A nutritional assessment of *Moringa oleifera* leaf meal for chickens commonly reared under extensive production systems: Effect on growth performance, serum biochemistry, and meat quality

Sebola Nthabiseng Amenda

(22954457)

Bachelor of Science Honours Degree in Agriculture (University of Limpopo, 2007)

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Supervisor: Prof. V. Mlambo

Co-Supervisors: Dr. H. K.Mokoboki

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DEDICATION

This thesis is dedicated to my mother, Ms Sebola Selina Motlatjo, sisters Khutso and Kgaugelo Sebola, Brother Franklin Sebola, My best friend Mashaba Khanya, My father Masilo Ratopola. Without your support, I wouldn't be where I am today. To my little angel Angelisa Lesedi, a big thanks you for understanding that mommy has to study.

An education isn't how much you have committed to memory, or even how much you know. It's being able to differentiate between what you do know and what you don't. It's knowing where to go to find out what you need to know; and its knowing how to use the information that you get.

William Feather

DECLARATION

I confirm that this is my own work and the use of all material from other sources has been properly and fully acknowledged.

Signed: …….. Date: ……

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ABSTRACT

Poultry production in most rural parts of South Africa is characterized by small scavenging operations. Indigenous chickens which are considered to be of low productivity due to poor growth rate, few eggs produced, high mortalities, susceptibility to diseases and long brooding period (Tadelle *et al.*, 2000). The major reasons for the poor productivity of indigenous village chickens are poor feed resource base, limited foraging ranges and poor management practices (Alders *et al.*, 2001; Swatson *et al.*, 2001). Proximate, minerals and fatty acids composition of *Moringa oleifera* leaves at different stages of maturity as well as the apparent digestibility of *M. oleifera* leaf meal (MOLM)-based diets in three chicken strains were determined. The leaves were harvested green, air-dried in a well-ventilated laboratory and milled into powder using a hammer mill to pass through a 1 mm sieve, to produce *M. oleifera* leaf meal (MOLM). The leaf meal was chemically analysed and used to dilute a commercial broiler finisher diet at 0 (MOLM0), 25 (MOLM25), 50 (MOLM50), and 100 (MOLM100) g/kg DM, producing four isoenergetic and isonitrogenous dietary treatments. A 90-day feeding trial was conducted to determine the effect of *Moringa oleifera* leaf meal supplementation on productivity, carcass characteristics, meat quality and haematology and biochemical indices of three chicken strains. Two hundred and sixteen (216) Potchefstroom Koekoek (PK), Ovambo (OV) and Black Australorp (BA) chickens were raised on a commercial starter mash for four weeks. On the fourth week, experimental diets were offered and growth performance data were collected for 13 weeks. The data obtained from the present study indicate that tender *M. oleifera* leaves can be utilised as feed for poultry due to its high quality protein and low crude fibre content. Digestibility data indicate that inclusion of MOLM in chicken diets did not negatively affect nutrient digestibility. Maximum feed intake was achieved at dietary MOLM inclusion levels between 50 and 70 g/kg DM. Black Australorp chickens had the highest feed conversion

efficiency (FCE) of 2.35, while OV and PK chickens had lower FCE values of 2.09 and 2.05, respectively. Male chickens attained higher ($P<0.05$) carcass weight, leg and thigh weight, dressing percent, and breast mass than female chickens ($P<0.001$). Inclusion of MOLM up to 10 g/kg had no adverse effect on the health and nutritional status of the three chicken strains. Macroscopic examination showed normal morphology of liver tissues in all chicken strains across all MOLM inclusion levels. Diet MOLM50 resulted in lower shear force and lower cooking loss, which indicates good meat tenderness. However, inclusion of MOLM did not affect fatty acid profile of the meat. Inclusion of MOLM in chicken diets positively affected growth performance carcass characteristics, haematological parameters, biochemical indices and meat quality of chickens. In conclusion, MOLM could be of great benefit to both feed millers and farmers due to its health benefits and in turn will reduce feed costs.

Keywords: *Moringa oleifera* leaf meal, digestibility, productivity, carcass characteristics, meat quality, haematological, serum, biochemical indices

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

a*	Redness
ADF	Acid Detergent Fiber
ADL	Acid Detergent Lignin
ANOVA	Analysis of Variance
AOAC	Association of Analytical Chemistry
b*	Yellowness
BA	Black Australorp
CLA	Conjugated Linoleic Acid
CP	Crude Protein
CF	Crude fiber
CL	Cooking loss
DM	Dry Matter
EE	Ether Extract
FA	Fatty Acid
Fe	Iron
g	Grammes
GLM	General Linear Model
kg	Kilogrammes
L*	Lightness
mg	Milligrammes
MUFA	Mono unsaturated fatty acids
N	Nitrogen
NDF	Nutrient Detergent Fiber
NWU	North West University
OV	Ovambo
PDIFF	Probability of Difference
pH	Potential of Hydrogen
PK	Potchefstroom Koekoek
PUFA	Polyunsaturated Fatty Acids
SAS	Statistical Analysis System
SFA	Saturated fatty acids

SLW	Slaughter weight
WBS	Warner - Bratzler Shear force

1. GENERAL INTRODUCTION

1.1 Background

South Africa's population is growing at almost 2% per year (Agricultural Statistics, 2008). The population of 49 million in 2009 is expected to grow to 82 million by the year 2035 (Agricultural Statistics, 2008). Food production must be more than doubled to feed the expanding population, using the same or fewer natural resources (Agricultural Statistics, 2008). Poultry is now by far the largest livestock species worldwide (FAO, 2004), accounting for more than 30% of all animal protein consumption (Permin & Pedersen, 2000). The contribution of local poultry production to the nutritional and economic status of rural households is well recognized (Norris & Ng'ambi, 2006). Indigenous breeds of chickens have been a product of their environment and have survived under harsh conditions for many generations (Umesiobi, 2000; Fourie *et al.*, 2004). However, there has been a decline in the number of indigenous chickens, mainly due to their poor productive and reproductive performance (Larbi *et al.*, 2013). According to Teketel (1986), the productivity of indigenous chickens, expressed in terms of egg production, egg size, growth and survivability of chicks under rural production systems is very low. The low productivity may be attributed to inferior genetics, predators, diseases, poor feeding and management factors (Alemu, 1995; Alemu & Tadelle, 1997). Mohammad & Sohail (2008) stated that balanced nutrition is an important factor in determining performance and productivity of chickens.

Rural households maintain their poultry by feeding them food leftovers due to limited resources. The high feed prices necessitate the search for cheaper high energy and protein feed ingredients (Moustafa *et al.*, 2008). Therefore, efficient utilization of alternative feed resources that cannot be utilised as food for humans should be fully explored. Indigenous plants rich in protein and minerals can be utilised as alternative feed. Protein is an essential key ingredient

of animal feeds required for growth, body maintenance, reproduction, milk, eggs and wool. In traditional low output farming systems, the protein supply can be met from plants and crops grown locally. The use of leaf meals from multipurpose plants as alternatives to conventional feed resources is a novel area of research. One such tropical plant that shows nutritional potential is *Moringa Oleifera* (Drumstick tree). The plant is known for its high leaf and seed protein (27%) content, adequate amino acid profile, high level of vitamins A and E, and low level of anti-nutritional compounds (Yang *et al.*, 2006). Olugbemi et al. (2011) noticed that supplementation of *Moringa oleifera* leaf meal at levels of up to 10% in a cassava chip-based diet offered to laying hens had no significant effect on feed intake, feed conversion ratio, and laying percentage. Egg weight significantly increased as a result of the supplementation of *Moringa oleifera* leaf meal with cassava chip when compared to a control diet (free of *Moringa oleifera* leaf meal and cassava chip). Therefore this plant, Moringa, make an ideal candidate for improving poultry nutrition, especially that dietary amino acid composition is important in non-ruminants.

1.2 Problem statement

Poultry production in most rural parts of South Africa is characterized by small scavenging operations. Indigenous chickens which are considered to be of low productivity due to poor growth rate, few eggs produced, high mortalities, susceptibility to diseases and long brooding period (Tadelle *et al.*, 2000). The major reasons for the poor productivity of indigenous village chickens are poor feed resource base, limited foraging ranges and poor management practices (Alders *et al.*, 2001; Swatson *et al.*, 2001). The growth of human and livestock populations has increased the competition for food and feed between animals and man. This calls for identification and evaluation of alternative feed resources for livestock must be identified and evaluated (Nworgu *et al.*, 2007). In evaluating such unconventional feed resources, it is also

important to assess the effects of such feed resources on the health status of the livestock and the quality of products.

1.3 Justification

Commercial broiler feed in South African is expensive and therefore out of reach for small-holder farmers. The major ingredients of broiler feed are maize and imported protein concentrates based on soybean concentrates, fish or animal meal. Data on nutrient requirements of the indigenous chickens is limited, particularly on energy and crude protein requirements. Knowing requirements of these nutrients will help in the formulation of diets to optimize productivity of the birds. The scarcity of locally produced protein supplements for animal diets in the tropics has created a need for finding alternative feed resources (Nworgu *et al.*, 2007). Edible wild indigenous plants have become an alternative source of feed with high potential of vitamins, minerals and other interesting elements particularly during seasonal food shortages (Glew *et al.*, 2005). Kakengi *et al.* (2007) revealed high pepsin and total soluble protein in *Moringa oleifera* leaf meal (MOLM). The high pepsin and total soluble protein makes MOLM more suitable feed or additive to monogastric animal diets such as poultry.

However, to sustain poultry production based on such feedstuffs, more research is necessary to characterise these feedstuff with regard to their digestibility, amino acid profile and content of anti-nutritional factors especially if high inclusion levels will be used (Teguia & Beynen, 2005).

1.4 Objectives

The broad objective of this study was to determine the effect of supplementing *M. oleifera* leaf meal on productivity, carcass characteristics and biochemical indices of indigenous chickens raised under a confined production system.

The specific objectives of the study were to:

1. To determine chemical characterisation of *Moringa oleifera* leaves and apparent nutrient digestibility of *M. oleifera* leaf meal-based diets in three chicken strains.
2. To determine the effect of *M. oleifera* leaf meal supplementation on productivity and carcass characteristics of extensively-reared chickens.
3. To examine the haematological, serum biochemical indices and histopathology of indigenous chickens as affected by dietary *M. oleifera* leaf meal.
4. To determine the effect of *M. oleifera* leaf meal on meat quality and fatty acid composition of three extensively-reared chickens.

1.5 Research questions

The research questions of the study were to:

1. Is there variation in chemical characterisation of *Moringa oleifera* leaves and apparent nutrient digestibility of *M. oleifera* leaf meal-based diets in three chicken strains?
2. Does the inclusion of *M. oleifera* leaf meal in poultry diets affect growth and carcass characteristics of indigenous chicken?
3. Does *M. oleifera* leaf meal affect haematological, serum biochemical indices and histopathology of indigenous chickens?
4. Is there variation in meat quality and fatty acid composition of indigenous chickens fed incremental levels of *M. oleifera* leaf meal?

2 LITERATURE REVIEW

2.1 Importance of poultry in rural economies

Indigenous chickens (*Gallus domesticus*) are the predominant poultry species in the rural areas of Africa (Andrews, 1990; Jalaludin, 1992). Indigenous poultry characterized by lack of regular health control programmes, unimproved shelter and scavenging to meet the nutritional needs (Yongolo, 1996). Local chickens are an important source of high quality protein (meat and eggs) and they also provide small cash income (Tadelle *et al.*, 2000).

The poultry industry in developing countries such as South Africa can be divided into two sub-sectors, namely commercial and traditional sub-sectors (John, 1995; Gueye, 1998). The commercial breeds are confined to the urban and peri-urban areas where the infrastructure necessary for the production and market of produce exists. However, the traditional sub-sector, on the other hand, consists mainly of indigenous birds which are made up of different breeds and or lines such as the Koekoek chicken. This sub-sector is important for the livelihood of most rural households (Sonaiya, 2001). This sub-sector, currently, constitutes about 80 % of the country's rural poultry flock and is a major source of readily available protein in the form of eggs and meat as well as for cash money for 90 % of the rural households (Gueye, 1998). However, when compared to commercial layer and broiler chickens, the indigenous chickens produce fewer eggs and have smaller body weights (Ebangi & Ibe, 1994; Safalaoh, 2001). Furthermore, the indigenous chickens tend to have lower feed efficiency (Kingori *et al.*, 2003; Tadelle *et al.*, 2003). According to Teketel (1986), the productivity of indigenous chickens, expressed in terms of egg production, egg size, growth and survivability of chicks under the rural production systems is low. This low productivity may be attributed to lack of improved poultry breeds, the presence of predators, and the high incidence of chicken diseases, poor feeding and management factors (Alemu, 1995; Alemu & Tadelle, 1997). Formulation of

alternative feed resources which meet protein and energy requirements of indigenous chickens should be well researched to ensure adequate food security for the increasing population. However, formulation of these feed should be vital to both livestock and consumers.

2.2 Nutrient requirements of indigenous and exotic poultry

Dramatic improvement in the productivity of poultry can be partially attributed to improvements in formulation of diets. Detailed knowledge of nutrient requirements is necessary for continued improvements in productivity (Lamberson & Firman, 2002). Chinrasri (2004) and Laohakaset (1997) defined nutrient requirement as the amount of nutrients needed by animals to maintain their activities, maximize growth and feed utilization efficiency, improve laying capacity and hatchability and optimize fat accumulation. Carbohydrates, lipids and protein that the chicken utilizes as sources of energy or as parts of its metabolic machinery are essential requirements for growth. Growth involves deposition of bones, muscle and fat, each exhibiting an individual pattern of development (Carlson, 1969).

2.3 Protein requirements in chickens

Protein is made up of amino acids. The need for the essential amino acids determines the need for protein, and a reduction in dietary protein that results in deficiencies of several essential amino acids. Feed proteins are complex amino acid polymers which are broken down in the gut into amino acids (NRC, 1994). These amino acids are absorbed and assembled into body proteins which are used in the construction of body tissue e.g. muscles, nerves, skin and feathers. Protein and amino acid requirements vary considerably according to the productive state of the bird, that is, the rate of growth or egg production. If dietary protein is inadequate, there is a reduction or cessation of growth or productivity and a withdrawal of protein from less vital body tissues to maintain the functions of more vital tissues (NRC, 1994).

Amino acid requirements differ among types, breeds, sex and strains of poultry. For example, male broiler chickens have higher protein requirements than females (Thomas *et al.*, 1986; Han & Baker, 1993), because male chickens contain more protein and less fat in their body tissue (Edwards *et al.*, 1973; Han & Baker, 1991). Genetic differences in amino acid requirements may occur because of differences in efficiency of digestion, nutrient absorption, and metabolism of absorbed nutrients (NRC, 1994). NRC (1994) recommended 23, 20 and 18 % dietary protein levels, respectively, for the broiler chickens during the starter, grower and finisher phases, for optimal growth and maximum productivity. In contrast, Tadelle and Ogle (1996) observed that the protein requirement of growing indigenous chickens varies between 16 and 18 % during the growing phase for optimal performance.

According to Geraert *et al.* (2005), amino acid requirements for broilers have been historically determined in dose-response trials, with the concentration of amino acid that produces maximum weight gain chosen as the requirement. However, recommended dietary amino acid levels may vary according to performance and carcass parameters.

2.4 Nutritional and biological effects of dietary fibre on poultry

Dietary fiber has been considered a diluent of the diet and can results in negative effects exerted on nutrient utilization and performance such as decrease in body weight gain and feed conversion. Feeding animals diets high in dietary fibre, particularly soluble fibre alters the rate of faecal passage, microbiota, metabolites, and efficacy of digestion (Bach Knudsen & Jørgensen, 2001). Soluble fibre increases viscosity in the small intestine (Choct *et al.*, 1996), and subsequently inhibits digestion and absorption. The rate of digesta passage is reduced; feed intake is decreased, creating favourable conditions for proliferation of microbes in the intestine (Smiths & Annison, 1996; Choct *et al.*, 1996; Langhout, 1998). Since diets high in insoluble fibre contain low energy, birds tend to increase feed consumption as a way to compensate for the reduced nutrient concentration in feed (Hill & Dansky, 1954). Insoluble fibre have some

beneficial effects. As long as insoluble fibre is included in poultry diets at moderate concentrations, performance of birds will not be affected despite the fact that the nutrient concentration of the diet is reduced (Hetland & Svihus, 2001; Hetland *et al.*, 2002). Fibre in chicken diets also influences the behaviour of birds by reducing cannibalism as birds spend more time eating than pecking each other (Hughes & Duncan, 1972). Therefore, including feed ingredients that contain fibre in diets of chickens such as Moringa may be beneficial.

Table 2-1. NRC (1994) requirement for crude protein and the most rate limiting amino acids for broilers

	Weeks of age		
Nutrient, %	0-3	3-6	6-8
Crude protein	23.00	20.00	18.00
Methionine	0.50	0.38	0.32
Total sulphur amino acids	0.90	0.72	0.60
Lysine	1.10	1.00	0.85
Threonine	0.80	0.74	0.68
Tryptophan	0.20	0.18	0.16
Isoleucine	0.80	0.73	0.62
Arginine	1.25	1.10	1.00
Valine	0.90	0.82	0.70

2.5 Moringa oleifera

2.5.1 Occurrence

In recent times *Moringa oleifera* is one of the most widely cultivated species of the monogenic family *moringaceae*. Thus the so called "Miracle tree" originate from sub-Himalayan tracts of India, Pakistan, Bangladesh and Afghanistan (Somali *et al.*, 1984; Mughal *et al.*, 1999). *Moringa oleifera* tree was first introduced in Eastern Africa from India at the beginning of 20th century. It is widely distributed in different parts of the continent including Rwanda and Uganda, Ghana and South Africa. It is a perennial softwood tree with timber of low quality, due to its rapid growing nature. It has become one of the world's most useful plants for human nutrition, traditional medicine, nutraceutical purposes, water purifying and industrial uses (Fahey, 2005; Anwar *et al.*, 2007).

Moringa oleifera can be used as alternative to some leguminous seeds as a source of high-quality protein, oil and antioxidant compounds and a way to treat water in rural areas where appropriate water resources are not available (Ferreira *et al.*, 2008). It is a medicinal and functional food for both human and livestock. It is also used as livestock feed and its twigs are highly palatable to ruminants and have substantial crude protein levels (Sutherland *et al.*, 1990; Kimoro, 2002; Sarwatt *et al.*, 2002). *Moringa oleifera* is a valuable component in human and animal feed due to its adequate amino acid profile, crude protein content, high level of vitamin A, E and its low level of anti-nutritional compounds (Yang *et al.*, 2006). However, there is limited information on its potential as an animal feed.

2.6 Chemical composition of Moringa oleifera leaves

Moyo *et al.* (2011) reported that *M. oleifera* leaves had a CP content of 30.3% with 19 amino acids. The highest value of the amino acids was alanine, which had a value of 3.033% and the least content was cysteine with 0.01%. Calcium (3.65%), pottassium (1.5%) and phosphorus

(0.30%) among the macro-elements. The highest value among the micro-minerals was Fe (490 mg/kg) followed by Se with (3.63 mg/kg). Copper had the least value of 8.25 mg/kg. The dried Moringa leaves were found to contain 17 fatty acids and α-linolenic acid (44.57%) had the highest value followed by heneicosanoic (14.41%), g-linolenic (0.20%) palmiteic (0.17%) and capric acid (0.07%). Vitamin E had the highest level with 77 mg/100 g, while Beta-carotene had 18.5 mg/100 g. The fiber content been NDF, ADF, ADL and ADC of the leaves were 11.4, 8.49, 1.8 and 4.01%, respectively.

Table 2-2. Chemical composition of dried leaves of Moringa (M. oleifera Lam.)

Nutritive value	Dry leaf
Moisture (%)	9.533
Crude protein (%)	30.29
Fat (%)	6.50
Ash (%)	7.64
Neutral detergent fibre (%)	11.40
Acid detergent fibre (%)	8.49
Acid detergent lignin (%)	1.8
Acid detergent cellulose (%)	4.01
Condensed tannins (mg/g)	3.12
Total polyphenols (%)	2.02

Moyo *et al* 2011

2.6.1 Antioxidants

Free radicals play an important role in the pathogenesis of several human diseases, such as cancer, rheumatoid arthritis, and cardiovascular diseases (Hertog *et al.*, 1997). Natural antioxidants present in food of plant origin protect against these radicals and are therefore important tools in obtaining and preserving good health (Dell Agli *et al.*, 2004; Soorbrattee *et al.*, 2005). *Moringa oleifera* contain high concentrations of antioxidants which reduce lipid

oxidation in chicken muscle, and substantial antioxidative activity has been demonstrated in various *in vitro* model systems (Deighton *et al.*, 1993; Dorman *et al.*, 2000; Milos *et al.*, 2000).

Moringa contains the following antioxidants and anti-inflammatory compounds or compounds with antioxidant and anti-inflammatory characteristics such as vitamin A, Vitamin C, Vitamin E, Vitamin K, Vitamin B (Choline), Vitamin B1 (Thiamin), Vitamin B2 (Riboflavin), Vitamin B3 (Niacin), Vitamin B6, Alanine, Alpha-Carotene, Arginine, Beta-Carotene, Beta-sitosterol, Caffeoylquinic Acid, Campesterol, Carotenoids, Chlorophyll, Chromium, Delta-5-Avenasterol, Delta-7-Avenasterol, Glutathione, Histidine, Indole Acetic Acid, Indoleacetonitrile, Kaempferal, Leucine, Lutein, Methionine, Myristic-Acid, Palmitic-Acid, Prolamine, Proline, Quercetin, Rutin, Selenium, Threonine, Tryptophan, Xanthins, Xanthophyll, Zeatin, Zeaxanthin, Zinc (Deighton *et al.*, 1993; Dorman *et al.*, 2000; Milos *et al.*, 2000).

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2.6.2 Secondary plant material

Natural products from plants, called secondary metabolites, are the end products of primary metabolites such as carbohydrates, amino acids and lipids (Harborne, 1984). They are synthesis large variety of chemical substances known as secondary metabolites which include alkaloids, steroids, flavonoids, terpernoids and glycosides. Unlike primary metabolites, these substances are accumulated by plants, they have no apparent functions in the life of the plants and are not necessarily involve in essential metabolism of the cell (Sesta *et al.*, 2006) . Some of these secondary metabolites have pronounced physiological effects on man, other animals and some possess' therapeutic properties which have and still being utilized in the treatment and cure of both human and animal diseases (Sesta *et al.*, 2006).

Rajanandh & Kavitha (2010) observed that 8 and 27 µg/mL of total phenolic and flavonoid compounds respectively are present in the hydroalcoholic extract of the leaves of *M. oleifera*. Moyo *et al.* (2011) reported total phenols that amount to 2.02%, 3.12% of condensed tannins, while Foidl *et al.* (2001) reported 1.4% of tannins and did not detect the condensed tannins.

Drying reduces or removes extractable condensed tannins by 15 to 30% relative to fresh foliage (Vitti *et al.*, 2005). The decrease of condensed tannins after drying may be due to decomplexation between tannins and proteins and depolymerisation and oxidation of tannins (Makkar, 2003).

2.7 *Uses of* Moringa oleifera

Moringa oleifera leaves are rich in protein, carotene, iron and ascorbic acid while the pods are rich in amino acids lysine (CSIR, 1962). These excellent nutritional characteristics would make suitable as forage for feeding animals (Nuhu, 2010).

2.7.1 Chemical composition

Moringa oleifera is a multipurpose tree. All the parts of the tree can be utilised in a variety of ways. It can be utilised as human food and also as livestock feed. Its leaves are an excellent source of vitamin A (four times the amount in carrots), vitamin C (seven times the amount in oranges), vitamin B, calcium (four times the amount in milk), protein (twice the amount in milk), and potassium (three times the amount in bananas).

Moyo *et al.* (2011) reported the crude protein content of Moringa to be 30.3%, which is lower than sunflower seed cake's CP of 35.9% (Mapiye *et al.*, 2010). Sunflower seed cake is commonly used as protein concentrate. Other studies have reported variable protein contents ranging from 16, to 40% (Gidamis *et al.*, 2003; Sarwatt *et al.*, 2004; Nouala *et al.*, 2006; Reyes-Sanchez *et al.*, 2006; Oduro *et al.*, 2008; Sanchez-Machado et al., 2009). Estrella *et al.* (2000) reported that *Moringa oleifera* leaf meal (MOLM) increased breast milk production among mothers (Estrella *et al.*, 2000). Most of the Philippines women consume *Moringa* leaves mixed in chicken or shellfish soups to enhance breast milk production. In southern India, village

people use the fresh leaves to prepare cow and buffalo ghee from butter fat. There is a significant increase in the shelf life of ghee.

2.7.2 Feed supplement for livestock

Kakengi *et al.* (2007) reported that MOLM inclusion levels in poultry diets influenced egg weight at different magnitude. Feed and dry matter intake trials demonstrated that MOLM is palatable and highly preferred by chickens. Kakengi *et al.* (2003) evaluated and compared nutritive value of different morphological components of *M. oleifera* with *Leucaena leucocephala* leaf meal in Tanzania and observed high pepsin and total soluble protein in *Moringa oleifera* leaf meal (MOLM) than other parts of the plant. The high pepsin and total soluble protein suggest that MOLM may be more suitable to monogastric animals. Adegun *et al.* (2011) compared *Moringa oleifera* favourably with *Gliciridia sepium* and *leucocephala*. *Moringa oleifera* also enhanced the performance of sheep as protein supplements. Ravindran *et al.* (1986), Osei *et al.* (1990) and Bhatnagar *et al.* (1996) observed a depression in intake when laying chickens were fed diets containing various levels of *leucocephala* (LLLM) when compared to MOLM. These variations probably suggest lower anti-nutritional factors and toxic materials in MOLM (Makker & Backer, 1997) than in other leaf meals. Increase in feed intake is usually associated with compensatory mechanism to energy demand (Smith, 1999).

Teguia *et al.* (2003) observed that replacement of meat meal in the starter diet of broiler chickens by meals of common black bean and cowpea induced deteriorating effects on growth rate. Nworgu *et al.* (2007) also reported that fluted pumpkin can be utilised as alternative valuable protein and mineral supplement for broiler chickens. The use of alternative protein resources including Moringa should be fully explored.

Also, Melesse *et al.* (2011) reported that use of *Moringa stenopetala* leaf meal in the diet of Rhode Island Red chicks produced significant ($P < 0.05$) increase in feed and crude protein

intake, average weight gain, feed efficiency ratios, and protein efficiency ratios when compared to a control diet.

Nuhu (2010) observed that offering weaner rabbits a diet containing *Moringa* leaf meal significantly ($P < 0.05$) increased dry matter and protein digestibility, daily weight gain, and crude protein of meat, and it reduced ether extract of meat when compared to a control diet. Diets containing *Moringa* leaf meal had no significant ($P > 0.05$) effect on crude fiber and ether extract digestibility, daily feed intake, feed conversion ratio, carcass characteristics, and blood components (hemoglobin, packed cell volume, red blood cells, white blood cells, neutrophils, lymphocytes, eosinophils, cholesterol, total protein, albumin, and globulin).

Abou-Elezz *et al.* (2011) stated that inclusion of different levels of *Moringa oleifera* leaf meal (0%, 5%, 10%, and 15%) in the laying hens' diets linearly decreased egg-laying percentage and egg mass, while egg weight and feed intake showed a quadratic trend with the increased levels of Moringa oleifera leaf meal with the absence of a significant effect on feed conversion ratio. Generally, Kakengi et al. (2007), Olugbemi *et al.* (2010), and Abou-Elezz *et al.* (2011) agreed that use of *Moringa oleifera* leaf meal up to a level of 10% had no negative effect on the productive performance of laying hens, but levels above that (15% and 20%) are expected to produce adverse effects.

2.7.3 Medicinal properties of Moringa oleifera

Leaves of *M. oleifera* have various biological activities, including hypolipidaemic, antiatherosclerotic, prevention of cardiovascular diseases and antioxidant (Chumark *et al.*, 2008; Iqbal & Bhanger, 2006), immune boosting effect, hypotensive (Faizi *et al.*, 1994) and tumour suppressive effect (Murakami *et al.*, 1998). Meda *et al.* (2008) reported that wild fruits and plants have nutritional and medicinal properties that can be attributed to their antioxidant effects and they can be used to fortify staple foods, particularly for malnourished children. The

antioxidants could attenuate this oxidative damage of a tissue indirectly by enhancing natural defences of cell and/or directly by scavenging the free radical species. Several epidemiological studies (Aruoma, 1998; Triantaphyllou *et al.*, 2001) have shown that carotenoids, tocopherols, ascobates and dietary intake of natural phenolic antioxidants correlates with the reduced risk of cancers, cardiovascular diseases, neurodegenerative diseases, aging, asthma and inflammation. *Moringa oleifera* is also rich in phytochemicals such as the carotenoids (including β-carotene or pro-vitamin A) (Aruoma, 1998; Triantaphyllou *et al.*, 2001). Beta-carotene as an antioxidant is a highly effective quencher of singlet oxygen and a direct scavenger of free radicals (Gaby & Singh, 1991). Extracts of various *Moringa* tissues have been used as anti-cancer (Guevarra *et al.*, 1999), anti-trypanosomal (Mekonnen *et al.*, 1999), antimicrobial (Caceres *et al.*, 1991), anti-inflammatory and hepatoprotective (Kurma & Mishra, 1998) agents.

Leaf extracts have been shown to regulate thyroid status (Tahiliani *et al.*, 2000) and cholesterol levels in rats (Ghasi *et al.*, 2000). Hennekens (1992) reported that *Moringa* may offer some protection against the oxidative damage associated with low density lipoproteins (LDL), which transport cholesterol through the arteries and contribute to blocked vessels. Polyphenols also help prevent atherosclerosis by boosting the activity of vitamin C, which, in turn, increases the levels of vitamin E. This synergy increases the overall resistance to oxidative stress (Very Berry & Grape too, 2001).

2.7.4 Effect of Moringa oleifera on haematological parameters

Haematology refers to the study of the numbers and morphology of the cellular elements of the blood – the red cells (erythrocytes), white cells (leucocytes), and the platelets (thrombocytes) and the use of these results in the diagnosis and monitoring of disease (Merck Manual, 2012). Blood act as a pathological reflector of the status of exposed animals to toxicant and other conditions (Olafedehan *et al.*, 2010). Ghasi *et al.* (1999) reported that Wister rat, that

even when Moringa juice extract was given at the relatively low dose of 1mg/g, co-administered with a high fat diet daily over a period of 30 days, cholesterol was reduced in serum. The serum enzymes activities assessed (ALT, AST and ALP) of rabbits fed Moringa diets were within the normal range reported by CCAC (1980). This result corroborates with the report of Ewuola *et al.* (2011) who observed that serum enzyme activities of gestating and lactating rabbits administered crude Moringa extract were not significantly different from the control rabbits. This implies that animals fed test diets were not adversely affected because of no indication of organ toxicity from the serum enzymes assessed. In addition to mitigating against oxidative damage, effects of polyphenols have been associated with improvement in meat quality and other meat characteristics.

2.7.5 Meat quality traits

Consumer concerns on the quality of meat and meat products have greatly increased during past decades. "Quality" and "healthfulness" are most important factors for influencing consumer's choice for foods (Lennernas *et al.*, 1997). Increased chicken meat production and augmented interest of food-store chains to market standardized products are the reasons for making greater efforts to evaluate selected physical indicators, such as colour and tenderness of poultry meat (Abeni & Bergoglio, 2001).

2.8 Fatty acid composition

Fat is an unpopular constituent of meat by consumers, being considered unhealthy. Yet fat and fatty acids, whether in adipose tissue or muscle, contribute to various aspects of meat quality and are central to the nutritional value of meat. The fatty acids can be classified into harmful and healthier dietary fat. Consuming food that are rich in polyunsaturated and monounsaturated fats improves blood cholesterol levels unlike consuming diet rich in saturated and *trans* fat. The origin of animals, carcass characteristics and its meat quality are important criteria for butchers and consumers when it comes to making purchasing decisions (Orellana *et al.*, 2009).

The fatty acid composition and cholesterol levels in meat have received increasing attention owing to their implications in human health and product quality. Animal feed enriched by nutritionally important various fatty acids can improve the nutritive value of animal fat. Meat should have a favourable balance between poly unsaturated fatty acids (PUFA) and saturated fatty acids (SFA) (P: S) which is 0.4 and the desirable omega 3 and omega 6 (n-3: n-6 PUFA) ratio which is below 4.0 (Wood & Enser, 1997; Wood *et al.*, 2003). Therefore, the PUFA/SFA and n-6/n-3 PUFA ratios have become some of the most important parameters in evaluating the nutritional value and healthiness of foods (Mapiye *et al.*, 2011). To preserve fatty acids integrity, anti-oxidants may be used.

2.8.1 Lipid peroxidation

Lipid peroxidation refers to the oxidative degradation of lipids and is a primary cause of quality deterioration in meat and meat products. Free radical chain reaction is the mechanism of lipid peroxidation and reactive oxygen species (ROS) such as hydroxyl radical and hydroperoxyl radical are the major initiators of the chain reaction (Ahn, 1993). Lipid peroxyl radical and alkoxyl radical formed from the initial reactions are also capable of abstracting a hydrogen atom from lipid molecules to initiate the chain reaction and propagating the chain reaction. Heme proteins such as myoglobin and hemoglobin and "free" iron have been regarded as major catalysts for initiation, and iron-oxygen complexes (ferryl and perferryl radical) are even considered as initiators of lipid peroxidation in meat and meat products (Ahn, 1993).

Lipid peroxidation results in free radicals by "stealing" electrons from the lipids in cell membranes, resulting in cell damage. This process proceeds by a free radical chain reaction mechanism. It most often affects polyunsaturated fatty acids, because they contain multiple double bonds in between which lie methylenes-CH2- groups that possess highly reactive hydrogens. Qwele (2011) observed a decrease in lipid peroxidation level in broiler breast meat

fed *M. oleifera* leaf meal which indicates the role of *M. oleifera* leaves as an antioxidant. This is in agreement with the study conducted by Kumar & Pari (2003), who observed that *M. oleifera* inhibited lipid peroxidation against anti-tubercular drugs induced lipid peroxidation in rats. Besides preventing lipid peroxidation, antioxidants are believed to impact positive effects on meat quality including meat colour and tenderness, especially vitamin A. Therefore the use of Moringa may fill that purpose.

2.9 Meat colour and tenderness

Three sensory quality characteristics appearance/colour, texture, and flavour are the main quality attributes that affect consumer acceptance of meat. Inherent characteristics of animal, long and short-term environmental influences on animal and processing parameters that affect the carcass or meat directly are all factors that influence meat colour, texture and flavour (Lyon *et al.*, 2004). Low ultimate pH reduces the importance of myoglobin, resulting in meat that appears less red and more yellow (Castellini *et al.*, 2002). The quality of meat is determined using biochemical, physical-chemical and bacteriological processes. A high ultimate pH is generally indicative of pre-slaughter stress in animals (Dhanda *et al.*, 2003; Muchenje *et al.*, 2009). The rate of pH decline is a good predictor of the colour and drip loss of meat (Aberle *et al.*, 2001; Muchenje *et al.*, 2008). Higher ultimate pH (pHu) in animals can be associated with low glycogen reserve due to insufficient nutrition (Mushi *et al.*, 2009).

Fletcher *et al.* (2000) reported significant linear relationships between raw meat colour and its pH, as well as the highest R^2 for lightness (L* value) as a function of pH. Thus, because of the good correlations among colour, pH, and cook loss it is possible to influence the PSE in meat by colour evaluation or pH (Barbut, 2009) through feeding of green plants such as *M. oleifera* leaves with natural pigments.

Age and genetic strain are two inherent factors that affect meat colour and texture. Age of the animal may be important because myoglobin, the primary muscle pigment, tends to increase with age in chicken (Lyon *et al.*, 2004). However, Smith *et al.* (2002) reported that the colour of broiler breast meat was not affected by age, whereas Lyon *et al.* (2004) reported that meat texture may be affected by age.

2.10 Summary

Improved nutrition management is necessary to assist in achieving optimum performance in poultry/livestock. Use of leaf meals of plants as feed ingredients as alternative to conventional feed resources is a novel area of research in animal nutrition. *Moringa oleifera* is a major source of natural antioxidants and enhancing animal feed with natural supplements will improve the health status of livestock and the consumer, as well as improving meat quality. The objective of this study was to determine the effect of supplementing *M. oleifera* leaf meal on productivity, carcass characteristics and biochemical indices of indigenous chickens raised under a confined production system.

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3 CHEMICAL CHARACTERISATION OF *MORINGA OLEIFERA* LEAVES AND DIGESTIBILITY OF THE LEAF MEAL-BASED DIETS IN THREE CHICKEN STRAINS

3.1 Abstract

Proximate, minerals and fatty acids composition of *Moringa oleifera* leaves at different stages of maturity as well as the apparent digestibility of *M. oleifera* leaf meal (MOLM)-based diets in three chicken strains were determined. From each individual tree, *M. oleifera* leaves were at two stages of maturity (tender and mature). The leaves were, air-dried in a well-ventilated laboratory and milled using a hammer mill to pass through a 1 mm sieve. The leaf meal was chemically analysed and used to dilute a commercial broiler finisher diet at 0 (MOLM0), 25 (MOLM25), 50 (MOLM50), and 100 (MOLM100) g/kg DM, producing four isoenergetic and isonitrogenous dietary treatments. Seventy two PK, OV and BA chickens were used for the digestibility trial. Apparent digestibility was measured when the chickens were between 87 and 90 days old. The crude protein (CP) content was significantly higher in tender (324.63 g/kg DM) than in mature (285.2 g/kg DM) leaves. Tender leaves had higher concentrations of calcium (19.15 g/kg) and phosphorus (4.15 g/kg). Iron content for mature leaves (150.5 dpm) was higher compared to tender leaves (110.5 dpm). The level of phenolics increased with maturity of the leaves. In BA, diet MOLM0 (87.0 %) had highest crude protein digestibility followed by MOLM100 (85.4 %). In OV and PK strains, incremental levels of MOLM resulted in higher crude protein digestibility than the control (MOLM0). Inclusion of MOLM in chicken diets did not negatively affect nutrient digestibility. The presence of tannins and other phenolics in MOLM provides challenges and opportunities in the exploitation of this feed resource for improved chicken productivity.

Key words: Stage of maturity, *Moringa oleifera* leaves, nutritional value, nutrient digestibility, chemical composition.

3.2 Introduction

The use of relatively low cost non-conventional feed resources is vital for animal agriculture. Efficient use of these non-conventional feed resources depends on their chemical and physical properties, which influence the quantity and quality of outputs from animal production systems. Some trees provide an alternative source of feed with high levels of vitamins, minerals, and other useful elements, particularly during seasonal feed shortages (Glew *et al.*, 2005). Tree foliage have nutritional and medicinal properties that can be attributed to plant compounds with antioxidant properties (Meda *et al.*, 2008). One of the determinants of forage quality is stage of maturity of the foliage when harvested. Stage of maturity and growing conditions interact to influence biomass yield and nutritive value. Information on the dynamics of forage quality during the plant's growth cycle can help optimize harvesting or foraging to meet specific animal requirements (Valente *et al.*, 2000). Nutritional parameters of forage that need to be defined include mineral composition, concentration of crude protein, fibre, and extend of digestion (Smit *et al.*, 2005; Arzani *et al.*, 2006; Conaghan *et al.*, 2008). Chemical and physical analyses contribute to the determination of the nutritive value of feedstuffs (Van Soest, 1983). *Moringa oleifera* (family: Moringaceae) commonly known as horse radish tree or drumstick tree is of nutritional and medicinal value and contains some useful minerals, vitamins, amino acids and bioactive compounds (Yang *et al.*, 2006, Fahey, 2005). Plant maturity can have a negative influence on the nutritional composition of forage, especially protein, and favours the accumulation of anti-nutritional compounds such as tannins (Callow *et al.*, 2003; Contreras-Govea *et al.*, 2009), which can result in low digestibility of nutrients. Plants generally contain biologically active chemical compounds, such as saponins, tannins, oxalates, phytates, trypsin inhibitors and cyanogenic glycosides, which are also known as secondary plant metabolites (Soetan & Oyewole, 2009). Secondary plant metabolites have beneficial applications in nutrition and as pharmacologically-active agents (Soetan & Oyewole, 2009). Evaluating the

potential of *M. oleifera* leaf meal (MOLM), as a non-conventional feedstuff in diets of chickens, is important since the cost of feeding chickens for optimum growth performance has become high. Furthermore, an investigation into the effect of stage of maturity is important to make recommendations when the leaves can be harvested to minimize possible antinutritional effects of some constituents in the leaves. Therefore, the current study was designed to evaluate the chemical composition of *M. oleifera* at different stages of maturity as well as the digestibility of MOLM-based diets when fed to three chicken strains (Potchefstroom Koekoek, Ovambo, and Black Australorp).

3.3 Materials and methods

3.3.1 Harvesting of Moringa oleifera leaves

Fresh green *M. oleifera* leaves were harvested from Lekgonyane Vendam farm, North-West Province, South Africa (25.6200° S, 27.9800° E). The soil on this farm is classified as sandy loam. The mean rainfall of the area is approximately 250 mm and the mean annual temperature is 15^0C. The plants were established in a 2 hectare plot and 5 trees were randomly selected. From each individual tree, *M. oleifera* leaves were simultaneously harvested at two stages of maturity (tender and mature). The leaves were harvested green, air-dried in a well-ventilated laboratory and milled into powder using a hammer mill to pass through a 1 mm sieve, to produce *M. oleifera* leaf meal (MOLM). Individual tree leaf meal samples were subjected to chemical analyses as described below.

3.3.2 Bulk /composite leaf sample

A mixture of both tender and mature leaves were harvested from all the five individual trees in Limpopo province, air-dried in a well-ventilated laboratory and milled into powder using a hammer mill to pass through a 1 mm sieve, to produce *M. oleifera* leaf meal (MOLM) bulk sample for use in the digestibility trial.

Tender leaves *Mature leaves*

3.3.3 Chemical analyses

Chemical analyses on the individual tree and bulk leaf samples were conducted in the Animal Nutrition laboratory at the North-West University Experimental Farm (Molelwane). Moisture and dry matter contents were determined after drying samples in an oven at 105°C to constant weight. Determination of ash content was done by ashing at 550°C for 6 hours in a muffle furnace. After ashing, crucibles were removed, placed in a desiccator to cool and weighed. The loss in weight was measured as OM content. Total nitrogen content was determined by the standard macro-Kjeldahl method (AOAC 978.04, 2005) and was converted to crude protein by multiplying percentage N content by 6.25. Neutral detergent fibre (NDF) and acid detergent fibre (ADF) were determined by refluxing 0.45g samples with neutral detergent and acid detergent solutions, respectively, for 1 h using the ANKOM^{2000} Fibre Analyzer (ANKOM Technology, New York) according to Lewis et al. (1991, 3588). Heat-stable α-amylase was used for NDF analysis. The fibre fractions were expressed inclusive of residual ash.

3.3.4 Minerals

The mineral (calcium (Ca), magnesium (Mg), potassium (K), sodium (Na), iron (Fe), zinc (Zn), manganese (Mn), phosphorus (P) and copper (Cu) content of *M. oleifera* leaves was determined using the atomic ICP spectrophotometer (AAS-Buck 205) according to AOAC (2005).

3.3.5 Phenolics

Dried (finely ground) plant material (200 mg) was taken in a glass beaker of approximately 25 ml capacity. Ten mililiters of 70 % aqueous acetone was added to the sample and the beaker was suspended in an ultrasonic water bath for 20 minutes at room temperature. The contents of the beaker were then transferred to centrifuge tubes kept on ice and then centrifuge at 3000g using an ordinary clinical centrifuge). The supernatant was decanted and kept on ice pending analysis. The residue was used to quantify insoluble phenolics.

Soluble phenolics (SPh) were estimated using Folin-Ciocalteau reagent, after extraction of a 40 mg sample three times with 10 ml of 70 % aqueous acetone for a total of 15 minutes. A Folin standard solution (Folin and Ciocalteau, 1927) was diluted 10 times and 5 ml was mixed with 0.1 ml of the acetone extract in a test-tube. Four millilitres of sodium carbonate (7.5 % w/v) was added to the mixture and the entire contents vortexed. A blank was prepared as described above; with 0.1 ml of 70 % aqueous acetone being used in place of the plant extract. Absorbance measurements were taken after 2 h using a spectrophotometer at 675 nm wavelength. Tannic acid was used to generate a standard curve from which the concentration of phenolics in leaves was estimated. SPh was, therefore, expressed as tannic acid equivalents (TAE).

The same acetone extract used in the soluble phenolics assay was used to assay for soluble/extractable-condensed tannins (SCT) using the butanol-HCl reagent (95:5 v/v) (Porter *et al.* 1986). Aqueous acetone extract (0.5 ml) was pipetted into a glass screw cap test-tube and

5 ml butanol-HCl reagent added. The test-tube was closed and then placed on a heating block at 100 °C for 1 hour. Absorbance was measured after the test tubes had cooled to room temperature. The measurements were reported as absorbance units (au) at 550 nm. Insoluble/unextractable-condensed tannin (ICT) content was determined in the sample residue remaining after acetone extraction. The residues were dried at 40 °C for 48 h, after which about 40 mg was weighed into test tubes to which 5 ml butanol-HCl reagent was added. Absorbance was measured as described above for SCT.

3.3.6 Fatty acid profiles

Total lipids from the bulk leaf sample were quantitatively extracted using Soxhlet apparatus (AOAC, 2005). The extracted fats were stored in a polytop (glass vial, with a push-in top) under a blanket of nitrogen and frozen at -20°C, pending analyses. Approximately 10 mg of extracted lipids were transferred into a Teflon-lined screw-top test tube by means of a disposable glass Pasteur pipette. Fatty acid methyl esters (FAME) were prepared for gas chromatography by methylation of the extracted fat, using methanol–BF3 (Christie *et al.*, 2001). Fatty acid methyl esters were quantified using a Varian GX 3400 flame ionization GC, with a fused silica capillary column, Chrompack CPSIL 88 (100 m length, 0.25 mm ID, 0.2 μm film thickness). Analysis was performed using an initial isothermic period (40°C for 2 min). Thereafter, the temperature was increased at a rate of 4°C/min to 230°C. Finally, an isothermic period of 230°C for 10 min followed. Fatty acid methyl esters in *n*-hexane (1 μl) were injected into the column using a Varian 8200 CX Auto sampler with a split ratio of 100:1. The injection port and detector were both maintained at 250°C. Hydrogen, at 45 psi, functioned as the carrier gas, while nitrogen was employed as the makeup gas. Varian Star Chromatography Software recorded the chromatograms. Fatty acid methyl ester samples were identified by comparing the retention times of FAME peaks from samples with those of standards obtained from Supelco

(Supelco 37 Component Fame Mix 47885-U, Sigma-Aldrich Aston Manor, South Africa). The following fatty acid combinations and ratios were calculated: total saturated fatty acids (SFA), total monounsaturated fatty acids (MUFA), polyunsaturated fatty acids (PUFA), PUFA/SFA ratio (P/S) and *n*-6/*n*-3 ratio. All other reagents and solvents were of the analytical grade and obtained from Merck Chemicals (Pty) Ltd Halfway House, South Africa.

3.4 Diet formulation

Four diets were constituted by diluting commercial broiler finisher diet with graded levels (0, 25, 50 and 100 g/kg) of air-dried and milled MOLM. The composition of MOLM and experimental diets are shown in Table 3-1, 3-2 & 3-3. The experimental diet formulation was done at a commercial feed manufacturing company, NutriFeed (Mafikeng). These experimental diets were formulated to be iso-nitrogenous and isoenergetic.

Table 3-1. Gross composition of *Moringa oleifera* leaf meal (MOLM)-based experimental diets

	Diet[1]			
	MOLM0	MOLM25	MOLM50	MOLM100
MOLM (g/kg diet)	0	25.0	50.0	100.0
Yellow maize	670.6	658.8	647.1	623.6
Prime gluten 60	50.0	50.0	50.0	50.0
Full fat soya meal	70.0	70.0	70.0	70.0
Soya bean meal	85.3	71.8	58.2	31.1
Sunflower oilcake	80.0	80.0	80.0	80.0
Limestone powder	12.3	9.7	7.1	1.8
Potassium carbonate	1.2	1.0	0.9	0.5
Mono calcium phosphate	9.8	9.9	10.0	10.3
Salt	3.2	3.17	3.15	3.11
Soya oil	7.8	10.6	13.5	19.1
Premix	6.8	6.8	6.8	6.7
Lysine	2.7	2.7	2.7	2.7
Methionine	0.3	0.5	0.7	1.0
Total	1000	1000	1000	1000

[1]Diet: MOLM0 = broiler finisher without MOLM inclusion; MOLM25 = broiler finisher diluted at 25 g/kg MOLM MOLM50 = broiler finisher diluted at 50 g/kg MOLM; MOLM100 = broiler finisher diluted at 100 g/kg MOLM.

Table 3-2. Chemical analysis of diets on an 'as fed basis' and chemical composition of dried *Moringa oleifera* leaf meal (MOLM)

	Diet[1]			
	MOLM0	MOLM25	MOLM50	MOLM100
MOLM	0	25.0	50.0	100.0
Dry matter	896.0	874.0	851.0	807.0
Crude protein	189.0	189.0	189.0	189.0
Ether Extract	52.0	57.0	61.0	69.0
Ash	49.0	47.0	45.0	42.0
Acid detergent fibre	36.0	42.0	47.0	57.0
Neutral detergent fibre	96.0	100.1	106.0	116.0
Crude Fibre	36.0	35.0	34.0	33.0
Metabolisable energy (KCal/kg)	3157.6	3157.4	3157.2	3156.8
Lysine	9.7	9.7	9.7	9.7
Methionine	4.0	4.2	4.3	4.5

[1]Diet: MOLM0 = broiler finisher without MOLM inclusion; MOLM25 = broiler finisher diluted with 25 g MOLM/kg; MOLM50 = broiler finisher diluted with 50 g MOLM/kg; MOLM100 = broiler finisher diluted at 100 g MOLM/kg.

Table 3-3. Fatty acid composition of the four experimental diets offered to chickens.

	Diet				
Fatty acids (%)	MOLM0	MOLM25	MOLM50	MOLM100	MOL
Myristic (C14:0)	0.07	0.07	0.10	0.11	4.76
Palmitic (C16:0)	15.91	10.98	10.92	10.24	31.19
Palmitoleic (C16:1c9)	0.17	0.13	0.11	0.09	0.35
Margaric (C17:0)	0.15	0.08	0.08	0.07	0.27
Heptadecenoic (C17:1c10)	0.03	0.02	0.02	0.02	0.00
Stearic acid (C18:0)	5.61	4.06	4.74	4.59	4.18
Oleic (C18:1c9)	28.16	23.63	22.16	22.27	5.97
Vaccenic(C18:1c7)	0.00	0.00	0.00	0.00	0.76
Linoleic (C18:2c9,12 (n-6))	45.44	56.71	57.29	57.97	8.93
Arachidic (C20:0)	0.56	0.41	0.43	0.44	1.78
γ-Linolenic (C18:3c6,9,12 (n-3))	0.00	0.00	0.00	0.00	0.51
Eicosenoic (C20:1c11)	0.18	0.13	0.12	0.12	0.00
α-Linolenic (C18:3c9,12,15 (n-3))	2.64	2.95	3.16	3.15	33.06
Eicosadienoic (C20:2c11,14 (n-6))	0.07	0.01	0.01	0.01	0.00
Behenic (C22:0)	0.56	0.46	0.50	0.54	2.62
Eicosatrienoic (C20:3c8,11,14 (n-6))	0.00	0.00	0.00	0.00	0.19
Tricosanoic (C23:0)	0.04	0.03	0.03	0.03	0.29
Eicosopentaenoic (C20:5c5,8,11,14,17 (n-3))	0.26	0.24	0.25	0.28	4.85
Nervonic (C24:1c15)	0.17	0.10	0.08	0.06	0.28
Fatty acid ratios:					
Total Saturated Fatty Acids (SFA)	22.90	16.08	16.80	16.02	45.10
Total Mono Unsaturated Fatty Acids (MUFA)	28.71	24.01	22.49	22.57	7.37
Total Poly Unsaturated Fatty Acids	48.39	59.91	60.71	61.41	47.54
Total Poly Unsaturated Fatty Acids (PUFA)	48.39	59.91	60.71	61.41	47.54
Total Omega- 6 Fatty Acids (n-6)	45.50	56.72	57.30	57.97	9.12
Total Omega- 3 Fatty Acids (n-3)	2.89	3.19	3.41	3.43	38.41
PUFA:SFA	2.11	3.73	3.61	3.83	1.05
n-6/n-3	15.73	17.78	16.80	16.90	0.24

[1]Diet: MOLM0 = broiler finisher without MOLM inclusion; MOLM25 = broiler finisher diluted at 25 g/kg MOLM MOLM50 = broiler finisher diluted at 50 g/kg MOLM; MOLM100 = broiler finisher diluted at 100 g/kg MOLM.

3.5 Nutrient digestibility

Seventy two chickens from Potchefstroom Koekoek, Ovambo and Black Australorp strains were used for this digestibility trial. A 3 (chicken strains) x 4 (diets) factorial treatment arrangement in a complete randomised design (CRD) was used for this experiment, replicated three times. Apparent digestibility was measured when the chickens were between 87 and 90 days old. Digestibility was conducted in specially designed metabolic cages. Birds (three per treatment combination) were randomly selected and housed individually in metabolic cages for measurement of apparent digestibility. A three-day acclimatization period was allowed prior to a three-day collection period. The excreta were collected from each replicate and stored at -15°C during the collection period pending proximate analyses. Feed offered and feed refusals were weighed. Apparent digestibility of the nutrients was calculated according to McDonald *et al.* (2004):

$$Apparent\ digestibility\ \% = \frac{(\text{Nutrient in feed} - \text{nutrient in feacal}) \times 100}{Nutrient\ in\ feed}$$

3.6 Statistical analyses

Variation in chemical composition data due to stage of maturity was analysed based on one-way ANOVA using SAS (2010) software. The linear model employed was:

$$Y_{ij} = \mu + SM_i + E_{ij},$$

where Y_{ijk} = observation of the dependent variable *ijk*, μ = fixed effect of population mean for the variable, and SM_i= stage of maturity of leaves (i = 2; tender and mature).

A two-way ANOVA was used to account for chicken strain, diet and chicken strain × diet interaction effects on apparent digestibility data. The general linear models (GLM) procedures

of SAS (2010) software were employed in this statistical analysis. The linear model employed was:

$$Y_{ijk} = \mu + S_i + D_j + (S \times D)_{ij} + E_{ijk},$$

where Y_{ijk} = observation of the dependent variable *ijk*, μ = fixed effect of population mean for the variable, S_i= effect of chicken strain (i = 3; Potchefstroom Koekoek, Ovambo, and Black Australorp), D_j = effect of diet (j = 4; MOLM0, MOLM25, MOLM50, and MOLM100), $(S \times D)_{ij}$= effect of interaction between strain at level i and diet at level j, E_{ijk} = random error associated with observation *ijk*. Where significant variation was detected, multiple comparisons of treatment means were carried out using the probability of difference (pdiff) option of the General Linear Models (GLM) procedures of SAS (2010). For all statistical tests, significance was declared at $P < 0.05$. No statistical analyses were carried out on the chemical composition data of the bulk/composite leaf sample.

3.7 Results

3.7.1 Tender and mature leaves

Proximate composition for leaves harvested at different stages of plant maturity is shown in Table 3-3. No variation ($P>0.05$) in dry matter (DM) was observed in *M. oleifera* mature and tender leaves. Higher ($P<0.05$) ash content was observed in mature leaves compared to tender leaves. The CP content was significantly higher in tender (324.6 g/kg DM) leaves than mature (285.2 g/kg DM) leaves. Crude fibre content (38.7 g/kg DM) was higher ($P<0.05$) in mature, whilst tender leaves exhibited lower fibre content (33.2 g/kg DM). Tender leaves had lower EE content (27.0 g/kg DM) than mature leaves (38.1 g/kg DM). Tender leaves significantly had lower detergent fibre contents than mature leaves.

Table 3-4. Chemical composition of tender and mature leaves of *Moringa oleifera*

Variable	Tender	Mature	SE
Dry matter (g/kg)	948.7[a]	937.9[a]	5.199
Organic matter (g/kg)	880.9[a]	855.9[b]	5.187
Ash (g/kg)	67.7[b]	82.0[a]	0.032
Neutral detergent fiber(g/kg DM)	533.4[b]	714.7[a]	161.81
Acid detergent fiber (g/kg DM)	43.1[b]	44.0[a]	0.0315
Ether extract (g/kg DM)	27.0[b]	38.1[a]	0.0315
Crude protein (g/kg DM)	324.6[a]	285.4[b]	0.0315
Crude fiber (g/kg DM)	33.1[b]	38.7[a]	0.0223
Condensed tannins (AU_{550}nm/10 mg)	0.107[b]	0.149[a]	0.0037
Total phenolic (AU_{725}nm/10 mg)	0.424[b]	0.652[a]	0.0174
Total carbohydrates[1] (%)	47.9[a]	43.1[b]	0.513
Energy value[2] (Kcal/g)	346.5[a]	320.0[b]	1.677

[a, b, c] Means within rows with different superscripts differ significantly ($P<0.05$).

SE, Standard error;

[1]$Total\ carbohydrate = 100\% - [\%\ crude\ protein + crude\ fiber + \%\ crude\ total\ ash]$;

[2]$Energy\ value = [\%\ crude\ protein \times 4.0] + [\%\ crude\ fat \times 9.0] + [\%\ carbohydrate \times 4.0]$

Tender leaves contained higher ($P<0.05$) total carbohydrates (475 g/kg) and energy value (344.3 Kcal/g) compared to mature leaves. The mineral analysis of tender and mature leaves are shown in Table 3-4. Tender leaves showed significantly higher ($P<0.05$) concentrations of calcium (19.15 g/kg DM), phosphorus (4.15 g/kg DM) and zinc (35.05 dpm). Iron content for mature leaves was higher (150.5 dpm) ($P<0.05$) compared to tender leaves. No significant differences were observed in selenium content of the leaves, even though tender leaves tended to have slightly higher values. Higher ($P<0.05$) total phenolic content was observed in mature leaves (0.652 AU_{725}/10 mg) compared to tender leaves (0.424 AU_{725}/10 mg). Mature leaves also contained higher ($P<0.05$) condensed tannins (0.149 AU_{550}/ 10 mg) than tender leaves (0.107 AU_{550}/ 10 mg).

Table 3-5. Mineral content of tender and mature *Moringa oleifera* leaves

Mineral	Tender	Mature	SE
Calcium %	1.915[a]	1.505[b]	0.0112
Phosphorus %	0.415[a]	0.245[b]	0.005
Magnesium %	0.255[a]	0.20[b]	0.0035
Potassium %	1.705[a]	1.60[b]	0.0035
Sodium %	0.061	0.06	0.0004
Sulphur (dpm)	9401[a]	6100[b]	353.339
Copper (dpm)	9.50[b]	9.90[a]	0.0035
Zinc (dpm)	35.05[a]	25.05[b]	0.050
Manganese (dpm)	46.50[b]	140.5[a]	0.500
Selenium (dpm)	0.195	0.190	0.004
Iron (dpm)	110.5[b]	150.5[a]	0.500

[a, b, c] Means within rows with no common superscripts differ significantly ($P<0.05$).

SE, Standard error

3.7.2 Moringa oleifera bulk leaf sample

The proximate composition of *M. oleifera* bulk leaf meal is shown in Table 3-5. The bulk sample had a crude protein content of 263.4 g/kg DM and crude fibre content of 54.9 g/kg DM. The leaves contained 42.3 g/kg DM EE and 80 g/kg DM ash. Total soluble carbohydrate content was found to be 561 g/kg DM while the energy value was 367.4 Kcal/100kg. As sown in Table 3-6, the bulk sample also contained 16.05 g/kg DM potassium, 13.05 g/kg DM calcium, 250 dpm iron and 47.10 dpm zinc.

The fatty acid composition of the bulk sample is shown in Table 3-7. The sample contained 17 fatty acids of which α-linolenic (33.06%) and palmitic (31.19%) acids were the predominant fatty acids, followed by linoleic (8.93%), oleic (5.97%), eicosopentaenoic (4.85%), myristic (4.76%), stearic (4.18%) and behenic (2.62%) acids. The major saturated fatty acids, including myristic (C14:0), palmitic (C16:0), stearic (C18:0), arachidic (C20:0) and behenic (C22:0), constituted 45.10% of the total fatty acids, while unsaturated fatty acids (oleic (C18:1), γ-linoleic (C18:2), α-linolenic (C18:3), palmitoleic (C16:1), and nervonic (C24:1) made up the difference (54.91%).

Table 3-6. Proximate compositions of bulk (mixture of tender and mature leaves) *Moringa oleifera* leaf meal on dry matter basis

Component	Quantity
Dry matter (g/kg)	950.8
Moisture (g/kg)	49.5
Organic matter (g/kg)	870.5
Ash (g/kg)	80.4
Ether extract (g/kg)	53.9
Neutral detergent fiber (g/kg DM)	761.7
Acid detergent fiber (g/kg DM)	52.1
Fat (g/kg DM)	42.3
Crude protein (g/kg DM)	263.4
Crude fiber (g/kg DM)	54.9
Condensed tannins ($AU_{550\ nm}$/10 mg)	0.33
Total Phenolic ($AU_{725\ nm}$/10 mg)	0.989
Total carbohydrates (%)	56.1
Energy value (Kcal/g)	367.4

Total carbohydrate and energy value: calculated as:

[1] $Total\ carbohydrate = 100\% - [\%\ crude\ protein + crude\ fiber + \%\ crude\ total\ ash]$

[2] $Energy\ value = [\%\ crude\ protein\ x\ 4.0] + [\%\ crude\ fat\ x\ 9.0] + [\%\ carbohydrate x\ 4.0]$

Table 3-7. Mineral content of bulk *Moringa oleifera* leaves

Mineral	Quantity
Calcium %	1.305
Phosphorus %	0.255
Magnesium %	0.445
Potassium %	1.605
Sodium %	0.065
Sulphur (dpm)	8500
Copper (dpm)	9.20
Zinc (dpm)	47.10
Manganese (dpm)	0.445
Selenium (dpm)	0.235
Iron (dpm)	250.5

Table 3-8. Fatty acid composition and ratios in bulk *Moringa oleifera* leaves

Fatty acid	Quantity (%)
Myristic (C14:0)	4.76
Palmitic (C16:0)	31.19
Palmitoleic (C16:1c9)	0.35
Margaric (C17:0)	0.27
Stearic acid (C18:0)	4.18
Oleic (C18:1c9)	5.97
Vaccenic(C18:1c7)	0.76
Linoleic (C18:2c9,12 (n-6))	8.93
Arachidic (C20:0)	1.78
γ-Linolenic (C18:3c6,9,12 (n-3))	0.51
α-Linolenic (C18:3c9,12,15 (n-3))	33.06
Behenic (C22:0)	2.62
Eicosatrienoic (C20:3c8,11,14 (n-6))	0.19
Tricosanoic (C23:0)	0.29
Eicosopentaenoic (C20:5c5,8,11,14,17 (n-3))	4.85
Nervonic (C24:1c15)	0.28
Fatty acid ratios	
Total saturated fatty Acids (SFA)	45.10
Total mono unsaturated fatty acids (MUFA)	7.37
Total poly unsaturated fatty acids (PUFA)	47.54
Total omega- 6 fatty acids (n-6)	9.12
Total omega- 3 fatty acids (n-3)	38.41
PUFA:SFA	1.05
n-6/n-3	0.24

3.7.3 Nutrient digestibility

There was a significant 'diet × strain' interaction on apparent digestibility of CP, NDF, ADF, CF and EE (Figures 1-5). In BA, diet MOLM0 (87.0 %) had highest crude protein digestibility followed by MOLM100 (85.4 %). In OV and PK strains, incremental levels of MOLM resulted in higher crude protein digestibility than the control. Black Australorp strains had highest ($P<0.05$) EE digestibility (72.1 %) followed by PK (69.1 %) and OV strain (60.6 %) having the lowest EE digestibility. In all strains, incremental level of MOLM resulted in lowest ($P<0.05$) CF digestibility compared to control diet (MOLM0). Black Australorp had highest ($P<0.05$) CF digestibility across all diets followed by OV strain and PK strain being the lowest. In all strains, highest inclusion (MOLM100) had highest ADF and NDF digestibility than control diet (MOLM0). Potchefstroom Koekoek had lowest ($P<0.05$) ADF and NDF digestibility across all diets compared to BA and OV chicken strains.

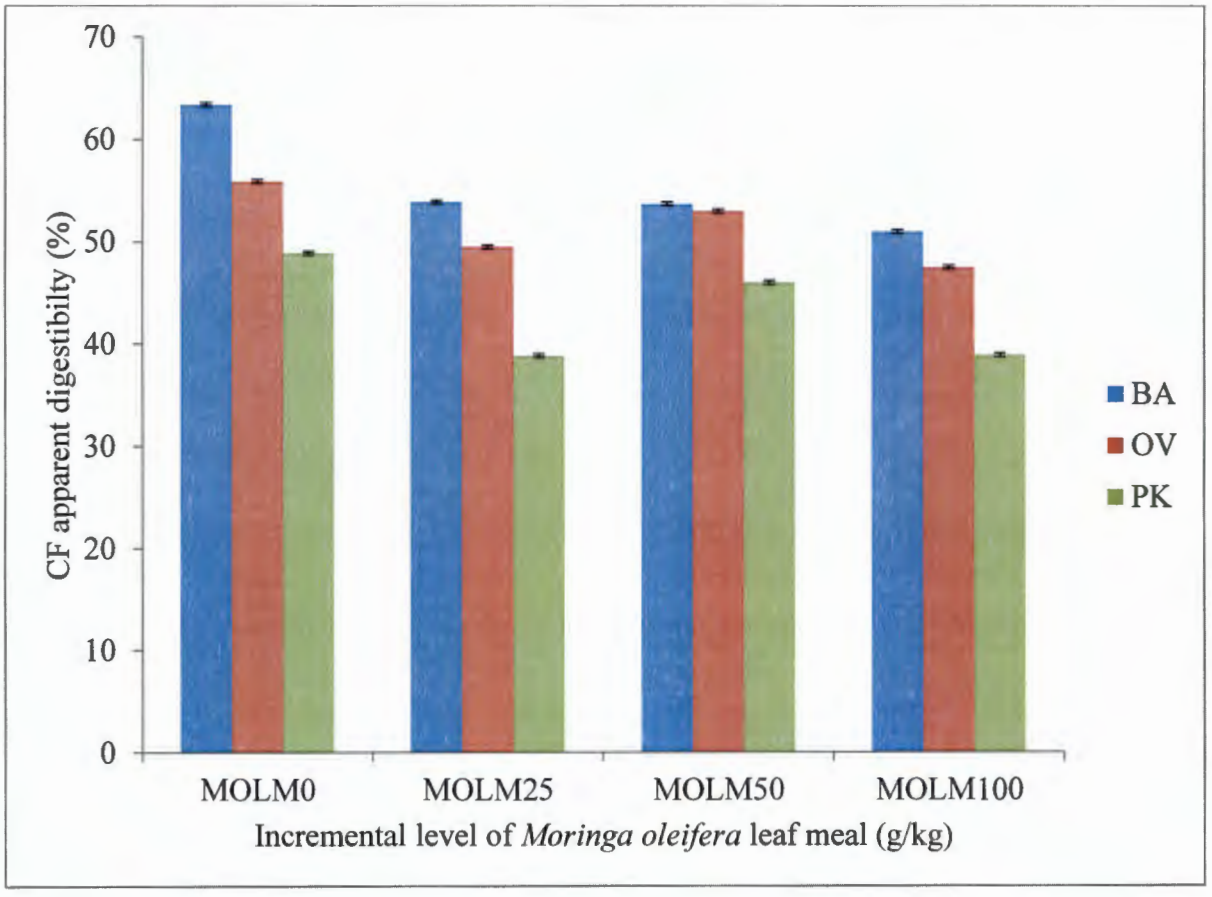

Figure 3-1. Effect of dietary *Moringa oleifera* leaf meal inclusion rate (%) on crude fiber (CF) apparent digestibility of Black Australorp (BA), Ovambo (OV) and Potchefstroom Koekoek (PK) chicken strain between at 13 weeks of age.

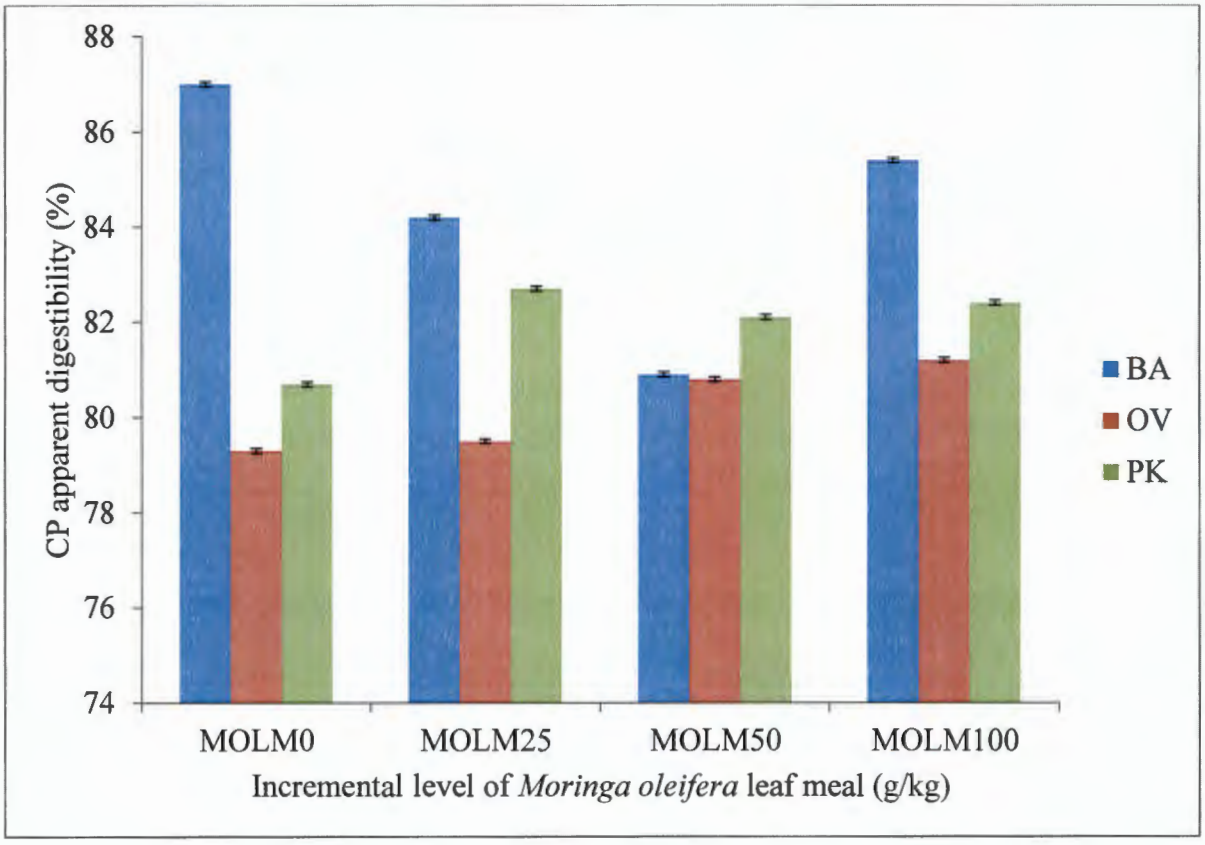

Figure 3-2. Effect of dietary *Moringa oleifera* leaf meal inclusion rate (%) on crude protein (CP) apparent digestibility of Black Australorp (BA), Ovambo (OV) and Potchefstroom Koekoek (PK) chicken strain between at 13 weeks of age.

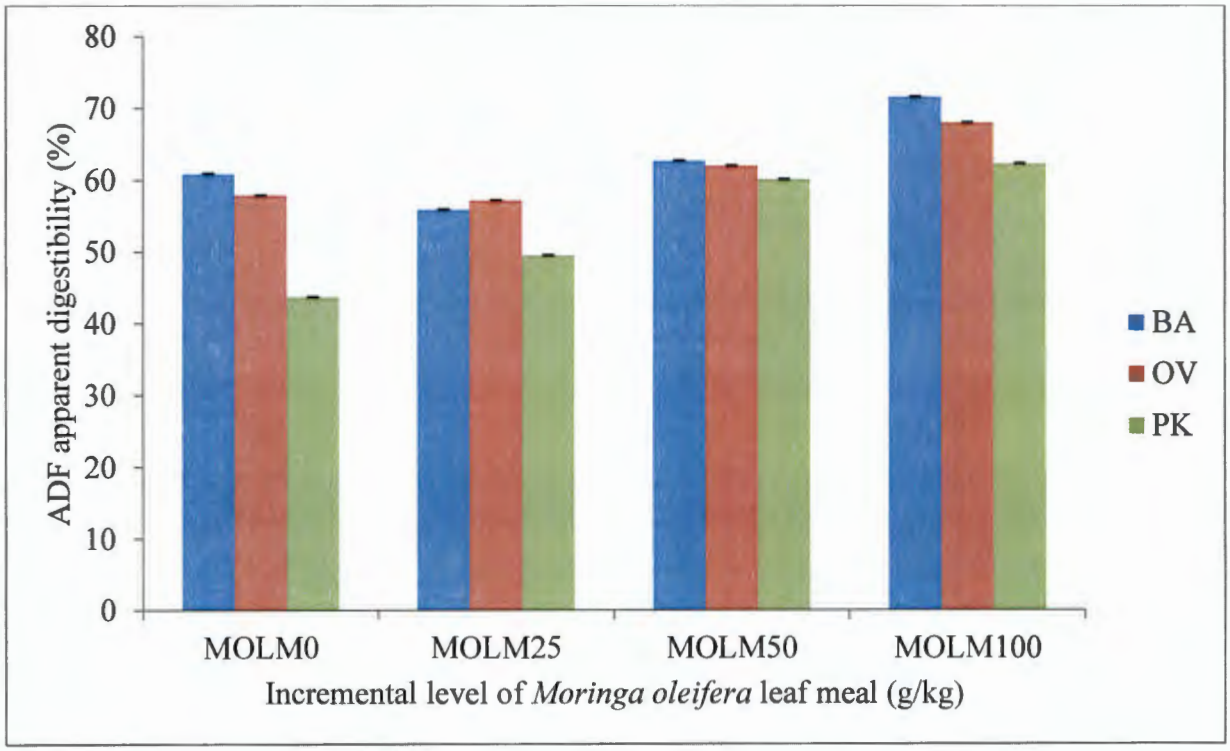

Figure 3-3. Effect of dietary *Moringa oleifera* leaf meal inclusion rate (%) on acid detergent fiber (ADF) apparent digestibility of Black Australorp (BA), Ovambo (OV) and Potchefstroom Koekoek (PK) chicken strain between at 13 weeks of age.

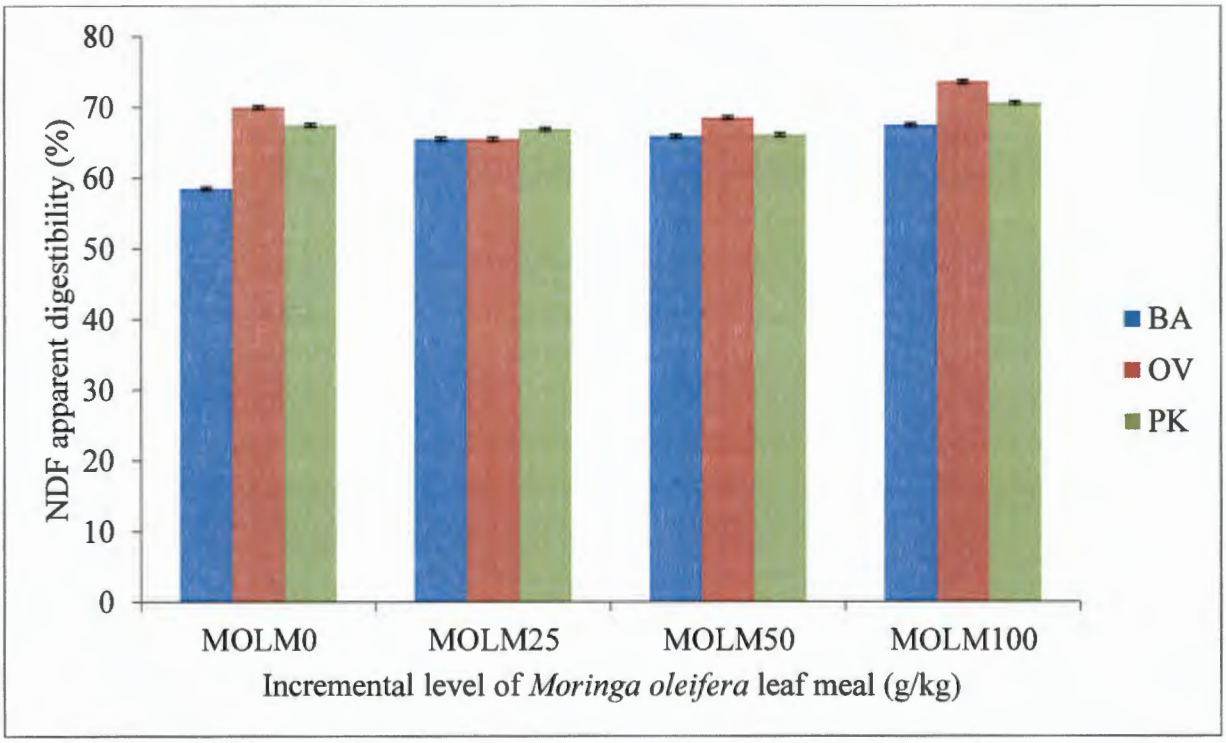

Figure 3-4. Effect of dietary *Moringa oleifera* leaf meal inclusion rate (%) on neutral detergent fiber (ADF) apparent digestibility of Black Australorp (BA), Ovambo (OV) and Potchefstroom Koekoek (PK) chicken strain between at 13 weeks of age.

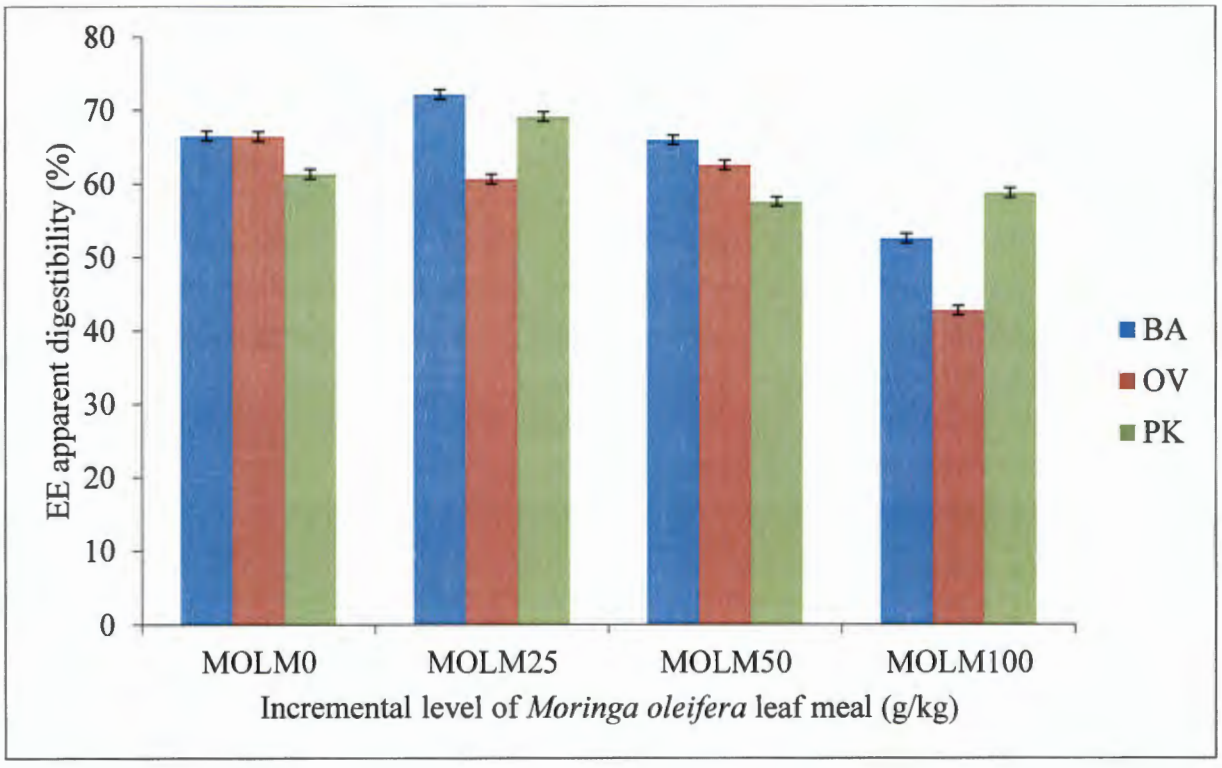

Figure 3-5. Effect of dietary *Moringa oleifera* leaf meal inclusion rate (%) on ether extract (EE) apparent digestibility of Black Australorp (BA), Ovambo (OV) and Potchefstroom Koekoek (PK) chicken strain between at 13 weeks of age.

3.8 Discussion

3.8.1 Chemical characterization

Genotype, stage of maturity and growing conditions interact to influence biomass yield and nutritive value of forage. *Moringa oleifera* leaf protein content was influenced by the stage of plant development. The deterioration in CP and escalation in fibre contents with advance in plant growth has been reported by several researchers (Callow *et al.*, 2003; Contreras-Govea *et al.*, 2009). The current finding contradict with Yang *et al.* (2006) whom indicated that mature leaves contained more CP than young shoots. *Moringa oleifera* tender (324.6 g/kg DM) and mature (285.2 g/kg DM) leaves has still sufficient protein content for production requirements for growing poultry. Generally, tender leaves contain high protein content and less fiber which will be suitable for high poultry performance due to limited ability of chickens to digest diets rich in fiber. In the present study total soluble carbohydrate content of the plant leaves decreases as the leaf matures. As plants mature, photosynthetic products are converted to structural components, thus having the effect of decreasing protein and soluble carbohydrate and increasing the structural cell wall components (Ammar *et al.*, 2004). Carbohydrates serve as a source of energy. Both NDF and ADF concentration increased with advancing maturity. Similar observations were reported by Turgut *et al.* (2008) and Ammar *et al.* (2010).

The moderate amount of EE at both stages of maturity is expected since plant leaves are not a major source of lipids. The ash content was lower in tender leaves (67.7 g/kg) compared to mature leaves (82.0 g/kg), this indicates the total amount of minerals since the ash content of a plant material is an index of total mineral content. The calcium and phosphorus content was higher in tender leaves compared to mature leaves. Dietary P plays an important role in the utilization of carbohydrates and fats and in the synthesis of protein for the growth, maintenance and repair of cells and tissues. Calcium is required for normal growth, activities of muscles and skeletal development. Calcium and phosphorus are the minerals present in the largest quantity

in the structure of the body and bones. Calcium is especially important in laying hens for the formation of egg shells. The use of both tender and mature leaves as feed for chickens will therefore ensure additional Ca in their diets necessary for optimal growth. Tender leaves had slightly higher selenium content. Selenium plays an important role in preventing cell damage and thus aids in protecting the body from the poisonous effects of heavy metals and other harmful substances. Mature leaves had higher Fe, which is an essential nutritional element. Fe is a necessary component of haemoglobin and myoglobin for oxygen transport and cellular processes of growth and division (Kozat, 2007). Magnesium was abundant in tender leaves than in mature leaves. Mg is responsible for chemical reactions in the body and intestinal absorption of Zinc (Muhammad *et al.*, 2011). Deficiency of these nutrients and minerals are known to affect the performance and health of poultry (Merck, 2005).

Tannins are plant derived compounds that are being successfully used as additives in poultry feed to control diseases and to improve animal performance (Acamovic and Brooker, 2005). Mature leaves had higher tannin content that tender leaves which can hinder growth performance in chicks. Dietary tannins are said to reduce feed efficiency and weight gain in chicks (Armstrong *et al.*, 1974; Dei *et al.*, 2007) when fed in large quantities. Generally, the composition of phenolic compounds can be affected by stage of maturity, post-harvest handling, processing and storage (Sreelatha & Padma, 2009). Polyphenols also help prevent atherosclerosis by boosting the activity of vitamin C, which in turn increases the levels of vitamin E. This synergy increases the overall resistance to oxidative stress and improve meat quality in poultry (Very Berry- and Grape too, 2001).

The bulk *M. oleifera* leaf meal had CP content of 263.4 g/kg DM, which is in agreement with the findings of Makkar & Becker (1997). However, Soliva *et al.* (2005) and Moyo *et al* (2011)

reported higher crude protein values (321 g/kg DM and 302.9 g/kg DM, respectively). The variations in CP contents of the reported values may be due to differences in agro-climatic conditions or to different ages of trees, and possibly due to different stages of maturity. The EE and ash contents reported by Gupta *et al.* (1989) and Makkar and Becker (1996, 1997) for *M. oleifera* leaves are in agreement with the current results. The CF content of the bulk *M. oleifera* leaf meal in the current study is comparable to that reported by Sodamade *et al.* (2013) but lower than the values (192.5 and 92.5 g/kg DM) reported by Oduro *et al.* (2008) and Ibok *et al.* (2008), respectively. The total soluble carbohydrate content obtained from bulk sample (561 g/kg) in the current study is higher than the 438.8 g/kg DM reported by Oduro *et al.* (2008). *Moringa oleifera* leaves consist of higher amount of unsaturated fatty acids (PUFA) than saturated fatty acids (SFA). These findings are comparable with those of Moyo *et al.* (2011). Polyunsaturated fatty acid composition of the diet is important for animal health. Hargis *et al.* (1993) reported that the FA composition of broiler chicken carcasses may be influenced considerably by diet.

To maintain optimal egg size, laying hens require a minimum of 1% LA in the diet (NRC, 1994), which can be satisfied by *Moringa oleifera* leaf (MOL) diet containing 8.93 % LA. *Moringa oleifera* leaves are rich in PUFA, which may aid in producing lower abdominal fat deposition than saturated or monounsaturated fatty acids (Kirchgessner *et al.* (1993). In addition, PUFA reduce the incident of narcotizing enterocolitis by modulating platelet activating factor and endotoxin translocation (Caplan & Jilling, 2001). In laying hens, decreased egg size, lowered egg weight and changes in egg yolk fatty acids follow linoleic acid deficiency. Both embryonic viability and hatchability are compromised during essential fatty acids (EFA) deficiency.

3.8.2 Apparent nutrient digestibility

Moringa oleifera leaves are a good source of protein, fibre, minerals, fatty acids profile and other elements important for the growth of chickens. The apparent digestibility of dietary nutrients decreases with fiber supplementation due to the replacement of digestible nutrients with components that are not digested or absorbed in the small intestine, and possibly to an increase in endogenous secretions in response to some types of fiber (Larsen *et al.*, 1993; Mosenthin *et al.*, 1994). Different chicken strain exhibited different apparent digestibility coefficients. This could be attributed to genetic differences in ability of each strain to utilise high fiber feeds. Higher crude protein digestibility observed at higher levels of MOLM inclusion may be due to the highly digestible nature of *Moringa oleifera* leaf. Indeed, Fahey *et al.* (2001) reported that *Moringa* contains highly digestible protein. Protein digestibility in *Moringa*-based diets was higher, possibly due to a greater percentage (82–91%) of pepsin-soluble protein and only 1–2% of acid-detergent insoluble protein (Makkar & Becker 1996). Fiber digestibility decreased in chickens consuming diets with fiber (MOLM) compared with the control diet. Generally, fiber ratios (insoluble vs. soluble fiber) play a critical role on rate of digestion and absorption of nutrients. Higher fiber digestibility on control diet indicates that soyabean meal fiber is highly digestible compared to MOLM fibre.

3.9 Conclusions

Significant variations were observed in chemical composition in tender and mature *M. oleifera* leaves. Based on the data observed, *M. oleifera* is a good source of minerals, crude protein and fatty acids profile. *Moringa oleifera* leaves have potential as a beneficial source of feed for animals. The presence of these important nutrients means *M. oleifera* leaves could be used as a nutritionally valuable and healthy ingredient to improve poultry health and growth performance. However, both tender and mature leaves meet nutrient requirements for poultry by NRC. Digestibility data indicate that inclusion of MOLM in chicken diets did not negatively

affect nutrient digestibility. It is, therefore, important to investigate the effect of MOLM on growth performance, carcass characteristics and other parameters in chickens.

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4 GROWTH PERFORMANCE AND CARCASS CHARACTERISTICS OF THREE CHICKEN STRAINS IN RESPONSE TO INCREMENTAL LEVELS OF DIETARY *MORINGA OLEIFERA* LEAF MEAL

4.1 Abstract

A 90-day feeding trial was conducted to determine the effect of *Moringa oleifera* leaf meal (MOLM) supplementation on growth performance and carcass characteristics of three chicken strains (male and female). The MOLM was chemically analysed and used to dilute a commercial broiler finisher diet at 0 (MOLM0), 25 (MOLM25), 50 (MOLM50), and 100 (MOLM100) g/kg DM, producing four dietary treatments. Two hundred and sixteen Potchefstroom Koekoek (PK), Ovambo (OV) and Black Australorp (BA) chickens were raised on a commercial starter mash for four weeks. On the fourth week, experimental diets were offered and growth performance data were collected over a period of 13 weeks. Carcass characteristics were measured upon slaughter at the end of the 13-week feeding period. Feed intake responded to incremental levels of MOLM in an asymptotic fashion. Maximum feed intake was achieved at dietary MOLM inclusion levels between 50 and 70 g/kg DM. Black Australorp chickens had the highest feed conversion efficiency (FCE) of 2.35, while OV and PK chickens had lower FCE values of 2.09 and 2.05, respectively. Diet, strain and gender, all had significant effects on dressing percentage, leg and thigh weight ($P<0.05$), and wing weight ($P<0.05$). Male chickens attained higher ($P<0.05$) carcass weight, leg and thigh weight, dressing percentage, and breast mass than female chickens ($P<0.001$). In female chickens, diets containing MOLM resulted in chickens with better carcass weight, leg and thigh weight, dressing percentage, and breast mass compared to the control. In conclusion, Black Australorp chickens can better utilize diets with higher levels of MOLM compared to OV and PK strains. Inclusion of MOLM in chicken diets positively affected growth performance and carcass characteristics of the birds.

Keywords: *Moringa oleifera* leaf meal, indigenous chickens, feed conversion efficiency, feed intake, carcass weight

4.2 Introduction

Extensively-reared chickens play a major role in ensuring food security in rural communities of most developing countries (Tadelle *et al.*, 2000). However, due to direct competition for food between man and non-ruminants, the cost of feeding chickens for optimum growth performance has become high. As a result, during the past few decades, developing countries have seen a decline in the contribution of indigenous poultry to food security (Bhatti *et al.*, 1990). This is mainly attributed to their relatively poor productive performance (Bhatti *et al.*, 1990) and an increase in poultry meat and eggs from commercially produced exotic poultry breeds (Gueye, 2000). Exotic poultry breeds have the distinct advantage of being highly productive and thus ensuring a quick return on investment. This increased productivity was achieved through improved management strategies, improved genetics, and research into nutrition and growth of the imported chicken breeds. The nutrient requirements of indigenous chickens differs from that of imported breeds, with the latter requiring feed of such high quality that it can also be used directly as human food. As a result, farmers in resource-poor rural communities find rearing of broilers to be unsustainable and prefer indigenous chicken strains that are adapted to extensive rearing systems. These chicken strains are products of their own environment and can be produced at a low cost. However, research to improve the productivity of indigenous chickens under intensive management systems is still limited. To contribute effectively to poverty alleviation, it is essential to improve and promote the production of local chickens. Due to high costs of poultry feed, formulating feed using cheap local resources is essential for sustainable production of indigenous chickens. Horsted (2006) reported that hens are capable of finding and utilizing a considerable amount of nutrients from forages. Abou-Elezz *et al.* (2011) and Kakengi *et al.* (2007) reported that inclusion of 5% *Moringa oleifera* leaf meal (MOLM) in the diet of Rhode Island Red hens improved egg mass production and egg laying rate. Leguminous leaves are important food resources because they provide

additional nutrients as well as bioactive plant compounds with beneficial effects on animal health and productivity. *Moringa oleifera* is currently being produced on a large scale in South Africa. While there have been a few studies investigating the use of *M. oleifera* leaves in poultry diets (Abou-Elezz *et al.*, 2011; Kakengi *et al.*, 2007), most of these have been carried out with broilers. The practical application of this intervention in broiler production is very low since the broiler precise nutritional requirements. Dilution of broiler diets with plant material is most likely to result in sub-optimal productivity. On the other hand, extensively-reared chicken strains are likely to have some capacity to utilize plant material but this has not been investigated extensively. Therefore, the purpose of this study was to investigate the growth performance and carcass characteristics of three chicken strains (Potchefstroom Koekoek, Ovambo (indigenous) and Black Australorp (imported), which are normally reared extensively in South Africa, in response to incremental levels of dietary MOLM.

4.3 Materials and methods

4.3.1 Study sites

This study was conducted at the North-West University Experimental Farm (Molelwane), Mafikeng (25.8° S and 25.5° E), South Africa. *Moringa oleifera* leaves were obtained from Patience Wellness Centre in Limpopo Province (24.305° S 29.565° E). The ambient temperature in this area ranges from 27 to 37°C during summer and between 11 and 17°C during winter. The annual rainfall ranges between 500 mm and 800 mm. The leaves were air-dried at a room temperature and then milled to pass through a 2 mm sieve.

4.3.2 Chicken strains

Ovambo (OV), Potchefstroom Koekoek (PK) and Black Australorp (BA) eggs were purchased from the Agricultural Research Council (ARC), (Irene, Pretoria) and hatched in an incubator at North West University farm. Temperature and humidity were, respectively, set at 37.5°C and 82.5% for incubation and 37°C and 85% for hatching. The OV strain originates from Ovamboland district of Namibia. The strain was brought to the Poultry Breeding Section of the ARC, Irene, South Africa, for conservation. The body conformation (small to medium) and colour patterns are typical of chickens found in rural communities of Southern Africa. The Potchefstroom Koekoek strain was bred at the Potchefstroom Agricultural College, South Africa, during the 1950s. It is a composite of the White Leghorn, Black Australorp and Barred Plymouth Rock. This multipurpose strain is, therefore, recognized as locally developed. The Black Australorp, an Australian chicken breed, is the most commonly used imported strain of chickens in communal production systems of South Africa. It was developed from the English Orpington.

4.3.3 Chemical analysis of Moringa leaf meal

The chemical analysis of Moringa leaf meal was done as described in Chapter 3.

4.3.4 Diet formulation

Diet formulation is described in Chapter 3 above and given in Tables 3.1 & 3.2.

4.3.5 Experimental design

Two hundred and sixteen chickens equally divided across the three strains, PK, OV, and BA), were raised on a commercial starter mash for 4 weeks. A 3 (chicken strains) x 4 (diets) factorial treatment arrangement in a complete randomised design (CRD) was used for the growth performance evaluation. However, A 3 (chicken strains) x 4 (diets) x 2 (gender) factorial treatment arrangement in a complete randomised design (CRD) was used for the carcass characteristics. The experimental unit was a pen holding 6 birds (3 males and 3 females), which

was replicated 3 times, resulting in a total of 36 floor pens measuring 3.5 m x 1.0 m x 1.85 m (L x B x H). At four weeks of age, the chickens from each strain were randomly allocated to the four experimental diets.

4.3.6 Feeding management and growth performance measurements

Feed and water was provided *ad libitum* during the 13-week experimental period under continuous lighting. Average daily feed intake per bird was measured from 4 -13 weeks of age by subtracting the weight of the feed refusals from that of the feed offered per day, and dividing the difference by the total number of birds in the pen. It is for this reason that performance parameters derived from feed intake data, such as feed conversion efficiency (FCE), could not be disaggregated by gender. The initial liveweight of the birds was measured at four weeks of age before the commencement of the experiment. Thereafter, average liveweight was measured weekly by weighing all the birds in each pen. These liveweights were used to calculate growth rates. A weekly FCE was calculated as follows:

$$FCE = \frac{Weight\ gained\ (g)}{Feed\ consumed\ (g)}$$

4.3.7 Carcass characteristics

At 13 weeks of age, the chickens (108 males and 108 females) were electrically stunned and killed by manual exsanguination. The feet, heads and intestines were manually removed. The carcasses were weighed to determine the slaughter yield (%) before being cut according to a standardized procedure (Uijttenboogaart & Gerrits, 1982) to determine the weight of breast (without skin), upper legs (thigh), lower legs (tibia and foot), and wings. These measurements were expressed as a percentage of the carcass weight.

4.4 Statistical analysis

Weekly feed intake, growth rate, and FCE data were analysed using the repeated measures procedure of SAS (SAS, 2008). Overall feed intake, weight gain, growth rate, and feed conversion efficiency data were analysed using the general linear models (GLM) procedure of SAS (2008) for a 4 (diets) × 3 (chicken strains) factorial treatment arrangement. The linear model employed was:

$$Y_{ijk} = \mu + D_i + S_j + (D \times S)_{ij} + E_{ijk} ,$$

where Y_{ijk} = observation of the dependent variable *ijk*, μ = fixed effect of population mean for the variable, D_i = effect of experimental diet (i = 4; MOLM0, MOLM25, MOLM50, and MOLM100), S_j = effect of chicken strain (j = 3; PK, OV and BA), $(D \times S)_{ij}$ = effect of interaction between diet at level *i* and chicken strain at level *j*, and E_{ijk} = random error associated with observation *ijk*.

Carcass characteristics data were analysed using the GLM procedure of SAS (2008) for a 4 (diets) × 3 (chicken strains) × 2 (gender) factorial treatment arrangement. The linear model employed was:

$$Y_{ijkl} = \mu + D_i + S_j + G_k + (D \times S)_{ij} + (D \times G)_{ik} + (S \times G)_{jk} + (D \times S \times G)_{ijk} + E_{ijkl} ,$$

where Y_{ijkl} = observation of the dependent variable *ijkl*, μ = fixed effect of population mean for the variable, D_i = effect of experimental diet (i = 4; MOLM0, MOLM25, MOLM50, and MOLM100), S_j = effect of chicken strain (j = 3; PK, OV and BA), G_k = effect of gender of chicken (k = 2; male and female), $(D \times S)_{ij}$ = effect of interaction between diet at level *i* and chicken strain at level *j*, $(D \times G)_{ik}$ = effect of interaction between diet at level *i* and gender at level *k*, $(S \times G)_{jk}$ = effect of interaction between chicken strain at level *j* and gender at level *k*, $(D \times S \times G)_{ijk}$ = effect of interaction between diet at level *i*, chicken strain at level *j* and gender

at level k, and E_{ijk} = random error associated with observation *ijkl*. For all statistical tests, significance was declared at $P \leq 0.05$. Least squares means were compared using Tukey's HSD.

The dose-related responses to incremental levels of MOLM were modelled using the following quadratic equation (SAS, 2008):

$$Y = a + b_1 x + b_2 x^2$$

Where y = response variable; a = intercept; b_1 and b_2 = coefficients of the quadratic equations, x = level of MOLM inclusion, and $\frac{-b_1}{2b_2}$ = MOLM level 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.

4.5 Results

4.5.1 Chemical composition of MOLM

The concentration (g/kg DM) of crude protein (CP), neutral detergent fibre (NDF), and acid detergent fibre (ADF) was found to be 284, 801, and 549, respectively.

4.5.2 Feed intake and growth performance

Statistical significance (P values) of the effect of main factors (diet, chicken strain and weeks) and their interaction on feed intake, feed conversion ratio and growth rate is presented in Table 4.1. The 3 way interaction (diet × strain × weeks) did not ($P>0.05$) affect feed intake, but significantly influenced growth rate and FCE.

Table 4-1. Statistical significance (P values) of the effects of main factors on the weekly (time) performance of three chicken strains (Black Australorp, Ovambo, and Potchefstroom Koekoek)offered four diets with graded levels of *Moringa oleifera* leaf meal(0, 25, 50, and 100 g/kg)

Parameter	Effect of treatment		Interaction		Interaction		
	Diet (D)	Strain (S)	D ×S	Time (T)	T × D	T ×S	T × D ×S
Feed intake	NS	*	NS	***	NS	***	***
Growth rate	NS	NS	NS	***	***	***	NS
FCE[1]	NS	NS	NS	***	NS	NS	*

[1]FCE = Feed conversion efficiency,

* P<0.05; ** P<0.01;*** P<0.001

In week 7 and 13, there was no significant effect of dietary treatments observed on feed intake. Chicken strain and weeks interacted significantly ($P< 0.05$) to influence feed intake. Feed intake significantly changed with time (weeks) as the chickens' digestive tracts adapted to experimental diets. Black Australorp had the highest ($P<0.05$) feed intake (564.9 g/week) while PK had lowest (485.5 g/week) during week 10 (Figure 4-1).

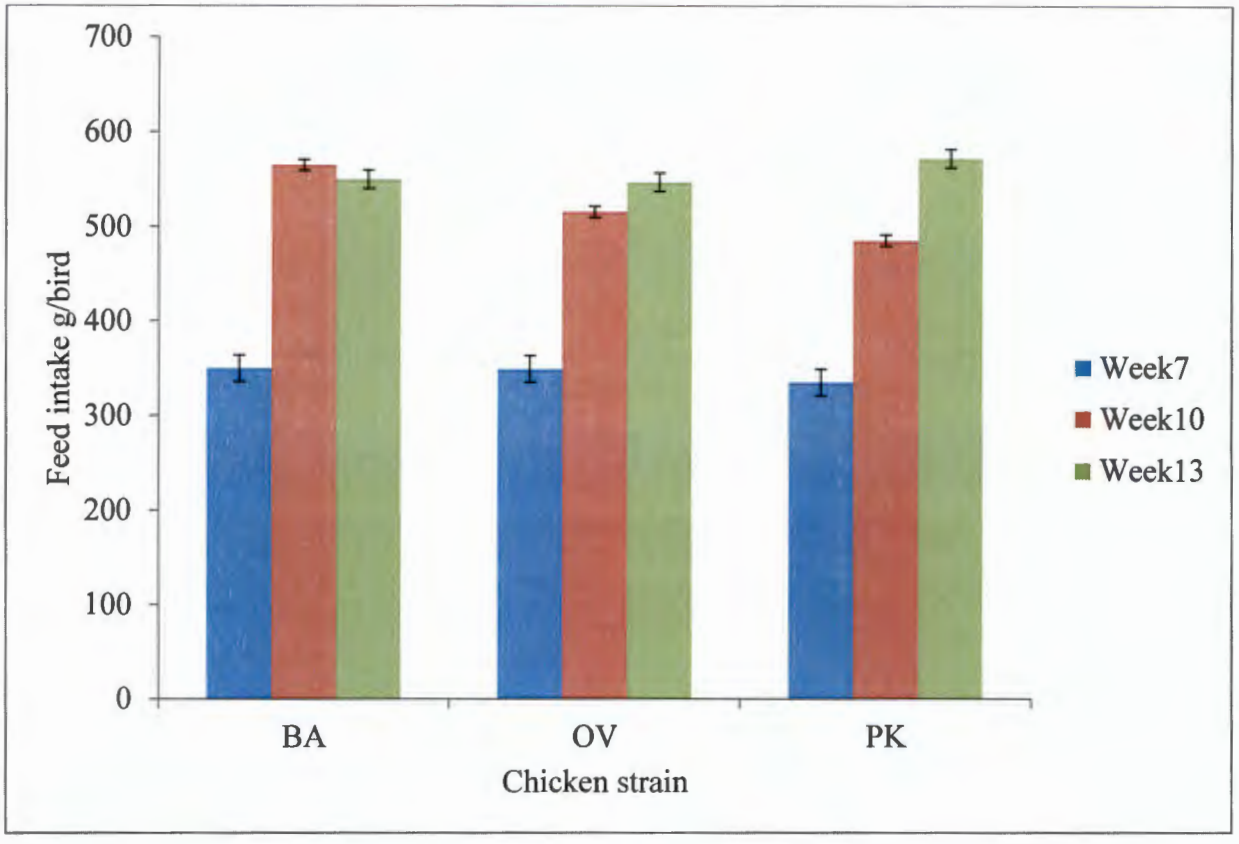

Figure 4-1. Weekly feed intake (g/bird/week) of 3 chicken strains fed incremental levels of *Moringa oleifera* leaf meal (0, 25, 50, and 100 g/kg).

Table 4-2. Statistical significance (P values) of the effects of main factors on overall feed intake and growth performance of three chicken strains(Black Australorp, Ovambo, and Potchefstroom Koekoek)offered four diets with graded levels of *Moringa* leaf meal (0, 25, 50, and 100 g/kg)

Parameter	Effect of treatment		Interaction
	Diet (D)	Strain (S)	D ×S
Feed intake	**	NS	***
Growth rate	NS	**	NS
FCE	NS	*	NS

* P<0.05; ** P<0.01;*** P<0.001

NS; Not significant

Table 4-3. Growth rate (g//bird/day) of three strains of chickens (Black Australorp, Ovambo, and Potchefstroom Koekoek) at 7, 10 and 13 weeks of age when fed incremental levels of *Moringa oleifera* leaf meal.

	Black Australorp			Ovambo			Potchefstroom Koekoek		
Diet	7	10	13	7	10	13	7	10	13
MOLM0	19.9	15.1	23.0	19.5^{a}	20.7^{ab}	28.9^{b}	15.5	19.4	20.6
MOLM25	26.8	21.2	21.9	19.0a	20.0ab	28.3^{b}	15.8	21.8	16.4
MOLM50	17.3^{a}	17.8^{a}	35.4^{b}	21.8^{a}	24.89^{ab}	32.18^{b}	19.9^{a}	23.7^{b}	25.9^{b}
MOLM100	13.5^{a}	18.2^{a}	31.4^{b}	18.5^{a}	23.1^{a}	33.4^{b}	17.3^{a}	22.2^{ab}	27.6^{b}
SEM	1.72	2.06	2.87	1.72	2.06	2.87	1.72	2.06	2.87

^{ab}Within each chicken strain, Means within the same row with different superscript differ ($P<0.05$)

Diets and chicken strains, did not affect (P>0.05) growth rate. Only week significantly affected growth rate and interacted significantly with diet and chicken strain. The growth rate of BA offered MOLM at the rate of 0 and 25 g/kg was similar (P>0.05) throughout the feeding period (Table 4-3). During week 13, BA chickens had higher growth rate (35.4 g/week) on MOLM50 compared to week 7 and 10. Similarly, in week 13, MOLM100 promoted higher (P<0.05) growth rate (31.4 g/week) in BA chickens compared to weeks 7 and 10. The growth rate of OV chickens in week 7 was lower than in weeks 10 and 13 for all diets. Ovambo chickens fed MOLM100 had higher (P<0.05) growth rate (33.4 g/week) during week 13 than week 7. Diets MOLM0 and MOLM25 had no significant effect on PK growth rate across all weeks. The growth rate of PK chickens offered MOLM100 was higher (P<0.05) in weeks 10 and 13 compared to week 7 growth rate.

Table 4-4. Feed conversion efficiency of three strains of chickens (Black Australorp, Ovambo, and Potchefstroom Koekoek) at 7, 10 and 13 weeks of age when fed incremental levels of *Moringa oleifera* leaf meal.

	Black Australorp			Ovambo			Potchefstroom Koekoek		
Diet	7	10	13	7	10	13	7	10	13
MOLM0	4.53[b]	1.83[a]	2.55[ab]	3.55	2.79	3.56	3.50	2.77	2.43
MOLM25	5.35[b]	2.64[a]	2.95[a]	3.36	2.8	3.65	3.23	3.16	3.14
MOLM50	3.49[ab]	2.23[a]	4.47[a]	3.96	3.48	4.19	3.21	3.43	3.16
MOLM100	2.53[ab]	2.26[a]	4.61[b]	6.49[b]	2.99[a]	4.36[b]	3.75	3.19	3.34
SEM	0.76	0.28	0.40	0.76	0.28	0.40	0.76	0.28	0.40

[ab]Within chicken strain, means within the same row with different superscript differ ($P<0.05$)

Feed conversion efficiency in Black Australorp chickens fed MOLM0 was highest in week 7 and 13 and lowest in week 10 (Table 4-4). Feeding MOLM25 resulted in higher FCE of 5.35 at 7 weeks of age compared to weeks 10 and 13, whose FCE values did not differ ($P>0.05$). Ovambo chickens offered MOLM0, MOLM25, and MOLM50 had similar FCE values throughout the feeding period. However, MOLM100 promoted higher ($P<0.05$) FCE in week 7 (6.49) and 13 (4.36). Feed conversion efficiency in PK chickens did not differ ($P>0.05$) across weeks. When offered the control diet (MOLM0), BA chickens had significantly lower overall feed intake (536 g/week) compared to OV (544) and PK (541) (Figure 4-2). Potchefstroom Koekoek had higher overall feed intake (626 g/week) when offered MOLM25 as compared to OV and PK strains.

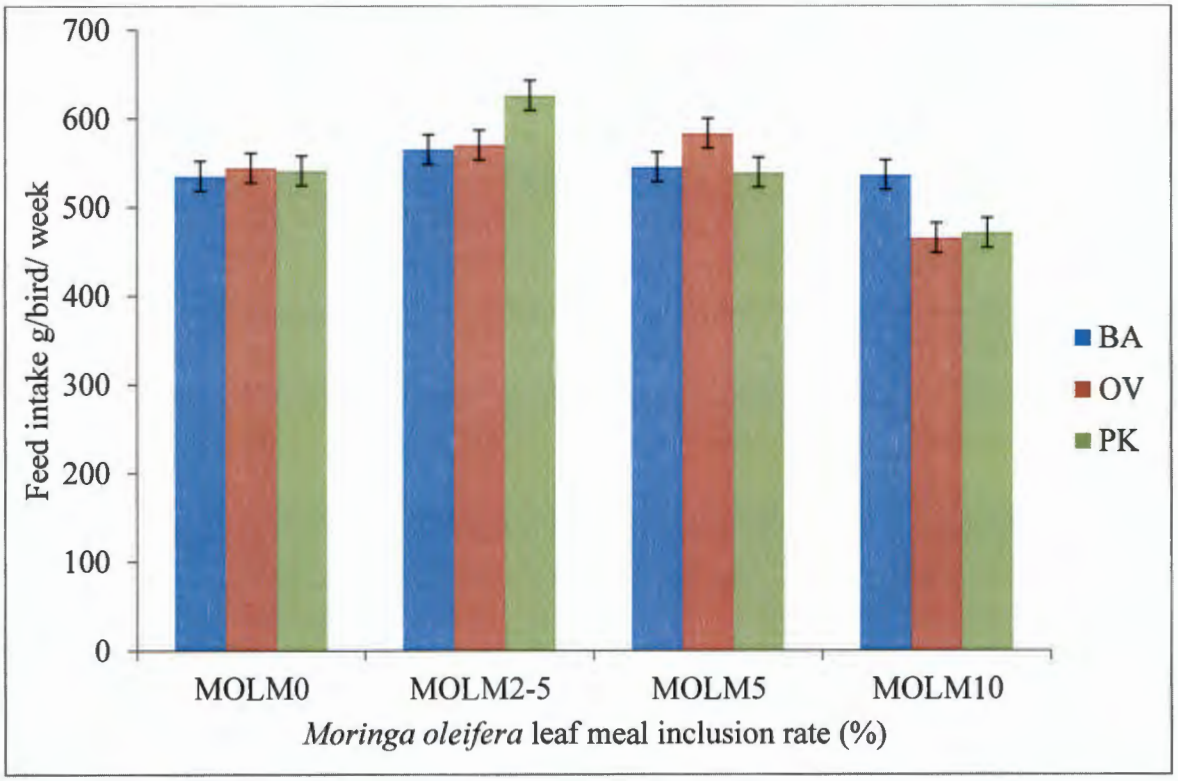

Figure 4-2. Overall feed intake (g/bird/week) of three strains of chickens fed incremental levels of *Moringa oleifera* leaf meal (0, 25, 50, and 100 g/kg).

BA - Black Australorp; OV – Ovambo; PK – Potchefstroom Koekoek

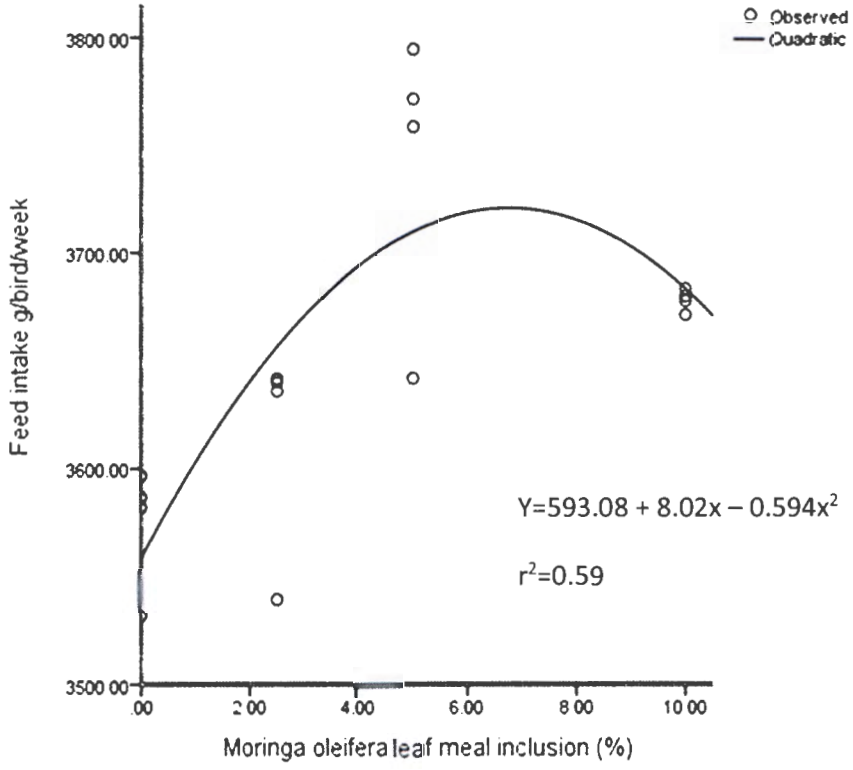

Figure 4-3. Effect of dietary *Moringa oleifera* leaf meal inclusion rate (%) on weekly DM feed intake in the Black Australorp (BA) chicken strain between five and thirteen weeks of age.

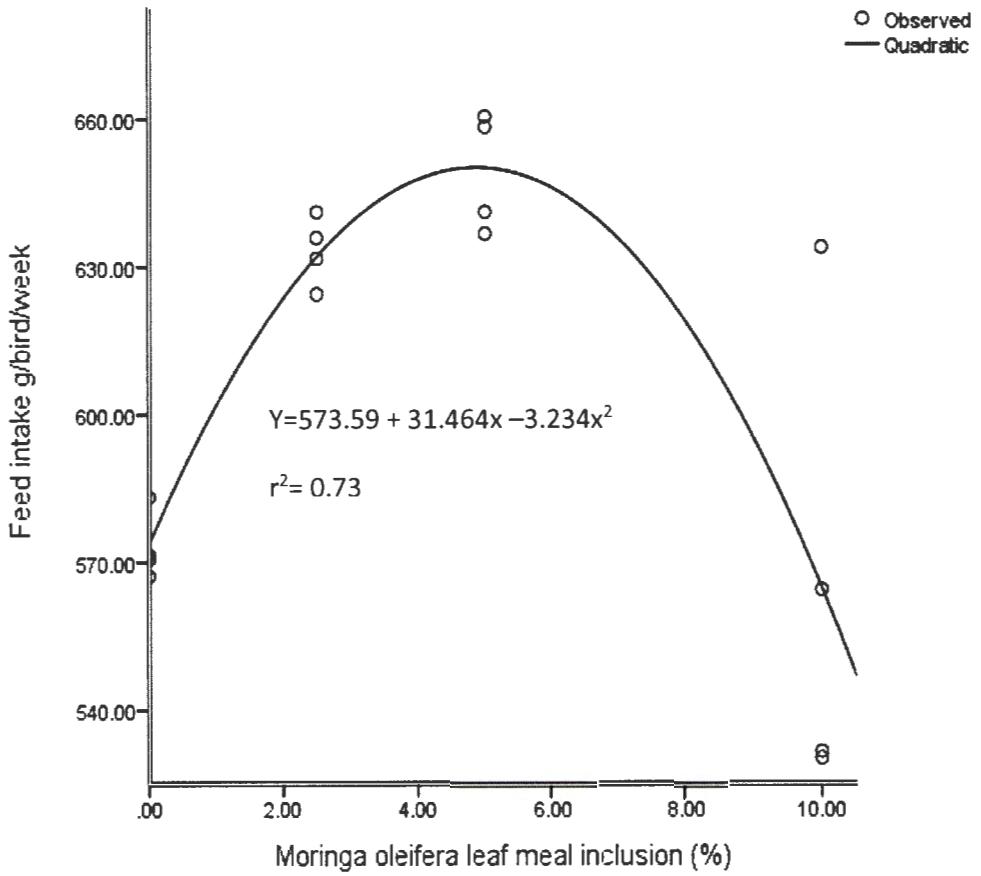

Figure 4-4. Effect of dietary *Moringa oleifera* leaf meal inclusion rate (%) on weekly DM feed intake in the Ovambo chicken strain between five and thirteen weeks of age.

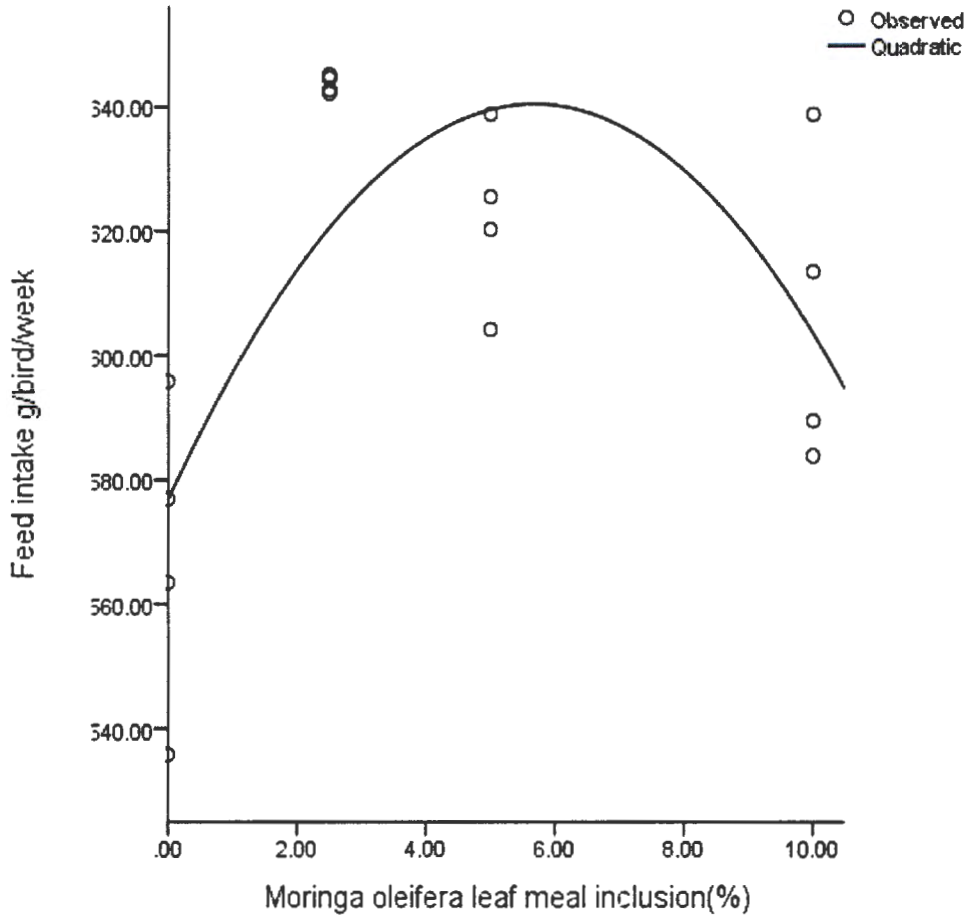

Figure 4-5. Effect of dietary *Moringa oleifera* leaf meal inclusion rate (%) on weekly DM feed intake in the Potchefstroom Koekoek chicken strain between five and thirteen weeks of age.

Ovambo had higher (P>0.05) intake (583 g/kg) of MOLM50 diet whilst BA chickens had higher intake (536 g/week) of MOLM100. Overall feed intake in the three chicken strains responded asymptotically to incremental inclusion levels of MOLM. Feed intake peaked at dietary MOLM levels between 50 and 70 g/kg DM inclusion levels (Figures, 4.31; 4.3.2 and 4.3.3). Both BA and OV had higher (P<0.05) growth rates than PK chickens (Figure 4-2).

Both BA (24 g/day) and OV (23.4 g/day) had higher (P<0.05) growth rates than PK chickens (21.5 g/day) (Figure 4-6). In addition, BA had the higher (P<0.05) overall FCE of 2.35 compared to OV (2.09) and PK (2.05) chickens.

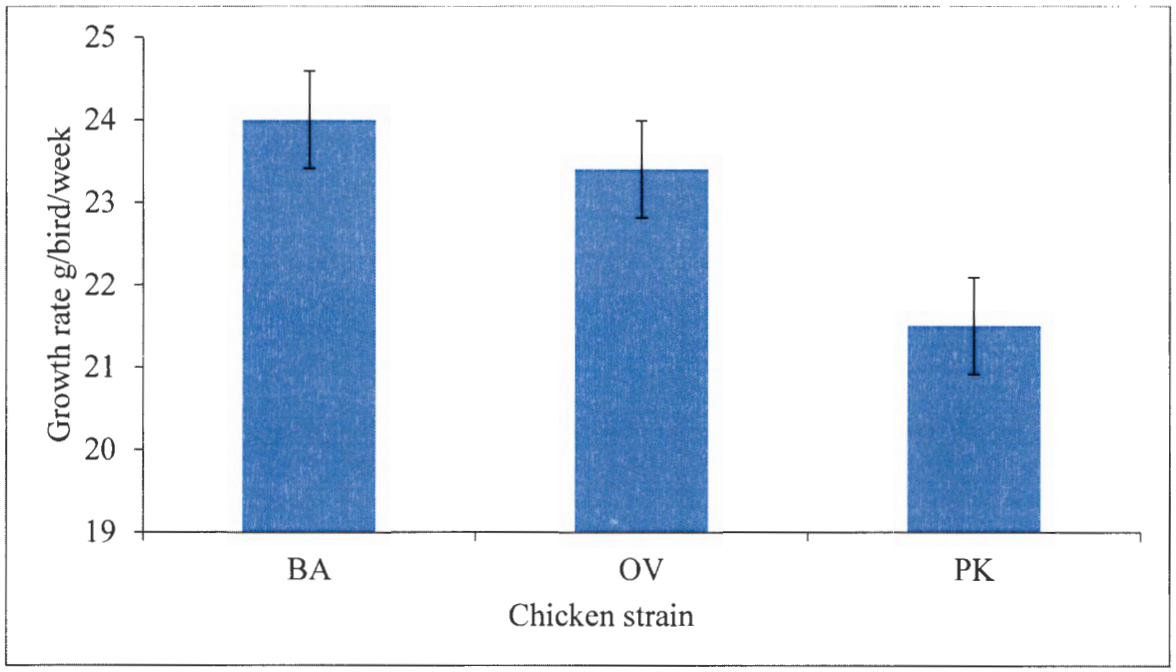

Figure 4-6. Overall growth rate (g/bird/week) of 3 strains of chickens fed incremental levels of *Moringa oleifera* leaf meal (0, 25, 50, and 100 g/kg).

BA- Black Australorp; OV – Ovambo; PK – Potchefstroom Koekoek

4.5.3 Carcass traits

The 3-way interaction term 'diet × strain × gender' did not ($P > 0.05$) affect carcass and breast weights but significantly influenced all the other carcass characteristics (Tabled 4-5). The interaction term 'diet × strain' did not affect any of the carcass characteristics. There was a significant diet × gender interaction for carcass ($P < 0.01$), thigh and drumstick ($P < 0.05$), and wing weight ($P<0.05$) (Table 4-6). Gender significantly affected all carcass characteristics. Chicken strain also significantly ($P < 0.05$) affected all carcass traits with the exception of breast weight (Table 4-6).

Table 4-5. Statistical significance (P values) of the effects of main factors (diet, chicken strain, and gender) and their interactions ondressing percent, and carcass and organ weights of chickens

Parameter	Diet[1] (D)	Strain[2] (S)	Gender[3] (G)	D×G	G×S	D×S	D×S×G
Carcass weight	NS	***	***	**	NS	NS	NS
Dressing percent	NS	***	***	NS	**	NS	***
Breast weight	NS	NS	***	NS	NS	NS	NS
Thigh & drumstick	NS	*	***	*	*	NS	*
Wing weight	NS	***	***	*	**	NS	*

[1]Diet = MOLM0, MOLM25, MOLM50, MOLM100; [2]Strain = Black Australorp, Ovambo and Potchefstroom Koekoek; [3]Gender = Male and female.

NS – Not significant

Black Australorp (1016.9 g) and OV (1012.5 g) chickens had higher ($P<0.05$) carcass weights compared to PK (963.8 g) strain. Carcass weight of male chickens was higher ($P<0.05$) than in female chickens when offered MOLM0, MOLM50, and MOLM100 diets. In PK, males had a higher dressing percentage (67.7%) compared to female chickens (63.4%). In BA and OV strains, there was no significant difference ($P>0.05$) between males and females in terms of dressing percentage. For female chickens, dressing percentage was greater in BA (64.7%) and lowest in OV (59.9 %). However for males, PK (67.7%) and BA (65.8%) had greater ($P<0.05$) dressing percentage compared to OV (59.2%) chickens. In BA, male chickens had greater thigh and drumstick weight (183.4 g) compared to female chickens (138.8 g).

No significant ($P>0.05$) differences in thigh and drumstick weight was observed between PK male and female chickens. Ovambo males had higher thigh and drumstick weight (161.9 g) compared to the female chickens (122.2 g). For female chickens, thigh and drumstick weight was highest in PK (148.4 g) and lowest in OV strain (95g). However for males, BA had the highest thigh and drumstick weight (183.4 g) compared to PK (163.4) and OV (161.9 g) strains. Male chicken strains had higher ($P>0.05$) breast weight as compared to female chicken strains. In BA, males had greater wing weight (88.8 g) compared to female chickens (76.3 g). Similarly, OV male chickens attained higher wing weight (80 g) compared to females (63.1 g). However, in PK chickens, there was no difference ($P>0.05$) in wing weight between males and females. For female chickens, wing weight was greater in BA (76.3 g) and PK (75.6 g) and lowest in OV (63.1 g). However for male chickens, BA had greater wing weight (88.8 g) while OV (80 g) and PK (78.1 g) had lower and similar ($P > 0.05$) weights.

Table 4-6. Carcass weight, dressing percent, drumstick and thigh weight, and wing weight in three strains of 13-week old chickens fed incremental levels of *Moringa oleifera* leaf meal.

		Black Australorp		Ovambo		Potchefstroom Koekoek	
Parameter	Diet[1](g/kg)	Female	Male	Female	Male	Female	Male
Carcass weight (g)	MOLM0	820^{aD}	1175.0^{aAB}	830.0^{bB}	1115.0^{aA}	900^{bC}	1105^{aAB}
	MOLM25	955.0^{bC}	1075.0^{aB}	850.0^{cB}	1065^{aA}	915^{bcC}	1045^{aB}
	MOLM50	895.0^{cCD}	1185.0^{aA}	865.0^{cB}	1050^{bA}	920^{cC}	1140^{aA}
	MOLM100	910.0^{cC}	1120^{abAB}	895.0^{cB}	1040^{bA}	920^{cC}	1155^{aA}
Dressing percent (%)	MOLM0	64.0^{abAB}	66.1^{aAB}	59.1^{cAB}	60.4^{bcAB}	63.4^{abBC}	66.4^{aB}
	MOLM25	67.5^{aA}	63.4^{bB}	60.4^{bcAB}	58.6^{cAB}	63.9^{abBC}	64.2^{abBC}
	MOLM50	63.5^{bcB}	67.8^{aA}	59.0^{dAB}	60.6^{cdAB}	61.2^{cdC}	66.2^{abB}
	MOLM100	63.8^{cB}	65.4^{bAB}	61.2^{cA}	57.0^{dB}	65.2^{bB}	73.6^{aA}
Drumstick & thigh (g)	MOLM0	120.0^{cdD}	202.5^{aA}	95^{dC}	157.5^{bAB}	140.0^{bcA}	165.0^{bA}
	MOLM25	150.0^{abCD}	166.3^{aBC}	127.0^{bBC}	167.5^{aAB}	151.0^{abA}	173.8^{aA}
	MOLM50	141.3^{cCD}	187.5^{aAB}	120.0^{cAB}	$180.^{abA}$	146.3^{bcA}	143.8^{cA}
	MOLM100	143.8^{aCD}	$177.8a^{ABC}$	142.5^{aB}	146.3^{aAB}	156.3^{aA}	171.3^{aA}
Wing (g)	MOLM0	63.8^{bcC}	86.3^{aAB}	60.0^{cC}	86.3^{aA}	75.0^{abAB}	80.0^{aAB}
	MOLM25	78.8^{aB}	82.5^{aAB}	61.3^{bBC}	77.5^{aA}	71.3^{abB}	82.5^{aAB}
	MOLM50	81.3^{abB}	91.3^{aAB}	57.5^{cC}	81.3^{abA}	70.0^{bcB}	71.3^{bB}
	MOLM100	81.3^{bB}	95.0^{aA}	73.8^{bAB}	75.0^{bA}	86.3^{abA}	78.8^{bAB}

[ab]Means within the same row with different lowercase superscripts differ ($P<0.05$);

[AB]Means within the same column with different uppercase superscripts differ ($P<0.05$); [1]Diet: MOLM0 = broiler finisher without MOLM inclusion; MOLM25 = broiler finisher diluted at 25 g/kg MOLM MOLM50 = broiler finisher diluted at 50 g/kg MOLM; MOLM100 = broiler finisher diluted at 100 g/kg MOLM.

4.6 Discussion

4.6.1 Feed intake and growth performance

From the current study it would seem that *Moringa oleifera* leaves are a good source of protein, fibre, minerals and other elements important for the growth of chickens. This is because in general MOLM positively affected feed intake and growth performance of birds. However, CP (284 g/kg), ADF (549 g/kg) and NDF (801 g/kg) content of *M. oleifera* leaves in this study is lower than the values reported by Moyo *et al.* (2011) and Kakengi *et al.* (2000). The variation could be attributed to differences in agro-climatic conditions or stage of maturity of the leaves at harvest in Moyo *et al.* (2011); Kakengi *et al.* (2000) study and the present study. Nevertheless, the values obtained in this study confirm that while MOLM may contain moderate to high levels of protein, the fibre content may negatively impact on protein utility as an alternative feed resource for the simple-stomached avian species.

In the present study, there were significant differences in feed intake, FCE and growth rate of the imported BA strain and that of the indigenous chickens, OV and PK. These results support the view that BA is a fast-growing strain compared to other extensively-reared chickens in South Africa. The genetics of a chicken affects its feed intake, digestibility, feed conversion efficiency and growth rate at different ages (Leeson *et al.*, 1997; Rondelli *et al.*, 2003). Growth potential of chickens can also be influenced by gender. Results from the present study indicate that capacity to utilize fibre-containing diets differed among chicken strains. These findings indicate that intake of fibre-containing diets in different chicken strains is regulated by the chicken's energy requirements. Feed intake showed a curvilinear response with increasing dietary MOLM levels. Feed intake of PK, OV, and BA strains reached a maximum at dietary MOLM inclusion levels of 3.1, 4, and 5%, respectively. Body size has been considered as a possible mechanism for interspecies differences in feed intake (Bell 1970; Hanley & Hanley1980; Sinclair, 1977; Demment, 1980; Van Soest, 1982). The BA strain had the highest

weight gain compared to OV and PK. Being the bigger strain, the energy requirements for growth for the BA strain are likely to be greater than for the other two strains under investigation. Higher energy requirements translate into higher feed intake in simple non-ruminants and pronounced compensatory feeding behaviour when a diet's energy concentration is diluted (Conrad, 1966), as was done in this study. According to Teguia & Beynen (2005), high fibre levels in poultry diets lead to poor digestibility of the diets and are associated with a higher feed intake. Conrad (1966) and NRC (1988) indicated that the increase in dietary fiber levels is associated with low energy density that may stimulate increased feed intake as a compensatory feeding behaviour. The mechanism regulating feed intake involves glucose level in blood stream. Richards (2003) suggested that when blood glucose drops, the blood releases the fat destroying hormones (growth hormones, glucagon and cholescystokinin) and suppresses energy storing insulin. Such transient changes in plasma glucose level do not appear to alter feed intake in chickens (Maclean & Luo, 2004; Richards, 2003). Indeed, Burnham *et al.* (1992) and Gous *et al.* (1987) observed that chickens increased their feed intake as the limiting nutrient in the feed decreased in an attempt to obtain more of the limiting nutrient to satisfy their requirements for that nutrient. As a result, the inclusion of insoluble fiber in poultry diets at moderate concentrations, does not affect the performance of birds despite the fact that the nutrient concentration of the diet is reduced (Hetland & Svihus, 2001; Hetland *et al.*, 2002). However, fibre dilutions beyond the optimal inclusion levels will ultimately result in lower feed intake possibly due to an increase in digesta viscosity and a longer retention time of the digesta in the gastro intestinal tract (GIT) (Conrad, 1966; NRC, 1988). This explains the asymptotic response of feed intake to incremental levels of MOLM that was observed in this study.

The variation in growth rate across weeks could be attributed to digestive tract adaptability to feed with high fibre content. Indeed, Horsted (2006) has reported that hens are capable of

finding and utilizing a considerable amount of nutrients from forages. The weekly increase in the intake could be explained by the growth rate of the chickens. As chickens increase in size, their nutritional requirements also increase and since they eat to satisfy their nutrient needs, feed intake increases accordingly (NRC, 1988). Black Australorp and OV chickens had better overall weight gain than PK strain. This response may be attributed to genetic and growth potential of different strains.

The present results are in agreement with those of Moyo *et al.* (2011) and Kakengi *et al.* (2007) who observed satisfactory growth rate, FCE, egg mass production and egg laying rate in broiler chickens at inclusion level of 5% MOLM. Despite the fibre content of the diet, different strains utilised the feed efficiently, which resulted in both good growth rate and feed conversion efficiency.

4.6.2 Carcass characteristics

Black Australorp and PK chickens had higher dressing percentage compared to OV at higher inclusion levels of MOLM. This could be ascribed to genetic variation and growth potential of the different strains. Male chickens had higher ($P<0.05$) carcass weights than female chickens strains. These finding are in agreement with Lazzari & Paganni (1999), who noticed a significant difference ($P<0.05$) in carcass weights between gender and strains of broilers. In the current study, male chickens had higher carcass weight, dressing percentage, wing, and leg and thigh weight than female chickens across all diets. Similar results were obtained by Negesse & Tera (2010) with Rhode Island Red chickens; Nikolova & Pavlovski (2009) with commercial broilers; and Aberra *et al.* (2013) with Koekoek chickens. These researchers reported higher weights of dressed carcass, thighs, and drumsticks in males than females. Scanes (2003) reported that higher values in carcass traits observed in male chickens might be attributed to the presence of male sex hormones that enhance muscle development more than estrogen in females. Estrogen is mostly responsible for fat deposition rather than muscle tissue

development. The superiority of male chickens in terms of overall weight gain corroborates the findings of Laseinde & Olayemi (1994), who reported that male broilers grow faster and weigh heavier than the females under various rearing conditions. In addition, Meijerhof (1988) reported that male broilers utilized feed more efficiently than the female broilers. Indeed, in the present study, this gender effect resulted in males attaining higher breast weight than female chickens. The observations are in agreement with Scheuermann *et al.* (2003) who reported that males were superior to females for body weight and breast weight. Genetic and gender-related variations in breast muscle yield of broiler chickens may be attributed to differences in number and size of muscle cells (myofibers) (Scheuermann *et al.*, 2003). However, other researchers have reported that the development of breast muscle in female chickens is faster than in male chickens of the same age. Thus the weight of the pectoralis muscle in females is higher than in males of the same age resulting in higher breast muscle yield in the former. Aberra *et al.* (2013) indicated that higher breast yield in the female chicken might be due to females approaching sexual maturity at the time of the measurement. However, in the current study the breast measurements were done seven weeks before the chicken's estimated sexual maturity and this could explain why males had higher breast meat yield than females. In female chickens, diets diluted with MOLM resulted in higher carcass traits compared to the control. However, in male chickens the response was the similar in all diets.

The positive response of chickens to the dilution of a commercial broiler finisher diet with MOLM reported in this study confirms that MOLM can be used as a source of feed for extensively-reared chickens without any negative effects on growth performance and carcass characteristics. The utility of MOLM for this purpose is supported by Kakengi *et al.* (2003), who observed high pepsin and total soluble protein in *M. oleifera* leaf meal, which makes it suitable dietary protein source for simple non-ruminant animals.

4.7 Conclusion

Extensively–reared chickens have the ability to utilise different levels of forages and convert it efficiently into body mass. All the three chicken strains investigated in this study can utilise MOLM efficiently at different levels. The inclusion of MOLM improved the growth performance and carcass characteristics of chickens and these findings indicate that MOLM can be used as a potential feed resource for poultry. Feed intake peaked at dietary MOLM levels between 50 and 70 g/kg suggesting that MOLM can be included at these levels to reduce feed costs and improve performance and carcass characteristics. However, the effect of MOLM on haematological, biochemical indices and toxicology should be explored to ensure product safety for consumers.

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5 EFFECT OF FEEDING *MORINGA OLEIFERA* LEAF MEAL ON WEIGHT OF INTERNAL ORGANS, HAEMATOLOGICAL PARAMETERS, AND SERUM BIOCHEMICAL INDICES IN THREE CHICKEN STRAINS

5.1 Abstract

Continuous supplementation of chicken diets with plant-based alternative feed resources, such as *Moringa oleifera*, has the potential to modify the birds' anatomy and physiology. A 90-day feeding trial was conducted to determine the effect of *M. oleifera* leaf meal (MOLM) supplementation on weight of internal organs, haematological parameters and serum biochemical indices in three chicken strains. The leaf meal was used to dilute a commercial broiler finisher diet at 0 (MOLM0), 25 (MOLM25), 50 (MOLM50), and 100 (MOLM100) g/kg DM, producing four isoenergetic and isonitrogenous dietary treatments. Two hundred and sixteen Potchefstroom Koekoek (PK), Ovambo (OV) and Black Australorp (BA) chickens were raised on a commercial starter mash for three weeks. On the fourth week, experimental diets were offered until 13 weeks of age. At thirteen weeks of age, blood samples were taken from 6 chickens (3 males and 3 females) per treatment and used for biochemical and haematological analysis. Also, at 13 weeks of age, all chickens were electrically stunned and slaughtered by manual exsanguination. Higher inclusion levels of MOLM resulted in longer small intestines and larger gizzards. Male BA chickens on MOLM0 diet had the least red blood cell (RBC) and haematocrit counts compared to other diets. When offered MOLM50, female OV chicken strain had lower aspartate transaminase (AST) and alkaline phosphate (ALKP) (156.9 U/L) compared to BA and PK chicken strains. Incremental levels of MOLM resulted in higher total protein (TP) in female chickens. In male chickens low levels of alanine transaminase (ALT) were observed when offered MOLM50 (10.0 U/L) and MOLM100 (11.0 U/L). It was concluded that inclusion of MOLM at levels up to 10 g/kg had no adverse effect on the health and nutritional status of chickens.

Keywords: gastrointestinal tract, haematology, indigenous chickens, internal organs, *Moringa oleifera* leaf meal, serum biochemistry

5.2 Introduction

Extensively-reared chickens contribute more than 50% of the total eggs and meat consumed by people living in rural areas of South Africa (Mukherjee, 1992). As such, the contribution of these chickens to food and nutrition security in resource-poor communities is unequivocal. However, their productivity lags behind that of the genetically improved strains used to provide meat and eggs in commercial production enterprises. The result is that intensive production of indigenous chicken strains remains an unattractive option for many smallscale farmers since the returns are relatively lower. The major stumbling block is the cost of commercial feeds, which are required in larger quantities for indigenous chickens whose growth rates are significantly lower than in improved chicken strains. A possible solution is the use of non-conventional feedstuffs as alternatives or supplements to the commercial diets. Locally available, plant-based non-conventional feedstuffs represent a cheaper. One such plant, being grown on a large scale in South Africa, is *Moringa oleifera*.

The leaves of *M. oleifera* can be used to make a leaf meal (MOLM) that may have potential as a low-cost feed supplement. *M. oleifera* is a good source of vitamins and amino acids (Olugbemi *et al.*, 2010). The plant boosts the immune system in broilers (Jayavardhanan *et al.*, 1994; Fuglier 1999; Olugbemi *et al.*, 2010). In evaluating the nutritive value of non-conventional feed resources such as *M. oleifera*, it is also important to assess the anatomical, physiological and health effects that such feed resources may have on the target animal. Several factors, such as nutrition, age, gender, breed, health and physiological status, may influence the normal blood values of various species (Jain, 1993). Esonu *et al.* (2001) reported that haematological constituents reflect the physiological responsiveness of an animal to its internal and external environments, which include feed and feeding. Nickon *et al* (2008) reported that *M. oleifera* extract has antibacterial properties and antifungal activities. The extract is also said to have hypotensive (Naznin *et al.* 2008), hypoglycemic and hypocholesterolemic (Dangi *et al.*

2002; Ghasi *et al.* 2000; Naznin *et al.* 2008), anti-inflammatory, anti-hepatotoxic, and anti-helminthic properties (Nikkon *et al.* 2003). *Moringa oleifera* leaf meal contains iron (23 mg/100g), which is necessary for many functions in the body including the formation of haemoglobin and myoglobin. The anti-nutritional compounds present in *M. oleifera* leaf may have detrimental effect on blood parameters and liver function in chickens. Liver enzymes are found in the hepatocytes where they carry out different functions ranging from metabolism, detoxification, synthesis and regulation. Transaminases or amino transferases, alanine transferase (ALT), aspartate transferase (AST) and alkaline phosphatase are membrane bound enzymes whose concentration in blood indicates the health status of liver cells (Bruraimoh *et al.* 2011). Therefore, alteration of these compounds due to feeding alternative diets may be indicative of change in health status.

For a comprehensive nutritional assessment of MOLM in chickens, it is imperative that its anatomical and physiological effects be evaluated. There is a dearth of information on haematological, electrolyte and serum biochemical parameters in domestic indigenous chicken strains. This study was, therefore, designed to examine the haematological and serum biochemical indices of one improved (Black Australorp) and two indigenous (Potchefstroom Koekoek, and Ovambo) chicken strains, supplemented with incremental levels of *M. oleifera* leaf meal.

5.3 Materials and methods

The study site, chicken strains, diet formulation and experimental design of the feeding trial are as described in Chapter 4.

5.4 Blood collection and analysis

At the end of the 13-week feeding trial, blood samples were collected from all 6 birds (3 males and 3 females) in each feeding trial replicate. Bleeding was done from a punctured wing vein

with a 5 ml scalp vein needle set. About 2 ml of blood was collected from each bird into two sets of sterilised bottles, one containing ethylene diamine tetra acetic acid (EDTA) as the anti–coagulant. Haematological parameters (haemoglobin concentration (Hb), red blood cells count (RBC), white blood cellscount (WBC), haematocrit (Hct), mean corpuscular volume (MCV), and mean corpuscular haemoglobin (MCH) were determined using an automated Idexx Laser Cyte Haematology (IDEXX Laboratories, Inc) and the values were recorded in g/100 ml (WHO, 1980). The mean corpuscular haemoglobin concentration (MCHC) was calculated as:

$MCHC = \frac{MCH}{MCV}$, where MCH is mean corpuscular haemoglobin and MCV is the mean corpuscular volume. Clotted blood (collected in red top tubes) was centrifuged in a macro centrifuge to generate serum for biochemical analysis. Total protein (TP), urea, creatinine, albumin, serum cholesterol, aspartate transaminase (AST), alanine transaminase (ALT), alkaline phosphate (ALKP) were analysed using an automated Idexx Vet Test Chemistry Analyser (IDEXX Laboratories, Inc).

5.5 Internal organs

At 13 weeks of age, all chickens were electrically stunned and slaughtered by manual exsanguination. Weights of the liver, gizzard (cleaned), heart, lungs, and pancreas as well as the length of small intestines were determined using a sensitive weighing balance and measuring tape (cm), respectively.

5.6 Histological procedures and analysis

Histological assessment was done as described by Saalu *et al.* (2008). Briefly, the liver was cut on slabs about 0.5cm thick and fixed in 10% formal saline for a day after which they were transferred to 70% alcohol for dehydration. The tissues were passed through 90% alcohol and chloroform for different durations before they were transferred into two chambers of molten paraffin wax for 20 minutes. Serial sections of 5μm thick were obtained from a solid block of

tissue and were stained with haematoxylin and eosin stains, after which they were passed through a mixture of equal concentration of xylene and alcohol. Photomicrographs were taken with a JVC colour video digital camera (JVC, China) mounted on an Olympus light microscope (Olympus UK Ltd, Essex, UK).

5.7 Statistical analysis

Data were statistically analysed separately for male and female chickens since gender differences in terms of haematology and biochemical indices are well established in literature (Peters *et al.*, 2011; Addass *et al.*, 2012). Thus, for each gender, the experiment took the form of a 3 (chicken strains) x 4 (experimental diets) factorial treatment arrangement in a completely randomized design. Variation in organ size, haematological and serum biochemical indices data was analysed using SAS (2008) software according to the following general linear model:

$$Y_{ijk} = \mu + S_i\ (i = 1-2) + D_j\ (j = 1-5) + (S \times D)_{ij} + E_{ijk}$$

Where Y_{ijk} = dependent variable (organ size, haematological and serum biochemical indices), μ = overall mean, S_i = effect of bird strain level i, D_j = effect of experimental diet level j, $(S \times D)_{ij}$ = interactive effect of bird strain and diet and E_{ijk} = random error, assumed to be normally and independently distributed. The level of significance was set at $P < 0.05$. For parameters where significant variation was detected, multiple comparisons of treatment means were carried out using the probability of difference (pdiff) option of the General Linear Models (GLM) procedures of SAS. Nonlinear regression analysis was used to determine the response relationship of organ size, haematological and serum biochemical indices to incremental levels of MOLM.

5.8 Results

5.8.1 Haematological parameters in female chicken strains

The interaction term 'diet × strain' significantly influenced ($P < 0.05$) haemoglobin (Hb), red blood cells (RBC), white blood cells (WBC), haematocrit (Hct), mean corpuscular volume (MCV), and mean corpuscular haemoglobin (MCH), lymphocytes, neutrophils, monocytes, eosinophils and basophils of female chicken strains (Table 5-1). In OV and PK strain, incremental level of MOLM had highest ($P<0.05$) RBC, Hb, MCH and MCHC compared to control diet. MOLM100 resulted in lower ($P<0.05$) RBC count compared to control diet (Table 5-2). No variation ($P>0.05$) was observed in Hct, Hb, MCH and MCHC of BA chicken strains when offered incremental level of MOLM. In the OV chicken strain, incremental level of MOLM had higher ($P<0.05$) WBC count than control diet (MOLM0). When offered incremental level of MOLM, OV and PK strain exhibited higher WBC count.

Table 5-1. Statistical significance (P values) of the effects of main factors on female haematological parameter of three strains (BA, OV, and PK) supplemented with four diets (MOLM0, MOLM 25, MOLM 50, and MOLM100)

Parameter	Diet	Strain	Diet × Strain
Red blood cell (10^{12}/L)	**	***	***
Haematocrit (%)	**	NS	***
Haemoglobin (g/L)	NS	*	**
Mean corpuscular volume (fl)	NS	***	**
Mean cell haemoglobin (fl)	***	*	***
Mean cell haemoglobin concentration (%)	*	*	**
White blood cell (10^{9}/L)	***	***	***
Lymphocytes (%)	NS	***	*
Neutrophils (%)	*	NS	*
Monocytes (%)	*	***	***
Eosinophils (%)	***	***	**
Basophils (%)	***	***	**

* P<0.05; ** P<0.01;*** P<0.001

NS – Not significant

No change (P>0.05) was observed in lymphocytes in BA and PK chicken strain across all diets, whilst OV strain had a lower (P<0.05) lymphocytes with incremental level of MOLM. In OV and PK strain, MOLM25 and MOLM50 had higher neutrophils than control diet, whilst BA strain had higher (P<0.05) neutrophils with MOLM incremental level. In PK and BA strains, incremental level of MOLM resulted in lower eosinophils compared MOLM0. Potchefstroom Koekoek strain had the highest (P<0.05) RBC (2.9 x 10^{12}/L), Hb (3.2 g/L) MCH (37.3 pg), MCHC (36.4 %) but lower eosinophils (2.84 %) than OV and BA chicken strains. No difference (P>0.05) was observed in Hct and neutrophils in all chicken strains.

Table 5-2. Haematological parameters in 13-week old Ovambo (OV), Potchefstroom Koekoek (PK), and Black Australorp (BA) female chickens fed incremental levels of *Moringa oleifera* leaf meal (MOLM).

Parameter	Strain	MOLM0	MOLM25	MOLM50	MOLM100	SE
RBC(10^{12}/L)	OV	2.7^{bA}	2.8^{aB}	2.8^{aA}	2.8^{aA}	0.049
	PK	2.5^{bC}	2.9^{aA}	2.9^{aB}	3.2^{aA}	
	BA	2.7^{abB}	2.9^{aA}	2.5^{bB}	2.6^{cB}	
Haematocrit (%)	OV	27.6^{A}	26.9^{B}	26.3^{B}	27.0^{B}	0.498
	PK	25.2^{cB}	26.5^{bcB}	27.7^{aA}	29.9^{aA}	
	BA	25.9^{bB}	29.5^{aA}	26.4^{baB}	27.1^{bB}	
Haemoglobin (g/L)	OV	8.4^{b}	6.5^{Cc}	9.7^{ab}	10.2^{a}	0.576
	PK	8.9^{b}	11.1^{aA}	9.8^{ab}	9.9^{ab}	
	BA	9.2	8.5^{B}	9.1	8.2	
MCV(fl)	OV	99.3	98.0^{A}	99.0^{B}	98.0^{BC}	1.544
	PK	99.2^{a}	92.2^{bB}	92.3^{bC}	93.8^{bC}	
	BA	96.8^{b}	101.6^{aA}	104.2^{aA}	106.2^{aA}	
MCH(pg)	OV	36.7^{bB}	24.3^{bC}	37.9^{aB}	41.3^{aA}	0.990
	PK	43.8^{aA}	38.9^{aA}	32.7^{bC}	34.0^{bC}	
	BA	35.1^{bB}	38.3^{aA}	33.5^{bB}	34.8^{bB}	
MCHC (%)	OV	36.9^{a}	24.1^{bB}	36.8^{a}	30.9^{a}	2.055
	PK	35.3^{b}	34.1^{aA}	35.3^{b}	33.0^{b}	
	BA	35.5^{a}	28.6^{bB}	34.3^{ab}	30.2^{ab}	
WBC(10^{9}/L)	OV	10.8^{bA}	11.9^{aA}	11.6^{aA}	11.6^{aA}	0.185
	PK	6.8^{bC}	5.1^{cC}	6.8^{bC}	10.9^{aB}	
	BA	9.3^{aB}	6.5^{cB}	7.5^{bB}	6.9^{bcC}	
Lymphocytes (%)	OV	65.6^{aA}	67.9^{aA}	47.0^{b}	54.4^{bA}	2.942
	PK	$44.^{6B}$	42.2^{C}	46.9	44.9^{B}	
	BA	51.2^{B}	53.5^{B}	51.5	54.6^{A}	
Neutrophils (%)	OV	15.8^{cB}	45.7^{aA}	32.8^{abAB}	22.5^{cB}	5.551
	PK	33.6^{abA}	46.0^{aA}	24.3^{bB}	25.3^{bB}	
	BA	23.5^{bAB}	27.3^{bB}	45.1^{aA}	33.4^{abA}	
Monocytes (%)	OV	13.9^{aA}	11.3^{bA}	12.5^{bA}	10.6^{bA}	0.440
	PK	9.8^{aB}	10.2^{aA}	8.2^{bB}	7.0^{bB}	
	BA	8.0^{bC}	8.3^{bB}	7.7^{bB}	10.1^{aA}	
Eosinophils (%)	OV	4.5^{abB}	3.8^{bA}	3.8^{bA}	4.7^{aA}	0.249
	PK	3.6^{aC}	3.1^{abAB}	2.5^{bcB}	2.2^{cC}	
	BA	5.4^{aA}	2.8^{bB}	2.6^{bB}	3.2^{bB}	
Basophils (%)	OV	1.0^{aB}	0.6^{bA}	0.9^{aA}	1.0^{aA}	0.038
	PK	1.8^{aA}	0.7^{bA}	0.7^{bB}	0.7^{bB}	
	BA	1.8^{aA}	0.55^{bB}	0.6^{bC}	0.7^{aB}	

[ab]In a row, lowercase superscripts compare strains within diet ,AB In column, uppercase superscripts compare diets within strains (P<0.05); 1Diet: MOLM0 = broiler finisher without MOLM inclusion; MOLM25 = broiler finisher diluted at 25 g/kg MOLM MOLM50 = broiler finisher diluted at 50 g/kg MOLM; MOLM100 = broiler finisher diluted at 100 g/kg MOLM.

Black Australorp had lower lymphocytes (52.7 %), eosinophils (3.47 %), basophils (0.64 %) and higher (P<0.05) MCV (102.2 fl) than PK and OV strains. Ovambo strain had higher WBC count (11.5 x 10^9/L), monocytes (12.1 %), eosinophils (4.2 %) and basophils (0.89 %) than PK and BA strains.

5.8.2 Haematological parameters in male chicken strains

The interaction term 'diet × strain' did not (P > 0.05) affect RBC, Hct, Hb and MCHC in male chickens but significantly influenced MCH, WBC, lymphocytes, neutrophils, monocytes, eosinophils and basophils (Table 5-3)

Table 5-3. Statistical significance (P values) of the effects of main factors on male haematological parameter of three strains (BA, OV, and PK) supplemented with four diets (MOLM0, MOLM 25, MOLM 50, and MOLM100)

Parameter	Diet	Strain	Diet × Strain
Red blood cell (10^{12}/L)	NS	NS	NS
Haematocrit (%)	NS	NS	NS
Haemoglobin (g/L)	NS	NS	NS
Mean corpuscular volume (fl)	NS	NS	*
Mean cell haemoglobin (pg)	NS	*	*
Mean cell haemoglobin concentration (%)	NS	*	NS
White blood cell (%)	***	***	***
Lymphocytes (%)	**	***	***
Neutrophils (%)	NS	**	NS
Monocytes (%)	**	***	**
Eosiphils (%)	***	NS	***
Basophils (%)	**	***	*

* P<0.05; ** P<0.01;*** P<0.001

NS – Not significant

Incremental level of MOLM resulted in higher (P<0.05) MCH in OV and PK chicken strain, whist BA strain had lower (P<0.05) MCH when offered incremental level of MOLM (Table 5-4). In OV strain, incremental level of MOLM resulted in higher WBC count than control diet (MOLM0). When offered MOLM incremental level, PK and OV strains exhibited in lower lymphocytes compared to MOLM0 whilst no difference (P>0.05) was observed in BA chicken strain.

Table 5-4. Haematological parameters in 13-week old Ovambo (OV), Potchefstroom Koekoek (PK), and Black Australorp (BA) male chickens fed incremental levels of *Moringa oleifera* leaf meal (MOLM).

Parameter	Strain	MOLM0	MOLM25	MOLM50	MOLM100	SE
RBC(10^{12}/L)	OV	2.89^{A}	2.79^{A}	2.92^{A}	3.05^{A}	0.119
	PK	2.89^{A}	2.72^{A}	2.90^{A}	2.90^{A}	
	BA	2.76^{B}	2.79^{A}	2.65^{B}	2.72^{B}	
Haematocrit (%)	OV	27.9	27.4	28.4^{AB}	28.2	0.985
	PK	27.4^{ab}	26.5^{b}	31.2^{aA}	27.7^{ab}	
	BA	26.7^{a}	26.5^{a}	25.8^{bB}	27.5^{a}	
Haemoglobin (g/L)	OV	8.7^{bB}	10.4^{a}	8.4^{bB}	9.2^{abB}	0.637
	PK	8.5^{B}	9.0	8.7^{B}	9.4^{B}	
	BA	10.3^{aA}	9.0^{a}	10.6^{aA}	10.4^{aA}	
MCV (fl)	OV	95.5^{aAB}	98.2^{aA}	97.4^{aA}	92.5^{bB}	1.513
	PK	94.8^{bA}	97.4^{aA}	94.6^{bA}	95.6^{abA}	
	BA	96.7^{aAB}	94.3^{aB}	$97.2a^{AB}$	101.2^{aA}	
MCH (pg)	OV	29.4^{bB}	37.8^{a}	30.5^{bB}	36.6^{aA}	2.521
	PK	29.4^{bB}	36.3^{a}	36.8^{aA}	34.2^{abA}	
	BA	42.5^{aA}	35.2^{b}	34.5^{bAB}	35.3^{bA}	
MCHC (%)	OV	31.0^{aB}	37.9^{aA}	29.5^{bB}	32.8^{abB}	2.537
	PK	30.9^{aB}	33.9^{aB}	27.9^{bB}	33.8^{aB}	
	BA	38.5^{aA}	33.9^{bB}	40.9^{aA}	38.0^{aA}	
WBC (10^{9}/L)	OV	6.4^{cB}	7.3^{bcB}	8.9^{bB}	10.7^{a}	0.220
	PK	9.3^{A}	10.5^{A}	10.9^{A}	11.3	
	BA	9.8^{A}	10.6^{A}	10.9^{A}	11.3	
Lymphocytes (%)	OV	66.1^{abA}	70.5^{aA}	44.6^{cB}	61.7^{bA}	1.999
	PK	50.2^{aB}	49.2^{aB}	46.9^{bB}	44.9^{bB}	
	BA	52.4^{abB}	49.2^{bB}	54.1^{abA}	56.9^{aA}	
Neutrophils (%)	OV	12.7^{aB}	13.8^{aC}	11.1^{cB}	17.8^{bC}	5.160
	PK	26.0^{bA}	20.1^{bB}	34.1^{abA}	40.6^{aA}	
	BA	27.1^{aA}	25.9^{aA}	32.8^{aA}	27.9^{aB}	
Monocytes (%)	OV	13.4^{aA}	11.5^{bA}	10.6^{bcA}	9.1^{cA}	0.564
	PK	10.4^{aB}	9.1^{aB}	9.8^{aA}	7.0^{bB}	
	BA	8.1^{bC}	9.1^{abB}	9.9^{aA}	9.3^{abA}	
Eosophils (%)	OV	0.6^{bB}	4.2^{aA}	0.7^{bB}	4.7^{aA}	0.242
	PK	1.9^{bA}	3.1^{aB}	2.4^{abA}	1.9^{bC}	
	BA	1.7^{bA}	2.3^{abC}	2.5^{aA}	2.8^{aA}	
Basophils (%)	OV	1.0^{aA}	0.9^{aA}	0.8^{bA}	0.7^{bA}	0.043
	PK	0.6^{aC}	0.6^{aB}	0.6^{aB}	0.4^{bB}	
	BA	0.9^{aB}	0.7^{bB}	0.7^{bAB}	0.8^{abA}	

[ab]In a row, lowercase superscripts compare strains within diet

[AB] In column, uppercase superscripts compare diets within strains ($P<0.05$);

[1]Diet: MOLM0 = broiler finisher without MOLM inclusion; MOLM25 = broiler finisher diluted at 25 g/kg MOLM MOLM50 = broiler finisher diluted at 50 g/kg MOLM; MOLM100 = broiler finisher diluted at 100 g/kg MOLM.

Lower (P<0.05) neutrophils were observed with MOLM incremental level in OV strain. Highest MOLM inclusion MOLM100 had higher eosinophils on both OV and BA strains compared to MOLM0. Black Australorp had lower (P<0.05) RBC (2.7x 10^{12}/L) and higher Hbg (10.1 fl), MCH (37.1 pg), MCHC (37.9 %), and MCV (97.4 fl) compared to OV and PK chicken strains. Higher RBC count was observed in PK and OV strain (2.95 and 2.90 x 10^{12}/L, respectively) than BA strain. Potchefstroom Koekoek had higher Hct (28.2), lymphocyets (47.8 %) and basophils (0.54 %). OV had lower WBC count than BA and OV strains. Overall, OV chickens had lower (P<0.05) neutrophils compared to BA and PK chickens.

5.8.3 Blood chemistry in female chicken strains

The interaction term 'diet × strain' did not (P > 0.05) affect female cholesterol but significantly influenced all the other serum biochemical indices (Table 5-5).

Table 5-5. Statistical significance (P values) of the effects of main factors on three strains (BA, OV, and PK) supplemented with four diets (MOLM0, MOLM 25, MOLM 50, and MOLM100)

Parameter	Diet	Strain	Diet × Strain
Urea (mmol/L)	*	NS	***
Creatinine (µmol/L)	***	***	***
Uric (µmol/L)	***	***	***
Total protein (g/L)	***	***	***
Albumin (g/L)	***	***	***
Globulin (g/L)	NS	***	**
Cholesterol (mmol/L)	NS	NS	NS
ALT (U/L)	***	***	***
AST (U/L)	***	***	***
ALKP (U/L)	***	***	***

* P<0.05; ** P<0.01;*** P<0.001

NS – Not significant

Incremental level of MOLM resulted in lower ($P<0.05$) urea and uric acid in all chicken strains (Table 5-6). Creatinine increased ($P<0.05$) with MOLM incremental level across all strains. In all strain, incremental level resulted in higher albumin and globulin level than MOLM0. When offered MOLM incremental level, all chicken strains resulted in lower ($P<0.05$) ALT, AST and ALKP concentration compared to MOLM0. Black Australorp strain had lower uric acid (279.0 µmol/L), AST (127.3 U/L) and higher ($P<0.05$) total protein (43.1 g/L), albumin (13.3 g/L) and globulin (29.88 g/L) than OV and PK chicken strains. Potchefstroom Koekoek had lower creatinine and ALKP than other strains. Ovambo strain had higher urea (0.49 mmol/L) and ALKP (143.8 U/L) level than other strains. No variation ($P>0.05$) in cholesterol was observed across all strains.

Table 5-6. Serum biochemical indices in 13-week old Ovambo (OV), Potchefstroom Koekoek (PK), and Black Australorp (BA) female chickens fed incremental levels of *Moringa oleifera* leaf meal (MOLM).

Parameter	Strain	MOLM0	MOLM25	MOLM50	MOLM100	SE
Urea (mmol/L)	OV	0.50^{aB}	0.45^{bA}	0.50^{aA}	0.50^{a}	0.014
	PK	0.50^{aA}	0.40^{cB}	0.40^{cB}	0.50^{a}	
	BA	0.40^{bC}	0.50^{aA}	0.50^{aA}	0.50^{a}	
Creatinine (µmol/L)	OV	10.0^{c}	12.0^{aA}	11.0^{bB}	12.0^{a}	0.144
	PK	10.0^{c}	12.0^{aA}	11.0^{bB}	12.0^{a}	
	BA	10.0^{c}	10.5^{bB}	12.0^{aA}	12.0^{a}	
Uric (µmol/L)	OV	311.0^{aA}	279.7^{bB}	277.5^{bA}	276.0^{bB}	1.12
	PK	300.2^{bB}	306.0^{aA}	258.9^{dB}	290.4^{cA}	
	BA	310.0^{aA}	270.0^{cC}	257.0^{dB}	279.0^{bB}	
Total protein (g/L)	OV	40.0	39.0^{B}	40.0^{B}	39.0^{B}	0.59
	PK	41.0	39.0^{B}	40.0^{B}	39.0^{B}	
	BA	41.50^{b}	41.0^{bA}	48.0^{aA}	42.0^{bA}	
Albumin (g/L)	OV	11.5^{b}	12.0^{b}	13.0^{aA}	13.0^{a}	0.204
	PK	11.5^{b}	12.0^{b}	13.0^{aA}	13.0^{a}	
	BA	12.0^{b}	12.0^{b}	12.0^{bB}	13.0^{a}	
Globulin (g/L)	OV	28.5^{a}	27.0^{bB}	27.0^{bB}	28.0^{ab}	0.479
	PK	28.5^{a}	27.0^{bB}	27.0^{bB}	28.0^{ab}	
	BA	29.5^{b}	29.0^{bA}	32.0^{aA}	31.0^{a}	
Cholesterol (mmol/L)	OV	2.74	3.09^{A}	2.63	2.99	0.239
	PK	2.72	2.28^{B}	2.77	2.49	
	BA	2.25^{b}	2.54^{aB}	2.99^{a}	2.26^{a}	
ALT (U/L)	OV	12.15^{aB}	11.4^{b}	10.26^{cAB}	10.0^{cB}	0.199
	PK	13.6^{aA}	11.0^{b}	10.65^{bA}	13.0^{aA}	
	BA	13.0^{aA}	11.0^{b}	10.0^{cB}	10.0^{cB}	
AST (U/L)	OV	133.0^{aB}	130.9^{bB}	127.0^{dB}	130.5^{cA}	0.138
	PK	131.6^{bB}	134.4^{aA}	130.2^{cA}	129.9^{cB}	
	BA	134.0^{aA}	129.0^{bC}	125.0^{cC}	121.0^{dC}	
ALKP (U/L)	OV	177.8^{aC}	170.2^{bA}	156.9^{cB}	169.1^{bA}	0.867
	PK	190.0^{aA}	143.0^{bB}	117.0^{dC}	125.0^{cC}	
	BA	180.4^{aB}	170.9^{bA}	160.8^{cA}	156.6^{dB}	

[ab]In a row, lowercase superscripts compare diets within strain ($P<0.05$)

[ABC]In a column, uppercase superscripts compare strains within diet ($P<0.05$);

[1]Diet: MOLM0 = broiler finisher without MOLM inclusion; MOLM25 = broiler finisher diluted at 25 g/kg MOLM MOLM50 = broiler finisher diluted at 50 g/kg MOLM; MOLM100 = broiler finisher diluted at 100 g/kg MOLM.

5.8.4 Blood chemistry parameters in male chicken strains

Diet x strain interaction did not ($P>0.05$) affect male albumin, ALT and cholesterol levels but significantly influenced all other serum biochemical indices (Table 5-7).

When offered MOLM incremental level BA strain resulted in lower ($P<0.05$) urea than MOLM0 whilst no variation ($P>0.05$) was observed in PK and OV chicken strains (Table 5.8). In all strains, incremental level MOLM resulted in higher creatinine content than MOLM0. Lower ($P<0.05$) uric acid level was observed with MOLM incremental level across all chicken strains.

Table 5-7. Statistical significance (P values) of the effects of main factors on male serum biochemical indices of three strains (BA, OV, and PK) supplemented with four diets (MOLM0, MOLM 25, MOLM 50, and MOLM100).

Parameter	Diet	Strain	Diet × Strain
Urea (mmol/L)	*	NS	**
Creatinine (μmol/L)	***	NS	***
Uric (μmol/L)	***	***	***
Total protein (g/L)	***	***	***
Albumin (g/L)	NS	NS	NS
Globulin (g/L)	NS	*	*
Cholesterol (mmol/L)	*	NS	NS
ALT (U/L)	NS	NS	NS
AST (U/L)	***	***	***
ALKP (U/L)	***	***	***

* P<0.05; ** P<0.01;*** P<0.001

NS – Not significant

In BA strain, MOLM incremental level resulted in higher total protein and albumin level compared to MOLM0. When offered MOLM incremental level, all chicken strains resulted in lower ($P<0.05$) ALT, AST and ALKP concentration compared to MOLM0. Black Australorp and PK strain had higher total protein (27.0 and 28.9 g/L), albumin (41.5 and 38.3 g/L) and globulin (12.5 and 11.5 g/L), respectively than PK strain. No variation ($P>0.05$) in creatinine, AST and ALT across all strains. Lower ($P<0.05$) level of urea was observed in OV strain than other strains.

Table 5-8. Serum biochemical indices in 13-week old Ovambo (OV), Potchefstroom Koekoek (PK), and Black Australorp (BA) male chickens fed incremental levels of *Moringa oleifera* leaf meal (MOLM).

Parameter	Strain	MOLM0	MOLM25	MOLM50	MOLM100	SE
Urea (mmol/L)	OV	0.4^{bB}	0.45^{ab}	0.50^{aA}	0.40^{bB}	
	PK	0.55^{aA}	0.45^{bc}	0.40^{cB}	0.50^{abA}	0.029
	BA	0.6^{aA}	0.50^{b}	0.45^{bcAB}	0.40^{cB}	
Creatinine (µmol/L)	OV	10.7^{dA}	14.5^{aB}	13.0^{c}	13.7^{bA}	
	PK	11.0^{bA}	10.2^{cC}	13.0^{a}	12.7^{aC}	0.127
	BA	10.0^{cB}	15.0^{aA}	13.0^{b}	13.3^{bB}	
Uric (µmol/L)	OV	275.0^{aA}	269.5^{bB}	269.0^{bA}	257.0^{cA}	
	PK	285.0^{aA}	277.7^{bA}	192.0^{dB}	207.0^{cC}	1.546
	BA	278.0^{aAB}	195.0^{cC}	195.0^{bB}	229.0^{bB}	
Total protein (g/L)	OV	41.0^{A}	35.5	40.5	36.0^{B}	
	PK	41.0^{A}	35.5	40.5	36.0^{B}	1.936
	BA	38.0^{bB}	40.0^{ab}	44.0^{a}	44.0^{aA}	
Albumin (g/L)	OV	12.5^{a}	10.5^{ab}	10.0^{bB}	10.5^{abB}	
	PK	12.5	10.5	12.5^{A}	10.5^{B}	0.69
	BA	11.0^{b}	11.0^{b}	14.0^{aA}	14.0^{aA}	
Globulin (g/L)	OV	28.5	25.5	25.5^{B}	26.0^{B}	
	PK	28.5	25.5	27.5^{AB}	26.0^{B}	1.137
	BA	27.0	28.0	30.0^{A}	30.0^{A}	
Cholesterol (mmol/L)	OV	2.85	3.18	3.10	2.76	
	PK	2.61	2.66	2.81	2.60	0.293
	BA	2.66	3.0	2.88	3.32	
ALT (U/L)	OV	12.4^{aB}	11.3^{bB}	10.85^{cA}	11.6^{bA}	
	PK	13.0^{aA}	11.0^{bB}	10.0^{cB}	11.0^{bB}	0.113
	BA	13.0^{aA}	12.4^{bA}	10.0^{dB}	11.0^{cB}	
AST (U/L)	OV	132.0^{aB}	129.0^{bC}	125.0^{cB}	121.2^{dB}	
	PK	134.8^{aA}	135.2^{aA}	130.0^{bA}	124.7^{cA}	0.256
	BA	131.2^{aC}	130.4^{aB}	125.7^{bB}	120.5^{cB}	
ALKP (U/L)	OV	185.2^{aA}	172.9^{bB}	152.7^{dB}	166.2^{cB}	
	PK	183.2^{aB}	174.9^{bA}	155.6^{dA}	166.7^{cB}	0.471
	BA	177.9^{aC}	169.7^{bC}	153.3^{cB}	170.5^{bA}	

[ab]In a row, lowercase superscripts compare strains within diet, [AB] In column, uppercase superscripts compare diets within strains ($P<0.05$); [1]Diet: MOLM0 = broiler finisher without MOLM inclusion; MOLM25 = broiler finisher diluted at 25 g/kg MOLM MOLM50 = broiler finisher diluted at 50 g/kg MOLM; MOLM100 = broiler finisher diluted at 100 g/kg MOLM.

5.8.5 Liver histology

Morphological patterns of the liver from 3 different strains are presented in Plates (1a – 3b). Macroscopic examination showed normal morphology of liver tissues in all chicken strains. The livers did not show any signs of cellular necrosis and fatty degeneration. In BA strain, livers from chickens offered MOLM100 showed hypertrophy of smooth muscle in arteries, which was not observed in the chickens on the control diet. Ovambo and PK chickens fed control diet (MOLM0) showed focal infiltration of mononuclear cells while BA fed MOLM0 showed a diffuse mononuclear infiltration. Hypertrophy of muscular bile duct, arterial endothelial cells and smooth muscle in artery was observed in OV strain offered MOLM0 and in BA strain offered MOLM100.

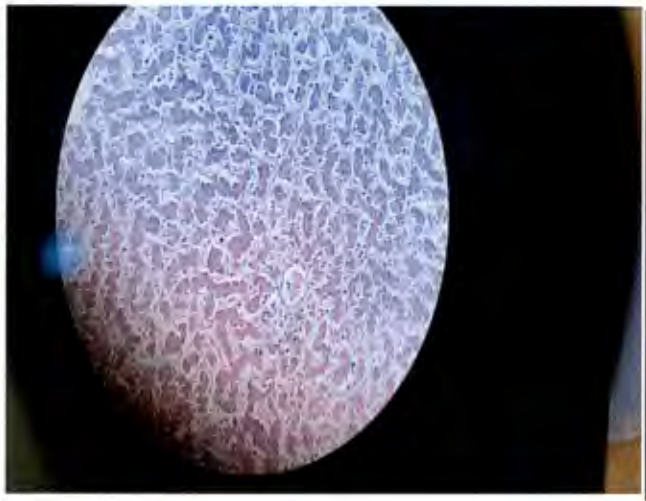

Plate 1a. MOLM0 (BA). Showing mononuclear diffuse infiltration and hepatocytes radiating out from the central vein.

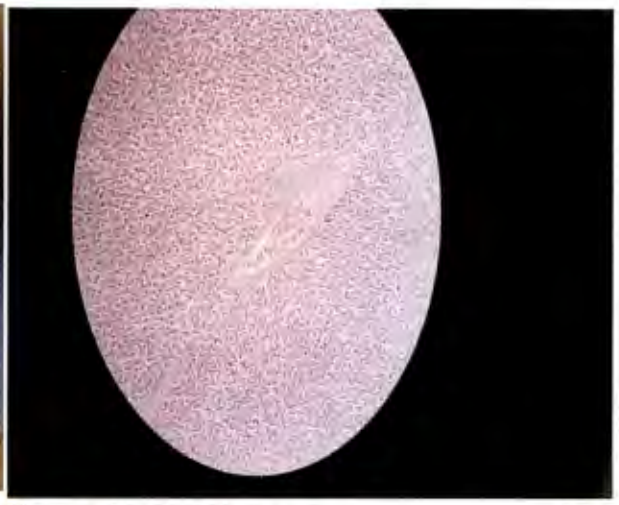

Plate 1b. MOLM100 (BA). Showing hypertrophy of the endothelial cells in artery, hypertrophy of smooth muscle in artery.

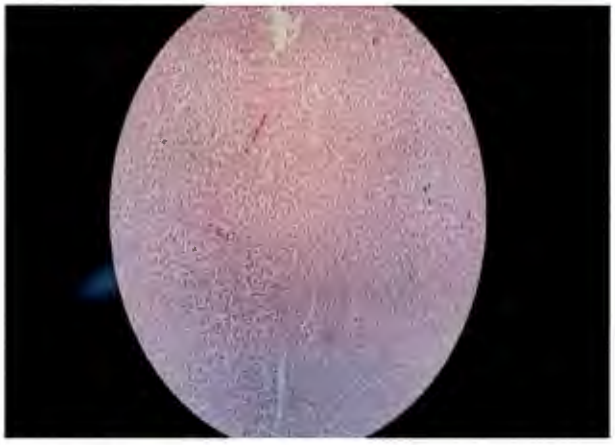

Plate 2a. MOLM0 (PK). Showing the connective tissue around the lobule, hepatocytes, central vein and portal tracts.

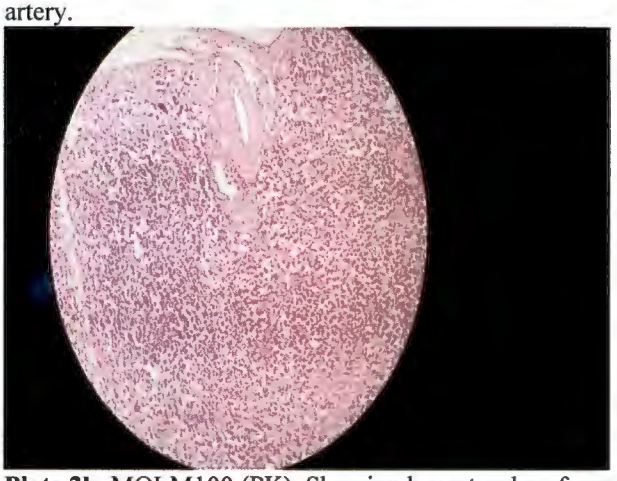

Plate 2b. MOLM100 (PK). Showing hypertrophy of smooth muscle in artery, focal infiltration of mononuclear.

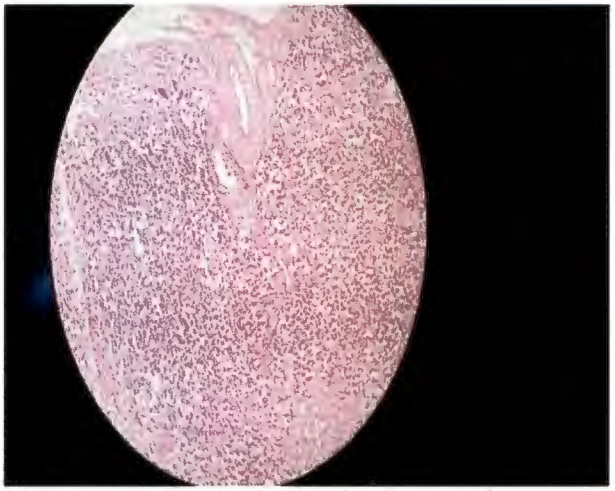

Plate 3a. MOLM0 (OV). Showing hypertrophy muscular bile duct, hypertrophy of the endothelial cells in artery, hypertrophy of smooth muscle in artery

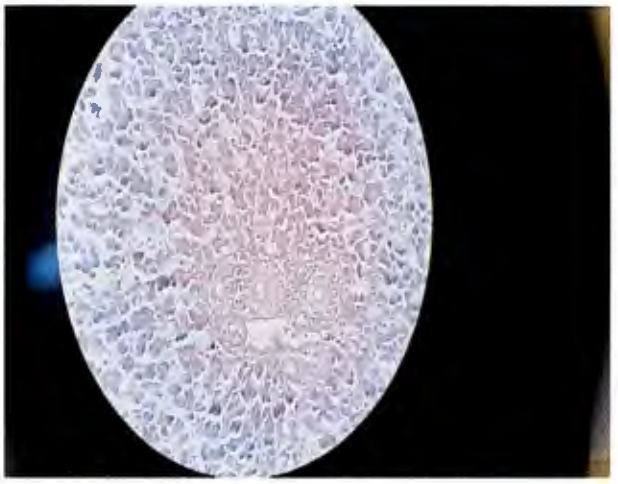

Plate 3b. MOLM100 (OV). Showing formation of hepatocytes

Figure 5-1. Effect of feeding *Moringa oleifera* leaf meal (0 and 100 g/kg) on liver morphology of three chicken strains.

5.8.6 Internal organs

In male chickens, the interaction term 'diet × strain' significantly influenced ($P < 0.05$) length of small intestines (Figure 5-2 A) and gizzard weight (Figure 5-2 B) , but not the size of the heart, liver and pancreas. Black Australorp (128.5 cm), OV (118.5 cm) and PK (111.5 cm) chickens fed MOLM0 had the shortest ($P<0.05$) intestinal length compared to other diets. Higher levels of MOLM resulted in longer intestinal length in all chicken strains.

Diet had no effect ($P>0.05$) on heart, liver and pancreas weights of three chicken strains. Diet MOLM25 resulted in lower gizzard weights in BA (30.5 g) and PK (32.5 g) chicken strains compared to other diets. Male chickens offered MOLM100 had the longest small intestines (144 cm) and highest gizzard weight (42.8 g). Similarly, in female chickens, birds offered MOLM100 had the longest small intestines (130.8 cm) and the highest gizzard weights (40.7 g). In female chickens, the interaction term 'diet × strain' significantly influenced ($P < 0.05$) liver weight only. Diet significantly affected ($P<0.05$) the size of the heart, liver, gizzard (Figure 5-3 A) and small intestine (Figure 5-3 B) in female chickens. Diet had no effect ($P>0.05$) on pancreas weights of BA, OV female chickens.

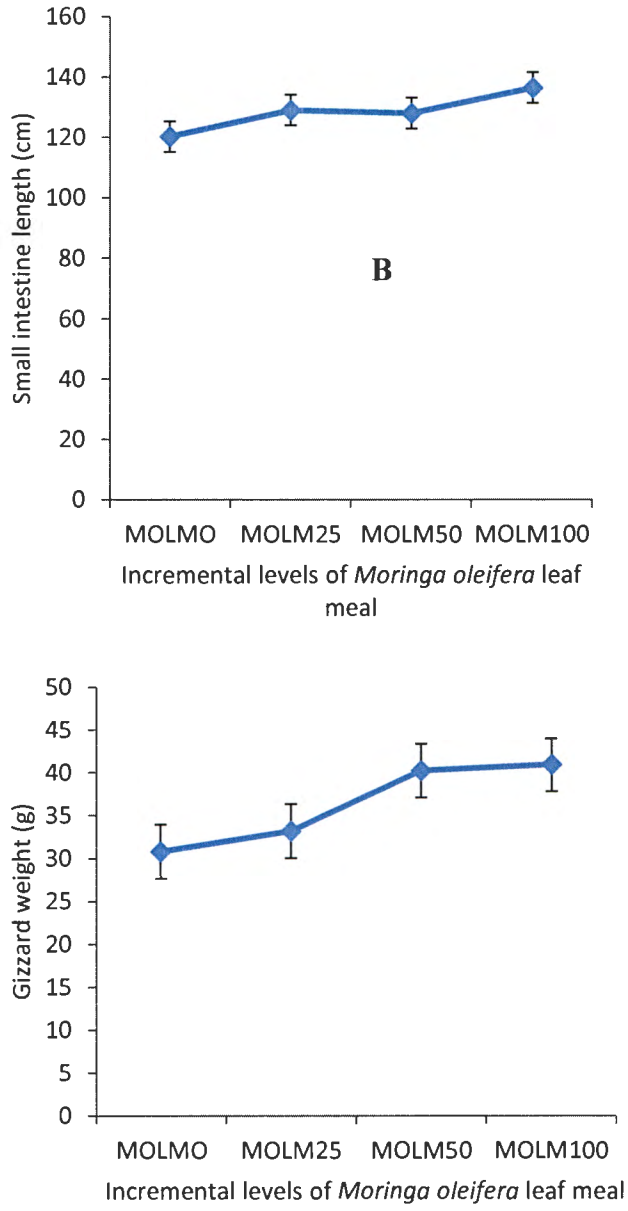

Figure 5-2. Effect of incremental levels of *Moringa oleifera* leaf meal on small intestine length (A) and gizzard weight (B) of male chickens. (MOLM0 = broiler finisher without MOLM inclusion; MOLM25 = broiler finisher diluted at 25 g/kg MOLM MOLM50 = broiler finisher diluted at 50 g/kg MOLM; MOLM100 = broiler finisher diluted at 100 g/kg MOLM).

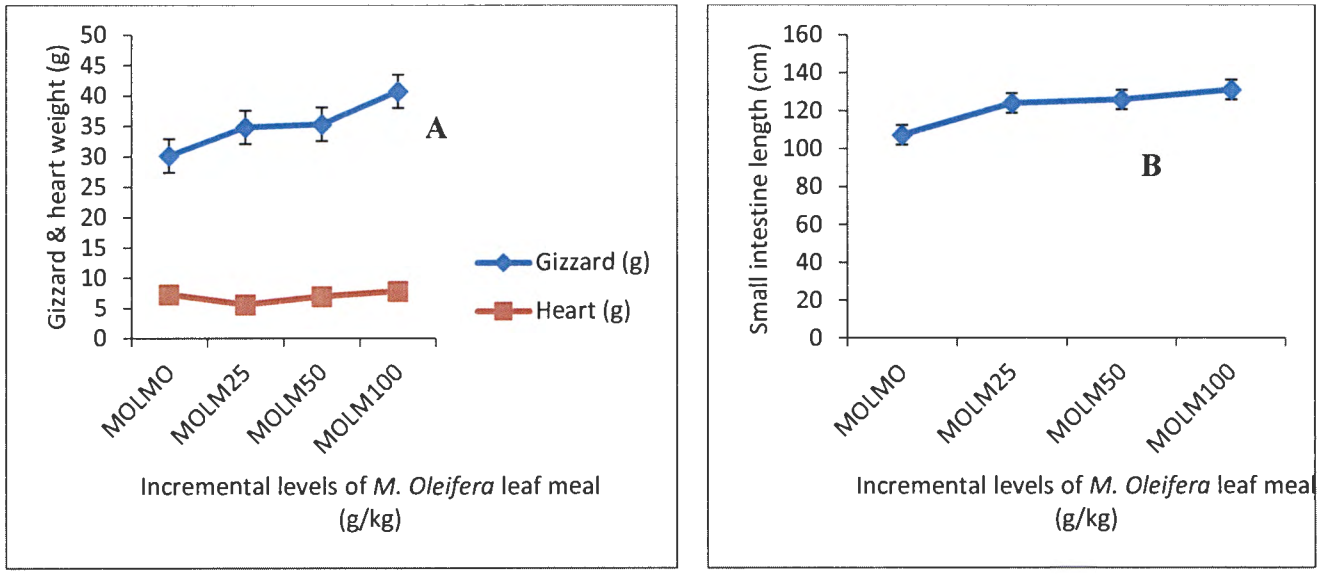

Figure 5-3. Effect of incremental levels of *Moringa oleifera* leaf meal on small intestine length (B), heart and gizzard weight (A) of female chickens. (MOLM0 = broiler finisher without MOLM inclusion; MOLM25 = broiler finisher diluted at 25 g/kg MOLM MOLM50 = broiler finisher diluted at 50 g/kg MOLM; MOLM100 = broiler finisher diluted at 100 g/kg MOLM).

5.9 Discussion

5.9.1 Haematological and biochemical indices of 3 strains of chickens

Haematological parameters are good indicators of the physiological status of animals (Khan & Zafar, 2005). Addass *et al.* (2012) reported that the majority of haematological parameters for indigenous chickens increase with advancing age with male chickens generally exhibiting higher values than female chickens. Haematological values of blood cells of the three different chicken strains were within the normal range reported for growing chickens (Jain, 1993). The differences between male and female haematological parameters have been fully established in literature (Peters *et al.*, 2011; Addass *et al.*, 2012). In addition Peters *et al.* (2011) reported that male chickens generally had higher mean values than females across all genotypes.

The results of the present study also reveal that haematological values vary among chicken strains. Different strains responded differently to incremental levels of MOLM. For instance, diet MOLM25 promoted higher RBC counts than other diets in female BA chickens. In males, BA strain had lower RBC counts while OV and PK strains had higher counts. Possibly this observation could be attributed to genetic variation of strains. Red blood cells (erythrocytes) serve as a carrier of haemoglobin. It is this haemoglobin that reacts with oxygen carried in the blood to form oxyhaemoglobin during respiration (Johnston & Morris 1996; Chineke *et al.*, 2006). Brown *et al.* (2000) opined that increased RBC values are associated with high quality dietary protein and with disease free animals.

In addition, this could be attributed to *Moringa oleifera* leaf meal protein content compared to control diet (Fuglie, 2005 & Oduro *et al.*, 2008). Incremental levels of MOLM resulted in elevated Hb in all chicken strains. Inclusion of *M. oleifera* leaf meal in chicken diets may have resulted in higher iron intake, which promotes synthesis of haemoglobin and increases

production of red blood cells. Indeed iron content of Moringa leaves from study 1 in this thesis was found to be 110.5 and 150.5 dpm for tender and mature leaves, respectively. Peters *et al.* (2011) reported variation in haematological parameters of Nigerian native chickens; normal-feathered birds had higher mean values compared to frizzled feather and native neck genotype. In both male and female chickens, the highest Hct counts were observed at higher inclusion levels of *M. oleifera* leaf meal. This observation could still be related to the higher dietary protein intake in chickens offered MOLM. Haemoglobin, hematocrit (PCV) and mean corpuscular haemoglobin concentration (MCHC) are very responsive to protein deficiency or low protein intake (Edozien & Switzer, 1977). Therefore, MOLM provided sufficient quality dietary protein which resulted in optimum concentration of blood constituents.

According to Isaac *et al.* (2013) haematocrit (Hct) is involved in the transport of oxygen and absorbed nutrients. In female chickens, the total WBC count was highest in OV and PK strain fed with higher levels of MOLM. Animals with low white blood cell count are at high risk of disease infection, while those with high counts are capable of generating antibodies and have high degree of resistance to diseases (Soetan *et al.*, 2013). The highest inclusion level of Moringa leaf meal (MOLM100) elevated WBC count, possibly due to the presence of antioxidants, which can improve the immune response of chickens and thus reduce mortality (Siddhuraju & Becker, 2003). In all strains incremental levels of MOLM caused a curvilinear response in total protein, albumin and globulin. Albumin functions as an osmotic pressure regulator and transport protein in birds while globulin transport nutrients to the muscles. Increased levels of MOLM in the diet significantly elevated the serum total protein, which is consistent with the findings of Teye *et al.* (2013) and Aberra *et al.* (2013).

Total serum protein has been reported as an indication of the protein retained in the animal body (Akinola and Abiola, 1991; Esonu *et al.*, 2001), while total blood protein and creatinine contents have been shown to depend on the quantity and quality of dietary protein ingested

(Eggum, 1970; Iyayi, 1998; Awosanya *et al.*, 1999; Esonu *et al.*, 2001). It is evident from the present findings that total serum protein differed among the strains. This is similar to the report of Ladokun *et al.* (2008) who reported a higher total serum protein in normally feathered than in naked neck chickens. Higher levels of MOLM promoted the highest creatinine concentration than the control. Serum creatinine concentrations are directly related to muscle volume and activity. Creatinine concentration was higher with incremental level of MOLM, and (Bishop *et al.*, 2005) established a direct relation between the amount of ingested protein and creatinine serum level. However, elevation of creatinine was highest within the normal ranges and control diet was at lower range within the normal ranges. Haematocrit values increased as the dietary MOLM ratio increased, possibly due to the additional amino acids in *Moringa oleifera* leaf, which may have increased the quantity and quality of dietary protein available to the birds. This is in agreement with the results of Edozien & Switzer (1977) who stated that haemoglobin, hematocrit (Hct) and mean corpuscular haemoglobin concentration (MCHC) are very responsive to protein intake.

The lowest level of blood urea was observed in female BA chickens at higher inclusion levels of MOLM. The same response was observed in all male chickens. The fact that higher levels of MOLM inclusion reduced blood urea concentration may be an indication of better absorption and efficient utilization of dietary protein compared to control diet. Possibly this observation could be stimulated by quality dietary protein in MOLM. Furthermore, digestion and absorption are essential parts of protein quality. The lowest values of ALT and AST were observed with high inclusion levels of MOLM in both male and female chicken strains. This indicates that MOLM had no toxic effect within the liver parenchyma of the birds. These results are in agreement with Olugbemi *et al.* (2010), who reported that *Moringa oleifera* leaves have no negative effect on the health of broilers. Instead they reported beneficial effects such as enhanced immune responses of the birds. Djuricic *et al.* (2011) also indicated that the higher

activities of these enzymes normally occur as the result of accelerated muscular tissue turnover. However, it was not significant to the current study with chickens fed MOLM. Cholesterol levels in chickens strains was not affected by MOLM inclusion, this affirms its potential as a hypocholesterolemic agent (Ghasi *et al.*, 2000). However, increase in AST may have resulted from handling or muscle injury during the collection of samples, which may have resulted in the leakage of intracellular AST into the blood. No signs of toxicity were observed in the liver of all chicken strains. The variation in photomicrographs is possibly due to tissues taken from different lobes. Changes in hypertrophy of the endothelial cells and smooth muscle maybe to changing blood pressure and blood flow (Julian (2007). However no signs of infection were observed in the current study. The current results are in agreement with the *in vitro* cytotoxicity study done by Mekonnen *et al.*, (2005), who reported that the aqueous extract of leaves from *M. oleifera* on hepatocytes did not affect cell viability.

5.9.2 Internal organs (GIT)

Birds respond quickly to changes in dietary fiber content as seen by changes in the intestinal length and weight of internal organs and probably due to change in the rate of passage through the different segments of the GIT (Mateos *et al.*, 2012). Male BA chickens had longer intestinal length as compared to OV and PK male chickens. This could be attributed to genetic variation between the strains. Diets MOLM25, MOLM50 and MOLM100 resulted in chickens with longer small intestine length than those offered the control diet (MOLM0) in both male and female chickens. This is in agreement with Borin *et al.*, (2005) and Rubio and Brenes (1988) who reported that high amounts of undigested materials in the digesta increased the lengths of the intestinal sections. Possibly due to stretching of the intestinal wall, following increased contents of digesta in the small intestine. However, these results contradict the findings by Sklan *et al.*, (2003) and Amerah *et al.* (2009) who observed a reduction in the length of small intestines with incremental levels of insoluble dietary fiber. Longer intestines are assumed to

digest feed efficiently and provide greater surface area for nutrient absorption. Chickens offered diet MOLM100 had larger gizzard weight compared to diets with lower proportions of MOLM. Musa *et al.* (2006) indicated that benefits of a larger gizzard include improved gut motility and improved digestibility of nutrients through effective grinding in the gizzard (Amerah *et al.*, 2007). In addition, these authors also reported that feed with high fiber content increased the gizzard weight. Fiber particles are, in general, harder to grind than other dietary components so they tend to accumulate in the gizzard (Hetland *et al.*, 2005) thus stimulating muscle development and functioning of this organ.

5.10 Conclusion

M. oleifera proved to have hepatoprotective influence and thus had favourable effects on some haematological, blood biochemical parameters and stimulated development and function of the gizzard and small intestine of male and female chicken strains. All chicken strains exhibited beneficial responses with higher inclusion level of MOLM. It is, therefore, concluded that MOLM can be used as a feed supplement for the investigated chicken strains without a risk of toxicity, compromised immunity or suboptimal nutritional supply.

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6 QUALITY PARAMETERS AND FATTY ACID COMPOSITION OF MEAT FROM THREE CHICKEN STRAINS FED *MORINGA OLEIFERA* LEAF MEAL-BASED DIETS

6.1 Abstract

The effect of *Moringa oleifera* leaf meal as a dietary supplement on quality and fatty acids profile of meat from three chicken strains was evaluated. *Moringa* leaves were harvested by hand, air-dried and milled into *M. oleifera* leaf meal (MOLM). The MOLM was used to dilute a commercial broiler finisher diet at 0 (MOLM0), 25 (MOLM25), 50 (MOLM50), and 100 (MOLM100) g/kg DM, producing four dietary treatments. Two hundred and sixteen Potchefstroom Koekoek (PK), Ovambo (OV) and Black Australorp (BA) chickens were raised on a commercial starter mash for three weeks. On the fourth week, experimental diets were offered until 13 weeks of age after which the chickens were slaughtered and the quality and fatty acid composition of the meat measured. The 3 way interaction (diet × strain × gender) term did not ($P>0.05$) affect redness (a*), yellowness (b*), pH, temperature or meat cooking loss, but significantly influenced ($P<0.001$) lightness (L*) and shear force. There was no effect ($P<0.05$) on meat pH across strains in all diets. In BA chickens, MOLM50 and MOLM100 resulted in higher ($P<0.05$) b* (21.42 and 19.92, respectively) compared to MOLM0 (15.62). In OV, b* increased in response to incremental levels of dietary MOLM. Diet MOLM50 resulted in lower shear force and lower cooking loss, which indicates good meat tenderness. However, inclusion of MOLM did not affect fatty acid profile of the meat. Chicken strains varied ($P<0.05$) in terms of their content of eicosatrienoic, docosahexanoic and palmitic acid. Meat from BA (0.789 %) had the highest ($P<0.05$) content of eicosatrienoic (C20:3C8, 11, 14 (n-6)) followed by PK (0.668) and OV (0.599) meat. It can be concluded that using *M. oleifera* leaf as feed supplement resulted in lower shear force and stabilised the fatty acid profile of the meat.

Keywords: *Moringa oleifera*, chicken strains, meat quality, fatty acids, leaf meal

6.2 Introduction

The consumption of chicken has steadily increased due to its low price and rare religious restrictions (Jaturasitha *et al.*, 2008). Due to its affordability, chicken meat is now by far the most consumed meat, eclipsing both beef and pork products. In rural communities of many developing nations, chicken meat is supplied from chicken strains that are adapted to extensive rearing. Most of these strains are native to these regions and their productivity tends to be lower than genetically improved exotic strains. The indigenous chickens are known for their tough, lean and tasty quality meat. However, because of their slower growth rates it is uneconomical to use commercially available feeds to boost productivity. It is, therefore, prudent to make use of inexpensive, locally available non-conventional feed resources. There are several success stories on the use of non-conventional feed resources to improve growth performance of chickens, although most of these are improved chicken strains. The nutritional evaluation of nonconventional feed resources, by necessity, must include their possible effect on the quality of the meat produced.

Moringa oleifera leaves are good source of nutrients for various types of chickens. *Moringa oleifera* leaves have high antioxidants content, fatty acids profile and nutritional value (Bennett *et al.*, 2003). Qwele *et al.* (2011) observed that broilers supplemented with *M. oleifera* leaves had the highest L* value. High L* values in meat are preferable because the lightness of broiler meat is more attractive and acceptable by consumers. Also, Karthivashan *et al.* (2015) observed meat tenderness in Cobb 500 broilers when offered 0.5 % MOLM. Meat colour is usually associated with factors such as breed (Ekiz *et al.*, 2010; Santos *et al.*, 2007; Muchenje *et al.*, 2009a; 2009b), slaughter weight (Martínez-Cerezo *et al.*, 2005), production system and pHu (Ekiz *et al.*, 2010). Chicken muscle that is enriched with polyunsaturated fatty acids may be

susceptible fat oxidation resulting in impaired organoleptic characteristics and decreased food shelf life (Mili´cevi´c et al. 2014). *Moringa oleifera* is rich in antioxidants, which diminish lipid oxidation and are utilized as growth promoters at sub-therapeutic levels and for treatment of poultry diseases. The use of natural antioxidants to stabilize meat has gained much attention from consumers because they are considered to be safer than synthetic antioxidants (Jung *et al.*, 2010) such as butylated hydroxytoluene (BHT) and tertiary butyl hydroquinone (TBHQ).

Increased chicken meat production and augmented interest of food-store chains to market standardized products are the reasons for making greater efforts to evaluate selected physical indicators, such as colour and tenderness of poultry meat (Abeni & Bergoglio, 2001). Meat quality is a term used to describe a range of attributes of meat. Consumer research suggests that tenderness is a very important element of eating quality and that variations in tenderness affect the decision to repurchase. Therefore, the aim of this study was to determine the meat fatty acid composition and quality parameters such as colour, cooking loss and pH, in three strains of chickens that are normally reared in extensive production systems when supplemented with *M. oleifera* leaf meal.

6.3 Materials and methods

The study site, chicken strains, diet formulation and experimental design of the feeding trial are as described in Chapter 4.

6.3.1 Cooking loss and pH changes in breast muscle

After slaughter, breast meat samples were cut from each bird using a knife and they were stored at 4°C before pH measurements were taken. The post-mortem pH was measured on the breast muscle of each bird 24 hours after slaughter using a portable digital pH meter (CRISON pH25, CRISON Instruments SA, Spain) with a piercing electrode.

The pH of cooked breast meat was measured after heat treatment in plastic bags in a water bath (82 °C), the core temperature of samples was kept at 80°C for 30 minutes. Samples were then cooled at room temperature and stored overnight at 4 ± 2 °C (Coró *et al.*, 2003). Cooking loss was calculated as the loss in sample weight after cooking, which was expressed as a proportion of the sample weight before cooking as follows:

$$\text{Cooking loss (\%)} = \frac{\text{Weight before cooking} - \text{Weight after cooking}}{\text{Weight before cooking}} \times 100$$

6.3.2 Meat colour measurement

Colour of the meat (L* = Lightness, a* = Redness and b* = Yellowness) was determined on breast meat, 24 hours after slaughter, using a colour-guide 45/0 BYK-Gardener GmbH machine with a 20 mm diameter measurement area and illuminant D65-day light, 10° standard observer. Three readings were taken by rotating the Colour Guide 90° between each measurement, to obtain a representative average value of the colour. The guide was calibrated before each day's measurements using the green standard.

6.3.3 Fatty acid profile determination

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

Conjugated linoleic acid (CLA) standards (cis-9, trans-11; cis-9, cis-11, trans-9, trans-11 and trans-10, cis-12isomers) were obtained from Sigma-Aldrich. Fatty acids were expressed as the proportion of each individual fatty acid to the total fatty acids present in the sample. The following fatty acid combinations and ratios were calculated: total saturated fatty acids (SFA), total mono-unsaturated fatty acids (MUFA), polyunsaturated fatty acids (PUFA), PUFA/SFA ratio (P/S) and n-6/n-3 ratio.

6.4 Statistical analysis

The experiment took the form of a 2 (male and female) x 3 (strains of indigenous birds) x 4 (experimental diets) factorial treatment arrangement in a completely randomized design. Variation in color, cooking loss, pH changes and fatty acids profile data was analysed using SAS (2007) software according to the following general linear model:

$$Y_{ijkl} = \mu + D_i + S_j + G_k + (D \times S)_{ij} + (D \times G)_{ik} + (S \times G)_{jk} + (D \times S \times G)_{ijk} + E_{ijkl}$$

where Y_{ijkl} = observation of the dependent variable *ijkl*, μ = fixed effect of population mean for the variable, D_i = effect of experimental diet (i = 4; MOLM0, MOLM25, MOLM50, and MOLM100), S_j = effect of chicken strain (j = 3; PK, OV and BA), G_k = effect of gender of chicken (k = 2; male and female), $(D \times S)_{ij}$ = effect of interaction between diet at level *i* and chicken strain at level *j*, $(D \times G)_{ik}$ = effect of interaction between diet at level *i* and gender at level *k*, $(S \times G)_{jk}$ = effect of interaction between chicken strain at level *j* and gender at level *k*, $(D \times S \times G)_{ijk}$ = effect of interaction between diet at level *i*, chicken strain at level *j* and gender at level *k*, and E_{ijk} = random error associated with observation *ijkl*. For all statistical tests, significance was declared at $P \leq 0.05$. Least squares means were compared using Tukey's HSD.

6.5 Results

6.5.1 Meat quality

The 3 way interaction (diet × strain × gender) did not (P>0.05) affect a*, b*, pH, temperature and meat cooking loss, but significantly influenced (P<0.001) L* and shear force (Table 6-1). In males and females, diet and strain interacted significantly to influenced meat lightness (L*) and shear force (Table 6-2). When fed the control diet, meat from female OV chickens had the lowest (P<0.05) L* value (48.278) compared to PK and BA female chickens. No variation (P>0.05) between strains was observed in L* when female chickens were offered either MOLM25 or MOLM100. Among male chickens fed MOLM0 and MOLM25, meat from the strain OV had higher L* compared to meat from BA and PK.

However, when fed MOLM50, meat from male BA chickens had the highest (P<0.05) L* value (54.64) compared to males from other strains. In BA strain, meat from males had lower L* (46.87) than female chickens when offered the control diet. In OV strain, meat from males had higher L* than females when offered MOLM25 but no gender variation was observed with other diets. In PK, meat from females had higher (P<0.05) L* value (55.59) than males when offered MOLM50. Female BA chicken strain had lower (P<0.05) shear force (45.88) at MOLM0 compared to other female chicken strains. At MOLM25 female BA chicken had higher shear force (60.72) compared to female PK and OV strains.

Table 6-1. Statistical significance (P values) of the effects of main factors on meat quality of three strains (BA, OV, and PK) supplemented with four diets (MOLM0, MOLM 25, MOLM 50, and MOLM100)

Parameter	Effect of treatment			Interaction			
	Strain (S)	Diet(D)	Gender(G)	D*G	B*G	D*B	D*B*S
Lightness (L*)	NS	*	NS	NS	***	*	***
Redness (a*)	**	NS	*	***	***	***	NS
Yellowness (b*)	*	***	*	NS	*	**	NS
pH	***	NS	NS	*	NS	NS	NS
Temperature	***	*	NS	*	NS	***	NS
Shear force	***	***	NS	NS	NS	***	***
Cooking loss	***	**	***	NS	NS	*	NS

* P<0.05; ** P<0.01;*** P<0.001

NS – Not significant

Table 6-2. Effect of feeding incremental levels of *M. oleifera* leaf meal on meat lightness (L*) and shear force of three chicken strains.

		BA		OV		PK	
Parameter	Gender	F	M	F	M	F	M
Lightness (L*)	MOLM0	50.78^{abAB}	46.87^{bC}	48.27^{b}	52.150^{aB}	53.06^{aABC}	48.79^{abD}
	MOLM25	51.94^{aAB}	50.22^{bB}	51.81^{a}	57.49^{aA}	53.37^{aAB}	49.17^{bCD}
	MOLM50	49.81^{bBC}	54.64^{aA}	51.09^{b}	49.99^{bB}	55.59^{aA}	50.26^{bBCD}
	MOLM100	52.33^{AB}	51.49^{AB}	48.59	52.03^{B}	52.37^{ABCD}	49.40^{aBCD}
Shear force (SF)	MOLM0	45.58^{bD}	80.65^{aAB}	63.02^{aC}	85.79^{aA}	56.54^{aBC}	84.65^{aA}
	MOLM25	60.72^{aC}	77.13^{aB}	50.22^{bD}	80.12^{aAB}	50.79^{bC}	78.08^{aA}
	MOLM50	40.31^{bD}	86.82^{aA}	46.94^{bD}	61.03^{cC}	60.39^{aB}	77.66^{bA}
	MOLM100	62.87^{aC}	78.98^{aAB}	41.29^{bD}	74.13^{aB}	56.06^{aBC}	82.49^{aA}

[ab]In a row, lowercase superscripts compare strains within diet while uppercase superscripts compare diets within strains, for each gender ($P<0.05$);

[1]Diet: MOLM0 = broiler finisher without MOLM inclusion; MOLM25 = broiler finisher diluted at 25 g/kg MOLM MOLM50 = broiler finisher diluted at 50 g/kg MOLM; MOLM100 = broiler finisher diluted at 100 g/kg MOLM.

Table 6-3. Effect of feeding incremental levels of *M. oleifera* leaf meal on meat lightness (L*), redness (a), yellowness (b), pH, temperature, cooking loss and shear force of meat from three chicken strains.

		Diet (g/kg)				
Parameters	Strain	MOLM0	MOLM25	MOLM50	MOLM100	S.E
L*	BA	48.83^{b}	51.08^{abB}	52.23^{a}	51.91^{a}	
	OV	50.21^{b}	54.65^{aA}	50.55^{b}	50.31^{b}	1.031
	PK	50.93	51.27^{B}	50.92	50.89	
a*	BA	3.27^{cB}	4.88^{aA}	4.10^{bB}	4.32^{abAB}	
	OV	5.04^{aA}	3.67^{cB}	5.37^{aA}	4.64^{bA}	0.211
	PK	4.49^{aA}	4.65^{aA}	4.26^{aB}	3.93^{aB}	
b*	BA	15.62^{bB}	15.84^{bB}	21.42^{bA}	19.92^{aB}	0.951
	OV	19.96^{bcA}	17.46^{cAB}	20.21^{abAB}	22.82^{aA}	
	PK	16.49^{bB}	19.14^{bA}	18.49^{bB}	22.40^{aAB}	
pH_{24h}	BA	5.45^{b}	5.67^{a}	5.46^{b}	5.66^{a}	
	OV	5.49^{b}	5.69^{a}	5.49^{b}	5.69^{a}	0.019
	PK	5.47^{b}	5.67^{a}	5.41^{b}	5.61^{ab}	
Temperature (^{0}C)	BA	21.43^{aB}	20.73^{abB}	19.86^{abB}	19.78^{bC}	
	OV	27.88^{aA}	28.25^{aA}	23.22^{cA}	26.15^{bA}	0.578
	PK	18.15^{cC}	20.73^{bB}	22.52^{aA}	21.71^{abB}	
Cooking loss%	BA	30.98^{b}	34.46^{aA}	32.58^{bA}	32.25^{b}	
	OV	29.79^{b}	31.49^{abB}	31.50^{abAB}	32.41^{a}	0.635
	PK	30.85	30.89^{B}	29.97^{B}	31.36	
Shear force (N)	BA	63.12^{bB}	68.93^{ab}	63.56^{bA}	70.93^{aA}	
	OV	74.41^{aA}	65.17^{b}	53.98^{cB}	57.71^{cB}	2.276
	PK	70.93^{A}	64.44	69.02^{A}	69.28^{A}	

^{abc}In a row, lowercase superscripts compare diets within strain; ^{ABC}In a column, uppercase superscripts compare strains within diet. Means with similar superscripts do not differ (P > 0.05).

[1]Diet: MOLM0 = broiler finisher without MOLM inclusion; MOLM25 = broiler finisher diluted at 25 g/kg MOLM MOLM50 = broiler finisher diluted at 50 g/kg MOLM; MOLM100 = broiler finisher diluted at 100 g/kg MOLM.

At MOLM50 female PK had higher shear force (60.39) than OV and BA strains. When offered MOLM100, female OV had lower shear force (41.29) than other strains. When offered MOLM0, 25 and 100 no variation ($P>0.05$) in shear force was observed across different male strains. At MOLM50 male OV strain had lower ($P<0.05$) shear force (61.03) than BA and PK strains. In BA, OV and PK female chickens had lower ($P<0.05$) shear force across all diets than male chicken strains.

The 2-way interaction term 'diet × strain' did not ($P>0.05$) affect pH but significantly influenced all the other colour characteristics, shear force, temperature and cooking loss (Table 6-3). There was no variation ($P>0.05$) in a* for PK chickens across all diets. When offered MOLM25, meat from BA and PK chickens had higher a* values (4.88 and 4.65, respectively) compared to OV meat (3.67). When offered incremental levels of MOLM (25, 50 & 100), OV and BA chickens produced meat with higher ($P<0.05$) a* values (4.88, 4.64 and 4.32, respectively) than PK chickens.

There was no effect ($P<0.05$) on meat pH across strains in all diets. In BA strain, MOLM50 and MOLM100 resulted in meat with higher ($P<0.05$) b* values (21.42 and 19.92, respectively) compared to lower inclusion levels of MOLM. In OV chickens, feeding MOLM0 and MOLM25 resulted in meat with lower (19.96 and 17.46, respectively) b* values while these values were increased at higher inclusion levels of MOLM. Similarly, in PK, incremental levels of MOLM resulted in meat with higher b* values.

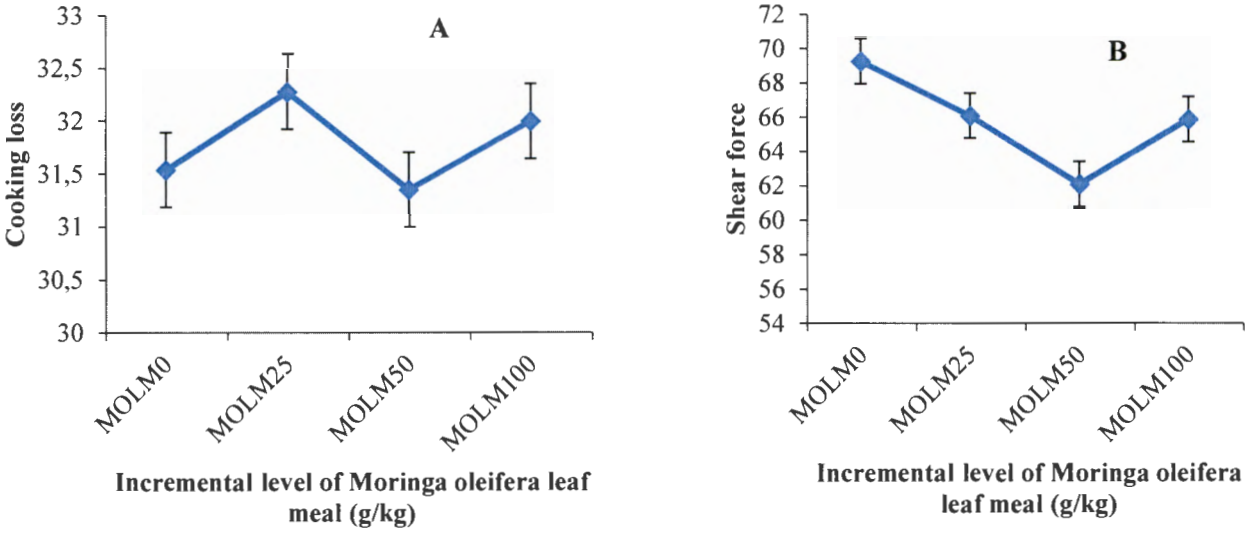


Figure 6-1. Effect of feeding incremental level of *Moringa oleifera* leaf meal on cooking loss (**A**) and shear force (**B**) of three chicken strains (MOLM0 = broiler finisher without MOLM inclusion; MOLM25 = broiler finisher diluted at 25 g/kg MOLM MOLM50 = broiler finisher diluted at 50 g/kg MOLM; MOLM100 = broiler finisher diluted at 100 g/kg MOLM).

When offered MOLM0, OV chickens had meat with higher (19.96) b* values compared to PK (16.49) and BA (15.62). In BA and OV strains, feeding incremental levels of MOLM produced meat with correspondingly higher L* values. No variation ($P>0.05$) was observed in meat lightness in strains across all diets. In PK strain, incremental levels of MOLM resulted in meat with higher temperatures, while OV had lower meat temperatures with incremental levels of MOLM. No variation ($P>0.05$) was observed in meat temperatures across all diets. In BA chickens, feeding MOLM25 resulted in meat with higher ($P<0.05$) cooking losses (34.46 %) compared to all the other diets. No dietary variation ($P>0.05$) in cooking loss was observed in meat from OV and PK chickens. In BA, meat from chickens fed MOLM100 higher shear force (70.93) compared to other diets. However, in OV chickens, shear force decreased with incremental levels of MOLM.

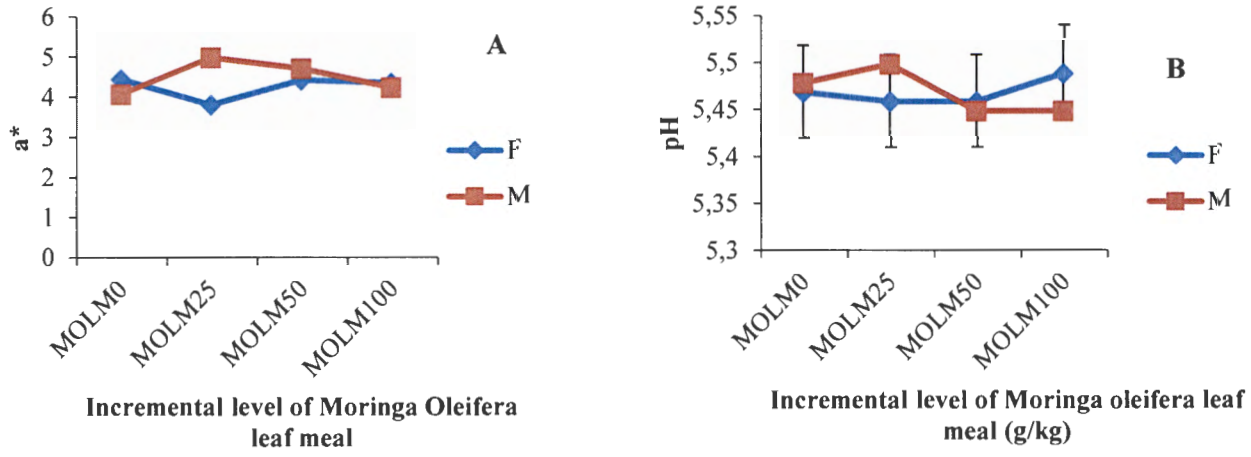
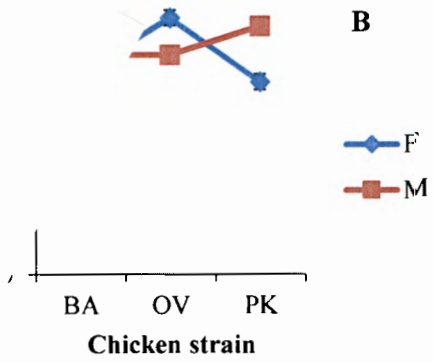
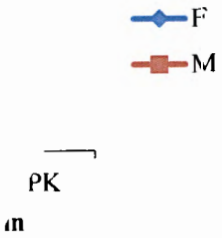


Figure 6-2. Effect of feeding incremental level of *Moringa oleifera* leaf meal on a*(**A**) and pH (**B**) of three chicken strains. (MOLM0 = broiler finisher without MOLM inclusion; MOLM25 = broiler finisher diluted at 25 g/kg MOLM MOLM50 = broiler finisher diluted at 50 g/kg MOLM; MOLM100 = broiler finisher diluted at 100 g/kg MOLM).

The 2-way interaction term 'diet × gender' did not ($P>0.05$) affect L*, b*, shear force and cooking loss but significantly ($P<0.05$) influenced a*, pH and temperature (Figure 6-5 A, B & C). Incremental levels of MOLM resulted in higher ($P<0.05$) a* values for meat from male chickens. Meat from females had the lowest a* value when the chickens were offered MOLM25. Highest pH was observed in in meat from male chickens offered MOLM25, whilst meat from females had highest pH when offered MOLM100 (Figures 6-4 A & B). Incremental levels of MOLM increased meat temperature in males however; the opposite was true in female chickens. Strain and gender interaction is presented in figures (6-5 A, B & C). In BA, meat from male and female chickens had similar ($P>0.05$) L* values but significant from other strains. In OV chickens, meat from males had higher ($P>0.05$) L* than OV females.





of strain and gender on lightness (A), redness (B) and temperature (C)

6.5.2 The proximate composition of breast meat

The proximate composition of breast meat from BA, OV and PK is presented in Table 6-4. Black Australorp contained a lower ($P<0.05$) fat free dry matter (FFDM) (224 g/kg) and higher moisture (766.0 g/kg) content compared with OV and PK chicken strains. However, there was no difference ($P<0.05$) in FFDM and moisture content between PK and OV chicken strains. Incremental levels of MOLM resulted in higher breast fat content compared to the control diet. Male chickens showed lower breast fat content than female breast meat.

Table 6-4. Moisture and free fat dry matter content (g/kg) of breast meat from Black Australorp (BA), Ovambo (OV) and Potchefstroom Koekoek (PK) chicken strains.

Strain	FFDM	Moisture
BA	224.0[b]	766.2[a]
OV	232.0[a]	757.0[b]
PK	237.0[a]	752.0[b]
S.E	0.247	0.289

[ab]Means within the same column with different lowercase superscripts differ ($P<0.05$);

Table 6-5. Effect of feeding incremental levels of *M. oleifera* leaf meal on breast fat from Black Australorp (BA), Ovambo (OV) and Potchefstroom Koekoek (PK) male (M) and female (F) chicken strains

	BA		OV		PK	
Diet (g/kg)	F	M	F	M	F	M
MOLM0	1.16^{aB}	0.96^{aB}	0.96^{aB}	0.75^{aB}	0.89^{aC}	0.79^{aC}
MOLM25	1.09^{aB}	0.90^{aB}	1.28^{aA}	0.87^{aB}	1.02^{aBC}	0.93^{aC}
MOLM50	1.17^{bB}	0.90^{ab}	0.99^{bB}	0.96^{aB}	1.88^{aA}	1.02^{aBC}
MOLM100	1.57^{aA}	0.81^{aB}	1.17^{bA}	0.91^{aB}	1.27^{bB}	0.88^{aC}

[1]Diet: MOLM0 = broiler finisher without MOLM inclusion; MOLM25 = broiler finisher diluted at 25 g/kg MOLM MOLM50 = broiler finisher diluted at 50 g/kg MOLM; MOLM100 = broiler finisher diluted at 100 g/kg MOLM.

The 3-way interaction term 'diet × gender x strain' significantly (P<0.05) influenced breast fat (Table 6.5). No significant variations (P>0.05) were observed when male chicken strains were offered incremental levels of MOLM.

Meat from female PK chickens had higher breast fat content when offered MOLM50, whereas meat from female BA chickens had higher fat content when offered MOLM100. In BA and OV chickens, meat from females had higher (P<0.05) fat content (15.7 and 11.7 %, respectively) when offered the highest level of MOLM compared to male chickens. Whilst in PK chickens offered MOLM 50, meat from females had higher fat content (18.8 g/kg) compared to male chickens.

6.5.3 Fatty acid composition of breast meat

Fatty acid composition of breast meat of BA, OV and PK chicken strains was not affected by diet. The fatty acid profile of meat from BA, OV and PK chickens differed (P < 0.05). Meat from BA had the highest (P<0.05) proportion (0.789 %) of eicosatrienoic acid (C20:3C8, 11, 14 (n-6) followed by PK (0.668 %) and OV (0.599 %). High proportion of docosahexanoic was observed in meat from OV (2.14 %), followed by BA (1.81 %) and then PK (1.43 %). Black Australorp breast meat exhibited higher (P<0.05) palmitic acid content (20.29 %) followed by PK (19.97 %) and OV (19.09 %) strains.

6.6 Discussion

The ultimate pH values reached after 24 h across the three different strains, in the current study, ranged between 5.41- 5.69. Generally, the pH of boneless-skinless chicken breast meat is determined by how much glycogen is in the breast muscle prior to slaughter and how rapidly the remaining glycogen is converted to lactic acid after slaughter (Fletcher, 1995). Incremental levels of MOLM in OV chickens resulted in higher meat pH after 24h. In addition, meat from

OV chickens had higher pH than from BA and PK chickens. Muscle pH decreases after slaughter, and a low pH can inhibit water holding capacity (WHC) and other muscular functions (Owens *et al.*, 2000; Woelfel *et al.*, 2002). Higher pH negatively affects meat quality, because it creates a more favourable environment for bacterial growth (Fanatico *et al.*, 2007a). Variation in pH might be associated with individual differences in bird strains. In the present study no variation was observed in pH between male and female breast muscle. However, Yates *et al.* (1976) reported that breast muscle from male birds had a higher pH value than that from female birds. The physical attributes are vital indicators of meat quality. Colour is generally influenced by animal related factors, mainly the genotype (Fletcher, 1995) and the age of the animals (Fanatico *et al.*, 2005b). In this study, diet had a significant effect on meat colour in the meat from the three chicken strains, a finding similar to reports by Fanatico *et al.* (2005b) and Ponte *et al.* (2008a). The breast meat of females was significantly lighter and more yellow than males. The current findings are in agreement with Saláková *et al.* (2009) & Wapi *et al.* (2013). This could be attributed to the higher myoglobin red type fibres content, due to increased physical activity of male chickens (Bogosavljević-Bosković *et al.*, 2009).

Incremental levels of MOLM resulted in higher yellowness (b*) of breast meat, which is a result of high carotene found in plant leaves such as *M. oleifera*. Prince (2000) reported that *M. oleifera* leaves contain 16.3 mg carotene/100 grams. Shear force is used to assess meat tenderness with higher shear force values indicating tougher meat quality (Cavitt *et al.*, 2004). Meat tenderness is affected by the amount and quality of connective tissue and by the contractile state of muscle fibers and bundles (Forrest *et al.*, 1972; Koohmaraie *et al.*, 2002). The shear force of the breast meat was lowest in OV followed by BA and PK strains, which had higher shear force. This could be attributed to differences in muscle fiber size and genetic variation among strains (Mahon, 1999) and suggest that OV would have tender breast muscle meat. When MOLM50 was offered, the chickens produced meat with lower shear values,

indicating dietary influence on meat tenderness. Karthivashan *et al.* (2015) showed that a low percentage (0.5%w/w) MOLM inclusion in broiler diets significantly improved meat tenderness. Male chickens produced meat with higher shear force than female chickens. This supports previous results by Lyon *et al.* (1992), Musa *et al.* (2006) and Yin *et al.* (2013) who reported that breast fillets from females had more tender meat than those from males. Male chickens are known for their high physical activities and according to Lewis *et al.* (2005), muscles from chickens with a high level of physical activity will result in tougher meat due to increased intramuscular collagen content. Cooking loss percentage was significantly affected by diet and type of strain. In addition, lower cooking losses were generally associated with decreased shear values. Omojola *et al.* (2004) stated that meat with less cooking losses would give a higher yield per unit cut.

Eicosatrienoic acid, also known as γ-linolenic acid (GLA), is an omega 6-fatty acid known to exert clinical efficacy in a variety of diseases, including suppression of chronic inflammation, vasodilation and lowering of blood pressure, and the inhibition of smooth muscle cell proliferation associated with atherosclerotic plaque development (Fan et al. 1995, Zurier et al. 1996). A high proportion of docosahexanoic (DHA; C22:6) was observed in OV (2.14 %) and BA (1.81) chickens while PK chickens had the least amount (1.43). Docosahexanoic acid, also known as cervonic acid, is an omega-3 fatty acid, which promotes cell cycle exit in retinal neuroprogenitor cells in culture (Insua, 2003) and promotes differentiation of neural stem cells into neurons by promoting cell-cycle exit and suppressing cell death (Kawakita, 2006). High level of docosahexanoic acid may be a result of chickens reaching sexual maturity at the time of slaughter. Black Australorp breast meat exhibited higher ($P<0.05$) palmitic acid content (20.29 %) than in PK (19.97) and OV (19.09) strains. Sung *et al.* (2000) reported that chicken meat contains palmitic acid (C16:0) as one of the major fatty acids; this finding was in agreement with the current results. Since essential fatty acids should be provided through the

diet because of its low biosynthesis in the human body (Cho *et al.*, 2009), the higher eicosatrienoic and docosahexanoic acid contents of meat from the three chicken strains is an attractive nutritional quality for health-conscious consumers. Breed-related differences in fatty acid composition of chickens have previously been reported by Van Marle-Köster & Webb (2000).

6.7 Conclusion

In conclusion, incremental levels of MOLM resulted in higher breast fat content than control diet, with females exhibiting higher fat content than males. Diet had a significant effect on meat colour in the meat from the three chicken strains. The breast meat of females was significantly lighter and more yellow than males. Diet MOLM50 resulted in lower shear force and lower cooking losses. However, incremental levels of *M. oleifera* leaf meal did not affect the fatty acid profile of the meat, which was unexpected due to high fatty acid composition of *Moringa oleifera* leaf. Ovambo chickens had meat with low shear force. However, differences observed in fatty acid composition among chicken strains could also be genetically based. The higher eicosatrienoic and docosahexanoic acid contents of meat from the three chicken strains are an attractive nutritional quality for health-conscious consumers.

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7 GENERAL DISCUSSION

Chemical characterisation of *M. oleifera* leaves and the digestibility of *M. oleifera* leaf meal-based diets in three chicken strains were determined. *Moringa oleifera* leaf protein content was significantly influenced by the stage of plant development. The deterioration in CP and escalation in fibre contents with advance in plant growth has been reported by several researchers (Callow *et al.*, 2003; Contreras-Govea *et al.*, 2009). Generally, tender leaves contain high protein content and less fiber, which would be suitable for high poultry performance due to limited ability of chickens to digest diets rich in fiber. *Moringa oleifera* leaves are a good source of protein, fibre, minerals, fatty acids profile and other elements important for the growth of chickens. The apparent digestibility of dietary nutrients decreases with fiber supplementation due to the replacement of digestible nutrients with components that are not digested or absorbed in the small intestine, and possibly to an increase in endogenous secretions in response to some types of fiber (Larsen *et al.*, 1993; Mosenthin *et al.*, 1994). Digestibility data indicated that inclusion of MOLM in chicken diets did not negatively affect nutrient digestibility.

A 90-day feeding trial was conducted to determine the effect of *M. oleifera* leaf meal supplementation on growth performance and carcass characteristics of three chicken strains (male and female) that are normally reared under extensive production systems. The leaf meal was used to dilute a commercial broiler finisher diet at 0 (MOLM0), 25 (MOLM25), 50 (MOLM50), and 100 (MOLM100) g/kg DM, producing four dietary treatments, which were fed to 216 Potchefstroom Koekoek (PK), Ovambo (OV) and Black Australorp (BA) chickens. Growth performance data were collected over a period of 13 weeks. At 13 weeks of age blood samples were taken from 6 chickens (3 males and 3 females) per treatment and used for biochemical and haematological analysis. Carcass characteristics of the chickens were

evaluated. Significant differences in feed intake, FCE and growth rate were observed between the imported BA strain and indigenous chickens, OV and PK. The genetics of a chicken affects its feed intake, digestibility, feed conversion efficiency and growth rate at different ages (Rondelli *et al.*, 2003). Results also support the view that BA is a fast-growing chicken strain compared to PK and OV strains. Results from the present study indicate that the capacity to utilize fibre-containing diets differed among chicken strains. The intake of fibre-containing diets in different chicken strains is known to be regulated by the chicken's energy requirements (Van Krimpen *et al.*, 2009). Feed intake showed a curvilinear response with increasing dietary MOLM levels. Feed intake of PK, OV, and BA strains reached a maximum at dietary MOLM inclusion levels between 30 and 50 g/kg DM. Black Australorp chickens had the highest feed conversion efficiency (FCE) of 2.35, while OV and PK chickens had lower FCE values of 2.09 and 2.05, respectively.

Black Australorp and PK chickens had higher dressing percentage compared to OV at higher inclusion levels of MOLM. This could be ascribed to genetic variation and growth potential of the different strains. In female chickens, diets containing MOLM resulted in chickens with better carcass weight, leg and thigh weight, dressing percent, and breast mass compared to the control. Since haematological parameters are good indicators of the physiological status of animals (Khan & Zafar, 2005), a comprehensive nutritional assessment of MOLM in chickens was performed to evaluate its anatomical and physiological effects. Diet MOLM25 promoted higher RBC counts than other diets in female BA chickens. In males, BA strain had lower RBC counts while OV and PK strains had higher counts. Possibly this observation could be attributed to genetic variation of strains. Red blood cells (erythrocytes) serve as a carrier of haemoglobin. It is this haemoglobin that reacts with oxygen carried in the blood to form oxyhaemoglobin during respiration (Johnston and Morris 1996; Chineke *et al.* 2006). Brown *et al.* (2000) opined that increased RBC values are associated with high quality dietary protein and with disease

free animals. Incremental levels of MOLM resulted in elevated Hb in all chicken strains. Inclusion of *M. oleifera* leaf meal in chicken diets may have resulted in higher iron intake, which promotes synthesis of haemoglobin and increases production of red blood cells. The highest inclusion level of Moringa leaf meal (MOLM100) elevated WBC count, possibly due to the presence of antioxidants, which can improve the immune response of chickens and thus reduce mortality (Siddhuraju & Becker, 2003). In both strains incremental levels of MOLM caused a curvilinear response in total protein, albumin and globulin. The lowest values of ALT and AST were observed with high inclusion levels of MOLM in both male and female chicken strains. This indicates that MOLM had no toxic effect within the liver parenchyma of the birds. These results are in agreement with Olugbemi *et al.* (2010), who reported that *Moringa oleifera* leaves have no negative effect on the health of broilers. Instead they reported beneficial effects such as enhanced immune responses of the birds. No signs of toxicity were observed in the liver of all chicken strains.

Internal organs were measured at 13 weeks of age. Chickens offered diet MOLM100 had larger gizzard weight and longer small intestine compared to diets with lower proportions of MOLM. Amerah *et al.* (2007) indicated that benefits of a larger gizzard include improved gut motility and improved digestibility of nutrients through effective grinding in the gizzard. The longer intestine may possibly be due to stretching of the intestinal wall, in response to increased contents of digesta in the small intestine. Quality parameters and fatty acid composition of meat from three chicken strains fed *Moringa oleifera* leaf meal-based diets were also evaluated. Incremental levels of MOLM in OV chickens resulted in higher meat pH after 24h. Muscle pH decreases after slaughter, and a low pH can inhibit water holding capacity (WHC) and other muscular functions (Owens *et al.*, 2000; Woelfel *et al.*, 2002). Higher pH negatively affects meat quality, because it creates a more favourable environment for bacterial growth (Fanatico *et al.*, 2007a). In this study, diet had a significant effect on meat colour from the three chicken

strains, a finding similar to reports by Fanatico *et al.* (2005b) and Ponte *et al.* (2008a). The breast meat of females was significantly lighter and more yellow than males. The current findings are in agreement with Saláková *et al.* (2009) & Wapi *et al.* (2013). This could be attributed to the higher myoglobin red type fibres content, due to increased physical activity of male chickens (Bogosavljević-Bosković, 2009). Male chickens produced meat with higher shear force than female chickens. This supports previous results by Lyon *et al.* (1992), Musa *et al.* (2006) and Yin *et al.* (2013) who reported that breast fillets from females had more tender meat than those from males. Male chickens are known for their high physical activities and according Lewis *et al.* (2005), muscles with a high level of physical activity will result in tougher meat due to increased intramuscular collagen content.

A high proportion of docosahexanoic (DHA; C22:6) was observed in OV (2.14 %) and BA (1.81) chickens while PK chickens had the least amount (1.43). Docosahexanoic acid, also known as cervonic acid, is an omega-3 fatty acid, which promotes cell cycle exit in retinal neuroprogenitor cells in culture (Insua, 2003) and promotes differentiation of neural stem cells into neurons by promoting cell-cycle exit and suppressing cell death (Kawakita, 2006). Since essential fatty acids should be provided through the diet because of its low biosynthesis in the human body (Cho *et al.*, 2009), the higher eicosatrienoic and docosahexanoic acid contents of meat from the three chicken strains is an attractive nutritional quality for health-conscious consumers.

8 CONCLUSIONS AND RECOMMENDATIONS

Significant variations were observed in chemical composition in tender and mature *M. oleifera* leaves. Based on the data observed, *M. oleifera* leaf meal is a good source of minerals, crude protein and fatty acids. The inclusion of MOLM improved the growth performance and carcass characteristics of chickens and these findings indicate that MOLM can be used as a potential

feed resource for poultry. *Moringa oleifera* had a hepatoprotective influence and proved to have favourable effects on some haematological, blood biochemical parameters and stimulated development and function of the gizzard and small intestine of male and female chicken strains. Diet MOLM50 resulted in lower shear force and lower cooking losses. Ovambo also chickens had breast muscle with low shear force. However, incremental level of *M. oleifera* leaf meal did not affect fatty acid profile of the meat, which was unexpected due to high fatty acid composition of *Moringa oleifera* leaf.

Extensively-reared chickens play a major role in ensuring food security in rural communities of most developing countries. However, due to direct competition for food between man and simple non-ruminants, the cost of feeding chickens for optimum growth performance has become high. As a result, during the past few decades, developing countries have seen a decline in the contribution of indigenous poultry to food security. Results from this project will improve poultry production in both small scale and commercial farming. *Moringa oleifera* is known for its high digestible nutrients and ascertained to improve performance in chickens. Farmers can use *Moringa oleifera* as feed for poultry. Furthermore, dilution of commercial broiler diet with MOLM enhances productivity of these indigenous chickens and reduces feed cost.

- Quantification of antioxidants and anti-nutritional factors in *Moringa oleifera* leaves should be fully researched as antibiotics in indigenous poultry.
- Detailed research on liver histopathology and intestinal morphology should be fully explored.
- Improved production techniques of *Moringa oleifera* leaf meal in large quantities and processing which are easily accessible to farmers should be research.

9 LIST OF APPENDICES:

Appendix 1. Peer-reviewed articles and papers to be produced from this thesis

1. Growth performance and carcass characteristics of three chicken trains in response to incremental levels of dietary *Moringa oleifera* leaf meal. *Livestock Science* (**in press**).
2. Effect of feeding *Moringa oleifera* leaf meal on weight of internal organs, haematological parameters, and serum biochemical indices in three chicken strains. *Journal of Animal Physiology and Animal Nutrition* (**Submitted**).
3. Quality parameters and fatty acid composition of meat from three chicken strains fed *Moringa oleifera* leaf meal-based diets. *Tropical Animal Production and Health* (**In preparation**).
4. Chemical characterisation and the digestibility of *M. oleifera* leaf meal-based diets in three chicken strains. *Journal of Animal Physiology and Animal Nutrition* (**In preparation**).