Effects of feeding Moringa oleifera leaf meal as an additive on growth performance of chicken, physico-chemical shelf-life indicators, fatty acids profiles and lipid oxidation of broiler meat

By

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Dissertation submitted in fulfillment of the requirements for the degree of

Masters of Science in Agriculture (Animal Science)

in the

Department of Livestock and Pasture Science

Faculty of Science and Agriculture

University of Fort Hare
Together in Excellence

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: Prof. V. Muchenje
Declaration
I, Cwayita Wapi, vow that this dissertation has not been submitted to any University and that it is my original work conducted under the supervision of Miss T.T. Nkukwana and Prof V. Muchenje. All assistance towards the production of this work and all the references contained herein have been duly accredited.

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Cwayita Wapi Date

........................................... ...........................................
Miss T.T. Nkukwana (Supervisor) Prof V. Muchenje (Co-supervisor)
Abstract

Effects of feeding *Moringa oleifera* leaf meal as an additive on growth performance of chicken, physico-chemical shelf-life indicators, fatty acids profiles and lipid oxidation of broiler meat

The main objective of the study was to determine the effect of *M. oleifera* leaf meal (MOLM) as an additive on growth performance, carcass characteristics, physico-chemical shelf-life indicators (colour, ultimate pH, driploss), fatty acids profiles and lipid oxidation of meat from broilers. A total of 432 1 day old unsexed broiler chicks (Aviance 48) were randomly allocated to four dietary treatments (TRTS) in 72 cages. There were 18 cages per treatment and each cage allocated 6 chicks. Water and feed was provided at *ad libitum*. The feeding phases were, pre-starter (0-7 Days), starter (8-18 Days), grower (19-28 Days), finisher (29-35 Days). The four TRTS contained graded levels of MOLM at 1000g/ton, 750g/ton, 500g/ton, and 0g/ton (control), respectively. The birds were slaughtered at 35 days of age. Breast muscles were sampled for meat, ultimate pH (pH_u), colour, drip loss over a 7 days shelf-life test. After each day’s test sub-samples were dipped in liquid nitrogen and kept at -18\(^{0}\) C for thiobarbituric acid reactive substances determination. On Day1 and Day 7 extra sub-samples were also kept at -18\(^{0}\) C for fatty acids analysis. The TRTS had no effect on average feed intake (AFI), feed conversion efficiency (FCE), and on average daily gain (ADG). Slaughter weight (SW), carcass weight (CW), dressing percentage (%) and gizzard weight (GW) values were similar in all TRTS. Liver weight (LW), heart weight (HW), and gastro-intestinal fat (GIF) differed in all the TRTS, with treatment 2 having the highest value of HW (28.3±2.55), and LW (44.2±1.60) was the highest on treatment 4 . The pH values in all TRTS were constant from Day1 to Day5, reached peak on Day6, and then declined on Day7. Meat from broilers given treatment 1 with MOLM (1000g/ton) had the highest lightness (L*) values. The redness (a*)
values were the highest in meat from treatment 2 (750g/ton MOLM). Treatments had no effect on yellowness (b*) values and on drip loss of the breasts. During storage L* values were high from Day1 to Day5 and decreased from Day6 to Day7. Drip loss increased with storage time as expected. Treatment 4 (control) had the highest proportions of poly-unsaturated fatty acids (PUFA) (30.3±1.87). Treatment 1 (1000g/ton) had the highest proportion of saturated fatty acids (SFA) (60.9±1.87). Treatment 1 (1000g/ton) had the highest proportion of SFA (60.9±4.30). Treatment 2 (750g/ton) had the highest n-6/n-3 ratio than other TRTS. Days had no effect (P>0.05) on PUFA, SFA, and n-6/n-3 ratio. Treatment 1 had a highest amount of malondialdehyde (MDA), treatment 4 had no effect (P>0.05) on MDA. Storage time had an effect (P<0.05) on MDA levels, except for on Day1 and Day7. Day2 had the highest amount of MDA (0.7±0.08). The use of MOLM as an additive in broiler diets reduced lipid oxidation in meat, and maintained the quality of the broiler meat during storage. It also did not have any adverse effects on the growth performance of broilers. Therefore, it has the potential to be used as an additive in broiler diets.

**Keywords:** *Moringa oleifera*, growth performance, meat colour, ultimate pH, driploss, storage time, fatty acids profile, lipid oxidation
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>a</td>
<td>Redness of meat</td>
</tr>
<tr>
<td>ADG</td>
<td>Average daily gain</td>
</tr>
<tr>
<td>AFI</td>
<td>Average feed intake</td>
</tr>
<tr>
<td>b</td>
<td>Yellowness of meat</td>
</tr>
<tr>
<td>CW</td>
<td>Carcass weight</td>
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<tr>
<td>D%</td>
<td>Dressing percentage</td>
</tr>
<tr>
<td>FCE</td>
<td>Feed conversion efficiency</td>
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<td>GIF</td>
<td>Gastro-intestinal fat</td>
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<tr>
<td>GW</td>
<td>Gizzard weight</td>
</tr>
<tr>
<td>HW</td>
<td>Heart weight</td>
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<tr>
<td>L</td>
<td>Lightness of meat</td>
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<tr>
<td>LW</td>
<td>Liver weight</td>
</tr>
<tr>
<td>MDA</td>
<td>Malondialdehyde</td>
</tr>
<tr>
<td>MOLM</td>
<td><em>Moringa oleifera</em> leaf meal</td>
</tr>
<tr>
<td>PUFA</td>
<td>Polyunsaturated fatty acids</td>
</tr>
<tr>
<td>SFA</td>
<td>Saturated fatty acids</td>
</tr>
<tr>
<td>TBARS</td>
<td>Thiobarbituric acid reactive substances</td>
</tr>
</tbody>
</table>
Acknowledgements

I owe sincere thankfulness to my Lord and saviour Jesus Christ who strengthened me through this study. My sincere gratitude goes to my supervisors Miss T.T. Nkukwana and Prof V. Muchenje they made me believe in myself and guided me through the whole process of dissertation writing. Their support, understanding and encouragement I felt when writing this dissertation. It is with great pleasure to thank Mr T. Mabusela and Dr B.Moyo for their ideas during this study. I would also like to thank Stellenbosch University for allowing me to conduct my experiments using their facilities. Thank you to Mr O. Tada and Mr P.O. Fayemi for assisting me with data analysis.

I would like to show my gratitude to my mom (Somikazi), sister (Funeka) and my baby brother (Sive) for their moral support and encouragement up until now. I am also thankful to my fellow colleagues and caring friends for their help, support and helpful ideas.
Abstract

3.1. Introduction ........................................................................................................... 32

3.2. Materials and Methods ........................................................................................ 34
   3.2.1. Study site description ....................................................................................... 34
   3.2.2. Experimental setup, housing and feeding ....................................................... 36

3.3. Statistical analysis .................................................................................................. 45

3.4. Results and discussion .......................................................................................... 46

3.6. References .............................................................................................................. 56

Chapter 4: Physico-chemical shelf life indicators of meat from broilers given *Moringa oleifera* leaf meal as an additive. ................................................................. 61

Abstract .......................................................................................................................... 61

4.1. Introduction .............................................................................................................. 62

4.2. Material and Methods ............................................................................................ 65
   4.2.1. Study site and management of broiler chickens. ............................................. 65
   4.2.2. Procedures after slaughter ............................................................................. 65

4.3. Meat quality measurements .................................................................................. 65
   4.3.1. Drip loss measurements .................................................................................. 65
   4.3.2. Ultimate pH ..................................................................................................... 66
   4.3.3. Determination of colour ................................................................................ 66

4.4. Statistical analysis ................................................................................................ 66

4.5. Results and discussion .......................................................................................... 67

4.6. Conclusion .............................................................................................................. 72

4.7. References .............................................................................................................. 74

Chapter 5: Effect of *Moringa oleifera* leaf meal supplementation on fatty acids profile of broiler meat .................................................................................................................. 79

Abstract .......................................................................................................................... 79

5.1. Introduction .............................................................................................................. 80
5.2. Materials and methods .................................................................83
  5.2.1. Study site and management of broiler chickens ........................................83
  5.2.2. Determination of fatty acid content .........................................................83
  5.2.3. Estimation of lipid peroxidation ...............................................................84
5.3. Statistical analysis ..............................................................................85
5.4. Results and Discussion .......................................................................86
5.5. Conclusion .........................................................................................93
5.6. References .........................................................................................94
Chapter 6: General discussion, Conclusions and Recommendations ...............101
  6.1. General discussion ...........................................................................101
  6.2. Conclusion ......................................................................................103
  6.3. Recommendations ............................................................................103
  6.4. References ......................................................................................104
List of Table
Table 3.1: Treatment structure .............................................. Error! Bookmark not defined.
Table 3.2: The ingredient (kg) and chemical composition of pre-starter, starter, grower and finisher .............................. 40
Table 3.3: Nutrient composition (%) of the treatments on prestarter phase .......................... 41
Table 3.4: Nutrient composition (%) of the treatments on starter phase .......................... 42
Table 3.5: Nutrient composition (%) of treatments on grower phase .......................... 43
Table 3.6: Nutrient composition (%) of treatments on finisher phase .......................... 44
Table 3.7: Effect of Moringa oleifera leaf meal as an additive on feed conversion efficiency (FCE) of broilers at 7, 18, 28 and 35 days ................................................................. 47
Table 3.8: Effect of Moringa oleifera leaf meal supplementation on feed intake (FI) of broilers at 7, 18, 28, and 35 days ................................................................. Error! Bookmark not defined.
Table 3.9: Effect of Moringa oleifera leaf meal as an additive on average daily gain (ADG) of broilers at 7, 18, 28 and 35 days ................................................................. 51
Table 3.10: Effect of treatments on meat portions of broilers 24hours after slaughter .......... 52
Table 3.11: Effect of Moringa oleifera as an additive on carcass characteristics of broilers .. 54
Table 4.1: Least square means and standard errors for L*, a*, b* and pH, Drip loss of chicken meat as affected by days ................................................................................ 70
Table 4.2: Least square means and standard errors for L*, a*, b* and drip loss of meat samples (chicken) as affected by treatment ............................................................................... 72
Table 5.1: Effect of Moringa oleifera leaf meal as an additive on fatty acid profile of chicken meat ........................................................................................................... 87
Table 5.2: Effect of days on fatty acids profiles of chicken meat ........................................... 89
Table 5.3: Means (±SE) for average TBARS (mg MDA/kg meat) of chicken breasts as affected by treatments ....................................................................................... 92
Table 5.4: Means (±SE) for average TBARS (mg MDA/kg meat) of chicken breasts as affected by days ................................................................................................. 92
List of Figure
Figure 3.1: Effect of treatment overtime (days) on the pH levels of chicken........................69
Chapter 1: Introduction

1.1 Background of the study

Growth in broilers is a very complex phenomenon which is influenced by genotype as well as by environmental factors, including nutrition. Genotype plays a major role in carcass fatness and the quality of meat (Jaturasitha et al., 2004; Musa et al., 2006). Therefore, as the genetic potential and the characteristics of poultry have evolved, so has the manipulation of diet specifications to suit market needs and meet the nutrient requirements of birds, for purposes of optimizing immune responsiveness, rather than simply growth rate or classical feed efficiency. Developments in poultry nutrition have generally been driven by the need to sustain genetic potential within the confines of ever evolving systems of poultry production (Leeson, 2008). Owing to genetic selection, the modern broiler has lower feed intake per unit of body weight (BW) gain, with the potential to increase recognition of white meat in comparison to commercial broilers of the past (Dozier et al., 2008). Consequently, over the last fifty years nutritionists have developed quite sophisticated systems for quantifying the available nutrients in both ingredients and diets, thus providing birds with precise levels of nutrients required for production (Leeson, 2008).

Additives in poultry diets are primarily included to improve efficiency of the bird’s growth, prevent diseases and improve feed utilisation. This leads to improved production, such as meat quality. For an example, antimicrobials which are common feed additive used in poultry diets, have been used extensively in intensive poultry operations in order to minimise diseases, and improve growth and feed utilisation.
The prophylactic use of antibiotics (as growth promoters) in animal feeds has made intensive farming possible and improved feed conversion in these animals (Herna’ndez et al., 2004). Until recently, gain in protein deposition (based on an improved feed conversion rate enhanced by antibiotic usage) has facilitated improvements in production efficiency, thereby allowing the consumer to purchase (at a reasonable cost) high quality meat and eggs (Donoghue, 2003). Antibiotic growth promoters in the poultry industry has been banned because of harmful effects on human health. This was observed by the development of microbial resistance to these products (Botsoglou and Fletouris, 2001; Williams and Losa, 2001; McCartney, 2002).

Consequently; herbs, spices, and various plant extracts considered to be natural products that consumers would accept have received increased attention as possible feed additives such as antibiotic growth promoter replacements following their ban by the European Union in 2006 (Shahid et al., 1992; Herna’ndez et al., 2004).

Several alternatives to these growth promoters have been proposed and organic acids, medicinal plants as natural feed additives are now recently used in poultry diet to enhance the performance of the immune response of birds (Ali Asghar Saki et al., 2012). One such plant is Moringa oleifera, commonly known as the drumstick tree (Makker and Becker, 1997; Sarwatt et al., 2002). This plant is widely cultivated in Africa, Thailand, Burma, Singapore, India, Sri-Lanka (Reyes-Sanchezi et al., 2006), in South Africa this plant is a bit scarce, especially in the Eastern Cape province. It is only available in few provinces which makes it difficult and quite expensive to get it.

However Moringa Oleífera has been reported to posses quality sources of several nutrients including protein, calcium, Magnesium, Potassium, Iron, Vitamin A, and Vitamin C, Vitamin
E (Foidl et al., 2001; Marcu and Pharm., 2005; Rweyemamu, 2006). The presence of vitamin C, vitamin E, carotenoids, flavonoids and selenium make *M. oleifera* a potential antioxidant (Moyo et al., 2012). The antioxidant compounds (phenols, Vitamin C, Vitamin E, β carotene, zinc, selenium, flavonoids) in *M. oleifera* have been reported (in some studies) to improve shelf-life and the quality of meat products in the pre-slaughter or post-slaughter stages (Valeria and Williams, 2011); that is incorporating natural antioxidants in animal diets or onto the meat surface or active packaging.

According to Sarwatt et al. (2004), *M. oleifera* foliages are a potential inexpensive source for livestock feeding. Aregheore (2001) reported that the use of *M. oleifera* as a supplement can improve voluntary feed intake, digestibility and animal performance. It is also reported to have some health benefits in terms of healing and prevention in humans, such as diabetes relief, healthy skin, decreased depression and anxiety (Donovan., 2007), improved immune system, encourages balanced metabolism, healthy digestion (Life in Health., 2011). Its medicinal properties in animals has also been tested on previous studies for instance in goats by Moyo et al., (2012) and in broiler chickens by Qwele et al., (2013). Equally important is the fact that few parts of the tree contain any toxins and other anti-nutritional factors that might decrease its potential as a source of food for animals or humans. For instance its bark contains tannins, alkaloids, saponnins and inhibitors (Makkar, Singh and Negi, 1990). This then make this plant to be similar to some plant extracts, hence essential oils which contain same factors. In monogastric animals, saponins are reported to be bound to cholesterol, thus hampering its absorption in the intestine (Sidhu and Dakenfull,1986) and that they could reduce the accumulation of cholesterol in meat (Brogna et al., 1985). Condensed tannins are said to have a positive influence on the meat fatty acids composition (Min et al., 2005; Vasta et al, 2009).
Despite the high nutritional composition of *M. oleifera* leaf meal, there is little information available on the use of this feed resource, especially on its potential antioxidant properties of when used as an additive in broiler diets. The aim of the present study was to assess the effect of *Moringa oleifera* leaf meal (MOLM), as an additive on growth performance, carcass characteristics, physico-chemical shelf-life indicators and fatty acids profiles of broiler chickens.

1.2. Problem statement

There is little information regarding the utilisation of Moringa leaves as an additive in poultry feeding for both growth and health benefits, as well as meat quality. The problem is further worsened by the fact that chicken, particularly those reared by small scale producers in communal areas are susceptible to nutritional deficiency and other diseases, such that their performance and productivity is lowered.

Another problem is that some additives such as vitamin E and selenium, which are usually added in mono-gastric feeds, are very expensive. However, due to high feeding costs and low revenue generated, there is often inadequate funds to buy drugs, which are expensive but may be mandatory, especially if antibiotics were to be banned in South Africa. Therefore, these farmers can benefit more from plants with immense nutritional value and medicinal properties such as *M. oleifera* leaves.

1.3. Justification

Despite the high nutritional content of *M. oleifera*, there is little information regarding its utilisation in poultry feeding as an additive for growth performance, physico-chemical shelf-life indicators of chicken meat, fatty acids profiles and lipid oxidation. Such information is
needed in identifying feeding strategies to improve growth and meat quality of broilers in limited resource farmers, especially since other additives have been banned because of their toxicity and their counterparts on meat products. Since chickens tend to suffer from nutritional deficiency and other diseases, which lower their performance and productivity, and also due to high prices of animal feeds, there is a need to identify alternative feed resources. This is even more crucial for small-scale farmers undertaking farm-based feed formulation, who are constantly finding it hard to produce with commercial feeds.

However, with the removal of antibiotics as feed additives, plants have been identified to have both antibiotics and antioxidants properties. Wood and Enser (1997) recommended the use of dietary antioxidants to reduce lipid peroxidation in the feed and animal, so as to preserve product quality.

Therefore, research on the use of *M. oleifera* as an additive in broiler diets and its effect on growth performance, meat characteristics and meat quality of broiler chickens; will be of importance. The widespread claim of *M. oleifera*’s nutritional and medicinal properties on humans can be extended and further investigated as an additive in chickens. This research will also improve small-scale production systems by providing evidence for the use of *M.oleifera* in chicken diet that will produce better carcass characteristics and meat quality of broilers, that is also affordable to consumers, thus leading to higher returns.

### 1.4. Objectives

The broad objective of the study was to determine the effects of *M. oleifera* leaf meal on growth performance, carcass characteristics, shelf-life indicators and fatty acids profiles and lipid oxidation of meat from broilers. The specific objectives were to:
1. Determine the effect of feeding *M. Oleifera* leaf meal to broilers on feed intake, growth rate, and feed conversion efficiency.

2. To determine the effect of feeding *M. Oleifera* leaf meal to broilers on slaughter weight, carcass weight, dressing percentage and physico-chemical shelf-life indicators (pHu, colour, drip loss) of the meat.

3. To determine the anti-oxidant effect of *M.oleifera* leaf meal fed as an additive on fatty acids profiles and lipid oxidation of meat from broilers.

**1.5. Hypothesis**

The hypothesis tested were:

1. Feeding of *M.oleifera* leaf meal as an additive to broilers has no effect on feed intake, growth rate and feed conversion efficiency.

2. Feeding *M.oleifera* leaf meal as an additive to broilers has no effect on slaughter weight, carcass weight, dressing percentage and physico-chemical shelf-life indicators (pHu, colour, drip loss) of the meat.

3. *Moringa oleifera* leaf meal fed as an additive to broilers has no effect on fatty acids profiles and lipid oxidation of the meat.
1.6. References


Chapter 2: Literature review

2.1. Introduction
Poultry in the world, is the most abundant livestock species and is reported to account for more than 90% of the total poultry population of the world (Biswas et al. 2011). It contributes as the source of income and employment among people of Africa (Yami, 1995; Food and Agriculture Organisation, 2009). In South Africa, over the past ten years the estimated number of birds in the South African poultry industry has increased from 107.065 million in 2001 by 49.190 million birds (+45.9%) to 156.255 million in 2011 (Strydom et al., 2012). However over the last few years the poultry industry in South Africa has experienced a major crisis of over-supply from big exporters (like Argentina, Brazil, the EU etc) at about 20 000 tons per month level (Lovell, 2012). Therefore that is the problem since grain costs are also high and broiler chickens are reported to have a high feed intake and fast growth rate (Richards, 2003).

Moreover there is an emphasis on improving growth and carcass yield, mainly by increasing breast proportion and reducing abdominal fat (Musa et al., 2006). Fat in chickens reduces carcass quality and feed efficiency (Oyedeji and Atteh, 2005). Improved feeding strategies in growing broiler chickens should be aimed at optimising lean carcass tissue, feed conversion efficiency (FCE) and body weight gain (Gous and Cherry, 2004).
2.2. Use of plant extracts as feed additives

The addition of plants and their extracts into diets is aimed at improving the productivity of livestock through amelioration of undesired feed properties, promotion of the animal’s production performance and improving the quality of food derived from those animals (Kolodziej-Skalska et al., 2011). Herbs such as herbs, spices, and various plant extracts have received increased attention as possible antibiotic growth promoters (Shahidi et al., 1992; Frankic et al., 2009). Figen et al., (2011) proposed that these herbs and spices could be used as feed additives in animal nutrition; and that these additives may have more than one mode of action, including improving feed intake, flavour and anti oxidative activity. These plant extracts are reported to contain some anti-microbial phytochemicals like phenolics and polyphenols (simple phenols and phenolics acids, quinines, flavones, tannins and coumarins), essential oils, alkaloids, and lectins and polypeptides (Cowan, 1999; Moyo et al., 2012). It is reported that when animals consume plant products containing antioxidant such as phenols, vitamins C, vitamins E, β-carotene, zinc, selenium and flavonoids, have these antioxidants passed into the meat (Lahucky et al., 2010; Middleton et al., 2000; Moyo et al., 2012). These natural antioxidants are considered to be safer than the synthetic antioxidants, and have greater application potential for consumer’s acceptability, palatability, stability and shelf-life of meat products (Jung et al., 2010). Chicken meat is very susceptible to oxidation, particularly during and after frozen storage. However, dietary vitamin E has been reported to reduce or prevent the process of lipid oxidation (major cause of quality deterioration in meat) occurring in broiler meat during storage (De Winne and Dirinck, 1996). Some studies have demonstrated that shelf life and meat quality (drip loss, pH and colour, etc) can be improved by using natural antioxidants in some stages of meat production (Valeria and Williams, 2011).
2.3. Uses of *Moringa oleifera* as the feed additive

*Moringa oleifera* plant extracts posses some antioxidant compounds and nutrients (carbohydrates, proteins, and lipids) which are essential requirements for chicken growth. These antioxidant properties are reported to be safe and have received remarkable attention due to their ability to preserve foodstuffs and prevent rancidity caused by oxidation (Moyo *et al*., 2012). *Moringa oleifera* seed oil contains all the main fatty acids found in olive oil. It also possesses behenic acid (C\textsubscript{22:0}), lignoceric acid (C\textsubscript{24:0}) and traces of lauric n-pentadecanoic and pentadecenoid acids (Dahot and Memo, 1985, Ferrao and Ferrao, 1970). Variation in fatty acid composition is reported to have an important effect on firmness or softness of the fat in meat, especially the subcutaneous and intermuscular (carcass fats), but also the intramuscular (marbling) fat (Wood *et al*., 2003).

A number of feed additives have been used world wide in the poultry industry for so many years (Jang *et al*., 2007). The common feed additives used in poultry diets include antimicrobials, antioxidants, emulsifiers, binders, pH control agents and enzymes (Poultry Consultancy, 2012). Moringa (commonly called drumstick tree) has been reported to posses quality sources of several nutrients including protein, calcium, Magnesium, Potassium, Iron, Vitamin A, and Vitamin C (Foidl *et al*., 2001; Marcu, 2005; Rweyemamu, 2006) and is also reported to be inexpensive for livestock feeding compared to other leaf meals (Sarwatt *et al*., 2004). This plant also possess some antioxidants which makes it suitable to be utilized as an additive in broiler diets. The presence of vitamin C, vitamin E, carotenoids, flavonoids and selenium makes *Moringa oleifera* a potential antioxidant (Moyo *et al*., 2012). The plant has also been advocated for traditional medicines for centuries (Marcu and Pharm, 2005). According to Mwale and Masika (2011), medicinal plants are easy to use, cheap, readily
available and easily accessible to the communal farmers. Therefore research on the effect of *Moringa oleifera* leaf meal as an additive on growth performance, fatty acid profile, and physico-chemical shelf life indicators of broiler chickens would be of importance.

2.4. Medicinal uses of *Moringa oleifera*

2.4.1. Antioxidants
*Moringa oleifera* has been reported to possess some antioxidant properties (Sreelatha and Padma, 2009; Atawodi *et al.*, 2010). Although there are several enzyme systems within the body which scavenge free radicals, the natural (vitamin) antioxidants are vitamin E, béta-carotene, and vitamin C (Nair *et al.*, 2003). These micronutrient antioxidants may be used as defence system to prevent free radicals from damaging the animal’s body. This therefore provides protection to animals against infections and degenerative diseases (Sreelatha and Padma, 2009; Verma *et al.*, 2009). A survey conducted by Yang *et al.* (2006) and Jung *et al.* (2010), on 120 edible plant species showed that *M. oleifera* was among the most promising species based on their high antioxidant activity, high contents of micro-nutrients and phytochemicals, processing properties, ease of growing, and also on palatability, stability and shelf life of meat products.

2.5. Nutritional composition of *Moringa oleifera* Lam leaves
According to Moyo *et al.* (2011), there is quite a lot of literature on the nutritional value of *M. oleifera* Lam leaves with varying nutritional content. *Moringa oleifera* has been reported to possess several nutrients, including: Calcium, Magnesium, Potassium, Iron, Vitamin A, and Vitamin C and a crude protein content that varies from 16 to 40% (Foidl *et al.*, 2001; Marcu and Pharm, 2005; Rweyemamu, 2006). Areghore (2001) reported that the use of *M. oleifera* as a supplement can improve voluntary intake, digestibility and livestock performance. According to Rubanza *et al.*, (2005) the extent and rate of feed digestibility is defined by the
fiber content. Therefore, *M. oleifera* leaves could be highly digestible because of its immense nutritional qualities such as its chemical composition (neutral detergent fiber (NDF); acid detergent fiber (ADF); crude protein (CP); gross energy (Gross En); (EE) ether extract) and amino acids profile (Table 2.2). The seeds of this tree are rich in oil and protein source, these seeds can also be used for the purification of water (Becker and Makkar, 1999; Foidl et al., 2001). According to Makkar and Becker (1997), *M. oleifera* Lam leaves are rich in carotenoids, ascorbic acid and iron. The leaves are widely recognised as a food source for humans and a dry season feed for animals because of the nutrient contents it contains (Table 2.1). Equally important is the fact that some parts of the tree contain toxins and other anti-nutritional factors that might decrease its potential as a source of food for animals or humans (Table 2.2). For instance its bark contains tannins, alkaloids, saponins and inhibitors (Makkar et al., 1990).
Table 2.1: Mineral contents of dried *Moringa oleifera* leaves

<table>
<thead>
<tr>
<th>Mineral</th>
<th>Dry leaf</th>
<th>Standard error</th>
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<tbody>
<tr>
<td><strong>Macro elements (%)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Calcium (%)</td>
<td>3.65</td>
<td>0.36</td>
</tr>
<tr>
<td>Phosphorus (%)</td>
<td>0.30</td>
<td>0.004</td>
</tr>
<tr>
<td>Magnesium (%)</td>
<td>0.50</td>
<td>0.005</td>
</tr>
<tr>
<td>Potassium (%)</td>
<td>1.50</td>
<td>0.019</td>
</tr>
<tr>
<td>Sodium (%)</td>
<td>0.164</td>
<td>0.017</td>
</tr>
<tr>
<td>Sulphur (%)</td>
<td>0.63</td>
<td>0.146</td>
</tr>
<tr>
<td><strong>Micro elements (mg/kg)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Zinc (mg/kg)</td>
<td>31.03</td>
<td>3.410</td>
</tr>
<tr>
<td>Copper (mg/kg)</td>
<td>8.25</td>
<td>0.143</td>
</tr>
<tr>
<td>Iron (mg/kg)</td>
<td>490</td>
<td>3.940</td>
</tr>
<tr>
<td>Manganese (mg/kg)</td>
<td>86.8</td>
<td>49.645</td>
</tr>
<tr>
<td>Selenium (mg/kg)</td>
<td>363.00</td>
<td>0.413</td>
</tr>
<tr>
<td>Boron (mg/kg)</td>
<td>49.93</td>
<td>2.302</td>
</tr>
</tbody>
</table>

Source: Moyo *et al.* (2011)

Table 2.2. Nutritional qualities of *M.oleifera* leaf meal
<table>
<thead>
<tr>
<th>Nutritive value</th>
<th>Dry leaves</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Chemical composition (% DM)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Crude protein</td>
<td>25.1-30.29</td>
<td>Foidl <em>et al.</em> 2001; Moyo <em>et al.</em>, 2011</td>
</tr>
<tr>
<td>Acid detergent fiber</td>
<td>8.49-11.4</td>
<td>Moyo <em>et al.</em>, 2011; Richter <em>et al.</em> 2003; Foidl <em>et al.</em> 2001</td>
</tr>
<tr>
<td>Gross energy (MJ/kg DM)</td>
<td>18.7</td>
<td>Foidl <em>et al.</em>, 2001</td>
</tr>
<tr>
<td>Ether extract</td>
<td>5.4</td>
<td>Foidl <em>et al.</em>, 2001</td>
</tr>
<tr>
<td><strong>Amino acid profile (% DM)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lysine</td>
<td>1.1-1.64</td>
<td>Richter <em>et al.</em> 2003; Moyo <em>et al.</em>, 2011</td>
</tr>
<tr>
<td>Histidine</td>
<td>0.6-0.72</td>
<td>Richter <em>et al.</em> 2003; Moyo <em>et al.</em>, 2011</td>
</tr>
<tr>
<td>Threonine</td>
<td>0.8-1.36</td>
<td>Richter <em>et al.</em> 2003; Moyo <em>et al.</em>, 2011</td>
</tr>
<tr>
<td>Arginine</td>
<td>1.2-1.78</td>
<td>Richter <em>et al.</em> 2003; Moyo <em>et al.</em>, 2011</td>
</tr>
<tr>
<td>Methionine</td>
<td>0.30</td>
<td>Moyo <em>et al.</em>, 2011</td>
</tr>
<tr>
<td><strong>Antinutrients (%)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total phenolics</td>
<td>2.02-2.74</td>
<td>Moyo <em>et al.</em>, 2011; Richter <em>et al.</em> 2003</td>
</tr>
<tr>
<td>Tannins</td>
<td>0.53</td>
<td>Richter <em>et al.</em> 2003</td>
</tr>
<tr>
<td>Condensed tannins (mg/g)</td>
<td>3.12</td>
<td>Moyo <em>et al.</em>, 2011</td>
</tr>
</tbody>
</table>
2.6. Growth promoting properties of plant extracts on performance and carcass characteristics of broiler chickens.

The optimal performance in terms of diet intake, growth rate, feed conversion efficiency (FCE), live weight and high meat yield can be improved by nutritional management. According to Chinrasri (2004) and Laohakaset (1997), nutrient requirement is the amount of nutrients needed by the animals to maintain their activities, maximize growth and feed utilization efficiency. Nutrients like carbohydrates, lipids, and proteins that chickens utilize as a source of energy are essential requirements for growth. Approximately up to 80% of domestic animals have been fed synthetic compounds for the purpose of either medication or growth promotion (Lee et al., 2001). However, there are recent concerns about possible antibiotic residues and disease resistance that have aroused great caution in the usage of antibiotics in the animal industry. Therefore, supplementing broilers with plant extracts since they contain most of the nutrients will enhance feed intake, growth and FCE. Plant extracts have been used as an alternative to antibiotics, for this reason these plant extracts are becoming more important due to their anti-microbial effects as well as stimulating effect on the digestive system of the animal (Osman et al., 2005). They also contain many active materials, including essential oils, which boost a wide range of pharmacological activities (Cowan, 1999). Kamel (2001) also reported evidence suggesting that herbs, spices and various plant extracts have appetite and digestion stimulating properties, as well as anti-microbial effects.
2.7. Meat quality characteristics of broiler meat

2.7.1. pH, colour and drip loss
There are a range of factors that contribute to the deterioration in quality and loss of shelf life as a consequence of lipid oxidation occurring in meat and meat products (Marusich et al., 1975). These factors include the state and content of pro-oxidants (iron, myoglobin), level of antioxidants present in muscle, composition and amount of muscle lipids and the storage conditions of meat. It has also been shown that meat colour can be affected by some supplements given to meat animals. For instance, high levels of iron contained in some plants including Moringa could have an effect on redness (a*) of the meat. Also, some antioxidant properties that Moringa also contain can have an effect on meat quality. For example, dietary vitamin E supplementation in meat animals is reported to be the best known method of improving meat quality by reducing lipid oxidation in fresh meat and meat products (Marusich et al., 1975). Vitamin E supplementation has been effectively used to improve the quality of poultry and poultry products.

Meat quality is determined by a combination of chemical and sensory attributes and a carcass with better fat or muscle proportions (Dhana et al., 1999; Madruga et al., 2009). Consumers are interested in meat that can contribute to their personal satisfaction. The quality measures related to visual aspect (colour, water holding capacity and fatness) and the palatability (juiciness, flavour and aroma) are regarded as the key measures that determine consumers initial interest in meat (Muchenje et al., 2009a). Meat quality is influenced, to a large extent, by the rate of ultimate pH (pH\textsubscript{u}), decline in the muscles after slaughter and by the ultimate pH (Sales and Millet, 1996; Muchenje et al., 2009a).
Meat quality can also be influenced by feeding, for an example in the study conducted by Xazela et al., (2011), goats supplemented with sunflower cake had a lower pH than non-supplemented goats. The reason being the supplemented goats were likely to have higher glycogen levels than non-supplemented. Normally, low pH in meat increases lightness (L*) and reduces redness (a*) of the meat. According to Dhanda et al. (1999) and Santos et al. (2007), redness of the meat is normally related to the pHu of meat. An ideal pH for meat ranges from 5.5 to 5.8 therefore, if the pH is above 6 after slaughter, it will lead to a reduced shelf life of the meat, since such pH encourages microbial growth. There are some indications that pH also has an effect on chicken meat (Bruce and Ball, 2000). For instance in chicken, studies of pH have shown that high pH meat is darker but less consistently tender than normal pH meat (Dyubele et al., 2010). However, ideal pH results in acceptably tender and normal colour of the meat. High pH also promotes growth of micro-organisms which lead to the development of off-odours, and often slime formation (Bruce and Ball, 2000).

The pH that is less than 5.0 results in pale coloured meat. The colour of the meat depends on several individual factors and their interactions.

According to Insausti and Berian (2008), oxidative processes have negative effect on both colour and flavour and lipid oxidation products contribute to the development of off-flavours, especially at storage. In addition, Thiansilakul et al. (2011), reported that myoglobin oxidation leads to meat discoloration.

Drip loss is the loss of fluid from the meat tissue. Fluid loss may cause an increase in the concentration of the solutes, which results in pH decrease as reported by Offer and Trinick (1983) where fast decline in pH or low pH leads to high drip loss.
Some minerals like selenium which are also found in Moringa, has been shown to significantly improve meat quality by decreasing cell membrane oxidation leading to reduced muscle drip loss (Rotruck et al., 1973). It has also been reported that the influence of selenium supplementation on reducing drip loss in pigs is somewhat less clear compared to the reduced drip loss observed in broilers.

2.7.2. Fatty acids profiles and lipid oxidation in meat quality
Meat is often wrongly identified as food with high fat content and an undesirable balance of fatty acids. Lean meat is very low in fat. It is reported that poultry and pork have a favourable balance between polyunsaturated fatty acids (PUFA) and saturated fatty acids (SFA) (P: S) (Wood and Esner, 1997) and that when grain based or grass based diets are fed they normally lead to a relatively more n-6 or n-3 fatty acids, respectively. In meat containing more unsaturated fatty acids there is a risk that their profile will change during storage (Jeremiah, 1980).

According to Muchenje et al. (2009a), fatty acid composition of meat is affected by the fatness level of the animal, which maybe enhanced by the type of feed the animal consumed, age and genotype. Similarly, changes in fatty acid composition of body fats are primarily due to the respective fatty acid content of the diet (Muchenje et al., 2009b). Flavonoids, carotenoids, essential oils and other plant substances may cause some changes during storage of meat and affect lipid accumulation in tissue (Koreveski and Swiatkiewicz, 2007).
Kishowar et al. (2004) reported that chicken meat is enriched with PUFA, including the key $n$-3 fatty acids in comparison to other meats. However, these polyunsaturated fatty acids are reported to increase the susceptibility of meat to lipid oxidation which causes loss of nutritional and sensory values as well as the formation of potentially toxic compounds that compromise meat quality and reduce its shelf life (Maraschiello et al., 1999; Ruiz et al., 1999; Grau et al., 2001). Antioxidants delay or prevent lipid oxidation in meat.

It has also been reported that the effect of fatty acids on shelf life is explained by the propensity of unsaturated fatty acids to oxidise, leading to the development of rancidity as display times increases (Wood et al., 2004). There will also be colour change, which is due to the oxidation of red myoglobin to brown metmyoglobin.

Poultry meat is particularly prone to oxidative deterioration due to its high concentration of polyunsaturated fatty acids (Luna et al., 2010). A lot of studies (Botsologou et al., 2002; Lee et al. 2004), have shown the improvement in the oxidative stability of meat after feeding chickens with antioxidants factors, added in the feed. Synthetic antioxidants such as butylated hydroxytoluene (BHT) have been utilised as antioxidants to reduce oxidation.

However, there is an interest on natural antioxidants from plants since the oxidative quality deterioration in meat can be reduced by the use of natural antioxidants. Natural antioxidants are various substances with different chemical characteristics, which are widely present in plants (Valasco and Williams., 2011). According to Pennington and Fisher (2009), the total antioxidant capacity of these plants reflects concentrations of ascorbic acid (vitamin C), alpha-tocopherol (vitamin E), beta carotene (vitamin A precursor), various flavonoids, and other phenolic compounds. *Moringa oleifera* has been revealed to be a source of high levels of vitamin E and beta carotene (Moyo et al., 2011) which have been reported against
oxidation (Yeum et al., 2009). With many positive effects of natural antioxidants on meat characteristics, retarding lipid oxidation is one of those positive effects (Camo et al., 2008). Therefore *moringa oleifera* can be used as an additive in broiler diets.

2.6. References


Chapter 3: Effect of feeding *Moringa oleifera* leaf meal as an additive on growth performance and carcass characteristics of broilers

Abstract
The objective of the current study was to determine the effect of *Moringa oleifera* leaf meal (MOLM) as feed additive on growth performance (average feed intake, body weight gain, average daily gain, feed conversion efficiency) and carcass characteristics of broilers. A total of 432 1-day-old unsexed broiler chicks (Aviane 48) were randomly allocated to four dietary treatments (TRTS) in 72 cages, each cage having 6 birds. Water and feed were provided ad
libitum. The feeding phases were: prestarter (0-7days), starter (8-18days), grower (19-28days) finisher (29-35days). Diets contained graded levels of the MOLM, ranging between 0 and 1000g/ton depending on treatment (TRT1=1000g/ton MOLM; TRT2=750g/ton MOLM; TRT3=500g/ton MOLM; TRT4=0 MOLM). At day 35 all the chickens were slaughtered and the slaughter weight, carcass weight, and internal organs weights were taken. The body weight gain (BWG) differed significantly with TRTS at Day 18, 28 and 35 with treatment 1 having highest value on Day 18, treatment 2 having the highest on Day 28 and treatment 2 having the highest on Day 35. The TRTS at Day 18 and Day 35 had a significant effect on average daily gain (ADG). At Day 18 chickens supplemented with treatment 1 (1000g/ton MOLM) had the highest ADG and at Day 35 treatment 2 was the highest. At Day 7 FI and FCE differed significantly with TRTS, with chickens supplemented with treatment 4 (0 MOLM) having high FI and FCE values. Slaughter weight (SW), carcass weight (CW), dressing percentage (D %) and gizzard weight (GW) values were similar in all the treatments. The liver weight (LW), heart weight (HW) and gastro intestinal fat (GIF) differed significantly in all the TRTS, with treatment 2 (750g/ton MOLM) having highest value of HW and GIF, and LW was the highest in treatment 4 (0 MOLM). In conclusion the use of MOLM as an additive had a significant effect on AFI, BWG, ADG, FCE of broiler chickens and on LW, HW and GIF.

**Keywords:** moringa oleifera, broilers, average feed intake, growth, dietary additive

3.1. Introduction

Recently, there is a trend of rising prices of animal feeds. Vitamin E and selenium (both organic and inorganic) usually used as additives in mono-gastric feeds are very expensive. On the other hand, there is a ban on the use of antibiotic growth promoters in the poultry industry, because of the harmful effects on humans ocassioned by the development of
microbial resistance to these products (Mc Cartney, 2002). However, organic acids and medicinal plants as natural feed additives have been used recently in poultry diet to enhance the performance and immune response of birds (Asghar et al., 2012). In addition, new commercial additives derived from plants, including aromatic plant extracts and their purified constituents, have been examined as part of alternative feed strategies for the future (Brenes and Roura, 2010). It has also been reported that such products have several advantages over commonly used commercial antibiotics, since they are residue free and are also generally recognized as safe and commonly used in the food industry (Varel, 2002).

Due to the present trend of rising prices of feed stuffs, considerable attention is therefore on the search for non-conventional feedstuffs (Esmail, 2002). For example, leaf meals made from shrubs have been useful to small scale farmers (WAC, 2006). Various leaf meals have been used in poultry diets, including those of cassava (Ogbma and Oredein, 1998) and Hoodia gordonii (Mohlapo et al., 2009).

Recently there has been an interest in plant extracts which have been used in poultry feed for improving growth performance, preventing some specific pathogenic micro-organism because of the antioxidant content they contain (Tekeli et al., 2012), but their influence as additives on growth performance of farm animals have not been sufficiently documented (Cowan, 1999).

These plant extracts are also reported to contain many active components, including essential oils, which boast a wide range of pharmacological activities (Lewis et al., 2003) and have been used as alternatives to antibiotics. There is also evidence of some plant extracts which
contain some antioxidant properties and minerals which could be valuable for poultry feeding.

*Moringa oleifera* is one such plant which has been used in poultry diets. It has been used as a substitute of fishmeal and soyabean meals in broiler diets by Zanu *et al.* (2011), and as a supplement (growth performance) of broilers (Du *et al.*, 2007). It has also been used in other livestock as feed (Anjorn *et al.*, 2010), more so in feeding dairy animals, goats and poultry layers (Mendieta-Araica *et al.*, 2011; Moyo *et al.*, 2012). Moringa leaves has been reported to have immense nutritional value as shown by the amino acid profile and crude protein content (Anwar *et al.*, 2007), its high level of vitamin A, E and low level of anti-nutritional compounds (Yang *et al.*, 2006). *Moringa oleifera* contain some health-promoting phytochemicals such as phenolics, flavonoids, carotenoids, alkaloids, sterols, which are reported to be authentic antioxidants that are safe and bio-active (Sreelatha and Padma, 2009). Despite the nutritive values of Moringa and its use in other animal species, its use or effect as broiler feed additive to improve the growth and carcass characteristics is not well known.

### 3.2. Materials and Methods

#### 3.2.1. Study site description

The experiment was conducted at Stellenboch University’s Mariendahl Research Farm, in Elsenburg, Western Cape. The experimental site is situated at longitude 18°50’E and latitude 33°51’S at an altitude of 177m above sea level. The climate at Mariendahl is typically Mediterranean, with an extreme annual rainfall of 622.7mm (30 year average) of which 84%
occurs between April and October (Labuschagne, 2005). Temperatures per year are between 4.8\(^\circ\)C (low) and 29.0\(^\circ\)C (high), humidity between 20% (lowest) and 98% (highest).
3.2.2. Experimental procedures

A total of four hundred and thirty two 1-day-old unsexed broiler chicks (Aviane 48) were used in this experiment. All the chicks were housed in the same brooding house and allocated to four dietary treatments (TRTS) with graded levels of MOLM (Table 3.1) in 72 cages. There were 18 cages per treatment and each cage allocated 6 chicks, that made 108 chicks per treatment. The cages permitted four-fold stocking density and each cage was equipped with an aluminium flooding pan feeder (300mm) and mini plastic drinkers of the same size (300mm).
Table 3.1: Treatment structure

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Basal diet (4-phase)</th>
<th>Inclusion rates of MOLM</th>
</tr>
</thead>
<tbody>
<tr>
<td>TRT&lt;sub&gt;1&lt;/sub&gt;</td>
<td><em>Moringa oleifera</em> leaf powder as additive</td>
<td>1000 g/ton of feed</td>
</tr>
<tr>
<td>TRT&lt;sub&gt;2&lt;/sub&gt;</td>
<td><em>Moringa oleifera</em> leaf powder as additive</td>
<td>750 g/ton of feed</td>
</tr>
<tr>
<td>TRT&lt;sub&gt;3&lt;/sub&gt;</td>
<td><em>Moringa oleifera</em> leaf powder as additive</td>
<td>500 g/ton of feed</td>
</tr>
<tr>
<td>TRT&lt;sub&gt;4&lt;/sub&gt;</td>
<td>Negative control</td>
<td>No MOLM added</td>
</tr>
</tbody>
</table>

MOLM=*Moringa oleifera* leaf meal
On arrival chicks were given water and the feed at *ad libitum* but with small amount of feed sprinkled inside cages for easier consumption and recognition of feed by chicks up to ten days of age. Temperatures were kept at 32°C for the first 7 days and monitored frequently for about 3 times/day i.e. in the morning, during the day, and at night. Sufficient air exchange was also allowed, and after 7 days temperatures were reduced gradually and equipment adjustments were done until the end of the experiment.

The chicks in groups and feeds were weighed weekly according to cage numbers using a normal scale. Body weight gain (BWG), feed intake (FI), average daily gain (ADG) and feed conversion efficiency (FCE) of the chicks were recorded at the beginning of each week, starting from placement until slaughter. Body weight gain was determined by subtracting the final body weight (g) from the initial body weight (g); average daily gain (ADG) was determined by dividing average body weight (g) by 7 (days); feed conversion efficiency (FCE) by dividing average feed intake (g) by the average body weights (g); and feed intake (FI) was determined by subtracting given feed (g/day) from the remaining feed (g/day). The faecal trays were cleaned weekly to avoid the build-up of diseases and contamination of feed. At eighteen days, all the chickens were moved from the brooding house, since they had grown, to the grower house and divided into 45 cages (10 chickens/cage). The cages contained a water channel system with drinking nipples and an aluminium flooding pan feeder (1500mm).

The diets were formulated to meet all the bird’s dietary nutrient requirements for pre-starter (0 to 7 days), starter (8 to 18 days), grower (19 to 28 days), and finisher (29 to 35 days) phases (National Research Council, 1994). The ingredient and chemical composition of all the
phases is shown on (Table 3.2.) and nutrient composition for pre-starter (Table 3.3.), starter
(Table 3.4.), grower (Table 3.5.) and finisher is shown on Table 3.6.
Table 3.2: The ingredient (kg) composition of pre-starter, starter, grower and finisher diets

<table>
<thead>
<tr>
<th>Ingredient (kg)</th>
<th>Prestarter (0-7Days)</th>
<th>Starter (8-18Days)</th>
<th>Grower (19-28Days)</th>
<th>Finisher (29-35Days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fine maize</td>
<td>89.69</td>
<td>244.12</td>
<td>398.63</td>
<td>528.35</td>
</tr>
<tr>
<td>Soya oil cake</td>
<td>42.32</td>
<td>124.61</td>
<td>149.45</td>
<td>202.76</td>
</tr>
<tr>
<td>Fish meal</td>
<td>10.8</td>
<td>10.14</td>
<td>19.95</td>
<td>32</td>
</tr>
<tr>
<td>Sunflower oil</td>
<td>3.22</td>
<td>8</td>
<td>15</td>
<td>13.52</td>
</tr>
<tr>
<td>Limestone</td>
<td>1.86</td>
<td>5.91</td>
<td>8.42</td>
<td>10.85</td>
</tr>
<tr>
<td>Monocalcium phosphate</td>
<td>0.76</td>
<td>3.44</td>
<td>4.25</td>
<td>6.11</td>
</tr>
<tr>
<td>Methionine</td>
<td>0.39</td>
<td>1.25</td>
<td>1.05</td>
<td>1.89</td>
</tr>
<tr>
<td>Salt</td>
<td>0.32</td>
<td>1.32</td>
<td>1.77</td>
<td>2.49</td>
</tr>
<tr>
<td>Vit+mineral premix</td>
<td>0.18</td>
<td>0.47</td>
<td>0.7</td>
<td>0.93</td>
</tr>
<tr>
<td>Sodium Bicarbonate</td>
<td>0.13</td>
<td>0.34</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Choline Chloride</td>
<td>0.15</td>
<td>0.31</td>
<td>0.29</td>
<td>0.2</td>
</tr>
<tr>
<td>Lysine</td>
<td>0.13</td>
<td>0.07</td>
<td>0.35</td>
<td>0.62</td>
</tr>
<tr>
<td>Treonine</td>
<td>0.65</td>
<td>0.02</td>
<td>0.14</td>
<td>0.27</td>
</tr>
</tbody>
</table>
**Table 3.3:** Nutrient composition (%) of the treatments on prestarter phase

<table>
<thead>
<tr>
<th>Nutrients</th>
<th>Treatment 1 (1000g/ton MOLM)</th>
<th>Treatment 2 (750g/ton MOLM)</th>
<th>Treatment 3 (500g/ton MOLM)</th>
<th>Treatment 4 (0MOLM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ash%</td>
<td>5.02</td>
<td>4.78</td>
<td>5.52</td>
<td>5.34</td>
</tr>
<tr>
<td>Ca%</td>
<td>0.86</td>
<td>0.64</td>
<td>0.90</td>
<td>0.84</td>
</tr>
<tr>
<td>CI%</td>
<td>0.31</td>
<td>0.32</td>
<td>0.34</td>
<td>0.34</td>
</tr>
<tr>
<td>Dry Matter%</td>
<td>90.12</td>
<td>90.46</td>
<td>90.29</td>
<td>90.09</td>
</tr>
<tr>
<td>Fat%</td>
<td>4.77</td>
<td>4.96</td>
<td>5.32</td>
<td>5.22</td>
</tr>
<tr>
<td>Fibre-Crude%</td>
<td>2.15</td>
<td>2.01</td>
<td>2.24</td>
<td>2.23</td>
</tr>
<tr>
<td>Moisture 103 °C %</td>
<td>9.88</td>
<td>9.54</td>
<td>9.71</td>
<td>9.91</td>
</tr>
<tr>
<td>Na%</td>
<td>0.17</td>
<td>0.17</td>
<td>0.20</td>
<td>0.20</td>
</tr>
<tr>
<td>P%</td>
<td>0.58</td>
<td>0.61</td>
<td>0.58</td>
<td>0.60</td>
</tr>
<tr>
<td>Protein CrudeN*6.25</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(Dumas)%</td>
<td>21.98</td>
<td>24.35</td>
<td>21.36</td>
<td>22.87</td>
</tr>
<tr>
<td>Se mg/kg</td>
<td>1.60</td>
<td>1.42</td>
<td>1.38</td>
<td>1.43</td>
</tr>
</tbody>
</table>
**Table 3.4:** Nutrient composition (%) of the treatments on starter phase

<table>
<thead>
<tr>
<th>Nutrients</th>
<th>Treatment 1 (1000g/tonMOLM)</th>
<th>Treatment 2 (750g/tonMOLM)</th>
<th>Treatment 3 (500g/tonMOLM)</th>
<th>Treatment 4 (0MOLM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ash%</td>
<td>4.88</td>
<td>4.53</td>
<td>4.88</td>
<td>4.62</td>
</tr>
<tr>
<td>Ca%</td>
<td>0.82</td>
<td>0.70</td>
<td>0.84</td>
<td>0.71</td>
</tr>
<tr>
<td>Cl%</td>
<td>0.30</td>
<td>0.31</td>
<td>0.33</td>
<td>0.31</td>
</tr>
<tr>
<td>Dry Matter%</td>
<td>89.66</td>
<td>89.67</td>
<td>89.8</td>
<td>89.63</td>
</tr>
<tr>
<td>Fat%</td>
<td>4.61</td>
<td>4.04</td>
<td>4.36</td>
<td>4.52</td>
</tr>
<tr>
<td>Fibre-Crude%</td>
<td>2.23</td>
<td>2.21</td>
<td>2.08</td>
<td>2.14</td>
</tr>
<tr>
<td>Moisture 103C%</td>
<td>10.34</td>
<td>10.33</td>
<td>10.2</td>
<td>10.37</td>
</tr>
<tr>
<td>Na%</td>
<td>0.19</td>
<td>0.17</td>
<td>0.19</td>
<td>0.20</td>
</tr>
<tr>
<td>P%</td>
<td>0.57</td>
<td>0.53</td>
<td>0.55</td>
<td>0.54</td>
</tr>
<tr>
<td>Protein Crude N*6.25 (Dumas)%</td>
<td>21.41</td>
<td>18.79</td>
<td>19.18</td>
<td>18.64</td>
</tr>
<tr>
<td>Se mg/kg</td>
<td>0.14</td>
<td>1.50</td>
<td>1.34</td>
<td>0.54</td>
</tr>
</tbody>
</table>
Table 3.5: Nutrient composition (%) of treatments on grower phase

<table>
<thead>
<tr>
<th>Nutrients</th>
<th>Treatment1 (1000g/tonMOLM)</th>
<th>Treatment2 (750g/tonMOLM)</th>
<th>Treatment3 (500g/tonMOLM)</th>
<th>Treatment4 (0MOLM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ash%</td>
<td>4.89</td>
<td>4.77</td>
<td>4.75</td>
<td>4.71</td>
</tr>
<tr>
<td>Ca%</td>
<td>0.85</td>
<td>0.75</td>
<td>0.67</td>
<td>0.66</td>
</tr>
<tr>
<td>Cl%</td>
<td>0.32</td>
<td>0.34</td>
<td>0.32</td>
<td>0.33</td>
</tr>
<tr>
<td>Dry Matter%</td>
<td>89.67</td>
<td>90.26</td>
<td>89.69</td>
<td>90.4</td>
</tr>
<tr>
<td>Fat%</td>
<td>5.13</td>
<td>5.36</td>
<td>5.32</td>
<td>4.69</td>
</tr>
<tr>
<td>Fibre-Crude%</td>
<td>2.27</td>
<td>2.25</td>
<td>1.96</td>
<td>2.05</td>
</tr>
<tr>
<td>Moisture 103C%</td>
<td>10.33</td>
<td>9.74</td>
<td>10.31</td>
<td>9.6</td>
</tr>
<tr>
<td>Na%</td>
<td>0.19</td>
<td>0.20</td>
<td>0.19</td>
<td>0.16</td>
</tr>
<tr>
<td>P%</td>
<td>0.50</td>
<td>0.67</td>
<td>0.49</td>
<td>0.49</td>
</tr>
<tr>
<td>Protein Crude</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N*6.25(Dumas)%</td>
<td>19.08</td>
<td>19.01</td>
<td>18.44</td>
<td>19.63</td>
</tr>
<tr>
<td>Se mg/kg</td>
<td>1.38</td>
<td>1.43</td>
<td>1.2</td>
<td>1.27</td>
</tr>
</tbody>
</table>
**Table 3.6:** Nutrient composition (%) of treatments on finisher phase

<table>
<thead>
<tr>
<th>Nutrients</th>
<th>Treatment 1 (1000g/tonMOLM)</th>
<th>Treatment 2 (750g/tonMOLM)</th>
<th>Treatment 3 (500g/tonMOLM)</th>
<th>Treatment 4 (0MOLM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ash%</td>
<td>5.03</td>
<td>4.78</td>
<td>5.08</td>
<td>4.94</td>
</tr>
<tr>
<td>Ca%</td>
<td>0.83</td>
<td>0.71</td>
<td>0.71</td>
<td>0.71</td>
</tr>
<tr>
<td>Cl%</td>
<td>0.37</td>
<td>0.31</td>
<td>0.36</td>
<td>0.32</td>
</tr>
<tr>
<td>Dry Matter%</td>
<td>90.04</td>
<td>89.86</td>
<td>90.05</td>
<td>90.06</td>
</tr>
<tr>
<td>Fat%</td>
<td>4.7</td>
<td>4.18</td>
<td>4.33</td>
<td>4.84</td>
</tr>
<tr>
<td>Fibre-Crude%</td>
<td>2.09</td>
<td>1.92</td>
<td>2.26</td>
<td>2.36</td>
</tr>
<tr>
<td>Moisture 103C%</td>
<td>9.96</td>
<td>10.14</td>
<td>9.95</td>
<td>9.94</td>
</tr>
<tr>
<td>Na%</td>
<td>0.23</td>
<td>0.16</td>
<td>0.16</td>
<td>0.1</td>
</tr>
<tr>
<td>P%</td>
<td>0.51</td>
<td>0.5</td>
<td>0.52</td>
<td>0.48</td>
</tr>
<tr>
<td>Protein Crude</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N*6.25(Dumas)%</td>
<td>18.04</td>
<td>19.02</td>
<td>18</td>
<td>17.67</td>
</tr>
<tr>
<td>Se mg/kg</td>
<td>1.56</td>
<td>1.25</td>
<td>1.9</td>
<td>1.50</td>
</tr>
</tbody>
</table>
3.2.3. Slaughter procedures and determination of carcass and organ weights

A day before slaughter all the chickens were weighed and not given feed overnight. After weighing 6 birds/treatment were randomly selected and kept separately, 24 birds were therefore used for the experiment. Before slaughtering, the slaughter weights were taken. Slaughtering was done following the normal procedures of the abattoir, whereby they were first stunned with an electrical stunner (50-70volts) under the beak for 5 seconds to render them unconscious before being slaughtered. The unconscious chickens were then shackled by their legs onto a conveyor line. While hanging, the throats were cut using a sharp knife, only one person was responsible for the throat cutting and one person for stunning. After throat cutting, tags having treatment numbers were tagged on their feet, this also gave time for the chickens to bleed. After plucking the feathers, the internal organs (liver, heart, gastrointestinal fat, gizzards) were removed from carcasses and weighed, so are the carcasses.

3.3. Statistical analysis
The AFI, BWG, ADG, FCE and the carcass characteristics (slaughter weight, carcass weight, dressing %) and intestinal organs (liver weight, heart weight, gizzard weight, gastrointestinal fat weight) were analyzed using one-way analysis of variance (ANOVA) SAS (2003). Where there was a significant P-test (P<0.05), the least significant difference (LSD) method was used to compare the means.
The statistical model that was used is, \( Y_{ij} = \mu + T_i + E_{ij} \).

Where,

*\( Y_{ij} \) = response variables (AFI, BWG, ADG & FCE), carcass characteristics and internal organs

*\( \mu \) = overall mean

*\( T_i \) = treatment effect

*\( E_{ij} \) = standard error

3.4. Results and discussion

The use of MOLM as an additive on broiler chickens had no significant effect (\( P>0.05 \)) on AFI and FCE (Table 3.7 and 3.8). However, when they were 7 Days old, they differed (\( P<0.05 \)) between treatments, with treatment 4 having the highest value. These observations are similar to the ones reported by Tekeli et al. (2011), indicating that antibiotics or plant extract supplementation in a broiler experiment did not influence body weight gain, feed intake and feed conversion efficiency of the chickens. Similarly, there are other research findings showing that ration supplemented with plant extract and propolis additives did not have significant effect on the improvement of FCE of poultry (Demir et al. 2003; Botsoglou et al., 2004). Botsoglou et al. (2002) and Hernandez et al. (2004) also reported that feeding oregano essential oil as a supplement did not have any effect on the growth performance of broiler chicks.

Folorunso and Onibi (2012), also observed no differences on ADG, AFI and FCE of broilers when fed with diets containing different levels of protein, reason being due to varying dietary protein levels showing that the birds were able to consume at fairly the same level regardless
of the quantity of protein in the diet. However, in our observations it may be due to the fact that the nutrients in the diets provided to the broilers were not in same quantities and could have affected the performance of the chickens. Another reason could be that the level of *M. oleifera* provided was too little to have any effects on AFI, BWG, FCE and ADG. The anti-nutritional factors, such as condensed tannins in MOLM could also play a significant role in the nutrition of animals, causing either adverse or beneficial effects on nutrient utilization, health and production (Waller and Thamsborg, 2004; Hoste et al., 2006). Some anti-nutritional factors are reported to affect palatability of diets which in turn will affect feed intake.

<table>
<thead>
<tr>
<th>Age(days)</th>
<th>Treatment1 (1000g/tonMOLM)</th>
<th>Treatment2 (750g/tonMOLM)</th>
<th>Treatment3 (500g/tonMOLM)</th>
<th>Treatment4 (0MOLM)</th>
<th>Significance</th>
</tr>
</thead>
</table>

**Table 3.7**: Effect of Moringa oleifera leaf meal as an additive on average feed intake (AFIg) of broilers at 7, 18, 28 and 35 days
<table>
<thead>
<tr>
<th></th>
<th>7</th>
<th>18</th>
<th>28</th>
<th>35</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>61.5\textsuperscript{ab}±4.49</td>
<td>73.6\textsuperscript{ab}±4.32</td>
<td>56.4\textsuperscript{a}±5.74</td>
<td>87.7\textsuperscript{b}±16.98</td>
<td>*</td>
<td></td>
</tr>
<tr>
<td>18</td>
<td>384.9±14.68</td>
<td>436.3±27.40</td>
<td>437.9±35.85</td>
<td>366.7±36.77</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>28</td>
<td>1032.4±52.53</td>
<td>981.5±122.48</td>
<td>1132.1±25.16</td>
<td>1036.6±19.67</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>35</td>
<td>1199.9±48.77</td>
<td>1165.6±141.45</td>
<td>1344.6±18.74</td>
<td>1203.5±25.44</td>
<td>NS</td>
<td></td>
</tr>
</tbody>
</table>

\textsuperscript{a,b,c}Means, ±se in the same row with similar superscripts are not significantly different (P >0.05) from each other.

*=P>0.05

SE=standard errors
Table 3.8: Effect of feeding *Moringa oleifera* leaf meal as an additive on feed conversion efficiency (FCE) of broilers at 7, 18, 28 and 35 days

<table>
<thead>
<tr>
<th>Age(days)</th>
<th>Treatment1 (1000g/tonMOLM)</th>
<th>Treatment2 (750g/tonMOLM)</th>
<th>Treatment3 (500g/tonMOLM)</th>
<th>Treatment4 (0MOLM)</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>7</td>
<td>0.8(^b)±0.06</td>
<td>1.0(^ab)±0.06</td>
<td>0.8(^b)±0.09</td>
<td>1.1(^a)±0.10</td>
<td>*</td>
</tr>
<tr>
<td>18</td>
<td>1.5±0.41</td>
<td>1.4±0.09</td>
<td>1.6±0.22</td>
<td>1.3±0.08</td>
<td>NS</td>
</tr>
<tr>
<td>28</td>
<td>1.8±0.15</td>
<td>2.1±0.35</td>
<td>2.0±0.01</td>
<td>1.8±0.15</td>
<td>NS</td>
</tr>
<tr>
<td>35</td>
<td>1.3±0.17</td>
<td>1.1±0.17</td>
<td>1.3±0.17</td>
<td>1.0±0.01</td>
<td>NS</td>
</tr>
</tbody>
</table>

\(^ab\) Means, ±se in the same row with similar superscripts are not significantly different \((P >0.05)\) from each other.

*\(=P>0.05\)

±SE=standard error
The body weight gain (BWG) differed significantly with TRTS at Day 18, 28 and 35 with treatment 1 having highest value on Day 18, treatment 2 having the highest on Day 28 and treatment 2 having the highest on Day 35 (Table 3.9). ADG on Day 18 and Day 35 differed ($P<0.05$) between TRTS (Table 3.10). The none significant differences in all the TRTS on ADG could be due to the fact that MOLM was added in small amounts so there was not much difference between the TRTS with MOLM and treatment 4 which had no MOLM. These findings contradict that of Denil et al. (2003) that showed supplementation of additives in broiler diets enhanced nutrient utilization, growth and feed conversion efficiency (FCE) of broilers. Ayssiwede et al. (2011) observed that the inclusion of *M. oleifera* leaf meal in the diet of growing traditional Senegal chickens had no negative impact on live body weight, average daily weight gain, feed conversion ratio, carcass and organ characteristics in birds. However, they reported a significant decrease in daily feed intake in treatments that contained different levels of *M. oleifera* leaf meal. All the TRTS had no effect on meat portions, but treatment 4 had highest values of thighs, breasts and drum sticks than the other TRTS though not statistically significant (Table 3.11).
Table 3.9: Effect of Moringa oleifera leaf meal as an additive on body weight gain (BWGg) of broilers at 7, 18, 28 and 35 days

<table>
<thead>
<tr>
<th>Age(days)</th>
<th>Treatment1 (1000g/tonMOLM)</th>
<th>Treatment2 (750g/tonMOLM)</th>
<th>Treatment3 (500g/tonMOLM)</th>
<th>Treatment4 (0MOLM)</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>7</td>
<td>83±2.60</td>
<td>74±2.66</td>
<td>75±2.56</td>
<td>81±12.69</td>
<td>NS</td>
</tr>
<tr>
<td>18</td>
<td>356a±20.45</td>
<td>311ab±4.87</td>
<td>289b±18.22</td>
<td>285b±17.25</td>
<td>*</td>
</tr>
<tr>
<td>28</td>
<td>638ab±17.78</td>
<td>572b±41.24</td>
<td>603ab±17.22</td>
<td>659a±12.61</td>
<td>*</td>
</tr>
<tr>
<td>35</td>
<td>890bc±23.47</td>
<td>1049ab±71.61</td>
<td>888bc±14.16</td>
<td>916bc±23.98</td>
<td>*</td>
</tr>
</tbody>
</table>

abc Means, ±se in the same row with similar superscripts are not significantly different (P>0.05) from each other.

*= P >0.05
±SE= Standard error

Table 3.10: Effect of Moringa oleifera leaf meal as an additive on average daily gain (ADGg) of broilers at 7, 18, 28 and 35 days

<table>
<thead>
<tr>
<th>Age(days)</th>
<th>Treatment1 (1000g/tonMOLM)</th>
<th>Treatment2 (750g/tonMOLM)</th>
<th>Treatment3 (500g/tonMOLM)</th>
<th>Treatment4 (0MOLM)</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>7</td>
<td>18.3±0.45</td>
<td>17.1±0.36</td>
<td>17.1±0.37</td>
<td>17.6±1.50</td>
<td>NS</td>
</tr>
<tr>
<td>Treatments</td>
<td>Breasts</td>
<td>Thighs</td>
<td>Wings</td>
<td>Drums</td>
<td></td>
</tr>
<tr>
<td>--------------------</td>
<td>------------------</td>
<td>-----------------</td>
<td>-----------------</td>
<td>-----------------</td>
<td></td>
</tr>
<tr>
<td>Treatment 1 (1000g/tonMOLM)</td>
<td>496</td>
<td>321</td>
<td>162</td>
<td>166</td>
<td></td>
</tr>
<tr>
<td>Treatment 2 (750g/tonMOLM)</td>
<td>510</td>
<td>321</td>
<td>183</td>
<td>167</td>
<td></td>
</tr>
<tr>
<td>Treatment 3 (500g/tonMOLM)</td>
<td>452</td>
<td>322</td>
<td>166</td>
<td>158</td>
<td></td>
</tr>
<tr>
<td>Treatment 4 (0MOLM)</td>
<td>518</td>
<td>342</td>
<td>174</td>
<td>177</td>
<td></td>
</tr>
</tbody>
</table>

S.E= standard errors

Sig= Significance
In Table 3.12, carcass characteristics, carcass weight (CW), slaughter weight (SW), dressing percentage (D %) and gizzard weight (GW) values were similar in all the treatments. The liver weight (LW), heart weight (HW) and gastro intestinal fat (GIF) differed significantly ($P < 0.05$) in all the treatments, with LW having highest value on treatment 4, and HW having highest value on treatment 2 ($750$g/ton MOLm) and the GIF also higher on treatment 2. It is reported that the enhancement of plant extracts such as metabolism oil in the major organs would increase the growth rate of internal organs (Mellor, 2000). In some studies plant extracts added in supplements are reported to improve D% (Alcicek et al., 2004). However the significance ($P < 0.05$) of internal organs (LW, HW, GIF) agrees with the results reported by Al-Kassie (2009), that different levels of oil extract derived from thyme and cinnamon also had significant effects on internal organs percentages (liver, heart and gizzard).
Table 3.12: Effect of Moringa oleifera as an additive on carcass characteristics of broilers

<table>
<thead>
<tr>
<th>Weights(g)</th>
<th>Treatment1 (1000g/tonMOLM)</th>
<th>Treatment2 (750g/tonMOLM)</th>
<th>Treatment3 (500g/tonMOLM)</th>
<th>Treatment4 (0MOLM)</th>
<th>SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart</td>
<td>12.3</td>
<td>28.3</td>
<td>11.5</td>
<td>12.1</td>
<td>2.55</td>
</tr>
<tr>
<td>GI fat</td>
<td>7.4</td>
<td>11.4</td>
<td>7.8</td>
<td>7.4</td>
<td>1.12</td>
</tr>
<tr>
<td>Liver</td>
<td>43.1</td>
<td>42.6</td>
<td>37.7</td>
<td>44.2</td>
<td>1.60</td>
</tr>
<tr>
<td>Carcass</td>
<td>1.2</td>
<td>1.2</td>
<td>1.1</td>
<td>1.2</td>
<td>0.04</td>
</tr>
<tr>
<td>Slaughter</td>
<td>1.6</td>
<td>1.6</td>
<td>1.5</td>
<td>1.6</td>
<td>0.05</td>
</tr>
<tr>
<td>Dressing%</td>
<td>75.9</td>
<td>76.8</td>
<td>76.4</td>
<td>75.7</td>
<td>0.85</td>
</tr>
<tr>
<td>Gizzard</td>
<td>18.5</td>
<td>17.8</td>
<td>17.2</td>
<td>18.1</td>
<td>1.03</td>
</tr>
</tbody>
</table>

*Means in the same row with similar superscripts are not significantly different (*P* >0.05)

GI Fat = Gastro intestinal fat
SE= Standard error
3.5. Conclusion

*Moringa oleifera* leaf meal as an additive had no effect on growth performance of broiler chickens. However, it had an effect on carcass characteristics by improving Liver weight (LW), heart weight (HW) and gastro intestinal fat (GIF). Since inclusion of *Moringa oleifera* leaf meal at different levels in broiler diets did not have adverse effect on performance of broilers, therefore it is vital to determine the effect of *M.oleifera* leaf meal as an additive on the meat quality of these broilers.
3.6. References


Mellor, S., 2000a. Antibiotics are not the only growth promoters. World Poult.16:30-33.


Chapter 4: Effect of feeding Moringa oleifera leaf meal as an additive on physico-chemical shelf life indicators of broiler meat.

Abstract
The objective of the current study was to determine the effect of using Moringa oleifera leaf meal (MOLM) as an additive on the physico-chemical shelf life indicators of meat from broilers. A total of 432 1-day-old chicks were randomly allocated to four treatments (TRTS). Feed and water was provided *ad libitum*. The feeding phases were pre-starter (0 to 7 days), starter (8 to 18 days), grower (19 to 28 days) and finisher (29 to 35 days). The four TRTS contained graded levels of the MOLM at 1000g/ton, 750g/ton, 500g/ton, and 0g/ton (control), respectively. The birds were slaughtered at 35 days of age and the breast muscle was sampled for meat pH, colour and drip loss measurements over a 7 days shelf-life test. The pH levels in all the TRTS were constant from Day 1 to Day 5, peaking on Day 6, then declining on Day 7.

Using MOLM as an additive had a significant effect on broiler meat colour, with TRT1 having the highest lightness (L*) values. The redness (a*) values were highest in TRT2. However, MOLM as an additive did not have an effect on the yellowness (b*) values or the drip loss of broiler chicken. Using MOLM as an additive in broiler feeds produced chicken breast with a light (L*) appearance while shelf life indicators generally remained constant in the first 5 days of storage.

**Key words:** drip loss, meat colour, meat pH, freshness, breast
4.1. Introduction

Chicken meat has many desirable nutritional characteristics. Chicken meat and its products have a vast consumer market and are making a significant contribution to the supply of high protein quality (Mothershaw et al. 2009), low lipid contents or cholesterol hence reported to be healthier than red meat. Meat quality attributes, such as juiciness, tenderness, drip loss, cooking-loss, ultimate pH and shelf-life are the major parameters considered in the assessment of meat (Muchenje et al. 2008; Muchenje et al., 2009) and they are important to the consumer as well as to the processor when producing value-added meat products (Allen et al., 1998). In addition, consumers consider several characteristics (sensory characteristics, nutritional value and impact on health) to determine the acceptance of meat or food products (Muchenje et al., 2009a; 2008b).

Poultry meat quality is receiving considerable attention recently due to the emergence of problems associated with poor waterholding capacity, poor texture, and pale colour called pale soft exudative (PSA) (Fletcher, 1999; Baeza, 2004). Meat quality attributes are dependent on certain factors such as genotype, sex, age and non-genetic factors such as nutrition. Diet composition and feed consumption (see chapter 3) can affect the chemical composition of muscle tissue such as pH, colour and tenderness to a greater or lesser extent (Qwele, 2011).
Spoilage of meat is influenced by packaging, temperatures of storage and storage time. Shelf-life is defined as the period of time between packaging of a product and its end use while the product properties remain acceptable for the product user (Lorenzo and Gomez, 2012). The shelf-life properties may include colour, appearance, texture, flavour and nutritive value (Singh and Singh, 2005). Good or better packaging of meat assures a maximum shelf-life. The vacuum packed meat have a maximum shelf life of 15-25 days depending on the microbiological quality of the meat, which is a direct reflection of sanitation during slaughter and packaging (Charley, 1982; Simons, 1980). It has been reported by Lorenzo and Gomez (2012) that bacteria like Pseudomonas spp., psychrotrophic aerobic bacteria, lactic acid bacteria are common in low temperature environments and are major causes of spoilage of chilled meats, fish and poultry. It has also been reported that unfrozen poultry at low temperatures around 0°C extend shelf-life quite markedly and temperature of -18°C or lower for frozen poultry are useful to maintain colour and minimize freezer burn.

Poultry maybe susceptible to antemortem and post-mortem stressors such as environmental temperatures, stunning methods (Backstrom and Kauffman, 1995), and chilling regimes (Offer, 1991). These stressors are reported to cause accelerated rigor development in some carcasses and have an effect in meat quality. For instance, it has been shown that higher post-mortem carcass temperatures (>20°C) in turkey resulted in lighter meat with higher drip-loss and cooking-loss (McKee and Sams, 1998).
Nutrition has considerable effect on certain meat quality traits. Minerals such as Selenium usually added in mono-gastric feeds are quite expensive and these minerals have been defined as essential dietary supplements which are important for improving health and performance of the birds and improving meat quality for human consumption (Haug et al., 2007; Yoon et al; 2007). However, there is an interest on the use of natural antioxidants which are reported to be safer than synthetic antioxidants (Moyo et al., 2011). These natural antioxidants especially of plant source such as vitamin E and selenium (Khalafalla et al, 2010), have greater application potential for stability and shelf-life of meat products when added in diets (Jung et al., 2010). These natural antioxidants have a potential to decrease lipid and pigment oxidation (Legonie et al. 2012), hence oxidation reduces shelf-life and leads to meat quality loss.

One such plant that contains these minerals and has a potential to be used as an antioxidant additive is Moringa oleifera. Moringa oleifera is drought tolerant, and it is considered as one of useful trees because it is prophylitic meaning it can be used both as medicine and feed (Reyes-Sanchez et al., 2006). This tree is commonly known as the horse-radish tree or drumstick tree. According to Qwele et al., (2011) it is highly recognised for having some useful minerals, vitamins, selenium and amino-acids. The high nutritive value of Moringa oleifera makes it more suitable as a feed additive of monogastric animals such as poultry. Therefore, this study was conducted to determine the effect of MOLM as an additive on physico-chemical shelf-life indicators of broiler meat.
4.2. Material and Methods

4.2.1. Study site and management of broiler chickens.
The study site, experimental animals and management of the chickens are as described in Sections 3.2.1 and 3.2.2.

4.2.2. Procedures after slaughter
The chickens were slaughtered at the end of the experiment (day 35). After plucking, evisceration and dressing, the carcasses were stored at 4°C overnight. On the following day 6 carcasses per treatment were randomly selected for meat quality measurements. The left breasts were deboned, the skins removed and cut into halves longitudinally for the shelf-life trial. Each of the half left breasts were weighed (WB) and packed in polystyrene trays and wrapped in 10 micron thick oxygen permeable cling film (Versafilm, Crown National, Montague Gardens, Cape Town, South Africa) with a moisture vapour transfer rate of 585 g.m⁻².24 h⁻¹.1 atm⁻¹, O₂ permeability of 25 000 cm³.m⁻².24 h⁻¹.1 atm⁻¹ and a CO₂ permeability of 180 000 cm³.m⁻².24 h⁻¹.1 atm⁻¹. Each tray was marked according to the treatment number and sample number, which then gave 12 trays per treatment. The trays were then stored at 4°C over 7 days. Every day, three trays per treatment were randomly removed from the cooler.

4.3. Meat quality measurements

4.3.1. Drip loss measurements
To determine drip loss three fillets breast trays per treatment were randomly selected from the cooler and patted dry using an absorbent paper towel and weighed (WA). The drip loss was then calculated as WB-WA/WB*100%.
4.3.2. **Ultimate pH**
The pH of the fillets was determined with a CRISON pH 25 (CRISON Instruments SA, Spain) which was calibrated before each measurement using pH4, pH7, and pH9 standard solutions. The measurements were done on the same fillets by inserting the probe throughout the fillet, approximately 24 hours after slaughter in breasts that were refrigerated at 4°C.

4.3.3. **Determination of colour**
Colour was measured according to the CIE L* a* b* colour system using a colour-guide 45°/0° colorimeter (BYK-Gardner GmbH, Geretsried, Germany) with a 20mm diameter measurement area and illuminant D65-day light and 10° standard observer on the same fillets. Three readings were taken by rotating the Colour Guide 90° between each measurement, in order to obtain a representative average value of the colour. The machine was first calibrated using the green standard before each measurement.

4.4. **Statistical analysis**
The effect of dietary supplementation on meat colour, pH and drip loss was analyzed using analysis of variance (ANOVA) GenStat of (2008). The least significant difference (LSD) method was used to separate the means.

The following model was used: $Y_{ij} = \mu + \alpha_i + \beta_j + E_{ij}$ Where:

- $Y_{ij}$=variables (meat colour, pH and drip loss)
- $\mu$ = constant
- $\alpha_i$ = effect of diet
- $\beta_j$=effect of day
- $E_{ij}$ = random error
4.5. Results and discussion

Figure 4.1 shows the effect of the treatment over time (days) on pH of chicken meat. The pH levels on meat from chickens supplemented with treatment 2 on day 7 were higher (7.00) than the ones for meat from chickens fed with treatment 1, treatment 3 and treatment 4 which were (5.9). This could be due to the high levels of vitamin C (ascorbic acid) contained in MOLM which is reported to be about 17.3mg/100g DM (Rweyemamu, 2006). Some studies have shown that breeds supplemented with vitamin C produce meat with high levels of pH (>5.5) at different time intervals. For example, in a study conducted by Kremer et al. (1999), the muscle pH measured in the right longissimus (LM) muscle post-mortem from pigs supplemented with vitamin C had significantly higher pH (p<0.13) than muscles from controls, which explains the low pH (5.2) at day1 in treatment 4 (0g/ton MOLM) negative control in the present study.

The pH levels in all the treatments were generally constant from day1 to day5 (5.8-6.00). The pH values reached their peak on day 6, and then declined on day 7 except for treatment 2. This observation is similar to the one by Jang et al. (2011), where they tested the effect of different basal diets on thighs of broiler meats. They observed that diets with antibiotics and the one with vitamin E increased pH value when measured on day1, 3, and 5 on chicken thighs compared to the meat from the chickens supplemented with basal diet only during a 5 day storage. Price and Schweigert (1987) reported that meat with a pH greater than 5.8 may be more conductive to spoilage and result in decreased shelf life. However, according to Greer and Murray (1988) lower pH product will visually have a shorter shelf life compared to higher pH product, which may be due to less enzymatic reduction and faster rate of myoglobin oxidation which is favoured at lower pH (Ledward, 1984). There were treatment
differences at day 1 and day 7, with treatment 4 having low pH (5.2) while the other treatments had a pH >5.5 at Day 1 and constant decrease at Day 7. The differences could be due to different levels of MOLM in the. The high pH meat with lower lightness values and higher redness values observed in this study supports the observations of Yang and Chen (1993), who observed ground chicken meat adjusted to a high pH was darker and redder in colour. However, the explanation for these observations is not clear and merits further investigation.
**Figure 4.1:** Effect of treatment overtime (days) on the pH levels of broiler breast muscle
Table 4.1 shows the effect of days on colour (L*, a*, b*) and drip loss of chicken meat. The lightness (L*) values were within the normal range of 50-56 for chicken meat (Petracci et al., 2004) from day 1-5, but then decreased on day 6 to 49.3. Lorenzo and Gomez (2012) also observed an increase in L* values during the early days of meat storage followed by a decrease from day 7. Other studies have also shown that L* values decrease towards the end of meat storage (Bingol and Ergun, 2011). As expected, the drip loss values increased with storage time. Accelerated pHu decline (Figure 4.1) are related to unacceptable drip loss increase (Table 4.1). This is in agreement with the findings by Muchenje et al. (2009).
Table 4.1: Least square means and standard errors for L*, a*, b* and pH, Drip loss of chicken meat as affected by storage time (Days)

<table>
<thead>
<tr>
<th>Attributes</th>
<th>DAYS</th>
<th>SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>L*</td>
<td>54.9d</td>
<td>54.0d</td>
</tr>
<tr>
<td>a*</td>
<td>4.8d</td>
<td>3.2a</td>
</tr>
<tr>
<td>b*</td>
<td>12.9c</td>
<td>11.6a</td>
</tr>
<tr>
<td>Drip loss</td>
<td>1.1a</td>
<td>1.5b</td>
</tr>
</tbody>
</table>

abc means with different superscripts in a row are different (P < 0.05)

SE=standard error of means

L* =lightness, a* =redness, b* =yellowness
Table 4.2 shows the effect of MOLM on post slaughter colour and drip loss of chicken breast meat. Treatment had an effect \( P<0.05 \) on the colour of the broiler breast meat which could be attributed to the antioxidant activity in MOLM (Moyo et al., 2012). Buckley and Morrissey (1992) reported that feeding poultry higher levels of natural dietary antioxidants provides the poultry industry with a simple method for improving the oxidative stability and shelf-life of poultry meats. The highest b* values were observed in broiler meat from treatment 1. This could be due to high beta carotene content in MOLM consumed by chicken in this treatment (Moyo et al. 2011; Richter et al. 2003; Reyes-Sanchez et al. 2006) which had the highest inclusion of MOLM (1000g/ton) than the other treatments. These observations could be due to vitamin E since it is reported to prevent discolouration and is known of extending colour display-life of meat (Faustman et al. 1989; Arnold et al., 1992). Dietary treatments had no \( P>0.05 \) effect on drip loss. This concurs with the findings by Lawrie (1998) that diet does not seem to affect the drip loss whilst Comale et al. (2011) reported that drip and cooking losses are not globally influenced by the use of the phytotherapeutic compound in the diets.

Table 4.2: Least square means and standard errors for L*, a*, b* and drip loss of meat samples (chicken) as affected by treatment

<table>
<thead>
<tr>
<th>Attributes</th>
<th>Attributes</th>
<th>1 (1000g/tonMOLM)</th>
<th>2 (750g/tonMOLM)</th>
<th>3 (500g/tonMOLM)</th>
<th>4 (0MOLM)</th>
<th>SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>L*</td>
<td></td>
<td>55.4b</td>
<td>51.8a</td>
<td>51.7a</td>
<td>51.4a</td>
<td>0.97</td>
</tr>
<tr>
<td>a*</td>
<td></td>
<td>4.1b</td>
<td>3.7a</td>
<td>5.2d</td>
<td>4.8c</td>
<td>0.40</td>
</tr>
<tr>
<td>b*</td>
<td></td>
<td>14.1d</td>
<td>12.4a</td>
<td>13.3c</td>
<td>12.9b</td>
<td>0.47</td>
</tr>
<tr>
<td>Drip loss</td>
<td></td>
<td>2.4</td>
<td>2.3</td>
<td>2.0</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
**4.6. Conclusion**

Using MOLM as an additive affected the colour, drip loss and pH of broiler breast meat as well as the stability of these parameters over time, the effects being most pronounced with the highest level of MOLM (TRT1; 1000g/ton MOLM) inclusion. The colour (lightness) and the pH of the meat were stable over time until day 6 when it started decreasing. Drip loss from the chicken breasts increased during storage. Antioxidants in MOLM seems to be effective in improving oxidation and shelf-life of broiler meat. Therefore, further research on the effect of
MOLM as an additive on fatty acids profiles and lipid oxidation of meat from broilers is recommended as presented in Chapter 5.

4.7. References


Chapter 5: Effect of Moringa oleifera leaf meal supplementation on fatty acids profile and lipid oxidation of broiler meat

Abstract
The objective of the study was to determine the effect of Moringa oleifera leaf meal (MOLM) as an additive on fatty acids profiles and lipid oxidation in broiler meat. A total of 432 1-day old chicks (Aviane 48) were randomly allocated to four dietary treatments (TRTS).
Water and feed was provided ad libitum. The four TRTS contained graded levels of the MOLM at 1000g/ton, 750g/ton, 500g/ton, and 0g/ton (control), respectively. Birds were slaughtered at 35 days of age and the left breast muscles were sampled for meat pH, colour and drip loss measurements over a 7 days shelf-life test. Sub- samples were kept at -18\(^\circ\) C for thiobarbituric acid reactive substances (TBARS) determination. On day1 and day7 extra sub- samples were also stored at -18\(^\circ\) C for fatty acids analysis. Treatments had an effect on C16:0, C18n6t, C18n9t, C20:1c11 with treatment 1 having the highest value. Treatment 4 had highest proportions of polyunsaturated fatty acids (PUFA). Treatments had an effect on saturated fatty acids (SFA) with treatment 1 having highest proportion. Treatment 2 had a highest n-6/n-3 ratio. Days had no significant effect on PUFA, SFA, and n-6/n-3 ratio. Using MOLM as an additive had a significant effect on lipid oxidation, with treatment 1 having higher levels of malondialdehyde (MDA). The 1000g/ton of MOLM (TRT1) in feed was more effective than 750g/ton (TRT2) and 500g/ton (TRT3) of MOLM whereas Treatment 4 had no effect (P>0.05) on MDA levels. Storage time had a significant effect on MDA levels, except for day1 and day7, with Day2 having the highest amount of MDA than Day3 and Day4. It can be concluded that TRTS with MOLM prevented lipid oxidation, however its effect on fatty acids was not clear, therefore meriting further investigations.

**Keywords:** Dietary TRTS, fatty acids profiles, thiobarbituric acid, breast meat

5.1. Introduction

To determine the acceptance of meat or food products, consumers consider several characteristics, including sensory characteristics, nutritional value and impact on health (Muchenje *et al.*, 2008; 2009). Chicken meat has low levels of fat, cholesterol and high levels of iron (Jarusitha *et al.*, 2008). There are several studies concerning the enrichment of chicken meat with polyunsaturated fatty acids (PUFA) by the addition of polyunsaturated fats
to the diet (Lin et al., 1989; Ajutah et al., 1993; Lopez-Ferrer et al., 1999, 2001). Chicken meat enriched with PUFA contains longer fatty acids (FA) with a high number of double bonds, which increases the susceptibility of meat to oxidation (Grau et al., 2001a; 2001b).

Lipid oxidation and discolouration are believed to be major causes of quality deterioration in meat during refrigerated storage (Ryu et al., 2005). Chicken meat is sensitive to oxidative deterioration due to high content of PUFA (Ryu et al., 2005). The rate of meat discolouration is believed to be related to the effectiveness of the oxidation processes (Ryu et al., 2005). There is an interest in foods containing higher levels of PUFA because of their beneficial effects on human health, mainly in the prevention of cardiovascular diseases (Krauss et al., 2001). Feeding diets that are supplemented with oilseeds or vegetable oils increases the PUFA concentration in edible tissue and inevitably reduces their oxidative stability (Skrivan et al., 2012). Increased degree of unsaturated fatty acids (USFA) in diets has been reported to be a positive feature with respect to human health, however it may have a negative effect on shelf-life of poultry (Skrivan et al., 2012).

For this reason, there is an interest in dietary antioxidants, particularly in Vitamin E and Selenium which are normally used as additives in animal diets. Antioxidants have an ability to prevent or reduce the oxidative damage of a tissue indirectly by enhancing natural defences of cell and/or directly by scavenging the free radical species (Verma et al., 2009). The effect of dietary supplementation with various antioxidants on chicken meat oxidation has been studied (Grau et al. 2001; Bou et al., 2004). There is a growing interest on natural antioxidants from plants such as Moringa oleifera.
These plants contain phenolic compounds such as simple phenolics or tannins, saponins or essential oils, which are said to improve some aspects of meat (Vista and Luciano, 2011). The antioxidative properties of flavonoids, carotenoids, essential oils and other plant substances fed to chickens may also affect the composition of fatty acids in tissue lipids and oxidative stability of meat (Koreleski and Swiatkiewicz, 2006), they are also known of reducing the changes in meat quality during frozen storage (see chapter 3). In monogastric animals saponins are reported to be bound to cholesterol, thus hampering its absorption in the intestine (Sidhu and Dakenfull,1986). According to Brogna et al. (2011), dietary saponins could reduce the accumulation of cholesterol in meat. Condensed tannins have also been reported to have a positive influence on meat fatty acids composition (Min et al., 2005; Vasta et al, 2009). However, most studies on fatty acids have been conducted on the ability of a lipid-rich plant extract to enhance levels of PUFA in meat (Kim et al., 2008).

Therefore, manipulation of fatty acid composition by introducing higher tissue concentrations of n-3 PUFA (optimum ratio of n-6/n-3) can be advantageous to human health and its supplementation can be successfully implemented in broiler diets through scientifically managed programmes (Malan, 2003). The addition of antioxidants such as vitamin E, improves oxidative stability and delays the development of rancidity. Therefore, manipulation of dietary additives to improve fatty acid of meat is of importance. This will reduce negative effects caused by feedstuffs such as fish flavour effects and excessive carcass fatness that can be perceived negatively by consumers (Malan, 2003). The objective of this study therefore, is to determine the potential of Moringa oleifera leaf meal as an additive on fatty acids profile and lipid oxidation of broiler meat.
5.2. Materials and methods

5.2.1. Study site and management of broiler chickens
The study site and experimental procedures are described in chapter 3, Sections 3.2.1 and 3.2.2.

5.2.2. Determination of fatty acid content
After slaughtering, plucking, evisceration and dressing of the broilers, the carcasses were stored at 4°C overnight. On the following day 6 carcasses per treatment were randomly selected for meat quality measurements. The left breasts were deboned, the skins removed and cut into halves longitudinally for 7 day shelf-life trial. Each day drip loss, colour and pH measurements were measured and sub samples were kept at -18°C for thiobarbituric acid reactive substances determination. On day 1 and day 7 sub-sample were also kept, wrapped in tinfoil and snap frozen (-18°C) for fatty acids analysis. After seven days, the samples were then removed from the freezer and allowed to defrost. After defrosting, 2 g meat sample was extracted with a chloroform:methanol (2:1; v/v) solution according to a modified method of Folch, Lees, and Stanley (1957). All the extraction solvents contained 0.01% butylated hydroxytoluene (BHT) as an antioxidant.

A polytron mixer (WiggenHauser, D-500 Homogenizer) was used to homogenise the sample with the extraction solvent. Heptadecanoic acid (C17:0) was used as an internal standard (catalogue number H3500, Sigma–Aldrich Inc., 3050 Spruce Street, St. Louis, MO 63103, USA) to quantify the individual fatty acids. A sub-sample of the extracted lipids was transmethylated for 2 hrs at 70 °C using a methanol/sulphuric acid (19:1; v/v) solution as transmethylating agent. After cooling to room temperature, the resulting fatty acid methyl esters (FAMEs) were extracted with water and hexane. The top hexane phase was transferred
to a spotting tube and dried under nitrogen (Tichelaar, Smuts, Van Stuijvenberg, Faber, and Benade.1998).

Analysis was done on a Thermo Focus GC equipped with a flame ionized detector using a BPX70 capillary column (60 m x 0.25 mm internal diameter, 0.25 μm film, SGE [SGE International Pty Ltd, 7 Argent Place, Ringwood, Victoria 3134] Australia). Gas flow rates were 30ml/min for the hydrogen carrier gas. Temperature programming was linear at 7 °C/min, with an initial temperature of 60 °C, a final temperature of 160 °C, an injector temperature of 220 °C and a detector temperature of 260 °C. The FAMEs were identified by comparing the retention times to those of a standard FAME mixture (Supelco™ 37 Component FAME Mix, 10 mg/ml in CH₂Cl₂, Catalogue Number 47885-U. Supelco, North Harrison Road, Bellefonte, PA 16823-0048, USA). The following indexes, useful for evaluating nutritional quality and healthiness of lipid profile, 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 (P/S) and n-6/n-3 ratio. (Thermo: Thermo Electron S.p.A, Strada Rivoltana, 20090 Rodana, Milan, Italy).

5.2.3. Estimation of lipid peroxidation
Lipid peroxidation was estimated in terms of thiobarbituric acid reactive species (TBARS) using Malondialdehyde (MDA) as standard. The homogenized breast tissue (0.1ml) was treated with 2 ml of (1:1:1 ratio) TBATCA-HCl reagent (thiobarbituric acid 0.37%, 15% trichloroacetic acid and 0.25 N HCl). All the tubes were placed in a boiling water bath for 30 minutes and allowed to cool. The amount of malonaldehyde (MDA) formed in each of the samples was assessed by measuring the optical density of supernatant at 535 nm using a spectrophotometer against a reagent blank. Percentage inhibition was calculated using the equation:

\[
\text{Percentage Inhibition} = \frac{\text{OD of blank} - \text{OD of sample}}{\text{OD of blank}} \times 100
\]
% of lipid oxidation Inhibition = \{Ao - A1\}/ A0*100

Where; Ao is the absorbance of the control and A1 is the absorbance of the sample extract.

5.3. Statistical analysis
The fatty acids were analyzed using one-way analysis of variance (ANOVA) in SAS 2003. Where there was a significant F-test ($P<0.05$), the least significant difference (LSD) method was used to separate the means. The statistical model that was used to check the effect of treatment on fatty acid profile of meat is,

\[ Y_{ij} = \mu + \alpha_i + \beta_{ij} + E_{ij} \]

Where,

- $Y_{ij}$ = response variables
- $\mu$ = overall mean
- $\alpha_i$ = effect of treatments and Days
- $\beta_{ij}$ = effect of Days
- $E_{ij}$ = random error

The lipid oxidation was analysed using the Proc GLM of SAS (2006). Comparisons of means were analysed using the Tukey’s HSD procedure in SAS (2006). Statistical model used was:

\[ Y_{ij} = \mu + \alpha_i + E_{ij} \]

where;

- $Y_{ij}$ = response variable
- $\mu$ = overall mean
- $\alpha_i$ = effect of treatments (TRT1, TRT2, TRT3, TRT4) and Days(1-7)
- $E_{ij}$ = random error
5.4. Results and Discussion

Table 5.1. presents the effects of *M. oleifera* leaf meal on fatty acid profile of broiler chicken meat. The TRTS had an effect (P<0.05) on C16:00, C18n6t, C18n9t, C20:1c11 fatty acids with TRT1(1000g/ton MOLM) having the highest proportions (Table 5.1). The treatments had an effect (P<0.05) on PUFA with chicken breast from treatment 4 having the highest proportions of PUFA (30.3±1.87). Tannins contained in *Moringa oleifera* leaf meal could also be the reason hence they are reported to have a positive effect on meat fatty acid composition (Min *et al*., 2005; Vasta *et al*., 2009b). The TRTS had an effect on SFA with treatment 1 having highest proportion of SFA. The treatments had no effect (P>0.05) on n-6/n-3 ratio, however in all the TRTS the n-6/n-3 values were higher than the recommended value of <4.0% (British Department of Health, 1994). However, TRTS had an effect on PUFA/SFA ratio with meat from treatment 1 having a favourable balance (0.3 ± 0.08) which is close to the recommended ratio 0.4 (Wood *et al*., 2003).
Table 5.1: Effect of Moringa oleifera leaf meal as an additive on fatty acid profile of chicken meat

<table>
<thead>
<tr>
<th>Fatty Acids</th>
<th>TRT1 (1000g/tonMOLM)</th>
<th>TRT2 (750g/tonMOLM)</th>
<th>TRT3 (500g/tonMOLM)</th>
<th>TRT4 (0MOLM)</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>C14:0</td>
<td>0.6±0.10</td>
<td>0.6±0.08</td>
<td>0.5±0.08</td>
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<tr>
<td>C14:1</td>
<td>0.1±0.02</td>
<td>0.1±0.02</td>
<td>0.1±0.02</td>
<td>0.1±0.02</td>
<td>NS</td>
</tr>
<tr>
<td>C15:0</td>
<td>0.2±0.08</td>
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<td>0.1±0.06</td>
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<td>NS</td>
</tr>
<tr>
<td>C16:0</td>
<td>40.5±2.70</td>
<td>23.8±2.28</td>
<td>25.0±2.28</td>
<td>23.6±2.28</td>
<td>*</td>
</tr>
<tr>
<td>C16:1c9</td>
<td>1.6±0.59</td>
<td>3.7±0.50</td>
<td>3.3±0.50</td>
<td>3.2±0.50</td>
<td>NS</td>
</tr>
<tr>
<td>C18:0</td>
<td>18.0±1.95</td>
<td>10.9±1.64</td>
<td>12.4±1.64</td>
<td>12.4±1.64</td>
<td>NS</td>
</tr>
<tr>
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<td>0.4±0.02</td>
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</tr>
<tr>
<td>C18n6</td>
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</tr>
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<tr>
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<td>0.3±0.05</td>
<td>0.4±0.04</td>
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<tr>
<td>C20t</td>
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<tr>
<td>C210</td>
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<tr>
<td>C220</td>
<td>1.1±0.39</td>
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</tr>
<tr>
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<td>0.1±0.02</td>
<td>0.1±0.02</td>
<td>0.1±0.02</td>
<td>NS</td>
</tr>
<tr>
<td>MUFA</td>
<td>20.5±3.09</td>
<td>33.9±2.60</td>
<td>30.8±2.60</td>
<td>30.5±2.60</td>
<td>NS</td>
</tr>
<tr>
<td>PS</td>
<td>0.3±0.08</td>
<td>0.8±0.07</td>
<td>0.7±0.07</td>
<td>0.8±0.07</td>
<td>*</td>
</tr>
<tr>
<td>PUFA</td>
<td>15.6±2.23</td>
<td>28.1±1.87</td>
<td>28.3±1.87</td>
<td>30.3±1.87</td>
<td>*</td>
</tr>
<tr>
<td>SFA</td>
<td>60.9±4.30</td>
<td>37.5±3.62</td>
<td>40.6±3.62</td>
<td>39.0±3.62</td>
<td>*</td>
</tr>
<tr>
<td>n-6/n-3</td>
<td>5.4±1.70</td>
<td>9.6±1.43</td>
<td>8.2±1.43</td>
<td>8.5±1.43</td>
<td>NS</td>
</tr>
</tbody>
</table>

**NS** Means in the same row with similar superscripts are not significantly different (P > 0.05)

MUFA=Total mono-unsaturated fatty acids

PUFA=Total poly-unsaturated fatty acids

SFA=Total saturated fatty acids

P:S=PUFA/SFA ratio

n-6/n-3=Total omega-6 and omega-3 fatty acids
Cold storage duration in Day 1 and Day 7 on C18n6t and C18n9t, with Day7 having the highest proportion (Table 5.2). Days of storage had no significant effect on PUFA, SFA, PUFA:SFA and n-6/n-3 ratio. Coetzee and Hoffman (2001) also did not find any changes of fatty acids proportions over storage time of meat from chickens fed diets with 120-200mg vitamin E. Koreleski and Swiatkiewicz (2007) also observed some differences in fatty acids profile of breast lipids in the initial month and after six months of cold storage on their study. They reported that the reason could likely be a result of changes in meat fat content, direction of oxidation and degree of fatty acids saturation or desaturation when meat was stored unfrozen and prepared for analyses.
Table 5.2: Effect of days on fatty acids profiles of chicken meat
<table>
<thead>
<tr>
<th>Fatty Acids</th>
<th>Day 1</th>
<th>Day 7</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>C140</td>
<td>0.5±0.06</td>
<td>0.5±0.07</td>
<td>NS</td>
</tr>
<tr>
<td>C141</td>
<td>0.1±0.01</td>
<td>0.1±0.01</td>
<td>NS</td>
</tr>
<tr>
<td>C150</td>
<td>0.1±0.04</td>
<td>0.1±0.05</td>
<td>NS</td>
</tr>
<tr>
<td>C160</td>
<td>25.9±1.51</td>
<td>31.5±1.94</td>
<td>NS</td>
</tr>
<tr>
<td>C161</td>
<td>3.1±0.3</td>
<td>2.7±0.4</td>
<td>NS</td>
</tr>
<tr>
<td>C180</td>
<td>12.8±1.09</td>
<td>14.4±1.40</td>
<td>NS</td>
</tr>
<tr>
<td>C18n3</td>
<td>0.4±0.02</td>
<td>0.3±0.02</td>
<td>NS</td>
</tr>
<tr>
<td>C18n6</td>
<td>0.4±0.03</td>
<td>0.4±0.04</td>
<td>NS</td>
</tr>
<tr>
<td>C18n6c</td>
<td>18.5±1.08</td>
<td>15.8±1.40</td>
<td>NS</td>
</tr>
<tr>
<td>C18n6t</td>
<td>0.1b±0.02</td>
<td>0.2a±0.02</td>
<td>*</td>
</tr>
<tr>
<td>C18n9c</td>
<td>26.7±1.46</td>
<td>23.8±1.88</td>
<td>NS</td>
</tr>
<tr>
<td>C18n9t</td>
<td>0.1b±0.02</td>
<td>0.2b±0.02</td>
<td>*</td>
</tr>
<tr>
<td>C200</td>
<td>0.4±0.03</td>
<td>0.4±0.3</td>
<td>NS</td>
</tr>
<tr>
<td>C201</td>
<td>0.1±0.01</td>
<td>0.1±0.01</td>
<td>NS</td>
</tr>
<tr>
<td>C202</td>
<td>0.6±0.05</td>
<td>0.5±0.07</td>
<td>NS</td>
</tr>
<tr>
<td>C20n3</td>
<td>0.13a±0.03</td>
<td>0.2b±0.04</td>
<td>*</td>
</tr>
<tr>
<td>C20n6</td>
<td>4.6±0.47</td>
<td>3.6±0.61</td>
<td>NS</td>
</tr>
<tr>
<td>C210</td>
<td>0.1±0.01</td>
<td>0.1±0.01</td>
<td>NS</td>
</tr>
<tr>
<td>C220</td>
<td>2.0±0.22</td>
<td>1.4±0.28</td>
<td>NS</td>
</tr>
<tr>
<td>C222</td>
<td>0.1±0.01</td>
<td>0.1±0.01</td>
<td>NS</td>
</tr>
<tr>
<td>C22n3</td>
<td>0.8±0.10</td>
<td>0.5±0.13</td>
<td>NS</td>
</tr>
<tr>
<td>C22n9</td>
<td>0.1±0.02</td>
<td>0.1±0.03</td>
<td>NS</td>
</tr>
<tr>
<td>C241</td>
<td>0.1±0.01</td>
<td>0.2±0.02</td>
<td>NS</td>
</tr>
<tr>
<td>MUFA</td>
<td>30.3±1.72</td>
<td>26.9±2.22</td>
<td>NS</td>
</tr>
<tr>
<td>P:S</td>
<td>0.7±0.04</td>
<td>0.6±0.06</td>
<td>NS</td>
</tr>
<tr>
<td>PUFA</td>
<td>27.3±1.24</td>
<td>23.2±1.60</td>
<td>NS</td>
</tr>
<tr>
<td>SFA</td>
<td>41.8±2.39</td>
<td>48.3±3.09</td>
<td>NS</td>
</tr>
<tr>
<td>n63</td>
<td>8.1±0.95</td>
<td>7.7±1.22</td>
<td>NS</td>
</tr>
</tbody>
</table>

**Means in the same row with similar superscripts are not significantly different (P >0.05) from each other.**

MUFA=Total mono-unsaturated fatty acids

PUFA=Total poly-unsaturated

SFA=Total saturated fatty acids

P:S=PUFA/SFA ratio

n-6/n-3=Total omega-6 and omega-3 fatty acids

*=P<0.05

±SE=standard error
The TRTS had a significant effect on lipid oxidation with treatment 1 having the highest values (Table 5.3). All the TRTS with MOLM were effective than treatment 4 (0g/ton MOLM) hence it had low amount of malondialdehyde (MDA) (P>0.05). These observation are similar with the ones by Botsoglou et al. (2002) and Lopez-Bote et al. (1998), whereby they investigated the effect of adding rosemary and sage extracts, and vitamin E to the broiler dietS on the meat and found a significant decrease in oxidation levels of the white muscle. Also, it has been reported that Vitamin E has a potential to reduce or prevent lipid oxidation in broiler meat during storage (De Winne and Dirinck, 1996). Therefore, the effectiveness of treatment1(1000g/ton MOLM) on MDA could be due to the antioxidant factors contained in Moringa such as vitamin E and selenium, phenols, flavonoids, vitamin C (Lahucky et al., 2010; Middleton et al., 2000; Moyo et al., 2012). The increase of unsaturated fatty acids levels in treatment 2 (Table 5.1) could be the reason fatty acids makes meat prone to lipid oxidation (Descalzo et al., 2007). However, high levels of PUFA in meat and meat products have been reported to present a challenge for the food industry to maintain lipid oxidation stability during a prolonged storage time (Narciso et al. 2011).
Table 5.3: Means (±SE) for average TBARS (mg MDA/kg meat) of chicken breasts as affected by treatments

<table>
<thead>
<tr>
<th>Treatments</th>
<th>TBARS (mg MDA/kg meat)</th>
<th>S.E</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 (1000g/ton MOLM)</td>
<td>0.37&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.06</td>
</tr>
<tr>
<td>2 (750g/ton MOLM)</td>
<td>0.15&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>0.06</td>
</tr>
<tr>
<td>3 (50g/ton MOLM)</td>
<td>0.25&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>0.06</td>
</tr>
<tr>
<td>4 (0g/ton MOLM)</td>
<td>0.12&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.06</td>
</tr>
</tbody>
</table>

<sup>a,b</sup> Means in the same column with similar superscripts are not significantly different (P > 0.05) from each other.

As expected, storage time had a significant effect on MDA levels, except for Day1 and Day7 (P > 0.05) with Day2 (0.73±0.08) having the highest amount of MDA (Table 5.4). These observations are similar with the ones by Luna et al. (2010), that showed that storage for 5 or 10 days significantly increased levels of MDA in broiler meat samples. Valesco and Williams (2011) reported that some plant extracts have a positive effect on lipid oxidation by reducing 2-thiobarbituric acid (TBAS) or malondialdehyde (MDA) formation on different types of meats during refrigeration storage.
Table 5.4: Means (±SE) for average TBARS (mg MDA/kg meat) of chicken breasts as affected by days

<table>
<thead>
<tr>
<th>Days</th>
<th>TBARS (mg MDA/kg meat)</th>
<th>S.E</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.03&lt;sup&gt;de&lt;/sup&gt;</td>
<td>0.08</td>
</tr>
<tr>
<td>2</td>
<td>0.73&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.08</td>
</tr>
<tr>
<td>3</td>
<td>0.26&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>0.08</td>
</tr>
<tr>
<td>4</td>
<td>0.70&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.08</td>
</tr>
<tr>
<td>5</td>
<td>0.04&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0.08</td>
</tr>
<tr>
<td>6</td>
<td>0.38&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>0.08</td>
</tr>
<tr>
<td>7</td>
<td>0.02&lt;sup&gt;e&lt;/sup&gt;</td>
<td>0.08</td>
</tr>
</tbody>
</table>

<sup>abc</sup>Means in the same column with similar superscripts are not significantly different (P>0.05) from each other.

TBARS= thiobarbituric acid reactive substances
SE= standard error of means
5.5. Conclusion
The observations from this study had shown that *Moringa oleifera* leaf meal (MOLM) inclusions at different levels have partially improved fatty acid composition of the chicken meat. Its inclusion also had similar effectiveness to retard lipid oxidation, therefore it can be considered as a useful natural additive that can be applied in poultry diets to improve meat quality.
5.6. References


Chapter 6: General discussion, Conclusions and Recommendations

6.1. General discussion

The objective of the study was to determine the growth performance, physico-chemical indicators of shelf-life and fatty acids profiles, lipid oxidation in meat from broilers given *Moringa oleifera* leaf meal (MOLM) as an additive. Four hundred and thirty two 1-Day old male broiler chicks (Aviane 48) were used in this study and reared for 35 Days. The growth performance of broilers given diets containing different levels of MOLM was determined in Chapter 3. The effect of MOLM as an additive on physico-chemical shelf-life indicators (colour, pH and drip loss) of broiler meat was determined in Chapter 4. In Chapter 5 the effect of MOLM as an additive on fatty acids profiles and lipid oxidation in broiler meat was determined.

In Chapter 3, average feed intake (AFI), average daily gain (ADG), feed conversion efficiency (FCE), and carcass characteristics (slaughter weight, carcass weight, dressing percentage, gizzard weight, liver weight, heart weight and gastrointestinal fat weight) were determined. Broiler chickens given diet with higher inclusion (TRT1 1000g/ton) of MOLM had higher values of AFI, ADG and FCE throughout the experiment compared to the control diet. Also chickens given TRT1 had similar values of carcass characteristics with the chickens given a diet with no MOLM (control). However, the liver weight, heart weight and gastrointestinal fat differed in all treatments with TRT2 (750g/ton MOLM) having highest value of heart weight, gastro-intestinal fat weight. The chickens given diets with MOLM inclusion did not differ much on growth performance with the ones given a control diet. However, the MOLM inclusion 500g/ton-1000g/ton in the diets had no adverse effects on the performance of broilers. Similarly, Ayssiwede *et al.* (2011) reported that inclusions of *Moringa oleifera* leaf meal in the diets up to 24% had not caused any adverse impact on live
body weight, average daily weight gain, feed conversion rate, carcass and organ
ccharacteristics in birds compared to their controls.

Results from Chapter 4 showed that chicken meat from treatment 1 (1000g/ton MOLM) fed
broilers had higher values for lightness ($L^*$) which could be due to the antioxidant activities
in *M. oleifera*, such as Vitamin E and selenium (Moyo *et al.*, 2011). It is reported that vitamin
E supplementation prevents discolouration and extends colour display life (Faustman *et al*.,
1989; Arnold *et al*., 1992). The redness ($a^*$) values were also higher in the diet which had
MOLM inclusion treatment 3. The reason could be due to the iron consumed by the broilers
on the MOLM diet, which could have increased haemoglobin and myoglobin concentrations
(Priolo *et al*., 2001; Sreelatha and Padma, 2009). There were no differences in drip loss in all
the treatments.

The pH levels of the stored meat from all the TRTS were generally constant from day 1 to
day 5 and then peaked on day 6 with a decrease on day 7 except for treatment 2. The reason
for this is not clear, hence further investigation is needed. The lightness ($L^*$) were within a
normal range during storage, from day 1 to day 5 but then at the end of storage on day 6 they
started to decrease. Previous studies also observed a decrease in $L^*$ values towards the end of
the meat storage (Bingol & Ergun, 2011). Drip loss increased with storage time as expected.
Offer and Knight (1988), described the changes in pH and temperature post-mortem as the
principle mechanism of drip development during storage of meat.
Chapter 5 determined the effect of MOLM as an additive on fatty acids profiles and lipid oxidation of broiler meat. It was observed that all TRTS had an effect on PUFA, SFA, but no effect on n-6/n-3 ratio. It has been reported that the quality and composition of fatty acids in meat are related to the presence of some of their precursors in the diet (Wood et al., 2004). Day1 and day7 had no effect on PUFA, SFA and n-6/n-3 ratio. However, TRTS with MOLM had a positive effect on lipid oxidation, with higher inclusion of MOLM treatment 1 having higher amount of MDA than control diet which had the lowest value. From these results one can objectively conclude that using MOLM as an additive on broiler diets has a beneficial effect on the oxidative stability of broiler meat. The reason behind this could be the antioxidant factors in MOLM. Moringa leaves have been reported to have a high content of vitamin E, a chain breaking antioxidants (Jyotsna Misha et al., 2007) and antioxidants are reported to delay lipid peroxidation in meat (Dirinck et al., 1996).

6.2. Conclusion

The use of MOLM as an additive on broiler diets had no adverse effects on the growth performance of broilers and its potential as an antioxidant seemed to be effective in improving physico-chemical shelf-life indicators of the meat from broilers. Feeding broilers diets with different levels of MOLM retarded lipid oxidation of the chicken meat compared to the diet with no MOLM. It was therefore, concluded that MOLM can be used in broiler diets as an additive with a potential to substitute commercial additives usually added in broiler diets, which are quite expensive for communal farmers.

6.3. Recommendations

- Although observations from this study showed that MOLM have a potential to be utilised as an additive in broiler diets, further investigations at higher inclusion rates
of MOLM in broiler diets is required, hence some of the findings in this study were not quite clear.

- *Moringa oleifera* is a bit scarce in South Africa, especially in the Eastern Cape province. It is only available in few provinces which makes it difficult and quite expensive to get it. Therefore, it is recommended that more studies or experiments on *Moringa oleifera* should be conducted such as growing its trees in large numbers so that the farmers will be aware of how highly nutritive this tree is.

- Further research should also be done to determine the effect of using *Moringa oleifera* seeds or leaf meal as an additive (in other livestock diets) on the performance and meat quality. These researches should be conducted in communal areas, working with farmers, in that way they will gain more knowledge about *Moringa oleifera* and its properties.

### 6.4. References


Effect of long- or short term feeding of α-tocopherol acetate to Holstein and
crossbreed beef steers upon performance, carcass characteristics and beef colour

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