

**RESPONSE OF BROILER CHICKENS TO TREATED
DIETARY *Moringa oleifera* SEED CAKE**

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CERTIFICATION

This is to certify that this thesis has been read and approved as meeting the requirements of the Department of Animal Production, University of Ilorin, Ilorin, Nigeria, for the Award of Doctor of Philosophy (Ph.D.) degree in Animal Production.

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DEDICATION

I dedicate this thesis to Almighty Allah for His numerous mercies on me in the course of my Doctoral programme in University of Ilorin.

The thesis is also dedicated to my darling wife; Mrs. Aremu K.F, my children and my mother for their moral and financial support.

DECLARATION

I, Jimoh Olalekan AREMU, a Ph.D. student in the Department of Animal Production, University of Ilorin, Ilorin, hereby declare that this thesis entitled “Response of broiler chickens to treated dietary *Moringa oleifera* seed cake” submitted by me is based on my actual and original work. Any materials obtained from other resources or work done by any other person or institutions have been duly acknowledged. In addition, the research work has been approved by the University of Ilorin Ethical Review Committee

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LIST OF ABBREVIATIONS

MOSC	<i>Moringa Oleifera</i> Seed Cake
ETMOSC	Ethanol Treated <i>Moringa Oleifera</i> Seed cake
GIMOSC	Gamma Irradiated <i>Moringa Oleifera</i> Cake
FKMOSC	Ferrous Sulphate Kaolin <i>Moringa Oleifera</i> Seed Cake
AHMOSC	Ammonium Hydroxide <i>Moringa Oleifera</i> Seed Cake
SBM	Soya Bean Meal

ABSTRACT

Moringa oleifera Seed Cake (MoSC) is a potential feedstuff for livestock but contains some antinutritional factors. Applications of technological treatments to MoSC could eliminate the antinutrients, improve its nutritional content, apparent nutrient digestibility coefficient and feeding values. The objectives of this study were to: (i) determine the proximate, secondary metabolites and mineral composition of raw MoSC (rMoSC), (ii) treat MoSC by soaking in 80% ethanol solution (ETMoSC), gamma irradiation at 10, 30 and 50K Gy (GIMoSC), addition of ferrous sulphate to kaolin (FKMoSC), and soaking in 10, 20 and 30% ammonium hydroxide solution (AHMoSC), (iii) investigate the effects of treated MoSC (tMoSC) on performance, haematology, serum chemistry and histology of broiler chickens.

Experiment 1, determined the proximate composition, phytochemical screening and quantification of rMoSC and studied the effects of ETMoSC on broiler chickens. Experiment 2 studied the effect of GIMoSC on broiler chickens. Experiment 3 evaluated the utilization of FKMoSC by broiler chickens. Experiment 4 investigated the response of broiler chickens to AHMoSC and determined the haematology, serum chemistry and histology of the broiler chickens fed tMoSC. A total of six-hundred-day old broiler chicks were used and each experiment lasted for 8 weeks. Data obtained were subjected to Completely Randomised Design, mean were separated using the Duncan Multiple range test at $p=0.05$.

The findings of the study were that:

- i. crude protein content of rMoSC was 33.82%, it contains 173.20, 132.16, and 36.08mg/kg of phytate, tannins and saponins, respectively.
- ii. the daily weight gain, feed intake and feed conversion ratio of broiler chickens fed ETMoSC were significantly ($p<0.05$) poorer than the control,
- iii. Broiler chickens fed GIMoSC had similar ($p>0.05$) DWG (59.03 to 59.64 vs. 59.69g/bird/day), FI (106.66 to 107.13 vs. 104.22g/bird/day) and FCR (1.81 to 1.84 vs. 1.75) with the control, those fed rMoSC had poorer DWG, FI and FCR,
- iv. Performance of broiler chickens fed FKMoSC were similar ($p>0.05$) to that of the control with DWG of 59.64 to 61.35 vs. 59.66g/bird/day, FI (101.98 to 105g vs. 103.43g/bird/day) and FCR (1.71 to 1.74 vs. 1.73), those fed rMoSC had significantly ($p<0.05$) poorer performance,
- v. Broiler fed graded level of AHMoSC had similar ($p<0.05$) DWG, FI and FCR with the control (61.73 to 61.99 vs. 59.99; 103.75 to 104.74 vs. 102.51 g/bird/day and 1.67 to 1.69 vs. 1.71, respectively, and were significantly ($p<0.05$) better than value for rMoSC,
- vi. The haematological indices and serum chemistry of chickens fed tMoSC were poorer than the control but significantly ($p<0.05$) better than those of rMoSC; histology of liver in broiler chickens fed tMoSC revealed degeneration of the hepatocytes, while those fed rMoSC showed coagulative necrosis.

The study concluded that the feeding value of the treated MoSC was better than raw; treatment of MoSC by soaking in ethanol was not effective in its detoxification. Treatments of MoSC with gamma Irradiation, Ferrous Sulphate–Kaolin and Ammonium hydroxide solution were more effective with performance results that are comparable to the control and their usage at higher level is recommended for effective detoxification of MoSC.

CHAPTER ONE

1.0 INTRODUCTION

Feeding in livestock production is majorly to provide various nutrients to meet the requirements of livestock and the importance of the feed can be from the point of view that feed accounts for 55-75% of cost of producing chickens, 45-65% of the cost of producing dairy cattle, 70-80% of the cost of feeding beef animals, 65-75% of the cost of producing pork, 40-60 % of the cost of producing sheep and goats and about 70% of the cost of raising rabbits (Atteh, 2002). However only certain portion of the nutrients and/or chemicals present in the feeds fulfill the nutrients roles. (Soetan and Oyewole, 2009). In Nigeria, the greatest challenge to livestock production especially poultry is feeding cost which is between 60 - 80% of the production cost in intensified poultry management (Oluyemi and Roberts, 2000). Essential feed ingredients for poultry diet formulation are also in great demand by man, animal and industries (Anike and Okeke, 2003) thus, the need to source for alternative feeding stuff to prevent the competition between these categories of users. Poultry protein supply is normally from two main origins viz plant and animal. Proteins from Plant sources are low cost, and using them in great amount will reduce the production cost as against animal proteins. Some nutrients chemicals possess well known toxic properties if consumed in excessive amounts or without adequate preparation. The toxic chemicals in feeds when consumed by animals can cause a lot of actions in the body or react in some adverse ways between the nutrients and toxins *in vivo*. For instance, some feed chemical toxins exert their effects by interfering with nutrient availability and retention, and some are also known to adversely affect the utilization of nutrients while some have been reported to influence negatively the metabolism of nutrients, all these chemicals which influence the metabolism

negatively are called anti-nutritional factors (ANF). Some of the antinutrients present in these feeds are: trypsin, chymotrypsin, aflatoxins, and polyphenols that have the tendency to hinder actions of enzymes responsible for digestion thereby resulting into digestion losses (Singh, 1988).

To reduce feed cost, there have been several efforts by researchers, nutritionists and producers to find alternative feed ingredients by considering novel feedstuffs which are safer, cheaper, locally available and nutritionally adequate and majorly that contain lesser toxin in which case their method of detoxification will be also be cheaper. It is for this reason that *Moringa oleifera* seed cake has been experimented in this project work.

Overwhelmed by the numerous economic benefits of *Moringa oleifera* tree in the tropical world where the plant is considered a wonderful tree due to its nutritive, industrial, and medicinal attributes, the area of its anti-nutritional or toxic factors remains to be fully dealt with. Ferrao and Ferrao, 1970; Ramachandran *et al.* (1980) reported that the seeds consist between 30-42% non-drying oil used for human consumption with minimal propensity for spoilage, stale or being gummed. The oil cake achieved as end-product of oil extraction has superior proteins and it is used as fertilizer and to offset the mixture in muddy or filthy water due to the negative electrical charges of the colloids. The protein of the cake meal is therefore used as an innocuous natural polypeptide coagulating agent for settling mineral particles, organics and cleaning of domestic water and purification of vegetable oil or for coagulating fibres in juice and beer companies. It is preferably used in place of industrial coagulating agents such as alum which can be poisonous and expensive. Most of the tree parts can be utilized in traditional medical practices particularly the leaves and root barks. Details on the curative and preventive features of *M. oleifera* are given in Trees for Life Journal (Fahey, 2005). In developing tropical countries, the tree unripe (green) pods are the most often and frequently utilized among the tree parts due to their nutritious content

with the essential nutrients. Its leaves can be consumed fresh or processed without losing its nutritional value. According to Optima of Africa (Donovan, 2007), 25g/day leaf powder provides 42% protein, 61% magnesium, 41% potassium, 125% calcium, 71% iron, 22% vitamins C and 272% Vitamin A. Details of other possible major nutritional benefits of *M. oleifera* tree can be obtained from Morton (1991), Makkar and Becker (1996a).

Phytochemistry of *Moringa oleifera* shows that the plant contains biologically-active organic compounds (Duke, 1983) which could elicit anti-nutritional and toxic responses. Anti-nutrients are generally contained by the plant kingdom particularly those used in human nutrition and as animal feeding stuff (Igile, 1996). Reports have shown that some of these chemicals, described as “secondary metabolites” are highly biologically active (Zenk, 1991). Other chemical elements like macro-, micro minerals and heavy metals which are neither useful nor poisonous at great amounts such as lead (Pb), mercury (Hg), boron (B), iron (Fe), cobalt (Co) in the tree are acquired from soil, fertilizers and many other naturally occurring chemicals. Oakenful and Sidhu, 1989; Soetan, (2008) reported that while some secondary metabolites cause adverse or very harmful effects, some are used in nutrition and others as pharmacologically-active agents Some of these metabolites include glucosinolates, protease inhibitors, tannins, saponins, lectins, alkaloids, cardiac glucosides, cyanogenic glycosides, flavonoids, etc. Research has shown that some of the phytochemicals when taken at reasonable quantities exerted deleterious effects on wellbeing or evidently helpful to normal health of man and animal (Kerston *et al.* 1991., Sugano *et al.* 1993; Soetan, 2008).

Considering the enormous nutrient composition of moringa seed cake and non-availability of information regarding its utilization in poultry nutrition, there is the need for a research on moringa seed cake for use in nutrition of poultry. Hence the need to investigate optimum

inclusion level of the seed cake in diets and proffer method(s) for detoxification in case of toxicity or anti-nutrient effects at higher inclusion level in diets.

1.1 General Objective

The general objective is to explore the effect of chemical treatment on the feeding value of *Moringa oleifera* seed cake (MoSC) for poultry production

1.2 The Specific Objectives are:

- i To determine the proximate, minerals and phytochemicals composition of raw and treated *moringa oleifera* seed cake
- ii To determine the optimum level at which the raw and the treated *moringa oleifera* seed cake could replace soyabean in diets of broilers.
- iii To determine the effect of selected treatment methods on the anti-nutrients and feeding potential of *moringa oleifera* seed cake in broilers
- iv To determine the profitability of using processed *moringa oleifera* seed cake in the diet of broilers.

CHAPTER TWO

2.0 REVIEW OF LITERATURE

2.1.1 *Moringa (Moringa Oleifera)* tree

Makkar and Becker (1997), reported *Moringa (Moringa oleifera)*, originating from the north-west zone of India, and South of the Himalayan mountaintop. It belongs to the Moringaceae family. Fahey *et al.*, (2001), reported *Moringa* to have adapted well and cultivated in several parts of the tropics. Kristin (2000) discovered that although there are thirteen species of *moringa* tree, it is only *Moringa oleifera* that is most widely grown. The tree has an average height of 5-12m, with a straight trunk of 10-30cm thick bark. It has fleshy tap root that made it to be drought resistant. *Moringa* can be cultivated easily by seeds and cuttings.



Figure 1: *Moringa Oleifera* Seeds With Seed Coating

Source: *Research Journal of Recent Sciences*(2012)



Figure 2: Moringa Oleifera Tree

Source: *Research Journal of Recent Sciences (2012)*



Figure 3: Moringa Oleifera Pod

Source: *Research Journal of Recent Sciences (2012)*

2.1.2 *Moringa (Moringa Oleifera)* as Medicinal Plant

History has it that earliest past kings and queens used *Moringa* parts in their diets to maintain their healthy skin and mental awareness. Jahn, (1996), discovered that *Moringa* dates back to 150 B.C. past Maurian fighters of India used *Moringa* Leaf Extract in their diet during war. The belief was that *Moringa* drink gave them additional energy and lessen the sufferings sustained in the war front. Report on *Moringa* drink made researchers to analyze its nutritional benefits. Makkar *et al.*, (1997), reported on the outstanding nutritional constituents of *Moringa* leaves and fruits.

Table 2.1: Analysis of Nutritional Value of *Moringa* Pods, Fresh (Raw) Leaves and Dried Leaf Powder per 100g of Edible Portion.

Components	Pod	Leaves	Leave powder
Moisture (%)	86.9	75.0	7.5
Calories	26	92	205
Protein (g)	2.5	6.7	27.1
Fat (g)	0.1	1.7	2.3
Carbohydrate (g)	3.7	13.4	38.2
Fiber (g)	4.8	0.9	19.2
Minerals (g)	2.0	2.3	-
Ca (mg)	30	440	2,003
Mg (mg)	24	24	368
P (mg)	110	70	204
K (mg)	259	259	1,324
Cu (mg)	3.1	1.1	0.57
Fe (mg)	5.3	7	28.2
S (mg)	137	137	870
Oxalic acid (mg)	10	101	1.6%
Vitamin A - B carotene (mg)	0.11	6.8	16.3
Vitamin B -choline (mg)	423	423	-
Vitamin B1 -thiamin (mg)	0.05	0.21	2.64
Vitamin B2 -riboflavin (mg)	0.07	0.05	20.5
Vitamin B3 -nicotinic acid (mg)	0.2	0.8	8.2

Source: Fahey (2005)

Table 2.1: Analysis of Nutritional Value of *Moringa* Pods, Fresh (Raw) Leaves and Dried Leaf Powder per 100g of Edible Portion.(contd)

Components	Pod	Leaves	Leave powder
Vitamin C -ascorbic acid (mg)	120	220	17.3
Vitamin E -tocopherol acetate (mg)	-	-	113
Arginine (g/16g N)	3.6	6.0	1.33%
Histidine (g/16g N)	1.1	2.1	0.61%
Lysine (g/16g N)	1.5	4.3	1.32%
Tryptophan (g/16g N)	0.8	1.9	0.43%
Phenylalanine (g/16g N)	4.3	6.4	1.39%
Methionine (g/16g N)	1.4	2.0	0.35%
Threonine (g/16g N)	3.9	4.9	1.19%
Leucine (g/16g N)	6.5	9.3	1.95%
Isoleucine (g/16g N)	4.4	6.3	0.83%
Valine (g/16g N)	5.4	7.1	1.06%

Source: Fahey (2005)

2.2 Phytochemicals in *Moringa* Tree

Reports have shown that *Moringa* species have notable amounts of compounds containing simple sugar, rahmnose, glucosinolates and isothiocyanates (Bennett *et al.*, 2003; Fahey *et al.*, 2001). Win and Jongen, (1996) also reported antioxidant activity of these compounds.

2.3 Anti-Nutrient Factors in Novel Feedstuffs and Their Effects on Animals

Anti-nutritional factors that can also be called ‘secondary metabolites’ are chemical compounds synthesized by the normal metabolism of species in natural foods or feedstuffs. Anti-nutritional factors can be categorized into two main groups. These are: (1). Proteins that are reactive to standard treatment temperatures eg lectins and protease inhibitors. (2). Other materials that are resistance to usual processing temperatures eg polyphenols (mainly condensed tannins), non-protein amino acids and galactomannan gums (Osagie, 1998). Habtamu and Nigussie, (2014) reported that these anti-nutritional factors have been implicated to be extremely organically active and lower utilization of nutrients. Toxicity caused by the intake of different forages is also frequent among the farm animals.

Intake of feed containing antinutrients in some cases cause, chronic intoxication, obstructs the usage of dietary protein and carbohydrate, hinders some minerals availability, thus growth rate, feed efficiency, and the production of edible products are negatively affected. Though antinutrients are present in many conventional feeds, Nityanand, (1997) reported that they are more prevalent in most of the non-conventional feed ingredients. Plants having multiple noxious compounds, emanating from the two sources, escalate the hinderance of their

decontamination.

2.4.1 Phytates

Phytates also known as inositol hexakisphosphate, are known to be an organically bound form of phosphorus in plants. Bello *et al.*, (2008) reported that phytates in foods complexed with vital minerals like calcium, magnesium in the process of digestion leading to mineral insufficiency. They complexed with inorganic substances forming insoluble salts, consequently lowering their rate of utilization (Thompson, 1993; Guil and Isasa, 1997; Muhammad *et al.*, 2011).

Walter *et al.*, (2002), reported that the availability of phytate in feeds is linked with reduction in mineral usage attributable to phytate structure that has high concentration of phosphate groups that are negatively charged that form very stable networks with mineral ions causing unavailability for intestinal absorption. Thava & James, (2001) reported that generally phytates are found in high fibre feeds especially legumes, whole grains, and wheat bran.

2.4.2 Tannins

Tannins are plant polyphenolic compounds that bind with several other organic compounds including amino acids and alkaloids. Presence of tannins in livestock feeds lower its productivity and weight gain in chicks (Armstrong *et al.*, 1974; Dei *et al.*, 2007). Tannins are reported to be the most widely occurring anti-nutrients in plants. Dube *et al.*, (2004), reported that these compounds are found in various trees, shrubs, seeds and industrial by-products.

Tannins are heat stable and reduced digestion of protein in humans and animals possibly by hindering enzymes of digestion and increasing fecal nitrogen or making protein partially unavailable. Reduced feed intake, feed efficiency, growth rate, and protein digestibility in experimental animals are known to be caused by tannins in feeds. Tannins also form insoluble complexes with proteins and the tannin-protein complexes may be responsible for the anti-nutritional effects of tannin containing foods (Habtamu and Nigussie, 2014).

2.4.3 Saponins

Dei *et al.*, (2007), discovered saponins to be organic compounds present in many plants which have soap character due to their surfactant properties. *Moringa* seed meal inclusion in diets lowers feed consumption as a result of the inherent anti-nutrients particularly mimosine and tannins (Atawodi *et al.*, 2008). Lower feed intake was due to the bitterness of saponins (Cheeke, 1971) and attributable to its unpleasant taste (Oleszek *et al.*, 1994). Over consumption of saponins leads to hypocholesterolaemia because they combined with cholesterol, rendering it inaccessible for absorption (Soetan and Oyewole, 2009).

Saponins have been reported to cause reduction in growth, feed efficiency in chickens and interfere the absorption of dietary lipids and vitamins (A & E) (Jenkins and Atwal, 1994).

2.4.4 Trypsin Inhibitor

Trypsin inhibitor has been discovered to hinder trypsin and chymotrypsin which perform vital task in broiler chickens protein digestion. Aleator and Fetuga, (1987), reported that trypsin inhibitors cause pancreatic enlargement and growth reduction. Hydrogen cyanide, present in

moringa seeds, is poisonous if taken by non ruminants in high amounts. Processing of plant products by soaking or boiling in water is reported to lower its deleterious consequences and enhance feed consumption and digestion of protein (Okai *et al.*, 1995; Dei *et al.*, 2007).

2.5 The Use of *Moringa* Seed Meal as Poultry Feed

Absolute absence of anti-nutrient content and the great protein, lipid and sulphur containing amino acid attributes support using *moringa* seed as an ingredient for animal feed. It is an outstanding source of protein for non-ruminant. (Ferreira *et al.*, 2008). Ferreira *et al.*, (2008) reported that given the seed meal to a week old broiler chickens to a status of 50 g/kg, growth rate, feed intake and efficiency of feed are enhanced.

2.6 Importance of Tree Crops to Feeding Animals

Recent studies have shown that *moringa* seed and leaf meals were utilized effectively in poultry diets as alternative to soyabean meal (Melesse *et al.*, 2011). Fuglie, (2000), also reported that reasonable numbers of research finding on the nutritional attributes of *moringa* are abundant in literature, such reports are however inconsistent. The seed protein contents are higher than those reported for important grain legumes and soybean varieties (Ferreira *et al.*, 2008). Reported analyses of the proximate composition of *M. oleifera* seeds have shown high levels of protein with the dry seeds usually containing 332.50 to 383.00 g kg⁻¹ of protein (Oliveira and Silveira, 1999; Abdulkarim *et al.*, 2005). EAA composition in *moringa* seed cake has high essential amino acid, especially the sulfur amino acid such as methionine, cystine, tryptophan (Makkar and Becker, 1996) except for lysine (15.3 g kg⁻¹ protein), threonine (30.8 g kg⁻¹) and valine (43.5 g kg⁻¹) (Oliveira and Silveira, 1999). These amino acids, however, are very low in soybean meal. It is found that methionine acid allows protein synthesis as well as being a reactant for homocysteine, cysteine, carnitine, creatine and choline.

2.7 Different Processing Methods to Lower the Feed Anti-Nutritional Contents

High level of anti-nutrients and noxious constituents in plant materials used for foods/feeds is of great concern to nutritionist and researchers in general. Hence, there is the need to find a way of removing or lowering these quantities to the lowest level (Soetan and Oyewole, 2009). Elimination of unwanted constituents is indispensable to promote the nutritive constituents of plant materials and judiciously use their full usefulness as ingredient for poultry feed. It is generally taken that modest and cheap treatment procedures are practical ways of obtaining required alterations in seeds constituents (Akande and Fabiyi, 2010).

Most of the toxic and anti-nutrient effects of these compounds in plants could be removed by several processing methods such as soaking, germination, boiling, autoclaving, fermentation, genetic manipulation and other processing methods (Soetan, 2008).

2.8 Some Physical Methods for Removing Anti-Nutritional Factors in Feed Stuffs.

2.8.1 Soaking

Soaking is one of the procedures being used to eliminate solvable anti-nutrients, that can be removed through the soaked discarded solution. Nevertheless, Vidal-Valverde *et al.*, (1992), discovered that in the course of soaking some metabolic reactions can occur that can influence some of the constituent compounds. Liener, (1980), reported that canavanine and canaline, anti-nutrients of jack were water soluble.

2.8.2 Cooking (Boiling)

Generally, cooking decontaminates heat-labile anti-nutrients like chymotrypsin inhibitors, trypsin and evaporable organic compounds. Cooked water may be thrown off, but some other dissolvable compounds could be eliminated. Bressani and Elias (1980) discovered that 30-40% of polyphenols can be eliminated by cooking *Phaseolus vulgaris*. Trugo *et al.*, (1990), reported that cooking *Phaseolus vulgaris* for 60 minutes at 100°C was adequate to decontaminate over 90% of the inherent trypsin inhibitor. Udedibie and Nwaiwu (1988) also discovered that cooking jackbean for 60 minutes was sufficient for the removal of most of the identified heat-sensitive anti-nutrients and alkaloids in raw jackbeans.

2.8.3 Toasting

Improvement in the nutritive quality of *jackbeans* using toasting technique was first discovered by Borchers and Ackerson (1950). Later, Udedibie *et al.*, (1994), observed that roasting alone can enhance its nutritive quality for broiler chickens to the maximum inclusion rate of 100g/kg dietary supplementation. Studies by Esonu *et al.* (1998) showed that toasting *jackbean* seeds singly as a treatment procedure did not significantly lower the quantity of its noxious constituents.

2.9 Some Chemical Methods for Removing Anti-Nutritional Factors in Feed Stuffs.

2.9.1 Gamma Irradiation

Hasnat *et al.*, (2014) found that a radiation of 9.33 KGy electron beam (EB) reduced hydrocyanic acid, phytic acid and tannic acid to the extent of 34, 63, and 22%, respectively.

Gamma irradiation process with its radiolytic action can eradicate glucosinolate molecules. Other researchers discovered that anti nutrients were seriously decontaminated by gamma irradiation. (Farag, 1989; Sattar et al., 1990; El-Morsi et al., 1992; Farag, 1998).

2.9.2 Kaolin

Of recent, the use of different substitute feed additives for animals like kaolin has been experimented. Mump ton, (1999), discovered that animal feed holding clay minerals such as kaolin enhanced weight gain and feed efficiency. Taugir and Nawaz, (2001), reported that kaolin lowered adulteration of the guts by bacterial and the deleterious consequence of contaminated diets by mycotoxin. Kaolin also defends the mucosa of the intestine, by clinging to pathogen and carefully advance their discharge (Droy-lefain et al., 1985).

2.9.3 Solvent Extraction

Extraction of oil from oil-bearing seeds using solvents has been disclosed to also enhance the nutritive quality of such seeds. For example, the anti-nutritional factors in cottonseed is soluble in organic solvents like hexane, ethyl alcohol, aqueous acetone and aliphatic amines. These substances have been disclosed to effectively lower the gossypol constituents in cotton seed meal. Hron et al., (1994), discovered that extraction of cotton seed meal with 95% ethanol resulted in reduction in total gossypol content by about 70%

CHAPTER THREE

EXPERIMENT ONE

3.0 EVALUATION OF THE CHEMICAL COMPOSITION AND FEEDING POTENTIAL OF ETHANOL REATED MORINGA OLEIFERA SEED CAKE IN BROILERS.

3.1 Introduction

Oil seeds, *Moringa oleifera* inclusive, contain a large amount of stored materials such as starch, storage proteins, oil and minerals required for seed and early plant growth. These stored materials make seeds highly susceptible to attack by micro-organisms, insects, birds and animals so that protection against predators is necessary. Protective mechanisms in *M. oleifera*, like in other plants, exist in the form of anti-nutritional factors and toxins. Plants by nature hold thousands of chemical organic compounds dependent on the circumstances, can have advantageous or hazardous influence on creatures taking them. More often, a single tree like *M. oleifera* may hold several or many noxious compounds which adds to the complication of using only simple detoxification technique. *M. oleifera* as a case study is one of such plants harbouring multiple anti-nutrients and toxic factors. Aletor (1993), observed that there are many anti-nutrients that are very notable in plants used for animal feeds and human foods. Anti-nutritional factors not only diminish human and animal output as well as induce toxins in the time of shortage or restraint when the feeds huge in them are taking by animals in great amounts (Kumar, 1992). Some of the anti-nutrients and toxic factors in *M. oleifera* are phytate, glucosinolate, saponin, haemagglutinin (lectin), alkaloids, tannin, oestrogen, cynogenic glucoside etc.

Besides the phytochemical composition of *M. oleifera*, the tree is known to be notable in elements like the heavy metals, which are either useful or poisonous at peak concentration such as Cadmium, Lead, Mercury, Nickel, Chromium, Arsenic, Iron (Magat *et al.* 2009). An understanding of the toxicity effect of the heavy metals is important because some are extremely toxic even at low concentration like lead (Pb). Thus the utility of moringa seed cake containing saponin, tannin, phytate and others may be limited in poultry by the existence of these anti-nutritional factors (Kumar, 1992).

3.2 Objective

The motive of this research was to determine the main phytochemicals in *Moringa oleifera* seed cake and the optimum level in which the broiler chickens can tolerate the raw and ethanol treated *Moringa oleifera* seed cake.

3.3 Materials and Methods

3.3.1 Experimental Site

The experiment was accomplished in the Poultry Unit of the Teaching and Research Farm, Kwara State University, Malete, Nigeria.

3.3.2 Source of Experimental Birds

A total of One hundred and fifty day-old Arbor acre breed of broiler birds was sourced from Novic Farms at Ibadan, Oyo State Nigeria.

3.3.3 Collection of *M. Oleifera* Seed Cake

50Kg of *Moringa oleifera* seed cake were obtained from Moringa processing plant at Afe Babalola University, Ado-Ekiti, Nigeria. 500g of the seed cake was used for the laboratory analyses.

3.3.4 Experimental Diets

Five experimental diets were formulated made of a corn-soy reference diet (control) and four other diets comprising ethanol treated seed cake. 15Kg of raw *Moringa oleifera* seed cake was soaked in 40 Litres of 80% ethanol for 72 hours. The seed cake was later removed from solution and properly sun dried to a constant weight and milled. The treated *Moringa oleifera* seed cake was added in the diet at graded level of 5, 10, 15, and 20% to replace soyabean which correspond to diets 1,2,3,4 and 5 respectively.

The composition of the experimental diets for the broilers on as fed basis is presented in Table 3.1. The proximate composition of the broilers diet is presented on Table 3.2.

Table 3.1: Composition of the Experimental diets (%)

	Diets				
	1	2	3	4	5
	Inclusion levels (%)				
Ingredients	0	5	10	15	20
Maize	58.25	57.75	58.75	59.00	58.00
SBM	38.40	32.90	26.90	21.65	17.65
ETMOSC	0.00	5.00	10.00	15.00	20.00
Bone meal	1.50	1.50	1.50	1.50	1.50
Oyster shell	0.50	0.50	0.50	0.50	0.50
Salt	0.50	0.50	0.50	0.50	0.50
Lysine	0.10	1.10	1.10	1.10	1.10
DL-methionine	0.25	0.25	0.25	0.25	0.25
*Vit/Min premix	0.50	0.50	0.50	0.50	0.50
Total	100.00	100.00	100.00	100.00	100.00
Calculated chemical analysis					
ME (Kcal/kg)	2954.39	2959.64	2942.97	2958.20	2945.85
CP (%)	23.18	23.13	23.15	23.18	23.12
CF (%)	3.20	4.11	3.13	3.58	4.23
EE (%)	4.65	9.13	5.85	6.23	4.67
Methionine (%)	0.49	0.58	0.35	0.48	0.45
Lysine (%)	1.25	1.50	1.08	1.45	1.1

SBM= soybean meal, ETMOSC= ethanol treated *Moringa oleifera* seed cake

0.50 premix supplied, per kilogram of diet: vitamin A, 12,000 IU; vitamin D3, 2,000 IU; vitamin E, 50 IU; vitamin B1, 1 mg; vitamin B2, 3 mg; vitamin B6, 1 mg; vitamin B12, 10 µg; vitamin K, 2 mg; copper (cupric sulphate), 75 mg; nicotinic acid, 12 mg; pantothenic acid, 10 mg; iron, 200 mg; cobalt, 0.5 mg; manganese, 40mg; zinc, 90 mg; iodine, 1 mg; selenium, 0.2 mg; calcium, 31.25 g; sodium, 10 g

3.3.5 Management of Experimental Birds and Data Collection

A total of 150-day old broiler chicks were used for the trial. The experiment was designed as a complete randomized design. The dietary treatments contained three replicates with 10 chicks per replicate. All the birds were raised under the same management practices all through the eight week experimental period except for the diets offered that differed. The birds were provided with feed and water *ad-libitum* and weekly weighed. Routine care was carried out as at when due.

3.3.6 Data Collection /Response Criteria

Proximate composition of the raw (untreated) and ethanol treated MOSC was conducted following the procedures of AOAC (1990). The cake was analysed for dry matter, nitrogen free extract, crude protein, ether extract, crude fibre and mineral matter.

The proximate analysis, macro and micro mineral element, phytochemical screening analysis of raw and ethanol treated *Moringa oleifera* seed cake were executed in the Department of Chemistry Laboratory, University of Ilorin.

Quantification of the phytochemicals in the raw *Moringa oleifera* seed cake was accomplished using the procedures outlined by Talamond *et al.*, (1998), Nwinuka *et al.*, (2005) for the detection of phytate, oxalate, saponins, tannins, phenolics, glycosides and flavonoids . The method described by AOAC (1990) was used for mineral analysis.

In the course of the experiment, data were collected on the following performance characteristics: feed consumption, daily body weight gain, growth rate, and feed conversion ratio and survival rate. At the end of the eight weeks feeding trial period, three birds per treatment were taken randomly, fasted for 12 hours before slaughtering and collection of blood samples for determination of biochemical and haematological constituents.

3.3.7 Nutrient Digestibility Trial

The study on apparent nutrient digestibility was accomplished at the expiration of the 8th week experimental period. Three birds per treatment were selected randomly and transferred to clean metabolic cages and fed the commensurate treatment diets. The test lasted for eight days. The first four days were taken as acclimatization period and the last four days as experimental period. During the last four days, data were taken on feed intake and faeces voided. The faeces were oven dried, and its samples were taken for chemical analysis. The samples were analyzed for proximate composition according to the methods of AOAC (1990) using the formula below;

$$\text{Apparent Nutrient Retention (\%)} = \frac{\text{Nutrient intake} - \text{Nutrient in faeces} \times 100}{\text{Nutrient intake}}$$

3.3.8

Serum Biochemical and Haematological Analysis

At the expiration of the eight week experimental study, one broiler per replicate in all the treatments was taken at random and blood collected by decapitation. Blood for biochemical indices was collected in bottles without EDTA for the analysis of the biochemical criterions. The blood samples were kept in cooled condition prior to analysis in the laboratory.

Ethylene diamine tetra-acetate (EDTA) was used to pretreat the collected blood samples for haematological parameters and were kept in cooled condition prior to analysis in the laboratory. Haematological indices examined include white blood cell (WBC), red blood cell (RBC), packed Cell Volume (PCV), haemoglobin (Hb). The mean cell volume (MCV), mean cell haemoglobin (MCH) and the mean cell haemoglobin concentration (MCHC) were calculated according to the methods of Jain (1986).

3.3.9

Histology of the Liver

Three birds were selected per treatment at the end of the feeding trial and sacrificed to obtain the liver. Sections were obtained from the anterior lobe of the liver, preserved in 10% formal-saline solution. The liver tissues from each replicate were processed using haematoxylin and eosin stains for histological studies. Digital microscope with a magnification of 400 was used to take photo micrographs. The analysis was accomplished at the histopathological Laboratory of Faculty of Veterinary Medicine, University of Ilorin.

3.4 Statistical Analysis

Data collected on the response criteria were subjected to analysis of variance according to the completely randomized design model and differences between treatment means were separated using the Duncan's multiple range test (Steel and Torrie, 1980).

3.5 Results

3.6.1 Chemical Evaluation of Raw *Moringa Oleifera* Seed Cake

Table 3.3 presents results of proximate composition of raw *Moringa oleifera* seed cake. Raw *Moringa oleifera* seed cake is shown to contain valuable nutrients. The macro and micro mineral contents of raw moringa seeds is given in Table 3.4. Raw *Moringa oleifera* seed cake contains valuable amounts of macro, micro minerals and heavy metals.

The phytochemical content of raw MOSC is shown on Table 3.5. Raw MOSC contains very high amount of alkaloids, phytate, tannins, oxalates, flavonoids as well as appreciable amounts of terpenoids, saponin and phenolic compounds.

3.6.2 Growth Performance Characteristics of Broilers Fed Ethanol Treated *Moringa Oleifera* Seed Cake Diet

Table 3.6 presents data on performance characteristic of broilers fed ethanol treated *Moringa oleifera* (MOSC) diet. The final live weights of the birds were significantly ($p < 0.05$) affected as the dietary ethanol treated MOSC diet increased. The final weights decreased with a corresponding increase in the dietary inclusion of the ethanol treated MOSC for the replacement of soyabean as protein source. The average daily weight gains of the birds were

significantly ($p < 0.05$) affected by the dietary inclusion of the ethanol treated MOSC diet. There was a decline in the average daily weight gains as the ethanol treated *Moringa oleifera* seed cake (ETMOSC) increased in the diet of the birds. The feed consumption values was very significantly ($p < 0.05$) affected by the dietary replacements of soyabean with ethanol treated MOSC. There was a decrease in feed consumption record per day as the level of treated MOSC escalate in the diet. The feed conversion ratio of the birds was significantly ($p < 0.05$) affected by the increase in the dietary incorporation of the ethanol treated MOSC in the diet of the birds. The feed conversion ratio favoured birds on the reference diet. There was no mortality recorded during the course of this trial.

Table 3.2: Analysed Nutrient Content of Experimental Diets for Broilers

Parameters %	Inclusion levels of MOSC (%)				
	0	5	10	15	20
Dry matter	90.15	90.37	90.13	90.18	90.67
Crude protein	23.20	21.91	22.41	21.98	22.78
Ether extract	2.47	2.90	2.70	2.71	2.46
Crude fibre	2.64	2.62	2.30	2.77	2.70
Ash	8.64	8.11	7.34	9.45	8.59
NFE	53.2	54.83	55.38	53.27	54.14

Table 3.3: Proximate Composition of Raw *Moringa Oleifera* Seed Cake

Parameters	(%)
Dry matter	89.31
Crude protein	33.82
Ether extract	13.37
Ash	4.78
Crude fibre	5.06
NFE	32.28

Table 3.4: Mineral Composition of Raw *Moringa Oleifera* Seed Cake

Parameters	mg/100g
Magnesium	84.60
Phosphorus	634.01
Aluminium	0.09
Boron	13.60
Cobalt	0.18
Manganese	85.32
Molybdenum	125.69
Iron	51.80
Nickel	3.20
Copper	2.86
Selenium	0.58
Zinc	0.08
Chromium	<0.01
Cadmium	<0.01
Arsenic (As)	0.00
Lead (Pb)	0.00

Table 3.5: Phytochemical Constituents of *Moringa Oleifera* Seed Cake

Parameters	Amounts
Saponin (%)	36.08
Phytate (mg/100g)	173.20
Tannins (mg/100g)	132.16
Oxalate (mg/100g)	109.40
Alkaloid (mg/100g)	283.58
Flavonoid (%)	86.42
Phenols (%)	38.16
Glycoside (%)	0.00
Terpenoids (%)	72.44

3.6.3. Serum Biochemical Indices of Broilers Fed Ethanol Treated *Moringa Oleifera* Seed Cake Diets

Table 3.7 presents the biochemical indices of the broiler chickens given dietary ethanol treated MOSC diet. There was a significant ($p<0.05$) rise in the serum urea as the ethanol treated MOSC increased in the diet. There was a high ($p<0.05$) rise in the creatinine levels of the birds as the inclusion of the ETMOSC increased in the diet of the birds.

There was a significant ($p<0.05$) decrease in the total protein levels and a decrease ($p<0.05$) in the globulin levels as the ethanol treated MOSC increased in the diet of the birds. The albumin levels revealed a decrease in the trend as the ethanol treated MOSC increased in the diet of the birds. The serum aspartate amino transaminase (AST), alanine amino transaminase (ALT) and alkaline phosphatase (ALP) activities of the birds increased ($p<0.05$) with increasing inclusion level of the ethanol treated MOSC.

3.6.4 Haematological Indices of Broilers Fed Ethanol Treated *Moringa Oleifera* Seed Cake Diets

The hematological indices of the birds fed with ethanol treated *Moringa oleifera* seed cake diet are presented in Table 3.8. The red blood cells, PCV, haemoglobin, white blood cell, and heterocyte were significantly ($p<0.05$) affected with an increase in the values as the incorporation of ethanol treated MOSC increased in the diet of the birds. The Lymphocyte, mean cell volume and the mean cell haemoglobin of the birds were not significantly ($p>0.05$) different by the inclusion of the ETMOSC in the diets of the birds. There was an irregular

pattern in the MCHC and platelets with significant ($p < 0.05$) differences as the ETMOSC increase in the diets of the birds.

Table 3.6: Performance Characteristics of Broiler Birds Fed Ethanol Treated *Moringa Oleifera* Seed Cake Diets

Parameters	Inclusion levels of ETMOSC (%)					SEM
	0	5	10	15	20	
Daily weight gain (g/bird/day)	44.72 ^a	26.46 ^b	19.87 ^c	14.57 ^d	12.75 ^e	0.608
Feed intake(g/bird/day)	81.88 ^a	53.37 ^b	41.96 ^b	33.61 ^c	30.86 ^c	0.532
Feed conversion ratio	1.83 ^e	2.02 ^d	2.11 ^c	2.31 ^b	2.42 ^a	0.001

SEM= Standard error of mean*=P<0.05 Means with different superscript along the same rows are significantly (P<0.05); NS=Non Significant (P>0.05); ETMOSC=Ethanol treated *Moringa oleifera*. Seed cake

3.6.5: Nutrient Retention of Broiler Birds Fed Ethanol Treated *Moringa Oleifera* Seed Cake Diets

Table 3.9 presents the nutrient retention of broilers fed with ethanol treated MOSC diet. The nutrient retention of the dry matter and fat were significantly ($p<0.05$) decreased as the ETMOSC increased in the diets of the birds. The crude protein retention was significantly ($p<0.05$) different among the treatment groups. There was a decline in the dry matter retention as the ETMOSC increased in the diet of the birds. The trend in the crude protein retention disclosed that the birds on the control diet had better crude protein digestibility compared with its counterparts in other treatment groups. There was reduction in the fat retention as the ETMOSC increased in the diet of the birds.

3.6.6 Histology of the Liver of Broiler Birds Fed Ethanol Treated *Moringa Oleifera* Seed Cake Diets

The photomicrographs showing the liver of the birds fed the ETMOSC diet are presented in Figure 1 (A-E). The sections of the liver revealed that, as the ETMOSC increased in the diet of the birds, the architecture of the liver in the birds were severely affected with severe hemorrhage of hepatic blood vessels.

Table 3.7: Serum Biochemical Indices of Broiler Birds Fed Ethanol Treated *Moringa Oleifera* Seed Cake Diets

Parameters	Inclusion levels of ETMOSC (%)					SEM
	0	5	10	15	20	
Urea (mmol/l)	1.40 ^c	1.45 ^c	2.60 ^b	3.20 ^b	4.35 ^a	0.15
Creatinine (mmol/l)	135.00 ^c	159.00 ^b	162.50 ^b	236.00 ^a	239.50 ^a	31.50
Total protein (g/dl)	39.00 ^a	24.50 ^b	23.50 ^c	22.00 ^c	18.50 ^c	11.80
Albumin (g/dl)	17.00 ^a	15.50 ^b	15.50 ^b	15.00 ^b	11.50 ^c	2.70
Globulin (g/dl)	12.00 ^a	9.00 ^{ab}	8.00 ^{ab}	7.00 ^b	7.00 ^{ab}	3.20
Albumin-Globulin ratio	1.4 ^a	1.72 ^a	1.93 ^b	2.14 ^c	1.64 ^c	0.20
Aspartate transaminase(IU/l)	40.50 ^c	42.50 ^c	46.00 ^b	53.50 ^{ab}	60.00 ^a	9.10
Alanine transaminase (IU/l)	53.00 ^c	57.50 ^c	79.00 ^b	83.50 ^b	95.50 ^a	14.70
Alkaline phosphatase(IU/l)	111.00 ^c	116.50 ^c	128.00 ^b	131.50 ^b	142.00 ^a	2.22

SEM= Standard error of mean*=P<0.05 Means with different superscript along the same rows are significantly (P<0.05); NS=Non Significant (P>0.05);. ETMOSC=Ethanol treated *Moringa oleifera* seed cake

Table 3.8: Blood Composition of Broiler Birds Fed Ethanol Treated *Moringa Oleifera* Seed Cake Diets.

Parameters	Inclusion levels of ETMOSC (%)					SEM
	0	5	10	15	20	
Red Blood Cell (x 10 ¹² /l)	3.37 ^a	3.32 ^a	3.20 ^b	2.12 ^c	1.48 ^c	0.54
Haemoglobin (g/dl)	5.60 ^a	4.90 ^a	4.40 ^b	3.55 ^b	2.15 ^c	1.55
Packed Cell Volume (%)	25.00 ^{ab}	23.00 ^{ab}	21.50 ^{ab}	20.50 ^b	16.50 ^c	4.50
Mean Cell Volume (fl)	58.50	61.00	63.00	59.00	63.00	6.90
Mean Cell Haemoglobin (pg)	16.70	16.80	17.40	15.75	16.35	0.60
Mean Cell Hae. Conc (g/dl)	28.45 ^b	27.55 ^b	27.45 ^b	34.10 ^a	26.45 ^b	3.12
White Blood Cell (x 10 ⁹ /l)	5.46 ^a	5.38 ^a	5.53 ^a	4.41 ^b	3.62 ^c	0.20
Lymphocyte (%)	58.00	58.50	58.50	47.00	47.50	18.70
Heterocyte (%)	39.50 ^d	39.50 ^d	48.00 ^c	60.00 ^a	53.50 ^b	2.17

SEM= Standard error of mean=P<0.05; Means with different superscript along the same rows are significantly (P<0.05); NS=Non Significant (P>0.05); Mean Cell Hae. Conc= Mean Cell Haemoglobin Concentration, ETMOSC=Ethanol treated *Moringa oleifera*. seed cake

Table 3.9: Nutrient Retention of Broiler Birds Fed Ethanol Treated Seed Cake Diet

Parameters (%)	Inclusion levels of MOSC (%)					SEM
	0	5	10	15	20	
Dry matter	80.39 ^a	42.66 ^b	40.28 ^b	34.87 ^{bc}	31.31 ^c	1.32
Crude protein	86.96 ^a	78.96 ^b	77.56 ^b	72.63 ^b	70.52 ^c	4.35
Fats	78.89 ^a	72.79 ^b	70.76 ^b	54.99 ^c	52.95 ^c	0.91

SEM= Standard error of mean; LOS= Level of Significance; *=P<0.05; **^c Means with different superscript along the same rows are significant (P<0.05), ETMOSC=Ethanol treated *Moringa oleifera* seed cake

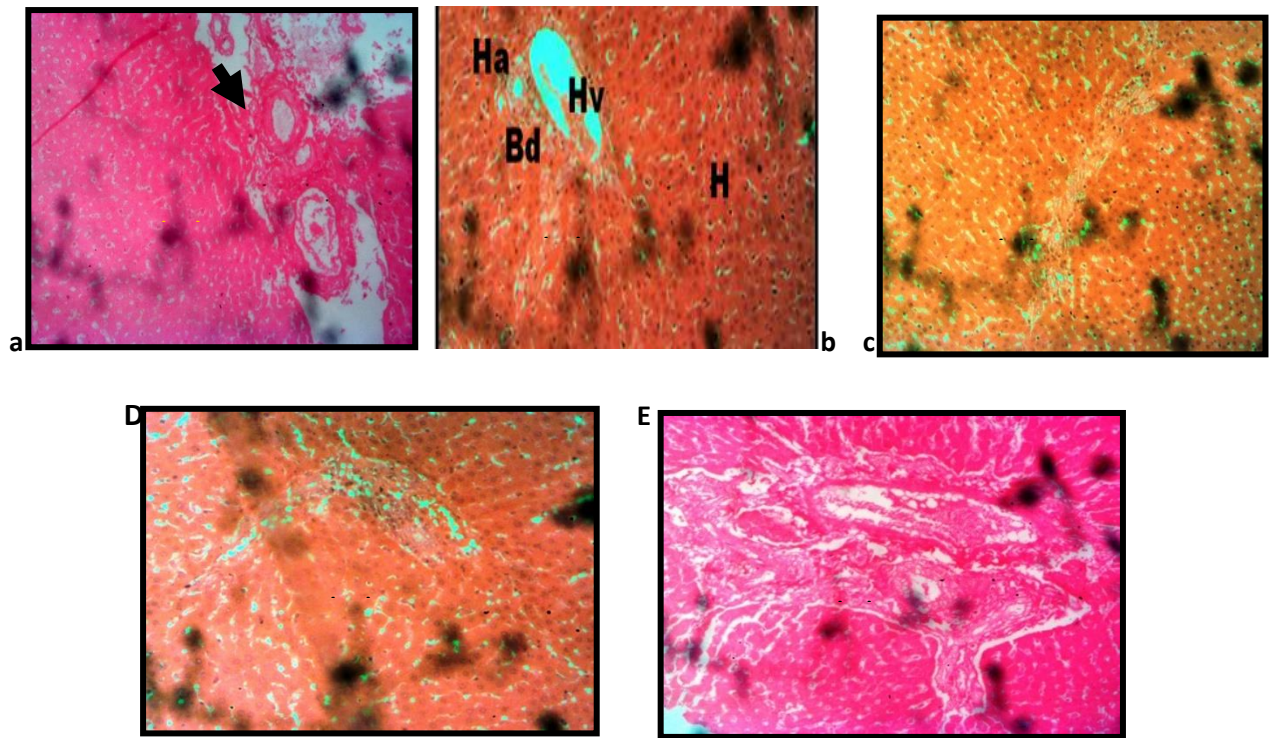


Plate 1 (A-E): Photomicrographs showing the liver of broilers fed the experimental diets, control (A) with normal hepatocyte, 5% ETMOSC (B) with normal hepatocyte, 10%ETMOSC (C) and 15% ETMOSC (D) with mild hemorrhage of hepatic blood vessel and 20% ETMOSC (E) with severe hemorrhage of hepatic blood vessel. Ha, hepatic artery; Hv, hepatic vein; Bd, Bile duct; H, hepatocyte.

H/E Stain Mag. X400

The Chemical composition: *Moringa* seed was established to be high in crude protein making it a suitable alternative feed ingredient to replace soyabean as a protein source in animal feeding. The crude protein values of 33.82 % obtained in this study for raw MOSC is within the span of 37.08- 44.40% reported by Van Eys *et al.*, (2004) for soyabean. The cake also has appreciable levels of minerals, fat and crude fibres which are similar to the values of 4.86-6.65%, 2.18-18.38%, and 5.12-6.75% reported by Van Eys *et al.* (2004) for the mineral, fat and crude fibre compositions of soybean respectively. Makkar and Becker (1997) worked extensively on the chemical composition of *M. oleifera* parts and reported that moringa leaves, the kernel and the fat free kernel meals contain 26.4%, 36.7% and 61.4% of crude protein, respectively. The raw MOSC was found to be high in phytate, tannins, oxalate, alkaloids, flavonoids and terpenoids necessitating the need for proper processing before its inclusion in diets for the acceptability and utilization of the fed animal.

Growth performance characteristics: A significant depression in growth performance of birds was observed as the dietary incorporation of ETMOSC replaced soyabean in the diets of the birds. Espe *et al.*, (2006), observed that diets which is plant protein based may lower growth. This is in line with the finding in the present study when reduction in growth rate could be as a result of decrease in feed.

The current result obtained could also be buttressed by the reports of Francis *et al.* (2001) who attributed growth depression to plant anti- nutrients (toxins) like saponins, cyanogenic glycoside, oxalate, phytate, tannins in MOSC. These phytotoxins are found to diminish the

nutrients bioavailability if present at high amounts. Similarly, Igile (1996) reported that the phytochemicals elicit toxic biological responses with possible physiological implications. The increase in level of replacement of soyabean by the ETMOSC in the diets of the birds might have elicited toxic biological responses in the birds with possible physiological implications such as the reduction in final live weights recorded in diets with 5%, 10%, 15% and 20% ETMOSC, relative to the control diet not containing ETMOSC.

The average daily weight gains of the birds was a reflection of their daily feed intakes as the birds on the control diet had superior average daily weight gains due to the highest feed intake while there was a corresponding decrease in the average daily weight gains in treatments with 5%, 10%, 15% and 20% ETMOSC, which caused decreased feed consumption. This view supports the findings of Feltwell and Fox (1979) who confirmed that growth may be regarded as a feed consumption function since live weight of birds increased in correlation to the amount of feed consumption above that which is needed for nurture.

The reduced weight gain observed in treatments with inclusion of 5,10,15,and 20% ETMOSC is in agreement with the investigations of Su and Guo (1986), Pant *et al.* (1989) that saponins reduce uptake of nutrients (carbohydrates, proteins) with concomitant decrease in rate of growth and efficiency of feed in animals. The significant ($p<0.05$) decrease in feed intake of birds fed 10%, 15%, and 20% ETMOSC agreed with the findings of Aletor (1993) that tannins do cause decrease in feed consumption especially in monogastrics. The decreased feed intake observed also supports the results of Tapiers *et al.* (2002) who discovered that tannins have astringent and other properties that negatively influence palatability, lower feed consumption and consequently body weight gain and growth.

There was an indication that birds fed the control diet and 5% ETMOSC had better feed conversion ratios. The best FCR observed in birds on the control diet which had no anti-nutrients followed by the birds fed 5% ETMOSC indicating that at levels higher than 5%, deleterious effects were observed. Dietary replacement of soyabean by ETMOSC beyond 5% inclusion led to very poor feed conversion ratios as observed with 10, 15 and 20% ETMOSC. This observation is in line with past works (Reddy *et al.* 1982; Cheeke and Shull, 1985; Su and Guo, 1986; Thompson 1993; Aletor 1993; Makkar and Becker 1997) who reported that anti-nutrients lower bioavailability of minerals in monogastric animals and negatively influence starch and protein digestion; that the phytochemicals, saponins, trypsin inhibitors are ANFs that affect monogastrics but do not affect ruminants because they are to some extent capable of degrading the toxins in their rumen. The ANFs present in the ETMOSC limited the usage of the nutrients present in the diets by the birds as saponins reduce uptake of nutrients; carbohydrates and proteins hence reducing growth rate and feed efficiency in monogastric animals, phytates inhibit protein digestion by complexing them and tannins lower feed intake in monogastrics, complex dietary proteins including digestive enzymes by forming complexes with them that are not readily digested.

The significant difference ($p < 0.05$) in dry matter retention in broilers in this research supports the findings of Aletor (1993) that high tannin in the diets of livestock induced low feed intake in animals especially monogastrics, binds dietary proteins including digestive enzymes by forming networks that are not easily digested hence increasing levels of tannins in the diet of the birds as the graded levels of the ETMOSC increased may have formed complexes with the digestive enzymes in the birds fed 5, 10, 15, and 20% (in contrast to their counterparts in the

control diet not fed the test ingredient) thus limiting the digestibility of dry matter and the undigested feed voided out without the nutrients utilized and retained by the animals.

Crude protein retention in the birds was significantly different ($p < 0.05$) significant, as dietary inclusion of the ETMOSC increased. The low protein retention recorded for the 10,15,and 20% ETMOSC diets could have contributed to lower PCV observed for the diets, because retained proteins furnish the body with amino acids that are used for the synthesis of body proteins including blood proteins.

The observed significant decrease ($p < 0.05$) in the fat retention as the ETMOSC incorporation levels rise in the diet of birds supports the discoveries of Su and Guo (1986); Pant *et al.*, (1989) that saponins interferes with the absorption of lipids, vitamin A, vitamin E and causes hypocholesteromia by binding cholesterol, making it unavailable for absorption. The increasing presence of the anti-nutrients in dietary treatments of 10,15 and 20% compared with their counterparts in treatment with 5% fed lower inclusion level of the ETMOSC limited the fat retentions in these treatment groups.

There is no mortality recorded in this study which suggests that the birds could tolerate inclusion of the test ingredient in diets up to 20% replacement with MOSC. This suggests that with proper processing methods of *Moringa oleifera* seed cake, it could be a good alternative novel feedstuff for animal feeding.

Serum biochemistry is a generalized medium of evaluating the health status of animals based on biochemical indices. The blood parameters are potent indices of the physiological, pathological and nutritional status of an organism (Babatunde and Olusanya 1992). Alterations in blood compositions are indirect indices to assess the metabolic state of an animal together with the feed quality.

Increase in serum urea as the ETMOSC increased in diets supported the findings of Omole and Sonaiya (1981) that increase in serum urea was a result of hydrolysed meal inclusion in diet. The authors opined that, the more the test feed was incorporated in the broilers diet, the more the birds exist at the detriment of their body reservoir. The significant increase in serum creatinine of the birds as the level of ETMOSC increased in diets gives evidence to the fact that the birds were surviving at the expense of their body protein reserves thus suggesting the reduced weight in the birds while ETMOSC increased in the diet. Ogbuewu *et al.* (2010) found

that high level of serum creatinine in the body signals muscle wastage. The decrease in total protein, globulin and albumin with increasing level of ETMOSC in diets indicate that ETMOSC could not meet the protein requirements of the chickens. Onifade and Tewe (1993) had earlier reported that serum protein, albumins and globulins are generally influenced by total protein intake.

ALP, AST and ALT were elevated when ETMOSC concentration in the diet was increased. These suggest the release of aminotransferases from cytoplasm to the blood stream and indicative of possible damage to the liver or other tissue. This is consistent with report of Muhammed and Oloyede (2010) who fed fermented *Teminalia cattapa* seed meal based diets to chicks. The increase in ALT values in birds with 5, 10, 15 and 20% seed cake compared with those on control suggest that there may be a likelihood of liver damage by the ETMOSC.

The range of $1.48-3.37 \times 10^{12}/l$ for red blood cells (RBC) achieved in this research was lower than the standard span of $3-6 \times 10^6/dl$ for chicken as established by Madubuike and Ekenyem (2006). It was found that when ETMOSC increased in diets, the red blood cells (RBC) decreased. The range of 2.15- 5.60g/dl haemoglobin values obtained in this study is lower than 7-18.6/dl reported for normal birds by Mitruka *et al.*, (1977). This implies that incorporation of moringa kernel meal in diet of broiler had obstructive influence on the health of the birds with regards to blood composition.

The range of PCV (16.5-25%) across all the experimental diets is below the normal values (30-33%) established for broilers at six weeks of age reported by Mitruka and Rawnseley (1977) and 28 - 30.70% reported by Ogbuewu *et al.*(2010). MCV reflects the size of RBC by

expressing the volume occupied by a single RBC. MCV of 58.50-63.00fl obtained in this research is contrary to the span between 115-127fl reported by Swenson (1993).

The mean cell haemoglobin (MCH) gives the average weight of haemoglobin in the red blood cells. The non-significant ($p>0.05$) effect observed for MCH among the dietary treatments implies that the inclusion of the processed MOSC in diets did not influence the average weight of the haemoglobin in the blood. However, the range of 15.75-17.40pg MCH reported in this study was lower than 25-27pg reported by Swenson (1993).

MCHC measures the average concentration of haemoglobin in red blood cells. It is the most valuable parameter in evaluating the therapy for anaemia because haemoglobin and haematocrit are used not RBC in its calculation. The mean cell haemoglobin concentration (MCHC) values of 26.45-28.45g/dl reported in this research are comparable with the standard values of 25.3-32.5g/dl reported by Mitruka and Rawsley (1977). Low MCHC means that a unit of packed RBC contains less haemoglobin than normal and a high MCHC means that there is more haemoglobin in a unit of RBC. Decreased MCHC levels may signify iron insufficiency, blood loss and B₆ deficiency. In this present study, the MCHC values indicate that the birds did not exhibit any anaemic symptom.

The high values for lymphocytes obtained is suggestive of the resistance of the birds to diseases. This implies that the inclusion of ETMOSC in diets of birds may be responsible for either high or low neutrophil counts. Therefore, the replacement of soyabean with ETMOSC meal negatively affect the physiological and pathological status of the birds.

The haematological results of the birds showed lower values of haemoglobin, red blood cells, packed cell volume and white blood cells of birds fed ETMOSC. This observation support the report of Apata (1990) that haemagglutinins in raw or partially detoxified grain legumes have the ability to agglutinate the erythrocytes of numerous animal species, thus leading to dysfunction of red blood cell haematopoiesis and a toxic induced red blood cell heamolysis and increase in the plasma volume.

Histology of the liver sections of the birds revealed that when ETMOSC expand in diets of the birds, degeneration of the hepatocytes and coagulative necrosis were noticed in birds fed 20% ETMOSC diets and were less obvious in birds fed lower levels of Ethanol- treated MOSC diets, whereas no changes were seen in birds fed with control diet.

The severe hepatocyte degradation and inflammation of the bile ducts observed in the liver of the birds fed 20% ETMOSC and mild hemorrhage of hepatic blood vessel in 5% ethanol treated MOSC diet supports the report of Ortiz *et al.*, (1994)who investigated that chicks fed tannin incorporated diets showed hydropic degeneration of hepatocytes of the liver. Aletor and Fetuga (1988) also reported cell necrosis and fatty acid changes in liver of rats fed raw lima bean.

CHAPTER FOUR

EXPERIMENT TWO

4.0 THE EFFECT OF REPLACING SOYBEAN WITH GAMMA IRRADIATED *MORINGA OLEIFERA* SEED CAKE IN DIETS OF BROILER CHICKENS

4.1 Introduction

Poultry production is among the major global agricultural business. Therefore, research on meat production world-wide shows chicken as the rapidly growing livestock farming particularly in developing countries. A major developmental challenge facing most developing countries like Nigeria in Africa is food insecurity due to the high population growth in Africa and low income. The request for eggs and poultry meat has drastically increased in recent years across large parts of the continent (WHO, 2010). The livestock producers appeared most affected in terms of exorbitant cost of feed ingredients. Apata and Ojo, (2000), reported that Poultry production will continue to exist as one of the genuine techniques of attaining verifiable and quick production of high quality animal protein to satisfy the escalating request of the Nigerians.

Because of limited supply of soyabean due to competition between man and animals many farmers have resolved to finding substitute feed ingredients for protein among which *Moringa oleifera* is an example. Food/feed processing by irradiation is relatively a new treatment technique evolved in the course of technological progression. Irradiation has been applied in numerous different uses and in various aspects of life including industries, medicine, and agriculture. In agriculture, it has been widely employed to produce improved varieties in plants and animals. Food or feed processing through this method involves treating a material with

radioactive particles from radioisotope sources of cobalt-60, Caesium-137 or with accelerated electrons with energies up to 10MeV or X-rays with energies up to 5MeV (Farkas, 2006). Radioactive rays are used in food pasteurization, sterilization, disinfection, disinfestations and inhibition of sprouting in cereals, nuts and seeds.

Past investigators (Farag, 1989; Sattar *et al.* 1990; El-Morsi *et al.* 1992; Farag, 1998) reported that gamma irradiation technique with its radiolytic reaction could decontaminate or significantly inactivate protease inhibitors. There is no data on the use of gamma irradiation for the process of *M. oleifera* seed cake for feeding broilers.

4.2 Objective

The aim of this experiment was to determine the potential of gamma irradiation in destruction of the phytochemicals in *M. oleifera* seed cake for enhanced suitability in broilers diet.

4.3 Materials and Methods

4.3.1 Experimental site

The experiment was accomplished in the Poultry Unit of the Teaching and Research Farm, Kwara State University, Malete, Nigeria.

4.3.2 Source of experimental birds

One hundred and fifty day-old Arbor acre breed of broiler birds were purchased from Novic Farms at Ibadan, Oyo State Nigeria.

4.3.3 Collection of Moringa Seed Cake

Moringa oleifera seed cake was obtained from Moringa processing plant at Afe Babalola University, Ado-Ekiti, Nigeria.

4.3.4 Experimental Diets

Moringa oleifera seed cake was properly sun dried to a permanent weight, milled and kept in airtight container before irradiation and included in the dietary mixture. 15kg of the milled *Moringa oleifera* seed cake was divided into four batches of equal parts and placed in opened polythene bags. A portion was left not irradiated representing raw or untreated *Moringa oleifera* seed cake sample. The other three bags of *Moringa oleifera* seed cake samples were irradiated

at a time in a gamma L-110 machine until the required total doses of 10, 30 and 50 kGy was achieved . Gamma irradiation was carried out at Central Research Laboratory, Tanke, Ilorin, Nigeria.

Five experimental diets were formulated made of a corn-soy reference diet (control diet) and four other diets having raw (untreated *Moringa oleifera* seed cake) and gamma irradiated *Moringa oleifera* seed cake at doses of 10, 30 and 50KGy respectively before incorporation in the diet to replace soyabean at the same 5% incorporation level corresponding to diets 1 ,2, 3, 4 and 5 respectively. The experimental diets composition for the broilers on as fed basis is presented on Table 4.1.

4.3.5 Management of Experimental Birds and Data Collection

One hundred and fifty day- old broiler chicks were used for the trial. The chicks were fed on standard starter diet for a week prior to the onset of this study. The experiment was designed as a complete randomized design. The dietary treatments contained three replicates with ten chicks per replicate. All the birds were raised under the same management practices all through the eight week experimental except for the diets offered that differed. Feed and water were proffered to the birds *ad-libitum* and weighed weekly. Standard vaccinations and medications were carried out as at when due.

Table 4.1: Composition (%) of Experimental Diets

Ingredients	Irradiation doses (KGy)				
	(control)	(untreated)			
	0	0	10	30	50
Maize	52.00	52.00	52.00	52.00	52.00
GIMOSC	0.00	5.00	5.00	5.00	5.00
Soya bean meal	31.00	26.00	26.00	26.00	26.00
Wheat offal	10.00	10.00	10.00	10.00	10.00
Palm oil	1.50	1.50	1.50	1.50	1.50
Lysine	0.10	0.10	0.10	0.10	0.10
Methionine	0.25	0.25	0.25	0.25	0.25
Bone meal	1.15	1.15	1.15	1.15	1.15
Oystershell	1.50	1.50	1.50	1.50	1.50
Salt	0.25	0.25	0.25	0.25	0.25
*Vit/Min premix	0.25	0.25	0.25	0.25	0.25
Fish meal	2.00	2.00	2.00	2.00	2.00
Total	100	100	100	100	100
Calculated chemical analysis					
ME (Kcal/kg)	2954.39	2959.64	2942.97	2958.20	2945.85
CP (%)	23.18	23.13	23.15	23.18	23.12
CF (%)	3.20	4.11	3.13	3.58	4.23
EE (%)	4.65	9.13	5.85	6.23	4.67
Methionine (%)	0.49	0.58	0.35	0.48	0.45
Lysine (%)	1.25	1.50	1.08	1.45	1.1

GIMOSC=Gamma irradiated *Moringa oleifera* seed cake

4.3.6 Data Collection/Response Criteria

In the course of the experiment, data were obtained on the performance characteristics: feed consumption, daily weight gain, feed conversion ratio, survival rate, and carcass evaluation at the end of eight week study period

4.3.7 Nutrient Digestibility Trial

A nutrient digestibility trial was accomplished at the 8th weeks of feeding period. Three birds were selected randomly per treatment. The trial lasted three days. Data were taken on feed intake and faecal discharge during the three days. The faecal samples were oven dried, and representative samples were taken for chemical analysis. Analysis of the proximate composition of the samples was done according to the methods of AOAC (1990).

$$\text{Apparent Nutrient Retention (\%)} = \frac{\text{Nutrient intake} - \text{Nutrient in faeces} \times 100}{\text{Nutrient intake}}$$

4.3.8 Serum Biochemical and Haematological Analysis

At the expiration of the research, one broiler was taken at random per replicate in all the treatments was taken at random and blood collected by decapitation. Blood for biochemical indices was collected in bottles with no EDTA for the analysis of the biochemical parameters. The specimen were kept in cooled condition before analysis in the laboratory.

The blood specimen for haematological parameters were preprocess with ethylene diamine tetra-acetate (EDTA), and were kept in cooled condition before analysis in the laboratory.

Haematological indices examined include white blood cell (WBC) red blood cell (RBC), packed Cell Volume (PCV), haemoglobin (Hb). The mean cell volume (MCV), mean cell haemoglobin (MCH) and the mean cell haemoglobin concentration (MCHC) were calculated according to the methods of Jain (1986).

4.3.9 Carcass Traits and Vital Internal Organs/Glands

At the end of the experimental period, three birds from each dietary treatment were randomly chosen, weighed and slaughtered. Just after complete bleeding, their carcasses were scalded, feather plucked and immediately processed.

4.3.10 Histology Examination of the Liver

Three birds were selected per treatment at the expiration of the feeding trial and sacrificed to obtain the liver. Sections were obtained from the anterior lobe of the liver, preserved in 10% formal-saline solution. The liver tissues from each replicate were processed using haematoxylin and eosin stains for histological studies. Digital microscope with a magnification of 400 was used to take photo micrographs. The analysis was accomplished at the histopathological Laboratory of Faculty of Veterinary Medicine, University of Ilorin.

4.4 Statistical Analysis

Data collected on the response criteria were put through analysis of variance according to the completely randomized design model and differences between treatment means were separated using the Duncan's multiple range test (Steel and Torrie, 1980).

4.5 Results

4.5.1 Growth Performance Characteristics of Gamma Irradiated *Moringa Oleifera* Seed Cake Diets

The proximate composition of the treated *Moringa oleifera* seed cake diet is presented in Table 4.2

Table 4.3 presents data on performance characteristic of broilers fed raw and graded doses of gamma irradiated *Moringa oleifera* (MOSC) diet. The average daily weight gains of the birds were greatly ($p<0.05$) affected by the dietary inclusion of raw and graded doses of gamma irradiated MOSC diet. There was a decrease in the average daily weight gains of birds fed with raw and a comparable average daily weight gains of birds fed with graded doses of the gamma irradiated diets. The feed intake values were very remarkably ($p<0.05$) affected by the dietary replacements of soyabean with graded doses of gamma irradiated MOSC. Except for raw diet (diet 2), there was an increase in the feed intake values of birds per day with graded doses of gamma irradiated MOSC in the diet. The feed conversion ratio of the birds was significantly ($p<0.05$) affected by the increase in the doses of the gamma irradiated treated MOSC in the diet of the birds. The feed conversion ratio was superior in birds on the reference diet. There was no mortality recorded during the course of this trial.

Table 4.2: Analysed Nutrient Content of Gamma Irradiated MOSC Diets for Broilers

Parameters %	Inclusion doses of MOSC (KGy)				
	0(control)	0(untreated)	10	30	50
Dry matter	86.69	92.71	91.84	92.51	93.19
Crude protein	22.20	22.60	22.20	21.75	21.80
Ether extract	3.64	3.84	3.19	2.83	3.43
Crude fibre	8.73	8.71	8.70	8.71	2.70
Ash	7.44	7.47	7.44	7.44	7.55
NFE	44.68	50.09	46.31	51.78	57.71

Table 4.3 Growth Performance Characteristics of Broiler Birds Fed Gamma Irradiated *Moringa Oleifera* Seed Cake Diets

Irradiation doses of MOSC (KGy)								
			(control)	(untreated)				
Parameters			0	0	10	30	50	SEM
Daily	weight	gains	59.69 ^a	47.007 ^b	59.030 ^a	59.64 ^a	58.18 ^a	1.99
(g/bird/day)								
Feed		intake	104.22 ^{ab}	98.473 ^b	106.66 ^a	108.57 ^a	107.13 ^a	3.94
(g/bird/day)								
Feed conversion ratio			1.75 ^b	2.100 ^a	1.807 ^b	1.82 ^b	1.84 ^b	0.05

SEM= Standard error of mean;*=P<0.05; Means with different superscript along the same rows are significantly (P<0.05);

MOSC=*Moringa oleifera* seed cake

4.5.2 Carcass Characteristics of Broilers Fed Gamma Irradiated *Moringa Oleifera* Seed Cake Diets

The carcass characteristics of broilers fed gamma irradiated *Moringa oleifera* seed cake diets are presented on Table 4.4. At the end of the experimental period, three birds from each dietary treatment were randomly chosen, weighed and slaughtered. Just after complete bleeding, their carcasses were scalded, feather plucked and immediately processed. The carcass characteristics of the birds fed the treated gamma irradiated (MOSC) diets were superior than the group fed with the raw diet. The differences in the live weights, carcass weights, and the dressing percentage were significantly ($p < 0.05$) affected across the treatment groups on the test feedstuff. There was a corresponding decrease in the live weights, carcass weights, and the dressing percentage of the birds with the inclusion of graded dose of gamma irradiated *Moringa oleifera* seed cake in the diets of the birds. The weights of the carcass components like the head, neck, wings, thigh, drumstick, breast, shank, back expressed as percentages of the live weight were lower in all the dietary groups fed the treated feedstuff compare with the control group. There were inconsistencies in the weights of the internal organs of the birds fed with the raw and treated groups and inclusion of the gamma irradiated MOSC could not be said to have either or not enhanced the weights of the organs. The weights of the lung, heart, kidney, proventriculus were not seriously ($P > 0.05$) affected except the gizzard that was totally ($p < 0.05$) affected.

Table 4.4 Carcass Characteristics of Broiler Birds Fed Gamma Irradiated *Moringa Oleifera* Seed Cake Diets

Parameters	Irradiation doses of MOSC (KGy)					SEM
	0(control)	0(untreated)	10	30	50	
Live weight (g/bird)	1,968.00 ^a	1,365.00 ^c	1,890.00 ^b	1,871.02 ^b	1,889.50 ^b	5.91
Carcass weights (g/bird)	1,275.00 ^a	672.00 ^b	1197.00 ^b	1178.02 ^b	1196.50 ^b	3.37
Dressing percentage (%)	64.78 ^a	49.23 ^c	63.33 ^b	62.96 ^b	63.32 ^b	2.53
Prime cut and Organ weights						
Head	2.97 ^a	2.51 ^b	3.05 ^a	2.39 ^b	3.09 ^a	0.37
Thigh	12.64 ^a	7.68 ^c	9.39 ^b	9.82 ^b	12.42 ^a	0.53
Breast	17.07 ^a	13.11 ^c	14.31 ^{ab}	15.32 ^{ab}	16.60 ^a	0.77
Wings	9.45 ^a	8.84 ^{ab}	7.48 ^d	8.68 ^{abc}	9.42 ^a	0.43
Shank	5.46 ^a	4.55 ^b	3.96 ^{cd}	4.11 ^{bc}	5.07 ^a	0.37
Neck	6.23 ^{ab}	5.68 ^{abc}	4.67 ^c	5.54 ^{abc}	5.54 ^{abc}	0.54
Drumstick	10.91 ^a	9.22 ^{ab}	7.45 ^{bc}	7.58 ^{bc}	9.71 ^{abc}	0.81
Back	11.64 ^a	9.94 ^b	11.54 ^a	11.49 ^a	11.46 ^a	0.69
Heart	0.49	0.39	0.45	0.50	0.47	0.22
Lungs	0.53	0.40	0.45	0.57	0.58	0.23
Liver	2.02	1.22	1.87	1.96	2.01	0.48
Kidney	0.52	0.29	0.40	0.47	0.44	0.23
Proventriculus	0.58	0.48	0.42	0.50	0.50	0.25
Gizzard	2.11 ^c	3.68 ^a	2.95 ^b	2.11 ^{abc}	2.25 ^{bc}	0.46

SEM= Standard error of mean; LOS= Level of Significance;*=P<0.05;**=P<0.01;***P<0.001;a,b,c,d,e,f,gMeans with different superscript along the same rows are significantly (P<0.05). Prime cut and Organ weights are expressed as % of live weight

4.5.3 Serum Biochemical Indices of Broilers Fed Gamma Irradiated *Moringa Oleifera* Seed Cake Diets.

Table 4.5 presents the biochemical indices of the broilers fed gamma irradiated treated MOSC diet. There was an increase ($p<0.05$) in the serum urea, albumin and total protein level with the inclusion of graded doses of gamma irradiated MOSC in the diet of the birds.

The serum aspartate amino transaminase (AST), alanine transaminase (ALT) and alkaline phosphatase (ALP) levels of the birds seriously ($p<0.05$) decreased linearly with the inclusion of graded dose of gamma irradiated MOSC in the diets of the birds.

4.5.4 Haematological Indices of Broilers Fed Gamma Irradiated Treated *Moringa Oleifera* Seed Cake Diets

The hematology indices of the birds fed gamma irradiated treated *Moringa oleifera* seed cake diet is presented on Table 4.6. Haemoglobin, white blood cell, and the mean cell volume were very significantly ($p<0.05$) affected with the inclusion of graded doses of gamma irradiated MOSC in the diet of the birds. Mean cell haemoglobin concentration of the birds was not statistically ($p\geq 0.05$) different with the addition of graded doses of gamma irradiated MOSC in the diet of the birds.

4.5.5 Nutrient Retention of Broilers Fed Gamma Irradiated Treated *Moringa Oleifera* Seed Cake Diets.

Table 4.7 presents the nutrient retention of broilers fed gamma irradiated *Moringa oleifera* seed cake diet.

The nutrient retention of crude protein, fat and crude fibre were significantly ($p < 0.05$) affected with the addition of doses of gamma irradiated MOSC in the diet of the birds. The trend in the crude protein, fat and fibre retention disclosed that the birds on the control diet had better nutrient retention compared with its counterparts in other treatment groups.

Table 4.5: Biochemical Indices of Broilers Fed Gamma Irradiated *Moringa Oleifera* Seed Cake Diets

		Irradiation doses of MOSC (KGy)					
Parameters		0(control)	0(untreated)	10	30	50	SEM
Total protein (g/l)		13.927 ^b	10.230 ^{ab}	12.600 ^a	13.383 ^{ab}	13.250 ^{ab}	0.280
Urea (mmol/l)		4.657 ^b	5.070 ^a	4.380 ^{ab}	4.233 ^{ab}	4.677 ^b	0.176
Albumin (g/l)		2.727 ^{ab}	2.963 ^{bc}	2.597 ^c	2.800 ^a	2.620 ^d	0.055
Aspartate	Amino	80.000 ^a	95.530 ^{bc}	71.843 ^b	81.843 ^d	74.210 ^c	3.935
transaminase(U/L)							
Alanine	Amino	19.480 ^b	25.443 ^a	18.960	19.230 ^b	19.710 ^b	1.716
transaminase (U/L)							
Alkaline		34.680 ^{ab}	37.287 ^a	27.600 ^{cd}	25.760 ^d	31.340 ^{cb}	2.486
phosphatase(U/L)							

SEM= Standard error of mean*= $P < 0.05$ Means with different superscript along the same rows are significantly ($P < 0.05$); NS=Non Significant ($P > 0.05$); MOSC=Moringa oleifera seed cake.

Table 4.6: Haematological Indices of Broilers Fed Gamma Irradiated *Moringa Oleifera* Seed Cake Diets

Irradiation doses of MOSC (KGy)						
Parameters	0(control)	0(untreated)	10	30	50	SEM
Red Blood Cell ($\times 10^{12}/l$)	2.360 ^a	2.12 ^b	2.27 ^a	2.50 ^a	2.39 ^a	0.174
Haemoglobin (g/dl)	8.75 ^a	7.30 ^b	8.45 ^a	9.30 ^a	8.45 ^a	0.606
Packed Cell Volume (%)	31.20 ^a	20.60 ^b	30.05 ^a	32.75 ^a	30.45 ^a	2.126
Mean Cell Volume (fl)	132.25 ^a	129.60 ^a	132.35 ^a	131.45 ^a	132.65 ^a	3.278
Mean Cell Haemoglobin (pg)	37.15 ^a	36.95 ^a	37.200 ^a	37.300 ^a	36.85 ^a	0.665
Mean Cell Hae. Conc (g/dl)	28.05 ^{ab}	28.55 ^a	28.15 ^a _b	28.350 ^a _b	27.80 ^b	0.332
White Blood Cell ($\times 10^9/l$)	231.00 ^a	203.85 ^a	229.80 ^a	238.80 ^{0^a}	238.80 ^a	6.472

SEM= Standard error of mean= $P<0.05$; Means with different superscript along the same rows are significantly ($P<0.05$); NS=Non Significant ($P>0.05$); Mean Cell Hae. Conc= Mean Cell Haemoglobin Concentration.

Table 4.7: Nutrient Retention of Broilers Fed Gamma Irradiated *Moringa Oleifera* Seed Cake Diets

Parameters	Irradiation doses of MOSC (KGy)					SEM
	0(control)	0(untreated)	10	30	50	
Crude protein	77.603 ^a	61.107 ^b	76.730 ^a	77.530 ^a	75.630 ^a	2.600
Crude fibre	65.667 ^a	51.703 ^b	64.930 ^a	65.600 ^a	63.990 ^a	2.200
Fats	73.423 ^a	57.817 ^b	72.600 ^a	73.353 ^a	71.557 ^a	2.460

SEM= Standard error of mean;*=P<0.05;*** Means with different superscript along the same rows are significantly (P<0.05) different

4.5.6 Histology of the Liver of Broilers Fed Gamma Irradiated *Moringa* *Oleifera* Seed Cake Diet.

The photomicrographs showing the liver of the birds fed the gamma irradiated MOSC diet are given in plate 2 (F-J). The sections of the liver revealed that with the addition of raw MOSC in the diets of the birds, the architecture of the liver in the birds were severely affected with severe hemorrhage of hepatic blood vessels. With the addition of graded amount of gamma irradiated MOSC in the rations of the birds, the density of the hepatocyte appears normal with characteristic delineation

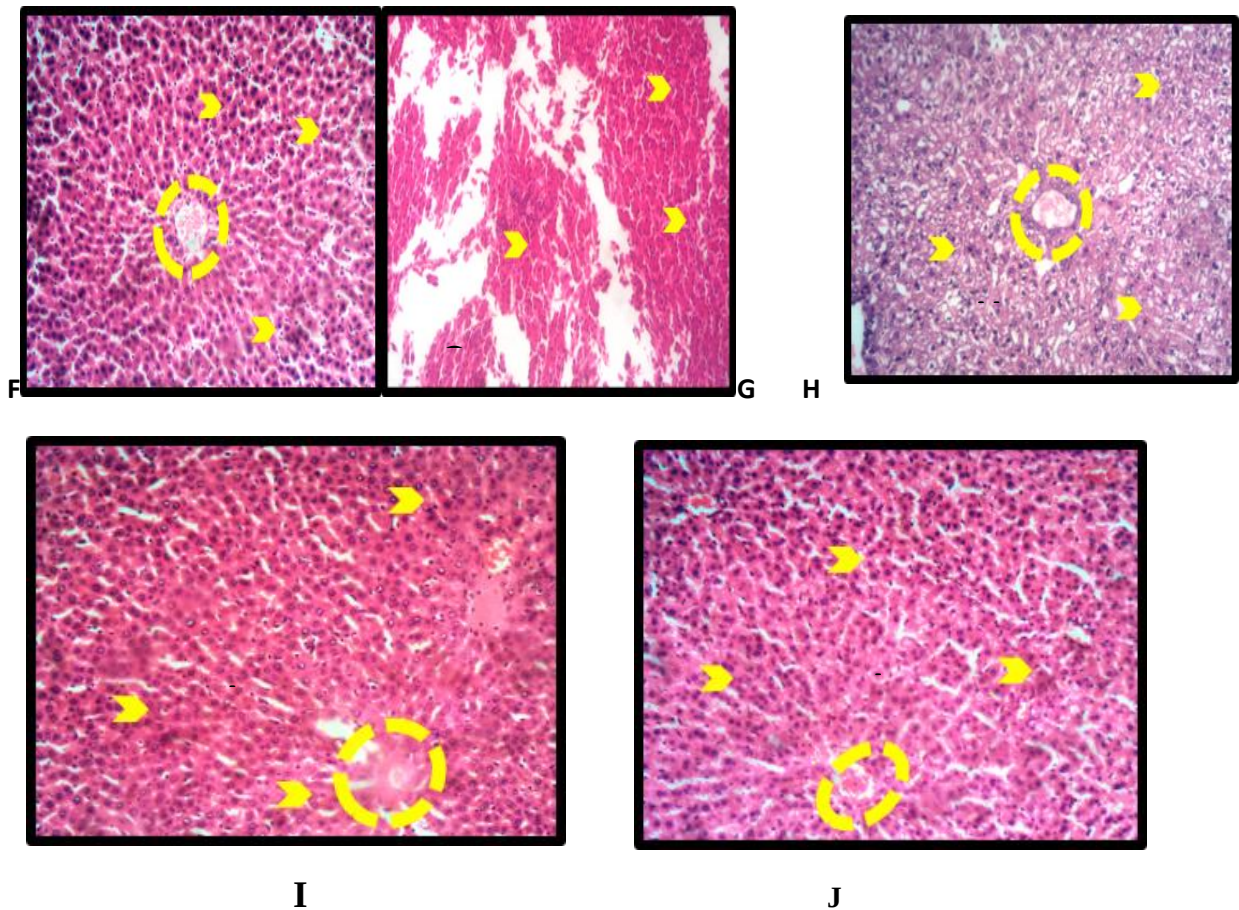


Plate 2 (F-J): Photomicrographs showing the liver of broilers fed the experimental diets, control (F) with normal hepatocyte, Raw MOSC (G) with severe haemorrhage of hepatic blood vessel, 10K Gy irradiated MOSC (H) with mild hemorrhage of hepatic blood vessel, 30 K Gy irradiated MOSC (I) with mild hemorrhage of hepatic blood vessel and 50 K Gy irradiated MOSC (J) with normal hepatocyte.

H/E Stain Mag. X400

4.5.7 Profitability of Broilers Fed Gamma Irradiated *Moringa Oleifera* Seed Cake Diets For Dietary Replacement Of Soyabean.

Table 4.8 shows the profitability of replacing soyabean using gamma irradiated *Moringa oleifera* seed cake in broilers diet. The total feed consumption and total cost of production per bird were higher in birds fed with gamma irradiated *Moringa oleifera* seed cake diet compared with the control. The reduction in cost of feed per kilogram obtained for the birds on raw MOSC diet as well as high cost of feed per kilogram obtained for the treated groups (treatments 3-5) could not enhance the profit on investment. The highest profit was achieved from the birds fed the control diet followed by those fed 30 and 50KGy gamma irradiated *Moringa oleifera* seed cake diet respectively. Losses in investment were recorded in birds fed raw and 10KGy gamma irradiated *Moringa oleifera* seed cake diets.

Table 4.8: Profitability of Broilers Fed Gamma Irradiated *Moringa Oleifera* Seed Cake Diets

		Inclusion doses of treated MOSC (KGy)					
Parameters		0(control)	0(Raw)	10	30	50	SEM
Total feed intake/bird/kg		5.83 ^a	5.51 ^b	5.97 ^a	6.08 ^a	5.99 ^a	3.94
Total weight gain/bird/kg		3.34 ^a	2.63 ^b	3.30 ^a	3.33 ^a	3.25 ^a	1.99
Cost of feed (₦/kg)		110.22	105.20	120.55	121.50	125.20	—
Cost of feeding a bird (₦)		642.58	579.65	719.68	738.72	749.94	—
Cost/weight gain (₦/kg)		192.38	220.39	218.08	221.84	230.75	—
Cost differential (₦/kg)		0.00	- 28.01	-25.70	-29.46	-38.37	—

SEM= Standard error of mean; Means with different superscript along the same rows are significantly (P<0.05);NS=Non Significant (P>0.05),MOSC= *Moringa oleifera* seed cake

Growth performance characteristics: Growth performance and feed usage were positively influenced by dietary treatment for all treatment diets. The comparable growth performance of birds on control diet and those fed gamma irradiated diets at doses 10, 30 and 50 KGy could be attributed to better use of the feed by the birds as a result of inactivation/removal of anti-nutrients in MOSC by the irradiation process. This view supports the reports of Taghinejad *et al.*, (2009) who discovered that gamma irradiation at 30 kGy totally eradicate phytate from full-fat soybeans.

Birds on the control diet had the highest average daily weight gains due to the highest feed intake while there was a corresponding decrease in the average daily weight gain in treatment 2 containing raw MOSC with the lowest feed intake. The comparable daily weight gains of the birds on control and those on treatments 3,4 and 5 may be as a result of improved feed usage by the birds. This supports the findings of Siddhuraju *et. al.*, (2002) who reported decrease in phytate content which the author attributed to the chemical disintegration to lower inositol phosphates and inositol by the action of free radicals generated by the irradiation

There was an indication that birds fed the control diet and those on gamma irradiated diets had better feed conversion ratios. The best FCR observed in birds on the control diet was due to the nonexistence of anti-nutrients in the control diet followed by the birds fed 10KGy gamma irradiated diet. Dietary replacement of soyabean by raw MOSC led to very poor feed conversion ratios as observed in untreated MOSC group. The result is in line with previous works (Makkar and Becker 1997) who reported that the saponins and trypsin inhibitors affect

monogastrics but do not affect ruminants because they are to some extent capable of degrading the toxins in their rumen. The ANFs present in the raw MOSC limited the usage of the dietary nutrients present in the diets by the birds as saponins reduce uptake of nutrients; carbohydrates and proteins hence reducing growth rate and feed efficiency in monogastric animals, phytates inhibit protein digestion by complexing them and tannins cause reduction in feed consumption in monogastrics, complex with dietary proteins including digestive enzymes by forming structures with them that are not easily digested.

Report on haematological parameters gives a mastery of the nutritional constituents of the poultry feed. It also give practical hint on the metabolic profile used to access bird's health condition (Suchy, 2000).

The observed significant ($p < 0.05$) rise in total protein, of the test diet confirms the nutritional requisite of gamma irradiated MOSC in satisfying the protein requirements of the birds. Onifade and Tewe (1993) had earlier reported that serum protein, albumins and globulins are generally influenced by total protein intake.

ALP, AST and ALT levels in this study were comparable in all the diets signifying normal organ function on feeding of *moringa* seed cake. These findings revealed that moringa seed cake fed groups were strong and healthful.

The range of $2.12 - 2.39 \times 10^{12}/l$ for red blood cells (RBC) obtained in this study was lower than $3 - 6 \times 10^6/dl$ for chicken as reported by Madubuike and Ekenyem (2006). Adamidous *et al.*, (2011), reported that substitute of 500 g kg^{-1} *Jatropha curcas* kernel meal by fish meal protein

in rainbow trout diets did not affect growth or nutrient usage.

The range in Hb values between 7.30-9.30% in this study is in line with the value of 6-13% obtained by past workers (Aiello and Mays 1998; Iheukwumere *et al.* 2008).

This can imply that incorporation of *moringa* seed cake meal in diet of broiler has no effect on the health condition of the birds with regards to blood composition. The result of this finding also recommends that the blood of the animals had a considerable oxygen carrying ability which in turn means that nutrients was not impaired when processed *moringa* seed meal is given in diet.

The range of PCV (20.6-32.75%) across all the experimental diets is within normal range of 22-35% reported Jain (1993) and 28 - 30.70% reported by Ogbuewu *et al.* (2010).

The MCV of 129.6-132.65fl obtained in this research is similar to the span between 115-127fl reported by Swenson (1993). Decreased MCV readings may signify microlytic anaemia attributable to iron insufficiency.

Similar values obtained for MCH on all the dietary treatments implies that the inclusion of the processed MOSC in diets did not influence the average weight of the haemoglobin in the blood. Lowered MCH value is linked with microlytic anaemia and increased MCH is linked with macrolytic anaemia. However, the range of 36.85-36.95pg MCH reported in this study are in the expected span of 25.4-33.4pg established by Mitruka and Rawsley (1977).

The mean cell haemoglobin concentration (MCHC) values of 27.8-28.55g/dl observed in this research are in the usual scope of 25.3-32.5g/dl reported by Mitruka and Rawsley (1977). Decreased MCHC levels may indicate iron and B₆ deficiencies and blood loss. In this present study the higher MCHC compared with the values reported by Mitruka and Rawsley (1977) implies that the birds did not exhibit any anaemic symptom.

The comparable haematological constituents could be related to the nutritional acceptability and trusty nature of the feedstuff. This shows that antinutrients discovered earlier and following gamma irradiated were condoned by the broilers up to 5% incorporation level in this research.

The considerable ($p<0.05$) difference in crude protein retention in the treated and raw MOSC in this work agrees with the findings of Aletor (1993) that ANFs like tannin in the diets of livestock causes lower feed intake in animals especially monogastrics, binds dietary proteins including digestive enzymes by forming networks that are not easily digested hence tannins in the raw diet of the birds (treatment 2) may have formed complexes with the digestive enzymes in the birds fed untreated MOSC in contrast to their counterparts in the control diet and those fed with the test ingredient thus limiting the digestibility of protein and the undigested feed voided out without the nutrients being utilized and retained by the animals.

Crude fibre retention in the birds is significantly different ($p<0.05$) among the treatments with the increasing inclusion of doses of irradiated MOSC. Panigrahi and Powell, (1991); Onifade (1993) have reported the implication of fibrous feed stuff on the nutrient retention by chickens.

Histology examination of the liver sections of the birds fed raw MOSC showed abnormal liver architecture attributable to the existence of the feed anti-nutritients. The observed alterations in the internal structure of organs and the related intracellular constituents attributable to feeding of untreated and processed MOSC diets indicate changes in the secretory and metabolic activities of these biological structures. The multifocal collections of liver lymphoid cells is consistent with the pathological demonstrations investigated by Aletor and Fetuga (1988) and Ologhobo *et al.* (1993).

The degeneration of the hepatocytes and coagulative necrosis in the liver were noticed in chickens fed untreated MOSC diet and were less observed in birds fed gamma irradiated treated diets, while no changes were discovered in birds fed with the control diet.

CHAPTER FIVE

EXPERIMENT THREE

5.0 THE EFFECT OF REPLACING SOYABEAN WITH FERROUS SULPHATE KAOLIN TREATED *MORINGA OLEIFERA* SEED CAKE IN DIETS OF BROILERS

5.1 Introduction

It is an established fact that feed represents the exorbitant item in any livestock industry, particularly poultry (Owen, et al. 2010). Any serious reduction or decline in the feed cost will lower the production cost and consequently the profit of the farm will be increased. Due the high cost of the conventional feed stuffs like groundnut cake meal, soya bean, Owen, et al. (2009) reported the focus of dietary research should tend towards feed manipulation

One of these non-conventional feed additives is kaolin or china clay which is a fine, white substance found by the weathering of aluminous minerals such as feldspar. Because of its adsorption attributes and non-existence of primary noxiousness (Anonymous 1998), kaolin usage is considered to be an easy and effective precaution against deleterious effects exerted by harmful substances present in the living organism and the environment (Heimann, 1984; Kasi, et al. 1995; Knezevich, 1998; Gebesh et al., 1999). Savory (1984) investigated dietary kaolin as a feed additive involving qualitative and/or quantitative manipulation of animal nutrition to influence growth performance without seriously upsetting nutritional requirements of values

Diamond *et al.* (1999) and Gilardi *et al.* (1999) reported reduction of biological absorption of toxic agents particularly alkaloids and tannins in seeds and various fruits by geophagic behaviour of free living birds. Kaolinites consumption being the most common soil mineral has also been used in rats suffering from acute diseases, gastrointestinal disorders, arthritis and stress (Burchfield *et al.* 1977; McCaffrey, 1985; Davey and Biederman, 1998).

Notwithstanding the merits of kaolin/geophagy in medicine and nutrition, clinical studies on the other hand showed that geophagy (kaolin consumption) caused mineral deficiency especially iron (Fe) suggested to be associated with anaemia occurrence (Halstead, 1968). In contrary, Paterson and Staszak, (1977) reported that intake of kaolin combined with iron (Fe) resulted in normal levels of haemoglobin, haematocrit and erythrocytes counts and live birth weight of the young. However, other authors (Geissler *et al.* 1998) stressed the beneficial effects of kaolin (geophagy) on haemoglobin and serum ferritin levels with the believe that intake of clay soil substances are vital sources of iron and other minerals in the diet. (John and Duquete, 1991; Abrahams, 1997; Geissler *et al.* 1998). There is no information on the use of ferrous sulphate with kaolin for the treatment of *Moringa oleifera* seed cake for feeding broilers.

5.2 Objective

The aim of this research was to determine the potential of ferrous sulphate kaolin in binding the phytochemicals in *Moringa oleifera* seed cake for enhanced suitability in broiler nutrition.

5.3 Materials and Methods

5.3.1 Experimental Site

The research was accomplished in the Poultry Unit of the Teaching and Research Farm, Kwara State University, Malete, Nigeria.

5.3.2 Source of Experimental Birds

One hundred and fifty day-old Arbor acre breed of broiler birds were sourced from Novic Farms at Ibadan, Oyo State Nigeria.

5.3.3 Collection of *M. Oleifera* Seed Cake

Moringa oleifera seed cake were obtained from *Moringa* processing plant at Afe Babalola University, Ado-Ekiti, Nigeria.

5.3.4 Experimental Diets

Moringa oleifera seed cake (MOSC) was properly sun dried to a permanent weight and milled for replacing soyabean in the diets. Five experimental diets were formulated made of a corn-soy reference diet (control) and four other diets containing raw (untreated MOSC) and ferrous sulphate kaolin treated *Moringa oleifera* seed cake for replacing soyabean at doses of 0.065, 0.130 and 0.260% ferrous sulphate and 0.01, 0.02 and 0.03% kaolin respectively corresponding to diets 1, 2, 3, 4 and 5 respectively at 5% level of inclusion The composition of the experimental diets for the broilers on as fed basis is presented on Table 5.1. The proximate composition of the broilers diet is presented on Table 5.2

5.3.5

Management of Experimental Birds and Data Collection

One hundred and fifty day- old broiler chicks were used for the trial. The chicks were fed on standard starter diet for a week prior to the onset of the experiment. The experiment was designed as a complete randomized design. The dietary treatments contained three replicates with ten chicks per replicate. All the birds were raised under similar management practices all through the eight week experimental period except for the diets offered that differed. The birds were provided with feed and water *ad-libitum* and weekly weighed. Routine vaccinations and medications were carried out as at when due.

Table 5.1: Composition (%) of the Experimental Diets

Ingredients	Inclusion levels of treated MOSC (%)				
	0(control)	Raw(untreated)	0.065	0.130	0.260
%	0	0	0.01	0.02	0.03
Maize	52.00	52.00	52.00	52.00	52.00
FKMOSC	0.00	5.00	5.00	5.00	5.00
Wheat offal	10.00	10.00	10.00	10.00	10.00
Soya bean meal	31.00	26.00	26.00	26.00	26.00
Palm oil	1.50	1.50	1.50	1.50	1.50
Lysine	0.10	0.10	0.10	0.10	0.10
Methionine	0.25	0.25	0.25	0.25	0.25
Bone meal	1.15	1.15	1.15	1.15	1.15
Oystershell	1.50	1.50	1.50	1.50	1.50
Salt	0.25	0.25	0.25	0.25	0.25
*Vit/Min premix	0.25	0.25	0.25	0.25	0.25
Fish meal	2.00	2.00	2.00	2.00	2.00
Total	100	100	100	100	100
Calculated chemical analysis					
ME (Kcal/kg)	2964.25	2959.14	2952.97	2954.20	2962.85
CP (%)	23.08	23.03	23.15	23.18	23.12
CF (%)	3.40	4.01	3.15	3.48	4.23
EE (%)	4.65	9.13	5.85	6.23	4.67
Methionine (%)	0.49	0.58	0.35	0.48	0.45
Lysine (%)	1.25	1.50	1.08	1.45	1.1

FKMOSC=Ferrous sulphate Kaolin Moringa oleifera seed cake.

Table 5.2: Analysed Nutrient Content of Ferrous Sulphate Kaolin MOSC Diets for Broilers

Parameters	Inclusion levels of MOSC (%)				
	0(control)	0(untreated)	0.065	0.130	0.260
			0.01	0.02	0.03
Dry matter	86.69	82.11	84.39	89.30	91.50
Crude protein	22.20	22.60	22.50	22.40	22.20
Ether extract	3.64	3.84	3.60	2.92	3.02
Crude fibre	5.03	6.41	5.18	5.50	5.24
Ash	7.44	7.47	7.51	7.56	7.57
NFE	48.38	41.79	45.60	50.92	53.47

5.3.6

Data Collection/Response Criteria

During the course of this research, data were collected on the performance characteristics: feed consumption, daily weight gain, growth rate, feed conversion ratio, survival rate, and carcass evaluation at the end of eight week study period

5.3.7

Nutrient Digestibility Trial

An apparent nutrient digestibility trial was executed at the 8th weeks of feeding period. Three birds per treatment were randomly selected. The trial lasted three days. Data were taken on feed intake and faeces voided during the three days. The droppings were oven dried, bulked and representative samples were taken for chemical analysis. The samples were analyzed for proximate composition according to the methods of AOAC (1990).

$$\text{Apparent Nutrient Retention (\%)} = \frac{\text{Nutrient intake} - \text{Nutrient in faeces} \times 100}{\text{Nutrient intake}}$$

5.3.8

Serum Biochemical and Haematological Analysis

At the expiration of the research, one broiler was taken at random per replicate in all the treatments and blood collected by decapitation. Blood for biochemical indices was collected in bottles with no EDTA for the analysis of the biochemical parameters. The specimen were kept in cooled condition before analysis in the laboratory.

The blood specimen for haematological parameters were preprocess with ethylene diamine tetra-acetate (EDTA), and were kept in cooled condition before analysis in the laboratory. Haematological indices were examined as in previous experiment.

5.3.9 Histology Examination of the Liver

Three birds were selected per treatment at the end of the feeding trial and sacrificed to obtain the liver. Sections were obtained from the anterior lobe of the liver, preserved in 10% formal-saline solution. The liver tissues from each replicate were processed for histological studies as in the previous experiment.

5.4 Statistical Analysis

Data collected on the response criteria were subjected to analysis of variance according to the completely randomized design model and differences between treatment means were separated using the Duncan's multiple range test (Steel and Torrie, 1980).

5.5 Results

5.5.1 Growth Performance Characteristics of Broilers Fed Ferrous Sulphate Kaolin Treated *Moringa Oleifera* Seed Cake Diets.

Table 5.3 presents data on performance characteristic of broilers fed ferrous sulphate kaolin treated *Moringa oleifera* seed cake (MOSC) diets. The feed intake values were remarkably ($p < 0.05$) affected by the dietary replacements of soyabean with raw MOSC but comparable between the birds on control group and treatment groups in the average daily feed consumption with the addition of graded amount of ferrous sulphate kaolin *Moringa oleifera* seed cake in the diet of the birds.

Table 5.3: Performance Characteristics of Broiler Birds Fed Ferrous Sulphate Kaolin Treated *Moringa Oleifera* Seed Cake Diets

Inclusion level of treated MOSC (%)							
Parameters	0(control)	0(Raw)	0.065	0.130	0.260	SEM	
			0.01	0.02	0.03		
Daily weight gain (g/bird/day)	59.66 ^b	41.01 ^c	61.35 ^a	60.20 ^{ab}	59.64 ^b	0.72	
Feed intake (g/bird/day)	103.43 ^a	93.10 ^b	104.76 ^a	105.00 ^a	101.98 ^a	2.32	
Feed conversion ratio	1.73 ^b	2.27 ^a	1.71 ^b	1.74 ^b	1.71 ^b	0.04	

SEM= Standard error of mean*=P<0.05 Means with different superscript along the same rows are significantly (P<0.05), MOSC=*Moringa oleifera* seed cake

5.5.2: **Carcass Characteristics of Broilers Fed Ferrous Sulphate Kaolin Treated *Moringa Oleifera* Seed Cake Diets**

The carcass characteristics of broilers fed ferrous sulphate kaolin treated *Moringa oleifera* seed cake diet is presented on Table 5.4.

At the end of the experimental period, three birds from each dietary treatment were randomly chosen, weighed and slaughtered. Just after complete bleeding, their carcasses were scalded, feather plucked and immediately processed.

The carcass characteristics of the birds fed the ferrous sulphate kaolin treated diets were superior to the group fed with the raw diet. The differences in the live weights, carcass weights, and the dressing percentage were notably ($p < 0.05$) affected across the treatment groups on the test feedstuff. There was a corresponding decrease in the live weights, carcass weights, and the dressing percentage of the birds fed with raw diet (treatment 2) and a comparable live weights, carcass weights, and the dressing percentage of the birds on the control and the birds fed with the ferrous sulphate kaolin treated *Moringa oleifera* seed cake diets. The weights of the carcass components like the head, neck, wings, thigh, drumstick, breast, shank, back expressed as percentages of the live weight were lower in all the dietary groups fed the treated feedstuff compare with the control group. There were inconsistencies in the weights of the internal organs of the birds fed with the raw and treated groups and inclusion of ferrous sulphate kaolin treated *Moringa oleifera* seed cake diet could not be said to have either or not enhanced the weights of the organs. The weights of the lung, heart, kidney, proventriculus were not significantly ($P > 0.05$) affected except the gizzard that was significantly ($p < 0.05$) affected.

Table 5.4: Carcass Characteristics of Broiler Birds Fed Ferrous Sulphate Kaolin *Moringa Oleifera* Seed Cake Diets

Parameters	Inclusion level of treated MOSC (%)					SEM
	0(control)	0(Raw)	0.065 0.01	0.130 0.02	0.260 0.03	
Live weight (g/bird)	1,952.00 ^a	1,255.00 ^c	1,890.00 ^b	1,900.02 ^b	1,889.50 ^b	5.91
Carcass weights (g/bird)	1,275.00 ^a	760.00 ^b	1213.00 ^b	1223.00 ^b	1212.00 ^b	3.37
Dressing percentage (%)	66.79 ^a	60.55 ^c	64.17 ^b	64.36 ^b	64.14 ^b	2.53
Prime cut and organ weights						
Head	3.97 ^c	3.11 ^a	3.55 ^b	3.29 ^a	3.09 ^b	0.37
Thigh	12.74 ^a	7.68 ^c	10.39 ^b	9.82 ^c	11.42 ^a	0.53
Breast	17.07 ^a	13.11 ^c	14.31 ^{ab}	15.32 ^{ab}	16.60 ^a	0.77
Wings	9.45 ^a	6.84 ^c	8.48 ^b	8.68 ^{abc}	9.42 ^a	0.43
Shank	5.46 ^a	3.55 ^b	4.96 ^{cd}	4.11 ^{bc}	5.07 ^a	0.37
Neck	6.23 ^{ab}	4.68 ^{abc}	5.67 ^c	5.54 ^{abc}	5.54 ^{abc}	0.54
Drumstick	10.91 ^a	6.22 ^{ab}	7.45 ^{bc}	7.58 ^{bc}	7.71 ^{abc}	0.81
Back	11.64 ^a	9.94 ^{ab}	11.54 ^a	11.99 ^c	11.46 ^a	0.69
Heart	0.49	0.39	0.45	0.50	0.47	0.22
Lungs	0.53	0.40	0.45	0.57	0.48	0.23
Liver	2.02	2.22	1.87	1.96	2.01	0.48
Kidney	0.52	0.29	0.40	0.47	0.44	0.23
Proventriculus	0.38	0.48	0.42	0.50	0.50	0.25
Gizzard	2.11 ^c	3.78 ^a	2.95 ^{abc}	2.11 ^{abc}	2.25 ^{bc}	0.46

SEM= Standard error of mean;;*=P<0.05;;a,b,c Means with different superscript along the same rows are significantly (P<0.05)different, Prime cut and organ weights Expressed as % of live weight

5.5.3 Serum Biochemical Indices of Broilers Fed Ferrous Sulphate Kaolin *Moringa Oleifera* Seed Cake Diets

Table 5.5 presents the biochemical indices of the broiler chickens fed dietary ferrous sulphate kaolin treated MOSC diet. There were increased ($p<0.05$) in the serum urea, total protein, and albumin with the inclusion of graded level of ferrous sulphate kaolin treated *Moringa oleifera* seed cake in the diet of the birds.

The serum aspartate amino transaminase (AST), alanine amino transaminase (ALT) and alkaline phosphatase (ALP) activities of the birds decreased ($p<0.05$) with the incorporation of graded level of ferrous sulphate kaolin treated *Moringa oleifera* seed cake in the diet of the birds.

5.5.4 Haematological Indices of Broilers Fed Ferrous Sulphate Kaolin Treated *Moringa Oleifera* Seed Cake Diet

The hematological indices of the birds fed with ferrous sulphate kaolin treated *Moringa oleifera* seed cake diet is presented on Table 5.6. The red blood cells, mean cell volume were affected ($p<0.05$) with the inclusion of graded level of ferrous sulphate kaolin treated *Moringa oleifera* seed cake in the diet of the birds. WBC, haemoglobin, and PCV, were significantly ($p<0.05$) different among the treatment groups and birds on the raw diets.

5.5.5 Nutrient Retention of Broiler Birds Fed Ferrous Sulphate Kaolin Treated *Moringa Oleifera* Seed Cake Diets

Table 5.7 presents the nutrient retention of broilers fed with ferrous sulphate kaolin treated MOSC diet. The nutrient retention of protein, fat and fibre were significantly ($p<0.05$) affected

with the incorporation of graded level of ferrous sulphate kaolin treated *Moringa oleifera* seed cake in the diet of the birds. The trend in the crude protein, fat and fibre retention showed that the birds on the raw diet (treatment 2) had poor crude protein, fat and fibre digestibility compared with its counterparts in other treatment groups.

Table 5.5: Serum Biochemical Indices of Broiler Birds Fed Ferrous Sulphate Kaolin Treated *Moringa Oleifera* Seed Cake Diets

Parameters	Inclusion level of treated MOSC (%)					SEM
	0(control)	0(Raw)	0.065	0.130	0.260	
			0.01	0.02	0.03	
Urea (mmol/l)	4.657 ^b	8.070 ^a	4.767 ^b	4.727 ^b	4.663 ^b	0.115
Total protein (g/l)	13.927 ^b	10.230 ^c	12.020 ^b	13.440 ^a	13.003 ^a	0.186
Albumin (g/l)	3.727 ^a	2.463 ^b	3.500 ^a	3.703 ^a	3.667 ^a	0.239
Aspartate Aminotransaminase(U/L)	70.000 ^b	90.030 ^a	62.107 ^c	68.840 ^c	70.843 ^b	6.779
Alanine Aminotransaminase(U/L)	19.480 ^b	30.443 ^a	19.867 ^d	18.037 ^c	19.460 ^c	1.059
Alkaline phosphatase(U/L)	35.680 ^a	46.287 ^a	34.660 ^c	32.540 ^b	30.840 ^b	3.839

SEM= Standard error of mean*=P<0.05 Means with different superscript along the same rows are significantly (P<0.05), MOSC=*Moringa oleifera* seed cake

Table 5.6: Blood Composition of Broiler Birds Fed Ferrous Sulphate Kaolin Treated *Moringa Oleifera* Seed Cake diets.

Inclusion level of treated MOSC (%)						
Parameters	0(control)	0(Raw)	0.065	0.130	0.260	SEM
			0.0	0.130	0.260	
Red Blood Cell (x 10 ¹² /l)	2.36 ^{ab}	2.52 ^a	2.30 ^b	2.18 ^b	2.24 ^b	0.106
Haemoglobin	8.75 ^a	9.30 ^a	9.00 ^a	8.50 ^b	8.70 ^a	0.415
Packed Cell Volume (%)	31.20 ^a	25.60 ^a	31.30 ^a	29.85 ^a	30.60 ^a	1.405
Mean Cell Volume (fl)	132.25 ^{ab}	129.60 ^b	136.10 ^a	137.00 ^a	136.55 ^a	3.372
Mean Cell Haemoglobin (pg)	37.15 ^a	36.95 ^a	39.15 ^a	39.00 ^a	38.80 ^a	1.216
Mean Cell Hae. Conc (g/dl)	28.05 ^a	28.55 ^a	28.75 ^a	28.50 ^a	28.45 ^a	0.402
White Blood Cell (x 10 ⁹ /l)	231.00 ^a	233.85 ^a	229.65 ^a	226.10 ^a	231.25 ^a	4.706

SEM= Standard error of mean=P<0.05; Means with different superscript along the same rows are significantly (P<0.05);NS=Non Significant (P>0.05);Mean Cell Hae. Conc= Mean Cell Haemoglobin Concentration.

**Table 5.7: Nutrient Retention of Broiler Birds Fed Ferrous Sulphate Kaolin Treated Seed
Cake diets**

Parameters (%)	Inclusion level of treated MOSC (%)					SEM
	0(control)	0(Raw)	0.065	0.130	0.260	
			0.01	0.02	0.03	
Crude protein	71.600 ^b	49.210 ^c	73.620 ^a	72.240 ^{ab}	71.570 ^b	0.863
Crude fibre	65.570 ^b	70.063 ^c	67.420 ^a	66.160 ^{ab}	65.540 ^b	0.791
Fats	65.300 ^a	55.103 ^c	67.490 ^a	66.220 ^{ab}	65.600 ^b	0.7971

SEM= Standard error of mean; Means with different superscript along the same rows are significant (P<0.05),MOSC=Moringa oleifera seed cake, Raw=untreated

5.5.6 Histology of the Liver of Broiler Birds Fed Ferrous Sulphate Treated *Moringa Oleifera* Seed Cake Diets

The photomicrographs showing the liver of the birds fed the FKMOSC diet are given in Figure 3(K-O). The sections of the liver revealed that with the addition of raw MOSC in the diets of the birds, the architecture of the liver in the birds were severely affected with severe hemorrhage of hepatic blood vessels. With the inclusion of graded level of FKMOSC in the diets of the birds, the density of the hepatocyte appears normal with characteristic delineation with no sign of pathological perturbation

5.5.7 Profitability of Broilers Fed Ferrous Sulphate Kaolin Treated *Moringa Oleifera* Seed Cake for Dietary Replacement of Soyabean.

Table 5.8 shows the profitability of replacing soyabean using ferrous sulphate kaolin treated *Moringa oleifera* seed cake in broilers diet. The total feed intake and total cost of production per bird were higher in birds fed ferrous sulphate kaolin treated *Moringa oleifera* seed cake in broilers diet compared with the control. The reduction in cost of feed per kilogram obtained for the birds on raw MOSC diet (treatment 2) as well as high cost of feed per kilogram obtained for the treated groups (treatments 3-5) could not enhance the profit on investment. This is attributed to the poor palatability/poor feed utilization by the birds. This implies that farmers/investors will have higher returns on investment from the use of soyabeans compare with ferrous sulphate kaolin treated MOSC.

Table 5.8: Profitability of Broilers Fed Ferrous Sulphate Kaolin Treated *Moringa Oleifera* Seed Cake Diets

		Inclusion doses of treated MOSC (%)					
Parameters		0(control)	0(Raw)	0.065	0.130	0.260	SEM
				0.01	0.02	0.03	
Total feed intake/bird/kg		5.80 ^a	5.21 ^b	5.86 ^a	5.88 ^a	5.71 ^a	2.32
Total weight gain/bird/kg		3.34 ^b	2.29 ^c	3.43 ^a	3.37 ^{ab}	3.33 ^b	0.72
Cost of feed (₦/kg)		115.00	105.20	120.51	125.20	130.00	—
Cost of feeding a bird (₦)		667.00	548.09	706.19	736.18	742.30	—
Cost/weight gain (₦/kg)		199.70	239.34	205.89	218.45	222.90	—
Cost differential (₦/kg)		0.00	- 39.64	-6.19	-18.75	-23.20	—

SEM= Standard error of mean; Means with different superscript along the same rows are significantly (P<0.05); NS=Non Significant (P>0.05), MOSC= *Moringa oleifera* seed cake

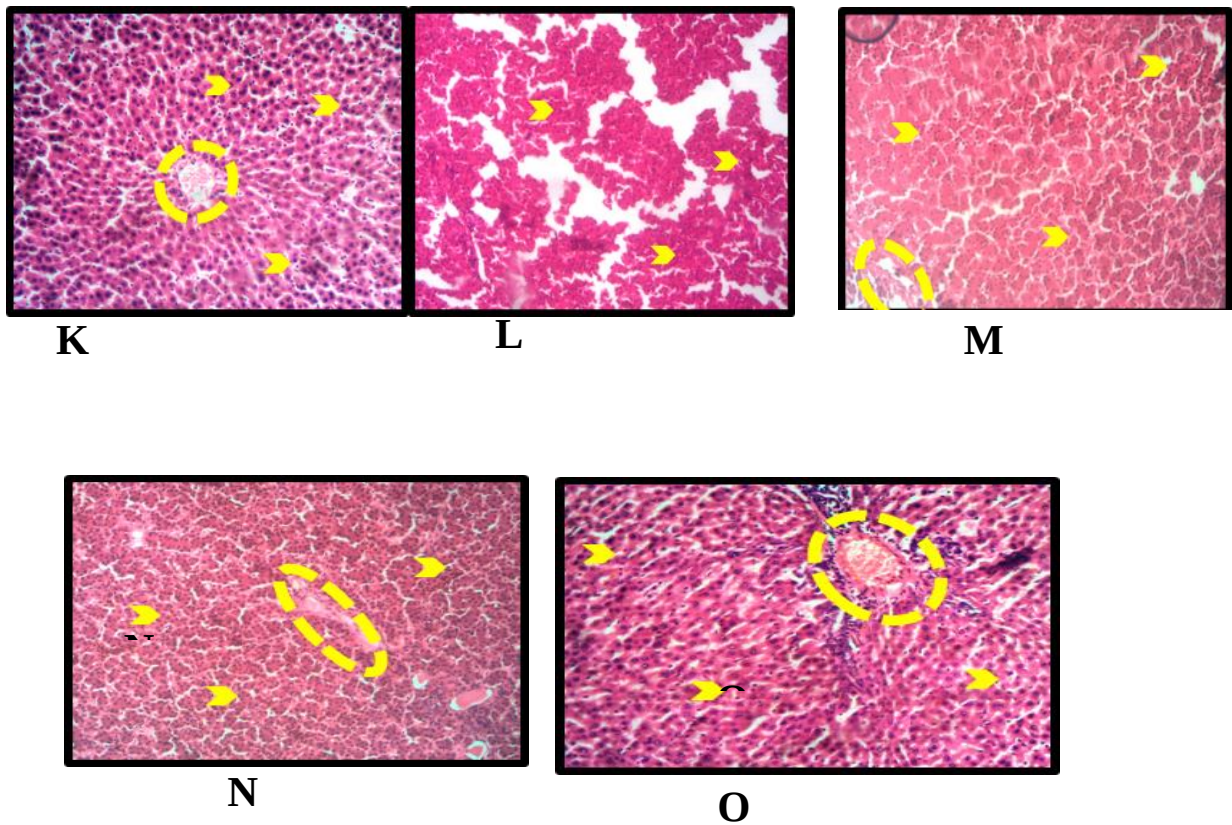


Plate 3(K-O): Photomicrographs showing the liver of broilers fed the experimental diets, control (K) with normal hepatocyte, Raw MOSC (L) with severe haemorrhage of hepatic blood vessel, 0.01%FKMOSC (M) with mild hemorrhage of hepatic blood vessel, 0.02%FK MOSC (N) with mild hemorrhage of hepatic blood vessel and 0.03%FKMOSC (O) with normal hepatocyte.

H/E Stain Mag. X400

Growth performance characteristics: The improved growth performance of birds fed with the graded level of ferrous sulphate kaolin in the diets of the birds indicates the efficiency of the feedstuff in poultry feeding. This result is consistent with the reports of Tauqir and Nawaz (2001) and Xia *et al.*, (2004) who reported that rations augmented with 10-30g/kg clay enhance feed efficiency in birds. It has also been shown that dietary augmentation with clay enhanced digestibility of the nutrient and enzymatic action of gastrointestinal secretion (Ouhida *et al.*, 2000; Alzueta *et al.*, 2002)

Lowest feed intake (93.10g/bird/day) and weight gain (41.01 g/bird/day) were recorded in birds fed untreated diet (diet 2). This findings may be a result of intrinsic anti-nutritients in *Moringa oleifera* seed cake.

Increased feed intake observed in birds fed treated *Moringa oleifera* seed cake diets could be attributed to adequate nutrient utilization occasioned by the relatively low dietary fibre and reduced anti-nutritional factor (NRC, 1994).

The feed intake values were remarkably ($p < 0.05$) affected by the dietary replacements of soyabean with raw MOSC but comparable in the average daily feed consumption of the birds with the addition of graded amount of ferrous sulphate kaolin *Moringa oleifera* seed cake in the diet of the birds.

The average daily weight gains of the birds were exceptionally ($p < 0.05$) affected by the dietary incorporation of raw MOSC for replacement of soyabean. Except for the raw diet (diet 2), there was a decrease but comparable average daily weight gains of the birds ($p > 0.05$) with the inclusion of graded level of kaolin-ferrous sulphate treated *Moringa oleifera* seed cake in the diet of the birds. The feed conversion ratio is superior with birds on the reference diet.

It was also reported that birds fed with the inclusion of graded level of ferrous sulphate kaolin in their diets ate smaller feed in contrast to the control group. This was indicated in the feed conversion ratio though similar weight gain was discovered in the treated and the control group.

Mumpton, (1999), reported that animal feed having clay minerals such as kaolin enhanced weight gain and feed efficiency. Taugir and Nawaz, (2001) observed reduction in guts contamination by bacteria as well as the detrimental effects of mycotoxin in contaminated diets with the inclusion of kaolin in animal diet. Kaolin also safeguards the intestinal mucosa, by clinging to pathogen and selectively aid their discharge (Droy-lefain *et al.*, 1985). This might have explained the comparable feed consumption and better feed conversion ratio in the treated groups probably due to elimination of the pathogens from the gut that would have competed for nutrients with the birds.

No record of mortality in this study is an evidence that suggests that most of the anti-nutrients in the treated MOSC diets might have been removed or eliminated. Some of these anti-nutrients are recognized to induce death in animals. Tannins bound with and lowered absorption of iron in the gut (Butler, 1989, 1992).

Nworgu and Fasogbon (2007) recorded no casualty of pullet chicks when fed with diets having different amounts of moringa seed meal. Likely reasons for the lack of death of animals when fed moringa seed meal in diet might be ascribed to the existence of antioxidants in the seeds which could strengthen the immune systems of the broiler chickens. (Yang *et al.*, 2006, Du *et al.*, 2007).

The non-significant ($p>0.05$) difference in serum urea between the control and the treated group agrees with the results of Eggum (1976); Esonu *et al.*, (2001); Iyayi and Taiwo (2003) who reported that total protein and serum urea contents depend on the quality and quantity of the provided protein in the diet. The increase in serum urea observed in raw diet (treatment 2) is an indication of the presence of ANFs that impaired nutrient utilization.

The increase in albumin and total protein with the inclusion of graded level of ferrous sulphate kaolin in the diets of the birds underscore the nutritional acceptability of treated MOSC in satisfying the protein requirement of the chickens.

ALP, AST and ALT decreased with the inclusion of graded level of ferrous sulphate kaolin in the diets of the birds. The increase in ALT values in birds in treatments 2, compared with the control and treated group suggest that there may be a likelihood of liver damage by the raw MOSC.

Haematological estimates are major pointers to the health of livestock (Jain, 1986) and are further modified by several other factors including diets (Talebi *et al.*, 2005).

The range of $2.18\text{-}2.52 \times 10^{12}/\text{l}$ for red blood cells (RBC) achieved in this work is near the standard span of $3\text{-}6 \times 10^6/\text{dl}$ for chicken as reported by Madubuike and Ekenyem (2006). It was observed that with the inclusion of graded level of ferrous sulphate kaolin in the diets of the birds, the red blood cells (RBC) increased. The high iron content of moringa kernels may have influenced erythropoiesis which in turn favoured the production of RBC. The observed increase ($p < 0.05$) in the haemoglobin content (Hb) with the inclusion of graded level of ferrous sulphate kaolin in the bird's diet could also be ascribed to the high level of iron in the MOSC. The range in Hb values between 8.50-9.30% reported in this study was within the range of

6-13% investigated by Iheukwumere *et al.*, (2002) but lower than the normal value of 9-13% reported by (MVM, 1986).

The range of PCV (25.6-31.3%) across all the experimental diets support the findings of Nworgu *et al.* (1999) and 28 - 30.70% reported by Ogbuewu *et al.* (2010).

MCV of 129.6-137.00fl obtained in this work falls within the normal span of 115-127fl reported by Swenson (1993). The observed significant ($p < 0.05$) increase in the MCV with the inclusion of graded level of ferrous sulphate kaolin in the diets of the birds suggests that the size of the red blood cells were enhanced by the volume occupied by each red blood cell. The non-significant ($p > 0.05$) difference observed for MCH in all the dietary treatments implies that the inclusion of the processed MOSC in diets did not influence the average weight of the haemoglobin in the blood. However, the range of 37.15-39.15pg MCH recorded in this work is higher than 25.4-33.4pg reported by Mitruka *et al.*, (1977) for normal birds.

The mean cell haemoglobin concentration (MCHC) value of 28.05-28.75g/dl reported in this study is within the established values of 25.3-32.5g/dl reported by Mitruka and Rawsley (1977). Low MCHC means that a unit of packed RBC contains less haemoglobin than normal and a high MCHC means that there is more haemoglobin in a unit of RBC. In this present study the higher MCHC compared with the values investigated by Mitruka and Rawsley (1977) implies that the birds did not exhibit any anaemic symptom.

White blood rates for the chickens fed kaolin –ferrous sulphate incorporated diets followed similar trend with RBC. This shows that the bird's capacity to combat disease attack was not hindered by the diets.

Crude fat and fibre retention; The observed decrease in fat and fibre retention of the birds fed the raw and the treated MOSC groups were due to poor diet usage by the birds on raw diet containing various anti-nutrients. Saponins were discovered to cling to the cells of the small intestine and consequently affecting the absorption of nutrients across the wall of the intestine (Annongu, 2012) The implication of fibrous ingredients on the nutrient retention by birds has also been well investigated (Panigrahi and Powell,1991; Onifade,1993).

In this study, the nutrient retention of the birds fed the treated MOSC was better than those fed with raw MOSC diet. The nutrient retention observed in birds fed with the graded level of ferrous sulphate kaolin treated MOSC implies the effectiveness of the processing of MOSC for broilers.

Histological examination of the liver sections of the birds fed raw MOSC showed abnormal liver architecture with severe hemorrhage of hepatic blood caused by the existence of the feed antinutrients. The degradation of the hepatocytes and cell necrosis in the liver was noticed in birds fed untreated MOSC diet and were not obvious in birds fed treated diets, while changes were not noticed in birds fed with the control. Non-existence of histological lacerations in these birds could be due to the reduced amount of residual anti-nutrients in the treated MOSC diets.

King et al. (1980) observed modification in the measurable structure acutely poisoned with seed haemagglutinins in the liver of rats. Aletor and Fetuga (1988) also reported focal infection and fatty acid changes in livers of rats when fed with untreated lima bean.

CHAPTER SIX

EXPERIMENT FOUR

6.0 EFFECTS OF REPLACING SOYABEAN WITH *MORINGA OLEIFERA* SEED CAKE TREATED WITH AMMONIUM HYDROXIDE IN BROILERS DIETS

6.1 Introduction

In the developing countries, Nigeria inclusive, the major factor militating against the development of livestock industry is the scarcity and inadequate availability of conventional feedstuff throughout the year. A high demand for these feed ingredients has resulted in an increase in their prices and consequently, cost of poultry feed and its products (Akinmutimi *et al.*, 2002). Hence, the need to source for alternative but promising feedstuffs. Condensed tannins in MOSC could be removed using aqueous ammonium hydroxide. Prabhu *et al.*, (2002) also reported that acid and alkali treatment were adopted to detoxify karanja cake. Ammonia reacts with the aflatoxin molecule by breaking an oxygen bond making the resulting products far less toxic (PER, 2003). There is no information on the use of ammonium hydroxide for the processing of *Moringa oleifera* seed cake in the diet of broilers.

6.2 Objective

The aim of this research was to evaluate the potential of ammonium hydroxide in the detoxification of phytochemicals present in *Moringa oleifera* seed cake for enhanced suitability in broilers diet

6.3 Materials and Methods

6.3.1 Experimental Site

The experiment was accomplished in the Poultry Unit of the Teaching and Research Farm, Kwara State University, Malete, Nigeria.

6.3.2 Source of Experimental birds

A total of One hundred and fifty day-old Arbor acre breed of broiler birds were sourced from Novic Farms at Ibadan, Oyo State Nigeria.

6.3.3 Collection of *M. Oleifera* Seed Cake

Moringa oleifera seed cake were obtained from Moringa processing plant at Afe Babalola University, Ado-Ekiti, Nigeria.

6.3.4 Experimental Diets

Raw (untreated) *Moringa oleifera* seed cake was divided into four uniform batches of 3Kg each. A batch was left untreated, while each of the other three batches was treated by soaking in 5Litres of 10, 20 and 30% ammonium hydroxide solution respectively for 24hours. The seed

cake was later removed from solution and properly sun dried to a permanent weight and milled for replacing soyabean in the diets

Five experimental diets were compounded made of a corn-soy reference diet (control) and four other diets having raw and treated *Moringa oleifera* seed cake using ammonium hydroxide before introducing into the diet at the same 5% level of incorporation corresponding to diets 1, 2, 3, 4 and 5 respectively. The composition of experimental diets for the broilers on as fed basis is presented on Table 6.1.

6.3.5 Management of Experimental Birds and Data Collection

A total of One hundred and fifty day- old broiler chicks were used for the trial. The chicks were fed on standard starter diet for a week prior to the onset of the research. The experiment was designed as a complete randomized design. The dietary treatments contained three replicates with 10 chicks per replicate. The birds were raised under similar management practices all through the eight week experimental duration except for the diets offered that differed. The birds were provided with feed and water *ad-libitum* and weekly weighed. Routine vaccinations and medications were carried out as at when due.

Table 6.1: Composition (%) of the Experimental Diets

Ingredients (%)	Inclusion doses of treated AHMOSC (%)				
	0 (control)	0 (Raw)	10	20	30
Maize	52.00	52.00	52.00	52.00	52.00
AHMOSC	0.00	5.00	5.00	5.00	5.00
Wheat offal	10.00	10.00	10.00	10.00	10.00
Soya bean meal	31.00	26.00	26.00	26.00	26.00
Palm oil	1.50	1.50	1.50	1.50	1.50
Lysine	0.10	0.10	0.10	0.10	0.10
Methionine	0.25	0.25	0.25	0.25	0.25
Bone meal	1.15	1.15	1.15	1.15	1.15
Oystershell	1.50	1.50	1.50	1.50	1.50
Salt	0.25	0.25	0.25	0.25	0.25
Fish meal	2.00	2.00	2.00	2.00	2.00
*Vit/Min premix	0.25	0.25	0.25	0.25	0.25
Total	100	100	100	100	100
Calculated chemical analysis					
ME (Kcal/kg)	2994.25	2979.40	2962.97	2934.20	2992.85
CP (%)	23.16	23.03	23.17	23.28	23.12
CF (%)	3.45	4.01	3.15	3.48	4.23
EE (%)	4.65	9.16	5.85	6.23	4.67
Methionine (%)	0.49	0.58	0.35	0.48	0.45
Lysine (%)	1.25	1.50	1.08	1.45	1.

AHMOSC=Ammonium hydroxide *Moringa oleifera* seed cake

6.3.6 Data Collection/Response Criteria

In the course of the experiment, data were obtained on the performance characteristics: feed consumption, daily weight gain, feed conversion ratio, and carcass evaluation at the expiration of eight week study period

6.3.7 Nutrient Digestibility Trial

An apparent nutrient digestibility trial was performed at the 8th weeks of feeding period. Three birds per treatment were randomly selected. The trial lasted for three days. Records of feed intake and faeces voided were taken during the three days. The droppings were oven dried, bulked and representative samples were taken for chemical analysis. The samples were analyzed for proximate composition according to the methods of AOAC (1990).

$$\text{Apparent Nutrient Retention (\%)} = \frac{\text{Nutrient intake} - \text{Nutrient in faeces} \times 100}{\text{Nutrient intake}}$$

6.3.8 Serum Biochemical and Haematological Analysis

At the end of the experiment, one broiler per replicate in all the treatments was taken at random and blood collected by decapitation. Blood for biochemical indices was collected in bottles without EDTA for the analysis of the biochemical parameters of total protein, blood glucose level, albumin, globulin, transaminase activities- AST, ALT ALP etc.

The blood samples for haematological indices were pretreated with ethylene diamine tetra-acetic acid (EDTA). Haematological indices examined include white blood cell (WBC), red blood cell (RBC), packed Cell Volume (PCV), haemoglobin (Hb). The mean cell volume (MCV), mean cell haemoglobin (MCH) and the mean cell haemoglobin concentration (MCHC) were calculated according to the methods of Jain (1986).

6.3.9 Histology of the Liver

Three birds were selected per treatment at the end of the experimental period and sacrificed to obtain the liver. Sections were obtained from the anterior lobe of the liver, preserved in 10% formal saline solution. The liver tissues from each replicate were processed for histological studies using haematoxylin and eosin stains. Digital microscope with a magnification of 400 was used to take photomicrographs. The analysis was accomplished at the histopathological laboratory of Central Research Laboratory, Tanke Ilorin.

6.4 Statistical Analysis

Data collected on the response criteria were subjected to analysis of variance according to the completely randomized design model and differences between treatment means were separated using the Duncan's multiple range test (Steel and Torrie, 1980).

6.5 Results

6.5.1 Growth Performance Characteristics of Broilers Fed Ammonium Hydroxide Treated *Moringa Oleifera* Seed Cake Diets.

The proximate composition of the treated *Moringa oleifera* seed cake diet is presented on Table 6.2

Table 6.3 presents data on performance characteristic of broilers fed ammonium hydroxide treated *Moringa oleifera* seed cake (MOSC) diet. Except for birds on raw diet (treatment 2), the average daily weight gains, the feed intake values and the feed conversion ratio of the birds were not significantly ($p>0.05$) affected by the dietary inclusion of the graded level of ammonium hydroxide treated MOSC for the replacement of soyabean. The feed conversion ratio favoured birds on the 20% ammonium hydroxide treated *Moringa oleifera* seed cake (MOSC) diet having the least mean (1.67) compared with the control . There was no death of birds recorded during the course of this trial.

Table 6.2: Analysed Nutrient Content of Ammonium Hydroxide MOSC Diets for Broilers

Parameters	Inclusion levels of AHMOSC (%)				
	0(control)	0(untreated)	10	20	50
Dry matter	86.69	92.71	93.25	94.31	93.58
Crude protein	22.20	22.60	21.70	20.65	24.12
Ether extract	3.64	3.84	3.43	3.98	2.89
Crude fibre	8.73	8.71	8.68	8.50	8.74
Ash	7.44	7.47	7.50	7.54	7.48
NFE	44.68	50.09	51.94	53.64	50.35

Table 6.3: Performance Characteristics of Broiler Birds Fed Ammonium Hydroxide Treated *Moringa Oleifera* Seed Cake diets

Parameters	0(control)	0(Raw)	10	20	30	SEM
Daily weight gains (g/bird/day)	59.993 ^a	42.340 ^b	61.763 ^a	61.873 ^a	61.993 ^a	1.366
Feed intake (g/bird/day)	102.507 ^a	92.307 ^b	104.477 ^a	103.747 ^a	104.737 ^a	3.972
Feed conversion ratio	1.707 ^b	2.180 ^a	1.693 ^b	1.677 ^b	1.687 ^b	0.045

SEM= Standard error of mean*=P<0.05 Means with different superscript along the same rows are significantly (P<0.05), MOSC=*Moringa oleifera* seed cake

6.5.2 Carcass Characteristics of Broilers Fed Ammonium Hydroxide Treated *Moringa Oleifera* Seed Cake Diets

At the end of the experimental period, three birds from each dietary treatment were randomly chosen, weighed and slaughtered. Just after complete bleeding, their carcasses were scalded, feather plucked and immediately processed.

The carcass characteristics of broilers fed ammonium hydroxide treated *Moringa oleifera* seed cake diets are presented in Table 6.4.

The carcass characteristics of the birds fed ammonium hydroxide treated diets were superior to the group fed with the raw diet. The differences in the live weights, carcass weights, and the dressing percentage were affected ($p < 0.05$) across the treatment groups on the test feedstuff. There was a corresponding decline in the live weights, carcass weights, and the dressing percentage of the birds fed with raw diet (treatment 2) and a comparable live weights, carcass weights, and the dressing percentage of the group of birds on control and the treated with the inclusion of graded level of ammonium hydroxide treated *Moringa oleifera* seed cake in their diets. The weights of the carcass components like the head, neck, wings, thigh, drumstick, breast, shank, back expressed as percentages of the live weight were lower in all the dietary groups fed the treated feedstuff compare with the control group.

Table 6.4: Carcass Characteristics of Broiler Birds Fed Ammonium Hydroxide *Moringa Oleifera* Seed Cake Diets

Inclusion doses of ammonium hydroxide treated MOSC (%)						SEM
Parameters	(control)	0(Raw)	10	30	50	
Live weight (g/bird)	1,952.00 ^a	1,255.00 ^c	1,890.00 ^b	1,900.02 ^b	1,889.50 ^b	5.91
Carcass weights (g/bird)	1,275.00 ^a	760.00 ^b	1213.00 ^b	1223.00 ^b	1212.00 ^b	3.37
Dressing percentage (%)	66.79 ^a	60.55 ^c	64.17 ^b	64.36 ^b	64.14 ^b	2.53
Prime cut and Organ weights						
Head	2.97 ^a	3.51 ^a	3.05 ^a	3.29 ^a	3.09 ^a	0.37
Thigh	12.74 ^a	7.68 ^c	11.39 ^a	11.82 ^a	11.42 ^a	0.53
Breast	17.07 ^a	13.11 ^c	15.31 ^b	15.32 ^b	16.60 ^a	0.77
Wings	9.45 ^a	8.84 ^{ab}	9.48 ^d	8.68 ^{abc}	9.42 ^a	0.43
Shank	5.46 ^a	4.55 ^b	5.26 ^a	5.11 ^a	5.07 ^a	0.37
Neck	6.23 ^a	5.68 ^b	6.17 ^a	6.54 ^a	6.44 ^a	0.54
Drumstick	10.91 ^a	9.22 ^b	10.45 ^a	11.08 ^a	10.71 ^a	0.81
Back	11.64 ^a	9.94 ^b	11.54 ^a	11.99 ^a	11.46 ^a	0.69
Heart	0.49	0.39	0.45	0.50	0.47	0.22
Lungs	0.53	0.40	0.45	0.57	0.48	0.23
Liver	2.02	2.22	1.87	1.96	2.01	0.48
Kidney	0.52	0.29	0.40	0.47	0.44	0.23
Proventriculus	0.38	0.48	0.42	0.50	0.50	0.25
Gizzard	2.11 ^b	3.68 ^a	2.95 ^b	2.11 ^b	2.25	0.46

SEM= Standard error of mean;;*=P<0.05;;a,b,c Means with different superscript along the same rows are significantly (P<0.05)different, Prime cut and Organ weights Expressed as % of live weight

6.5.3

Serum Biochemical Indices of Broilers Fed Ammonium Hydroxide Treated *Moringa Oleifera* Seed Cake Diets

Table 6.5 presents the biochemical indices of the broiler chickens given dietary ammonium hydroxide treated MOSC diet.

There was an increase ($p < 0.05$) in the total protein level with the inclusion of ammonium hydroxide treated *Moringa oleifera* seed cake (MOSC) in the diets of the birds. The albumin level revealed an increase in the trend with the inclusion of ammonium hydroxide treated *Moringa oleifera* seed cake (MOSC) in the diets of the birds. The serum alanine amino transaminase (ALT) and alkaline phosphatase (ALP) activities of the birds significantly ($p < 0.05$) decreased except raw diet (treatment 2) with the inclusion of ammonium hydroxide treated *Moringa oleifera* seed cake (MOSC) in the diets of the birds.

6.5.4

Haematological Indices of Broilers Fed Ammonium Hydroxide Treated *Moringa Oleifera* Seed Cake Diets

The hematological indices of the birds fed with graded level of ammonium hydroxide treated *Moringa oleifera* seed cake diet is presented on Table 6.6. The red blood cells, WBC PCV, MCH and MCHC were affected ($p < 0.05$) with an increase in the values with the incorporation of graded level of ammonium hydroxide treated MOSC in the diet of the birds.

6.5.5: Nutrient Retention of Broiler Birds Fed Ammonium Hydroxide Treated *Moringa Oleifera* Seed Cake Diets

Table 6.7 presents the nutrient retention of broilers fed with graded level of ammonium hydroxide treated MOSC diet. The nutrient retention of crude protein, fat and fibre of birds on control diet and the ammonium hydroxide treated groups were significantly ($p < 0.05$) different with the addition of graded level of ammonium hydroxide treated MOSC in the diet of the birds. The trend in the crude protein, fat and fibre retention revealed that the birds on 30% ammonium hydroxide treatment (diet 5) had the best nutrient retention compared with its counterparts, the raw and the control diet.

**Table 6.5: Serum Biochemical Indices of Broiler Birds Fed Ammonium Hydroxide
Treated *Moringa Oleifera* Seed Cake Diets**

Inclusion doses of treated MOSC (%)							
Parameters		0(control)	0(Raw)	10	30	50	SEM
Urea (mmol/l)		4.657 ^b	8.070 ^a	4.707 ^b	4.520 ^b	4.657 ^b	0.127
Total protein (g/l)		13.927 ^a	11.230 ^b	13.817 ^a	13.620 ^a	14.060 ^a	0.230
Albumin (g/l)		2.727 ^a	1.163 ^b	2.307 ^a	2.020 ^a	2.157 ^a	0.261
Aspartate	Amino	70.000 ^b	90.030 ^a	64.210 ^{bc}	54.737 ^c	61.053 ^{bc}	6.894
transaminase(U/L)							
Alanine	Amino	19.480 ^b	30.443 ^a	19.827 ^b	19.480 ^b	19.830 ^b	1.136
transaminase(U/L)							
Alkaline	phosphatase	25.680 ^a	36.287 ^a	25.020 ^b	25.580 ^b	25.960 ^b	3.948
(U/L)							

SEM= Standard error of mean*= $P < 0.05$ Means with different superscript along the same rows are significantly ($P < 0.05$); NS=Non Significant ($P > 0.05$); MOSC=Moringa oleifera seed cake.

Table 6.6: Blood Composition of Broiler Birds Fed Ammonium Hydroxide Treated *Moringa Oleifera* Seed Cake Diets

Inclusion doses of treated MOSC (%)							
Parameters							SEM
		0(control)	0(Raw)	10	20	30	
Red Blood Cell (x 10 ¹² /l)		2.360 ^{ab}	1.520 ^a	2.215 ^b	2.355 ^{ab}	2.180 ^b	0.148
Haemoglobin (g/dl)		8.750 ^a	7.300 ^b	9.000 ^a	8.950 ^a	8.650 ^a	0.397
Packed cell Volume (%)		31.200 ^a	28.600 ^b	30.250 ^a	31.400 ^a	31.800 ^a	1.412
Mean Cell Volume (fl)		132.250 ^a	129.600 ^b	132.550 ^a	133.350 ^a	132.500 ^a	4.048
Mean cell Haemoglobin (pg)		37.150 ^a	34.950 ^b	40.600 ^a	38.050 ^a	39.950 ^a	1.264
Mean Cell Hae. Conc (g/dl)		28.050 ^a	28.550 ^a	29.750 ^a	28.500 ^a	29.050 ^a	0.4147
White Blood Cell (x 10 ⁹ /l)		231.000 ^a	233.850 ^a	234.750 ^a	238.900 ^a	232.500 ^a	4.482

SEM= Standard error of mean=P<0.05; Means with different superscript along the same rows are significantly (P<0.05); Mean Cell Hae. Conc= Mean Cell Haemoglobin Concentration, MOSC=Moringa oleifera seed cake

**Table 6.7: Nutrient Retention of Broiler Birds Fed Ammonium Hydroxide Treated
Moringa Oleifera Seed Cake Diets**

Parameters(%)	Inclusion doses of treated MOSC (%)					SEM
	0(control)	0(Raw)	10	20	30	
Crude protein	76.870 ^a	55.300 ^b	76.107 ^a	75.440 ^a	76.007 ^a	1.432
Crude fibre	68.993 ^a	48.690 ^b	61.023 ^a	65.153 ^a	68.293 ^a	1.571
Fats	65.997 ^a	46.570 ^b	67.937 ^a	68.057 ^a	68.197 ^a	1.503

SEM= Standard error of mean;p<.05;***c Means with different superscript along the same rows are significant (P<0.05),
.MOSC=Moringa oleifera seed cake.

6.5.6 Histology of the Liver of Broiler Birds Fed Ammonium Hydroxide Treated *Moringa Oleifera* Seed Cake Diets

The photomicrographs showing the liver of the birds fed the ammonium hydroxide treated *Moringa oleifera* seed cake (AHMOSC) diet are given in plate 4 (P-T). The sections of the liver revealed deterioration of the hepatocytes and cell necrosis with the inclusion of raw MOSC in the diets of the birds, and were less distinct in birds fed treated diets, while no alterations were observed in birds fed control and 30% ammonium hydroxide treated MOSC diet. . With the inclusion of graded level of AHMOSC in the diets of the birds, the density of the hepatocyte appears normal with characteristic delineation.

6.5.7 Profitability of Broilers Fed Ammonium Hydroxide Treated *Moringa Oleifera* Seed Cake For Dietary Replacement of Soyabean.

Table 6.8 shows the profitability of replacing soyabean using ammonium hydroxide treated *Moringa oleifera* seed cake in broilers diet. The total feed intake and total cost of production per bird were higher in birds fed with ammonium hydroxide treated *Moringa oleifera* seed cake compared with the control. The reduction in cost of feed per kilogram obtained for the birds on raw MOSC diet as well as high cost of feed per kilogram obtained for the treated groups (treatments 3-5) could not enhance the profit on investment. The highest profit was achieved from the birds fed the control diet followed by those fed with 10% and 20% ammonium hydroxide treated *Moringa oleifera* seed cake diet respectively. Losses in investment were recorded in birds fed with raw MOSC and 30% ammonium hydroxide treated *Moringa oleifera* seed cake diet respectively.

Table 6.8: Profitability of Broilers Fed Ammonium Hydroxide *Moringa Oleifera* Seed Cake Diets

Inclusion doses of treated AHMOSC (%)							
Parameters		0(control)	0(Raw)	10	20	30	SEM
Total	feed	5.74 ^a	5.16 ^b	5.85 ^a	5.81 ^a	5.86 ^a	3.972
intake/bird/kg							
Total	weight	3.36 ^a	2.37 ^b	3.45 ^a	3.46 ^a	3.47 ^a	1.366
gain/bird/kg							
Cost of feed (₦/kg)		110.00	100.00	115.00	120.00	125.00	—
Cost of feeding a bird		631.40	516.00	672.75	697.20	732.50	—
(₦)							
Cost/weight	gain (₦	187.91	217.72	195.00	201.50	211.10	—
/kg)							
Cost differential (₦/kg)		0.00	- 29.81	-7.09	-13.59	-23.19	—

SEM= Standard error of mean; Means with different superscript along the same rows are significantly (P<0.05);NS=Non Significant (P>0.05),MOSC= *Moringa oleifera* seed cake

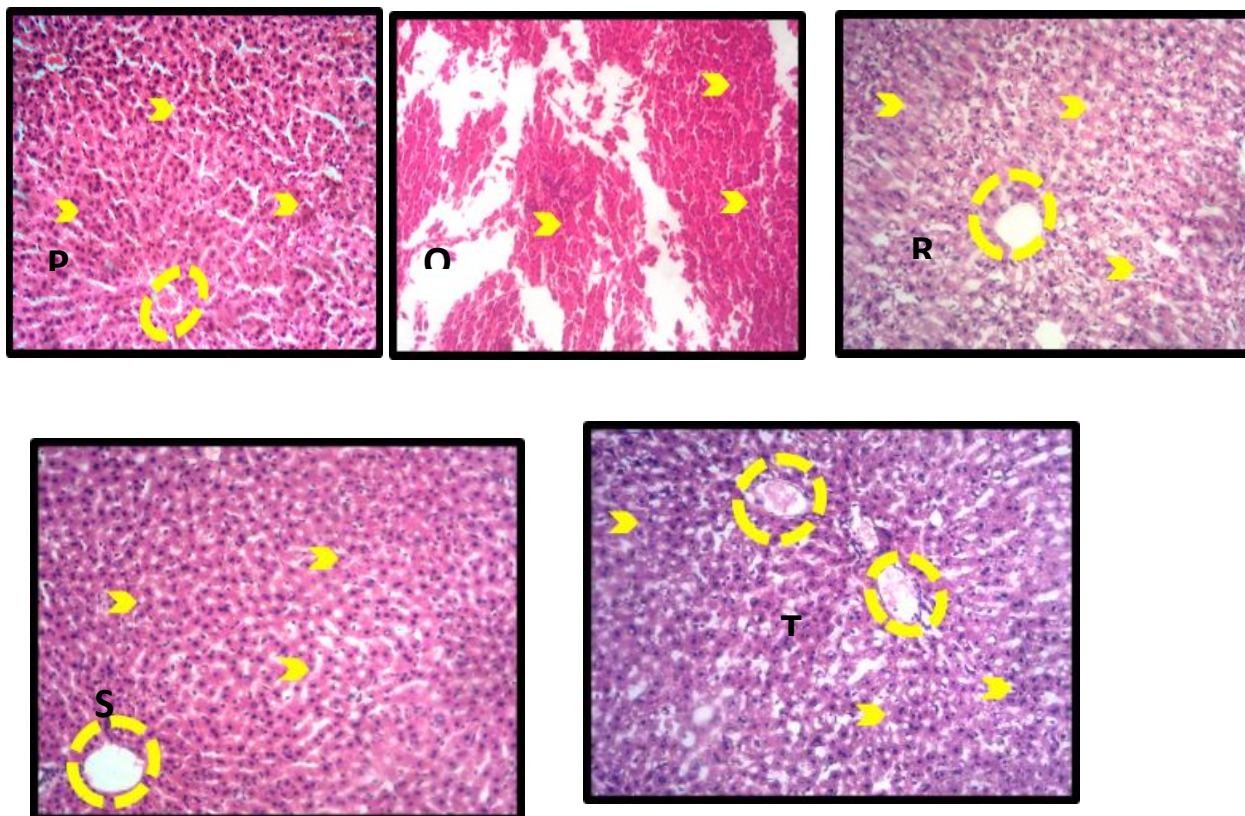


Plate 4 (P-T): Photomicrographs showing the liver of broilers fed the experimental diets, control (P) with normal hepatocyte, Raw MOSC (Q) with severe haemorrhage of hepatic blood vessel, 10%AHMOSC (R) with mild hemorrhage of hepatic blood vessel, 20%AH MOSC (S) with mild hemorrhage of hepatic blood vessel and 30%AHMOSC (T) with normal hepatocyte..

H/E Stain Mag. X400

Growth performance characteristics: The observed improved growth performance of birds with the addition of graded level of ammonium hydroxide in the diets of the birds indicate the efficiency of the processing method to reduce the various anti-nutrients inherent in MOSC thereby enhancing its better utilization by the birds. This present study supports the findings of Barlett (1998) who reported reduction in toxicity of mustard meal after alkali treatment in broilers.

The increase in the feed consumption of the animals fed ammonium hydroxide treated MOSC diet in contrast to those on the untreated MOSC may be due to decrease in the content of tannins in the treated feed sample. Tannins form insoluble complexes with proteins and the tannin-protein complexes may be responsible for the anti-nutritional effects of tannin containing foods (Habtamu and Nigussie, 2014).

The average daily weight gains of the birds was a reflection of their daily feed intake as the birds on the treated groups (treatments with 10, 20 and 30% ammonium hydroxide) had the highest average daily weight gains due to the highest feed intake while there was a corresponding decrease in the average daily weight gain in treatments 2 containing raw MOSC which caused decreased feed intake. Ferreira *et al.*, (2008) reported that greater amounts of 7.5 and 100 g/kg *moringa* seed meal inclusion reduced intake, growth rate and live weights of the birds. The authors were of the view that the decline in intake of feed, rate of growth and live weights of the birds were attributable to the negative influence of anti-nutritional

factors present in *moringa* seed meal. Makkar and Becker (1997) found that *moringa* seed kernel and seed meal had greater amounts of anti-nutrients.

The improved feed conversion ratio of the birds fed ammonium hydroxide treated MOSC based diet, in contrast to those on the raw MOSC diet, underscore a better use of the feed resource. There was an indication that birds fed the control diet and the treated groups had better feed conversion ratios. The best FCR observed in birds on the control diet and the treated groups was attributable to the nonexistence of anti-nutrients in the control diet and subsequent reduction in the ANFs present in MOSC by the treated method used.

This was due to better usage of the nutrients in the control diet without anti-nutritional factors and 30% ammonium hydroxide based diet with reduced contents of ANFs than 10% and 20% ammonium hydroxide based diets. This report agrees with earlier discovery of Emiola *et al.*, (2003) that reduction in ANFs as a result of better treatment procedures promotes the performance of the birds.

There were inconsistencies in the weights of the internal organs of the birds fed with the raw and treated groups and inclusion of ammonium hydroxide treated *Moringa oleifera* seed cake diet could not be said to have either or not enhanced the weights of the organs. The weights of the lung, heart, kidney, proventriculus were not completely ($P>0.05$) affected except the gizzard that was significantly ($p<0.05$) affected.

Nonexistence of mortality in this study is an evidence that most of the anti-nutrients in the treated MOSC diets have been destroyed or greatly reduced. Some of these anti-nutrients are

known to be responsible for the death of farm animals.

The observed significant ($p < 0.05$) difference in serum urea between the treated and the control groups support the findings of Eggum (1976); Esonu *et al*; (2001); Iyayi and Taiwo (2003) who reported that total protein and serum urea content depend on the quality and quantity of the protein contained in the diet. The increase in serum urea observed in raw diet (treatment 2) is an indication of ANFs that impaired utilization of feed resource in broilers.

The observed increase in albumin and total protein with the inclusion of graded level of ammonium hydroxide in the diets of the birds indicates nutritional acceptability of treated MOSC in satisfying the protein requirements of the birds. Onifade and Tewe (1993) had earlier reported that serum protein, albumins and globulins are generally influenced by total protein intake.

ALP, AST and ALT decreased with the inclusion of graded level of ammonium hydroxide in the diets of the birds. The increase in ALT values in birds in treatments 2, compared with the control and treated group suggest that there may be a likelihood of liver damage by the raw MOSC.

Haematological indices like Packed Cell Volume (PCV), Haemoglobin (Hb) and Total Protein (TP) tended to be higher for the control diet than for the MOSC diets although the differences were not significant ($p > 0.05$). Similar findings have been reported by Apata (2004) who treated *Prosopis africana* seeds in the diets of laying hens. The author concluded that grain legume seeds if properly processed can be included in poultry ration with no deleterious consequence.

The range of $1.52\text{-}2.36 \times 10^{12}/\text{l}$ for red blood cells (RBC) observed in this study is lower than $3\text{-}6 \times 10^6/\text{dl}$ standard for chicken as reported by Madubuike and Ekenyem (2006). It was observed that with the inclusion of graded level of ammonium hydroxide in the diets of the birds, the red blood cells (RBC) increased. The high iron content of *moringa* kernels may have influenced erythropoiesis which in turn favoured the production of RBC. The increase in the haemoglobin concentration (Hb) with the inclusion of graded level of ammonium hydroxide in the diets of the birds could also be related to the superior content of iron in the MOSC. The range in Hb values between 7.30-9.3% recorded in this research was within the range of 7.00-18.6g/dl reported by previous investigators (Mitruka *et al.*, 1977) as normal values of Hb for chickens. This implies that addition of *moringa* kernel meal in diet of broiler has no noxious consequence on the health condition of the birds with regards to blood composition. Finding in this study indicate that the blood of the animals had a substantial oxygen carrying capability which in turn means that nutrients was not impaired when processed *moringa* seed meal was included in broiler's diet.

The range of PCV (28.6-31.8) across all the experimental diets is similar to the normal values of 30-33% reported by Swensor (1970); 25-45% reported for broilers at six weeks of age by Mitruka and Rawnsely (1997) and 28 - 30.70% reported by Ogbuewu *et al.* (2010).

MCV of 129.6-132.55fl obtained in this work is higher than the normal range of 115-127fl reported by Swenson (1993). The observed increase in the MCV with the inclusion of graded level of ammonium hydroxide in the diets of the birds suggests that the size of the red blood cells were enhanced by the volume occupied by each red blood cell.

Similar MCH values observed in all the dietary treatments implies that the inclusion of the processed MOSC in diets did not influence the average weight of the haemoglobin in the blood. However, the range of 34.95-40.600pg MCH obtained in this study compared with 25.4-33.4pg reported by Mitruka *et al.* (1977) for typical chickens.

The mean cell haemoglobin concentration (MCHC) value of 28.05-29.75g/dl recorded in this work is within the stipulated values of 26-35g/dl reported by Mitruka and Rawsley (1997). Low MCHC implies that a component of packed RBC contains fewer haemoglobin than standard and an elevated MCHC implies that there is higher haemoglobin in a component of RBC. Decreased MCHC content may indicate blood loss, iron and B₆ deficiency. In this present study the higher MCHC compared with the values reported by Mitruka and Rawsley (1997) implies that the birds did not exhibit any anaemic symptom.

White blood parameters for the broiler chicken fed ammonium hydroxide based diets followed similar trend with RBC. This suggests that the bird's capacity to combat disease attack was not hindered by the diets.

The significant ($p < 0.05$) increase in the white blood cells (WBC) of the birds fed control and raw MOSC compared with their counterparts fed graded level of ammonium hydroxide treated MOSC could be as a result of the limited exposure of the birds to the anti-nutrients in MOSC. However, Chineke *et al.* (2006) reported that dissimilarities in haematological indices may be induced by nutrition, age, sex, genotype environment, and hormonal factors. Soetan *et al.* (2013) had earlier reported that animals with few white blood cell counts are susceptible to

high risks of disease attack while those with high counts are capable of generating antibodies in the process of phagocytosis and have a high degree of resistance to diseases.

The significant ($p < 0.05$) increase in crude protein retention in the control and treated groups may be attributable to reduction in anti-nutrients existing in MOSC that enhanced better feed intake and efficient usage of the diet by the birds resulting in increased nutrient retention.

Crude fat retention; Ether extract was uniformly conserved by the birds of the control and the treated groups probably due to high fat content of the test ingredient and the very effective usage of dietary fat by the birds .

The observed decrease in fat and fibre retention of the birds fed the raw MOSC groups was due to defective usage of the diet by the birds on raw diet containing various anti-nutrients.

Annongu, (2012), reported that saponins cling to the cells of the small intestine and eventually affecting the absorption of nutrients across the wall of the intestine The implication of fibrous ingredients on the nutrient retention by birds has also been well established (Panigrahi and Powell,1991; Onifade,1993).

In this study, the nutrient retention of the birds fed the treated MOSC was better than those fed with raw MOSC diet and the better nutrient retention of the birds fed with the graded level of ammonium hydroxide treated MOSC implies the effectiveness of the processing of raw MOSC for broilers. Histology examination of the liver sections of the birds fed with raw MOSC in diet of the birds showed abnormal liver architecture attributable to the existence of

anti-nutritients in the feed. With the inclusion of the graded level of ammonium hydroxide treated MOSC in diet of the birds, the liver architecture appears normal with characteristic delineation.

CHAPTER SEVEN

7.0

CONCLUSION AND RECOMMENDATIONS

From this study, broilers fed treated MOSC diet performed better than those fed raw MOSC diet while those fed the control diet performed significantly better than those fed raw and treated MOSC diet respectively. Tannin, phytate and saponins were the main anti-nutritional factors that limit MOSC use in poultry diet. The treatment methods used in this study were not sufficient to totally detoxify the anti-nutrients present in MOSC. The profitability of replacing soyabean protein with MOSC in diets did not favour its inclusion in broiler's diet. It can be concluded that soyabean meal is superior to *Moringa oleifera* seed cake diet based on the unavailability of the nutrients to the birds. The best result obtained from replacing soyabean with treated *Moringa oleifera* seed cake diet was 30% Ammonium hydroxide treatment at 5% inclusion level.

7.1

Recommendation

This study therefore recommends further studies be carried out on the treatment methods for effective detoxification of MOSC for its usage at higher level of inclusion in broiler's diet.

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