EFFECT OF *MORINGA OLEIFERA* AND PROBIOTIC INCLUSION ON GROWTH PERFOMANCE, CARCASS CHARACTERISTICS AND COST BENEFIT ANALYSIS IN BROILER CHICKEN PRODUCTION

BY

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A dissertation submitted in fulfilment of the requirements for the degree of Master of Science in Agriculture (Animal Science)

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2019

DECLARATION

I, Ramathithi Tshilidzi of student number: 14004351, hereby declare that this dissertation submitted in fulfilment of the requirement for the Master of Science in Agriculture Department of Animal Science at the School of Agriculture, University of Venda, has not been previously in part or in its entirety been submitted to any university for any other degree. I further declare that this is my own work in design and execution and that all references material contained herein has been duly acknowledged.

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DEDICATION

This dissertation is dedicated to my parents, Mr. M.N and Mrs. R. Ramathithi, to my brothers and sister Mulalo, Khathutshelo and Wavhuthu, my uncle and his wife Mr. M.E. and Mrs. M. Tshikondela and my cousins. Family and relatives to the excellent love and care they have given to me during the period of my study. To my grandmother, I really miss you, but I know that you are happy and smiling down on me where you are now.

ACKNOWLEDGEMENT

This dissertation reflects the work of many people who deserve recognition. I would like to acknowledge the following:

1. Special thanks to the **Almighty God** for love, strength, guidance and wisdom accorded to me during the period of the study.
2. **My supervisor**, Dr. E Bhebhe and **Co-supervisor**, Prof J.J Baloyi. I am grateful for your guidance, skilful contribution to this dissertation and close follow up throughout my study, and priceless assistance.
3. **Department of Animals Science**: Mr A.J. Netshipale and Mr M.E. Nyathi, thank you for your constant support and advices.
4. **Department of Food Science**: Mr M.E. Mashau and Ms B. Moyo, thank you for your support in data collection.
5. **Sponsors**: Special thanks to:
   i. University of Venda Research and Publication Committee Funds (Project No: SARDF/19/ANS/01/2404) ii. National Research Foundation for Scarce Skills Scholarship (Grand No: 118698) for offering funds on my first and second year.
6. **Family**: To my beloved family, thank you for your support and prayer.
7. **Colleagues and friends**: Rakau M.V., Lubisi M.W., Mkhonto Z.D., Mukosi, R., Madilindi M.A., Mariba, N., and Muavha, N.R. thank you for your constant support and advices, you are appreciated.

ABSTRACT

One of the ways to minimise cost and promote health in humans and animals is to use natural feed additives instead of antibiotics. *Moringa oleifera* (Moringa) is a phytobiotic which possess anti-microbial and immune-modulatory properties and contains high levels of nutrients and it can be used as a feed additive. Probiotics are feed additives which consist of living microorganisms that have beneficial effects on the physiology and health of other organisms. The objective of the study was to determine the effect of various levels of *Moringa oleifera* and probiotics inclusion on growth performance, carcass characteristics and cost benefit analysis for broiler chicken production. *Moringa oleifera* leaf powder was purchased from Bethel Farm No:683 Bethel mission Gucksdadt Vryheid in Zululand district AbaQulusi municipality. The study was a 5x2 factorial design with five levels of Moringa and two levels of probiotic. Six hundred (600) Ross 308-day old chicks were received and fed commercial starter. The experimental treatments were randomly divided into five levels of Moringa with and without probiotics introduced through drinking water at grower to finisher phase. The diets were supplemented with different inclusion levels of *Moringa* (Mo) as follows: 0g/kg (M_0), 0.6g/kg (M_3), 1.2g/kg (M_6), 1.8g/kg (M_9), 2.4g/kg (M_{12}) of *Moringa oleifera* leaf meal (MOLM) and probiotic at P_0 (0ml/bird/week), P_1 (1 ml/bird/week) of a commercial probiotic administered in water for the first three weeks (starter phase). The experiment had three replications with 20 birds per replicate. MOLM and PRB interaction had no significant effect ($P>0.05$) on any of growth parameters and same findings with MOLM. Probiotics treatments had significantly reduced ($P < 0.05$) mortality rate (MTRT) in the grower phase. MOLM×PRB had significant effect on ($P<0.01$) feed intake (FI), MOLM had significantly reduced ($P< 0.05$) average bodyweight gain (ABWG) and FI in finisher phase. MOLM and PRB interaction had significant effect ($P< 0.05$) on dressed weight only and non-significant effect ($P>0.05$) on the rest of carcass parameters. MOLM inclusion levels did not significantly affect ($P>0.05$) back fat weight. However, MOLM inclusion level significantly reduced ($P< 0.05$) dressed weight, shank length, wing weight, drum and thigh weight, back weight and breast muscle weight. PRB inclusion in the diets significantly increased ($P< 0.01$) shank size and drum + thigh weights ($P<0.05$). MOLM and PRB inclusion level had no significant effect ($P>0.05$) on giblets parameters. MOLM and PRB interaction had significant effect on ($P<0.05$) water holding capacity (WHC) and hardness. MOLM had significant effect ($P<0.05$) on pH and dripping loss. A significant effect between (MOLM×PRB) *Moringa oleifera* leaf meal and probiotics ($P<0.01$) was observed on C_D, C* and b*. MOLM had significant effect ($P<0.01$) on C_D, C*, a*, b*, L*, PRB had significant effect ($P<0.01$) on h*(increased) and a*(reduced) on colour parameters.

MOLM fed at P0 resulted in higher mortality at grower stage. It is concluded that MOLM can be added up to 12% with or without PRB without affecting growth performance at finisher phase and carcass characteristics. Inclusion of MOLM up to 12% had good impact on hardness and water holding capacity in the meat. PRB presence improved the growth performance of birds supplemented with MOLM up to 12% inclusion level. MOLM diets were not economically profitable compared to control diet due to high price level of *Moringa oleifera* powder supplemented in the diets and no best return weight gain per rand invested amongst the diets.

Keywords: Broiler, Growth parameters, Carcass characteristics, *Moringa oleifera*, Probiotics

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LIST OF ABBREVIATIONS

º	Degree
ºC	Degree Celsius
%	Percentage
a*	CIE red (+)/ green (-) colour attribute
ABWG	Average Body Weight Gain
ADF	Acid Detergent Fiber
ALBW	Average Live Body Weight
AOAC	Association of Official Analytical Chemists
b*	CIE yellow (+)/ blue (-) colour attribute
Ca	Calcium
C*	Chroma
C_D	Colour difference
cfu/g	Colony-forming unit per gram
Cm	Centimetre
Cu	Copper

Fe	Iron
FCR	Feed conversion ratio
FI	Feed intake g
	Grams
g/kg	Gram per kilogram
h*	Hue angel
IBW	Initial body weight
K	Potassium
Kg	Kilogram
L*	CIE lightness coordinate
M	Moringa level (0, 3, 6, 9 and 12) %
M	Molecule
M	Metre
M^2 Square Metre Mg	Magnesium
Mg	Milligram
Ml	Millilitre
Mm	Millimetre
Mm^2	Millimetre square
Mn	Manganese
MOLM	*Moringa oleifera* leaf meal
MTRT	Mortality rate
N	Number of observations
Na	Sodium
NDF	Neutral Detergent fibre
OC	Oil cake
P	Phosphorus
P0	probiotics level 0=No probiotic
P1	Probiotics level 1=Probiotics as per manufacturer's

	recommendations
SEM	Standard error mean
Wt	Weight
Zn	Zinc

LIST OF APPENDICES

CHAPTER 1: INTRODUCTION

1.1. Background

Poultry production, during the past two decades, has been one of the most expanding sectors in the world (Alkhalf *et al.*, 2010). In South Africa, the broiler industry is an important source of animal protein (Boer *et al.*, 2001). Broiler meat cost less compared to other livestock meat and is the animal protein which most people in developing countries can afford. Production of poultry plays an important role in addressing the gap between requirement and availability of affordable animal protein for human consumption.

Productivity in the poultry sector is very sensitive to various infectious diseases such as avian influenza, infectious bursal disease, infectious bronchitis and others. These kinds of diseases can potentially compromise poultry production through reduced growth performance of the birds resulting in economic loss (Alkhalf *et al*., 2010). Infectious diseases are an ever-present concern in the poultry industry resulting in wide therapeutic use of antibiotics over the years. However, the continuous use of antibiotics has led to the presence of antibiotic residues in meat and meat products as well as microbial resistance (Agashe, 2016). The use of antibiotics as an additive in animal production has been curtailed in many countries and it has led to investigation of alternative feed additives for improving growth performance under intensive systems to produce natural poultry products.

Feed is the major component which affects the profitability of poultry enterprises (Agashe, 2016) and consequently, different feed additive strategies are used to ensure better profit margins. The profitability of broiler enterprises largely depends on efficiency of feed utilization, improved body weight gain, prevention of diseases and minimization of mortality rate. Natural alternative feed additives that can be used instead of antibiotics include *Moringa oleifera* and probiotics. Moringa is the most widely cultivated species of the genus in the family *Morinaceae*, common name drumstick tree. This plant is mostly grown in tropical and sub-tropical climates and every part of Moringa has beneficial properties. The plant contains natural antioxidants (Siddhuraju and Becker, 2003). The antioxidant properties of *Moringa oleifera* leaves are due to presence of tannins, polyphenols, anthocyanin, glycosides and thiocarbamates, which remove free radicals, active antioxidant enzymes and inhibit oxidases (Luqman *et al.*, 2012).

Moringa leaf meal contains 27.51% crude protein (Oduro *et al*., 2008), making it a good source of this essential nutrient.

The main flavonoids found in *Moringa oleifera* leaves are myrecytin, quercetin and kaempferol, in concentrations of 5.8, 0.207 and 7.57 mg/g, respectively. Quercetin is found in dried *M. oleifera* leaves, at concentrations of 100 mg/100 g, as quercetin-3-O-β-dglucoside (isoquercetin or isotrifolin). Quercetin is a strong antioxidant, with multiple therapeutic properties (Vergara-Jimenez *et al*., 2017) Probiotics are feed additives consisting of living microorganisms that have beneficial effects on the physiology and health of organisms (Ledezma-Torres *et al.*, 2015). In poultry, probiotics are known for improving production parameters and general health (Koenen *et al.*, 2004; Mountzouris *et al.*, 2007; Vicente *et al.*, 2007; Botlhoko, 2009)

1.2. Problem statement

The broiler chicken industry is one of the fastest growing livestock industries in South Africa with potential to yield profit in a relatively short period compared to other livestock enterprises. Modern commercial broiler chickens are characterised by very high growth rates and an efficient feed conversion ratio. Broiler growth rate is influenced by feeds and nutrient availability in the feed. The high cost of quality broiler feeds is a key constraint towards achieving profitable livestock farming in South Africa (Nkosi *et al.*, 2011). High incidences of metabolic diseases, low growth rate and high mortality force farmers to increase the rearing period and concomitantly the feed cost for broiler chickens to reach target mass for the market. Once farmers are facing problems such as low growth rate, they must supplement some nutrients for growth promotion. Antibiotics have been used as growth promoters in past years, however, their use in animal production increases microbial resistance and residues in animal products can be harmful to consumers (Jin *et al*., 1998) hence the need for investigations to identify alternative and safe additives needed for broiler production.

1.3. Justification

Productivity and general health of livestock depends on good quality feeds and balance of nutrients in the feed together with feed additives. Natural growth promoters (feed additives) are believed not to be harmful and are relatively inexpensive. The use of natural feed additives for production is on the increase and is considered safer than antibiotics and other non-natural feed additives (Agashe, 2016).

Moringa oleifera is a plant which is rich in protein and vitamins and it has been found to be useful as an animal feed additive and for protein supplementation in livestock production (Nkukwana *et al*., 2014). *Moringa oleifera* is a perennial plant that can be harvested several times in one growing season and when used in broiler feeds it has the potential to reduce feed cost (Sarwatt *et al.*, 2004). Despite *Moringa oleifera* being high in nutrient content, there is little information regarding its utilization in poultry feeds in general and specifically as an additive or protein source in broiler rations. Such information is needed for designing feeding strategies to improve broiler production and help smallholder farmers undertaking farm-based feed formulation. The information generated will help farmers who find it difficult or expensive to procure commercial feeds for their poultry enterprises or those who want to mix their own poultry rations using locally available resources.

Probiotics are live microorganisms, non-pathogenic bacteria that contribute to the health and bring about physiological balance in the intestinal tract of the animal. Commercially available probiotics can be found in the form of powder, capsules or liquid. It has been reported that probiotics have a positive impact on poultry performance (Koenen *et al.*, 2004; Mountzouris *et al.*, 2007), improve microbial balance, and synthesize vitamins (Fuller, 1989). Numerous researchers have partially replaced antibiotics with probiotics as therapeutic and growth promoting agents with positive results. However, limited information is available on the effect of level of administering probiotics on growth parameters, mortality and carcass components of broilers. Therefore, this study was undertaken to evaluate the effect of *Moringa oleifera* leaf meal and probiotics on the growth performance, carcass components and cost benefit analysis of their use in a broiler enterprise

1.4. Objectives

1.4.1. Main objective

The broad objective of the study was to determine the effect of various levels of *Moringa oleifera* leaf meal (MOLM) and probiotic inclusion in broiler diet as supplements on growth performance, carcass characteristics and cost benefit analysis of their inclusion in a broiler enterprise.

1.4.2. Specific objectives

The specific objectives of the study were to determine:

i. The effect of various levels of *Moringa oleifera* leaf meal (MOLM) and probiotic inclusion on growth parameters, mortality and carcass characteristics of broiler chickens.
ii. Cost benefit analysis of different inclusion levels of *Moringa oleifera* leaf meal (MOLM) and probiotic in a broiler enterprise.
iii. Effect of *Moringa oleifera* leaf meal and probiotic inclusion on meat quality parameters (water holding capacity, hardness, pH and dripping loss) and colour parameters (C_D, c*, a*, b*, h* and L*).

1.5. Hypotheses

1.5.1. Null hypotheses

i. *Moringa oleifera* leaf meal and probiotic inclusion levels have no significant effect on growth parameters, mortality and carcass characteristics of broiler chickens.
ii. Different levels of MOLM and probiotic combinations do not significantly enhance the economic efficiency of a broiler enterprise.
iii. Inclusion of *Moringa oleifera* and probiotic have no significant effect on meat quality (water holding capacity, colour, hardness and dripping loss).

CHAPTER 2: LITERATURE REVIEW

2.1. General introduction

Moringa oleifera is a tree with many uses. It is found throughout most of the tropics and is of great economic importance because of its nutritious pods, edible leaves, flowers and can also be utilized in medicines, cosmetic oil or forage for livestock. *Moringa oleifera* belongs to the family of *Moringaceae* (Navie and Csurches, 2010). It is a deciduous perennial evergreen tree that originated in India but has spread to other regions. It is one of the fastest growing trees in the world with high biomass yield, high crude protein of 31%, and an equitable level of other nutrients in the leaves (Moyo *et al.*, 2011; Gopalakrishnan *et al.*, 2016). The leaves of the moringa tree in the form of a leaf meal are the preferred part for use in animal diets. The Moringa leaf meal contains quality protein that can compete with the likes of soya bean because it contains all the essential amino acids in the right proportion. The leaves are known to be excellent source of protein with great potential, vital B-complex vitamins, minerals and low percentage of phenols (3-4%) (Moyo *et al.*, 2011). Probiotics can be used as safe growth promoters for poultry production. This chapter reviews the information pertaining to effect of probiotics on the performance and carcass characteristics of birds and the potential in poultry production enterprises. According to Patidar and Prajapati (1999), probiotics improved weight gain and feed utilization of broiler chickens, however, other studies have reported no increase in body weight gain after supplementing broiler diets with probiotics (Khan *et al*., 1992).

2.2. Economic importance of broilers

The industry is one of the fastest growing livestock industries in South Africa and is the process by which broiler chickens are raised and prepared for meat. Modern broiler chickens are characterized by very high growth rates and very good feed conversion ratios (Molepo, 2014). Broiler chickens are considered to be one of the most popular options for reducing the incidence of malnutrition particularly protein deficiency in human diet. Chickens play an important role in many rural households of South Africa and the world. They are an important source of income and employment. Broiler production is a source of income, it is a good source of protein and offers quick returns on investment (Kekocha, 1994). However, the broiler industry in developing countries is facing some challenges including but not limited to high feed to gain ratio and the ever-increasing cost of feed due to the high prices of feed ingredients (Abbas, 2013).

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 quantity and 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.

2.3. Origin, production and utilisation of *Moringa oleifera*

Moringa oleifera is the most widely cultivated species of the tropical flowering plant family *Moringaceae* containing thirteen diverse species (Shahzad *et al*., 2013). It is a medium-sized agroforestry tree that originated from south Asia but has become naturalised in many countries globally. *Moringa oleifera* has gained importance due to its multipurpose uses and good adaptability to both humid and dry climates. *Moringa oleifera* thrives well in a variety of soil conditions preferring well-drained sandy or loamy soil that is slightly alkaline. It can grow well in the humid tropics or hot dry lands and can survive in less fertile soils. *Moringa oleifera* is a drought-tolerant plant and grows best with rainfall of 250-1500 mm per year (Martin, 2007).

Moringa oleifera is the cheapest and credible alternative not only providing good nutrition, but also to cure and prevent a lot of diseases (Paliwal and Sharma, 2011). It is an important source of food in many parts of the world and it can be grown easily and cheaply, hence it is used in many feeding programs across African countries to address malnutrition (Anwar *et al*., 2007). *Moringa oleifera* has multipurpose uses and it has the ability to guarantee a good yield (Leone *et al*., 2015). Rich in nutrients such as protein and minerals, *Moringa oleifera* is a highly valuable plant found in many tropical and subtropical countries and is one of those plants that have not been studied for many years but is now being investigated for its fast growth, higher nutritional value, and increasing utilisation as a livestock fodder crop (Nouman *et al*., 2013).

2.4. Moringa and the nutrient requirements for growing broilers

Moringa oleifera is a multipurpose plant which is exploited as food and for various medicinal purposes. *Moringa oleifera* is one of the plants that can be utilized in the preparation of poultry feeds. Apart from being a good source of vitamins and amino acids, Moringa has medicinal uses (Makkar and Becker 1997; Francis *et al*., 2005). *Moringa oleifera* leaves are rich in protein, minerals, beta-carotene and antioxidant compounds, which are often deficient among the diets of developing countries (Leone *et al*., 2015). *Moringa oleifera* leaves incorporated into maize meal-based poultry feed led to better growth performance of the chicks and a significant increase in the serum level of biochemical minerals compared to the maize meal fed alone. Preparing a balanced diet can be a complex and possibly costly process especially for producers with little background in nutrition.

An important part of raising chickens is feeding, it makes up the major cost of production and good nutrition is reflected in the bird's performance and its products (Fanatico, 2003). Starter rations are high in protein-an expensive feed ingredient. However, grower and finisher rations can be lower in protein since older birds require less of this ingredient. A starter diet is about 24% protein, grower diet 20% protein, and finisher diet 18% protein (Cheeke, 1991).

2.5. Nutritional composition of *Moringa oleifera* leaf meal

Moringa is especially promising as a feed source in the tropics because the tree is in full leaf at the end of the dry season when other feeds are typically scarce (Kushwaha *et al*., 2015). It is a fast-growing drought-resistant tree whose morphological parts are an outstanding source of nutrients. They contain cytokinins in the form of zeatin as well as other beneficial phytochemicals such as vanillin, beta-sitosterol, caffeoylquinic acids, kaempferol, quercetin and carotenes (Kushwaha *et al*., 2012). *Moringa oleifera* leaves are a storehouse of important nutrients which are available at no cost and are very rich in all the micronutrients (Kushwaha and Chawla, 2015). Thus, the leaves are rich in minerals like calcium, potassium, zinc, magnesium, iron and copper. In addition, *Moringa oleifera* has high levels of crude protein, crude fibre, crude fat, iron, calcium and beta-carotene.

The chemical content of plants is affected by the type of soil or climatic condition in which they grow and hence it would be expected that there will be variation in the chemical composition reported by different researchers. Oduro *et al*., (2008) reported that *Moringa oleifera* leaves contains 27.51% crude protein, 19.25% crude fibre, 2.23% crude fat, 7.13% ash, 76.53% moisture, 43.88% carbohydrates and a calorific value of 1296.00 kJ/g (305.62 cal/g). Seshadri *et al*., (1997) analysed fresh *Moringa oleifera* leaves and revealed that they contain 27.1mg of total carotene, 17.4 mg of beta carotene, and 143.6 mg of ascorbic acid per 100g. Quarcoo (2008) developed a beverage from leaves of *Moringa oleifera* and the validated product contains 50-52% *Moringa oleifera,* 38-40% pineapple and 10-12% carrots. The optimized beverage contained 2.9 g/100ml protein, 1.02 mg/100ml iron and 159.14 mg/100ml vitamin C. After 8 weeks of storage 78% of vitamin C was still retained even under the most severe storage conditions (sunlight).

2.6. Anti-nutrients in *Moringa oleifera* leaf meal

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). Anti-nutrients are substances which interfere with the metabolism and utilization of nutrients by the body; examples are phytates, oxalates, tannins and saponins. Moringa leaves have been found to contain small quantities of anti-nutrients (Moyo *et al*., 2011). From a plant health perspective, the higher values of anti-nutrients (oxalates, phytates and saponins) obtained in leaves of manure-fertilized moringa plants suggests positive influence of poultry manure on the composition of the anti-nutrients in the plants. *Moringa oleifera* has been reported to be a good source of vitamins and amino acids (Olugbemi *et al*., 2010).

2.7. *Moringa oleifera* leaf meal in poultry diets

Poultry production is constrained by inadequate supply of good quality feed and escalating costs. This is due to poor availability and expensive raw materials especially the proteins. Plants in general are good sources of dietary fibre, have low fat content, particularly saturated fats and generally deficient in one or more of the essential amino acids (Dousa *et al*., 2011). Soyabean is one of the important conventional plant protein sources in the poultry industry, but its increasing price is of great concern (Catootjie, 2009). As a result, it has become necessary to evaluate alternative protein sources, among which are the *Moringa oleifera* leaf meals. Presently, research is on-going into the viability of *Moringa oleifera* leaf meal as a protein source for poultry especially in view of the quality and quantity of its nutrient content which includes crude protein, water- and fat-soluble vitamins, calcium, phosphorus and iron (John and Kenaleone, 2014).

Moringa oleifera is among the plants that can be integrated into livestock production feedstock to increase feed quality and availability. *Moringa oleifera* has been widely esteemed as a versatile plant due to its multipurpose uses. The leaves, fruits, flowers and immature pods of the species are edible, and they form a part of traditional diets in many tropical and sub-tropical countries (Siddhuraju and Berker, 2003; Anhwange *et al*., 2004). The leaves are highly nutritious and contain significant quantities of vitamins (A, B and C), calcium, iron, phosphorus and protein (Murro *et al*., 2003). *Moringa oleifera* fresh leaves contain 0.06 mg Vitamin B1, 220 mg Vitamin C, 448 mg Vitamin E, 440 mg Calcium, 42 mg Magnesium, 70 mg Phosphorus and 0.85 mg Iron (Gopalakrishnan *et al*., 2016)

Moringa oleifera seems to reduce the activity of pathogenic bacteria and moulds and improves the digestibility of other foods, thus helping chickens to express their natural genetic potential. Gaia, (2005) and Ayssiwede *et al*., (2011) assessed the effects of MOLM inclusion in poultry diets on growth performances, carcass and organs' characteristics and production performance. Inclusion of *Moringa oleifera* meal as a protein supplement in broiler diets at 25% inclusion level produced broilers of similar weight and growth rate compared to those fed under conventional commercial feeds but had improve feed conversion ratio (Gadzirayi *et al*., 2012). *Moringa oleifera* leaf meal inclusion at 5% improved feed intake, feed conversion ratio and body weight (Safa and Tazi, 2012)

Some studies indicate that *Moringa oleifera* leaf meal can be successfully used in poultry rations to substitute soyabean meal (Melesse *et al*., 2011). One of the reports on the nutritional qualities of *Moringa oleifera* now exist in literature (Fuglie, 2000). However, such reports are lacking information on inclusion levels. Therefore, this study was conducted to determine the effect of *Moringa oleifera* leaf meal supplementation levels on growth parameters, carcass characteristics as well as to carry out a cost benefit analysis on different inclusion levels of Moringa in broiler grower and finisher diets.

2.8. Effect of *Moringa oleifera* leaf meal on growth parameters and carcass characteristics

Gadzirayi *et al.*, (2012) studied the effects of supplementing soyabeans based broiler diets with *Moringa oleifera* leaf meal, as a protein source with different level of supplementation in poultry production using Hubbard® broiler chicks. The researchers reported that there were no significant differences in feed intake for the various Moringa leaf meal inclusion levels, however significant differences in feed conversion ratios were noted. The authors concluded that inclusion of *Moringa oleifera* meal as a protein source in broiler diets at levels of up to 25% produces broilers of similar weight and growth rate compared to those fed under conventional commercial feeds.

Dey and De, (2013) showed that hand ground *Moringa oleifera* leaf meal (MOLM) as a feed additive incorporated at 0.25% and 0.40% levels significantly improved the body weight and feed efficiency of broilers. The cost of broiler production was reduced (15%) due to supplementation with MOLM, the observation of the study is similar to reports by Safa and Tazi, (2012) who showed that *Moringa oleifera* leaf meal (MOLM) inclusion at various levels had a significant and positive effect on broiler live weight. Birds fed on MOLM gained significantly more weight and had superior FCR compare to birds fed on a control diet, with birds fed on the (5% MOLM) diet showing the heaviest body weight, highest total feed intake and had the best feed conversion ratio. In addition, the study of Dey and De, (2013) also reported a significant ($P<0.05$) decrease in mortality rates. Aderinola *et al.,* (2013) conducted an experiment with broilers using *Moringa oleifera* leaf meal (MOLM) as a feed supplement at various inclusion levels, the results revealed that the control diet had higher total weight gain and feed conversion ratio than MOLM based diets. The authors concluded that utilization of MOLM in broiler diet as a supplement could be adopted when the motive is production of broiler meat with low fat content.

Bolu *et al*., (2013) conducted a study on broiler chickens to determine the suitability of feeding *Moringa oleifera* leaf meal and showed that reduction in performance was observed as levels of MOLM increased above 5% of the ration but there was no significant difference in final live weight between birds on the experimental (MOLM) and those on the control diet.

2.9. Probiotics in poultry diets

Antibiotics have been used as additives in poultry feed to enhance the growth performance and protect birds from the negative consequences of pathogenic and non-pathogenic enteric microorganisms. Antibiotic feed additives were banned by the European Union in 2006 due to concerns over the rise of widespread antibiotic resistance in human pathogens (Mirza, 2009). Therefore, the need for alternative techniques for poultry production is increasing and the contribution of probiotics may be considerable (Patterson and Burkholder, 2003). Probiotics are live microorganisms, non-pathogenic bacteria that contribute to the health and balance the dynamics of the intestinal tract of the animal, (Koenen *et al*., 2004; Mountzouris *et al*., 2007) reported that probiotics have a good impact on poultry performance hence they can be used as a replacement for antibiotics.

The short lifespan of broiler chickens and delay in microbial colonization of the intestinal tract can leave the bird's intestine vulnerable to diseases. In the natural environment, the hen is always responsible for feeding and warming up the recently hatched chicks. Feeding may involve use of feed which the parent birds would have temporarily stored in their crop. The feed from the parent's crop will contain beneficial gut microflora which serve as probiotics in the young chicks. Another way for hatched chicks to get beneficial microbes was for those chicks to eat the hen's faeces. The acquired microbes served to protect the chicks from pathogens (Fuller, 2001). The commercially reared chicks are hatched in incubators, those incubators are thoroughly cleaned for hygiene purposes and hence they do not usually contain organisms from the chicken's gut. For the young chicks, lack of contact with the natural environment makes colonization of the intestinal tract with beneficial microbes to be a prolonged process taking around 21 days for broiler chickens to develop balanced intestinal flora (Amit-Romach *et al.*, 2004). After 21 days of the production cycle challenges such as feed formulation changes, stress and diseases can upset the gastrointestinal flora and that can lead to poor growth performance (Gasson *et al.*, 2004).

2.10. Effect of probiotic supplementation on growth parameters and carcass characteristics

Several reviews discuss the effect of probiotics on growth parameters of broiler chickens (Kumprechtova *et al*. 2000; Koenen *et al*., 2004; Vicente *et al.,* 2007 and Botlhoko, 2009). The gut microflora affects the digestion, absorption and metabolism of nutrients (Jin *et al.*, 1997) and most volatile fatty acids formed by intestinal bacterial are absorbed and metabolised by the host contributing to its energy requirements. Maintaining a balance of good gut health is a key aspect of ensuring the best bird performance and health. Alkhalf *et al.*, (2010) showed that there is a significant increase in final body weight, daily weight gain and feed conversion ratio (FCR) by broilers in the last stage of production (week 3 to week 6) when the birds were fed diets supplemented with probiotics. Odefemi, (2016) observed an increase in body weight of broiler chickens fed diets supplemented with probiotics and with the highest body weight gain reported as 1218,15g while the controls weighed in at 1163,68g at six weeks of age. Shirisha, (2016) showed that the overall gain at 42 days of production with dietary supplementation of probiotic and antibiotic yielded significantly ($P<0.05$) higher body weight gain (1700g) followed by commercial probiotic (1691 g) and non-commercial probiotic (1685g) compared to the control (1514 g). However, it was reported that an increase in the inclusion levels of the commercial probiotic were associated with increased mortality. Taklimi *et al*, (2012) stated that feed intake and body weight gain was significantly improved by inclusion of Biomin Imbo® probiotic (BIOMIN Holding GmbH, Herzogenburg, Austria) at 0.1, 0.05 and 0.025% cfu/g.

Karaoglu and Durdag, (2005) investigated the influence of a dietary probiotic on growth, slaughter and carcass characteristics of broiler chickens. The authors reported that live performance was not significantly different between birds on the control diet and those supplemented with probiotics. Similarly, probiotic treatment had no significant effect on the hot and cold carcass weight, carcass yield, weight of carcass cuts and abdominal fat pad.

Anjum *et al*, (2005) investigated the effects of a multi-strain probiotic (Protexin® Somerset, United Kingdom) on broiler growth performance, carcass parameters and economic efficiency and reported that weight gain and feed conversion ratio were significantly ($p<0.05$) improved in chicks fed on Protexin®-supplemented diets compared to a control diet. Further, weight gain of the chick fed on diet C (110g/t of Protexin®) was significantly ($p<0.05$) better than chicks on diet B (100 g/t of Protexin®). Feed conversion ratio was not significantly different between the Protexin®-supplemented groups. Differences in feed intake, meat composition, dressing percentage and empty organ weights among all the diets were nonsignificant. However, abdominal fat content was reduced significantly ($P<0.05$) in Protexin® supplemented groups. The study suggests that Protexin® supplementation is beneficial for better weight gains, feed efficiency and economic efficiency in broiler chicks.

2.11. Effect of *Moringa oleifera* and probiotic inclusion on meat quality

Meat quality is a generic term used to describe properties and perceptions of meat. Generally, meat by being a nutrient (protein), with adequate moisture it becomes a possible substrate for bacterial proliferation, which also gets exacerbated by the inclusion of poor quality feed-grade, fat blends particularly in the starter ration thus creating an environment for pathogenic bacteria to flourish Ferndandez, (2008). It is believed that genetic progress, although beneficial to the industry, has put more stress on growing birds, resulting in historical and biochemical modification of the muscle tissue that are presumed to compromise some meat quality traits (Petracci and Cavani., 2012). Nutrition of birds has significant impact on poultry meat quality (Mir *et al*., 2017). `

Colour is a key factor which influence consumer's selection of fresh meat at the retail level, determining consumer's final evaluation and acceptance of a meat product at time of consumption (Fletcher *et al*., 2000; Muchenje *et al*., 2009a; Dyubele *et al*., 2010). Chicken meat colour is influenced by factors such as diet, age, strain, sex, moisture content (meat), pre-slaughter conditions and processing variables. The observable colour is the results of numerous physical and chemical process in the muscle (Barbut, 2009). Breast meat is expected to have a pale pink colour when it is raw, while thigh and leg meat are expected to be dark red when raw (Fletcher *et al*., 2000).

Nkukwana, (2012) studied the effect of *Moringa oleifera* leaf meal on growth performance, gut integrity, bone strength, quality and oxidative stability of meat from broiler chickens. The study showed that inclusion of *Moringa oleifera* at 3% and 5% level had a significant effect ($p< 0.05$) on colour (L^*,a^* and b^*), highest values of a^* and b^* were found on broiler breast meat of chickens fed diet with 3% and 5% of *Moringa oleifera* leaf meal but there was no significant (P>0.05) treatment effects on h*. Further, it was found that drip loss increased as L* increases.

CHAPTER 3: MATERIALS AND METHODS

3.1. Location of the study

The experiment was conducted at the Experimental Farm at the School of Agriculture University of Venda, situated in Thohoyandou (22°58'32"S, 30°26'45"E; 596m above sea level) in Limpopo Province of South Africa. Thohoyandou falls under Thulamela municipality in Vhembe district. The area experiences daily temperatures varying from 25˚C to 40˚C in summer and between 12˚C to 26˚C in winter. Rainfall is highly seasonal with 95% occurring between October and March. The average rainfall is about 800 mm but varies temporarily (Mzezewa and Gwata, 2012). Thohoyandou is located within a sub-tropical climate region.

3.2. Experimental birds and housing management

The experiment was conducted on 600-day old commercial broiler chicks of Ross 308 strain procured from Alpha company Pty Ltd (Alpha chicks, Pretoria, South Africa). The experimental broiler house and equipment for broiler production management was cleaned and disinfected before arrival of the day-old chicks. Fresh saw dust was provided as bedding, during the study, wet sawdust was removed immediately for good comfort of the birds and to prevent growth of microorganisms which may be harmful to the birds. During the first six days after their arrival, the birds received a poultry vitamin stress pack (Virbac© Samrand Business Park, Centurion, Pretoria, South Africa) mixed in their drinking water.

On day seven, the birds were randomly divided into 30 groups of 20 chicks each, randomly allocated to 30 experimental pens measuring 0.75 × 1.43 m, thereby allowing 0.21 m^2 of floor space per bird. Each pen was equipped with one 175-Watt infrared bulb for heating, one tube feeder and one manual drinker, allowing each bird 12.4 cm feeder space and 9.4 cm drinker space.

3.3. Preparation of Moringa

Moringa oleifera leaf meal (MOLM) was purchased from Bethel Farm No:683 Bethel Mission Gucksdadt Vryheid in Zululand district AbaQulusi municipality. Samples of procured *Moringa oleifera* leaf meal was used in the laboratory analyses to determine the chemical composition of the MOLM.

3.4. Preparation of probiotics

A commercial probiotic (Biosin) was supplied by Nandrea Health Products (Pty) Ltd (Edms) Bpk H/A Biorem and Kosmos (Oudtshoorn, Western Cape, South Africa) and inclusion was as per the manufacturer's recommendations of 1ml/bird/week for the first three weeks.

3.5. Experimental feed diet/ration

From day one up to day twenty-one, the birds were fed on a standard broiler starter crumbs (Meadow feeds, Randfontein, South Africa) with half of the birds not receiving a probiotic (P_0) and the other half receiving a probiotic at 1ml/bird/week (P_1). From day twenty-two, the birds were fed on a standard grower and finisher diet supplemented with different levels of MOLM as follows: M_0 (0 g/kg), M_3 (0.6 g/kg), M_6 (1.2 g/kg), M_9 (1.8 g/kg), M_{12} (2.4 g/kg) as illustrated in Table 3.1 below

Table 3.1: Feeding regime of the Moringa diet.

		Supplementation	
Stage (days)	**Diet**	**MOLM*²**	**PRB*¹**
1 – 21	Starter	0	0 or 1
22 – 35	Grower	0; 3; 6; 9; 12	0
36 – 42	Finisher	0; 3; 6; 9; 12	0

MOLM: *Moringa oleifera* leaf meal; M: *Moringa oleifera* Inclusion levels (M0:0%; M3: 3%; M6: 6%; M9: 9%; M12: 12%). PRB: Probiotic inclusion level (0 and 1)

Table 3.2. Composition of grower and finisher diets formulated with inclusion of *Moringa oleifera leaf meal* (%).

Ingredient Composition(g/kg)	Grower					Finisher				
	M0	M3	M6	M9	M12	M0	M3	M6	M9	M12
Maize	579	576	570	559	559	597.5	587.5	587.5	567.5	555
soya oil cake	180	180	160	140	140	132.5	120	110	105	105
Sunflower oil cake	90	80	80	80	50	72	50	30	30	22.5
Wheat bran	61	50	50	50	50	42	60	60	60	60
Gluten 20	30	30	30	30	30	43,5	40	40	35	35
hominy chop	47	41	40	40	40	100	100	100	100	90
BGM / BFM	3	3	9	1	1	3.5	3.5	3.5	3.5	3.5
Limestone	10	10	10	10	10	9	9	9	9	9
MOLM	0	30	60	90	120	0	30	60	90	120
Total	1000	1000	1000	1000	1000	1000	1000	1000	1000	1000

MOLM: *Moringa oleifera* leaf meal; M: *Moringa oleifera* Inclusion levels (M0:0%; M3: 3%; M6: 6%; M9: 9%; M12: 12%). BGM: broiler grower mash; BFM: broiler finisher mash

3.6. Experimental design, treatments and procedures

The experiment was a 5x2 factorial design (five levels for MOLM and two levels for the probiotic) with three replications for each treatment. Thus, there were 10 treatments, each replication having 20 experimental birds giving a total of 600 unsexed broiler birds for the study. The birds were feed *ad libitum* throughout the study and clean water was freely available all the time through manual bell type drinkers. For birds on treatments that required inclusion of a probiotic, it was administered through the drinking water.

3.7. Chemical analysis of nutrient and mineral composition of experimental diets supplemented with *Moringa oleifera* leaf meal

Analysis of feed was done for Dry matter (DM), crude protein (CP), ash, fat, neutral detergent fibre (NDF) and acid detergent fibre (NDF). Quantification of minerals was carried out for magnesium, potassium, sodium, phosphorus, zinc, copper and iron. The DM was determined according to AOAC (AOAC, 1990; method 930.15). Ash was analysed by combusting at 550°C overnight (AOAC, 1990; method 942.05). The N content was determined using a Kjeldahl procedure (AOAC, 2000; method 976.05) and the CP was calculated as N x 6.25. Neutral detergent fibre and acid detergent fibre were determined using the technique of Van Soest *et al*. (1991). The mineral contents (Calcium (Ca), Magnesium (Mg), Potassium (K), Sodium (Na), Phosphorus (P), Zinc (Zn), Manganese (Mn), Copper (Cu), and Iron (Fe)) of the samples were determined by atomic absorption spectrophotometry (Varian Techtron Pty. Ltd, Springvale, Australia) according to Rowe (1973).

3.8. Vaccination programme of the chickens during the experiment

Table 3.3. The vaccination programme for the birds followed the procedures used at the University of Venda Experimental Farm

Age in days	Vaccination	Administration route
8	Infectious Bronchitis	Drinking water
12	Gumboro	Drinking water
18	Gumboro	Drinking water

23	Newcastle Disease	Drinking water
30	Newcastle Disease	Drinking water

3.9. The effect of various levels of *Moringa oleifera* leaf meal (MOLM) and probiotic inclusion on growth parameters, mortality and carcass characteristics of broiler chickens

3.9.1. Average weekly live body weight and weight gain of chickens fed supplementary *Moringa oleifera* and probiotics

Once every week, a random sample of five birds per replicate was individually weighed using an electronic weighing scale and the weekly weight was recorded. The average weekly live weight of the five birds from each replicate was calculated and from these, the treatment mean was calculated by averaging the mean weekly weights for the three replicates for each treatment. Average weekly weight gain for each treatment was calculated as the difference between the average live weights for two adjacent weeks.

3.9.2. Weekly feed intake

Weekly feed intake was calculated by subtracting the weekly feed leftovers from total weekly feed offered. Birds were given feed *ad libitum* at eight in the morning (AM) daily for three weeks (grower and finisher phase respectively)

3.9.3. Weekly feed conversion ratio (FCR)

Feed conversion ratio per week was calculated using the following formula:

$$FCR = \frac{\text{Average weekly feed consumption per treatment}}{\text{Average weekly weight gain per treatment}}$$

3.9.4. Daily mortality (%) and Health status

Mortality was recorded daily and the total number of dead birds per treatment for a given week was expressed as a percentage of the total number of birds in that treatment at the beginning of each week.

3.9.5. Carcass evaluation

At the end of the experiment, birds were fasted for 12 hours, but they had free access to water all the time. Three birds were randomly selected from each replicate and slaughtered following the protocol described by Netshipale *et al*., (2012). The slaughtered birds were de-feathered, viscera and shanks were removed, and the carcasses were then put onto a carcass hanger and chilled overnight. The evisceration steps and cutting procedures were performed according to the methods of Brake *et al*. (1993). Briefly, each carcass was placed on its back, the drumsticks and thighs were separated and a slanted cut (about 45°) was made just under the keel to the backbone. Abdominal fat (fat surrounding the gizzard, rectum, cloaca and adjacent abdominal muscles) were removed and weighed. The carcass weight dressed out weight, weight of internal organs and other carcass components were determined and recorded.

3.9.6. Meat Quality

The breast was used to measure meat quality; namely pH, dripping loss, water holding capacity, colour and texture as follows:

3.9.6.1. pH

The pH values of chicken breast samples were measured in a homogenate prepared with 10 g of sample and 90 ml distilled water. Homogenization was done using the stomacher (pbinternational. L7) according to Zhang *et al*., (2012). The pH value of the homogenate was measured using a pH meter (basic 20 pH meter, CRISON INSTRUMENTS, SA EU). The pH meter was calibrated with standard 4.00, 7.00, and 9.00 pH buffer. All determinations were performed in triplicates.

3.9.6.2. Dripping Loss

Dripping loss was determined according to Bowker and Zhaung, (2015). Breast samples were weighed using a digital scale (Precision balance, J series RS323, South Africa) prior to being refrigerated for 3 days at 4˚C. After this period, all the samples were weighed once more and drip loss was calculated using the following equation, where W_c is the weight of chicken breast prior to storage and W_f is the weight of chicken breast after storage. Dripping loss (%) = $\frac{W_f - W_c}{W_f} \times 100$

3.9.6.3. Water Holding Capacity

For the determination of water holding capacity (WHC), 8 g of meat from the breast was mixed in 0.6 M sodium chloride solution (12 ml) in a test tube. After centrifugation of the sample at 4˚C for 30 minutes at 5000 rpm, the supernatant was collected and WHC was measured and expressed in percentage following the procedure of Wardlaw *et al*., (1973).

3.9.6.4. Colour

Colour measurements of the meat samples were carried out using a colour measuring system (Model Lab Scan-XE, Hunter Associates Laboratory Inc. USA). A glass cell containing the meat sample was placed above the light source and covered with a back cover and Lightness (L*) Redness, (a*) and Yellowness, (b*) values were recorded following the procedure of Mohamed *et al*., (2008).

3.9.6.5. Texture

TA-XT Plus Texture Analyser (Stable Micro System Ltd, Surrey, England) was used to determine broiler chicken breast texture. Each sample was immobilized between stainless steel plates and then compressed, perpendicular to muscle fibre orientation, in two consecutive cycles of 30% compression with 5 s between cycles, using a cylindrical probe of 4 cm diameter. The crosshead was programmed to move at a constant speed of 1 mm/s. Hardness (g) was calculated. This parameter was obtained by using computer software whereby: Hardness (toughness) is the maximum force reached during the first compressive cycle.

3.9.7. Cost benefit analysis of broiler chickens fed Moringa various diets

A cost benefit analysis was carried out in order to determine which of the dietary combinations gives the best return per rand invested. Kilogram of feed and kilogram of weight gain were calculated using formula:

Cost benefit analysis = Feed cost / kg weight gain

3.10. Statistical Analysis

An analysis of variance was conducted, using the Generalised Linear Models procedure of the Minitab statistical software version 17 (Minitab, 2014), and where necessary (i.e. significant main effects) this was followed by standard means separation using Tukey's procedure (Steel and Torrie, 1986) in Minitab (Minitab, 2014).

The following statistical model was used:

$Y_{ijk} = \mu + M_i + P_j + (MP)_{ij} + \varepsilon_{jkl}$

Where:

μ = overall mean

Y_{ijkl} = Observations on the l^{ith} bird assigned to i^{th} level of Moringa leaf meal, j^{h} probiotic level and measured in the k^{th} week of age

M_i = Effect associated with the i^{th} level of Moringa leaf meal; M=0, 1, 2, 3 and 4; (0%; 3% 6%, 9 and 12%)

P_j = Effect associated with the j^{h} probiotic level: P=0 and 1 (0g/litre and 0.5 g/litre)

$(MP)_{ij}$ = Effect of the interaction between Moringa leaf meal levels and probiotic levels

ε_{ijkl} = random error term; Assumed to be normally and independently distributed with mean 0 and variance equal to d^2

CHAPTER 4: RESULTS

4.1. Analysed nutrient composition of experimental diets

Analysed nutrient composition of experimental diets results are presented in Table 4.1. Nutrient composition of Moringa feed diets: In finisher feed phase, it could be observed that an increase in level of MOLM increased crude protein.

Table 4.1: Analysed nutrient composition of the experimental diets and *Moringa oleifera* leaf meal, on dry matter basis.

Dietary Treatments	DM	Ash	CP	Fat	NDF	ADF
	g/kg	g/kg DM				
Grower (22-35 days)						
M0	89.47	49.8	210	38.4	226.6	80.7
M3	86.53	56.2	208.1	9.9	268.9	76.5
M6	89.43	55.3	205.7	40.3	285.5	68.1
M9	89.22	58.6	207.7	37.3	283.3	73.1
M12	90.67	50.4	197.2	40.8	254	70.3
Finisher (36-42 days)						
M0	90.15	46.8	197.1	37.10	232	64.3

M3	92.41	45.4	193.7	38.50	220.1	61.1
M6	91.26	50.2	188.5	36.60	209.8	62.9
M9	91.61	52.5	203.6	37.20	252.4	74.6
M12	88.69	49.3	212.3	21.20	228	67.4
MOLM	93.57	121.2	301.7	61.7	170	113.2

MOLM: *Moringa oleifera* leaf meal; M: *Moringa oleifera* Inclusion levels (M0: 0%; M3: 3%; M6: 6%; M9: 9%; M12: 12%). DM: dry matter; CP: crude protein; NDF: neutral detergent fibre; ADF: acid detergent fibre.

4.2. Analysed mineral composition of experimental diets

Analysed Mineral composition of experimental diets results are presented in Table 4.2.

Table 4.2: Analysed mineral composition of the experimental diets and *Moringa oleifera* leaf meal, on dry matter basis.

Dietary Treatments	Ca	Mg	K	Na	P	Zn	Mn	Cu	Fe
			g/kg				mg/kg		
Grower (22-35 days)									
M0	7.7	2.5	9.5	1.5	5.6	154	116	16	266
M3	9.1	2.5	9.9	1.5	5.5	150	109	15	273
M6	8.2	2.5	9.7	1.5	5.1	152	103	15	306
M9	9.1	2.6	9.9	1.5	5.2	139	103	17	332
M12	8.8	2.5	9.3	1.4	5.1	132	93	15	293
Finisher (36-42 days)									
M0	6	2.4	8.7	1.4	5	153	102	12	870
M3	6.1	2.4	8.8	1.3	5	145	102	15	242
M6	5.5	2.3	8.8	1.3	4.9	134	92	14	798

M9	7.1	2.6	9.1	1,4	5	140	103	16	299
M12	7.1	2.5	9.3	1	4.7	129	90	10	221
MOLM	25.4	4.7	16.7	0.4	3.3	21	56	3	530

Inclusion levels (M0: 0%; M3: 3%; M6: 6%; M9: 9%; M12: 12%); Ca: calcium, Mg: magnesium, K:potassium, Na: sodium, P: phosphorus, Zn: Zinc, Mn: manganese, Cu: copper, Fe: iron

4.3. Growth parameters during 22-35-day grower phase

Effect of *Moringa oleifera* leaf meal and probiotic inclusion on average body weight gain, feed intake, feed conversion ratio, mortality rate and average live body weight of broiler chickens during the grower phase are presented in Table 4.3. There was no significant MOLM and PRB interaction effects (P>0.05) on all growth parameters, MOLM inclusion did not significantly affect all growth parameters (P>0.05), however, for means across treatments, PRB inclusion significantly reduced mortality rate (P<0.05).

Table 4.3: Effect of *Moringa oleifera* leaf meal and probiotic inclusion on growth parameters (average body weight gain, feed intake, feed conversion ratio, mortality rate and average live body weight) of broiler chickens during grower phase.

Growth Parameters			ABWG (g)	FCR	FI (kg)	MTRT (%)	ALBW (kg)
MOLM	PRB	N					
M0	P0	6	391.00	2.06	0.76	1.67	1.39
M3	P0	6	427.33	1.92	0.76	0.83	1.36
M6	P0	6	388.67	2.02	0.76	0.00	1.34
M9	P0	6	435.33	1.75	0.76	0.83	1.36
M12	P0	6	372.33	2.08	0.76	2.50	1.36
M0	P1	6	422.33	1.89	0.76	0.00	1.37
M3	P1	6	448.00	1.75	0.75	0.00	1.43
M6	P1	6	349.33	2.21	0.76	0.00	1.30
M9	P1	6	328.00	2.55	0.76	0.00	1.27
M12	P1	6	683.33	2.03	0.76	0.00	1.34
SEM			22.004	0.131	0.002	0.393	0.064
MOLM means							
M0		12	406.67	1.98	0.76	0.83	1.38
M3		12	437.67	1.87	0.75	0.42	1.39
M6		12	369.00	2.12	0.76	0.00	1.32
M9		12	381.67	2.15	0.76	0.42	1.32
M12		12	377.83	2.06	0.76	1.25	1.35
SEM			22.002	0.131	0.003	0.392	0.061
PRB means							
P0		30	402.93	1.97	0.76	1.17[a]	1.36
P1		30	386.20	2.09	0.76	0.00[b]	1.34
SEM			11.003	0.074	0.003	0.191	0.032
Significance							
MOLM			NS	NS	NS	NS	NS
PRB			NS	NS	NS	**	NS
MOLM×PRB			NS	NS	NS	NS	NS

**: (P <0.01); (NS) not significant: (P >0.05). [ab] Column Means with different superscript differ significantly at (P <0.01). MOLM: *Moringa oleifera* leaf meal; M: *Moringa oleifera* inclusion levels (M0: 0%; M3: 3%; M6: 6%; M9: 9%; M12: 12%). PRB: Probiotic; Probiotic inclusion levels (P0: No probiotic; P1: 1 ml/bird/week); N: Number of observations; (ABWG) average body weight gain, (FI) feed intake, (FCR) feed conversion ratio, (MTRT) mortality rate, (ALBW) average live body weight.

4.4. Growth parameters during 36 - 42-day finisher phase

Results on the effect of *Moringa oleifera* leaf meal and probiotic inclusion on growth parameters (average body weight gain, feed intake, feed conversion ratio, mortality rate and average live body weight) of broiler chickens during the finisher phase are presented in table 4.4. MOLM and PRB interaction had significant effect on feed intake with an increase mean value at M9P1. MOLM inclusion significantly affect (P<0.05) ABWG and FI with highest mean value at control diet (M0), MOLM inclusion levels had no significant effect (P >0.05) on FCR, MTRT and ALBW. PRB inclusion did not have any significant effect (P >0.05) on all growth parameters.

Table 4.4: Effect of *Moringa oleifera* leaf meal and probiotic inclusion on growth parameters (average body weight gain, feed intake, feed conversion ratio, mortality rate and average live body weight) of broiler chickens during finisher phase.

Growth Parameters			ABWG (g)	FCR	FI (kg)	MTRT (%)	ALBW (kg)
MOLM	PRB	N					
M0	P0	6	813.33	1.78	1.45^{ab}	0.00	2.39
M3	P0	6	560.67	2.57	1.43^{ab}	0.00	2.17
M6	P0	6	719.33	2.07	1.46^{ab}	0.00	2.25
M9	P0	6	617.33	2.28	1.39^{b}	0.00	2.30
M12	P0	6	722.67	1.99	1.41^{ab}	0.00	2.25
M0	P1	6	783.33	1.87	1.45^{ab}	0.00	2.37
M3	P1	6	686.67	2.07	1.38^{b}	0.00	2.33
M6	P1	6	764.00	1.88	1.43^{ab}	0.00.	2.24
M9	P1	6	699.33	2.13	1.47^{a}	0.00	2.13
M12	P1	6	645.33	2.15	1.38^{b}	0.00	2.19
SEM			34.500	0.110	0.010	0.002	0.053
MOLM means							
M0		12	798.33^{a}	1.82	1.45^{a}	0.00	2.38
M3		12	623.67^{b}	2.32	1.43^{ab}	0.00	2.25
M6		12	741.67^{ab}	1.97	1.44^{ab}	0.00	2.24
M9		12	658.33^{ab}	2.21	1.43^{ab}	0.00	2.16
M12		12	684.00^{ab}	2.07	1.40^{b}	0.00	2.22
SEM			34.500	0.113	0.012	0.004	0.051
PRB means							
P0		30	686.67	2.14	1.43	0.00	2.25
P1		30	715.73	2.02	1.42	0.00	2.25
SEM			17.301	0.053	0.014	0.003	0.032
Significance							
MOLM			*	NS	*	NS	NS
PRB			NS	NS	NS	NS	NS
MOLM×PRB			NS	NS	**	NS	NS

**: ($P<0.01$); *: ($P<0.05$); (NS) not significant: ($P>0.05$). [ab] Column Means with different superscript differ significantly at ($P<0.05$). MOLM: *Moringa oleifera* leaf meal; M: *Moringa oleifera* inclusion levels (M0: 0%; M3: 3%; M6: 6%; M9: 9%; M12: 12%). PRB: Probiotic; Probiotic inclusion levels (P0: No probiotic; P1: 1 ml/bird/week); N: Number of observations; (ABWG) average body weight gain, (FI) feed intake, (FCR) feed conversion ratio, (MTRT) mortality rate, (ALBW) average live body weight.

4.5. Carcass parameters

The effect of *Moringa oleifera* leaf meal and probiotic inclusion on carcass parameters of broiler chickens are presented in Table 4.5. MOLM and PRB interaction had significant effect (P<0.05) on dressed weight and non-significant effect (P>0.05) on the rest of parameters. MOLM inclusion levels did not significantly affect (P > 0.05) back fat weight. However, MOLM inclusion level significantly reduced (P<0.05) dressed weight, shank length, wing weight, drum and thigh weight, back weight and breast muscle weight. In all these instances, only the pairwise comparison between the control diet (M0) significantly differed from diets containing 9% MOLM (M0) while all other pairwise comparisons were not statistically different (P>0.05). PRB inclusion in the diets significantly increased (P<0.01) shank size and drum + thigh weights (P < 0.05).

Table 4.5: The effect of *Moringa oleifera* leaf meal and probiotic inclusion levels on carcass parameters of broiler chickens.

Carcass Parameter MOLM	PRB	N	Dress Wt (kg)	Shank (cm)	Back fat (g)	Wing (g)	Drum + Thigh (g)	Back Wt (g)	Breast Wt (g)
M0	P0	6	1.71[ab]	7.57	31.47	89.19[ab]	236.87[abc]	197.08	616.44[ab]
M3	P0	6	1.60[ab]	7.07	25.97	87.22[ab]	218.78[abc]	177.39	544.23[b]
M6	P0	6	1.64[ab]	6.97	28.62	83.40[b]	219.88[abc]	173.32	585.10[ab]
M9	P0	6	1.34[ab]	6.88	27.01	83.66[b]	214.69[bc]	181.50	555.42[b]
M12	P0	6	1.67[ab]	7.15	27.72	88.61[ab]	233.02[abc]	192.70	609.67[ab]
M0	P1	6	1.89[a]	7.80	32.96	100.12[a]	265.57[a]	203.08	707.54[a]
M3	P1	6	1.82[a]	7.42	33.34	92.41[ab]	247.65[abc]	195.41	626.72[ab]
M6	P1	6	1.73[ab]	7.56	27.89	86.45[ab]	253.65[ab]	185.45	630.84[ab]
M9	P1	6	1.48[b]	7.12	21.83	81.41[b]	201.66[c]	161.81	520.61[b]
M12	P1	6	1.58 [ab]	7.43	37.41	85.83[ab]	228.53[abc]	193.67	593.87[ab]
SEM			46.302	0.144	2.642	2.181	6.503	5.651	19.500
MOLM means									
	M0	12	1.80[a]	7.68[a]	32.21	94.65[a]	251.22[a]	200.08[a]	661.99[a]
	M3	12	1.71[ab]	7.24[ab]	29.65	89.81[ab]	233.22[ab]	186.40[ab]	585.47[ab]
	M6	12	1.69[ab]	7.27[ab]	28.25	84.90[ab]	236.77[ab]	179.39[ab]	607.97[ab]
	M9	12	1.56[b]	7.00[b]	24.42	82.51[b]	208.17[b]	171.66[b]	538.01[b]
	M12	12	1.62[ab]	7.29[ab]	32.56	87.22[ab]	230.78[ab]	193.18[ab]	601.78[ab]
SEM			46.302	0.131	2.644	2.191	6.503	5.653	19.512
PRB means									
	P0	30	1.65	7.13[b]	28.16	86.41	224.65[b]	184.40	582.17
	P1	30	1.70	7.47[a]	30.68	89.23	239.41[a]	187.89	615.91

SEM		23.101	0.072	1.324	1.091	3.251	2.830	9.762
Significance								
	MOLM	*	*	NS	**	**	*	**
	PRB	NS	**	NS	NS	*	NS	NS
	MOLM x PRB	*	NS	NS	NS	NS	NS	NS

**: (P<0.01); *: (P<0.05); (NS) not significant: (P>0.05). [abc] Column means with different superscripts differ significantly at (P <0.05; MOLM: *Moringa oleifera* leaf meal; M: *Moringa oleifera* inclusion levels (M0: 0%; M3: 3%; M6: 6%; M9: 9%; M12: 12%). PRB: Probiotic; Probiotic inclusion levels (P0: No probiotic; P1: 1 ml/bird/week); g: gram; cm: centimetre; wt: weight; N: Number of Observations; SEM: Standard Error Mean.

4.6. Giblets parameters

The effect of *Moringa oleifera* leaf meal and probiotic inclusion levels on giblets of broiler chickens are presented in Table 4.6. Both MOLM and PRB inclusion levels had no significant effect (P>0.05) on giblets characteristics.

4.6: *Moringa oleifera* leaf meal and probiotic inclusion levels on giblets of broiler chickens.

Giblets			Heart	Spleen	Gizzard	Liver	Abdominal fat
MOLM	PRB	N	G				
M0	P0	6	10.88	2.04	38.98	46.35	17.83
M3	P0	6	10.70	2.98	35.56	44.90	17.89
M6	P0	6	11.92	2.22	38.02	44.65	17.00
M9	P0	6	11.14	1.56	38.90	42.35	18.61
M12	P0	6	12.07	2.32	43.24	45.50	17.92
M0	P1	6	11.74	2.37	37.69	50.41	17.99
M3	P1	6	12.15	2.04	41.65	49.30	21.29
M6	P1	6	10.98	1.70	36.68	47.13	20.33
M9	P1	6	10.31	2.12	35.24	40.71	16.00
M12	P1	6	11.98	1.96	36.19	44.10	20.58
SEM			0.451	0.240	1.171	1.784	1.462
MOLM							
	M0	12	11.31	2.21	38.34	48.25	17.91
	M3	12	11.42	2.51	40.11	47.10	19.59
	M6	12	11.45	1.96	37.35	45.89	18.66
	M9	12	10.53	1.83	37.37	41.53	17.30
	M12	12	12.03	2.14	39.71	44.80	19.25
SEM			0.441	0.232	1.173	1.780	1.461
PRB means							
	P0	30	11.34	2.22	39.54	44.75	17.85
	P1	30	11.43	2.04	37.49	46.27	19.24
SEM			0.221	0.123	0.592	0.891	0.731
Significance							
	MOLM		NS	NS	NS	NS	NS
	PRB		NS	NS	NS	NS	NS

Table The effect of

MOLM x PRB	NS	NS	NS	NS	NS

(NS) not significant: (P>0.05); PRB: Probiotic; MOLM: *Moringa oleifera* leaf meal; M: *Moringa oleifera* inclusion levels (M0: 0%; M3: 3%; M6: 6%; M9: 9%; M12: 12%). PRB: Probiotic; Probiotic inclusion levels (P0: No probiotic; P1: 1 ml/bird/week); g: gram; N: Number of Observation; SEM: Standard Error Mean.

4.7. Meat quality parameters

The effect of *Moringa oleifera* leaf meal and probiotic inclusion on meat quality parameters of broiler chicken breast muscle is presented in Table 4.7. MOLM and PRB interaction had significant effect (P<0.05) on WHC and hardness but non-significant effect (P>0.05) on pH and dripping loss. MOLM had significant effect (P<0.05) on pH only. Across PRB inclusion levels none of the level had effect (P>0.05) on meat quality parameters.

4.7: *Moringa oleifera* leaf meal and probiotic inclusion on meat quality parameters of broiler chicken breast muscle.

Meat quality p ıeter MOLM	PRB	N	pH	WHC (ml)	Dripping Loss	Hardness N (kg/mm^2)
M0	P0	6	6.04[a]	11.12[ab]	1.31	10.22
M3	P0	6	5.76[ab]	11.07[ab]	2.22	11.91
M6	P0	6	5.92[ab]	11.15[ab]	-1.54	8.08
M9	P0	6	5.78[ab]	11.33[ab]	2.23	6.70
M12	P0	6	5.99[ab]	11.52[a]	-0.14	9.32
M0	P1	6	5.84[ab]	11.51[a]	1.40	9.48
M3	P0	6	5.89[ab]	11.39[ab]	-3.27	9.77
M6	P0	6	5.86[ab]	11.00[ab]	-3.45	10.51
M9	P0	6	5.70[b]	10.79[b]	0.25	14.12
M12	P0	6	5.88[ab]	11.38[ab]	-6.84	6.85
SEM			0.053	0.102	1.780	1.131
MOLM						
	M0	12	5.94[a]	11.32	1.35	9.85
	M3	12	5.83ab	11.23	-0.52	10.84
	M6	12	5.89ab	11.08	-2.49	9.30
	M9	12	5.74[b]	11.06	1.23	10.41
	M12	12	5.94[a]	11.55	-3.49	8.09
SEM			0.052	0.094	1.770	1.132
Probiotic						
	P0	30	5.90	11.24	0.82	10.14
	P1	30	5.84	11.22	-2.38	9.25
SEM			0.020	0.491	0.893	0.564
Significance						
	MOLM		*	NS	NS	NS
	PRB		NS	NS	NS	NS
	MOLM x PRB		NS	*	NS	*

Table The effect of

**: (P<0.01); *: (P<0.05); (NS) not significant: (P>0.05). [ab] Column means with different superscripts differ significantly at (P <0.05. MOLM: *Moringa oleifera* leaf meal; M: *Moringa oleifera* inclusion levels (M0: 0%; M3: 3%; M6: 6%; M9: 9%; M12: 12%). PRB: Probiotic; Probiotic inclusion levels (P0: No probiotic; P1: 1 ml/bird/week); WHC: Water Holding Capacity; ml: millilitre; N: Number of observation SEM: Standard Error Mean.

4.8. Meat colour parameters

The effect of *Moringa oleifera* leaf meal and probiotic inclusion on colour parameters of broiler chicken breast muscle is shown in Table 4.8. MOLM and PRB interaction significantly affect (P< 0.01) C_D, C* and b* mean values at the different levels, hence, the M12 at P0 inclusion increased (P< 0.01) C_D, C* and b* means. The MOLM and PRB interaction did not affect (P> 0.05) h*, a* and L*. However, the MOLM inclusion significantly affect (P< 0.01) C_D, C*, a*, b* and L*, hence M12 increased (P< 0.01) C_D, C*, a*, b* and M3 increased L*. The PRB inclusion significantly affect (P< 0.01) h*(increased) and a*(reduced), and did not significantly affected C*, C_D and L* at any level of inclusion.

4.8: *Moringa oleifera* leaf meal and probiotic inclusion on colour parameters of broiler chicken breast.

Colour Parameter			C_D	h*	C*	a*	b *	L*
	Treatments							
MOLM	PRB	N						
M0	P0	18	0.00^{c}	65.90^{b}	22.45^{c}	9.16^{bcd}	20.42^{d}	54.73^{bc}
M3	P0	18	5.43^{b}	67.21^{ab}	23.51^{c}	9.12^{bcd}	21.60^{cd}	58.13^{a}
M6	P0	18	7.51^{ab}	68.31^{ab}	28.19^{b}	10.37^{ab}	26.19^{b}	53.54^{c}
M9	P0	18	6.44^{b}	68.91^{ab}	28.19^{b}	10.15^{abc}	26.27^{b}	55.86^{abc}
M12	P0	18	9.93^{a}	68.50^{ab}	30.87^{a}	11.37^{a}	28.67^{a}	54.66^{bc}
M0	P1	18	5.20^{b}	69.88^{a}	23.16^{c}	7.98^{d}	21.67^{cd}	56.40^{ab}
M3	P1	18	4.78^{b}	70.35^{a}	24.32^{c}	8.33^{cd}	22.79^{c}	56.81^{ab}
M6	P1	18	7.64^{ab}	69.22^{ab}	29.00^{ab}	10.36^{ab}	27.02^{ab}	53.74^{c}
M9	P1	18	6.39^{b}	69.79^{a}	27.62^{b}	9.54^{abcd}	25.91^{b}	55.99^{abc}
M12	P1	18	7.81^{ab}	69.85^{a}	27.72^{b}	9.62^{abcd}	25.97^{b}	55.25^{bc}
SEM			0.431	0.502	0.374	0.281	0.320	0.374
MOLM								
	M0	36	2.60^{d}	67.89	22.80^{b}	8.57^{c}	21.05^{b}	55.57^{b}
	M3	36	5.11^{c}	68.78	23.91^{b}	8.73_{bc}	22.19^{b}	57.47^{a}
	M6	36	7.57_{ab}	68.77	28.60^{a}	10.37^{a}	26.61^{a}	53.64^{c}
	M9	36	6.41_{bc}	69.35	27.91^{a}	9.84_{ab}	26.10^{a}	55.93_{ab}
	M12	36	8.87^{a}	69.19	29.30^{a}	10.49^{a}	27.32^{a}	54.95_{bc}
SEM			0.423	0.512	0.370	0.271	0.323	0.370
PRB								

Table The effect of

	P0	90	5.86	67.77^{b}	26.64	10.03^{a}	24.63	55.38
	P1	90	6.36	69.82^{a}	26.36	9.17^{b}	24.67	55.64
SEM			0.210	0.252	0.184	0.141	0.163	0.184
Significance								
	MOLM		**	NS	**	**	**	**
	PRB		NS	**	NS	**	NS	NS
	MOLM x PRB		**	NS	**	NS	**	NS

**: (P<0.01); (NS) not significant: (P>0.05) ^{abcd} Column means with different superscripts differ significantly at (P<0.05). MOLM*: Moringa oleifera leaf meal;* M: *Moringa oleifera* inclusion levels (M0: 0%; M3: 3%; M6: 6%; M9: 9%; M12: 12%). PRB: Probiotic; Probiotic inclusion levels (P0: No probiotic; P1: 1 ml/bird/week); C_D: Colour Difference; h*: Hue angel; C*: Chroma; a*: CIE red (+)/ green (-) colour attribute; b*: CIE yellow (+)/ blue (-) colour attribute; L*: CIE lightness coordinate; g: grams; N: Number of Observation; SEM: Standard Error Mean.

4.9. Cost benefit analysis

The effect of MOLM and PRB inclusion on cost benefit analysis is presented on Table 4.9. The cost benefit analysis revealed that inclusion of *Moringa oleifera* leaf meal at all levels investigated in this study is not cost effective due to its high price.

Table 4.9. Effect of *Moringa oleifera* leaf meal on the cost of producing a unit weight (kg) of dressed broiler chickens.

Dietary Treatments	Kg feed/Kg gain	Feed cost(R)/kg gain
Grower (22-35 days)		
M0	1,94	9,54
M3	1,77	18,78
M6	1,96	31,94
M9	1,73	37,98
M12	2,05	56,86
Finisher (36-42 days)		
M0	1,78	8,22
M3	2,54	25,34
M6	2,02	31,40
M9	2,25	45,71
M12	1,96	50,52

M: *Moringa oleifera* inclusion levels (P0: 0%; P3: 3%; P6: 6%; P9: 9%; P12: 12%); kg: kilogram; R: Rands

CHAPTER 5: DISCUSSION

5.1. *Moringa oleifera* and probiotics inclusion on growth performance in the grower phase

The current study showed that there was no significant interaction between *Moringa oleifera* leaf meal and probiotic (MOLM×PRB) in the grower phase for average body weight gain, feed intake, mortality rate, feed conversion ratio and average live body weight indicating consistent but insignificant trends. These observations are similar to results of Onunkwo and George, (2015) who reported that there was no significant difference in growth performance parameters (average daily feed intake, average daily weight gain, feed conversion ratio). Gadzirayi *et al*., (2012) also reported that there were no significant differences in feed intake for the various inclusion level of *Moringa oleifera leaf meal*, the authors concluded that inclusion of *Moringa oleifera* leaf meal as a protein source in broiler diets at levels of up to 25% produces broilers of similar weight and growth rate compared to those fed conventional commercial feeds.

Bolu *et al*., (2013) observed a reduction in performance as level of MOLM increased above 5% but there was no significant difference in final live weight between birds. Dey and De, (2013) showed that hand ground *Moringa oleifera* leaf meal (MOLM) as a feed additive incorporated at 0.25% and 0.40% levels significantly improved the body weight and feed efficiency of broilers, the observation of the study was similar to reports by Safa and Tazi, (2012) who showed that *Moringa oleifera* leaf meal (MOLM) inclusion at various levels had a significant and positive effect on broiler live weight. Birds fed on MOLM gained significantly more weight and had superior FCR compared to birds fed on a control diet, with birds fed on the (5% MOLM) diet showing the heaviest body weight, highest total feed intake and had the best feed conversion ratio (Bolu *et al*., 2013). In addition, the study of Dey and De, (2013) also reported a significant decrease in mortality rates. The contradictory outcomes for the different studies may be attributed to a variety of factors including but not limited to the environmental conditions under which the *Moringa oleifera* grew, the processing of the leaf meal, the broiler strain and management of the birds in general.

Probiotic inclusion had significant reduced on mortality rate with a higher mortality in birds feds a diet that did not contain the probiotic compared to chickens which were fed probiotics. High mortality rate of untreated (P0) birds might be explained in terms of a delay in intestinal colonization by beneficial microbes leaving the bird's intestine vulnerable to colonization by disease causing organisms. Similar findings were observed by Vicente *et al*. (2007) and Deiver (2008) who observed that effective microorganism supplementation significantly reduced mortality in chickens. However, Wondmeneh *et al*., (2011) reported that supplementation of commercial probiotic to broiler chickens had no significant effect on mortality. Probiotics had no significant effect on average body weight gain, feed intake, mortality rate, feed conversion ratio and average live body weight, similar results were presented by Karaoglu and Durdag, (2005) who showed that live performance was not significantly different between birds on the control diet and those supplemented with probiotics.

Alkhalf *et al*., (2010) showed that there is a significant increase in final body weight, daily weight gain and feed conversion ratio (FCR) in broilers in the last stage of production (week 3 to week 6) when the birds were fed diets supplemented with probiotics. Odefemi, (2016) observed an increase in body weight of broiler chickens fed diets supplemented with probiotics and with the highest body weight gain reported as 1218,15g while the controls weighed 1163,68g at six weeks of age.

5.2. *Moringa oleifera* and probiotics inclusion on growth performance in the finisher phase

The results of the study showed that during the finisher phase there was a significant difference between chickens which were fed *Moringa oleifera* leaf meal and probiotic (MOLM×PRB) compared to the control group on feed intake with an increase at 9% *Moringa oleifera* leaf meal and inclusion of a probiotic. MOLM and PRB inclusion at level 9% together with probiotic (M9×P1) increased feed intake at finisher phase compared to the other treatments. However, the interaction between MOLM and PRB had no significant effect on average body weight gain, feed conversion ratio, mortality rate and average live body weight in finisher feed phase. Increase in feed intake of birds fed a probiotic supplemented diet might be due to a healthy gut leading to better digestion and hence to more feed consumption.

Inclusion of *Moringa oleifera* leaf meal without a probiotic had a significant effect on feed intake with the highest intake recorded for rations contained no MOLM while a lower intake was observed for birds fed diets containing 12% MOLM. The general trend observed was that an increase in level of MOLM lead to a decrease in feed intake. Similar results were observed by Bolu *et al*., (2013) who observed a reduction in performance as level of MOLM increased above 5% level. However, Tesfaye *et al*., (2013) found that there was a significant increase in feed intake in groups supplemented with MOLM compared to the control groups.

Moringa oleifera leaf meal significantly reduced feed intake and average body weight gain. The highest ABWG increase was observed for birds feed the MOLM free diet. These findings suggest that MOLM inclusion decreases average body weight gain. This contradicts the results reported by Nkukwana *et al*., (2014) who showed that birds supplemented with *Moringa oleifera* leaf had increased body weight than the birds fed the basal diets. Safa and Tazi, (2012) showed that *Moringa oleifera* leaf meal (MOLM) inclusion at various levels had a significant and positive effect on broiler live weight. Birds fed on MOLM gained significantly more weight and had superior FCR compare to birds fed on a control diet, with birds fed on the (5% MOLM) diet showing the heaviest body weight, highest total feed intake and had the best feed conversion ratio. The divergent results are most likely due to disparities in the levels of inclusion and possibly the quality of the MOLM. The current study deliberately set out to test inclusion levels on the upper end of those reported in literature in the hope that inclusion of a probiotic might enable higher levels of MOLM inclusion. Based on the results it appears that the inclusion of a probiotic did not positively affect the amount of MOLM that may be incorporated in broiler diets.

Probiotic inclusion had no significant effect on average body weight gain, feed conversion ratio, feed intake, mortality rate and average live body weight in finisher phase. These results are similar to those of Sarker *et al*., (2017) who reported no significant differences on body weight, weight gain, feed intake and FCR between the control and MOLM treatments. Contrary observation was reported by Taklimi *et al*., (2012) who stated that feed intake and body weight gain was significantly improved by inclusion of Biomin Imbo® probiotic (BIOMIN Holding GmbH, Herzogenburg, Austria) at 0.1, 0.05 and 0.025% cfu/g. Similarly, Shirisha, (2016) showed that the overall gain at 42 days of production with dietary supplementation with probiotic or antibiotic yielded significantly higher body weight gain (1700g) for the antibiotic, followed by those on a commercial probiotic (1691g) and on a commercial probiotic (1685g) compared to (15414g) for the control (1514 g).

5.3. *Moringa oleifera* and probiotic inclusion on carcass characteristics.

The interaction between MOLM and PRB had significant effect on dressed weight and nonsignificant effect on back fat weight, shank length, wing weight, drum + thigh weight, back weight and breast muscle weight. However, *Moringa oleifera* leaf meal (MOLM) inclusion had significantly reduced dressed weight, wing, shank, drum + thigh, back weight and breast weight with an increase at M0 level, which means inclusion of MOLM had decrease carcass mean values (dressed weight, wing, shank, drum + thigh, back weight and breast weight) similar to the findings of the study by Zanu *et al*., (2012) who indicated that none of the parameters measured for carcass characteristics in birds fed diets containing *Moringa oleifera* leaf meal was affected significantly by inclusion of Moringa leaf meal and different findings by Ologhobo *et al*., (2014) who found that, higher mean values of slaughter weights were recorded for birds fed diets containing *Moringa oleifera* leaf meal as compared to those fed on the control diet.

Probiotic inclusion significantly increased shank and drum + thigh with an increase in P1 which shows that inclusion of probiotic to broiler chickens increase shank size and drum + thigh, reason being probiotics in broiler chickens have good impact on the growth and general health of the chicken. Similar to Macelline *et al*., (2017) who showed that broiler chickens fed 1.4% *saccharomyces cerevisiae* (probiotic) supplemented in the diet showed increased in thigh muscle weight compared to broilers fed the 0.6% and 1% supplemented diets. Contrary to findings by Karaoglu and Durdag, (2005) the authors found that probiotic treatment had no significant effect on the hot and cold carcass weight, carcass yield, weight of carcass cuts and abdominal fat pad.

5.4. *Moringa oleifera* and probiotic inclusion on giblets parameters.

Moringa oleifera leaf meal and probiotic inclusion levels did not have significant effect on broiler giblets (heart, spleen, gizzard, liver and abdominal fat) parameters, Similar to findings by Hassan *et al*., (2015) who showed that different levels of MOLM had no significant effect on carcass relative weight, liver, gizzard, heart, abdominal fat, breast and thigh. Nkukwana *et al.,* (2014) observed that addition of MOLM (0.1-2.5%) to broiler diets have no significant effects on carcass weight, dressing percentage and the relative weights of the liver, gizzard, heart and spleen. Contrary results were observed by Ologhobo *et al*., (2014) who showed that the addition of *Moringa oleifera* leaf meal to the diets of broiler chickens had no significant effect on the carcass qualities of the birds, but higher mean values of slaughter weights (breast, drumstick, spleen and heart) were recorded for birds fed diets containing *Moringa oleifera* leaf meal. Similar to findings by Onunkwo and George, (2015) who observed significant difference in organ weights (wings, shank, drumsticks, kidney, liver, gizzard) and some cut parts between the experimental and control groups.

Sarker *et al*., (2017) found that organ weight like liver, heart, kidney, spleen, gizzard, abdominal fat and intestine weight also did not show any significant differences between the dietary treatments of control and Biofast (probiotics), Konca *et al*., (2009) showed that dietary treatments did not affect liver, heart, empty gizzard and abdominal fat weights. Different from findings obtained by Anjum *et al.,* (2005) who reported that abdominal fat content was reduced significantly in Protexin® supplemented groups compare to control.

5.5. *Moringa oleifera* and probiotic inclusion on meat quality parameters.

MOLM×PRB interaction at different inclusion levels had a significant effect on water holding capacity and hardness. The findings for interaction between *Moringa oleifera* leaf meal and probiotics inclusion at different levels significantly did not affect pH values, however, the MOLM increased with M0 and decline at M3, M6, M9 and then increased again at higher inclusion level of MOLM (M12) on pH, these findings are similar to results observed by Nkukwana *et al*., (2012) which showed that dietary treatments had no significant effects on breast meat pH measurements. Sarker *et al*., (2017) observed non-significant differences found in pH, cooking loss, meat color and TBA values between the treatments, similar to results by Al-Owaimer *et al*., (2014) who observed that treatment had no effect on pH value when measured 15 min post-mortem, similar, to results found by Pelicano *et al*,. (2003) reported that probiotic did not have effect on pH of broiler meat.

MOLM and PRB interaction had no significant effect on dripping loss, the findings are similar to the results observed by Al-Owaimer *et al*., (2014) observed non-significant effect of probiotics on dripping loss and contrary to water holding capacity, Ali, (2010) and Pelicano *et al*., (2003) found that probiotic treated broiler groups gave higher breast meat WHC compared to birds fed a probiotic absent diet at the age of 42 days and different to Macelline *et al*., (2017) who observed that broilers fed a diet with SC (probiotics) for short-term breast meat gave less WHC compared to the meat of broilers fed control diet.

5.6. *Moringa oleifera* and probiotic inclusion on meat colour parameters.

MOLM and PRB interaction significantly affect C_D, C* and b* at the different levels, hence, M12 at P0 inclusion increased C_D, C* and b* means, MOLM and PRB interaction did not affect h*, a* and L*. However, the MOLM inclusion significantly increased C_D, C*, a*, b* and L*, hence M12 increased C_D, C*, a*, b* and M3 increased L* but significantly did not affect h*, unfortunately information of *Moringa oleifera* on colour parameters is scarce but Qwele *et al*., (2013) found no significant effect on a* and b* but there was increase in L*. PRB significantly affect h* and a*, P1 increased h*and P0 increased a*, PRB inclusion did not affect C*, $C_{D,}$ b* and L* significantly at any level of inclusion results are similar to Sarker *et al*., (2017) who showed no significant were found in meat colour, L* means for control was numerically higher that probiotic means, Konca *et al*., (2009) showed that meat colour were not influenced by dietary MOS and SC supplementation, Al-Owalmer *et al*., (2014) observed non-significant effect on lightness (L*) and yellowness (b*) values of breast meat. Contrary to Pelicano *et al*., (2003) who found that probiotics inclusion increases L* value while no different were observed for a*and b*.

5.7. *Moringa oleifera* and probiotic inclusion on cost benefit analysis.

Different levels of MOLM in diet did not gave any cost benefit analysis, kilogram feed per kilogram gain increases with level of MOLM same results in feed cost per kilogram gain. The results show that in this study no diet gave best return per weight gain or per rand invested. Contradictory to results found by Onunkwo and George. (2015) who showed that costs of a kilogram weight gain of the *Moringa oleifera* leaf meal-based diets were lower than the control and Ayssiwede *et al*., (2011) showed that *Moringa oleifera* leaf treatments meal was most economically profitable compared to the control.

CHAPTER 6: CONCLUSION AND RECOMMENDATIONS

6.1. Conclusion

Feeding high level of MOLM (M12) without probiotics (P0) increases mortality, so addition of probiotic (P1) with MOLM up to M12 level reduces mortality and that makes *Moringa oleifera* leaf meal to be a good source of protein supplement in grower phase. On finisher phase MOLM can be fed with or without probiotic without affecting growth parameters and health of the birds which results to 0% mortality. *Moringa oleifera* leaf meal can make up to 12% of the ration with or without probiotics with no negative effects on carcass dressing weights and giblets parameters. Presence of MOLM and probiotics give normal level of pH which range from 5.7 to 6.0. WHC increases in presence of MOLM up to 12% without probiotics. MOLM can be added to broiler diets without inclusion of probiotics and it increases level of C_D, C* and b* which is consumer's preference meat colour. Based on the cost benefit analysis, inclusion of MOLM is not economically viable due to the high price of *moringa oleifera* leaf powder. It is possible that a different situation may prevail if the poultry producers are in a position to grow their own *Moringa oleifera.*

6.2. Recommendations

It is recommended to use *Moringa oleifera* and probiotics in broiler production as feed additives, MOLM as good source of protein up to 12% and 1ml/bird probiotics (Biosin), probiotics for beneficial of the gut and good health of the birds and reduction of mortality in broiler chicken production. Information on MOLM inclusion on meat colour parameters is limited or unavailable, hence further investigations need to be done. From this study, inclusion of *Moringa oleifera* leaf meal financially is not recommended due to high cost but for biological reasons it can be recommended. However, local farmers can plant their own Moringa trees and produce their own powder to be used in feed as a protein supplement and this will lower the cost of the MOLM.

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APPENDICES

Appendix 1: Calculation procedure

$$FCR = \frac{\text{Average weekly feed consumption per treatment}}{\text{Average weekly weight gain treatment}}$$

Appendix 2: Analysis of Variance for growth parameters in grower phase

Source	Df	IBW	ABWG	FI	FCR	MTRT	ALBW
MOLM	4	6805.7	9312	0.000069	0.1939	2.708	0.013074
PRB	1	24.0	4200	0.000038	0.2178	20.417**	0.005568
MOLM*PRB	4	14199.0	9898	0.000114	0.4904	2.708	0.008664
Error	50	8779.5	7274	0.000215	0.2663	2.250	0.057063

** P<0.01, df: Degrees of Freedom, IBW: Initial Body Weight, ABWG: Average Body Weight Gain, FI: Feed Intake, FCR: Feed Conversion Ratio, MTRT: Mortality Rate, ALBW: Average Live Body Weight, MOLM: *Moringa oleifera* Leaf meal, PRB: Probiotic.

Appendix 3: Analysis of Variance for growth parameters in finisher phase

Source	df	IBW	ABWG	FI	FCR	MTRT	ALBW
MOLM	4	0.08590	28826*	0.003509**	0.22786	ND	0.037120
PRB	1	0.04403	6337	0.000140	0,10326	ND	0.000203
MOLM*PRB	4	0.04164	10219	0.004128**	0.10131	ND	0.012632
Error	20	0.03134	8933	0.000769	0.09031	ND	0.018054

** P<0.01, P<0.05, df: Degrees of Freedom, IBW: Initial Body Weight, ABWG: Average Body Weight Gain, FI: Feed Intake, FCR: Feed Conversion Ratio, MTRT: Mortality Rate, ALBW: Average Live Body Weight, MOLM: *Moringa oleifera* Leaf meal, PRB: Probiotic, ND: Non defined.

Appendix 4: Analysis of Variance for carcass parameters

Source	Df	Dress W	Shank	Back Fat	Wing	Drum + Thigh	Back W	Breast
MOLM	4	98288*	0.73188*	132.16	262.96**	2888.9**	1497.8*	23875**
PRB	1	30917	1.75104**	95.91	119.51	3271.1*	182.4	17078
MOLM*PRB	4	85068*	0.06896	109.41	96.78	1422.6	626.8	9723
Error	50	32092	0.27254	104.73	71.43	633.1	479.2	5721

** P<0.01, P<0.05, df: Degrees of Freedom, W: Weight, MOLM: *Moringa oleifera* leaf meal, PRB: Probiotic.

Appendix 5: Analysis of Variance for giblets parameters

Source	Df	Spleen	Heart	Gizzard	Liver	Abdominal fat
MOLM	4	0.7935	2.5798	22.31	79.51	10.66
PRB	1	0.5264	0.1233	63.22	34.76	29.02
MOLM*PRB	4	1.1429	3.2966	41.31	24.73	20.26
Error	50	0.8830	2.9728	20.68	47.31	32.01

df: Degrees of Freedom, MOLM: *Moringa oleifera* leaf meal, PRB: Probiotic.

Appendix 6: Analysis of Variance for Meat quality parameters

Source	Df	Dripping Loss	Hardness	WHC	pH
MOLM	4	56.83	41321922	0.99908	0.24965*
PRB	1	153.23	36093087	0.03756	0.20944
MOLM*PRB	4	23.64	153276340*	1.32686	0.13587
Error	50	47.44	56953160	0.43245	0.09171

P<0.05, df: Degrees of Freedom, WHC: Water holding capacity, MOLM: *Moringa oleifera* leaf meal, PRB: Probiotic.

Appendix 7: Analysis of Variance for colour parameters

Source	df	C_D	h*	C*	a*	b*	L*
MOLM	4	208.740**	11.45	311.048**	29.401**	288.359**	70.381**
PRB	1	11.222	189.20**	3.454	33.835**	0.062	2.949
MOLM*PRB	4	69.030**	18.19	26.280**	3.742	24.897**	10.342
Error	170	8.223	11.44	6.020	3.600	4.621	6.008

** P<0.01, df: Degrees of Freedom, C_D: Colour Difference; h*: Hue angel; C*: Chroma; a*: CIE red (+)/ green (-) colour attribute; b*: CIE yellow (+)/ blue (-) colour attribute; L*: CIE lightness coordinate MOLM: *Moringa oleifera* leaf meal, PRB: Probiotic.