EFFECT OF MORINGA SEED MEAL SUPPLEMENTATION ON PRODUCTIVITY AND CARCASS CHARACTERISTICS OF ROSS 308 BROILER CHICKENS

MOLEPO LEPHAI SARAH

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BY

MOLEPO LEPHAI SARAH

B.SC AGRIC (ANIMAL SCIENCE) (UNIVERSITY OF VENDA)

A mini-dissertation submitted in partial fulfilment of the requirements for the degree of Master of Science in Agriculture (Animal Production), Department of Agricultural Economics and Animal Production, School of Agricultural and Environmental Sciences, Faculty of Science and Agriculture, University of Limpopo, South Africa

Supervisor : Prof J. W. Ng'ambi

Co-supervisor : Prof D. Norris

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DECLARATION

I declare that the dissertation hereby submitted to the University of Limpopo for the degree of Master of Science in Agriculture (Animal Production) has not previously been submitted by me for a degree at this or any other University, that it is my own work in design and execution, and that all material contained therein has been duly acknowledged.

Signature---------- Date ----------

Molepo Lephai Sarah

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DEDICATION

To my loving parents and supervisors whose prayers, encouragements and cooperation have enabled me to complete this work.

ABSTRACT

Two experiments were conducted to determine the effect of moringa seed meal supplementation on productivity and carcass characteristics of Ross 308 broiler chickens. The first experiment determined the effect of moringa seed meal supplementation on productivity of Ross 308 broiler chickens aged one to 21 days. Two hundred and fifty unsexed day-old Ross 308 broiler chicks were randomly allocated to five dietary treatments, replicated five times, and each replication having 10 chickens. A completely randomized design was used. The chickens were fed on a grower diet supplemented with 0 (M_0), 5 (M_5), 10 (M_{10}), 15 (M_{15}) and 20 (M_{20}) g of moringa seed meal/bird/day. Moringa seed meal supplementation had no effect ($P>0.05$) on feed intake, metabolisable energy intake, nitrogen retention, feed conversion ratio and live weight of unsexed Ross 308 broiler chickens. Moringa seed meal supplementation improved ($P<0.05$) growth rates of unsexed Ross 308 broiler chickens aged one to 21 days. A moringa seed meal supplementation level of 13.3 g/kg DM feed optimized growth rate of Ross 308 broiler chickens aged one to 21 days.

The second experiment determined the effect of moringa seed meal supplementation on productivity and carcass characteristics of female Ross 308 broiler chickens aged 22 to 42 days. The chickens weighing 558 ± 10 g/bird were randomly allocated to five treatments with five replications having 10 birds. The chickens, aged 21 days, were allocated to the treatments in a completely randomized design. The chickens were fed on a grower diet supplemented with 0 (FM_0), 5 (FM_5), 10 (FM_{10}), 15 (FM_{15}) and 20 (FM_{20}) g of moringa seed meal per kg DM. Moringa seed meal supplementation had no effect ($P>0.05$) on feed intake, growth rate, feed conversion ratio, live weight, metabolisable energy intake, carcass weight, breast meat weight, abdominal fat pad weight, liver weight, heart weight, thigh weight, meat flavour, juiciness and tenderness of female Ross 308 broiler chickens. However, moringa seed meal supplementation improved ($P<0.05$) nitrogen retention and gizzard weights of female Ross 308 broiler chickens.

It was concluded that moringa seed meal supplementation improved growth rate of unsexed Ross 308 broiler chickens aged one to 21 days. Similarly, moringa seed meal supplementation increased nitrogen retention and gizzard weights of female Ross 308 broiler chickens aged 22 to 42 days.

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CHAPTER 1
INTRODUCTION

1.1 Background

Chickens play an important role in many rural households of South Africa and the world as a whole. They are an important source of income and employment. They contribute substantially to food security among the rural people of Limpopo province (Yami, 1995). Broiler chicken production has developed extensively in the past 50 years and has become the major livestock system in many countries in terms of the quality of the meat produced. Additionally, broiler chicken production and processing technologies have become rapidly accessible and are being implemented worldwide, which allows continued expansion and competitiveness in this meat sector (Aho, 2001). The success of poultry meat production has been strongly related to improvements in growth and carcass yield. However, an improvement in carcass yield has also resulted in excessive carcass fat. This leads to obesity and various metabolic disorders in growing chickens (Richards *et al*., 2003). Excessive carcass fat is not liked by consumers (Appleby *et al*., 1992). Therefore, there is need to reduce fat content in broiler chicken carcasses.

1.2 Problem statement

The broiler chicken industry is one of the fastest growing livestock industries in South Africa. Modern broiler chickens are characterized by very high growth rates and very good feed conversion ratios. On the other hand, incidences of metabolic diseases, high mortalities, leg problems and an increased carcass fat deposition are typical for these selected lines (Leeson, 1997; Tadelle *et al.,* 2001; King'ori *et al.,* 2003;). These negative aspects are of major concern for the producers, because they can bring about important economic losses (Lippens *et al*., 2002). Excessive broiler chicken carcass fat is of concern to producers since it reduces feed efficiency and carcass quality (Oyedeji and Atteh, 2005). Excessive carcass fat may also cause rejection of the meat by the consumers (Macajova *et al., 2003*). There is evidence that moringa (*Moringa oleifera*) seed meal supplementation improves productivity and carcass characteristics of broiler chickens (Vohra, 1972).

1.3 Motivation

Results of this study will indicate the effects of supplementing moringa (moringaceae) seed meal on productivity and carcass characteristics of Ross 308 broiler chickens. Such results will add knowledge on the use of moringa seed meal to manipulate productivity and carcass characteristics of broiler chickens. Such information will be helpful to broiler chicken farmers and poultry industries when formulating diets aimed at reducing mortality and fat deposition, and optimizing productivity of Ross 308 broiler chickens. Optimization of productivity of the chickens will assist to improve socio-economic and nutritional status of broiler chickens farmers.

1.4 Objectives

The broad objective of this study was to determine moringa seed meal supplementation levels for optimal productivity and carcass characteristics of Ross 308 broiler chickens.

The objectives of the study were:

i. To determine the effect of moringa seed meal supplementation on feed intake, digestibility, growth rate, feed conversion ratio, mortality and carcass characteristics of Ross 308 broiler chickens from a day old up to 42 days of age.

ii. To determine moringa seed meal supplementation levels for optimal responses in feed intake, digestibility, growth rate, feed conversion ratio, body weight, mortality and carcass characteristics of Ross 308 broiler chickens aged one to 42 days.

1.5 Hypotheses

The hypotheses of this study were:

i. Moringa seed meal supplementation has no effect on feed intake, digestibility, growth rate, feed conversion ratio, mortality and carcass characteristics of Ross 308 broiler chickens aged one to 42 days.

ii. There are no Moringa seed meal supplementation levels for optimal responses in feed intake, digestibility, growth rate, feed conversion ratio, body weight, mortality and carcass characteristics of Ross 308 broiler chickens aged one to 42 days.

CHAPTER 2
LITERATURE REVIEW

2.1 Introduction

Foliages from drought tolerant multi-purpose trees could be used as alternative protein and energy sources during drought periods of tropical countries. Plants, generally, contain saponins, tannins, oxalates, phytates, trypsin inhibitors and cyanogenic glycosides known as secondary metabolites, which are biologically active (Soetan and Oyewole, 2009). Secondary metabolites may be applied in nutrition and as pharmacologically-active agents (Soetan and Oyewole, 2009). Plants also have high amounts of essential nutrients, vitamins, minerals, fatty acids and fibre (Gafar and Itodo, 2011). Among multi-purpose tree foliages, moringa tree foliages are known for better biomass yield, nutrient composition and drought tolerant in tropical and sub-tropical climates (Sanchez *et al*., 2006).

Moringa (*Moringa oleifera*), according to Makkar and Becker (1997), belongs to the *Moringaceae family*, and is considered to have its origin in the north-west region of India, south of the Himalayan mountains. It is now widely cultivated and has become naturalized in many locations in the tropics (Fahey *et al*., 2001). Kristin (2000) reported that there are thirteen species of moringa trees in the family *moringaceae* and that *Moringa oleifera* is the most widely cultivated species. It was further stated that they are native to India, the Red Sea area and/or parts of Africa including Madagascar. *Moringa oleifera* is indigenous to Northern India and Pakistan (Bosch, 2004) and was introduced throughout the tropics and sub-tropics, becoming naturalized in many African countries.

This rapidly-growing tree also known as horseradish tree or drumstick tree was utilized by the ancient Romans, Greeks and Egyptians (Bosch, 2004). All parts of the moringa tree are edible and have long been consumed by humans. Fuglie (1999) reported the many uses of moringa as follows: alley cropping (biomass production), animal forage (treated seed-cake), biogas (from leaves), domestic cleaning agent (crushed leaves), blue dye (wood), fertilizer (seed-cake), foliar nutrient (juice expressed from the leaves), green manure (from leaves), gum (from tree trunks), honey and sugar cane juice-clarifier (powdered seeds), honey (flower nectar), medicine (all plant parts), ornamental plantings, biopesticide (soil incorporation of leaves to prevent seedling damping off),

pulp (wood), rope (bark), tannins for tanning hides (bark and gum), and water purification (powdered seeds). In tropical countries, forage quality is often too low to meet the nutritional requirement of animals. Furthermore, supplementation with conventional concentrates is generally too costly and the levels of concentrate feeding are therefore low. New low-cost alternatives to commercial concentrates are needed and moringa has been shown to be one possible option. However, the first critical step in its general use in livestock diets is precise and reliable knowledge of its chemical composition, digestibility and nutritional value. Other practical issues in connection with the use of moringa as a feedstuff are the labour requirement and how well it can be conserved. Such information is particularly vital in the current context, where farmers are trying to achieve more sustainable production throughout the year (Aregheore, 2002).

2.2 Nutrient composition of moringa seeds

Moringa seeds have high levels of lipids and proteins, with minor variations. The major saturated fatty acids present in the seeds are palmitic, stearic, arachidic and benic acids (Abdulkarim *et al.,* 2005). Oleic acid is the main unsaturated fatty acid whose high concentration is desirable in terms of nutrition and stability during cooking and frying. Moreover, as a natural source of benic acid, the moringa seed oil is used as a solidifying agent in margarines and other foodstuffs containing solid and semi-solid fat, thus eliminating hydrogenation processes (Makkar and Becker, 1999; Francis *et al.,* 2005). A lot has been established between diets rich in trans-unsaturated and saturated fatty acids and increased risk of cardiovascular diseases caused by high blood cholesterol levels (Pal *et al*., 1995; Makonnen *et al.,* 1997; Ghasi *et al*., 2000; Matthew *et al.,* 2001).

Moringa seeds are also rich in carotenoids, calcium, methionine and cystine but generally deficient iron and minerals, and are used as vitamin A supplement. They are also reported to possess antioxidants, hepatoprotective 8, 9 antihypertensive 10 and hypoglycemic 11 effects. It has the potential as alternative animal feed resources during dry periods. The plant, apart from being a good source of vitamins and amino acids, has

medicinal uses (Makkar and Bekker 1999; Francis *et al,* 2005). *Moringa oleifera*, has also been used in the treatment of numerous diseases (Pal *et al*., 1995; Makomen *et al.*, 1997; Gbasi *et al*, 2000; Matthew *et al.*, 2001) including heart disease and obesity due to its hypocholesterolemic property (Gbasi *et al.*, 2001; Olugbemi *et al. (*2010a) also reported this quality. *Moringa oleifera* leaves have the calcium equivalent of 4 glasses of milk, 3 times the iron of spinach, 4 times the amount of vitamin A in carrot, and 2 times protein in milk (Loren, 2007). Moringa tree has in, recent times, been advocated as an outstanding indigenous source of highly digestible protein, Ca, Fe and carateroids suitable for utilization in many developing regions of the world where undernourishment is a major concern (Oduro *et al*., 2008).

Fully mature, dry seed are round or triangular shaped. The kernel surrounded by a light wooded shell with three paper wings. Moringa seeds contain between 330 and 410 g/kg oil. Moringa seed oil is highly resistant to oxidative rancification, which can explain its several industrial uses such as in the production of cosmetics. It is also used in machinery lubricants, cooking oil, and in the perfume industry due to its high odour retention capacity (Makkar and Becker, 1999; Francis *et al.,* 2005). Moringa seed meal has high essential amino acid contents, except for lysine, threonine and valine which are present in lower levels (Makkar and Becker, 1999; Francis *et al.,* 2005). The high methionine and cysteine contents are close to those of human and cow milk and chicken eggs (Oliveira *et al., 1999*). Inspite of being free of trypsin inhibiting materials and tannins, the seeds contain an acidic protein with haemagglutinating activity, glucosinolates (65.5 µmol/g) and phytates (41 g/kg). Phytate contents of the kernel samples are higher than those in the vegetative parts. Phytates reduce mineral bioavailability in monogastric animals, particularly Zn^{2+} and Ca^{2+}. Potassium and calcium levels are high. Ca helps in transporting of long chain fatty acids, which help in preventing heart diseases and high blood pressure (Anjorin *et al.,* 2010). Phytate reduces bioavailability of minerals (Reddy *et al.,* 1982). Lectins, on the other hand, are usually responsible for agglutinating cells, interacting with intestinal epithelium, interfering with nutrient digestion and absorption and reducing food efficiency (Thompson *et al., 1993)*. The seed's bitter taste is generally attributed to alkaloids,

saponins, cyanogenicglucosides and glucosinolates which are removed by heat treatment, suggesting that this taste would not limit the use of this material in animal diets (Oliveira *et al., 1999*).

The amount of available protein in the seeds may be greater than that of wheat bran, which diminishes the nitrogen loss as ammonia (Oliveira *et al., 1999*). The use of moringa seeds has been suggested to be a viable alternative source of proteins, vitamins and minerals for poultry feeding (Church, 1991). Oduro *et al*. (2008) revealed that moringa leaf meal contains 765.3, 275.1, 192.5, 71.3, 22.3 and 4338 g/kg of dry matter, crude protein, crude fibre, ash, ether extract and nitrogen free extract, respectively (Fuglie, 2000).The moringa tree has been widely used in feeding non-ruminants and, especially, poultry resulting in improvement of their productivity (Lopez, 1986; D'Mello *et al*., 1987). However, the uses of moringa seed meal are limited by their fibre contents and in some cases, presence of toxic factors or metabolic inhibitors (Bostock-Wood, 1992). Moringa seed meal inclusion in the broiler diets, globally, had no adverse effect on dressing carcass and carcass organs except the gizzard (Akouango *et al*., 2010). Ayssiwede *et al*. (2010) reported no improvements in carcass characteristics due to inclusion of moringa seed meal in the diets of growing indigenous Senegal chickens. Nworgu and Fasogbon (2007) observed no deaths of pullet chicks fed diets containing different levels of moringa seed meal.

2.3 Phytochemicals in moringa seeds

Moringa species are rich in compounds containing the simple sugar, rahmnose, and it is rich in a fairly unique group of compounds called glucosinolates and isothiocyanates (Bennett *et al*., 2003; Fahey *et al*., 2001). Some of the compounds that have been isolated from moringa preparations which are reported to have hypotensive, anticancer and antibacterial activity include 4-(4'-O-acetyl-a-L-rhamnopyranosyloxy) benzyl isothiocyanate, 4-(L-rhamnopyranosyloxy) benzyl isothiocyanate, niazimicin, pterygospermin, benzyl isothiocyanate, and 4-(a-L-rhamnopyranosyloxy) benzyl glucosinolate (Daxenbichler *et al*., 1991; Fahey *et al*., 2001; Bennett *et al.,* 2003; Mekonnen and Dräger, 2003). Antioxidant activity of these compounds has also been

reported (Win and Jongen, 1996). Seeds of moringa contain a glucosinolate that on hydrolysis yields 4-(a-L-rhamnosyloxy)-benzyl isothiocyanate, an active bactericide and fungicide (Grubben and Denton, 2004). Duke (1983) reported that moringa root-bark yields two alkaloids: moringine and moringinine.

The chemical composition of moringa seed meal is indicated in Table 2.1.

Table 2.1 Nutrient composition of moringa seed meal (on dry matter basis)

Nutrient	g/kg DM	mg/kg DM	MJ/kg DM
Crude Protein	197.6	-	-
Ca	21.3	-	-
ADF	271.1	-	-
NDF	444.2	-	-
Ash	96.1	-	-
Mg	24	-	-
P	2.4	-	-
Cu	3.1	-	-
Fe	5.3	-	-
GE	-	-	18.64
Vitamin A-B carotene	-	0.11	-
Vitamin B-choline	-	423	-
Vitamin B1-thiamin	-	0.05	-
Vitamin B2-riboflavin	-	0.07	-
Vitamin B3-nicotinic acid	-	0.2	-
Vitamin C-ascorbic acid	-	120	-

Source: Abou-Elezz *et al*. (2012)

2.4 Anti-nutrient factors in moringa seeds

Anti-nutritional factors are compounds mainly organic, which when present in a diet, may affect the health of the animal or interfere with normal feed utilization. Anti-nutritional factors may occur as natural constituents of plant and animal feeds, as artificial factors added during processing or as contaminants of the ecosystem (Barnes and Amega, 1984). Ingestion of feed containing such substances induce, in some cases, chronic intoxication and in others interferes with the digestion and utilization of dietary protein and carbohydrate and also interferes with the availability of some minerals, thus feed efficiency and growth rate and, consequently, the production of the edible products. Although anti-nutritional factors are present in many conventional feeds, they are more common in most of the non-conventional feeds (Nityanand, 1997).

Anti-nutritional factors are classified as tannins, phytates, trypsin inhibitors, saponins, oxalates and low levels of cyanide. Phytate is an organically bound form of phosphorus in plants. Phytates in foods bind with essential minerals such as calcium, iron, magnesium and zinc in digestive tract, resulting in mineral deficiencies (Bello *et al*., 2008). They bind minerals to form insoluble salts, thereby decreasing their bioavailability or absorption (Thompson, 1993; Guil and Isasa, 1997; Muhammad et al., 2011). Tannins are plant polyphenols, which have the ability to form complexes with metal ions and with macro-molecules such as proteins and polysaccharides (De-Bruyne *et al*., 1999; Dei *et al*., 2007). Dietary tannins reduce feed efficiency and weight gain in chicks (Armstrong et al., 1974; Dei *et al*., 2007. Saponins are glycosides, which are steroid saponins and triterpenoid saponins (Dei *et al*., 2007). High levels of saponins in feed adversely affect intake and growth rate in poultry (Sim *et al*., 1984; Potter *et al*., 1993; Dei *et al*., 2007). Moringa seed meal supplementation reduces feed intake due to the presence of anti-nutritional factors, especially mimosine and tannins (Atawodi *et al*., 2008). Ter Meulen *et al*. (1984) reported no improvements in feed intake, body weight and feed conversion ratio of broiler chickens. Reduction in feed intake has been ascribed to the bitter taste of saponins (Cheeke, 1971) and due to the irritating taste (Oleszek *et al*., 1994). Saponins, in excess, cause hypocholestrolaemia because they bind with cholesterol, making it unavailable for absorption (Soetan and Oyewole, 2009).

Saponins also have haemolytic activity against erythrocytes (Khalil and Eladawy, 1994). Saponin-protein complex formation reduces protein digestibility (Potter *et al*., 1993; Shimoyamada *et al*., 1998). Nworgu and Fasogbon (2007) observed enhanced body weight gain in growing pullets fed on diets containing 20, 40 and 60 g/kg *moringa* seed meal. On the other hand, Du *et al.* (2007) observed no significant difference in growth performance of 3 week old broiler chickens (Arbor Acres) that were fed on diets supplemented with 5, 10, 20 and 30 g/kg levels of *M. oleifera* seed meal.

Trypsin inhibitor inhibits trypsin and chymotrypsin, which play roles in digestion of protein in broiler chickens. Trypsin inhibitors also cause pancreatic enlargement and growth depression (Aletor and Fetuga, 1987). Hydrogen cyanide, found in moringa seeds, is toxic when ingested by monogastric animals in large quantities. The levels of these anti-nutrients and cyanide detected in the moringa leaves are low. Soaking of plant materials or boiling in water is said to reduce toxic effects and improve feed intake and protein digestibility (Okai *et al.,* 1995; Dei *et al.,* 2007). Environmental factors and the method of preparation of samples may influence the concentration of tannins present. Proper food processing would reduce anti-nutrients (Akinyeye *et al.,* 2011). The presence of essential nutrients and minerals in moringa imply they could be utilized to improve growth performance and health status of poultry. Certain bioactive chemical compounds (like saponins, tannins and other phytochemicals), which are known as secondary metabolites of plants are said to have pharmacologically active agents (Soetan and Oyewole, 2009). They have anti-bacterial and anti-parasitic properties. Nworgu and Fasogbon (2007) observed no mortality when pullet chicks were fed on diets containing different levels of *C. pubescens* leaf meal. Possible explanation for the absence of mortality in chicks might be due to the presence of antioxidants in moringa leaves, which enhance the immune system of animals (Yang *et al*., 2006; Du *et al.* 2007). Moreover, moringa leaf extracts exhibited anti-microbial activity including inhibition of the growth of *Staphylococcus aureus* strains isolated from feed and animal intestines.

2.5 Moringa seed meal as poultry feed

The relative lack of anti-nutritional components and the high protein, lipid and sulphur containing amino acid contents encourage the use of moringa seed as an animal feed. It is an excellent source of proteins for monogastric animals (Ferreira *et al.,* 2008). The antioxidant action of some compounds present in the plant, one of the most important physiological roles of food, can protect organisms against the deleterious effects of oxidation. When the seed meal is fed to one week old broiler chickens up to a level of 50 g/kg, growth rate , body weight , feed consumption and feed efficiency are improved (Ferreira *et al., 2008*) . However, higher levels of seed meal (75 and 100 g/kg) resulted in depressed body weight gain and feed efficiency, and increased feed consumption by the broiler chickens (Ferreira *et al., 2008*). It has the potential as alternative animal feed resources during dry periods.

Recent studies indicated that moringa seed meal and leaf meal have been successfully used in poultry rations to substitute soyabean meal (Melesse *et al*., 2011). A large number of reports on the nutritional qualities of moringa now exist in literature (Fuglie, 2000). However, such reports are contradictory. This study was, therefore, conducted to determine the effect of moringa seed meal supplementation on productivity and carcass characteristics of Ross 308 broiler chickens.

2.6 Moringa seed meal as medicine in poultry

The medicinal effect of the plant was ascribed to their possession of anti-oxidants, which suppress formation of reactive oxygen species (ROS) and free radicals (Ogbunugafor *et al.,* 2011). In many developing countries, sources of animal's drinking water may be contaminated with suspended materials and even bacteria but unknown to the animal owner(s). *Moringa oleifera* seeds are good and safe for water treatment; as synthetic chemical compounds (alum) may be carcinogenic (Ayotunde *et al.,* 2011). Plant substances that are foods are of little or no side effects. Most of the prescribed medicines today (about 25%) are substances derived from plants (Ngaski, 2006). However, information is scanty on the utilization of moringa seeds as feed supplement or medicine for poultry.. All parts of the moringa tree are edible and have long been

consumed by humans Fahey (2005). However, the seeds are mostly used for animal feed.

2.7 Microbial elimination with moringa seeds

Plant oil from seeds and leaves such as *Moringa oleifera* are in high demand for their medicinal value. Apart from the medicinal uses, *Moringa oleifera* is a good source of vitamins (A and E), amino acids and low level of anti-nutritional compounds (Olugbemi *et al*; 2010a; Yang *et al*., 2006). *Moringa oleifera* boosts the immune systems (Jayavardhanan *et al.,* 1994; Fuglier, 1999; Olugbemi *et al.,* 2010a). Also, Madsen *et al*. (1987) mentioned that the use of *Moringa oleifera* seeds reduced bacterial count of turbid Nile water in Sudan by 1-4 log units (90-99.9%) within the first 1-2 hours of treatment. Furthermore, Walter *et al*. (2011) reported that *Moringa oleifera* and *Moringa stenopetala* methanol and n-hexane seed extracts produced inhibition effect on *Salmonella typhii*, *Vibrio cholerae* and *Escherichia coli*, which normally cause water borne diseases. *Moringa oleifera* seeds are considered as a good source of fat, protein, antioxidants and minerals (Mg and Zn), so it can overcome malnutrition due to micronutrient deficiencies in children (Compaore *et al*., 2011). The moringa seed oil is high in (80.4%) polyunsaturated fatty acid (Anwar and Rashid, 2007; Ogbunugafor *et al.*, 2011).The edible leaves are nutritious and are consumed by humans. *Moringa oleifera* extract have antibacterial properties and its use on phytotherapeutic agent to combat infectious agents needs to be investigated.

Moringa seeds also possess antimicrobial properties (Olsen, 1987; Madsen *et al.*, 1987). Broin *et al.* (2002) reported that a recombinant protein in the seed is able to flocculate gram-positive and gram-negative bacteria cells. In this case, microorganisms can be removed by settling in the same manner as the removal of colloids in properly coagulated and flocculated water (Casey, 1997). On the other hand, the seeds may also act directly upon microorganisms and result in growth inhibition. Antimicrobial peptides disrupt the cell membrane or inhibit essential enzymes (Silvestro *et al.*, 2000; Suarez *et al.*, 2003). Sutherland *et al.* (1990) reported that moringa seeds could inhibit the

replication of bacteriophages. The antimicrobial effects of the seeds are attributed to the compound 4(α-L-rhamnosyloxy) benzyl isothiocynate (Eilert *et al.*, 1981).

2.8 Conclusions

Information on the effect of moringa seed meal supplementation on productivity, and carcass characteristics of Ross 308 broiler chickens is limited. It is, therefore, important to ascertain the effects of moringa seed meal on productivity and carcass characteristics of Ross 308 broiler chickens.

CHAPTER 3
MATERIALS AND METHODS

3.1 Study site

The study was conducted at the University of Limpopo Experimental Farm at Syferkuil. The farm is located at about 10 km northwest of the Turfloop campus of the University of Limpopo. The ambient temperatures around the study area range between 20 and 36 °C during summer and between -2 and 25 °C during winter seasons. The annual rainfall ranges between 446.8 and 468.4 mm (Shaker *et al*., 2009).

3.2 Preparation of the house

The broiler chicken house was thoroughly cleaned with water and then disinfected with formalin (NTK, Polokwane). All drinkers and feeders were thoroughly cleaned and disinfected before use. The house was left empty for at least two weeks after cleaning so as to break the life cycle of any disease causing organisms that were not killed by the disinfectant. Heating equipments were turned on 24 hours before the arrival of the chicks. Fresh saw dust was spread to a thickness of 7 cm high.

3.3 Acquisition and grinding of moringa seeds

Dry seeds were collected from Ga-Mathabatha, Limpopo Province of South Africa. The seeds were removed from the pods and kernels. The seeds were then ground to pass through a 2.5 cm sieve size.

3.4 Experimental designs, treatments and procedures

The first part of the study involved 250 unsexed day-old Ross 308 broiler chicks. The chicks (45 ± 2 g/chick) were randomly allocated to five dietary treatments, replicated five times and each replication having 10 birds. Thus, a total of 25 pens were used. A completely randomized design was used to determine the effect of moringa seed meal supplementation level on productivity and mortality of Ross 308 broiler chickens aged one to 21 days. Feed and water were provided *ad libitum* throughout the experiment. Light was provided 24 hours daily. The vaccination programme for the chicks followed the procedures used at the University of Limpopo Experimental Farm (Appendix A).

The treatments were as follows:

CM_0 Unsexed Ross 308 broiler chickens fed on a grower diet (20 % CP) without moringa seed meal supplementation

CM_5 Unsexed Ross 308 broiler chickens fed on a grower diet (20 % CP) supplemented with 5 g of moringa seed meal per kg DM diet

CM_{10} Unsexed Ross 308 broiler chickens fed on a grower diet (20 % CP) supplemented with 10 g of moringa seed meal per kg DM diet

CM_{15} Unsexed Ross 308 broiler chickens fed on a grower diet (20 % CP) supplemented with 15 g of moringa seed meal per kg DM diet

CM_{20} Unsexed Ross 308 broiler chickens fed on a grower diet (20 % CP) supplemented with 20 g of moringa seed meal per kg DM diet

The grower diet contained 880 g of DM/kg, 16.9 MJ of energy/kg DM, 200 g of crude protein/kg DM, 11.5 g of lysine/kg DM, 25 g of fat/kg DM, 10 g of calcium/kg DM and 5.5 g of phosphorus/kg DM (determined at LATS, University of Limpopo) (AOAC, 2010). The ingredients of the grower diet are presented in Table 3.2. The chemical composition of moringa seed meal contained 473 g of moisture/kg DM, 96.1 g of ash/kg DM, 197 g of crude protein/kg DM, 2.6 g potassium/kg DM, 21.3 g of calcium/kg DM, 271.1 g of ADF/kg DM, 444.2 g of NDF/kg DM, 2.4 g of phosphate/kg DM and 18.64 MJ of gross energy/kg DM.

The second part of the study involved 250 female Ross 308 broiler chickens weighing 558 ± 10 g/bird. The chickens were raised on a grower diet for 21 days before the commencement of the experiment. The experiment was terminated when the chickens were 42 days old. The chickens were assigned to five dietary treatments, replicated five times and each replication having five birds. Thus, a total of 25 pens were used. A completely randomized design was used. Feeds and clean water were provided *ad libitum* throughout the experiment. Light was provided 24 hours daily.

The treatments were as follows:

FM_0 Female Ross 308 broiler chickens fed on a grower diet (20 % CP) without moringa seed meal supplementation

FM_5 Female Ross 308 broiler chickens fed on a grower diet (20 % CP) supplemented with 5 g of moringa seed meal per kg DM

FM_{10} Female Ross 308 broiler chickens fed on a grower diet (20 % CP) supplemented with 10 g of moringa seed meal per kg DM

FM_{15} Female Ross 308 broiler chickens fed on a grower diet (20 % CP) supplemented with 15 g of moringa seed meal per kg DM

FM_{20} Female Ross 308 broiler chickens fed on a grower diet (20 % CP) supplemented with 20 g of moringa seed meal per kg DM

Table 3.1 Diet composition of grower mash for Ross 308 broiler chickens

Ingredient	Quantity (g/kg)
Yellow Maize	567
Sunflower meat	100
Full fat soya meal	290
Fish meal	10
Monocalcium phosphate	13.6
Limestone	13.6
Iodised salt	0.5
DL Methionine	0.3
L Threonine	0.0
Vitamin/ mineral premix	5.0
Total	1000
Crude Protein (g/kg)	200
Energy (MJ/kg DM)	16. 9

3.5 Data collection

Daily feed intake was measured by subtracting the weight of the refusals from that of the feed offered per day and the difference was divided by total number of the birds in the pen. Initial live weights of the birds were measured at the commencement of the experiment. Thereafter, weekly body weights were taken. These body weights were used to calculate growth rates. Feed conversion ratio was calculated as total amount of feed consumed divided by the weight gain of the birds in the pen.

Apparent digestibility of the diets was determined when the birds were between 19 and 21 days, and 40 and 42 days old. Apparent digestibility was conducted in specially designed metabolic cages having separated watering and feeding troughs. One bird was randomly selected from each replicate and transferred to the metabolic cages for measurement of apparent digestibility. A three-day acclimation period was allowed prior to three-day collection period. Droppings voided by each bird were collected on a daily basis at 08.00 hours. Care was taken to avoid contamination from feathers, scales, debris and feeds. Apparent digestibility of nutrients was calculated according to McDonald *et al*. (2004). All remaining chickens in each replicate were slaughtered at 42 days of age. Before slaughtering, each chicken was weighed by using an electronic weighing scale. After slaughter, carcass weight of each chicken was measured. Dressing percentage was determined by dividing carcass weight by body weight and then multiplied by 100. Breast, heart, gizzard, thigh, liver and fat pad weights were measured by using an electronic weighing scale.

3.6 Sensory evaluation

Sensory evaluations were conducted on meat samples. The meat was cut into 5 cm pieces. These pieces were baked for 30 minutes in a stove oven set at 71 ℃. The meat was then evaluated for its flavour, juiciness and tenderness preferences using 15 trained panelists to rank each part on a five point ranking scale (Table 3.2).

Table 3.2 Evaluation scores used by the sensory panel

	Meat characteristics		
Score	Tenderness	Juiciness	Flavour
1	Too tough	Too dry	Very bad flavor
2	Tough	Dry	Poor flavour
3	Neither tough nor tender	Neither dry nor juicy	Neither bad nor good
4	Tender	Juicy	Good flavour
5	Too tender	Too juicy	Very good flavour

3.7 Chemical analysis

Dry matter contents of feeds, feed refusals, faeces and meat were determined by drying the samples at 105 °C for 24 hours. Feed samples were also analysed for ash by placing the samples in the furnace at 300 °C for 48 hours. Nitrogen contents of feeds, feed refusals, faeces, and meat were determined by micro Kjeldahl method (AOAC, 2008). Lysine, Nitrogen detergent fibre (NDF), Acid detergent fibre (ADF), fat, calcium and phosphorus were determined according to the methods described by AOAC (2008). The apparent metabolisable energy contents of the diets were calculated according to AOAC (2008).

3.8 Statistical analysis

Data on feed intake, growth rate, feed conversion ratio, digestibility, and carcass characteristics of Ross 308 broiler chickens were analyzed using the General Linear Model procedures for statistical analysis of variance (SAS, 2008). Duncan's multiple range test was used to determine differences between treatment means ($P<0.05$) (SAS, 2008). The responses in feed intake, growth rate, feed conversion ratio, mortality and carcass characteristics of Ross 308 broiler chickens to moringa seed meal level of supplementation were modelled using the following quadratic equation (SAS, 2008):

$Y= a + b_1x + b_2x^2$

Where Y= optimum feed intake, growth rate, feed conversion ratio, body weight, apparent metabolisable energy, nitrogen retention, carcass, breast meat, heart, liver or abdominal fat; a = intercept; b_1 and b_2 = coefficients of the quadratic equation; x =

moringa seed meal level of supplementation and $-b_1/2b_2$ = x value for optimum response. The quadratic model was fitted to the experimental data by means of the NLIN procedure of SAS (SAS, 2008). The quadratic model was used because it gave the best fit.

The relationships between feed intake, liver weight, heart weight and thigh weight of female Ross 308 broiler chickens and moringa seed meal supplementation level were modelled using a linear regression equation (SAS, 2008) of the form:

$Y= a + bx$

Where Y = optimum carcass weight, a = intercept; b = coefficients of the linear equation; x = the level of supplementation.

CHAPTER 4
RESULTS

Results of the effect of moringa seed meal supplementation on feed intake, growth rate, feed conversion ratio, apparent metabolisable energy and body weight of unsexed Ross 308 broiler chickens aged one to 21 days are presented in Table 4.1. Moringa seed meal supplementation had no effect (P>0.05) on feed intake, apparent metabolisable energy, nitrogen retention, feed conversion ratio and live weight of unsexed Ross 308 broiler chickens. Moringa seed meal supplementation had effect (P<0.05) on growth rate of unsexed Ross 308 broiler chickens aged one to 21 days. Ross 308 broiler chickens supplemented with 10 g of moringa seed meal per kg DM feed had higher (P<0.05) growth rates than those feeding on a diet not supplemented with moringa seed meal. Chickens on an unsupplemented diet and those offered a diet supplemented with 5, 15 or 20 g of moringa seed meal per kg DM feed had similar (P>0.05) growth rates. Similarly, chickens supplemented with 5, 10, 15 or 20 g of moringa seed meal per kg DM feed had similar (P>0.05) growth rates. Growth rate of unsexed Ross 308 broiler chickens was optimized at a moringa seed meal supplementation level 13.3 (r^2 = 0.934) g per kg DM feed, respectively (Figure 4.1).

Results of the effect of moringa seed meal supplementation on feed intake, growth rate, feed conversion ratio, live weight, apparent metabolisable energy and nitrogen retention of female Ross 308 broiler chickens aged 21 to 42 days are shown in Table 4.2. Moringa seed meal supplementation had no effect (P>0.05) on feed intake, growth rate, feed conversion ratio, body weight and apparent metabolisable energy intake of the chickens. However, moringa seed meal supplementation had effect (P<0.05) on nitrogen retention of female Ross 308 broiler chickens. Broiler chickens supplemented with 15 g of moringa seed meal per kg DM feed had higher (P<0.05) N retention values than those not supplemented with moringa seed meal and those supplemented with 5 or 20 g of moringa seed meal per kg DM feed. Similarly, chickens supplemented with 10 g of moringa seed meal per kg DM feed had higher (P<0.05) N retention values than those supplemented with 5 or 20 g of moringa seed meal per kg DM feed. Female broiler chickens supplemented with 10 or 15 g of moringa seed meal per kg DM feed had similar (P>0.05) nitrogen retention values. Similarly, chickens on an unsupplemented diet and those offered 10 g of moringa seed meal per kg DM feed had

similar (P>0.05) nitrogen retention values. Female broiler chickens not supplemented with moringa seed meal and those supplemented with 5 or 20 g of moringa seed meal per kg DM feed had similar (P>0.05) nitrogen retention values.

Nitrogen retention of female Ross 308 broiler chickens was optimized at a moringa seed meal supplementation level of 15.13 (r = 0.610) g per kg DM feed (Figure 4.2).

The effect of moringa seed meal supplementation on carcass characteristics of female Ross 308 broiler chickens aged 42 days are presented in Table 4.3. Moringa seed meal supplementation had no effect (P>0.05) on carcass, breast meat, abdominal fat pad, liver, heart and thigh weights of female Ross 308 broiler chickens aged 42 days. However, moringa seed meal supplementation had effect (P<0.05) on gizzard weights of female Ross 308 broiler chickens aged 42 days. Broiler chickens supplemented with 15 g of moringa seed meal per kg DM feed had higher (P<0.05) gizzard weights than those not supplemented with moringa seed meal and those supplemented with 5 g of moringa seed meal per kg DM feed. Similarly, chickens supplemented with 10 or 20 g of moringa seed meal had higher (P<0.05) gizzard weights than those not supplemented with moringa seed meal. Female broiler chickens supplemented with 10, 15 or 20 g of moringa seed meal per kg DM feed had similar (P>0.05) gizzard weights. Also, broiler chickens supplemented with 5, 10 or 20 g of moringa seed meal per kg DM feed had similar (P>0.05) gizzard weights. Similarly, chickens on an unsupplemented diet and those offered 5 g of moringa seed meal per kg DM feed had similar (P>0.05) gizzard weights.

Gizzard weights of female Ross 308 broiler chickens were optimized at a level of 16.03 (r = 0.872) g per kg DM feed (Figure 4.3). Daily supplementation with moringa seed meal had no effect (P>0.05) on meat flavour, juiciness and tenderness of female Ross 308 broiler chickens aged 42 days (Table 4.4).

Table 4.1 Effect of moringa seed meal supplementation level (g/kg DM feed) on feed intake (g/bird/day), growth rate (g/bird/day), feed conversion ratio (FCR) (g DM feed/g live weight gain), live weight (g/bird at 21 days old), apparent metabolisable energy (AME) (MJ/kg DM) and nitrogen retention (g/bird/day) of unsexed Ross 308 broiler chickens aged one to 21 days

Variable	Treatment					SE
	M_O	M_5	M_{10}	M_{15}	M_{20}	
Feed intake	61	60	62	56	58	0.61
Growth rate	23b	24^{ab}	26^{a}	25^{ab}	25^{ab}	0.39
FCR	2.62	2.49	2.42	2.37	2.35	0.40
Body weight	530	546	593	576	568	11.5
AME	12.9	12.9	13.9	13.7	13.1	0.29
N-retention	1.2	1.7	1.7	0.9	1.3	0.15

a, b : Means in the same row not sharing a common superscript are significantly different ($P<0.05$)

SEM : Standard error of the mean

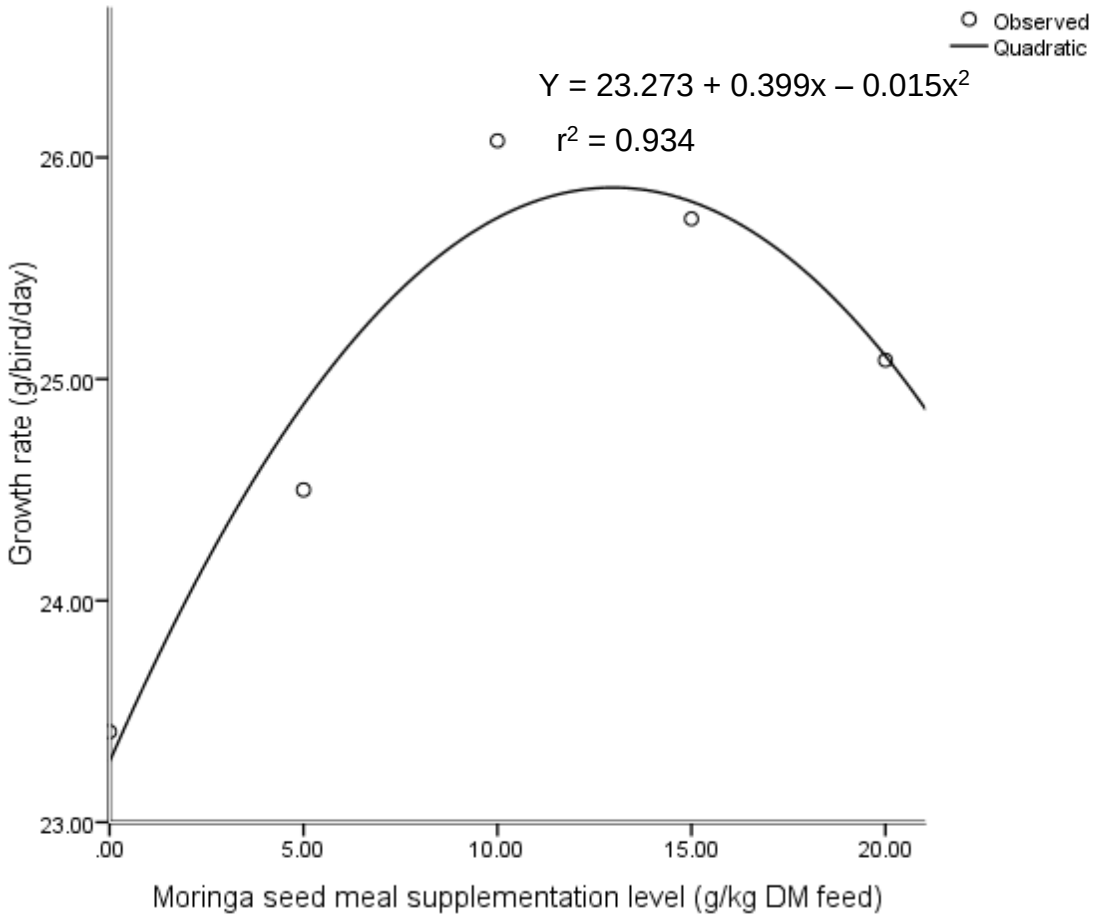

Figure 4.1 Effect of moringa seed meal supplementation level on growth rate of unsexed Ross 308 broiler chickens aged one to 21 days

Table 4.2 Effect of moringa seed meal supplementation level (g/kg DM feed) on intake (g/bird/day), growth rate (g/bird/day), feed conversion ratio (FCR) (g DM feed/g live weight gain), live weight (g/bird aged 42 days), apparent metabolisable energy (AME) (MJ/kg DM) and nitrogen retention (g/bird/day) of female Ross 308 broiler chickens aged 21 to 42 days

Variable	Treatment					SEM
	FM_0	FM_5	FM_{10}	FM_{15}	FM_{20}	
Intake	154	149	150	147	148	1.35
Growth rate	51.8	49.8	54.3	55.0	52.0	1.16
FCR	4.3	3.4	3.6	3.6	3.6	0.54
Live weight	1617	1590	1733	1730	1658	22.69
AME	11.3	10.9	13.0	13.0	12.0	0.28
N-retention	2.1^{bc}	1.8^{c}	2.4^{ab}	2.4^{a}	1.8^{c}	0.06

a, b, c : Means in the row not sharing a common superscript are significantly different ($P<0.05$)

SEM : Standard error of the mean

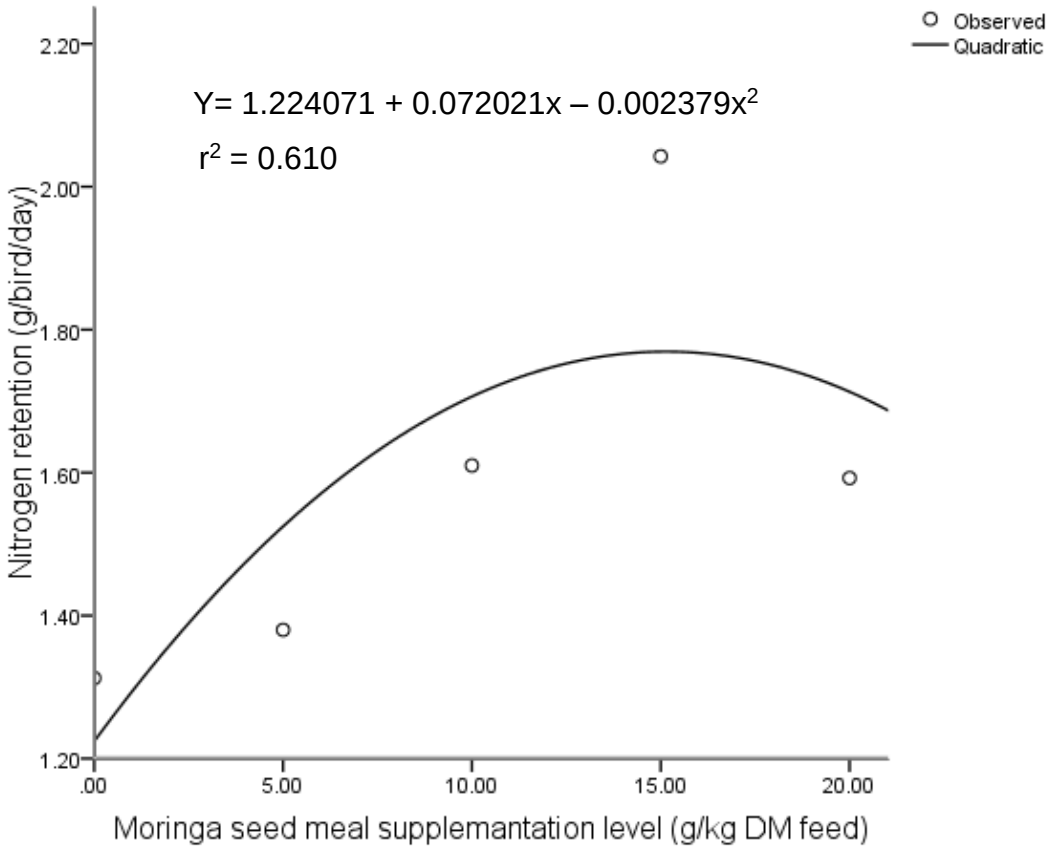

Figure 4.2 Effect of moringa seed meal supplementation level on nitrogen retention of female Ross 308 broiler chickens aged 6 weeks

Table 4.3 Effect of moringa seed meal supplementation level (g/kg DM feed) on carcass weight (g/bird) and carcass parts (g) of female Ross 308 broiler chickens aged 42 days

Variable	Treatments					SEM
	FM_0	FM_5	FM_{10}	FM_{15}	FM_{20}	
Carcass weight	1232	1198	1431	1392	1248	34.56
Breast weight	359	367	349	421	324	20.77
Heart weight	4	5	5	4	6	0.36
Liver weight	29	26	29	27	31	1.57
Abdominal fat weight	17	19	17	14	17	1.85
Thigh weight	185	173	189	205	224	8.2
Gizzard weight	15.7^{c}	18.4^{bc}	24.2^{ab}	30.1^{a}	24.5^{ab}	1.51

a, b, c : Means in the same row not sharing a common superscript are significantly different ($P<0.05$)

SEM : Standard error of the mean

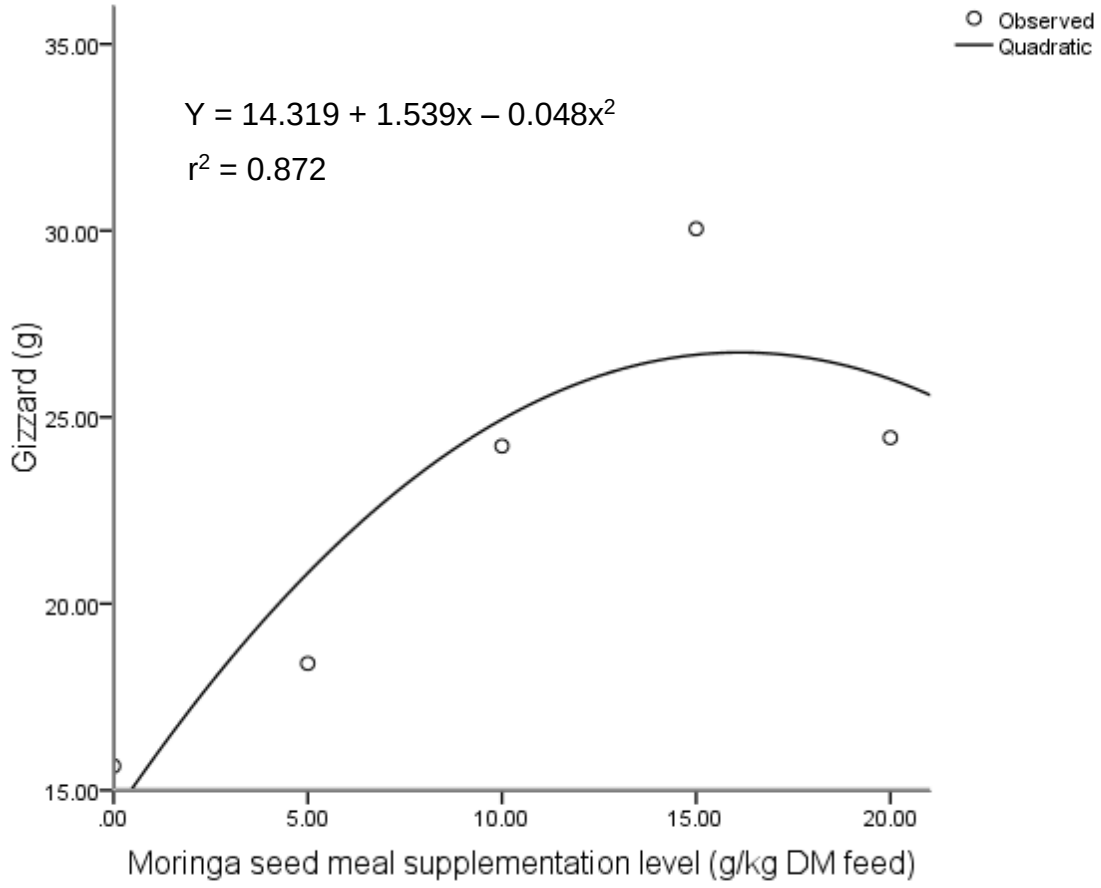

Figure 4.3 Effect of moringa seed meal supplementation level on gizzard weight of female Ross 308 broiler chickens aged 42 days

Table 4.4 Effect of moringa seed meal supplementation level (g/kg DM feed) on meat flavour, juiciness and tenderness of female Ross 308 broiler chickens aged 42 days

	Treatment					
Variable	FM_0	FM_5	FM_{10}	FM_{15}	FM_{20}	SEM
Flavour	2.7	2.9	2.9	3.0	2.9	0.08
Juiciness	3.0	3.1	3.2	3.0	2.9	0.20
Tenderness	3.4	3.5	3.6	3.4	3.4	0.19

SEM: Standard error of the mean

CHAPTER 5

DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

5.1 Discussion

Moringa seed meal supplementation did not affect feed intake, feed conversion ratio, metabolisable energy intake, nitrogen retention and body weight of unsexed Ross 308 broiler chickens aged one to 21 days. However, moringa seed meal supplementation improved growth rate of unsexed Ross 308 broiler chickens. Similar findings were observed by Soetan and Oyewole (2009) in broiler chickens. Quadratic analysis of the data on growth rate of unsexed Ross 308 broiler chickens indicated that a moringa seed meal supplementation level of 13.3 g/kg DM feed optimized growth rate. Ferreira *et al.* (2008) reported that a 50 g/kg supplementation level of moringa seed meal optimized intake, growth rate, feed conversion ratio and live weight of chickens aged one to 21 days. However, higher levels of 7.5 and 100 g/kg moringa seed meal supplementation depressed intake, growth rate and live weights of the chickens (Ferreira *et al.,* 2008). The authors suggested that the depressions in intake, growth rate and live weights of the chickens were due to the effects of antinutritional factors contained in moringa seed meal, for example, hydrogen cyanide. Makkar and Becker (1997) reported that moringa seed kernel and seed meal have high levels of antinutrients. Antinutrients reduce bioavailability of minerals in non-ruminant animals (Reddy *et al*., 1982) and adversely affect digestibility of starch and protein (Thompson, 1993). Moringa seed meal supplementation did not affect mortality of unsexed Ross 308 broiler chickens aged one to 21 days in the present study. In fact there were no deaths of chickens aged one to 21 days. However, Walter *et al.* (2011) noticed that *Moringa oleifera* and *Moringa stenopetala* seed extracts reduced bacteria numbers which cause water borne diseases, thus reducing mortality rates in chickens.

Moringa seed meal supplementation improved nitrogen retention of female Ross 308 broiler chickens aged 22 to 42 days. However, this improvement did not affect growth rate, feed conversion ratio and live weight of the chickens aged 22 to 42 days. These results are similar to those observed by Atawodi *et al*. (2008) who reported no improvements in dietary intake, feed conversion ratio and live weight of laying pullets when supplemented with moringa seed meal. Similar findings were observed by D' Mello *et al*. (1987) who stated that moringa seed meal supplementation did not improve

dietary feed intake, feed conversion ratio and body weight of broiler chickens aged 22 to 42 days. The present results are also similar to those obtained by Ter Meulen *et al*. (1984) who reported no improvements in feed intake, body weight gain and feed conversion ratio of broiler chickens supplemented with moringa seed meal. Similarly, Atuahene *et al*. (2008) found no significant effects of moringa seed meal supplementation at 2.5%, 5% or 7.5% levels on feed intake of broiler chickens. Du *et al.* (2007) observed no improvements in growth rates of broiler chickens supplemented with moringa seed meal. Quadratic analysis of the present data on nitrogen retention indicated that a moringa seed meal supplementation level of 15.13 g/kg DM feed optimized nitrogen retention of female Ross 308 broiler chickens aged 22 to 42 days. Supplementing female Ross 308 broiler chickens with moringa seed meal did not improve metabolisable energy intakes. Ossebi (2010) found that moringa seed meal supplementation reduced metabolisable energy of broiler chickens. However, Munguti *et al*. (2006) reported that moringa seed meal supplementation increased metabolisable energy intakes of broiler chickens.

Moringa seed meal supplementation did not improve carcass weight, breast weight, heart weight, liver weight, abdominal fat weight and thigh weights of female Ross 308 broiler chicken aged 42 days. However, moringa seed meal supplementation improved gizzard weight of female Ross 308 broiler chickens aged 42 days. Akouango *et al*. (2010) also found that moringa seed meal supplementation increased gizzard weights of broiler chickens. Iheukumere *et al*. (2008) found that broiler chickens supplemented with moringa seed meal had lower carcass weight. Ayssiwede *et al*. (2010) reported no improvements in carcass characteristics due to inclusion of moringa seed meal in the diets of growing indigenous Senegal chickens. Quadratic analysis of the present data indicated that a moringa seed meal supplementation level of 16.03 g/kg DM feed optimized gizzard weight of female Ross 308 broiler chickens aged 42 days. Moringa seed meal supplementation to the diets of Ross 308 broiler chickens did not have effect on fat pads. However, Compaore *et al*. (2011) and Akouango *et al*. (2010) reported that moringa seed meal supplementation increased fat pad of broiler chickens aged 42 days.

No chicken deaths were observed throughout the entire experiment. Nworgu and Fasogbon (2007) observed no deaths of pullet chicks fed diets containing different levels of moringa seed meal. Akouango *et al*. (2010) observed no deaths of indigenous chickens when supplemented with moringa seed meal. Possible reasons for the absence of deaths of chickens where moringa seed meal is supplemented might be due to the presence of antioxidants in moringa seeds, which enhance the immune systems of the chickens (Yang *et al*., 2006; Du *et al.,* 2007).

Moringa seed meal supplementation did not affect meat flavour, juiciness and tenderness of female Ross 308 broiler chickens aged 42 days. No information on flavour, juiciness and tenderness of the meat from chickens supplemented with moringa seed meal was found.

5.2 Conclusions

Moringa seed meal supplementation improved growth rates of Ross 308 broiler chickens aged one to 21 days. A moringa seed meal supplementation level of 13.3 g/kg DM feed optimized growth rate of Ross 308 broiler chickens aged one to 21 days.

Moringa seed meal supplementation improved nitrogen retention of female Ross 308 broiler chickens aged 22 to 42 days. However, moringa seed meal supplementation did not have any effect on dietary intake, growth rate, feed conversion ratio, metabolisable energy intake and body weight of female Ross 308 broiler chickens aged 22 to 42 days. Similarly, moringa seed meal supplementation affected gizzard weights.

5.3 Recommendations

It is recommended that a moringa seed meal supplementation level of 13.3 g/kg DM feed be used in ration formulations to optimize growth rate of Ross 308 broiler chickens aged one to 21 days. However, moringa seed meal supplementation to the diets of Ross 308 broiler chickens aged 22 to 42 days had no effect on growth rate. Thus, if growth rate is the parameter of interest, there may be no need to supplement.

More research is suggested to fully explore biological reasons for the effect of moringa seed meal supplementation level on productivity and carcass characteristics of Ross 308 broiler chickens.

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CHAPTER 7
APPENDIX

APENDIX A: VACCINATION PROGRAM

The vaccination programmes of the study were as indicated below:

Day one:	Chicks were vaccinated against New castle disease from the hatchery using Clone 30. Secondly, Vita stress was added in the drinking water immediately on arrival for the first two days to calm down the chicks due to stress they might have experienced through transportation and handling.
Day three:	Tylo Tad was added in drinking water for prevention of Escheria coli bacteria and other disease causing microorganisms.
Day seven:	Chicks were vaccinated against infectious Bronchitis using “IBH 120”.
Day twelve:	Chicks were vaccinated against Gumbora using D78 through drinking water.
Day eighteen:	Chickens were vaccinated against Gumbora using D78 through drinking water.
Day twenty one:	Tylo tad was added in the drinking water.
Day twenty three:	Chickens were vaccinated against New Castle disease using Clone 30.