	**1= Extremely dry** **2= Fairly dry** **3= Slightly dry** **4= Slightly juicy** **5= Fairly juicy** **6= Extremely juicy**	**LW1**		
		LW2		
		LW3		
		KB1		
		KB2		
		KB3		
3. First bite The impression that you form at first bite	**1= Extremely tough** **2= Fairly tough** **3= Slightly tough** **4= Slightly tender** **5= Fairly tender** **6= Extremely tender**	**LW1**		
		LW2		
		LW3		
		KB1		
		KB2		
		KB3		
4. Sustained impression of juiciness The impression of juiciness that you form as you start chewing	**1= Extremely dry** **2= Fairly dry** **3= Slightly dry** **4= Slightly juicy** **5= Fairly juicy** **6= Extremely juicy**	**LW1**		
		LW2		
		LW3		
		KB1		
		KB2		
		KB3		

5. Muscle fibre & overall tenderness Chew samples with a light chewing action	**1= Extremely tough** **2= Fairly tough** **3= Slightly tough** **4= Slightly tender** **5= Fairly tender** **6= Extremely tender**	**LW1**		
		LW2		
		LW3		
		KB1		
		KB2		
		KB3		
6. Amount of connective tissue The chewiness of the meat	**1= None** **2= Slight** **3= Moderate** **4= Extremely**	**LW1**		
		LW2		
		LW3		
		KB1		
		KB2		
		KB3		
7. Overall flavour intensity This is a combination of taste while chewing and swallowing	**1= Extremely bland** **2= Fairly bland** **3= Slightly bland** **4= Slightly intense** **5= Fairly intense** **6= Extremely intense**	**LW1**		
		LW2		
		LW3		
		KB1		
		KB2		
		KB3		
8. Off-flavour presence and intensity ** This refers to the flavour that is present over and above typical pork meat flavour	**1= None** **2= Moderate** **3= Slightly intense** **4= Extremely intense**	**LW1**		
		LW2		
		LW3		
		KB1		
		KB2		
		KB3		

If off flavour was detected, please tick relevant description of the off flavour

1	**Livery/bloody**		**6**	**Sour**	
2	**Cooked vegetable**		**7**	**Unpleasant**	
3	**Pasture/grassy**		**8**	**None**	
4	**Animal-like/kraal (manure)**				
5	**Metallic**				

CONSENT FORM

I hereby agree to participate in this survey regarding **sensory evaluation of pork.** I understand that I can participate freely without being forced in any way to do so. I also understand that I can stop this evaluation at any point should I not want to continue and that this decision will not in any way affect me negatively.

I understand that this is a research project whose purpose is not necessarily to benefit me personally.

I understand that my answers will remain confidential.

....

Signature of participant Date

Appendix 2. Ethical clearance certificate: MUC011 SNDU01

University of Fort Hare
Together in Excellence

ETHICAL CLEARANCE CERTIFICATE

Certificate Reference Number:	MUC011 SNDU01
Project title:	**Effects of dietary inclusion of *Moringa oleifera* leaf meal on growth performance, oxidative stability and the quality of pork from Kolbrock and Large White pigs**
Nature of Project:	Masters
Principal Researcher:	Xola Pauline Nduku
Supervisor: Co-supervisor:	Prof V Muchenje

On behalf of the University of Fort Hare's Research Ethics Committee (UREC) I hereby give ethical approval in respect of the undertakings contained in the above-mentioned project and research instrument(s). Should any other instruments be used, these require separate authorization. The Researcher may therefore commence with the research as from the date of this certificate, using the reference number indicated above.

Please note that the UREC must be informed immediately of

- Any material change in the conditions or undertakings mentioned in the document
- Any material breaches of ethical undertakings or events that impact upon the ethical conduct of the research