Antioxidant activity and the quality of meat from goats and broilers supplemented with Moringa (*Moringa oleifera*) leaves

By

Kumnandi Qwele

A dissertation submitted in fulfilment of the requirements for the degree of

Master of Science in Agriculture (Animal Science)

in the

Department of Livestock and Pasture Science

Faculty of Science and Agriculture

September 2011

Alice, South Africa

Supervised by:

Prof. V. Muchenje
Declaration

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

_________________________   __________________________

Kumnandi Qwele                  Date

Approved as to style and content by:

_________________________

Prof. V. Muchenje (Supervisor)

September 2010
Abstract

Antioxidant activity and the quality of meat from goats and broilers supplemented with Moringa (Moringa oleifera) leaves

By

Kumnandi Qwele

The objective of the study was to determine the antioxidant activity (AA) and the quality of meat from goats and chickens supplemented with Moringa (Moringa oleifera) leaves. For the first experimental chapter, eighteen 9 months old Xhosa lop-eared, castrated goats with an average body weight of 14 kg were used. There were three supplements namely, Moringa oleifera leaves, sunflower cake and grass hay. There were six goats per supplement. The goats were slaughtered after 60 days of supplementation and the Muscularis longissimus thoracis et lumborum (LTL) of the right side of each goat was used to determine fatty acid composition, total phenolic content (TPC), diphenylpicrylhydrazyl (DPPH) assay, 2, 2 azino-bis (3-ethylbenzothiazoline-6-sulphonic diammonium salt (ABTS) assay and reducing power assay of meat. Glutathione (GSH), catalase (CAT) and superoxide dismutase (SOD) and lipid peroxidation (thiobarbituric acid reactive substances, TBARS) were taken as parameters to evaluate in vivo AA of the meat. For the second experiment, twelve male broilers were used in four groups of dietary supplementation. Three broilers were allocated per group. After slaughter, the breast muscle was sampled for total phenols, flavonoids, proanthocyanidins, TPC, ABTS, GSH, CAT, SOD, lipid peroxidation, ultimate pH (pH24), colour (L* (lightness), a* (redness), b* (yellowness)), WB-shear force and cooking loss. In both chapters Moringa leaves exhibited the highest AA. Meat from goats supplement with Moringa exhibited the highest AA. Meat from broilers supplemented with a mixture of Moringa leaves, broiler finisher and crushed maize, had the highest (P < 0.05) pH24 and L* values. Moringa can therefore be used to preserve meat without changing the quality of meat.
Keywords: *Moringa oleifera*, Xhosa lop-eared goats, broilers, antioxidant potential, fatty acid profile, meat colour, ultimate pH
List of abbreviations

a* - Redness

AA - Antioxidant activity

ABTS - 2,2 azino-bis (3- ethylbenzothiazoline-6-sulphonic diammonium salt)

b* - Yellowness

BF - Broiler finisher

CAT - Catalase

CL - Cooking loss

CM - Crushed maize

CW - Carcass weight

DPPH - diphenylpicrylhydrazyl

GSH - Glutathione

L* - Lightness

M - Moringa oleifera

SAS - Statistical Analysis System

SLW - Slaughter weight

SOD - Superoxide dismutase

TBARS - Thiobarbituric acid reactive substances

WBSF - Warner - Bratzler Shear force

PUF - Polyunsaturated fatty acids

SFA- Saturated fatty acids

MUFA- Mono unsaturated fatty acids
# Table of Contents

Declaration.................................................................................................................. ii

Abstract......................................................................................................................... iii

List of abbreviations ..................................................................................................... v

List of Tables .................................................................................................................. viii

Acknowledgements ...................................................................................................... x

Chapter 1: Introduction ................................................................................................. 1
  1.1. Justification ........................................................................................................... 4
  1.2. Objectives ............................................................................................................ 5
  1.3. Hypothesis ........................................................................................................... 6
  1.4. References .......................................................................................................... 7

Chapter 2: Literature review ....................................................................................... 12
  2.1. Introduction .......................................................................................................... 12
  2.2. Antioxidants found in Moringa leaves ................................................................. 12
  2.3. Benefits of the leaves .......................................................................................... 13
    2.3.1. Feed supplement ............................................................................................ 13
    2.3.2. Nutritional composition of Moringa leaves .................................................. 14
    2.3.3. Feed supplement ............................................................................................ 14
    2.3.4. Fighting against cardiovascular diseases using Moringa .............................. 16
  2.6. Methods used to determine antioxidant activities .............................................. 17
    2.6.1. In vitro models for evaluating antioxidant activity ......................................... 17
    2.6.2. In vivo models for evaluating antioxidant activity .......................................... 20
  2.7. Meat quality characteristics as affected by supplements .................................... 24
    2.7.1. pH and the quality of meat ........................................................................... 25
    2.7.2. Meat colour .................................................................................................. 26
    2.7.3. Meat tenderness ........................................................................................... 27
    2.7.4. Cooking loss ................................................................................................. 29
  2.8. Fatty acids and the quality of meat ...................................................................... 29
  2.9. Summary of review .............................................................................................. 32
  2.10. References ......................................................................................................... 33

Chapter 3: Fatty acid composition and antioxidant activity of meat from goats supplemented with Moringa (*Moringa oleifera*) leaves, sunflower cake and grass hay .................................................. 46
  Abstract ....................................................................................................................... 46
  3.1. Introduction .......................................................................................................... 48
  3.2. Materials and methods ......................................................................................... 50
    3.2.1. Site description ............................................................................................... 50
    3.2.2. Meat samples ................................................................................................. 50
Chapter 5: General Discussion, Conclusions and Recommendations

5.1. General discussion ................................................................. 109
5.3. Recommendations .................................................................. 112
5.4. References ............................................................................. 113

Chapter 4: Effect of dietary mixtures of Moringa (Moringa oleifera) leaves, broiler finisher and crushed maize on antioxidative potential and physico-chemical characteristics of breast meat from broilers ........................................... 80

Abstract.......................................................................................... 80

4.1. Introduction ............................................................................. 82

4.2. Materials and Methods ............................................................ 84

4.2.1. Feeding Management of broilers .......................................... 84

4.2.2. Phenol content determination .............................................. 87

4.2.3. Total flavonoids ................................................................. 87

4.2.4. Total proanthocyanidins ..................................................... 87

4.2.5. DPPH radical scavenging activity ......................................... 88

4.2.6. ABTS+ reducing activity ..................................................... 88

4.2.7. Reducing power .................................................................. 88

4.2.8. Lipid peroxidation assay .................................................... 88

4.2.9. Glutathione (GSH) assay, Catalase (CAT) activity and Superoxide dismutase (SOD) assays ......................................................... 89

4.2.10. Broiler meat quality .......................................................... 89

4.3. Results and Discussion ............................................................ 92

4.3.1. Total Polyphenols ............................................................... 92

4.3.2. Antioxidative activity ........................................................ 94

4.3.3. Glutathione and enzymatic antioxidants ......................... 97

4.3.4. Carcass characteristics .................................................... 99

4.3.5. Physico - chemical quality characteristics ....................... 99

4.4. Conclusions ........................................................................... 102

4.5. References ............................................................................. 103

Chapter 3: Chemical quality characteristics of breast meat from broilers raised on Moringa leaves using different diet formulations ........................................... 74

3.1.4. Proximate analyses and cholesterol determination ...................... 54

3.2.4. Fatty acid profile determination ........................................... 55

3.2.5. Assaying methods ............................................................. 55

3.3. Statistical analysis .................................................................. 60

3.4. Results and Discussion ............................................................ 62

3.5. Conclusions ........................................................................... 73

3.7. References ............................................................................. 74

Chapter 2: Effect of dietary mixtures of Moringa (Moringa oleifera) leaves and crushed maize on chemical characteristics of breast meat from broilers .................. 47

2.1. Introduction ........................................................................... 47

2.2. Materials and Methods ............................................................ 47

2.3. Design of experiment .............................................................. 47

2.4. Results and Discussion ............................................................ 51

2.5. Conclusions ........................................................................... 52

2.7. References ............................................................................. 52
List of Tables
Table 2.1: Mean nutritional values of *M. oleifera* leaves. ................................................................. 15

Table 3.1: Nutritional composition of the experimental diets............................................................... 51

Table 3.2: Total polyphenolic contents of the leaf extracts of *M. oleifera*........................................... 52

Table 3.3: Diet composition of *M. oleifera* leaves, sunflower cake and grass hay with Least square means and standard error of means (s.e.m) of fatty acids composition (% total fatty cid)...................53

Table 3.4: Chemical composition and standard errors of *Muscularis longissimus thoracis et lumborum* muscle of goats supplemented with *Moringa oleifera* leaves, sunflower cake and grass hay diets.........................................................................................................................63

Table 3.5: Least square means and standard errors of fatty acid composition in percentage from the *longissimus thoracis et lumborum* meat from goats supplemented with *Moringa oleifera* leaves, sunflower cake and grass hay......................................................................................................................... 64

Table 3.6: Antioxidant potential (lsmeans ±se) of *Muscularis longissimus thoracis et Lumborum* meat from goats supplemented *Moringa oleifera* leaves, sunflower cake and grass hay..............66

Table 3.7: Activities of glutathione, enzymatic antioxidants and of lipid peroxidation (lsmeans ±se) of *Muscularis longissimus thoracis et lumborum* meat supplemented with *Moringa oleifera* leaves, sunflower cake and grass hay......................................................................................................................... 67

Table 4.1: Composition (%) and calculated chemical composition (%) of the control diet (broiler finisher).................................................................................................................................85

Table 4.2: Percentage composition of broiler finisher, crushed maize and *Moringa oleifera* leaves diet. ........................................................................................................................................86

Table 4.3: Polyphenol contents (mg gallic acid equivalent/g meat) of breast meat from broilers supplemented with broiler finisher, mixture of broiler finisher and crushed maize, *Moringa oleifera* leaves and broiler finisher, mixture of *Moringa leaves*, broiler finisher and crushed maize. .................................................................................. 93
Table 4. 4: Antioxidative potential of breast meat from broilers supplemented with broiler finisher, mixture of broiler finisher and crushed maize, *Moringa oleifera* leaves and broiler finisher, mixture of Moringa leaves, broiler finisher and crushed maize. ............................... 95

Table 4. 5: Activities of glutathione and enzymatic antioxidants of breast meat from broiler supplemented with broiler finisher, mixture of broiler finisher and crushed maize, *Moringa oleifera* leaves and broiler finisher, mixture of Moringa leaves, broiler finisher and crushed maize. ........................................................................................................................... 93

Table 4. 6: Eat quality characteristics, slaughter weight and carcass weight of broilers supplemented with broiler finisher, mixture of broiler finisher and crushed maize, *Moringa oleifera* leaves and broiler finisher, mixture of Moringa leaves, broiler finisher and crushed maize. .......................................................................................................................................... 100
Acknowledgements

I am obliged to give thanks to my Lord and personal savoir Jesus Christ for giving me strength to commence and complete this study. I would like to express my gratitude to my supervisor, Prof. V. Muchenje, whose help, stimulating suggestions and encouragement helped me in all the time of research and writing of this thesis. His ability to understand and work with people from diverse backgrounds is impressive. I would like to thank the staff at Fort Cox Agricultural College for allowing me to conduct my experiments using their facilities. Special thanks to Mr. Busani Moyo for his guidance, patience and ideas during this study. I have furthermore to thank Dr S.O. Oyedemi for assisting me all the way with my antioxidant experiments. I also want to thank Dr U. Marume and Mr Jubane for assisting me with data analysis.

I am deeply indebted to my family and good friends who have supported and encouraged me up to this stage of my studies. My colleagues, I want to thank them for their help, support and valuable hints.

Lastly, I am particularly grateful to my sponsors the National Research Foundation for their financial support during the study.
Chapter 1: Introduction

Free radicals are types of Reactive Oxygen Species (ROS), which include all highly reactive, oxygen-containing molecules such as the hydroxyl radical, the super oxide anion radical and hydrogen peroxide (Kohen and Gati, 2000). These molecules are continuously generated inside the animal body as a consequence of exposure to a plethora of exogenous chemicals in our ambient environment and/or a number of endogenous metabolic processes involving redox enzymes and bio-energetic electron transfer (Sreelatha and Padma, 2009). Under normal circumstances, the ROS generated are detoxified by the antioxidants present in the body and there is equilibrium between the ROS generated and the antioxidants present. However, owing to ROS overproduction and/or inadequate antioxidant defence, this equilibrium is hampered favouring the ROS upsurge that culminates in oxidative stress (Kohen and Gati, 2000). To protect the cells and organ systems of the body against reactive oxygen species, researchers have evolved a highly sophisticated and complex antioxidant protection system, that functions interactively and synergistically to neutralize free radicals. Thus, antioxidants are capable of stabilizing or deactivating free radicals before they attack cells (Beris, 1991).

Lipid peroxidation is a complex process occurring in aerobic cells and reflects the interaction between molecular oxygen and polyunsaturated fatty acids via a free radical chain mechanism, forming fatty acyl hydroperoxides, generally called peroxides or primary products of oxidation (Rasooli, 2007). The primary auto-oxidation is followed by a number of secondary reactions which lead to degradation of lipids and the development of oxidative rancidity (Ladikos and Lougovois, 1990). Lipid peroxidation is one of the primary causes of quality deterioration in meat and meat products, as it largely contributes to colour and flavour
deterioration, loss of nutritional value and safety, and generates compounds that may be detrimental to consumers (Min et al., 2008). Antioxidants act as radical-scavengers, and inhibit lipid peroxidation and other free radical-mediated processes: therefore, they are able to protect consumers from several diseases attributed to the reactions of radicals (Kumaran and Karunakaran, 2007). Use of synthetic antioxidants to prevent free radical damage has been reported to involve toxic side effects (Moure et al., 2001), making attractive the search for natural antioxidants and scavenger compounds. On the other hand, herb extracts have an advantage of being more acceptable by consumers and have legal requirements for market access as they are considered non or less toxic (Rasooli, 2007).

All meat is prone to oxidation. Amongst meat products, poultry meat is considered to be more prone to the development of oxidative rancidity compared to red meat (Ali and Zahran, 2010). This is due to higher content of phospholipids in poultry meat. Phospholipids are located in the membrane structure and are rich in poly-unsaturated fatty acids. It has been demonstrated that the oxidation of meat starts by a peroxidation of the phospholipid fraction (Fennema, 2008). Due to the high degree of poly-unsaturated lipids the phospholipids are most prone to oxidation. Unsaturation of fatty acids makes lipids susceptible to oxygen attack with negative implications on meat quality and consumer health due to lipid peroxidation (Kemin, 2009). However, autoxidation of lipids and the production of free radicals are natural processes occurring in meat biological systems leading to oxidative deterioration, drip loss (Jensen, 1998; Weber 2001), colour changes and off-flavours development. The formation of volatile lipid oxidation products strongly reduces the consumers’ acceptability of the product (Ladikos and Lougovois, 1990).
Antioxidants are critical for maintaining optimal cellular and systemic health and well-being. They are nutrients in foods which can prevent or slow the oxidative damage to our bodies (Tsang, 2007). These nutrients may protect cells against the effects of free radicals which have been reported to be responsible for tissue damage and loss of function in a number of tissues and organs (Zheng and Huang, 2001). Antioxidants help prevent molecular damage caused by free radicals through oxidation; this protection may help fend off many diseases including cancer, cardiovascular diseases, and muscular degeneration (Islam et al., 2002).

Numerous methods are used to evaluate antioxidant activities of natural compounds in foods or biological systems with varying results. Two free radicals that are commonly used to assess antioxidant activity in vitro are 2,2′-azinobis (3-ethylbenzothiazoline- 6-sulfonic acid) (ABTS+) and 2,2-diphenyl-1-picrylhydrazyl (DPPH). However, both of these radicals are foreign to biological systems (Miller and Rice-Evans, 1997). There are other methods which determine the resistance of lipid or lipid emulsions to oxidation in the presence of the antioxidant being tested. The malondialdehyde (MDA) or thiobarbituric acid-reactive substances (TBARS) (Miller et al., 2000) assays have been used extensively to estimate the peroxidation of lipids in membrane and biological systems. There are also in vivo methods which are used to determine enzymatic antioxidant activities, such as lipid peroxidation, glutathione, catalase and superoxide dismutase.

Various studies have shown that a number of plant products including polyphenolic substances and various plant or herb extracts exert potent antioxidant actions (Krishnaraju et al., 2009; Craig, 1999; Kaˇhkoˇnen et al., 2000). The natural antioxidants of spices and herbs are generally classified as vitamins, phenols including flavanoids and phenolic acids, and volatile compounds (Sreelatha and Padma, 2009; Wojdylo et al., 2007; Joshi et al., 2008). In
recent years, there has been increasing attention in the search of natural antioxidants from plants because they can protect the human body from the attack of free radicals, and retard the progress of many chronic diseases as well as retarding the lipid-oxidative rancidity in foods. Crude extracts of herbs and spices, and other plant materials rich in phenolics are of increasing interest in the food industry because they retard oxidative degradation of lipids and thereby improve the quality and nutritional value of food (Wojdyło et al., 2007). 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).

*Moringa oleifera* Lam. (Drumstick) of the family Moringaceae has been used as an herbal medicine in treating a wide variety of diseases in India (Fahey, 2005). Leaves of the plant are the main source of drug preparation. A number of variables including vitamins and amino acids (Nambiar and Seshadri, 2001; Makkar and Becker, 1996) have been identified as contributing to the observed medicinal effects of the plant. Leaves of this plant are traditionally known for or reported to have various biological activities, including cardiovascular action, liver disease (Kumar and Pari, 2003) and hypocholesterolemic agent (Faizi et al., 1995). The Moringa plant provides a rich and rare combination of zeatin, quercetin, kaempferom and many other phytochemicals (Kumar and Pari, 2003). Other important medicinal properties of the leaves include antioxidant, hepatoprotective, antibacterial and antifungal activities (Bukar et al., 2010).

1.1. Justification

Oxidative damage is associated with free radical formation and oxidative stress causes health deterioration. Apart from health deterioration, lipid peroxidation is also a major cause of meat
quality deterioration, affecting colour, flavour, texture, and nutritional value and hence affecting the consumers’ acceptance of meat (Giannenas et al., 2009). Synthetic antioxidants such as butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA) and tertiary butyl hydroquinone (TBHQ) have been widely used by the food industry because they are both powerful and inexpensive. However, BHA (Sherwin, 1990) and BHT (Sun and Fukuhara, 1997) are suspected carcinogens, and consumers require meat that is said to be preservative-free, treated with no chemicals during meat preservation (Fasseas et al., 2007).

This has led to food industries to focus their attention on the use of extracts from herbs and spices as natural antioxidants to improve sensory characteristics and extend the shelf life of food products (Arora and Kaur, 1999). Natural antioxidants such as herb extracts, tocopherols (vitamin E) and ascorbic acid (vitamin C) (Panagiotis et al., 2010) have an advantage of being more acceptable by consumers and have no legal requirements for market access as they are considered non or less toxic. Oxidative quality deterioration in meat can be reduced by the use of organic or natural antioxidants such as M. oleifera. Moringa oleifera has been revealed to be a source of phytochemicals such as carotenoids, vitamins, minerals, amino acids, sterols, glycosides, alkaloids, flavonoids (Iqbal and Bhanger, 2005) and phenolics (Makkar and Becker, 1996; Sidduraju and Becker, 2003; Anwar et al., 2007)

1.2. Objectives

The objectives of the study were to determine:

1. The antioxidant activity of meat from goats supplemented with Moringa (Moringa oleifera) leaves
2. The effect of dietary mixture of Moringa (*Moringa oleifera*) leaves, broiler finisher and crushed maize on antioxidative potential and physico-chemical characteristics of breast meat from broilers

1.3. Hypothesis

The hypothesis tested:

1. Supplementary feeding with Moringa (*Moringa oleifera*) has no effect on antioxidative activity of goat meat

2. Supplementary feeding with Moringa (*Moringa oleifera*) has no effect on antioxidative activity and physico-chemical characteristics of breast meat from broilers
1.4. References


Chapter 2: Literature review

2.1. Introduction

Historically, plants have provided a source of inspiration for novel drug compounds, as plant derived medicines have made large contributions to human health and well-being. Their role in the development of new drugs is two-fold: they may become the base for the development of a medicine, a natural blueprint for the development of new drugs, or; a phytomedicine to be used for the treatment of disease (Iwu et al., 1999). Moringa has been reported to be a valuable component in human and animal feed due to its adequate amino acid profile and crude protein content, its high level of vitamin A, E and its low level of anti-nutritional compounds (Yang et al., 2006). According to Fahey (2005) all parts of the Moringa tree are edible and have long been consumed by humans. However, the leaves and seeds are mostly used for animal feed.

2.2. Antioxidants found in Moringa leaves

Moringa has a host of antioxidants, in combinations, which are highly beneficial and easily absorbed such as Vitamin A, Vitamin C, Vitamin E, Vitamin K, Vitamin B (Choline), Vitamin B1 (Thiamin), Vitamin B2 (Riboflavin), Vitamin B3 (Niacin), Vitamin B6, Alanine, Alpha-Carotene, Arginine, Beta-Carotene, Beta-sitosterol, Caffeoylquinic Acid, Campesterol, Carotenoids, Chlorophyll, Chromium, Delta-5-Avenasterol, Delta-7-Avenasterol, Glutathione, Histidine, Indole Acetic Acid, Indoleacetonitrile, Kaempferal, Leucine, Lutein, Methionine, Myristic-Acid, Palmitic-Acid, Prolamine, Proline, Quercetin, Rutin, Selenium, Threonine, Tryptophan, Xanthins, Xanthophyll, Zeatin, Zeaxanthin, Zinc. A few important antioxidant enzymes the body naturally produces are superoxide dismutase (SOD), catalase and glutathione peroxidase. However, in order for the body to produce these it requires zinc, manganese, copper and selenium (Group, 2009).
2.3. Benefits of the leaves

2.3.1. Feed supplement

*Moringa oleifera* is a potential alternative feed resource. Reports from other tropical countries around the world have shown very encouraging biomass yields (Yang *et al.*, 2006). More recently, under high density cultivation, biomass yields in excess of 15 tonnes DM/ha in a 60-day growing cycle has been obtained at the International Trypanotolerance Centre, Banjul (Akinbamijo *et al.*, 2006). This volume of high quality biomass is simply high given the context of the semi arid ecologies in the Gambia. This is of particular interest to animal nutrition where dietary protein sources are becoming increasingly expensive and difficult to access (Akinbamijo *et al.*, 2006).

Moringa can be used as a feed supplements during the six months of dry period of each year in order to satisfy the nutritional requirements of animals. Fresh leaves were found to contain 23 % crude protein (CP) in dry matter (DM), 12.3 MJ ME/kg DM and had an *in vitro* DM digestibility of 79.7% (Becker, 1995) and Moringa can thus be a valuable supplement for animals. Moringa leaves, petioles and young stems have a slightly lower protein content than other fodder trees such as the *Gliricidia sepium* (26 %) and *Leucaena leucocephala* (25 %) (Becker, 1995). However, the CP of Moringa is of better quality for ruminants than the CP of leaves of Gliricidia or Leucaena because of its high content of bypass protein, 47 % versus 30 % and 41 % respectively (Becker, 1995). Moringa is also rich in carotene, ascorbic acid, iron and in the two amino acids, methionine and cystine generally deficient in other feeds (Makkar and Becker, 1996).

In one study, goats supplemented with Moringa had an average body weight gain of 78 g/day while non supplemented goats gained only 55 g/day (Aregheore, 2002). Dairy cows that were fed with *Hyparrenia rufa* grass and sorghum straw supplemented with different levels of
Moringa leaves readily accepted the diet and Moringa did not seem to have any toxic effect or contain any factors limiting intake (Aregheore, 2002). Supplementation with Moringa leaves at a level of 0.3 % of BW resulted in a milk yield of 5.7 kg cow per day, and this was 13 % higher than the yield of cows that were only grazing (Rocha and Mendieta, 1998).

2.3.2. Nutritional composition of Moringa leaves

The nutritional composition of Moringa leaves is summarized in Table 2.1
Table 2.1: Mean nutritional values of *M. oleifera* leaves.

<table>
<thead>
<tr>
<th>Nutrient</th>
<th>Mean value for 100g Dry Matter</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vitamin C* (mg)</td>
<td>773 ± 91</td>
</tr>
<tr>
<td>Vitamin A (IU)</td>
<td>15620 ± 6475</td>
</tr>
<tr>
<td>Zinc (mg)</td>
<td>2.5 ± 0.6</td>
</tr>
<tr>
<td>Manganese (mg)</td>
<td>8.4 ± 2.4</td>
</tr>
<tr>
<td>Phosphorus (mg)</td>
<td>267 ± 49</td>
</tr>
<tr>
<td>Magnesium (mg)</td>
<td>422 ± 52</td>
</tr>
<tr>
<td>Potassium (mg)</td>
<td>1384 ± 420</td>
</tr>
<tr>
<td>Iron (mg)</td>
<td>28 ± 6</td>
</tr>
<tr>
<td>Copper (mg)</td>
<td>1.0 ± 0.2</td>
</tr>
<tr>
<td>Calcium (mg)</td>
<td>1924 ± 288</td>
</tr>
<tr>
<td>Fibers (g)</td>
<td>10 ± 3</td>
</tr>
<tr>
<td>Carbohydrates (g)</td>
<td>38 ± 7</td>
</tr>
<tr>
<td>Fat (g)</td>
<td>8 ± 2.5</td>
</tr>
<tr>
<td>Minerals (g)</td>
<td>11 ± 2.2</td>
</tr>
<tr>
<td>Proteins (g)</td>
<td>29 ± 6</td>
</tr>
</tbody>
</table>

(Brion, 2011)
2.3.4. Fighting against cardiovascular diseases using Moringa

Granato (2003) reported that sufficient intake of dietary antioxidant found in Moringa leaves may help in the prevention of cardiovascular diseases. It is further indicated in the study that the aim of nutritional components and antioxidants is to eliminate cellular waste products that build up in the bloodstream. The antioxidants, found in Moringa leaves, quench free radicals by donating an electron and stabilizing the compound. Polyphenols, Flavones and carotenoids, which are found in the leaves, have been studied for their abilities to prevent lipid peroxidation, inhibiting the development of atherosclerosis and lowering blood pressure.

Beta-carotene as an antioxidant is a highly effective quencher of singlet oxygen and a direct scavenger of free radicals (Gaby and Singh, 1991). Also as reported by Hennekens (1992), it may offer some protection against the oxidative damage associated with low density lipoproteins (LDL), which transport cholesterol through the arteries and contribute to blocked vessels. Yet another way in which polyphenols help to prevent atherosclerosis is by boosting the activity of vitamin C, which in turn increases the levels of vitamin E. This synergy increases the overall resistance to oxidative stress (Very Berry- and Grape too, 2001).

According to Null (2004), a single molecule of beta-carotene can arrest up to 1,000 molecules of singlet oxygen. Some of the best-known sources of beta-carotene antioxidants are Moringa leaves.

These antioxidants found in Moringa leaves aid during digestion, inhibiting some of the oxidation of fats in gastric fluid (Granato, 2003). Furthermore, beta-carotene survives the process of absorbing singlet oxygen intact.
2.6. Methods used to determine antioxidant activities

2.6.1. In vitro models for evaluating antioxidant activity

2.6.1.1. ABTS 2,2-azinobis(3-ethyl benzothiazoline-6-sulfonicacid) diamonium salt

Method

This is a measure of antioxidant activity as opposed to antioxidant concentration which might include a proportion of biologically inactive antioxidants. It also permits the measurement of antioxidant activity of mixtures of substances and hence helps to distinguish between additive and synergistic effects. Also, the antioxidant activity of wines is measured by using this method. The assay is based on interaction between antioxidant and ABTS\(^+\) radical cation which has a characteristic colour showing maxima at 645, 734 and 815nm (Rice-Evans and Miller, 1994; Kanner et al, 1994; Simonetti et al., 1997; Vinson and Hontz, 1995).

The ABTS assay measures the relative ability of antioxidant to scavenge the ABTS\(^+\) generated in aqueous phase, as compared with a Trolox (water soluble vitamin E analogue) standard. The ABTS\(^+\) is generated by reacting a strong oxidizing agent (for example, potassium permanganate or potassium persulfate) with the ABTS salt. The reduction of the blue-green ABTS\(^+\) radical by hydrogen-donating antioxidant is measured by the suppression of its characteristic long wave absorption spectrum (Miller and Rice-Evans, 1997). The method is usually expressed as Trolox equivalent antioxidant capacity (TEAC). The method is rapid and can be used over a wide range of pH values (Arnao et al., 1999; Lemanska et al., 2001), in both aqueous and organic solvent systems. It also has good repeatability and is simple to perform; hence, it is widely reported. The method, however, has not been correlated with biological effects; hence, its actual relevance to in vivo antioxidant efficacy is unknown.
2.6.1.2. The DPPH method

The DPPH method is a rapid, simple, inexpensive and a stable free radical with an absorption band at 515 nm. It loses this absorption when reduced by an antioxidant or a free radical species. The DPPH method is widely used to determine antiradical/antioxidant activity of purified phenolic compounds as well as natural plant extracts (Brand-Williams et al., 1995; Sripriya et al., 1996; Bonnet et al., 1997; Mahinda and Shahidi, 2000; Peyrat-Maillard et al., 2000; Fukumoto and Mazza, 2000). Bonnet et al. (1997) found that most phenolic antioxidants react slowly with DPPH, reaching a steady state in 1-6 hours or longer. This suggests that antioxidant activity using DPPH should be evaluated over time. The method also has good repeatability and is used frequently. However, like ABTS, it has limited, if any, relevance to biological systems. Also, colour interference of DPPH with samples that contain anthocyanins leads to underestimation of antioxidant activity (Arnao et al., 1999).

These simple methods have been developed to determine the antioxidant activity of foods utilizing the stable 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical. The odd electron in the DPPH free radical gives a strong absorption maximum at 517 nm and is purple in colour. The colour turns from purple to yellow as the molar absorptivity of the DPPH radical at 517 nm reduces from 9660 to 1640 when the odd electron of DPPH radical becomes paired with a hydrogen from a free radical scavenging antioxidant to form the reduced DPPH-H (Prakash et al., 2007). The resulting decolorization is stoichiometric with respect to number of electrons captured. Antioxidant compounds may be water-soluble lipid-soluble, insoluble, or bound to cell walls. Hence, extraction efficiency is an important factor in quantification of antioxidant activity of foods. Trolox (as the reference standard) and the sample are reacted with DPPH solution in methanol or water for four hours at 35°C in a vessel mounted on a rotary shaker and the absorbance changes are measured at 517 nm (Prakash et al., 2007; Cao et al., 1995;
Vinson et al., 1998). The quantity of sample necessary to react with one half of the DPPH is expressed in terms of the relative amount of Trolox reacted. Antioxidant activity of a sample is expressed in terms of micromole equivalents of Trolox (TE) per 100 grams of sample, or simply (TE) per 100 gm of sample or, simply Trolox units per 100 gm or TE/100g (Cao et al., 1995; Vinson et al., 1998).

The popular DPPH antioxidant method have been through some thorough investigations due to widely use of different protocols which differed in the concentration of DPPH (22.5-250 µM), incubation time (5 minutes-1 hour), reaction solvent and pH of the reaction mixture. Light, oxygen and pH of the reaction mixture also affect the absorbance of DPPH (Ozcelik et al., 2003). The present investigation on the DPPH antioxidant assay was carried out for developing a standard protocol within the sensitivity range of spectrophotometric assays (Ayres, 1949; Sloane and William, 1977).

The revisited DPPH antioxidant method where they used methanol, ethanol and buffered methanol, the order of absorbance was highest in buffered methanolic solution, followed by methanolic and ethanolic solutions. Higher absorbance in methanolic solutions implies better sensitivity in relation to ethanolic solution of DPPH (Ayres, 1949). The recommended range of accuracy for spectrophotometric measurements falls within an absorbance of 0.221-0.698 which equals a transmittance of 20–60% (Ayres, 1949). This corresponds to the DPPH concentration reported by Sharma and Bhat (2009) of nearly 25-70 µM of 20-60% (Ayres, 1949). A number of workers have used DPPH concentrations far beyond the spectrophotometric accuracy, even up to 250 µM.
In the revisited DPPH assay a DPPH concentration of 50 µM was used, in consonance with the requirements of the accuracy of spectrophotometric measurements (Sloane and William, 1977). The absorbance of DPPH without any additions was stable over 30 min. In addition, the suitable solvent for then DPPH assay was methanol or buffered methanol for the assay of antioxidant activity of non-polar/less polar and polar compounds/extracts, respectively.

The antioxidant assay based on scavenging of DPPH radical at a DPPH concentration of 50 µM in methanol or buffered methanol, depending upon the solubility of the compound under investigation, is recommended. All operations must be done in dark or dim light (Ozcelik et al., 2003). The extent of inhibition is influenced by the solvent. The protocol described here, thus, takes care of the spectrophotometric sensitivity range, besides sensitivity of DPPH to light, pH and solubility of the compound.

2.6.2. In vivo models for evaluating antioxidant activity

2.6.2.1. The malondialdehyde (MDA) or thiobarbituric acid-reactive-substances (TBARS) assays

Lipid peroxidation is associated with the oxidative degradation of lipids. In meat it is the major cause of chemical muscle spoilage (Teets and Were, 2008). It involves free radicals stealing electrons from the lipids in cell membranes which results in cell damage. This process proceeds by a free radical chain reaction mechanism. It most often affects polyunsaturated fatty acids, because they contain multiple double bonds in between which lies methylene -CH2- groups that possess especially reactive hydrogens. As with any radical reaction the reaction consists of three major steps: initiation, propagation and termination (Lipid oxidation, FDSC 400).
Certain diagnostic tests are available for the quantification of the end products of lipid peroxidation, specifically malondialdehyde (MDA) (Miller et al., 2000). The most commonly used test is called a TBARS Assay (thiobarbituric acid reactive substances assay). Thiobarbituric acid reacts with malondialdehyde to yield a fluorescent product. However, there are other sources of malondialdehyde, so this test is not completely specific for lipid peroxidation (Hodges et al., 1999).

Thiobarbituric acid test is one of the most frequently used tests for measuring the peroxidation of lipids. Method involves isolation of microsomes from rat liver and induction of lipid peroxides with ferric ions leading to the production of small amount of Malonaldehyde (MDA). The TBA reacts with MDA to form a pink chromagen, which can be detected spectrophotometrically at 532 nm (Kimura et al., 1984; Gutteridge et al., 1986).

2.6.2.2. Superoxide dismutase

Superoxide Dismutase (SOD) is an enzyme that repairs cells and reduces the damage done to them by superoxide, the most common free radical in the body. The SOD is found in both the dermis and the epidermis, and is key to the production of healthy fibroblasts (skin-building cells) (Vitaminstuff.com, 2005). Superoxide dismutase (SOD) catalyzes the destruction of the O2- free radical.

\[2O_2^- + 2H^+ \rightarrow O_2 + H_2O_2\]

It protects oxygen-metabolizing cells against harmful effects of superoxide free-radicals (Catalase, 2010). Studies have shown that SOD acts as both an antioxidant and anti-inflammatory in the body, neutralizing the free radicals that can lead to wrinkles and precancerous cell changes (Salin and McCord, 1975). Superoxide Dismutase helps the body...
use zinc, copper, and manganese. There are two types of SOD, namely the copper/zinc (Cu/Zn) SOD and manganese (Mn) SOD. Each type of SOD plays a different role in keeping cells healthy. Cu/Zn SOD protects the cell’s cytoplasm, and Mn SOD protects their mitochondria from free radical damage (Bannister et al., 1987).

Superoxide dismutase is found in barley grass, broccoli, Brussels sprouts, cabbage, wheatgrass, and most green plants. The body needs plenty of vitamin C and copper to make this natural antioxidant. It is also available by injection, sublingual oral supplements, enteric-coated pills, and topical creams. However, this substance must be absorbed in the small intestines, so it is important to choose oral supplements that are either enteric coated or sublingual in order to bypass the stomach acid that destroys SOD before it can be absorbed by the body (Vitaminstuff.com, 2005; Johnson and Giulivi, 2005).

In order to determine the SOD activity, several direct and indirect methods have been developed. Among these methods, an indirect method using nitroblue tetrazolium (NBT) is commonly used due to its convenience and ease of use. However, there are several disadvantages to the NBT method, such as poor water solubility of the formazan dye and the interaction with the reduced form of xanthine oxidase (Fluka analytical, 2004). The SOD Assay Kit-WST allows very convenient SOD assaying by utilizing Dojindo’s highly water-soluble tetrazolium salt, WST-1 (2-(4-Iodophenyl)- 3-(4-nitrophenyl)-5-(2,4-disulfophenyl)-2H-tetrazolium, monosodium salt) that produces a water-soluble formazan dye upon reduction with a superoxide anion. The rate of the reduction with O$_2$ are linearly related to the xanthine oxidase (XO) activity, and is inhibited by SOD. Therefore, the IC50 (50% inhibition activity of SOD or SOD-like materials) can be determined by a colorimetric method (Fluka Analytical, 2004).

2.6.2.3. Catalase
Catalase is an antioxidant enzyme that, like superoxide dismutase (SOD) and glutathione peroxidase, is produced naturally within the body. It helps the body to convert hydrogen peroxide into water and oxygen, thus preventing the formation of carbon dioxide bubbles in the blood. Also, it has one of the highest turnover numbers of all enzymes; one catalase molecule can convert 40 million molecules of hydrogen peroxide to water and oxygen each second (Goodsell, 2004). Catalase also uses hydrogen peroxide to break down potentially harmful toxins in the body, including alcohol, phenol, and formaldehyde.

Catalase works closely with superoxide dismutase to prevent free radical damage to the body. SOD converts the dangerous superoxide radical to hydrogen peroxide, which catalase converts to harmless water and oxygen. Catalases are some of the most efficient enzymes found in cells, each catalase molecule can convert millions of hydrogen peroxide molecules every second (Nozik-Grayck et al., 2005)

Catalase, superoxide dismutase, methionine reductase, and glutathione peroxidase are all manufactured in the body. Oral supplements are available for SOD, catalase, and glutathione peroxidase; however, these substances may be digested in the intestine before they ever reach the bodily tissues. Giving the body extra amounts of the building blocks it requires to make these natural antioxidants, such as manganese, zinc, copper, and selenium, may be a more effective way to increasing their presence in the body (Vitaminstuff.com, 2005).

2.6.2.4. Glutathione

Reduced glutathione (GSH), a tripeptide (γ-glutamylcysteinylglycine), is the major free thiol in most living cells and is involved in many biological processes such as detoxification of xenobiotics, removal of hydroperoxides, and maintenance of the oxidation state of protein sulfhydryls. It is the key antioxidant in animal tissues (Akerboom and Sies, 1981).
Glutathione is present inside cells mainly in the reduced form (90-95% of the total glutathione). Oxidation of glutathione leads to the formation of glutathione disulfide (GSSG). Intracellular GSH status appears to be a sensitive indicator of the overall health of a cell, and of its ability to resist toxic challenge. High levels of GSH in the cell may indicate pathological changes (Nair et al., 1991).

In similar term, Glutathione is a very small molecule that is produced by the body and found in every cell (Bounous, 2001). Glutathione, in purified extracted form, is a white powder that is soluble in water and in alcohol. It is found naturally in many fruits, vegetables, and meats. However, absorption rates of glutathione from food sources in the human gastrointestinal tract are low (Uretsky, 2005).

Furthermore, the cells of the immune system produce many oxiradicals as a result of their normal functioning, resulting in a need for higher concentrations of antioxidants than most cells. Glutathione plays a crucial role in fulfilling this requirement (Gutman, 2010). Glutathione is an antioxidant and free radical scavenger, it plays a central protective role against the damaging effects of bacteria, viruses, pollutants and free radicals (Perlmutter, 2010).

2.7. Meat quality characteristics as affected by supplements

The quality of meat poultry meat is an extremely complex notion that can be assessed from different points of view. From the consumer perception and the slaughter industry, animals should not have only high slaughter yields and desirable carcass conformation scores but also good aesthetic, sensory and nutritional characteristics. 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 and continued interest in meat (Muchenje et al., 2009a). Meat is a valuable source of proteins, vitamins and minerals (Waskar et al., 2009), and has a relatively low fat content. In that respect, the chemical composition of muscle tissue of major primal cuts is an important element of broiler meat quality (Bogosavljević-Bošković et al., 2010). Chicken meat is considered better than red meat because of comparably low levels of fat, cholesterol and high levels of iron (Jaturasitha et al., 2008). Furthermore, chicken meat is relatively lowly priced, packaged in typically convenient portions, and lack religious restrictions against its consumption (Jaturasitha, 2004).

The quality traits mentioned above are dependent upon a number of factors. These factors include genotype, sex and age stand out among biological factors (Lewis et al., 1997; Bokkers and Koene, 2003). Among numerous nongenetic factors that substantially affect certain meat quality traits, nutrition plays an important role. Diet composition and feed consumption can affect the chemical composition of muscle tissue (such as pH, colour and tenderness) to a greater or lesser extent.

2.7.1. pH and the quality of meat

Nutritional stress can result in dehydration, electrolyte imbalances, negative energy balance, glycogen depletion in muscle, and catabolism of protein and fat, ultimately increasing the pHu (Dhanda et al., 2003; Mushi et al., 2009). Xazela et al. (2011) reported lower pH in supplemented goats than non-supplemented goats and this is associated with higher glycogen levels in supplemented animals.
The quality of meat is determined using biochemical, physical-chemical and bacteriological processes. A high ultimate pH is generally indicative of pre-slaughter stress in animals (Dhanda et al., 2003; Muchenje et al., 2009b). It may result from transportation, rough handling, inclement temperatures, or anything that causes the animal to draw on its glycogen reserves before slaughter. The rate of pH decline is a good predictor of the colour and drip loss of meat (Aberle et al., 2001; Muchenje et al., 2008). Higher ultimate pH (pHu) in animals can be associated with low glycogen reserve due to insufficient nutrition (Mushi et al., 2009).

2.7.2. Meat colour

Colour is one of the most important factors in consumer selection and the decision to purchase meat and meat products (Muchenje et al., 2009a; Waskar et al., 2009). Muscle colour is affected by several factors the most important of which are; age, sex, intramuscular fat, moisture content, pre-slaughtering conditions, processing, presence of muscle pigments (Northcutt et al., 2001) and storage time (Karaoglu, 2005). Differences in meat colour have been associated with variations in intramuscular fat and moisture content, age dependent changes in muscle myoglobin content, the pHu of the muscle (Muchenje et al., 2008). Friesen and Marcy (2011) add that red poultry muscle colour depends on myoglobin. Luciano et al. (2009) reported higher levels of myoglobin in supplemented lambs than non-supplemented.

Maintaining the red colour in meat is important and also increasing the shelf life of meat as it largely influences the consumer’s perception of meat at purchasing point (Faustman and Cassens, 1990; Muchenje et al., 2008). In pursuit of increased shelf life colour and healthiness of meat, meat producers more and more commonly apply natural feeding supplements, mainly herbs (Gardzielewska et al., 2003). Several evidence suggests that
dietary vitamin E supplementation of steers caused accumulation of \( \alpha \)-tocopherol in the muscle tissue, oxymyoglobin and lipid oxidation, thereby by prolonging the colour stability of beef (Arnold et al., 1993b; Chan et al., 1996a; Liu et al., 1996). Dietary delivery of \( \alpha \)-tocopherol through supplementation seems to be the most effective way in obtaining the colour stabilizing (Faustman et al., 1998). By utilizing vitamin E rich supplements, the colour stability of meat cuts sold commercially to consumers can be improved. An increase in the amount of vitamin E in the diet promotes an increase in shelf life and colour stability of meat cuts (Ali and Zahran, 2010; Liu et al., 1995).

Colour measurements may be used for various reasons such as to support visual appraisals; as a basis for production acceptance and rejection; to document colour deterioration over time; and to estimate the proportion of myoglobin in different chemical state. However, the most important aims for objective colour measurements are to support visual observations and to provide unbiased evidence of treatment effects that can be statistically analyzed (ASMA, 1991). To fulfil these aims data expression as colour co-ordinated is probably enough to represent relative colour differences. The use of colour measurements helps in prediction of functional properties of meat. Commission International De L’ Eclairage (1976) is based on three co-ordinates such as \( L^* \) (lightness); \( a^* \) (redness) and \( b^* \) (yellowness) that are used to determine differences in meat colour.

2.7.3. Meat tenderness

Tenderness of meat is another most important characteristic that also regulates acceptability of meat even though it is difficult to predict. Maltin et al. (2003) states that tenderness is a very important element of eating quality and that variations in tenderness affect the decision
to re-purchase. Tenderness is based on ease of chewing that is contributed by many factors. Among them, the fibrous nature of muscle contributes to chewing resistance (Gerrard and Grant, 2003).

Several researchers have associated tenderness of meat with the breakdown of myofibrillar proteins affected by the presence of calcium-dependent proteases or calpains (Boehm et al., 1998; Claeys et al., 2001; Huff-Lonergan et al., 1996; Ouali, 1990; Muchenje et al., 2009a). Hwang et al. (2003) also found that rapid cooling of meat with a relatively high pH resulted in tougher meat due to contraction of the sarcomeres and by altering the calpain activity. Slaughter and processing also have an impact on the tenderness of the meat (Fletcher, 2003).

Whether or not meat is tender also depends upon the rate and extent of the chemical and physical changes in conversion of muscle to meat. After an animal is slaughtered, there is no source of oxygen or nutrients being supplied to the muscles. Thus, the muscles run out of energy and began to contract and become stiff (rigor mortis). Eventually the muscles will relax and cause the meat to be tender when cooked. This process of rigor mortis can be affected by ante mortem stress. Animals that struggle before or during slaughter expend their energy quicker and cause rigor mortis to set in faster than normal. This increases the toughness of the meat (Fletcher, 2002). Swanek et al. (2009) reported that the levels of tenderness in beef were elevated by supplementation. Ali and Zahran (2010) also reported that supplementation improved chicken meat tenderness during storage. The improved tenderness could be attributed to the tenderizing effect of vitamin E when supplemented to chicken (Ali and Zahran, 2010). Vitamin E (α-tocopherol) actively improves immunity in poultry and other species, and protects against rancidity and prolongs shelf-life of meat.
through its antioxidative properties (Li et al., 2009). Li et al. (2009) also reported that vitamin E supplementation decreased shear force measurements thus improving tenderness in broilers.

2.7.4. Cooking loss

Cooking loss refers to the reduction in weight of meat during the cooking process (Jama et al., 2008). Major components of cooking losses are thawing, dripping and evaporation. Thawing loss refers to the loss of fluid in meat resulting from the formation of exudates following freezing and thawing (Jama et al., 2008; Muchenje et al., 2009a). Such losses are lower following a rapid freezing compared with slow freezing. This is because of small crystallization formed by the rapid freezing (Hui, 2004). Dripping is the loss of fluid from meat and water evaporation from the shrinkage of muscle proteins (actin and myosin) (Yu et al., 2005). Cooking loss in meat cuts is important for maintaining an attractive retail display of meat. Normally, fresh postrigor meat exudes fluid, or drip, from cut surfaces (Lawrie, 1991). Asghar et al. (1991) reported less drip loss from thawed pork chops obtained from pigs supplemented with vitamin E. High cooking loss in meat has a large financial impact in meat industry. For example, meat and their products are a rich source of proteins, essential minerals and vitamins. The increased loss of such nutrients deteriorates the meat nutritional quality and lowers its purchase (Jama et al., 2008).

2.8. Fatty acids and the quality of meat

Fatty acids may be saturated, monounsaturated and polyunsaturated. Saturated fatty acids (SFA) are the main and bad fatty acids found in meat. This is because they are implicated in
various diseases such as cardiovascular diseases (Wood and Enser, 1997). On the other hand, the essential fatty acids, polyunsaturated fatty acids (PUFA) which are transferred to the meat through diet, are considered as the healthiest dietary fats because their consumption is associated with lowering the risk of these diseases (Whetsell et al., 2003). Omega 6 and omega 3 fatty acids are a group of PUFA that are vitally important to be present in the diet in a proper balance for a good health. Meat should have a favourable balance between PUFA and SFA (P:S) which is 0.4 and the desirable omega 3 and omega 6 (n-3:n-6 PUFA) ratio which is below 4.0 (Wood and Enser, 1997; Wood et al., 2003). Therefore, the PUFA/SFA and n-6/n-3 PUFA ratios have become some of the most important parameters in evaluating the nutritional value and healthiness of foods (Mapiye et al., 2011).

Meat fat is a healthy essential dietary component providing both easily metabolizable energy and essential fatty acids. Fat also imparts pleasant palatability attributes to food hence the partiality (Casey, 1992). However fat, have been implicated in diseases, such as cancers and coronary heart disease. In addition, the healthiness of meat is largely related to its fat content and its fatty acid composition (Muchenje et al., 2009b). In Germany, it is recommended that people should decrease their intake of saturated fatty acids (10% of the total calories) and trans fatty acids (less than 1%) and to increase the intake of unsaturated fatty acids (0.5%) and to decrease the n-6/n-3 ratio in the diet to levels ≤5:1 (Nuernberg et al., 2005; Casey, 1992). The Department of Health (1994) in the UK, also recommend that saturated fats to be reduced from 15% to 10% of total energy intake whilst increasing the ratio of polyunsaturated to saturated fatty acids (P:S) to above 0.4. This is because some meats naturally have a P:S ratio of around 0.1, meat has been implicated in causing the imbalanced fatty acid intake of consumers (Wood et al., 2003).

In recent years awareness of the importance of diet in human health has increased. There are recommendations that it is important for diets to contain higher levels of n-3 poly unsaturated
fatty acids (PUFA) (Wood and Enser, 1997). However, researchers since have been studying the importance of meat as a natural supplier of these fatty acids to the diet. According to Enser (2001), the ratio of n-6/n-3 PUFA is particularly beneficial (low) in ruminant meats, especially from animals that have consumed grass which contains high levels of α-linolenic acid (18:3). Furthermore, ruminants naturally produce conjugated linoleic acids (CLAs) which may have a range of nutritional benefits in the diet.

According to Muchenje et al. (2009a, b) linoleic acid (18:2n-6) are one of the most important PUFA, which naturally occur in ruminant-derived food and to which various beneficial health effects are ascribed. Wood and Enser (1997) reported that the benefit of feeding diets rich in α-linolenic acid is that increased deposition could lead to increased synthesis of the long-chain polyunsaturated fatty acids such as eicosapentaenoic acid (EPA, 22:5) and docosahexaenoic acid (DHA, 22:6). These fatty acids have also been shown to exert various beneficial health effects and their increase in animal feed will improve the dietetic value of meat (Muchenje et al., 2009a, c). Kouba et al. (2003) reported increased and improved levels of n-3 PUFA and the PUFA: MUFA ratios in linseed fed pigs. In ruminants such as sheep and cattle, the concentrations of PUFA in total lipid are low because of hydrogenation in the rumen. Oleic acid (18:1) increases with fat content, also in pigs, but stearic acid (C18:0) falls, leading to a general increase in unsaturation and softness as fatness increases, unlike the situation in pigs (Wood and Enser 1997). Mapiye et al. (2011) reported higher content of α-linolenic acid and a more desirable n-6/n-3 ratio from supplemented Nguni beef.

Fat colour is another aspect of quality affected by fatty acids. The ability of UFA, especially those with more than two double bonds, to rapidly oxidize, is important in regulating the shelf life of meat (rancidity and colour deterioration). However, this propensity to oxidize is important in flavour development during cooking (Wood et al., 2004).
2.9. Summary of review

Moringa is a major source of natural antioxidants. With the natural antioxidants, cells are protected against oxidative stress by an interacting network of antioxidants and antioxidant enzymes. Enhancing animal feed with natural supplements improves the quality of desirable fatty acids, hence maintaining and improving the quality of meat.
2.10. References


Lipid oxidation, FDSC 400.


Wood, J. D., Richardson, R. I., Nute, G. R., Fisher, A. V., Campo, M. M., and Kasapidou, E.,


Chapter 3: Fatty acid composition and antioxidant activity of meat from goats supplemented with Moringa (Moringa oleifera) leaves, sunflower cake and grass hay

By Kumnandi Qwele

Abstract
The objective of the present study was to determine the fatty acid composition and antioxidant activity (AA) in the Muscularis longissimus thoracis et lumborum (LTL) from goats supplemented with Moringa oleifera leaves, sunflower cake or grass hay. Eighteen castrated goats aged 9 months, were fattened for 60 days and used in this study. After slaughter, samples from the muscle tissue were collected to determine fatty acid composition and antioxidant activity. The crude protein and polyphenols was lower in the grass hay supplement. The protein and fat contents in the meat from goat fed with Moringa or sunflower cake was higher than in meat from grass hay supplement. The ratio of unsaturated fatty acids/saturated fatty acids ratios obtained from meat supplemented with grass hay, Moringa and sunflower cake were 1.5, 1.39 and 2.17, respectively. Total phenolic content was high in the meat from goat supplemented with Moringa (10.62±0.27 GAE/g), followed by sunflower cake (5.21±0.05GAE/g) while that of grass hay-supplemented goats was very low (1.58±0.17GAE/g). Data obtained from meat supplemented with moringa showed effective and moderate reduction of ABTS (93.51±0.19) and DPPH (58.95±0.3) radicals than other supplements. Similarly, the ferric reducing power of meat from goat fed with Moringa, sunflower cake and grass hay was increased in descending order. The antioxidant activity of meat sample supplemented with Moringa on catalase (CAT), reduced glutathione (GSH), superoxide dismutase (SOD) and lipid oxidation (LO) was significantly (P<0.05) higher than GH and SC supplements. The current study indicated that Moringa supplementation
improved the fatty acid composition of meat from goats while also improving the antioxidant potential of goat meat.

**Keywords:** *Moringa oleifera*, fatty acid profile, total phenolic content, antioxidant potential, thiobarbituric acid, superoxide dismutase, catalase, goat meat
3.1. Introduction

Lipid peroxidation is a complex process occurring in aerobic cells and reflects the interaction between molecular oxygen and polyunsaturated fatty acids (Verma et al., 2009). It causes meat spoilage. It occurs during processing and storage when meat is exposed to oxygen, heat, and light (Fasseas et al., 2007). Lipid oxidation promotes production of rancid flavours and odours while also reducing the shelf-life, nutritional quality, and safety of food products. To prevent or delay the autoxidation process antioxidants have been utilized for many years (Lahucky et al., 2010). 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).

Fatty acids (saturated, polyunsaturated and mono unsaturated fatty acids) are involved in various technological aspects of meat quality. Because of their different melting points, variation in fatty acid composition has an important effect on firmness or softness of the fat in meat, especially the subcutaneous and intermuscular but also the intramuscular fat (Wood et al., 2003). Fatty acids contribute to a wide range of quality attributes for meat such as colour stability, drip loss and the development of oxidative rancidity (Kouba et al., 2003). The presence of polyunsaturated fatty acids such as linoleic and arachidonic acids found in red meats and poultry undergo the greatest oxidative damage during storage (Teets and Were, 2008).

Over the years, synthetic antioxidants such as hydroxyanisole, butylated hydroxytoluene and tertiary butyl hydroquinone have been widely used to preserve meat (Fasseas et al., 2007). The use of these antioxidants has been questionable since they have been discovered to possess toxic, pathenogic and carcinogenic effects to humans and animals (Hayes et al., 2010). Hence there has been a growing interest in the use of natural antioxidants as
alternative remedy for synthetic antioxidants. In addition, consumers have shifted their interest to natural antioxidants since they are considered safer than the synthetic antioxidants (Jung et al., 2010). It has also been reported that these natural antioxidants, especially of plant source, have greater application potential for consumer’s acceptability, palatability, stability and shelf-life of meat products (Jung et al., 2010). One such plant with a potential to be used as an antioxidant is Moringa (*Moringa oleifera*).

*Moringa oleifera*, native to the sub-Himalayan tracts of India, Pakistan, Bangladesh and Afghanistan, is the most widely cultivated species of the Moringaceae family. In addition to being the most nutritious tree, it has various functions, including pharmacological activities and antioxidant properties (Verma et al., 2009). Verma et al. (2009) reported the presence of some phytochemicals such as, carotenoids, vitamins, minerals, amino acids, sterols, glycosides, alkaloids, flavonoids and phenolics in moringa. Health-promoting phytochemicals, especially phenolics and flavonoids are some of the authentic antioxidants that have been reported to be safe and bioactive (Sreelatha and Padma, 2009). Sunflower cake and grass hay are commonly used to supplement ruminants.

No investigation on fatty acid profiles, enzymatic antioxidants and antioxidant potential of meat from goats supplemented with Moringa, sunflower cake and grass hay has been carried out. The objective of this study was therefore to determine the fatty acid profiles, antioxidant activities (AA) of meat from Xhosa lop-eared goat supplemented with *Moringa oleifera* leaves, sunflower cake and grass hay.
3.2. Materials and methods

3.2.1. Site description

The study was conducted at Honeydale Farm, University of Fort Hare, Alice, South Africa. Honeydale farm lies along longitude 32° 78' E and latitude 26° 85' S at an altitude of 450-500 m above sea-level. It is located in the False Thornveld of the Eastern Cape Province which is characterized by mean annual rainfall of 480 mm and mean annual temperature of 18.7°C, respectively.

3.2.2. Meat samples

Eighteen castrated Xhosa lop-eared goats of 9 months of age and 14 kg of weight were randomized into three groups consisting of six each. Group 1 animals were given 200 grams of dried Moringa leaves twice a day. Group 2 animals were given 170 grams of sunflower cake, also twice a day. Group 3 animals were given ad libitum grass hay. Estimation of nutrient requirements was based on Langston University Goat Research for a local goat weighing 17 kg and growing 100 g/day under tropical conditions according to Paul et al. (2003). The energy and protein requirements were estimated to be 6.4 MJ ME and 80 g per day to feed the goats for a 60 day period (NRC, 2007). The animals were fed for 60 days. Groups 1 and 2 were also given grass hay was a basal diet. The nutritional composition, polyphenolics and fatty acid profiles of the diets used are presented in Tables 3.1, 3.2 and 3.3, respectively. After slaughter, fresh meat samples were taken from the right side of the Muscularis longissimus thoracis et lumborum (LTL). Visible fat was removed. Meat samples were stored at 4 °C for 24 h before assays for antioxidant activity were performed. Ten grams of each meat sample was homogenized. A volume of 10 %w/v homogenate was prepared in 0.05 M phosphate buffer (pH 7) and centrifuged at 12,000 × g for 60 min at 4°C. The supernatant obtained was used for the estimation of total phenolic content, 1, 1-diphenyl-2-
Table 3.1: Nutritional composition of the experimental diets.

<table>
<thead>
<tr>
<th>Component</th>
<th>Grass hay</th>
<th>Sunflower Meal</th>
<th>M. oleifera leaf Meal</th>
<th>S.E</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dry matter</td>
<td>89.33&lt;sup&gt;a&lt;/sup&gt;</td>
<td>88.93&lt;sup&gt;b&lt;/sup&gt;</td>
<td>89.00&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.22</td>
</tr>
<tr>
<td>Crude protein (%)</td>
<td>14.08&lt;sup&gt;a&lt;/sup&gt;</td>
<td>23.27&lt;sup&gt;b&lt;/sup&gt;</td>
<td>23.76&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.37</td>
</tr>
<tr>
<td>Polyphenols (%)</td>
<td>0.433&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.67&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.77&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.02</td>
</tr>
</tbody>
</table>

<sup>abc</sup> means with different superscripts in a row are different ($p<0.05$).
Table 3. 2: Total polyphenolic contents of the leaf extracts of *M. oleifera*.

<table>
<thead>
<tr>
<th>Solvent extracts</th>
<th>Phenolics (TE/g)</th>
<th>Flavonoids (QE/g)</th>
<th>Flavonols (QE/g)</th>
<th>Proanthocyanidin (CE/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetone <em>M. oleifera</em> leaves</td>
<td>120.33 ± 0.76&lt;sup&gt;b&lt;/sup&gt;</td>
<td>295.01 ± 1.99&lt;sup&gt;b&lt;/sup&gt;</td>
<td>132.74 ± 0.83&lt;sup&gt;b&lt;/sup&gt;</td>
<td>32.59 ± 0.50&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Aqueous <em>M. oleifera</em> leaves</td>
<td>40.27 ± 0.99&lt;sup&gt;a&lt;/sup&gt;</td>
<td>45.1 ± 0.47&lt;sup&gt;a&lt;/sup&gt;</td>
<td>18.10 ± 0.18&lt;sup&gt;a&lt;/sup&gt;</td>
<td>16.91 ± 0.87&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Standard errors of mean (n=6)

<sup>a,b</sup> Means superscript with different letters in the same column differ significantly (P< 0.05)

QE = Quercetin equivalent

TE = Tannic acid equivalent

CE = Catechin equivalent
Table 3. 3: Fatty acids composition (% total fatty cid) of *M. oleifera* leaves, sunflower cake and grass hay diets with least square means and standard error of means (s.e.m)

<table>
<thead>
<tr>
<th>Fatty acid (% total fatty acid)</th>
<th>Grass hay</th>
<th><em>M. oleifera</em> leaves</th>
<th>Sunflower cake</th>
</tr>
</thead>
<tbody>
<tr>
<td>C13:0</td>
<td>0.40±0.021c</td>
<td>0.06±0.012b</td>
<td>0.00±0.000a</td>
</tr>
<tr>
<td>C14:0</td>
<td>0.77±0.021c</td>
<td>0.33±0.038b</td>
<td>0.18±0.012a</td>
</tr>
<tr>
<td>C15:0</td>
<td>0.31±0.040c</td>
<td>0.02±0.010a</td>
<td>0.10±0.006b</td>
</tr>
<tr>
<td>C16:0</td>
<td>23.63±0.271c</td>
<td>8.93±0.309a</td>
<td>17.77±0.417b</td>
</tr>
<tr>
<td>C17:0</td>
<td>0.52±0.030c</td>
<td>0.09±0.006a</td>
<td>0.13±0.010b</td>
</tr>
<tr>
<td>C18:0</td>
<td>5.22±0.136c</td>
<td>3.71±0.085b</td>
<td>1.87±0.052a</td>
</tr>
<tr>
<td>C20:0</td>
<td>3.70±0.139c</td>
<td>2.18±0.065b</td>
<td>0.49±0.036a</td>
</tr>
<tr>
<td>C21:0</td>
<td>0.25±0.035b</td>
<td>0.03±0.006a</td>
<td>0.04±0.006b</td>
</tr>
<tr>
<td>C22:0</td>
<td>6.26±0.343c</td>
<td>4.47±0.136b</td>
<td>0.67±0.066a</td>
</tr>
<tr>
<td>C23:0</td>
<td>0.59±0.049b</td>
<td>0.08±0.000a</td>
<td>0.09±0.012a</td>
</tr>
<tr>
<td>Total saturated fatty acids (SFA)</td>
<td>41.65±0.725b</td>
<td>19.90±0.565a</td>
<td>21.34±0.602a</td>
</tr>
<tr>
<td>C15:1c10</td>
<td>0.44±0.110b</td>
<td>0.07±0.020a</td>
<td>0.06±0.015a</td>
</tr>
<tr>
<td>C16:1c9</td>
<td>0.08±0.026a</td>
<td>0.38±0.586b</td>
<td>0.07±0.021a</td>
</tr>
<tr>
<td>C17:1c10</td>
<td>0.00±0.000a</td>
<td>0.01±0.015a</td>
<td>0.04±0.001b</td>
</tr>
<tr>
<td>C18:1c9</td>
<td>9.20±0.609a</td>
<td>63.40±2.196c</td>
<td>18.76±0.125b</td>
</tr>
<tr>
<td>C20:1c11</td>
<td>0.16±0.025b</td>
<td>0.05±0.006a</td>
<td>0.00±0.000a</td>
</tr>
<tr>
<td>C24:1c15</td>
<td>0.36±0.036c</td>
<td>0.00±0.000a</td>
<td>0.22±0.006b</td>
</tr>
<tr>
<td>Total monounsaturated fatty acids (MUFA)</td>
<td>10.24±0.396a</td>
<td>63.91±0.845c</td>
<td>19.15±0.097b</td>
</tr>
<tr>
<td>C18:2c9,12(n-6)</td>
<td>23.44±0.480b</td>
<td>11.86±0.555c</td>
<td>58.00±0.557c</td>
</tr>
<tr>
<td>C20:2c11,14(n-6)</td>
<td>0.00±0.000a</td>
<td>0.00±0.000a</td>
<td>0.08±0.003b</td>
</tr>
<tr>
<td>C20:3c8,11,14(n-6)</td>
<td>0.12±0.015b</td>
<td>0.08±0.010a</td>
<td>0.15±0.015b</td>
</tr>
<tr>
<td>Total omega-6 fatty acids (n-6)</td>
<td>23.56±0.495b</td>
<td>11.94±0.558a</td>
<td>58.23±0.571c</td>
</tr>
<tr>
<td>C18:3c9,12,15(n-3)</td>
<td>18.50±0.380c</td>
<td>1.64±0.050b</td>
<td>0.67±0.006a</td>
</tr>
<tr>
<td>C20:5c5,8,11,14,17(n-3)</td>
<td>5.37±0.240c</td>
<td>1.07±0.055b</td>
<td>0.59±0.057a</td>
</tr>
<tr>
<td>Total omega-3 fatty acids (n-3)</td>
<td>23.87±0.195c</td>
<td>2.71±0.157b</td>
<td>1.26±0.056a</td>
</tr>
<tr>
<td>Total polyunsaturated fatty acids (PUFA)</td>
<td>47.43±0.670b</td>
<td>14.65±1.238a</td>
<td>59.49±0.522c</td>
</tr>
<tr>
<td>PUFA/SFA</td>
<td>1.14±0.036b</td>
<td>0.74±0.081a</td>
<td>2.79±0.104c</td>
</tr>
</tbody>
</table>

Standard errors of mean (n=6)

abc Means in the same row, with different superscript differ significantly (P< 0.05)

picrylhydrazyl (DPPH), ABTS, lipid peroxidation, glutathione, catalase and superoxide dismutase.
3.2.3. Proximate analyses and cholesterol determination

A 50-g sample of the LTL was ground and freeze-dried for the determination of, protein, fat, moisture and ash contents; phosphorus, calcium, iron, copper and zinc as described by AOAC International (AOAC) (1985).

The extraction and quantification of cholesterol were carried out by the method of Al-Hasani et al. (1993), with modifications (Rowe et al., 1999). Meat samples weighing 5-10 g were placed in a 250 ml flat-bottom flask and dispersed in an ethanol-methanol-isopropanol (90:5:5, v/v/v) solution in an amount equivalent to 4 ml/g of sample. A 1-ml sample of 60% KOH in water was then added. The flask containing this mixture was connected to a water cooled condenser and refluxed for 1 h. After cooling to room temperature, 100 ml of n-hexane was added and the mixture was stirred for 10 min and finally 25 ml of deionised water was added and the mixture was stirred for a further 15 min. Layers were then separated and the n-hexane layer was collected in a flask. A supernatant of 25 ml of the n-hexane layer was evaporated to dryness under nitrogen. The residue was dissolved in 2 ml of n-hexane containing 0.2 mg of 5α-cholestane internal standard/ml and transferred to a vial. Approximately 3 ml were injected into a gas chromatograph.

A Shimadzu 14A instrument GC (Japan) fitted with a flame ionization detector (FID, 300 8C) and a split/splitless injector (260 8C, split 1: 150) was used for the analysis of cholesterol. Separation was carried out in a fused silica capillary column at 300 8C (25 m x 0.25 mm), coated with SE-30 (0.25 mm phase thickness) (Quadrex, USA). The carrier gas used was hydrogen (1.5 ml/min) and the make-up gas was nitrogen (25 ml/min). Cholesterol identification was made by comparing the relative retention time of peaks from samples with standards from SIGMA (U.S.A.). The internal standard used was 5αcholestane. For peak integration a CG 300 computing integrator (CG Instruments, Brazil) was used.
3.2.4. Fatty acid profile determination

Total lipid from muscle sample was quantitatively extracted, according to the method of Folch et al. (1957), using chloroform and methanol in a ratio of 2:1. An antioxidant, butylated hydroxytoluene was added at a concentration of 0.001% to the chloroform: methanol mixture. A rotary evaporator was used to dry the fat extracts under vacuum and the extracts were dried overnight in a vacuum oven at 50 °C, using phosphorus pentoxide as moisture adsorbent. Total extractable intramuscular fat was determined gravimetrically from the extracted fat and expressed as % fat (w/w) per 100 g tissue. The extracted fat was stored in a polytop (glass vial, with push-in top) under a blanket of nitrogen and frozen at -20 °C, pending analyses.

Conjugated linoleic acid (CLA) standards were obtained from Matreya Inc. (Pleasant Gap, United States). These standards include cis-9,trans-11; cis-9,cis-11, trans-9, trans-11 and trans-10, cis-12isomers. All other reagents and solvents were analytical grade and obtained from Merck Chemicals (Pty) Ltd., Halfway House, South Africa. Fatty acids were expressed as the proportion of each individual fatty acid to the total of all fatty acids present in the sample. The following fatty acid combinations and ratios were calculated: total saturated fatty acids (SFA), total mono-unsaturated fatty acids (MUFA), polyunsaturated fatty acids (PUFA), PUFA/SFA ratio (P/S) and n-6/n-3 ratio.

3.2.5. Assaying methods

3.2.5.1. Determination of total phenol content (TPC)

The total phenol content (TPC) in meat was determined colorimetrically using the Folin-Ciocalteau reagent, following the modified method of Gutfinger (1981). To 0.1 mL supernatant was added 0.2 mL of the Folin–Ciocalteu reagent, followed by the addition of 3
mL sodium carbonate solution (5%). The reaction mixture was vortexed after incubation at 23°C for 1 h. The absorbance of phenolic content was measured spectrophotometrically (Hewlett Packard, UV/visible light) at 765 nm. The quantification of phenolics was based on the standard curve generated with the use of gallic acid and expressed as gallic acid equivalent.

3.2.5.2. **DPPH free radical scavenging assay**

The DPPH (1, 1-diphenyl-2-picrylhydrazyl) radical-scavenging activity was estimated by the method of Blois (1958) with slight modifications. A 1mL methanolic DPPH solution (0.2 mM) was added to 200 μL of the supernatant and 800 μL distilled water. The mixture was vortexed and left to stand at room temperature (20–22 °C) for 30 min. A tube containing 1 mL of distilled water and 1 mL of methanolic DPPH solution (0.2 mM) served as the control. The absorbance of the solution was measured at 517 nm using a spectrophotometer (Hewlett Packard, UV/visible light). The DPPH scavenging capacity of the extract was expressed as percent of control and was calculated as:

\[
\text{ Radical scavenging activity} = [1− (\text{absorbance of sample} / \text{absorbance of control})] \times 100.
\]

3.2.5.3. **ABTS\(^+\) reducing activity**

The 2, 2 azino-bis (3- ethylbenzothiazoline-6-sulphonic) (ABTS) assay described by Erel (2004) was employed to measure the antioxidant activity in meat supplemented with *M. oleifera* leaves, sunflower cake and grass hay. The ABTS was dissolved in distilled water to a 7 mM concentration. The ABTS was dissolved in distilled water to 7 mM concentration and potassium persulphate added to a concentration of 2.45 mM. The reaction mixture was left to stand at room temperature overnight (12-16 h) in dark to allow the completion of radical generation. This solution was then diluted with ethanol to adjust its absorbance to 0.70±0.02
at 734 nm. The diluted ABTS\(^+\) solution (3 mL) were added to 20 \(\mu\)L aqueous supernatant and the absorbance was measured by a spectrophotometer (Beckman) at 734 nm using ethanol as blank. The percentage inhibition was calculated by the following equation:

\[
\text{ABTS}^+\text{ reducing activity (\%)} = \frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}} \times 100
\]

3.2.5.4. Reducing power

The reducing power adopted by Jung et al. (2010) was used to measure the antioxidant activity in meat supplemented with of \textit{M. oleifera} leaves. A 200 \(\mu\)L supernatant was mixed with 500 \(\mu\)L sodium phosphate buffer (0.2 M, pH 6.6) and 500 \(\mu\)L potassium ferricyanide (1%), and the resultant mixture was incubated at 50 °C for 20 min. After addition of 2.5 mL trichloroacetic acid [TCA, (10%)], the mixture was centrifuged (Hanil) at 2200\(\times\)g for 10 min. The upper layer (500 \(\mu\)L) was mixed with 500 \(\mu\)L distilled water and 100 \(\mu\)L ferric chloride (0.1%), and the absorbance was measured at 700 nm using a spectrophotometer. Increased absorbance of the reaction mixture indicated higher reducing power of the tissue extract.

3.2.5.5. Lipid peroxidation

Lipid peroxidation was estimated in terms of thiobarbituric acid reactive species (TBARS) using Malondialdehyde (MDA) as standard. The homogenized breast tissue (0.1 ml) 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 min 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:

\[
\text{% of lipid oxidation Inhibition} = \left\{ \frac{\text{Ao} - \text{A1}}{\text{Ao}} \right\} \times 100
\]

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

3.2.5.6. Glutathione (GSH) assay

Reduced Glutathione (GSH) was assayed by the method of Akerboom and Sies (1981). Preparation of glutathione standard solutions was done by serial dilutions of the 50 μM glutathione solution according to the manufacture instructions. The fresh meat was first deproteinized with the 5% 5-sulfosalicylic acid solution, centrifuged to remove the precipitated protein. The first 2 wells contained 10 μl of the 5% 5-sulfosalicylic acid solution as a reagent blank, followed by adding 10 μl duplicate samples of the prepared glutathione standard solutions, later added varying volumes of the unknown sample into separate wells up to 10 μl sample to desire concentrations (2, 4, 6, 8 and 10μl). Add 150 μl of the working mixture which comprises of 8 ml of 1X assay buffer added to 228 μl of the diluted enzyme solution (6 units/ml) and 228 μl of DTNB stock solution (1.5 mg/ml)) to each well and resuspended. After Incubating for 5 min at room temperature, 50 μl of the diluted NADPH solution was added and then mixed to generate yellow colour. The value reagent blank was subtracted from measurement. The values of the glutathione standard solutions were used to determine the standard curve and the ΔA412/min equivalent was calculated to 1 nmole of reduced glutathione per well. The nmoles of GSH in the unknown sample was calculated as follows:

\[
\text{nmoles GSH per ml of sample} = \frac{\Delta A412/\text{min (sample)}}{\text{dil}}
\]
ΔA412/min (1 nmole) x vol

Where,

ΔA412/min (sample) = slope generated by sample (after subtracting the values generated by the blank reaction).

ΔA412/min (1 nmole) = slope calculated from standard curve for 1 nmole of GSH

dil = dilution factor of original sample

vol = volume of sample in the reaction in ml

3.2.5.7. Catalase (CAT) activity

Catalase (CAT) activity was assayed by the method of Deisseroth and Dounce (1970). For the Hydrogen peroxide (H₂O₂) standard curve the reaction was initiated by 0, 125, 250, 500, and 750 ml of 10 mM H₂O₂ solution in microcentrifuge tubes. To this reaction was added 1x assay buffer to a final volume of 1.0 ml and mixed by inversion. The assay system consisted of 10 µl aliquot of each solution being transferred to a second tube and 1 ml of the colour reagent was added to each tubes. After 15 minutes the absorbance was read at 520 nm. A standard curve of the absorbance is plot at 520 nm versus the final amount of H₂O₂ in the reaction mixture. For catalase colorimetric enzymatic reaction, the assay reaction was performed at room temperature (~25 °C). The 1x assay buffer, colorimetric assay substrate solution (200 mM H₂O₂), and colour reagent were allowed to equilibrate to room temperature. A volume of 20 µl of the aliquot and 55 µl of 1x assay buffer was added to the microcentrifuge tube. The reaction was started by addition of 25 ml of the colorimetric assay substrate solution, mixed by inversion and then incubated for 1-5 minutes. A 900 ml of the stop solution was added and the tube was inverted. A 10 ml aliquot of the catalase enzymatic reaction mixture was added to another microcentrifuge tube and 1 ml of the colour reagent
was added. The mixture was left for 15 minutes at room temperature for colour development and the absorbance was measured at 520 nm.

3.2.5.8. Superoxide dismutase (SOD) assay

The reaction mixture comprised of 1 ml of working solution (WST) diluted with 19 ml of buffer solution, centrifuged enzyme solution tube for 5 sec then mixed by pipeting, and 15 μl of enzyme solution was diluted with 2.5 ml of dilution buffer, superoxide dismutase (SOD) solution, where SOD is diluted with dilution buffer to prepare SOD standard solution as follows: 200 U/ml, 100 U/ml, 50 U/ml, 20 U/ml, 10 U/ml, 5 U/ml, 1 U/ml, 0.1 U/ml, 0.05 U/ml, 0.01 U/ml, 0.001 U/ml. A 20 μl of sample solution was added to each sample and blank 2 well, and 20 μl of ddH₂O (double distilled water) was added to each blank 1 and blank 3 well. A 200 ml of WST was added to each well and mixed. To each blank 2 and blank 3 well, 20 ml of dilution buffer was added. Then 20 ml of enzyme working solution was added to each sample and blank 1 well, and then mixed thoroughly. After incubating the plate at 37 °C for 20 min, absorbance was read at 450 nm using a microplate reader. The SOD activity (inhibition rate %) was calculated using the following equation:

\[ \text{SOD activity (inhibition rate \%) = } \frac{[(A_{\text{blank 1}} - A_{\text{blank 3}}) - A_{\text{sample}} - A_{\text{blank 2}}]}{(A_{\text{blank 1}} - A_{\text{blank 3}})} \times 100 \]

3.3. Statistical analysis

Data for proximate composition, fatty acid profiles, in vivo and in vitro antioxidant parameters were analysed using the Proc Glm procedure of SAS (2003). Comparisons of means were analysed using the Tukey’s HSD procedure in SAS (2003). The statistical model used was as follows:

\[ Y_{ij} = \mu + \alpha_i + \epsilon_{ij} \]
Where $Y_{ij}$ is the proximate component, fatty acid profiles and AA variable

$\mu$ is the overall mean

$\alpha_i$ is the effect of dietary supplementation

$E_{ij}$ is the random error

In the model it was assumed that the data was normally distributed, with sum of errors equal to zero (0) and variances were identical ($I\sigma^2$).
3.4. Results and Discussion

The average slaughter weights for goats supplemented with *Moringa oleifera* leaves, sunflower cake and grass hay were 20.55, 20.75 and 17.46, respectively. The chemical composition, fatty acid profiles, antioxidant potential, glutathione and enzymatic antioxidants of meat from goats supplemented with *M. oleifera* leaves, sunflower cake and grass hay are shown in Tables 3.4, 3.5, 3.6 and 3.7, respectively.

The nutritional and fatty acid profiles of meat from goats are presented in Tables 3.4 and 3.5, respectively. The fatty acid profiles reported in this study are within the ranges reported by Muchenje *et al.* (2009a, b). The proportions of SFA and n-3 and MUFA/SFA and n-6/n-3 ratios, from samples of LTL muscle, were affected by supplementation. The proportion of SFA was higher in Moringa meat samples than in sunflower cake and grass hay meat samples. The ratios of PUFA/SFA in the current study ranged between 0.17 and 0.41 in LTL meat samples. Those values were lower than those reported by Peña *et al.* (2009) in goat kids. The ratio between n-6 and n-3 fatty acids has important roles in reducing the risk of coronary heart disease (American Heart Association, 2008).

The proportions of desirable fatty acids (18:0 + MUFA + PUFA) ranged within 74.81% and 76.38%, percentages slightly higher than those recorded by Santos *et al.* (2007). Devendra (1988) reported that unsaturated fatty acids predominate in goat meat (68.5% to 72.3%) The total unsaturated fatty acids of meat from goats supplemented with Moringa, sunflower cake and grass hay were 55.55%, 57.56% and 58.15% respectively. These values were similar to the findings of Eastridge (1990), who reported 50%, and were lower than those reported by the U.S. Handbook (1989) value of 69%. The presence of polyunsaturated fatty acids, such as, linoleic and arachidonic acids found in red meats and poultry undergo the greatest oxidative damage during storage (Lai *et al*., 1991).
Table 3. 4: Chemical composition and standard errors of *Muscularis longissimus thoracis et lumborum* muscle of goats supplemented with *Moringa oleifera* leaves, sunflower cake and grass hay diets.

<table>
<thead>
<tr>
<th>Nutrients</th>
<th>Grass hay</th>
<th><em>M. oleifera</em></th>
<th>sunflower cake</th>
<th>S.E</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moisture (%)</td>
<td>76.73&lt;sup&gt;b&lt;/sup&gt;</td>
<td>74.33&lt;sup&gt;a&lt;/sup&gt;</td>
<td>74.53&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.389</td>
</tr>
<tr>
<td>Protein content (%)</td>
<td>21.20&lt;sup&gt;a&lt;/sup&gt;</td>
<td>23.57&lt;sup&gt;b&lt;/sup&gt;</td>
<td>22.95&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.411</td>
</tr>
<tr>
<td>Fat content (%)</td>
<td>1.13&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.39&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.42&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.154</td>
</tr>
<tr>
<td>Cholesterol (mg/100g)</td>
<td>35.63&lt;sup&gt;a&lt;/sup&gt;</td>
<td>38.76&lt;sup&gt;a&lt;/sup&gt;</td>
<td>42.84&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.96</td>
</tr>
<tr>
<td>Ash (%)</td>
<td>1.29&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.62&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1.38&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.142</td>
</tr>
</tbody>
</table>

Standard errors of mean (n=6)

<sup>abc</sup> Means in the same row, with different superscript differ significantly (P< 0.05).
Table 3.5: Least square means and standard errors of fatty acid composition in percentage from the *longissimus thoracis et lumborum* meat from goats supplemented with *Moringa oleifera* leaves, sunflower cake and grass hay.

<table>
<thead>
<tr>
<th>Fatty acid (% total fatty acid)</th>
<th>Grass hay</th>
<th><em>M. oleifera</em> leaves</th>
<th>Sunflower cake</th>
</tr>
</thead>
<tbody>
<tr>
<td>C12:0</td>
<td>0.10</td>
<td>0.07</td>
<td>0.06</td>
</tr>
<tr>
<td>C14:0</td>
<td>1.78±0.123</td>
<td>1.80±0.107</td>
<td>2.52±0.107b</td>
</tr>
<tr>
<td>C15:0</td>
<td>0.62±0.033b</td>
<td>0.42±0.028a</td>
<td>0.49±0.028a</td>
</tr>
<tr>
<td>C16:0</td>
<td>21.72±0.545a</td>
<td>21.55±0.472a</td>
<td>21.64±0.470a</td>
</tr>
<tr>
<td>C17:0</td>
<td>1.40±0.079a</td>
<td>1.28±0.680a</td>
<td>1.38±0.068a</td>
</tr>
<tr>
<td>C18:0</td>
<td>19.21±0.838a</td>
<td>18.23±0.726a</td>
<td>17.30±0.726a</td>
</tr>
<tr>
<td>C20:0</td>
<td>0.21±0.007b</td>
<td>0.08±0.006a</td>
<td>0.06±0.006a</td>
</tr>
<tr>
<td>C21:0</td>
<td>1.94±0.069b</td>
<td>0.64±0.059a</td>
<td>0.70±0.059a</td>
</tr>
<tr>
<td>C22:0</td>
<td>0.93±0.024b</td>
<td>0.42±0.021a</td>
<td>0.49±0.021a</td>
</tr>
</tbody>
</table>

Total saturated fatty acids (SFA) 47.91±1.152a 44.49±0.998a 44.64±0.998a

C14:1c9                                        | 0.06±0.008a | 0.06±0.007a          | 0.07±0.007a   |
C16:1c9                                        | 1.67±0.097a | 1.87±0.084b          | 2.13±0.084b   |
C17:1c10                                       | 0.02±0.126a | 0.23±0.109b          | 0.09±0.009a   |
C18:1c11                                       | 0.17±0.016a | 0.15±0.011a          | 0.18±0.011a   |
C18:1c9                                        | 33.45±1.522a| 41.24±1.319b         | 46.12±1.319b  |
C18:1111                                       | 1.25±0.026a | 1.16±0.021a          | 1.18±0.021a   |

Total Monounsaturated Fatty Acids (MUFA) 36.62±1.355a 44.71±1.174b 49.77±1.174b

C18:2c9,12(n-6)                                 | 5.11±0.252a | 3.52±0.219a          | 3.02±0.219a   |
C18:3c9,12(n-6)                                 | 0.03±0.004a | 0.03±0.003a          | 0.04±0.003a   |
C18:2c9,11(n-6)(CLA)                            | 0.15±0.007b | 0.12±0.006a          | 0.11±0.006a   |
C20:2c11,14(n-6)                                | 0.55±0.027a | 0.51±0.024a          | 0.49±0.024a   |
C20:3c8,11,14(n-6)                              | 0.03±0.003a | 0.02±0.002a          | 0.01±0.002a   |
C20:4c5,8,11,14(n-6)                            | 5.45±0.212a | 3.59±0.184b          | 1.65±0.184a   |
C22:2c13,16(n-6)                                | 0.03±0.212a | 0.02±0.01a           | 0.01±0.01a    |

Total omega-6 fatty acids (n-6) 11.35±0.926b 7.81±0.802a 5.33±0.802a

Total omega-3 fatty acids (n-3) 7.58±0.712b 5.63±0.617b 2.46±0.617a

Total polyunsaturated fatty acids (PUFA) 18.93±0.539c 13.44±0.463b 7.79±0.467a

PUFA/SFA                                        | 0.41±0.016b | 0.30±0.014b          | 0.17±0.014a   |

PUFA/MUFA                                       | 0.52±0.012b | 0.30±0.026a          | 0.16±0.014a   |

n-6/n-3                                        | 1.50±0.092a | 1.39±0.080a          | 2.17±0.080b   |

18:0 + 18:1/16:0                                | 2.42±0.09a  | 2.80±0.05b           | 2.93±0.04a    |

Standard errors of mean (n=6)

abc Means in the same row, with different superscript differ significantly (P< 0.05)
aRatio of polyunsaturated fatty acids and saturated fatty acids.

bRatio of polyunsaturated fatty acids and saturated fatty acids.

cRatio of n-6 and n-3 fatty acids.
Table 3. 6: Antioxidant potential (lsmeans ±se) of *Muscularis longissimus thoracis et Lumborum* meat from goats supplemented *Moringa oleifera* leaves, sunflower cake and grass hay.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>DPPH (%)</th>
<th>ABTS (%)</th>
<th>Reducing power</th>
<th>Total phenolics (mg of gallic acid/g of meat)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>M. oleifera</em> leaves</td>
<td>58.95 ± 0.30&lt;sup&gt;c&lt;/sup&gt;</td>
<td>93.51 ± 0.19&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.44 ± 0.01&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1.62 ± 0.27&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Sunflower cake</td>
<td>38.35 ± 0.30&lt;sup&gt;b&lt;/sup&gt;</td>
<td>91.89 ± 0.19&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.38 ± 0.01&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.51 ± 0.27&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Grass hay</td>
<td>27.09 ± 0.30&lt;sup&gt;a&lt;/sup&gt;</td>
<td>90.15 ± 0.19&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.31 ± 0.01&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.48 ± 0.27&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Standard errors of mean (n=6)

<sup>abc</sup> Means within the same column with different superscripts differ significantly at P<0.05.

DPPH – diphenylpicrylhydrazyl, ABTS - 2, 2 azino-bis (3- ethylbenzothiazoline-6-sulphonic diammonium salt.
Table 3. 7: Activities of glutathione, enzymatic antioxidants and of lipid peroxidation (lsmeans ±se) of *Muscularis longissimus thoracis et lumborum* meat supplemented with *Moringa oleifera* leaves, sunflower cake and grass hay.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>GSH (nm/ml)</th>
<th>CAT (µm/min/ml)</th>
<th>SOD (%)</th>
<th>% of LOI</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>M. oleifera</em> leaves</td>
<td>172.02 ± 2.91&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.13 ± 0.01&lt;sup&gt;c&lt;/sup&gt;</td>
<td>93.13 ± 0.39&lt;sup&gt;c&lt;/sup&gt;</td>
<td>47.90 ± 0.35&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Sunflower cake</td>
<td>128.71 ± 2.91&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.08 ± 0.01&lt;sup&gt;b&lt;/sup&gt;</td>
<td>88.88 ± 0.39&lt;sup&gt;b&lt;/sup&gt;</td>
<td>20.27 ± 0.35&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Grass hay</td>
<td>107.14 ± 2.91&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.02 ± 0.01&lt;sup&gt;a&lt;/sup&gt;</td>
<td>67.53 ± 0.39&lt;sup&gt;a&lt;/sup&gt;</td>
<td>11.03 ± 0.35&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Standard errors of mean (n=6)

<sup>abc</sup>Means within the same column with different superscripts differ significantly at P<0.05.

GSH - glutathione, CAT – catalase, SOD - superoxide dismutase, LOI - lipid oxidation inhibition.
The profile of the long chain fatty acids of goat meat show oleic acid (C18:1) to be the most abundant, with palmitic (C16:0) and stearic acid (C18:0) being relatively high. Similar findings were reported by Casey (1992). Pena et al. (2009) suggested that palmitic acid increases blood cholesterol, stearic acid has no effect, and oleic acid decreases blood cholesterol content. Banskalieva et al. (2000) suggested that the ratio of (C18:0 + C18:1)/C16:0 could be useful in describing the potential health effects of different types of lipids. In the current study the ratios were 2.80 for Moringa supplemented meat samples, 2.93 for sunflower cake and for 2.42 grass hay.

The cholesterol levels, proximate analysis and mineral content from goats supplemented as described in this study are presented in Table 3.4, with the Moringa and sunflower cake supplemented LTL samples having lower (P < 0.05) moisture content, higher (P < 0.05) crude protein than the LTL samples from goats supplemented grass hay. Meat from goats supplemented with grass hay had the least (P < 0.05) protein content. Cholesterol levels were similar (P > 0.05) for meat supplemented with Moringa and sunflower cake. The fat content of meat samples from Moringa and sunflower cake supplemented goats were similar (P > 0.05) and were lower for grass hay. Cholesterol can be both good and bad for food consumers. Abnormally high levels of cholesterol and abnormal proportions of low-density lipoproteins (LDL) and high-density lipoproteins (HDL) are associated with cardiovascular diseases. Meat with high levels of intra-muscular fat (IMF) has high levels of cholesterol (Muchenje et al., 2009b). In addition, plasma cholesterol levels are influenced by the fatty acid composition of the diet (Flynn et al., 1985; Muchenje et al., 2009b), with high levels of some long-chain SFA’s such as lauric (C12:0), myristic (C14:0) and palmitic acid (C16:0) increasing serum cholesterol levels (Grundy and Denke, 1990; Rowe et al., 1999).
Both Tables 3.6 and 3.7 show that meat from goats supplemented with Moringa leaves had the highest ($P<0.05$) AA while those supplemented with grass hay had the lowest ($P<0.05$) AA. The high antioxidant activity of meat from Moringa-supplemented goats could be attributed to vitamins and antioxidant compounds available in Moringa such as the large amount of Vitamin E (113 mg per 100 g of dried leaf powder) (Moringa oleifera: The Miracle Tree, 2006; Dolcas Biotech LLC, 2008) which also assist in prevention of meat degradation by oxidation. Sunflower cake is also a good source of crude protein, Vitamin E and B but lacks Vitamin C which largely contributes to free radical scavenging by suppressing the chain initiation reaction and also recycles Vitamin E. Natural antioxidants, in particular polyphenols, are the major plant compounds which have the ability to attenuate the oxidative damage of a tissue indirectly by enhancing natural defences of cell and/or directly by scavenging the free radical species combat pathological disorders generated by phytochemical's Reactive Oxygen Species (ROS) (Du et al., 2010).

DPPH radical scavenging activity was quantified in terms of percentage inhibition of a pre-formed free radical by antioxidants in each sample. There was a significant variation in the percentage inhibition of the DPPH radical by the diets (27-59% inhibition) (Table 3.6). Meat from goats supplemented with Moringa leaves exhibited the highest (58.95%) antioxidant capacity with the diet inhibiting the radical, followed by sunflower cake (38.35%). Concurrently, grass hay also had a considerably lower DPPH scavenging capacity (27.09%). Interaction of antioxidants with DPPH, either the transfer of an electron or a hydrogen atom to DPPH, neutralises its free radical character (Naik et al., 2003). In addition, efficacies of antioxidants are often associated with their ability to scavenge stable free radicals (Sreelatha and Padma, 2009). Therefore, the high DPPH scavenging ability of the meat extract from goats supplemented with Moringa may be attributed to its high hydrogen donating ability.
ABTS activity was quantified in terms of percentage inhibition of the ABTS$^+$ radical cation by antioxidants in each sample. There was a significant variation in the percentage inhibition of the diets (90-94% inhibition) (Table 3.6). Moringa was once again the most efficient scavenger of the radical (93.51%). Meat from goats supplemented with sunflower cake and grass hay also exhibited high scavenging activity (92-90) respectively. The ABTS is a stable free radical cation applicable to both lipophilic and hydrophilic antioxidants that has been used to measure total antioxidative activity (Kim and Lee, 2009). Meat samples from all dietary supplementations showed a potential electron donating ability. The fact that meat from goats supplemented with Moringa leaves had the highest ABTS$^+$ reducing activity suggest that these samples had highest potential to donate electrons for neutralizing free radicals.

Variation in the reducing power was observed for all three supplements. The reducing power of various tissues of the three supplements are presented in Table 3.6 where it shows that the best reducing power obtained is significantly higher (p < 0.05) for M. oleifera leaves (0.44) than for sunflower cake (0.38) and grass hay (0.31). It has been found in the present study that M. oleifera leaves exhibit antioxidant properties and may be exploited as an important source of antioxidants. The reducing power of bioactive compounds serves as a significant reflection of antioxidant activity (Sidduraju et al., 2002). Compounds with reducing power indicate that they are electron donors and can reduce the oxidized intermediates of lipid peroxidation processes, so that they can act as primary and secondary antioxidants (Chanda and Dave, 2009).

All three supplements had a significant effect on the lipid oxidation in all meat samples (p ≤ 0.05). Meat from goats supplemented with Moringa leaves had the highest percentage (47) of lipid oxidation inhibition followed by sunflower cake (20) and meat from goats supplemented
with grass hay exhibited the lowest percentage (11) of lipid oxidation inhibition (Table 3.6). The percentage inhibition observed in meat from Moringa supplementation indicates the ability of antioxidant defense mechanism in the system to prevent the formation of excessive free radicals, therefore, Moringa leaves have a potential of minimizing lipid peroxidation in meat. Lipid peroxidation is the process whereby free radicals steal electrons from the lipids in cell membranes, resulting in cell damage. This process proceeds by a free radical chain reaction mechanism. It most often affects polyunsaturated fatty acids, because they contain multiple double bonds in between which lies methylene -CH\_2- groups that possess especially reactive hydrogens. The ability of antioxidants in meat to scavenge free radicals highly depends on the antioxidants the plant possesses, in particular the polyphenols. *In vitro*, there has been evidence that Moringa leaves exert strong and efficient antioxidant activities (Verma *et al.*, 2009). Polyphenols inhibit lipid peroxidation by acting as chain-breaking peroxyl-radical scavengers, and can protect meat from oxidation (Sreelatha and Padma, 2009).

The activity of the enzymes during post mortem processes can be influenced by pre-slaughter treatments, this was observed in this study. As with AA indicators, GSH was highest in meat from goats supplemented with Moringa leaves than sunflower cake and grass hay. Increased levels of GSH indicate the protective role of Moringa leaves in tissues by detoxification of xenobiotics. The GSH is a tripeptide (\(\gamma\)-glutamylcysteinylglycine), a major free thiol in most living cells and is involved in many biological processes such as detoxification of xenobiotics, removal of hydroperoxides, and maintenance of the oxidation state of protein sulphydryls (Akerboom and Sies, 1981). It is an antioxidant and free radical scavenger. This enzyme plays a central protective role against the damaging effects of bacteria, viruses, pollutants and free radicals (Perlmutter, 2010). It is the key antioxidant in animal tissues
(Akerboom and Sies, 1981). A protective role of *M. oleifera* leaf extract in rats has been reported (Kumar and Pari, 2003).

All meat samples from three diets showed a significant source of SOD with Moringa leaves showing (93%), sunflower cake (89%) and grass hay (68%) respectively (Table 3.7). The highest SOD activity in meat from goats supplemented with Moringa leaves indicates its ability to protect the animal body/cells by quenching free radicals so as to maintain meat quality. Curtis and Mortiz (1972) reported SOD as one of the most important enzymes in the enzymatic antioxidant defence system. It is an enzyme that repairs cells and reduces the damage done to them by superoxide convert them to more stable products and terminate radical chain reaction.

Catalase, which is one of the enzymatic antioxidant widely distributed in all animal tissues, was highest in meat from goats supplemented with Moringa leaves (0.13) compared to sunflower cake (0.08) and grass hay (0.02) (Table 3.7). Previous studies have indicated that endogenous antioxidant enzymes, especially catalase, could potentially delay the onset of oxidative rancidity in stored meat (Mei et al., 1994; Pradhan et al., 2000). There is limited information about goats supplementation on antioxidant enzyme activities. Catalase decomposes hydrogen peroxide and helps to protect tissues from highly reactive hydroxyl radicals (Sreelatha and Padma, 2009). This enzyme prevents the generation of hydroxyl radical and protects cellular constituents from oxidative damage in peroxisomes (Kumar and Pari, 2003). In our experiment, the differences among meat samples suggest some supplementation effect on antioxidant activity of goat meat.
3.5. Conclusions

Results from this study demonstrate that meat from goats supplement with Moringa had improved fatty acid composition. The present study also demonstrated that the antioxidative potential of goat meat can be improved by supplementing goats with Moringa leaves. This will ultimately help to preserve goat meat. It is also recommended to determine the effects of different levels of Moringa supplementation on physico-chemical characteristics of meat from different species as presented in Chapter 4.
3.7. References


Dolcas Biotech LLC. (2008). *Moringa oleifera*. info@dolcas-biotech.com


Chapter 4: Effect of dietary mixtures of Moringa (*Moringa oleifera*) leaves, broiler finisher and crushed maize on antioxidative potential and physico-chemical characteristics of breast meat from broilers

By Kumnandi Qwele

Abstract

This study was carried out to determine the effects of dietary mixtures of Moringa (*Moringa oleifera*) leaves and broiler finisher (M-BF); Moringa leaves, broiler finisher and crushed maize (M-BF-CM); broiler finisher and crushed maize (BF-CM); and broiler finisher (BF) on antioxidative potential and physico-chemical characteristics of breast meat from broilers. Antioxidant activity (AA), ultimate pH (pH_u), lightness (L*), redness (a*), yellowness (b*), Warner-Bratzler shear force (WBSF) and cooking loss (CL) were determined in breast meat samples from each group. The AA of the extract was evaluated using ferric reducing power and the radical scavenging activity against 1, 1-diphenyl-2-picrylhydrazyl (DPPH) and 2, 2’-azino-bis-(3-ethylbenzothiazoline-6-sulfonic-acid) (ABTS). Total phenols, flavonoids and proanthocyanidins contents of breast meat were also determined. Similarly, the in vivo antioxidant activity of the extract was evaluated in meat by determining the activity of glutathione (GSH), catalase (CAT), superoxide dismutase (SOD) and lipid peroxidation. Moringa fed samples exhibited the highest phenolics (15.5 ± 0.22) and flavonoids (29.9 ± 0.32) content. There were no significant differences (P > 0.05) observed on the broiler slaughter weight (SLW), pH_u, CL, a* and b* in all the meat samples. The highest carcass weight (CW), L* values were observed in breast samples of M-BF-CM and M-BF respectively. These findings suggest that Moringa supplementation could result in free
radicals inhibition thus enhancing the oxidative stability of meat without affecting the physico-chemical characteristics of meat.

**Keywords:** Natural antioxidants, broiler meat quality, polyphenols, lipid oxidation, enzymes
4.1. Introduction

Poultry meat and its products have a vast consumer market and are making a significant contribution to the supply of quality protein (Mothershaw et al., 2009), vitamins and minerals (Waskar et al., 2009). Chicken accounts for more than 90% of the total poultry population of the world (Biswas et al., 2011). The major parameters considered in the assessment of meat quality are appearance, juiciness, tenderness and flavour (Lawrie and Ledward, 2006; Muchenje et al., 2008; Muchenje et al., 2009a). The presence of adipose tissue as marbling fat between muscle fibre bundles can weaken the structure so that it is broken down more easily during chewing. Thus, marbling increases juiciness, tenderness, and flavour of the meat (Lawrie and Ledward, 2006; Muchenje et al., 2008b; 2009a, b). At buying point, appearance is the major parameter that influences purchase, selection and initial evaluation of meat quality (Waskar et al., 2009). These desirable meat parameters tend to be negatively affected by lipid peroxidation.

Besides health deterioration, lipid peroxidation is also a major cause of meat quality deterioration, affecting colour, flavour, texture and nutritional value (Giannenas et al., 2009). Antioxidants have been discovered to be efficient in diminishing lipid oxidation of meat (Chapter 3). However, the use of natural antioxidants to stabilize meat has gained much attention from consumers because they are considered to be safer than synthetic antioxidants (Jung et al., 2010) such as butylated hydroxytoluene (BHT) and tertiary butyl hydroquinone (TBHQ). Natural antioxidants also have the ability to increase the antioxidant capacity of the plasma and reduce the risk of certain diseases such as cancer, stroke and cardiovascular diseases (Chanda and Dave, 2009). It has also been reported that these natural antioxidants, especially of plant source, have greater application potential for consumer’s acceptability,
palatability, stability and shelf-life of meat products (Jung et al., 2010). One such plant with a potential to be used as an antioxidant is Moringa (*Moringa oleifera*).

*Moringa oleifera* (family: Moringaceae) is native to sub-Himalayan regions of North West India. It is commonly known as horse radish tree drumstick tree and highly recognised for its nutritional and medicinal properties with some useful minerals, vitamins and amino acids. It is the most economically important species of its kind followed by *Moringa stenopetala*. The leaves of the tree have been reported to bear antioxidant activity/contain a higher amount of polyphenols (Sreelatha and Padma, 2009; Verma et al., 2009). The polyphenolics content of the leaves are high comparable to vegetables and fruits of strawberries, hot pepper, carrot and soybean which are high in phenolics, ascorbate, carotene and α-tocopherol respectively (Yang et al., 2006).

A study conducted by Kakengi et al. (2007) revealed high pepsin and total soluble protein in *Moringa oleifera* leaf meal (MOLM). The high pepsin and total soluble protein makes MOLM more suitable to monogastric animals such as poultry. On the other hand, broiler finisher and crushed maize have been used for many years both commercially and communally to supply essential nutrients for growth and to reach maximum weight gain with lowest feed conversion ratio. Because this feed is medicated (Han and Rhee, 2005), there is a need to investigate alternative feed. However, there are limited reports on the effect and antioxidative potential of dietary mixtures of Moringa leaves with feeds such as broiler finisher and crushed maize on the quality of meat from broilers. Therefore the current study was designed to determine the effects of dietary mixtures of Moringa (*Moringa oleifera*) leaves, broiler finisher and crushed maize on antioxidative potential and the physico-chemical characteristics of breast meat from broilers.
4.2. Materials and Methods

4.2.1. Feeding Management of broilers

The study was conducted at Fort Cox College, Middle drift at the Eastern Cape, South Africa. It is situated 547 mm above sea level and is also located 32.45° latitude and 27.02° longitudes. The area receives approximately about 507 mm of rainfall per annum, mostly occurs in summer with the average temperature of 22.9°C. Twenty four day-old White Leghorn male broilers were purchased from an Agricultural Co-operation located in Berlin, South Africa. The birds were randomly assigned to four groups of dietary supplementation with each group having six birds. The first group of the birds were 100% fed broiler finisher (control); the second group, 80% broiler finisher and 20% crushed maize; the third group, 5% Moringa and 95% broiler finisher and the birds from the fourth group were supplemented with 5% Moringa, 80% broiler finisher and 15% crushed maize. Tables 4.1 and 4.2 present the composition and calculated chemical composition of the control diet (broiler finisher); and the percentage composition of broiler finisher, crushed maize and Moringa leaves, respectively. All groups of birds were housed in a brooding house under the same management and environmental conditions. The birds were supplied ad libitum feed and water for four weeks (until slaughter).

Slaughter was done by quickly decapitating the bird’s heads from the neck using a sharp knife. The carcass weights and the weights were measured using a digital scale. After slaughter, fresh breast samples were used. Visible fat was removed. Meat samples were stored at 4 °C for 24 h before assays for antioxidant activity were performed. Ten grams of each meat sample was homogenized. A volume of 10 %w/v homogenate was prepared in 0.05 M phosphate buffer (pH 7) and centrifuged at 12,000 × g for 60 min at 4°C. The supernatant obtained was used for the estimation of total polyphenols, 1, 1-diphenyl-2-
Table 4.1: Composition (%) and calculated chemical composition (%) of the control diet (broiler finisher).

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Inclusion %</th>
</tr>
</thead>
<tbody>
<tr>
<td>White maize</td>
<td>70.60</td>
</tr>
<tr>
<td>Soyabean meal</td>
<td>17.40</td>
</tr>
<tr>
<td>Sunflower cake</td>
<td>6.30</td>
</tr>
<tr>
<td>Fullfat soya</td>
<td>2.70</td>
</tr>
<tr>
<td>Limestone</td>
<td>1.78</td>
</tr>
<tr>
<td>Methionine</td>
<td>0.06</td>
</tr>
<tr>
<td>Lysine</td>
<td>0.11</td>
</tr>
<tr>
<td>Mono calcium phosphate</td>
<td>0.65</td>
</tr>
<tr>
<td>Salt</td>
<td>0.15</td>
</tr>
<tr>
<td>Premix*</td>
<td>0.25</td>
</tr>
<tr>
<td>Total</td>
<td>100.00</td>
</tr>
</tbody>
</table>

**Calculated chemical composition**

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Crude protein (%)</td>
<td>16.20</td>
</tr>
<tr>
<td>Calcium (%)</td>
<td>1.10</td>
</tr>
<tr>
<td>Phosphate (non phytate) (%)</td>
<td>0.46</td>
</tr>
<tr>
<td>Metabolizable energy (kcal/kg)</td>
<td>3100.00</td>
</tr>
</tbody>
</table>

*Vitamin/trace mineral premix/kg diet: Vitamin A - 12 000 IU; vitamin D3 - 1 500 IU; vitamin E - 50 mg; vitamin K3 - 5 mg; vitamin B1 - 3 mg; vitamin B2 - 6 mg; vitamin B6 - 5 mg; vitamin B12 - 0.03 mg; niacin - 25 mg; Ca-D-pantothenate - 12 mg; folic acid - 1 mg; D-biotin - 0.05 mg; apo-carotenoic acid ester - 2.5 mg; choline chloride - 400 mg; Mn - 80 mg; Fe - 60 mg; Zn – 60 mg; Cu - 5 mg; Co - 0.20 mg; I - 1 mg; Se - 0.15 mg.
Table 4.2: Percentage composition of broiler finisher, crushed maize and *Moringa oleifera* leaves diet.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>BF</th>
<th>CM</th>
<th>MO</th>
<th>T</th>
</tr>
</thead>
<tbody>
<tr>
<td>BF</td>
<td>100</td>
<td>0</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td>BF-CM</td>
<td>80</td>
<td>20</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td>M-BF</td>
<td>95</td>
<td>0</td>
<td>5</td>
<td>100</td>
</tr>
<tr>
<td>M-BF-CM</td>
<td>80</td>
<td>15</td>
<td>5</td>
<td>100</td>
</tr>
</tbody>
</table>

BF = Broiler finisher, CM = Crushed maize, MO = *Moringa oleifera*, T = Total.
picrylhydrazyl (DPPH), ABTS, lipid peroxidation, glutathione, catalase and superoxide dismutase. Broiler physico-chemical meat quality characteristics were also determined.

4.2.2. Phenol content determination

The total phenol content was determined by the Folin-Ciocalteau method (Subramanian et al., 1965) as described in Chapter 3.

4.2.3. Total flavonoids

The method of Ordon Ez et al. (2006) was used to determine the total flavonoid contents of the tissue. A volume of 0.5 ml of 2 % AlCl₃ ethanol solution was added to 0.5 ml of the supernatant. After 1 h of incubation at the room temperature, the absorbance was measured at 420 nm using UV-VIS spectrophotometer (Hewlett Packard, UV/visible light). A yellow colour indicated the presence of flavonoids. All determinations were done in triplicate and the total flavonoids content was calculated as quercetin (mg/g) using the following equation based on the calibration curve: $Y = 0.0255x$, $R^2 = 0.9812$, where $x$ was the absorbance and $Y$ was the quercetin equivalent (mg/g).

4.2.4. Total proanthocyanidins

Total proanthocyanidins was determined based on the procedure of Sun et al. (1998). A volume of 0.5 ml of 0.1 mg/ml of the supernatant was mixed with 3 ml of 4% vanillin-methanol solution and 1.5 ml hydrochloric acid then the mixture was vortexed. The absorbance of resulting mixture was measured at 500 nm after 15 min at room temperature. Total proanthocyanidin content was expressed as catechin equivalents (mg/g) using the
following equation from the calibration curve: \( Y = 0.5825x, R^2 = 0.9277 \), where \( x \) was the absorbance and \( Y \) the catechin equivalent (mg/g).

**4.2.5. DPPH radical scavenging activity**

DPPH radical scavenging activity was estimated according to the method of Blois (1958) with slight modifications as described in Chapter 3.

**4.2.6. ABTS\(^+\) reducing activity**

The free radical scavenging activity was determined by ABTS radical cation decolorization assay described by Erel (2004) (Chapter 3).

**4.2.7. Reducing power**

The reducing power of meat extract was evaluated according to the method of Oyaizu (1986) as described in Chapter 3.

**4.2.8. Lipid peroxidation assay**

Lipid oxidation was measured as increases in thiobarbituric acid-reactive substances (TBARS) (Ohkowa et al., 1979). Briefly, an egg-yolk homogenate was used as an egg rich media (Ruberto et al., 2000). A volume of 0.5 mL of egg yolk prepared in distilled water (10% v/v) and 0.1 mL of extract were mixed in a test tube and the volume was made up to 1 mL, by adding distilled water. Then 0.05 ml of FeSO\(_4\) (0.07 M) was added to the above mixture and incubated for 30 min to induce lipid peroxidation. Finally, 1.5 mL of 20% acetic acid (pH adjusted to 3.5 with NaOH) and 1.5 mL of 0.8% TBA (w/v) (prepared in 1.1% sodium dodecyl sulphate) and 0.05 mL 20% TCA was added. Tubes were heated (90 °C) in a boiling water bath for 60 min then cooled. After cooling, 5.0 mL of 1-butanol was added to each tube.
and then centrifuged at 3000×g for 10 min. Absorbance of the supernatant was measured at 532 nm with a spectrophotometer (Hewlett Packard, UV/visible light). Distilled water (0.1 mL) was used in place of the extract for the blank.

4.2.9. Glutathione (GSH) assay, Catalase (CAT) activity and Superoxide dismutase (SOD) assays

Glutathione, catalase and superoxide dismutase in meat were determined as described in Chapter 3.

4.2.10. Broiler meat quality

4.2.10.1. pH changes in breast muscle

After slaughter, breast samples were cut from each bird using a knife and they were stored at 4°C before the pH measurements were taken. The post-mortem pH was determined on the breast muscle of each bird using a digital pH meter with a piercing electrode. It was measured 24 hrs after slaughter. The measurement was carried out using a portable pH meter (CRISON pH25, CRISON Instruments SA, Spain). The pH meter was calibrated using pH 4, pH 7 and pH 9 standard solutions (CRISON Instruments, SA, Spain) before the measurements.

4.2.10.2. Colour (lightness, L*; redness, a*; yellowness, b*)

Colour of the meat (L* = Lightness, a* = Redness and b* = Yellowness) was determined on the breast 24 hrs after slaughter using a colour-guide 45/0 BYK-Gardener GmbH machine, with a 20 mm diameter measurement area and illuminant D65-day light, 10° standard observer. 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 guide was calibrated before each day’s measurements using the green standard.

4.2.10.3. Warner bratzler shear force (WBSF)

Triplicate 10 mm thick sub samples were cut from the centre of each breast muscle after measurement of cooking loss. The samples were sheared perpendicular to the fibre direction rather than across using a Warner Bratzler (WB) shear device mounted on an Instron (Model 3344) Universal testing apparatus, using a Texture Analyser TA-XT2 (Texture Technologies Corp., Scarsdale, NY, USA) (cross head speed at 400mm/min, one shear in the centre of each core). Maximum force measured to cut the core was expressed as kilogram (kg). For each steak, three cores were taken and the average of the maximum forces was used for data analysis.

4.2.10.4. Cooking loss

Cooking loss was determined in breast meat samples placed inside plastic bags in a water bath and cooked at 70°C for 90 min. Samples were cooled at room temperature and reweighed. Cooking loss (%) was calculated as follows:

\[
\text{Cooking loss (\%)} = \frac{\text{Weight before cooked} - \text{weight after cooked}}{\text{Weight before cooked}} \times 100
\]

4.2.11. Statistical analysis

The PROC GLM procedure of SAS (2003) was used to analyse in vivo and in vitro antioxidant parameters, the effect of diet on slaughter weight, carcass weight, pH, L*, a*, b*,
WBSF values, and cooking loss. Comparisons of means were analyzed using the Tukey’s HSD procedure in SAS (2003).

The model used was:

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

Where \( Y_{ij} \) = AA variable, slaughter weight, carcass weight, pH, L*, a*, b*, WBSF values, and cooking loss.

\( \mu \) is the overall mean

\( \alpha_i \) is the effect of dietary supplementation

\( E_{ij} \) is the random error

In the model it was assumed that the data was normally distributed, with sum of errors equal to zero (0) and variances were identical (\( I\sigma^2 \)).
4.3. Results and Discussion

The polyphenolic content, antioxidant potential, glutathione and enzymatic antioxidants and meat quality attributes of breast meat from broilers supplemented with broiler finisher, mixture of broiler finisher and crushed maize, *Moringa oleifera* leaves and broiler finisher, mixture of Moringa leaves, broiler finisher and crushed maize are shown in Tables 4.3, 4.4, 4.5 and 4.6 respectively.

4.3.1. Total Polyphenols

The polyphenolic contents of the leaf extracts of *M. oleifera* is shown in Table 3.3 (Chapter 3). The total phenolic contents in breast meat from broilers fed with BF, BF-CM, M-BF and M-BF-CM are presented in Table 4.1. The phenols compound were highest (P < 0.05) in breast meat of broilers fed with M-BF-CM followed by M-BF, BF and BF-CM. The flavonoids content was highest in breast meat of broilers fed M-BF-CM followed by M-BF, BF and BF-CM respectively. The breast meat from broilers fed with M-BF had the highest concentration of proanthocyanidins followed by BF, BF-CM and M-BF-CM. Findings in this study corroborated with other reports on the leaves of Moringa which suggest that Moringa can be used as an antioxidant to scavenge free radicals in order to prevent chronic, arterial and cardiovascular diseases (Iqbal and Bhanger, 2006; Kumar and Pari, 2003; Sreelatha and Padma, 2009; Verma *et al.*, 2009). Sreelatha and Padma (2009) also observed higher levels of total phenolics and total flavonoids in antioxidant activity of *Moringa oleifera* leaves in two stages of maturity. Similarly, higher amount of phenols were observed in antioxidant properties of different fractions of *M. oleifera* leaves (Verma *et al.*, 2009).

Among phytochemicals, flavonoids and phenols have been demonstrated to have significant antioxidant activity (Sreelatha & Padma, 2009). Phenols are well known to have anti-
Table 4. 3: Polyphenol contents (mg gallic acid equivalent/g meat) of breast meat from broilers supplemented with broiler finisher, mixture of broiler finisher and crushed maize, *Moringa oleifera* leaves and broiler finisher, mixture of Moringa leaves, broiler finisher and crushed maize.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Phenols</th>
<th>Flavonoids</th>
<th>Proanthocyanidins</th>
</tr>
</thead>
<tbody>
<tr>
<td>BF</td>
<td>12.0 ± 0.12&lt;sup&gt;a&lt;/sup&gt;</td>
<td>23.9 ± 0.28&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.5 ± 0.01&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>BF-CM</td>
<td>11.1 ± 0.15&lt;sup&gt;a&lt;/sup&gt;</td>
<td>23.7 ± 0.12&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.4 ± 0.02&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>M-BF</td>
<td>14.1 ± 0.36&lt;sup&gt;b&lt;/sup&gt;</td>
<td>26.7 ± 0.38&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.5 ± 0.02&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>M-BF-CM</td>
<td>15.5 ± 0.22&lt;sup&gt;c&lt;/sup&gt;</td>
<td>29.9 ± 0.32&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.4 ± 0.01&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Standard errors of mean (n=6).

Phenols are expressed as mg tannic acid/g of meat.

Flavonoids are expressed as mg quercetin/g of meat.

Proanthocyanidins are expressed as mg quercetin/g of meat.

<sup>abc</sup> Means with different letters within the same column significantly differ at p < 0.05.

inflammatory (Ezeamuzle et al., 1996; Jyotsna et al., 2007) and to prevent oxidative damage caused by Reactive Oxygen Species (ROS) in the tissues, DNA (Chanda and Dave, 2009) RNA, enzymes and proteins (Jyotsna Mishra et al., 2007). Flavonoids have also been reported to scavenge free radicals and combat pathological disorders generated by ROS (Jyotsna Mishra et al., 2007).

4.3.2. Antioxidative activity

The DPPH results are presented in Table 4.4. The DPPH was highest in BF-CM–fed broilers followed by BF then M-BF-CM and M-BF. Even though breast samples that were supplemented with Moringa leaves showed the lowest DPPH free radical scavenging activity, it did show proton-donating ability and could serve as free radical inhibitors or scavengers that can be used as antioxidants. The efficacies of antioxidants are often associated with their ability to scavenge stable free radicals of DPPH by donating electron to the unpaired valence electron at one atom of nitrogen bridge (Krishnaraju et al., 2009; Sharma and Bhat, 2008). The stable radical DPPH has been used widely for the determination of antioxidant activity. When it is mixed with antioxidants it donates hydrogen atom to form a stable DPPH-H (2,2-diphenyl-1-picrylhydrazine). The degree of discoloration indicates the scavenging potential of the antioxidant extract, which is due to the hydrogen donating or radical scavenging ability (Adedapo et al., 2008).

All supplements showed effective scavenging activity against ABTS radical as shown in Table 4.4. Meat from the M-BF-fed broilers produced the highest percentage inhibition followed by M-BF-CM then BF and BF-CM respectively. The high inhibitory concentration
Table 4.4: Antioxidative potential of breast meat from broilers supplemented with broiler finisher, mixture of broiler finisher and crushed maize, *Moringa oleifera* leaves and broiler finisher, mixture of *Moringa* leaves, broiler finisher and crushed maize.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>DPPH (%)</th>
<th>ABTS (%)</th>
<th>Reducing power</th>
<th>% of LOI (mg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BF</td>
<td>66.4 ± 0.45c</td>
<td>43.9 ± 0.67b</td>
<td>0.6 ± 0.01a</td>
<td>0.3 ± 0.01c</td>
</tr>
<tr>
<td>BF-CM</td>
<td>68.3 ± 0.45d</td>
<td>27.9 ± 0.67a</td>
<td>0.6 ± 0.01a</td>
<td>0.2 ± 0.01b</td>
</tr>
<tr>
<td>M-BF</td>
<td>57.2 ± 0.45a</td>
<td>68.6 ± 0.67d</td>
<td>0.7 ± 0.01b</td>
<td>0.1 ± 0.01a</td>
</tr>
<tr>
<td>M-BF-CM</td>
<td>62.3 ± 0.45b</td>
<td>52.4 ± 0.67c</td>
<td>0.7 ± 0.01b</td>
<td>0.1 ± 0.01a</td>
</tr>
</tbody>
</table>

Standard errors of mean (n=6)

abcd Means within the same column with different superscripts differ significantly at P<0.05.

BF = Broiler finisher, BF-CM = Broiler finisher & Crushed maize, M-BF = Moringa & Broiler finisher, M-BF-CM = Moringa+ Broiler finisher & Crushed maize

DPPH - diphenylpicrylhydrazyl, ABTS - 2, 2 azino-bis (3- ethylbenzothiazoline-6-sulphonic diammonium salt, LOI - lipid oxidation inhibition.
in breast samples of broilers supplemented with M-BF and M-BF-CM could be attributed to the presence of polyphenolics compounds in Moringa leaves (Sreelatha and Padma, 2009).

Even though DPPH and ABTS\(^+\) are both radical scavenging assays, percentage inhibition will vary due to different methods of preparation. The DPPH radical is stable at formation whereas the ABTS\(^+\) assay allows formation of a radical which remains stable for several days due to the optimised pH (Cano et al., 1998), hence lower antioxidant scavenging activity. Wootton-Beard et al. (2010) reported a weak correlation between DPPH and ABTS\(^+\) assays.

The reducing power of meat from M-BF-CM and M-BF-fed broilers was significantly (P < 0.05) higher than BF and BF-CM (Table 4.4). The presence of antioxidants in meat causes the reduction of Fe\(^{3+}\)/Ferric cyanide complex to the ferrous form (Fe\(^{2+}\)) (Chung et al., 2002). Higher absorbance exhibited in meat from M-BF-CM and M-BF-fed broilers is due to the high polyphenolic content in Moringa leaves. Results of scavenging activity also suggest the ability of Moringa supplement to minimize oxidative damage to some vital tissues in the body (Kojic et al., 1998; Weighand et al., 1999). Additionally, it has been reported that the reducing power of bioactive compounds is directly related to its antioxidant activity (Iqbal and Bhanger, 2005).

In the present investigation, levels of lipid peroxides in breast meat of broilers supplemented with BF were significantly high followed by BF-CM then M-BF and M-BF-CM (Table 4.4). The decrease in lipid peroxidation level in breast meat indicates the role of *Moringa oleifera* leaves as an antioxidant where meat from M-BF and M-BF-CM-fed showed higher inhibition than BF and BF-CM (Sreelatha and Padma, 2009). This is in agreement with the study conducted by Kumar and Pari (2003), who observed that *Moringa oleifera* inhibited lipid peroxidation against anti-tubercular drugs induced lipid peroxidation in rats. Previous studies
have shown that the negative outcome of lipid oxidation in chicken meat and eggs was diminished by the use of diets containing antioxidants such as medicinal herb mix (Jung et al., 2010).

4.3.3. Glutathione and enzymatic antioxidants

The levels of tissue glutathione in breast were significantly higher (P < 0.05) in breast meat from broilers supplemented with M-BF-CM followed by M-BF, BF-CM and BF (Table 4.5). Thus Moringa have demonstrated to have a protective role against oxidative damage and can be used as an antioxidant to inhibit tissue injury. Glutathione is a non-protein in thiol cells in a living organism which is responsible for cellular oxygen defence (Jyotsna Mishra et al., 2007). Administration of thiol compounds such as glutathione, cysteine and methionine have been shown to protect against oxidative stress in humans and animals (Krishnaraju et al., 2009).

Higher activity of catalase was observed in Moringa supplemented broiler meat samples compared to BF and BF-CM (Table 4.5). These results indicate that M-BF and M-BF-CM are able to protect meat against lipid peroxidation and ROS due to antioxidant property of Moringa. Catalase is an enzymatic antioxidant located in the mitochondria and cytosol of a living organism which is responsible for the removal of hydrogen peroxides (H$_2$O$_2$) (Jyotsna Mishra et al., 2007).
Table 4.5: Activities of glutathione and enzymatic antioxidants of breast meat from broiler supplemented with broiler finisher, mixture of broiler finisher and crushed maize, *Moringa oleifera* leaves and broiler finisher, mixture of *Moringa* leaves, broiler finisher and crushed maize.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>GSH (nm/ml)</th>
<th>CAT (µm/min/ml)</th>
<th>SOD (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BF</td>
<td>97.0 ± 0.77&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.07 ± 0.01&lt;sup&gt;a&lt;/sup&gt;</td>
<td>70.3 ± 0.55&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>BF-CM</td>
<td>99.0 ± 0.77&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.07 ± 0.01&lt;sup&gt;a&lt;/sup&gt;</td>
<td>73.0 ± 0.55&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>M-BF</td>
<td>110.0 ± 0.77&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.08 ± 0.01&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>75.0 ± 0.55&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>M-BF-CM</td>
<td>121.0 ± 0.77&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0.09 ± 0.01&lt;sup&gt;b&lt;/sup&gt;</td>
<td>79.0 ± 0.55&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Standard errors of mean (n=6)

<sup>abcd</sup>Means within the same column with different superscripts differ significantly at P<0.05.

BF = Broiler finisher, BF-CM = Broiler finisher & Crushed maize, M-BF = Moringa & Broiler finisher, M-BF-CM = Moringa+ Broiler finisher & Crushed maize

GSH - Glutathione, CAT - Catalase, SOD - Superoxide dismutase.
Table 4.5 also presents the effect of BF, BF-CM, M-BF and M-BF-CM on the activities of superoxide dismutase. Higher percentage inhibition of superoxide anion in meat from Moringa supplemented broilers was observed which implies an efficient protective mechanism of the plant. Superoxide anion is one of the main reactive oxygen species in the cell (Curtis and Mortiz, 1972) thus superoxide dismutase (SOD) would play a key antioxidant role. Although the fatty acid profiles and antioxidant activities of the Moringa mixtures could not be determined in this study, addition of Moringa leaves in broiler diets increases antioxidant activities in broiler meat with the potential to improve its shelf life.

4.3.4. Carcass characteristics

The effect of diet on SLW and CW are shown in Table 4.6. No significant differences (P > 0.05) were observed in SLW from broilers, although there was a different (P < 0.05) CW. The CW of broilers fed BF was significantly lower (P < 0.05) than broilers fed BF-CM, M-BF and M-BF-CM. High CW from broilers supplemented with M-BF and M-BF-CM could be attributed to high amount of nutrients and antioxidants contained in Moringa (Yang et al., 2006).

4.3.5. Physico - chemical quality characteristics

The pHu values of breast samples are presented in Table 4.6. Dietary supplementation had no effect on pHu. Similar results were reported by Perlo et al. (2010) in broiler breast fillets. When animals are slaughtered glycogen is broken down to glucose and glucose undergoes glycolysis. In the absence of oxygen, lactic acid is produced which is responsible for the drop of muscle pH. Such as drop aid in the conversion of muscle to meat (Muchenje et al., 2009b). Meat pH is vital characteristic that influences the acceptability of meat. Higher meat pH
Table 4. 6: Eat quality characteristics, slaughter weight and carcass weight of broilers supplemented with broiler finisher, mixture of broiler finisher and crushed maize, *Moringa oleifera* leaves and broiler finisher, mixture of Moringa leaves, broiler finisher and crushed maize.

<table>
<thead>
<tr>
<th></th>
<th>BF</th>
<th>BF-CM</th>
<th>M-BF</th>
<th>M-BF-CM</th>
<th>SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>SLW (kg)</td>
<td>4.0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.7&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.7&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.8&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.31</td>
</tr>
<tr>
<td>CW (kg)</td>
<td>2.9&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.5&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3.12&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3.6&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.38</td>
</tr>
<tr>
<td>CL (%)</td>
<td>15.4&lt;sup&gt;b&lt;/sup&gt;</td>
<td>14.3&lt;sup&gt;b&lt;/sup&gt;</td>
<td>12.9&lt;sup&gt;a&lt;/sup&gt;</td>
<td>12.3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.21</td>
</tr>
<tr>
<td>L&lt;sub&gt;24*&lt;sup&gt;24&lt;/sup&gt;&lt;/sub&gt;</td>
<td>49.4&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>48.9&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>47.5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>51.6&lt;sup&gt;b&lt;/sup&gt;</td>
<td>5.93</td>
</tr>
<tr>
<td>a&lt;sub&gt;24*&lt;sup&gt;24&lt;/sup&gt;&lt;/sub&gt;</td>
<td>4.2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.8&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.01&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.8&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.58</td>
</tr>
<tr>
<td>b&lt;sub&gt;24*&lt;sup&gt;24&lt;/sup&gt;&lt;/sub&gt;</td>
<td>18.3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>17.6&lt;sup&gt;a&lt;/sup&gt;</td>
<td>16.5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>17.8&lt;sup&gt;a&lt;/sup&gt;</td>
<td>6.12</td>
</tr>
<tr>
<td>WSBF (N)</td>
<td>14.9&lt;sup&gt;a&lt;/sup&gt;</td>
<td>11.7&lt;sup&gt;a&lt;/sup&gt;</td>
<td>13.3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>16.8&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.03</td>
</tr>
<tr>
<td>pH&lt;sub&gt;24&lt;/sub&gt;</td>
<td>5.7&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5.8&lt;sup&gt;a&lt;/sup&gt;</td>
<td>6.0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5.9&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.44</td>
</tr>
</tbody>
</table>

Means in the same row with different superscript are significantly different (P < 0.05).

BF = Broiler finisher, BF-CM = Broiler finisher & Crushed maize, M-BF = Moringa & Broiler finisher, M-BF-CM = Moringa+ Broiler finisher & Crushed maize

SLW = Slaughter weight, CW = carcass weight, CL = Cooking loss, L* = Lightness, a* = redness, b* = yellowness, WBF = Warner Bratzler Shear Force value.
results in lower L* (lightness), implying that high meat pH meat is darker than normal meat pH (Zhang et al., 2005; Muchenje et al., 2008b).

Meat samples from broilers supplemented with M-BF-CM had the highest L* values while no significant differences (P > 0.05) were observed for a*, b*, WBSF and cooking loss in all meat samples (Table 4.6). High L* values in meat are preferable because the lightness of broiler meat is more attractive and acceptable by consumers. Meat colour is usually associated with factors such as breed (Ekiz et al., 2010; Santos et al., 2007; Muchenje et al., 2008b; 2009a, c), slaughter weight (Martínez-Cerezo et al., 2005) production system and pHu (Ekiz et al., 2010). Colour is an important quality attribute that influences consumer acceptance of poultry meat. Faustman and Cassens (1991) found that meat discoloration was caused by oxidation processes and enzymatic reducing systems. Several studies have reported a significant relationship between colour and pH (Perlo et al., 2010; Mothershawa et al., 2009). Contrary, Muchenje et al. (2008a) reported poor correlations between L* and pHu.
4.4. Conclusions

Results from this study show that dietary supplementation of Moringa formulated diets for broilers was effective in enhancing the oxidative stability of chicken meat, but did not result in differences in the physico-chemical characteristics of meat.
4.5. References


Chapter 5: General Discussion, Conclusions and Recommendations

5.1. General discussion

The objective of the current study was to determine the antioxidant activity and quality of meat from goats and chickens supplemented with Moringa (*Moringa oleifera*) leaves. Eighteen Xhosa lop-eared goats and thirty day-old White Leghorn male broilers were used in this study. Antioxidant activity of meat from goats supplemented with Moringa (*Moringa oleifera*) leaves was determined in Chapter 3. In Chapter 4, the effect of dietary mixture of Moringa (*Moringa oleifera*) leaves, broiler finisher and crushed maize on antioxidative potential and physico-chemical quality attributes of breast meat from broilers were determined.

In Chapter 3, total phenolic content, DPPH free radical scavenging assay, ABTS$^+$ reducing activity, reducing power, lipid peroxidation, glutathione, catalase, superoxide dismutase were determined. Moringa supplemented goat had meat with the highest phenolic content. Meat samples from goats supplemented with Moringa leaves had the highest DPPH and ABTS$^+$ values which signify the ability of Moringa to scavenge and neutralize the action of free radicals. The effect of antioxidants on DPPH is thought to be due to their hydrogen donating ability, since Moringa exhibited proton-donating ability, it is evident that it could serve as free radical inhibitor or scavenger, acting possibly as a primary antioxidant. The observed result obtained in reducing power showed that the meat extract possessed antioxidant activity in a concentration dependent manner. This effect may suggest the ability of Moringa to minimize oxidative damage to some vital tissues in the body (Kojic *et al*., 1998).

Natural products of plant origin have been proposed as a potential source of natural antioxidants with strong activity. The high antioxidant activity of *Moringa oleifera* leaves is
mainly due to the presence of phenolic compounds such as flavonoids, phenols and proanthocyanidins (Rice-Evans et al., 1995) which have been observed in this study.

In Chapter 3, it was concluded that Moringa supplemented goats produced meat with higher lipid oxidation inhibition than those supplemented sunflower cake and hay. However, research need to be done on whether the reduction of oxidative activities in meat can also be accompanied by differences in meat quality of animals supplemented with Moringa.

Work in Chapter 4, therefore, sought to determine the effects of different levels of Moringa-broiler finisher-maize mixtures on the anti-oxidant activities and physico-chemical quality attributes of meat from broilers. Meat samples from broilers supplemented with M-BF and M-BF-CM showed higher lipid oxidation inhibition than BF and BF-CM. Lipid peroxidation depends upon the degree of unsaturation of the fatty acids and the levels of the antioxidant vitamin E (α-tocopherol) and prooxidants such as free iron (Panagiotis and Stelios, 2010). Moringa leaves have been reported to have a high content of vitamin E is a chain breaking antioxidants (Jyotsna Mishra et al., 2007) that contributes in prevention of meat quality degradation. Lipid peroxidation have shown to be inhibited by Moringa oleifera against antitubercular drugs induced lipid peroxidation in rats (Kumar and Pari, 2003).

It was also observed that supplementation in all meat samples had no effect on pHu, a*, b* and WBSF. Meat samples supplemented with M-BF-CM were observed to have the highest L* values and meat samples from M-BF having the lowest.
5.2. Conclusions

The highest antioxidant and activities were observed in all meat samples supplemented with Moringa. Supplementing livestock with Moringa may therefore assist in preserving meat. Moringa had inhibitory effect on lipid oxidation when compared to other treatment groups. Moringa exhibited a protective mechanism against free radicals in enzymatic antioxidants without necessarily affecting the quality of broiler meat.
5.3. Recommendations

Areas that require further research include:

- Since *Moringa oleifera* leaves have been found to possess antioxidant activity it is also important to investigate the anthelmintic effects of the leaves for treatment of diseases. Also, plant derived drug serve as a prototype to develop more effective and less toxic medicines.

- Meat quality is important for consumers when it comes to making purchasing decisions. The wealth of documentable evidence indicates that goat meat (chevon), regardless of age, breed, or region, will supply a high quality protein source along with a healthy fat (increased unsaturated fats/saturated fats ratio) with a minimal cholesterol intake risk. In developed countries chevon has gained acceptance mainly because of its low-fat content and it is, therefore, necessary and important to develop programmes to popularize chevon to consumers in under developed areas.

- Fatty acids especially polyunsaturated fatty acids, such as linoleic and arachidonic acids found in red meats and poultry undergo the greatest oxidative damage during storage. This leads to oxidative rancidity also results in nutritional losses when essential polyunsaturated fatty acids are oxidized and break down into potentially carcinogenic and mutagenic products. Hence important to determine the fatty acid profiles of chicken.
5.4. References


